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REVIEW

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Antigen presentation in autoimmunity and CNS inflammation: how T lymphocytes recognize the brain

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Abstract The central nervous system (CNS) is traditionally viewed as an immune privileged site in which overzealous immune cells are prevented from doing irreparable damage. It was believed that immune responses occurring within the CNS could potentially do more damage than the initial pathogenic insult itself. However, virtually every aspect of CNS tissue damage, including degeneration, tumors, infection, and of course autoimmunity, involves a significant cellular inflammatory component. While the blood–brain barrier (BBB) inhibits diffusion of hydrophilic (immune) molecules across brain capillaries, activated lymphocytes readily pass the endothelial layer of postcapillary venules without difficulty. In classic neuro-immune diseases such as multiple sclerosis or acute disseminated encephalomyelitis, it is thought that neuroantigen-reactive lymphocytes, which have escaped immune tolerance, now invade the CNS and are responsible for tissue damage, demyelination, and axonal degeneration. The developed animal model for these disorders, experimental autoimmune encephalomyelitis (EAE), reflects many aspects of the human conditions. Studies in EAE proved that auto-reactive encephalitogenic T helper (Th) cells are responsible for the onset of the disease. Th cells recognize their cognate antigen (Ag) only when presented by professional Ag-presenting cells in the context of major histocompatibility complex class II molecules. The apparent target structures of EAE immunity are myelinating oligodendrocytes, which are not capable of presenting Ag to invading encephalitogenic T cells. A compulsory third party is thus required to mediate between the attacking T cells and the myelin-expressing target. This review will discuss the recent advances in this field of research and we will discuss the journey of an auto-reactive T cell from its site of activation into perivascular spaces and further into the target tissue.

Keywords Experimental autoimmune encephalomyelitis · Multiple sclerosis · Autoimmunity · Inflammation · CNS · Ag-presentation

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Introduction

The central nervous system (CNS) is often referred to as a site of limited immune surveillance. This concept dates back to the seminal work of Medawar [1] and Barker and Billingham demonstrating that allografts fare better in the CNS compared to other tissues [2, 3]. Its "immunologically privileged" status has for long been attributed to the blood–brain barrier (BBB) and the lack of classic lymph vessels. However, the concept of a BBB describes a mechanical diffusion barrier for hydrophilic molecules (including plasma proteins such as antibodies and complement) formed by specialized endothelial cells at the level of capillaries, while recruitment of leukocytes takes place in postcapillary venules. In fact, it became apparent that leukocytes readily cross the brain endothelial cells to reside in perivascular spaces or move on into the neuropil (for a review, see [4, 5]. Moreover, it is now established that antigens efficiently drain into cervical lymph nodes via the cribroid plate and perineural sheath of cranial nerves [6], and recent data suggest similar routes for antigen-presenting cells [7–9]. In fact, the CNS is the apparent target of heavily studied autoimmune diseases such as multiple sclerosis (MS), acute disseminated encephalomyelitis (ADEM), and its animal model experimental autoimmune encephalomyelitis (EAE). Although the etiology of MS remains to be definitively established, the most widely accepted view of the pathogenesis of the disease implicates a cellular immune response as a central requirement (reviewed in [10]). This view is supported by the histopathologic observations of the presence of activated T cells in the perivascular spaces and the parenchyma in the early phases of the disease. These inflammatory lesions are the apparent substrates corresponding to the recurrent lesions seen with magnetic resonance imaging [11–13].

Studies of the EAE model have helped define the sequence of events involved in the development of autoimmune CNS-directed inflammatory disease. This sequence of immunopathological events can be subdivided into two major phases:

1. Initial T-cell priming/activation
2. Subsequent recruitment and effector phase

T-cell priming can be initiated by systemic immunization with myelin Ags [10]. Protein Ag and peptides are presented by Ag-presenting cells (APCs) within secondary lymphoid organs leading to the activation and expansion of neuroantigen-reactive T cells. The major antigens that can induce EAE include myelin basic protein (MBP), proteolipid protein, and myelin oligodendrocyte glycoprotein (MOG). Activated myelin-reactive T cells migrate then to the CNS where they reencounter their cognate Ag, in the context of major histocompatibility complex (MHC) class II molecules [14, 15]. Within the CNS, initial Ag presentation is likely to occur in Virchow–Robin spaces (Fig. 1). Perivascular macrophages (MΦs)/"microglia" as well as dendritic cells (DCs) located within this compartment [16, 17] are the only cells associated with the CNS expressing MHC class II molecules. The contribution of astroglia in Ag presentation during the early phase of disease is unlikely (reviewed in [18]). It is widely held that the activation and expansion of encephalitogenic T cells occurs within the systemic immune compartment and not within the CNS. This is certainly the case for EAE and ADEM and we will thus, in this review, not elaborate on T-cell priming during immunization-induced autoimmunity. We will, however, later discuss the hypothesis that CNS inflammation may be initiated within the CNS.

