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Sensing the microenvironment of the central nervous system: Immune cells in the central nervous system and their pharmacological manipulation

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Abstract

Immune responses are highly regulated in all organs and severely restricted in certain tissues such as the central nervous system (CNS). This phenomenon, called “immune privilege”, has been linked to the existence of multiple anatomical and physiological protective mechanisms. The finely balanced anti-inflammatory microenvironment within the CNS contributes to the immune privilege status of this tissue. The regulation of this compartment changes under pathological conditions when pro-inflammatory mediators might dominate. The past few years brought a wealth of novel information fostering our understanding of how CNS resident cells regulate the functions of immune cells, particularly helper T lymphocytes (Th) and dendritic cells (DC). These two cell types play a critical role in the initiation and maintenance of neuroinflammatory diseases. The change from anti- to pro-inflammatory microenvironment in the inflamed CNS affect Th and DC accumulation and function in the nervous tissue. A new era of dendritic cell targeted therapies has begun, with the possibility of designing novel immunomodulatory therapies to intervene with neuroinflammation in a wide range of neurological diseases.

Introduction

While CD4⁺ T lymphocytes play a critical role in the initiation and maintenance of autoimmune responses in the CNS, DCs have emerged as pivotal regulators of CNS autoimmune and inflammatory responses. In the absence of inflammation, DCs are rare and localize to vascular-rich areas in the CNS, suggesting possible roles for DCs in immune surveillance, antigen sampling and delivery to peripheral lymph nodes, and/or T cell activation. Under inflammatory conditions (e.g. autoimmunity, infectious disease), DCs together with CD4⁺ T cells accumulate in the CNS parenchyma. Thus, supporting that CNS DCs are critical for the initiation, regulation, and/or maintenance of CNS targeted immune responses. Within the CNS, the state of the local environment regulates the fate and function of these cells, as they receive cues regarding the normal or inflammatory milieu. Immune cells traffic through multiple compartments in the CNS (e.g. the blood-brain barrier, perivascular space, brain parenchyma, choroid plexus or cerebral spinal fluid) and receive different cues depending on their localization. Their ability to respond to the local environment may change depending on the status of their activation and lineage commitment. A systematic and thorough evaluation of

microenvironmental regulation of DC and CD4⁺ T cells within the CNS will be required to fully understand the immunoregulatory role of these cells in CNS inflammatory diseases and to design effective therapies to treat CNS autoimmune diseases such as MS.

In this review, we will focus on the environment-sensing molecules on CD4⁺ T cells and DCs, and regulatory signals in the local tissue milieu that affect activated CD4⁺ T lymphocytes or DCs in the CNS. The pharmacological manipulation of DCs as a new avenue to treat CNS autoimmunity is also discussed.

The non-inflamed CNS: a hostile environment for immune cells

Although immune cell traffic into the non-inflamed brain is severely limited, the immune surveillance of the healthy brain is critical for the protection of the CNS from infections and manifestation of cancer. This was recently reinforced by data showing that blocking this process by targeting adhesion molecules leads to the up-regulation of chronic (latent) viral infections in the CNS, such as the oligodendroglialopathy induced by the polyomavirus JCV that leads to progressive multifocal leukoencephalopathy (PML) [1]. The trafficking pathway for surveillance-providing cells is probably different from the migratory pathways of inflammatory cells. While effector or memory T cell surveillance of the CNS is regulated through the cerebral spinal fluid (CSF)-choroid plexus barrier [2] (Figure 1), the recruitment of inflammatory immune cells mostly takes place through post-capillary venules which are surrounded by perivascular space [3] (Figure 2). Thus far, three major pathways have been suggested for immune cell recruitment into the CNS: (1) migration from blood to CSF across the choroid plexus of the ventricles, (2) migration from blood to the subarachnoid space across the arachnoid epithelium forming the middle layer of the meninges, and (3) through the perivascular space that surrounds the tight junction forming blood–brain barrier (BBB) endothelial cells. However, these pathways were described by studying T lymphocyte trafficking into the CNS; the knowledge regarding DC trafficking in or out of the nervous tissue is much more limited. In the non-inflamed CNS, most of the T cell and DC traffic is probably regulated via route 1 (from blood to CSF across the choroid plexus) [4]. This notion is supported by data showing that when fluorescently labeled neuroantigen-activated T lymphocytes are injected into the blood circulation, these cells can be detected in the meninges and choroid plexus two hours following injection without any cell accumulation in the CNS parenchyma [4]. Similarly, CD11c⁺ cells, presumably dendritic cells, can be detected in the choroid plexus and around the ventricles in the normal CNS in CD11c EYFP transgenic mice in which the DCs are fluorescent [5•]. Once immune cells are in the CNS, the tissue microenvironment will influence whether they will survive or be eliminated from the CNS.

Low expression of major histocompatibility antigens on resident cells of the normal CNS limits T cell activation

In order to survive and further differentiate in the anti-inflammatory environment of the CNS, CD4⁺ T cells must first obtain additional stimulatory signals. T cells have microenvironment sensors that regulate this process [6•]. Once inside the CNS, CD4⁺ T cells must interact with other cells, including antigen-presenting cells (APCs), which possess the metabolic machinery necessary to take up, degrade and display antigen in the context of the MHC molecules. The expression of MHC class II molecules varies greatly from cell to cell, although these molecules are found primarily on cells of the lymphoid system, such as B cells, macrophages and DCs. In the absence of inflammation, there is a very low constitutive expression of these molecules on cells of the CNS, particularly on those that have very little capacity for regeneration, such as neurons.

