Interactions Between Microglia and T Cells in Multiple Sclerosis Pathobiology

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Brain-resident microglia and T lymphocytes recruited into the central nervous system both play important roles in the neuropathology of multiple sclerosis. The microglia and recruited T cells are in close proximity in lesions of multiple sclerosis and in animal models, suggesting their potential for interactions. In support, microglia and T cells express a number of molecules that permit their engagement. Here we describe the interactions between T cells and microglia and the myriad responses that can result. These interactions include antigen presentation by microglia to activate T cells, the T cell activation of microglia, their progressive stimulation of one another, and the production of injurious or neurotrophic outcomes in their vicinity. Important considerations for the future include the nature of the T helper cell subsets and the M1 and M2 polarized nature of microglia, as the interactions between different subsets likely result in particular functions and outcomes. That T cells and microglia are in proximity and that they interact in lesions in the central nervous system implicate them as modifiers of pathobiology in multiple sclerosis.

Introduction

Multiple sclerosis (MS) is an inflammatory disease of the central nervous system (CNS) characterized by debilitation resulting from loss of CNS components, particularly of myelin and axons. Most patients suffer from relapsing-remitting MS, which involves relapses followed by remission of symptoms. Over time, most patients progress into the secondary stage of the disease during which their disability progresses without apparent relapse or remission. Some patients enter directly into the progressive form of the disease from the beginning, and this is called primary progressive MS (Confavreux and others 2000).

MS is a heterogeneous disease in that lesions appear throughout the CNS (Ontaneda and others 2012). As a result, the disease course and symptoms are impossible to predict and can be very difficult to manage. Current therapies target the aberrant immune responses in an attempt to reduce relapses and slow progression of the disease; there are currently no therapies for individuals in the progressive stage of the disease (Bruck and others 2013).

Much of our understanding of MS has been gained through the use of animal models, including experimental autoimmune encephalomyelitis (EAE). A number of animal species can be used in EAE, but mice and rats are most often employed. The animal is commonly immunized with a myelin protein or peptide such as myelin oligodendrocyte glycoprotein, usually along with pertussis toxin and an adjuvant. This model mimics much of the immunology and neuropathology observed in human MS patients (Jones and others 2008; Rangachari and Kuchroo 2013).

Most immune cell subsets including T lymphocytes can be found in active lesions in the CNS of patients with MS. Correspondingly, the immune-competent cell intrinsic to the CNS, microglia, becomes activated. The infiltrated T cells and microglia can engage in significant crosstalk with the potential of eliciting myriad outcomes (Ransohoff and Brown 2012; Codarri and others 2013). Indeed, preactive MS lesions, defined by the presence of small clusters of activated microglia in normal appearing brain, often resolve until infiltrated by lymphocytes (van Noort and others 2011), highlighting the impact of T cells on microglia functions in MS. Here we describe the role of microglia and T cells in MS pathobiology focusing in particular on their interactions in the CNS in MS and in animal models. We first introduce microglia and T cells in isolation, discuss their potential for interactions, and then describe studies of the various outcomes resulting from their interactions with one another.

Microglia

Microglia are the innate immune cells intrinsic to the CNS. They populate the brain in early embryonic life in mice, from primitive myeloid progenitors found in the yolk sac (Ginhoux and others 2010). Microglia exhibit many

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properties similar to monocyte-derived macrophages that infiltrate into the CNS in response to injury, even though monocytes have a different lineage and are derived from precursors in the bone marrow throughout life (Schulz and others 2012). Indeed, the activated microglia and macrophages in the injured CNS are often indistinguishable from one another by histological or immunohistochemical analyses, and they are often collectively referred to as macrophages/microglia. Thus, some of the characteristics ascribed to microglia in T cell–microglia interactions could have been contaminated by data on macrophages.

In normal conditions, microglia are found throughout the CNS and, although they are described to possess a relatively quiescent phenotype, their processes are continually surveying the microenvironment for threats (Nimmerjahn and others 2005). Microglia express a variety of scavenger receptors and toll-like receptors important for activation and regulation of innate immunity (Goldmann and Prinz 2013). In response to injury or infection, therefore, microglia are rapidly activated and change their phenotype.