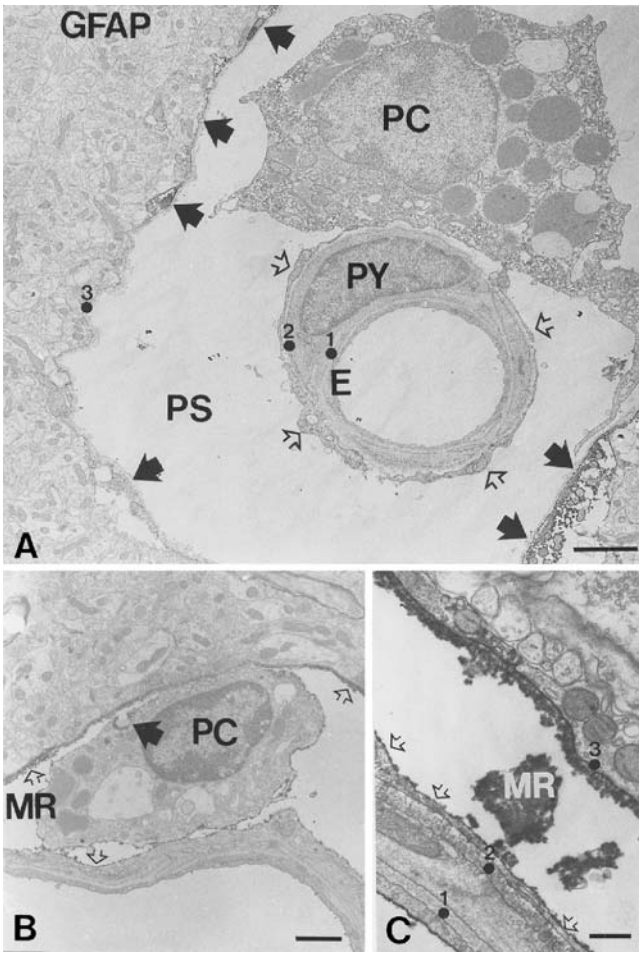

Fig. 1 Topography of the perivascular (Virchow–Robin spaces). **a** Three basal laminae separate the blood from the neuropil. The first one (*1*) is located around the endothelium. At regions where pericytes engulf these cells, the lamina splits and forms a second sheath (*2*). The third membrane (*3*) is associated with the astrocytic endfeet building the glia limitans (labeled with *GFAP* and indicated by *arrows*). The compartment between the second and the third basement membrane is the perivascular space. Note that pericytes (*PY*) and perivascular antigen-presenting cells (*PC*, a macrophage is shown here) belong to different compartments. *Open arrows* indicate cellular processes of the macrophage in vicinity to the vessel wall. **b**, **c** The Virchow–Robin space is connected to the subarachnoid space as evidenced by intraventricular tracer injections. At this point, a dextran-amine (mini-ruby, *MR*) was inserted. Within minutes, the tracer diffuses within the CSF along the perivascular spaces, where it is phagocytosed by perivascular cells (*arrow* in **b**) leading to phagocytosis-dependent labeling. The tracer remains confined to the compartment between the second and third basal lamina. The glia limitans apparently provides a tight barrier (**c**) [118]. *Scale bars*, 0.5 μm

After a few days, a substantially increased number of activated T cells enter the CNS, fostering subsequent T-cell migration deeper into the CNS parenchyma [19]. They can now cause tissue destruction leading to, depending on the model, demyelination and, ultimately, neurologic deficit. During this phase, microglial cells are thought to provide additional Ag-specific as well as MHC-independent signals for invading inflammatory T cells. We propose that, during both phases, the interactions of auto-reactive Th cells with CNS resident cells are critical determinants of disease development. We will focus here on the recent advances made in understanding the contribution of CNS resident and associated cells in autoimmune-mediated tissue injury.

Antigen presentation and T-cell immunity

Antigen presentation is a crucial process during immunity for the generation of protective T-cell responses against pathogens or other foreign structures. This requires APCs capable of engulfing foreign microorganisms and expressing the antigenic peptides in the major histocompatibility complex molecules on the cell surface. CD4 T cells recognize antigens that are associated with the MHC class II molecules, whereas CD8 T cells bind antigen–MHC class I complexes. Both naïve CD4 and CD8 T cells become fully activated only when receiving additional co-stimulatory signals from the APC, whereas the activation of T cells that have already been "primed" or exposed to their antigens may be less dependent on co-stimulation [20, 21]. There are "professional" APCs equipped to initiate a primary immune response by the presentation of antigen to naïve T cells and "non-professional" APCs that can only stimulate a secondary response by the presentation of antigen to primed T cells. The dendritic cells and macrophages of the immune system possess the former ability, whereas B cells and certain stromal cells may engage in secondary T-cell responses [22]. MS and EAE are thought to be mainly mediated by the actions of myelin-reactive CD4 T cells. This notion is most strikingly supported by the fact that the adoptive transfer of myelin-reactive CD4 T lymphocytes into naïve animals can initiate EAE. The role of CD8 T cells remains controversial [23, 24], while several groups could clearly demonstrate that CD8 T cells restricted to an MHC class I myelin antigen can transfer EAE [25–27]; the lack of CD8 T cells in actively immunized mice worsens EAE development [28, 29]. For the purpose of clarity, we will focus on CD4 T cells, but very similar rules apply to CD8 T cells.

Most tissues of the body contain APCs, which capture antigen and migrate to the draining lymph nodes for the initiation of T-cell activation. Once activated, T cells leave the lymphoreticular system, and infiltrate and inspect tissues for foreign antigens that are being displayed on resident APCs. If T cells were fully activated, they proliferate, differentiate, and acquire an increased sensitivity to Ag on restimulation in tissues. The most effective APC of the systemic immune compartment are DCs. DCs are currently being heavily investigated as they appear to conduct major control over the development of immunity. Several distinct subtypes of DCs have been identified and their distinct immune function is being investigated. Under steady-state conditions, DCs constantly capture Ag and present it to T cells. Under such conditions, DCs do not effectively prime naïve T cells due to the lack of appropriate secondary co-stimulatory signals. These "inefficient" T cell/APC encounters are likely to mediate peripheral immune tolerance [30]. The presence of danger signals such as microbial products, however, leads to DC activation, maturation, and ultimately licensing such DCs to prime naïve T cells. DC maturation leads to an arrest of MHC recycling and, hence, the formation of stable MHC/peptide complexes. Furthermore, DC maturation coincides with an increase in the expression of co-stimulatory signals required for efficient priming. We will apply this concept to CNS immunity and inflammation.

Encephalitogenicity

In addition to T-cell activation and expansion, APCs are also capable of regulating the differentiation of effector T cells. During their activation, CD4 T helper (Th) cells can differentiate into at least two subsets of effector cells: Th1 cells, which secrete predominantly IFNγ and TNFα, and Th2 cells which secrete IL4, IL5, and IL10. In general, Th1 cells are responsible for eliciting phagocyte-mediated defense against infections and for promoting the differentiation of CD8 T cells into active killer cells, whereas Th2 cells mediate humoral immune responses by stimulating the differentiation of B cells and can downregulate Th1 responses. As well as their protective roles in host defense, Th1 cells were proposed to also mediate organ-specific autoimmunity and have been implicated in the pathogenesis of EAE and MS [31]. Th2 cells can conversely suppress EAE and MS, although they may exacerbate these diseases in immuno-compromised hosts [32]. Thus, the final composition of the Th cell response to antigen can determine whether the outcome of infectious, inflammatory, and autoimmune responses is beneficial or detrimental. Many factors influence the development of Th1 or Th2 cells: for example, the cytokines (e.g., IL12 favors Th1 cells and IL4 favors Th2 cells), the Ag doses, the co-stimulatory molecules as well as the types of activated APCs. The Th1/Th2 concept has recently undergone a significant paradigm shift. This shift was brought about by the observation that the major Th1 cytokine IFNγ could be demonstrated to not exacerbate disease development. On the contrary, lack of IFNγ actually worsens the disease [33] and even permits disease development in otherwise resistant mouse strains [34, 35]. In addition, mice lacking the most critical Th1-inducing cytokine IL12 are also hyper-susceptible to EAE [36–38]. This and a host of additional data indicate that the Th1/Th2 paradigm does not apply to EAE but that a discrete population of Th_{IL17} cells driven by IL23 is important for the development of autoimmune diseases such as EAE or collagen-induced arthritis [39–41].

Immune privilege

Immunity is necessary to eliminate dangerous infectious agents and to support regeneration, but it also causes significant tissue damage. At sites harboring irreparable structures such as cornea and lens in the eye or neuronal networks in the brain, where regeneration for obvious reasons is limited, a strong evolutionary pressure may have caused organ-specific modulation of immunity (for a review, see [5, 42]). The anti-inflammatory environment of eye and brain became apparent in transplantation studies [1] and their state of relative tolerance to grafts has been termed "immunologically privileged" [2]. In the brain, immune privilege has been attributed to two morphological peculiarities of this organ, the absence of classical lymph vessels and the BBB, a mechanical diffusion barrier for hydrophilic blood molecules built by specialized tight junctions in brain capillaries [5]). However, antigens turned out to drain from brain to cervical lymph nodes [6], and leukocyte recruitment takes place in postcapillary venules [4]. The picture of the brain being ignored by the immune system has thus been shifted to a more dynamic scenario, in which immune tolerance is actively maintained involving various mechanisms such as peripheral depletion and tight control over the activation state of local APCs (for a review, see [42]). The former is achieved by the constitutive expression of CD95 ligand (FasL and Apo1L) allowing apoptotic elimination of activated T cells [43–45]. As for the latter, vasoactive intestinal peptide [46] and transforming growth factor-beta [47] seem to be key players (for a review, see [5]). Moreover, a negative feedback mechanism exists involving the induction of indolamine 2,3-dioxygenase on microglia through IFNγ secreted by encephalitogenic Th1 cells leading to arrest or apoptosis of T cells [48].