A number of both protective and detrimental roles have been attributed to activated microglia in the CNS (Czeh and others 2011). Upon activation, microglia release a variety of cytokines and chemokines involved in promoting inflammation and recruiting peripheral immune cells, including T cells and macrophages (Chastain and others 2011). Excessively activated microglia can be toxic to neurons through the elaboration of proteases, glutamate, free radicals, including nitric oxide, and other toxic molecules (Takeuchi 2010; Nikic and others 2011). In a recent study of cortical lesions in MS, patients with rims of activated microglia had a less favorable disease course than those that did not (Kooi and others 2012). Studies using transgenic and chimeric mice to affect microglia in the CNS revealed that arresting microglial function can prevent many inflammatory processes and abrogate EAE disease (Heppner and others 2005); it should be emphasized that monocytes/macrophages were also affected in this study. Nonetheless, in EAE, a combination of techniques including parabiosis and myeloablation to replace circulating progenitors without affecting CNS-resident microglia concluded that the infiltration of macrophages may be more important that microglia activation in the evolution and severity of disease (Ajami and others 2011).

Microglia have also been implicated in protection and repair during CNS inflammation. It has been demonstrated that microglia play an important role in the clearance of dead cells and debris (Napoli and Neumann 2010); the failure to phagocytose dead and damaged cells significantly impairs resolution of inflammatory processes and prevents remyelination (Miron and others 2013). Another mechanism accounting for the benefits of microglia is that they can produce significant amounts of growth factors that are trophic and protective for axons and neurons, including brain-derived neurotrophic factor (Stadelmann and others 2002). We refer the reader to a review that details extensively the benefits and detriments of activated microglia in MS and its models (Rawji and Yong 2013).

T Cells

It is generally accepted that T cells are very important in the inflammatory pathobiology of MS. Both CD4+and

CD8+T cells are found in MS lesions and are oligoclonally expanded, but CD4+T cells are often the focus in MS research. When CD4+T cells are activated after antigen presentation, they polarize to a specific effector phenotype dependent on the cytokine milieu in their microenvironment; commonly recognized T cell phenotypes include T helper (Th)1, Th2, Th17, and regulatory T cells (Kleinewietfeld and Hafler 2013). All of these subsets are encountered in MS, with an emphasis on the pathogenic potential of proinflammatory Th1 and Th17 cells, and the possible dysregulation of anti-inflammatory Th2 and regulatory T cell subsets (Dittel 2008). The exact contribution of specific T cell phenotypes in MS remains to be completely characterized. One way that T cells may influence MS pathobiology is through interactions with microglia.

Microglia and T Cells Are in Close Proximity in Lesions of MS and Its Animal Models

In the spinal cord of EAE-immunized mice, significant amounts of CD3+T cells can be found in regions of accumulation of macrophages/microglia identified by the marker, Iba1; this is the case also in relatively recent lesions where the myeloid cells are still of the ramified microglia morphology. Similarly, in MS, several studies have demonstrated that activated microglia and T cells can be found in close proximity in CNS lesions (Ferguson and others 1997; Kuhlmann and others 2002; Hucke and others 2012). As noted earlier, preactive MS lesions defined by the presence of small clusters of activated microglia often resolve until infiltrated by lymphocytes (van Noort and others 2011). A study of cortical biopsy samples from human patients with demyelination and inflammation, many of whom were later confirmed to have MS, found that activated microglia were present in all demyelinating lesions, and that T cells were in their proximity (Lucchinetti and others 2011).

Lysophosphatidylcholine (LPC) rapidly and briefly demyelinates axons and has been used to investigate demyelination and remyelination as a model of MS. The microinjection of LPC into the spinal cord led to a rapid influx of T cells for a duration of 6–12 h accompanied by activation of microglia/macrophages, and demyelination (Ghasemlou and others 2007). When LPC was injected into nude mice that do not possess any T cells, activation of microglia/macrophages was attenuated while recruitment and numbers of microglia and macrophages were unchanged (Ghasemlou and others 2007). In addition to the LPC model, it has been shown that the interaction between autoreactive T cells and macrophages/microglia is a critical step in the development and progression of EAE (Hucke and others 2012). Overall, there is ample data of the proximity of microglia with T cells in lesions of the CNS in MS and its models, providing the potential of both cell types to interact to affect outcomes.