As a consequence of such multilayered tolerance mechanisms, the CNS microenvironment appears inefficient for an immune-mediated inflammation, a necessary event to defeat pathogens. From an evolutionary point of view, it may in fact be less detrimental for the individual to tolerate certain neurotropic viruses (such as varicella zoster) than the elimination of all infected neurons [5, 42]. In mice, infection of peripheral tissues with lymphocytic choriomeningitis virus (LCMV) usually induces a massive expansion of virus-specific CD8 T cells, which results in efficient viral clearance and host survival [49]. When the virus is inoculated directly to the CNS, however, it causes a lethal disease. There is unequivocal evidence that the virus-specific T-cell response is implicated in the fatality of LCMV infection in the CNS [50, 51]. Should it be infected, the CNS would appear a defenseless victim, at the mercy of the peripheral immune system to control or end the attack. Nevertheless, T cells that control infections of the peripheral tissue without causing irreparable collateral damage cannot do so within the brain. This may be attributed to the simple fact that lost neurons can hardly be replaced and, therefore, dealing with viral infections in the brain is impossible without causing permanent or even lethal cell loss. However, a recent study in which patients were treated with a mAb against VLA4 demonstrates that restricting the CNS' immune surveillance (Fig. 2) could potentially lead to out-of-control expansion of pathogens such as the normally well-controlled JC virus (human polyoma virus) [52]. Under pathologic conditions, the action of CNS-associated APCs is absolutely essential for the immune invasion of the CNS. This also suggests that permanent elimination of infectious agents may take place within the brain in the absence of significant clinical symptoms.

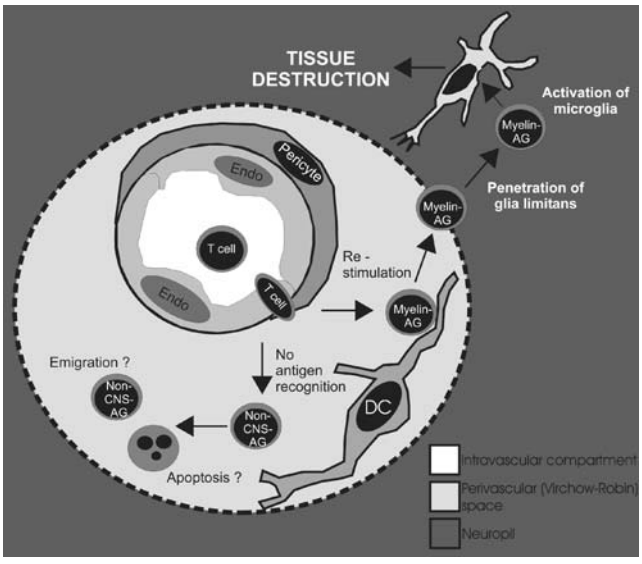

Fig. 2 There is vivid immune surveillance in perivascular spaces, as activated T cells pass the vessel wall of postcapillary venules even under apparently physiologic conditions. However, progression into the neuropil appears to depend on antigen recognition and re-stimulation by antigen-presenting (dendritic) cells located in Virchow–Robin spaces [17]. This reconfirmation and target recognition event apparently enables T lymphocytes to pass the glia limitans and to enter the brain parenchyma, where they can interact with microglial cells which drive the inflammatory cascade leading to tissue damage [99]. In the absence of APCs presenting the cognate antigen, T cells do not proceed across the glia limitans. They may either recirculate or undergo apoptosis

MHC and co-stimulatory molecules are indeed strongly upregulated in CNS infections and in virtually all other CNS pathologies, including ischemia, axonal degeneration, neoplasm, traumatic nerve injury, and neurodegenerative disorders, such as multiple sclerosis, HIV-associated dementia, and Alzheimer's, Parkinson's, and Creutzfedt–Jakob's disease [53–59]. Why and how such immune-associated reactivity occurs in the disturbed CNS remains speculative; such reactivity may reflect an attempt of the CNS to defend and repair. For example, CD4 T cells are recruited to sites of myelinated axon injury [60], and they promote regeneration of neurons via secretion of nerve growth factors after in situ antigen-specific activation [61–63], but secondary damage has also been reported [64, 65]. In MS, immune reactivity in the CNS also becomes destructive. CD4 T cells are blamed for wrongly attacking CNS myelin through secretion of pro-inflammatory cytokines and activation of cytopathic factors and cytotoxic CD8 T cells [31, 66, 67].