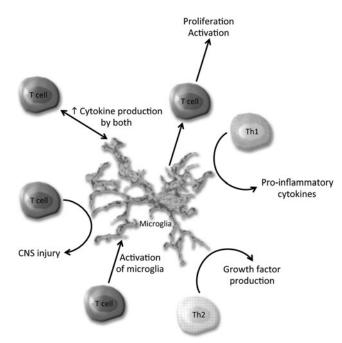## Microglia and T Cells Express Molecules That Facilitate Their Interactions

Antigen presentation is the phenomenon whereby antigen-presenting cells present a peptide within the groove of its major histocompatibility complex (MHC) molecule to engage a specific T cell receptor (TCR) of T cells; the process requires the involvement of a myriad of costimulatory

molecules for the productive activation of T cells. The genes encoding molecules of the immune system, including of costimulatory molecules, are risk factors for MS (International Multiple Sclerosis Genetics and others 2011), and MHC genes are the greatest known genetic risk factors for this disease (Dyment and others 2005). Microglia express MHC class I and II molecules in addition to a range of costimulatory molecules, and they are competent antigen presenting cells (Almolda and others 2011). In a rat EAE model of MS, microglia were described as the primary antigen presenting cells within the CNS (Almolda and others 2011). In live imaging cell tracking experiments, myelin basic protein (MBP)-specific T cells, but not ovalbumin-specific T cells, formed synapse-like contacts with other cells, presumably including microglia, within the CNS to subsequently produce EAE disease; this process was blocked by anti-MHC class II antibodies (Kawakami and others 2005).

One well-defined costimulatory pathway involves the molecules B7.1 and B7.2 on antigen-presenting cells that can interact with either CD28 or CTLA on T cells. Interaction of B7.1 or B7.2 with CD28 provides the T cell with a positive activating signal inducing proliferation, differentiation, and cytokine production; interaction of B7.1 or B7.2 with CTLA delivers a negative inhibitory signal to the T cell that can induce anergy or apoptosis (Salama and others 2003). It has been demonstrated that microglia express B7.1, and that this expression increases upon their activation (Raivich and Banati 2004). Many costimulatory molecules are carefully regulated throughout different stages of disease in a rat EAE model. Cells labeled with microglia markers expressed both MHC class I and II, and these cells were B7.2 positive during recovery and postrecovery of disease in the presence of T cells expressing CTLA-4; in contrast, levels of CTLA-4 were low during induction and early stages of disease (Almolda and others 2010).

Another important costimulatory molecule on T cells is CD40. CD40 knockouts are resistant to EAE, and deficiency of the CD40 ligand, CD154, also leads to EAE resistance (Becher and others 2001). The study of bone marrow chimeras showed that CD40 knockout specifically within the CNS significantly abrogated migration of peripheral macrophages to the CNS, and while the total number of T cells seemed unaffected, their activation within the CNS was significantly impaired; this correlated with an improvement in EAE clinical score (Ponomarev and others 2006). Another important costimulatory molecule for T cells is OX40 (CD134). Microglia/macrophages that express the OX40 ligand are closely correlated with T cells expressing OX40 during autoimmune inflammation (Weinberg and others 1999).

What Happens When T Cells and Microglia Interact?