All of these examples clearly show that immunity within the CNS does occur and emphasize the delicate balance of

costs over benefits during neuroinflammation. In the following, we will focus on the key question, where and how antigens are presented within the CNS.

Antigen presentation and target recognition in EAE

In EAE and ADEM, the priming phase occurs within the systemic immune compartment. After immunization-induced expansion of neuroantigen-restricted encephalitogenic Th cells, these T cells are now equipped to leave the lymphoreticular environment and invade the bodies' tissues scanning for APCs presenting their cognate Ag. Oligodendrocytes are ultimately the target of such Th cells in the case of myelin Ag immunization. They are the CNS' myelinating cells wrapping their cell membranes around axons to provide nerve insulation for optimal conductivity. However, while oligodendrocyte loss and demyelination are ultimately seen in EAE, oligodendrocytes cannot be directly recognized by CNS-invading T cells as they are incapable of expressing MHC class II molecules [68]. A compulsory third party APC is an absolute requirement for encephalitogenic Th cells to even recognize myelin Ag within the CNS.

Ag presentation likely occurs at the level of the BBB promoting entry of T cells into the CNS. Cerebral microvascular endothelial cells have been considered as APCs because of their large cumulative surface and their unique anatomical location between circulating T cells and the extra-vascular sites of antigen exposure. However, endothelial cells, unlike perivascular APCs, do not constitutively express MHC class II molecules in vivo or in vitro [69]. In two different models of antigen presentation, brain endothelial cells have failed to induce T-cell proliferation [69, 70, 71]. No data has causally implicated endothelial expression of class II MHC as an important element in disease.

Oligodendrocytes are the apparent target of the autoimmune attack in MS and EAE. It is feasible that MS is in contrast to EAE, a primary degenerative disease in which oligodendrocytes undergo premature death resulting in subsequent immune activation [72]. However, the data supporting this hypothesis are not numerous. In contrast, there is overwhelming evidence that immune cells from the systemic immune compartment become activated and that myelin-reactive lymphocytes are the prime and initial mediators of the disease. This is certainly the case in EAE, where immune tolerance to self (myelin) is broken resulting in the expansion of myelin-reactive T cells which subsequently migrate into the CNS to initiate an inflammatory cascade leading to demyelination, oligodendrocyte loss, and axonal degeneration. However, viewing the CNS only as the defenseless target of such an immune assault would be oversimplistic. There is now clear evidence that CNS-associated DCs play a vital role in target recognition for neuroantigen-reactive T cells and that CNS resident microglia are crucial for the maintenance of encephalitogenicity during an immune response. We will now discuss the different anatomic locations and cellular compositions of potential encounters between encephalitogenic T cells and their APCs.

Microglia, sentinels of the CNS parenchyma

Glial cells were initially defined as the glue (Greek: glia) keeping the neurons in the brain together [59]. Astroglia as well as oligodendroglia clearly have a supporting function for neuronal development, sprouting, and activity. In 1932, the neuropathologist del Rio-Hortega described a third type of glial cell, later named microglia [73]. Microglia comprise about 10% of the total glial population in the CNS parenchyma [59] and are in contrast to the other cells residing in the CNS parenchyma not of neuroectodermal but of mesodermic origin. While the origin of microglia has long been disputed, it has become definitively established that they are of hematopoietic origin. They share many properties with macrophages, having developed from a common precursor cell [74, 75]. The most widely held hypothesis is that, early in fetal development, myeloid cells infiltrate the CNS and develop into parenchymal microglia [76]. Unlike the CNS-associated phagocytes of the leptomeninges and the perivascular spaces, microglia are not readily repopulated by bone-marrow-derived monocytes during adulthood [77], but there is evidence for regional turnover at low rates and rapid transformation of blood monocytes into microglia during pathologies [5, 78].

Under virtually all inflammatory conditions in the CNS, parenchymal microglial cells rapidly upregulate MHC class II expression, suggesting that they may participate in Ag presentation that occurs during the inflammatory process [57, 75, 79, 80, 81]. Activated microglia also upregulate co-stimulatory molecules such as CD40, CD80, and CD86, which equip them for Ag presentation to T cells [16, 55, 70, 82].

Microglia, like the CNS-associated cells, phagocytose myelin [83–85], indicating that these cells can activate myelin-specific CD4 T cells. Microglia infected with Theiler's murine encephalomyelitis virus have been demonstrated to be activated to efficiently process and present not only endogenous viral epitopes but also exogenous myelin epitopes to CD4 T cells [86]. Human microglia, either immediately ex vivo or cultured for several days, have been reported to act as APCs and to induce both primary and secondary proliferative T-cell responses [79, 87]. A similar APC potential was also demonstrated for rat microglia isolated from neonatal brains [18, 88], but in these studies with neonatal glial cultures, extraneural, leptomeningeal, and perivascular CNS-associated APCs were not separated from microglia and may contribute to T-cell activation. Furthermore, recent reports have raised the concern on whether such neonatally derived cells faithfully represent the adult population in vivo. In fact, microglia isolated from adult mice fail to present antigen to naïve antigen-specific CD4 T cells [89], but those that are pretreated with IFNγ or isolated from mice with EAE can activate T cell lines in an antigen- and CD86-dependent manner in vitro [90]. Nevertheless, adult rodent microglia