Several outcomes occur after the interaction between T cells and microglia (Fig. 1 and Table 1). Earlier studies reported that microglia were inefficient antigen-presenting cells (Sedgwick and others 1991; Ford and others 1995) and, indeed, induced death to T cells rather than stimulating their proliferation (Ford and others 1996). Subsequently, others determined that both unactivated (CD45 low) or activated microglia (CD45 intermediate) cultured from normal or IL-2 transgenic brain, respectively, were weak stimulators of proliferation of naïve T cells but could promote their dif-

FIG. 1. The multiple outcomes that can result from the interactions between T cells and microglia. Please see text for details. CNS, central nervous system; Th, T helper.

ferentiation into Th1 effector cells that produced IFN-γ (Carson and others 1999). Microglia from an ovalbumin-specific TCR transgenic line stimulated with IFN-γ to express MHC class II, CD40, and ICAM-1 were found to have the capacity to present ovalbumin leading to T cell proliferation and their differentiation into Th1 and Th2 cells (Aloisi and others 1998). Comparative studies by the same group (Carbone and others 1999) determined that IFN-γ-preactivated microglia were less efficient than dendritic cells in inducing naive T cell proliferation, but more efficient than B lymphocytes or astrocytes. In an EAE study of the kinetics of myelin antigen uptake by cells in the CNS, microglia were determined to be the first cell type to contain myelin fragments within the CNS, followed later by infiltrating dendritic cells; the authors postulated that microglia may have an important local role in modulating T cell responses before the arrival of professional antigen-presenting cells (Sosa and others 2013). A multistep process involving cytokines (granulocyte macrophage–colony stimulatory factor and IFN-γ) and cognate signaling (B7–CD28 and CD40–CD40 ligand interactions) were found by others to be required for microglia to become professional antigen-presenting cells that presented MBP to both unprimed and primed T cells (Matyszak and others 1999). More recent studies have affirmed that microglia expressing both MHC class I and II molecules can prime or activate CD8 + and CD4 + T cells, respectively (Almolda and others 2011). Another study found that microglia could even present antigen to naïve CD8 + T cells that were injected into the brain, so long as the microglia were adequately stimulated (Jarry and others 2013).

While the above illustrates that microglia can stimulate T cells, it is notable that T cells can also stimulate microglia. In organotypic cultures, the introduction of myelin-reactive T cells upregulated MHC class I and II molecules on

TABLE 1. THE PRODUCTS OF MICROGLIA INTERACTIONS WITH T CELLS AND THEIR PRESUMED
OUTCOMES IN MULTIPLE SCLEROSIS PATHOBIOLOGY

Cell–cell interactions	Molecules produced	Presumed outcome in MS
Microglia-activated nonpolarized T cell	IFNγ (Carson and others 1999)	Promotes activation of microglia and T cells
	IL-10 (Chabot and others 1999)	Generates a regulatory/anti-inflammatory environment
	iNOS (Dasgupta and others 2002)	Produces nitric oxide, a free radical
	TNFα (Chabot and others 1997, 1999; Becher and others 2000)	Promotes inflammation
	IL-12 (Becher and others 2000)	Promotes a Th1 response
Microglia-Th1 Cell	IFNγ (Aloisi and others 1998, 1999b)	Promotes activation of microglia and T cells
	IL-12 (Aloisi and others 1999a)	Promotes a Th1 response
	TNFα (Aloisi and others 1999b)	Promotes inflammation
	Prostaglandin E_2 (Aloisi and others 1999a)	Anti-inflammatory
		Promotes a Th2 response
		Inhibits IL-12 production by APCs
	IL-2 (Aloisi and others 1998, 1999b)	Promotes T cell proliferation and survival
Microglia-Th2 Cells	Neurotrophin-3 (Roy and others 2007)	Supports cell survival
	IL-4 (Aloisi and others 1998, 1999b)	Generates an anti-inflammatory environment
		Promotes a Th2 phenotype
	Brain-derived neurotrophic factor (Roy and others 2007)	Supports cell survival

iNOS, inducible nitric-oxide synthase; MS, multiple sclerosis; Th, T helper; APC, antigen presenting cell.

microglia, and promoted the microglial phagocytosis of damaged axons (Gimsa and others 2000). MBP-primed T cells isolated from female and castrated male, but not from male mice, caused the expression of inducible nitric-oxide synthase (iNOS) and proinflammatory cytokines (IL-1, IL-6, and TNF-α) in microglia in culture by a cell–cell contact-dependent mechanism (Dasgupta and others 2005); these authors postulated that the gender-sensitive activation of microglia by neuroantigen-primed T cell contact could be one of the mechanisms behind the higher prevalence of MS in females. In EAE-afflicted mice, time-course studies show that the infiltration of Th1/Th17 cells into the CNS coincided with the activation of CD11b + microglia and the local production of IL-1, IL-6, and TNF-α (Murphy and others 2010). While Th1 cells induce the production of proinflammatory molecules by glia including microglia, MBP-reactive Th2 cells induced neurotrophins but not proinflammatory cytokines from microglia and astrocytes in culture (Roy and others 2007).