may not support a typical T-cell activation: T cells become blasts, express activation markers CD25 (IL2 receptor alpha chain) and CD134, and produce the cytokines TNFα and IFNγ, in an antigen-specific manner, but do not proliferate [7, 91, 92]. Even when MHC class II expression is enhanced by the induction of systemic graft vs host disease, the isolated microglia still do not promote proliferation of T cells but rather induce death of these cells through apoptotic mechanisms [91]. In line with this finding, many T cells in the CNS of rodents with EAE undergo apoptosis within the parenchyma [93], where they are in contact with microglia. There is now overwhelming evidence that microglia do not serve as initial APCs to invading myelin-reactive T cells [16, 17].

The activation and differentiation of CD4 T cells require MHC class II recognition and co-stimulation. Studies using adoptive transfer of primed myelin-reactive CD4 T cells into mice in which APCs can no longer deliver a specific co-stimulatory signal have identified CD40, CD80/CD86 (B7), and CD134 (OX40) as important for the reactivation of these T cells in the CNS [94, 95]. The observation that microglia are largely resistant to radiation while CNS-associated cells are radiation sensitive has allowed the generation of radiation bone marrow chimeric mice to further pinpoint on the most important source of these co-stimulatory molecules in the CNS in an in vivo setting. Recent work using such an approach to create bone marrow chimeric mice in which CD40 is deficient only in microglia but is intact in CNS-associated cells has revealed that microglial CD40 is most critical to the reactivation of peripherally primed encephalitogenic T cells for the production of Th1 cytokines and chemokines, the recruitment of leukocytes to the CNS parenchyma, and the progression of EAE [96]. Like murine microglia, human microglia can express CD40 and, in response to CD40 stimulation, produce IL12 [82, 87]. IL12 is a 70-kDa heterodimeric, secreted protein consisting of two disulfide-linked subunits, p35 and p40. The p40 subunit can also associate with a different p19 subunit to form IL23, which has recently been shown to be essential in the activation of the CNS-associated macrophages for autoimmune inflammation in the mouse brain [36, 37, 97]. This study and others [38, 98] have challenged the view that IL12 is central in autoimmune inflammation of the CNS. Though both microglia and CNS-associated macrophages can produce IL23, only macrophages can respond to this cytokine [37]. Lastly, it was shown by Heppner et al. that EAE was considerably repressed in mice in which microglial cells were transiently "paralyzed". The inhibited activation of microglia leads to a reduced inflammation of the CNS, suggesting that the release of reactive oxygen species intermediates in addition to cytokines and chemokines by activated microglial cells is crucial for the development and maintenance of EAE [99]. These observations collectively allow us to speculate that the interplay between microglia and macrophages via IL23 may regulate CNS inflammation triggered by primed autoimmune CD4 T cells. Infiltrating T cells expressing CD40 ligand (CD154) can stimulate microglial CD40 to upregulate IL23. This cytokine in turn can directly act on memory T cells [100] and activates CNS-associated macrophages to produce critical inflammatory cytokines (e.g., IL-17) and possibly other co-stimulatory molecules (e.g., CD80/CD86 and CD134). A better understanding of the molecular mechanisms regulating the expression of CD40 and its ligand in the CNS will undoubtedly cast new light on the functions of these co-stimulatory molecules beyond T-cell activation.

It remained unresolved whether microglia actually present antigen to naïve T cells in vivo during inflammation until recently. It is likely that T-cell activation and expansion are subjected to a tighter regulation in the CNS parenchyma than elsewhere. CNS APCs were indeed found to inhibit T-cell proliferation through the release of toxic levels of nitric oxide [92] and to induce T cell apoptosis through Fas–Fas ligand interaction [7, 91]. For a long time, microglia have appeared to be the most capable APCs to initiate and sustain a T-cell-mediated immune response within the CNS [101]. By combining bone marrow chimerism and gene targeting, we restricted the APC capability to either the CNS parenchyma (microglia) or the systemic immune compartment. Upon transfer of MOG-reactive T cells, chimeras with MHC class II expression restricted to the systemic immune compartment and CNS-associated cells were susceptible to EAE. However, mice with MHCII expression restricted to the CNS parenchyma were resistant to the development of disease [17]. Taken together, these findings provide evidence that cells within the CNS parenchyma such as microglia do not serve as APCs permitting lymphocyte entry. However, for the development of CNS inflammation and the effector phase during EAE, microglia are pivotal components. We will later discuss that the CNS-associated and not CNS residents permit the recognition of the cognate neuroantigen by CNS-invading T cells.