The above highlights articles of the microglia stimulation of T cells, and of articles that T cells can activate microglia. We have conducted studies to evaluate the propensity of microglia and T cells to mutually stimulate one another. Initial experiments demonstrated that polyclonally activated human T cells induce the microglial production of TNF-α through a cell–cell contact-dependient mechanism involving the interaction of VLA4 on T cells with its ligand VCAM-1 on microglia (Chabot and others 1997). In culture, adult human microglial cells assume different morphologies, although the majority is elongated in shape. However, when cocultured with activated T cells, microglia became amoeboid in appearance (Chabot and others 1997), indicating an elevated state of activation.

A similar pattern was established for IL-10, a cytokine with significant anti-inflammatory or regulatory properties. Although neither microglia nor T cells alone released substantial amounts of IL-10, coculturing the 2 induced significantly higher levels of the cytokine, compared with culturing microglia alone; the IL-10 production was dependent on cell–cell contact and involved the CD40, B7, and CD23 pathways (Chabot and others 1999). When analyzed by flow cytometry for cellular content, both T cells and microglia were found to be active in IL-10 expression. Thus, in T cell–microglia interactions, both the T cells and microglia receive the signals to mutually regulate cytokine production. Besides TNF-α and IL-10, cytokines that were produced in interactions of human T cells with human microglia included IL-1β, IL-4, IL-6, IL-12, and IL-13 (Chabot and others 2002).

Besides the aforementioned pathways, a number of other molecules have been identified that may have functions in mediating T cell interactions with microglia. The deficiency of peroxisome proliferator-activated receptor-γ (PPARγ) in mice led to exacerbation of disease pathology, whereas pharmacological activation of PPARγ reduced T cell interaction with myeloid cells within the CNS leading to reduced EAE severity and neurotoxicity (Hucke and others 2012). VLA4 is expressed by MBP-primed T cells and required for microglia activation and cytokine production through NF-κB and C/EBPβ transcription factors (Dasgupta and others 2003). The blockade of α5 and β3 integrins prevented Th2 cells from inducing microglial expression of neurotrophic factors (Roy and others 2007).

We contend that the interaction between T cells and microglia is of pathologic importance in MS (Table 1), owing to the large amounts of proinflammatory cytokines that are generated. In support, we have determined that immunomodulators that have established (glatiramer acetate and interferon-β) or purported (minocycline and intravenous immunoglobulins) value in the treatment of MS attenuate the microglia-T cell production of cytokines (Chabot and others 1997, 2002; Giuliani and others 2005; Janke and Yong 2006).

While microglia and T cell interactions can promote a proinflammatory milieu, microglia in culture can lead to the generation of FoxP3 + regulatory T cells, dependent on the

antigen dose and the concentration of cytokines such as IFNγ; the microglia-induced regulatory T cells ameliorated EAE when adoptively transferred into mice (Ebner and others 2013).

Impact of T Cell–Microglia Interactions on MS Neuropathology

In studies of MS autopsied specimens, the correspondence of activated macrophages/microglia and T cells occurs in close proximity to, or within, prominent areas of demyelination and axonal injury (Ferguson and others 1997; Kornek and others 2000; Kuhlmann and others 2002; Hucke and others 2012). In another study of MS specimens, regions displaying signs of oxidative damage in oligodendrocytes correlated significantly with the presence of both T cells and macrophages/microglia (Haider and others 2011). Thus, an outcome of interactions between activated T cells and microglia *in situ* is the production of damage to axons/neurons and oligodendrocyte/myelin. This can be modeled in tissue culture where activated T cells can kill neurons in an antigen-non-specific manner (Giuliani and others 2003; Nitsch and others 2004), and where the concurrent presence of microglia exacerbates neuronal death (unpublished observations).