Astrocytes

While numerous studies have supported the role of microglia in T-cell activation within the CNS, whether astrocytes, the major glial populations, also play such a role is a lively controversy [102–105]. On one hand, both in situ and in vivo evidence indicate that astrocytes express high levels of MHC class II and co-stimulatory molecules (e.g., CD80, CD86, CD40, ICAM-1, and VCAM-1) upon exposure to IFNγ [106] and are capable of sensing pathogens through toll-like receptors [107]. It is interesting to note that CD80 and CD86 are differentially regulated on astrocytes during the course of EAE: CD86 is upregulated at the peak disease, while CD80 dominates during remission [108]. Furthermore, astrocytes express elements involved in the MHC class II endocytic pathway and are capable of processing native MBP for presentation to myelin-specific T cells in vitro [109]. Myelin-specific T cells that were activated by astrocytes in vitro were indeed able to transfer EAE in SJL/J mice [110]. Although both untreated and IFNγ-treated astrocytes can initiate

secondary T-cell responses, only treated astrocytes activate naïve T cells [106]. However, many other studies do not support astrocytes as efficient APCs [18, 111, 112]. It is interesting that astrocyte-derived soluble factors suppress LPS induction of co-stimulatory molecules on microglia and macrophages [47], suggesting that astrocytes are not only themselves poor stimulators of CD4 T cells but can also inactivate other APCs.

Unlike microglia, astrocytes may require additional stimuli in combination with IFNγ to provide stronger synergistic activation of their APC function [113]. Because of their phenotypic and functional heterogeneity, astrocytes may differ in their APC function, and this may add to the controversy. Moreover, strain differences might influence the antigen-presenting potential of astrocytes. Upon in vitro stimulation with IFNγ, astrocytes that were isolated from EAE-susceptible Lewis rats, but not from EAE-resistant Brown Norway rats, expressed higher levels of MHC class II and induced proliferation of myelin-specific T cell lines [114]. A similar correlation between EAE susceptibility and astroglial MHC class II induction was also observed for certain mouse strains [115].

CNS-associated cells, sentinels at the gateway to the CNS

Compartments in association to the CNS, such as the Virchow–Robin's (perivascular) space, the leptomeninges, and the choroid plexus, contain phagocytes that have been identified as macrophages and dendritic cells, based on molecular and functional phenotypes [116, 117]. They are continuously replenished by bone-marrow-derived monocytes [77, 118]. Their normal turnover may be indeed exploited by pathogens, such as lentiviruses, to enter the CNS [117]. Under various pathological conditions, these CNS-associated cells can increase in number transiently or for longer periods [119]. Like peripheral macrophages, they constitutively express high levels of MHC class II, CD11b, and the leukocyte common antigen CD45, which distinguish them from the $CD45^{low}$ parenchymal microglia [79, 120]. Both in vitro and in vivo studies strongly point to the CNS-associated cells, rather than the microglia, as competent APCs. When isolated from adult rodent CNS, they were found to activate both naïve and primed CD4 T cells, as efficiently as splenic or lymph node APCs, and even more so than $CD45^{low}$ parenchymal microglia isolated from the same animals [7, 91, 121]. In vivo studies using infectious or autoimmune models also support the immune functions of the CNS-associated cells. For instance, Matyszak and Perry showed that an injection of the pathogen Bacillus Calmette–Guerin (BCG) to the meninges provokes a typical inflammation and a BCG antigen-specific T-cell response, as seen in the skin after a subcutaneous challenge [122]. By contrast, no such immune response was observed when the pathogen was delivered directly to the CNS parenchyma. Using different MHC-class-II-restricting T-cell elements, Hickey and Kimura could show that perivascular APCs "considered to be macrophages" are sufficient to provide stimulatory signals for T cells to infiltrate the CNS. However, to induce EAE, the rats were required to develop graft vs host disease [123] and thus changing the inflammatory environment and infiltrates of the CNS.

Due to the strategically important location, between the outermost basal lamina of the vessel wall endothelium and the one on top of the glia limitans CNS parenchyma, perivascular CNS-associated APCs are ideal candidates to present Ag to invading myelin-reactive T cells. These CNS-associated cells may engage in de novo processing of myelin antigen for in situ activation or tolerance induction of effector/memory as well as naïve T cells. They also seem strategically positioned for influencing T-cell trafficking to the CNS parenchyma. When their function is perturbed or absent, lymphocyte migration into the CNS parenchyma is blocked and EAE is prevented [124]. In fact, antigen recognition in perivascular spaces was proposed to be required for T cells to progress from the Virchow–Robin space across the glia limitans into the neuropil [125]. CNS-associated cells have been shown recently to be important sources of TNFα, which induces glial production of chemokines, such as TCA3 and MCP, to mediate leukocyte recruitment and initiation of inflammation in the parenchyma [126].