Conversely, T cells may have beneficial roles during neuroinflammation through their signaling to microglia. Regulatory T cells pre-exposed to microglia and then incubated with organotypic hippocampal slices ameliorated the ongoing neuronal death in the hippocampus (Kipnis and others 2004). In a model of spinal cord injury, immunization with a myelin peptide combined with transplantation of neural progenitor cells into the cerebrospinal fluid promoted recovery; the postulated mechanism involved an interaction between T cells and microglia, leading to a growth factor milieu that promoted the recruitment of neural progenitor cells to the lesion site (Ziv and others 2006a). In a model of Alzheimer's disease, immunization with the MS medication glatiramer acetate improved neurogenesis, and this was attributed to the generation of IL-4-producing T cells that interacted with microglia to switch the latter to a permissive phenotype (Butovsky and others 2006). The supernatant collected from the coculture of microglia with glatiramer acetate-reactive T cells reduced the death of retinal ganglion cells in culture, presumably through mechanisms that include the delivery of neurotrophic factors (Qian and others 2013).

There are other aspects of T cell–microglia interactions that are relevant to MS. MBP-specific T cells initiate a different microglial response compared with ovalbumin-specific T cells at the site of neuronal damage; in this regard, the autoreactive T cells produced a phagocytic phenotype in microglia resulting in the latter being better able to clear myelin debris compared with microglia interacting with T cells primed with nonself antigen (Nielsen and others 2009). The interaction between autoreactive T cells, but not T cells reactive to nonself antigen, with microglia is also important for neurogenesis and trafficking of neural stem cells (Ziv and others 2006b).

Refinements of T Cell–Microglia Interactions

In recent years, macrophages have been subdivided into those that are of the M1 phenotype that produce proinflammatory cytokines such as IL-1, IL-12, or TNF-α, or M2 cells that produce anti-inflammatory cytokines, including IL-10 and IL-1 receptor antagonist. Other markers that distinguish these cells include the enzymes iNOS for M1, and arginase 1 and YM1 for M2, and surface receptors FcRγ (CD16/32) and CD14 for M1, and macrophage scavenger receptor 1 (MSR1, CD204) and macrophage mannose receptor (CD206) for M2 cells (Mosser and Edwards 2008; Martinez and others 2009; Murray and Wynn 2011; Prinz and others 2011). Microglia have also been similarly classified, where the phagocytosis of myelin was greater in M2 human microglia compared with M1 cells (Durafourt and others 2012). In mice, the systemic administration of *ex vivo*-activated M2 monocytes ameliorated ongoing EAE and reduced proinflammatory cytokine expression pattern in lesions, emphasizing their role in the induction of recovery (Mikita and others 2011), Thus, the inflammatory or neuroprotective/injurious outcomes of the interaction of activated T cells with M1 or M2 microglia may potentially differ, but we are not aware of any studies in this regard. Similarly, the interactions of Th1, Th2, or Th17 lymphocytes with M1 or M2 microglia may result in grossly different outcomes, and this remains to be investigated.

Conclusions

The interactions between microglia and T cells are crucial in MS pathobiology. The results of these interactions are variable (Fig. 1) and the outcomes can be conflicting, largely because these interactions depend on a wide variety of factors. The role of the microenvironment where cells interact, as well as the phenotype of the cells, may be significant contributors to the resultant outcomes. Furthermore, the species, strain and gender, and the underlying genetics may also influence the T cell interaction with microglia. The nature of the antigen may also be an important contributing factor in these interactions, although T cells appear to ignore antigen specificity for interaction with microglia upon their activation. Future studies should consider these differential factors while also acknowledging that macrophages may contaminate the results. That the interaction of T cells with microglia can affect the outcomes of protection or injury to CNS elements, including neurons, makes it imperative to further define characteristics of T cell–microglia interactions, and how to sway these interactions toward benefits.

Author Disclosure Statement

No competing financial interests exist.

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