Using transgenic mice in which MHC class II is exclusively targeted to DCs, we showed that DCs alone are sufficient to present the cognate antigen to MOG-reactive T cell and are permissive for the development of CNS inflammation [17]. Furthermore, by augmenting the number of CNS-associated DCs, upon adoptive transfer with MOG-reactive T cells, the disease severity of EAE is increased. In MS lesions, analogous cells, $CD209^{+}$ DCs, were detected to be associated with vessels and in close proximity to invading T cells. These data indicate that CNS-associated DCs are the crucial APCs in conferring CNS inflammation of encephalitogenic T cells [17].

In agreement with these results, McMahon et al. demonstrated in two mouse models of EAE that epitope spreading occurs directly in the CNS and not in peripheral lymphoid tissues. They showed that DCs were the most crucial APC population of the CNS, initiating epitope spreading in the inflamed CNS [16]. Neither microglia nor macrophages isolated form the CNS were capable of stimulating naive myelin-specific T cells. These in vivo studies collectively strongly suggest that CNS-associated DCs are capable of initiating both primary and secondary T-cell responses locally. These CNS-associated APCs, like certain antigens in the brain, may drain to lymphoid organs to activate naïve T cells. The outflow of the normal brain can escape to the interstitial and cerebrospinal fluids, which follow cranial nerves to drain into nasal lymphatics and cervical lymph nodes or can drain to the spleen via blood. Studies tracing these outflow pathways have revealed that, contrary to the earlier view, both intracranially injected macrophages and antigens drain preferentially to the cervical lymph nodes [127–129]. Myelin basic proteins were indeed elevated in the cerebrospinal fluid of patients with relapsing–remitting MS [130] and myelin-ingested

APCs were also abundant in their cervical lymph nodes [128]. In addition, the cervical lymph nodes of marmoset and rhesus monkeys that were immunized with only myelin oligodendrocyte glycoprotein contained an accumulation of APCs that had engulfed other myelin antigens, such as myelin basic protein and proteolipid protein [128]. These observations indicate that myelin antigens must have drained or that APCs containing them must have migrated to the cervical lymph nodes from the inflamed CNS. However, it is unclear whether myelin antigen presentations in the cervical lymph nodes would result in the progression or modulation of immune responses in the CNS. In support of the cervical lymph node implication in disease, encephalitogenic T cells primed in these lymph nodes were found to preferentially target the brain [131] and an ablation of the cervical lymph nodes markedly attenuated the cryolesion-enhanced EAE in Lewis rats [132]. Although the drainage of CNS-derived antigens into the cervical lymph nodes generates local production of antigen-specific antibodies, it does not provoke a rapid destruction of the CNS [127]. Moreover, intracranially injected antigens in normal mice rather evoked a systemic immune deviation that suppresses normal T-cell effector activity [133]. Cervical lymph node cells from the protected mice were able to transfer such suppression to naïve recipients. However, alymphoplasia mice, which completely lack all lymph nodes, are fully susceptible to EAE induced by an adoptive transfer of MOG-reactive T cells, indicating that the presence of CNS antigen in the cervical lymph node is a result of CNS inflammation but not a driving force [17].

Concluding remarks

Immunization of naïve mice with myelin Ags results in the expansion of potentially pathogenic/encephalitogenic T cells. Immunization-induced expansion likely occurs within secondary lymphoid tissues, although recent data from our laboratory indicate that immunization-induced T-cell priming can occur outside of such dedicated structures (manuscript in preparation). Upon activation and maturation, myelin-reactive T cells migrate through the body's tissues in search of their cognate antigen. In perivascular spaces, T cells apparently re-encounter their cognate antigen presented by DCs, leading to reactivation enabling the T cells to cross the glia limitans and to invade the CNS parenchyma. This confirmation event is vital, as the encounter between encephalitogenic T cells and MHC-class-II-expressing DCs is an absolute requirement for disease development. T cells which recognize non-myelin Ags can also travel into this location but will not receive their reconfirmation signal [134]. After this second encounter with their cognate Ag, T cells cross the glia limitans and migrate deeper into the CNS parenchyma where they can activate CNS resident microglia. They in turn will produce a host of vasoactive substances, cytokines, and chemokines, leading to the invasion of other leukocytes such as poly- and monomorphonucleated phagocytes. The presence of such inflammatory mediators including IL23 ultimately leads to the development of a tissue lesion. While the precise factors that lead to the collateral tissue damage of myelinating tissue remain to be definitively established, our current knowledge on target recognition and the location of vital T cell/APC interactions could lead to the development of novel therapeutic strategies for the treatment of inflammatory CNS diseases.

Under steady-state and physiologic conditions, the CNS-associated APCs are likely to be vital for the maintenance of peripheral tolerance. Immune surveillance of the CNS under non-pathologic conditions appears to be essential for the control of CNS pathogens. The goal now would be to use this knowledge and to target these APCs to reestablish peripheral immune tolerance against self and to use this approach as a therapeutic strategy for the treatment of neuroimmune disorders.

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