Thus, in the absence of histocompatibility molecules, the survival or further differentiation of activated T cells in the CNS is suboptimal. It is even less likely that naïve T cells would gain access to the non-inflamed CNS, as it is widely accepted that naïve T cells cannot accumulate in this tissue [7]. Although naïve myelin basic protein (MBP)-specific T cells were isolated from the CNS of T cell receptor (TCR) transgenic mice, these cells inhibited MBP-specific immune responses *in vivo*, indicating that naïve T cells may contribute to *in situ* tolerance in the brain [8]. In the absence of inflammation the CNS microenvironment stimulates the development of regulatory or suppressor T cells. The access of activated myelin oligodendrocyte glycoprotein (MOG)-specific Th1 cells alone to the un-inflamed CNS is not sufficient to induce autoimmune pathology. Other signals, such as Toll-like receptor 9 (TLR9)-stimulating CpG oligonucleotide microbial products are necessary to induce CNS autoimmunity [9].

In summary, T cell survival and re-stimulation within the naïve CNS is very limited. Aside from the lack of MHC antigens, other anti-inflammatory mediators will also shape the repertoire of immune cells within the naïve CNS, as we will discuss below.

CNS cells produce a plethora of micro environmental anti-inflammatory modulators, including neuropeptides and cytokines

Microenvironmental variations in different tissues modulate immune responses in their own ways. In the non-diseased CNS, numerous mediators are constitutively expressed that prevent the differentiation of Th cells and might promote the development of regulatory T lymphocytes (Treg) and DCs (Figure 1). One of the many neuropeptides found in brain tissue that has been shown to regulate immune responses and contribute to the anti-inflammatory environment within the CNS is the 28 amino acid peptide, vasoactive intestinal peptide (VIP). VIP has strong inhibitory effects on microglia and macrophages, as well as on T cells and has been shown to up-regulate Treg cell functions [10] and induce the differentiation of tolerogenic DCs [11]. The expression of the G protein-coupled receptor VIP receptor-1 (VPAC1) and VPAC2 on CD4⁺ T cells changes reciprocally in the context of the activation state of these cells, and this directly affects the responsiveness of CD4⁺ T cells [12]. Melanocyte-stimulating hormones produced by cells in the intermediate lobe of the pituitary gland and in the hypothalamus have also been shown to have anti-inflammatory effects. One of these mediators, α -MSH, a tridecapeptide derived from pro-opiomelanocortin by post-translational processing, has direct effects on some cells of the immune system as well as indirect effects by affecting resident CNS cells, such as microglia and astrocytes. α -MSH affects several pathways implicated in regulation of inflammatory responses such as NF- κ B activation, expression of adhesion molecules and chemokine receptors, production of pro-inflammatory cytokines and other mediators. It was also demonstrated that one of the mechanisms by which α -MSH regulates T cell activity and inhibits IFN- γ production is through mediating a change in the pattern of protein ubiquitination in IFN- γ producing T cells [13]. These two examples highlight the importance of the neuropeptide-rich environment in the down-regulation of immunity within the CNS parenchyma.

Immune cells in the CNS are further controlled by potent anti-inflammatory cytokines produced by resident CNS cells. For example, transforming growth factor beta (TGF- β) is secreted within the CNS by astrocytes and glial cells and has been shown to induce the differentiation of regulatory T cells (Treg) [14]. However, in inflammation, this cytokine in combination with IL-6 or IL-21 induces the differentiation of IL-17-producing T cells (Th17) that express the transcription factors retinoic acid receptor-related orphan receptor (ROR)- γ and - α [15]. These cells have very important roles in human autoimmune diseases, and we will discuss them in the context of an inflammatory environment within the CNS.

Specific direct signals in the CNS microenvironment that regulate the function of immune cells

Aside from soluble anti-inflammatory mediators, multiple cell surface molecules on cells of the CNS have been described that can directly interact with immune cells in this tissue. For example, CD200, a member of the Ig superfamily of integral membrane glycoproteins, that is ubiquitously expressed on neurons, their axons, cerebral endothelial cells and macrophages in the choroid plexus, delivers a powerful inhibitory signal via its receptor (CD200R) to macrophages, microglia, DCs, neutrophils, eosinophils and mast cells [16,17]. The CD200-CD200R-mediated inhibitory signaling suppresses IFN- β , NO, IL-5, IL-13 and IL-6 production in CNS infiltrating myeloid cells, thus protecting neurons from inflammatory damage.

The FasL (CD95L) molecule and its receptor Fas (CD95) might also contribute to maintaining the anti-inflammatory environment within the CNS. Both tissue damage and the elimination of T cells leading to the termination of inflammation, crucially depend on FasL-induced cell death [18]. It was shown that one of the major sites for this effect was at the glia limitans, where astrocytes probably can eliminate activated T cells in FasL-dependent way [19]. Constitutive expression of this death ligand beyond the BBB appears to provide an immunological brain barrier capable of eliminating infiltrating cells.

Environmental sensors on effector CD4 T lymphocytes and DCs that perceive the CNS inflammatory microenvironment

CNS infiltrating helper T cells in autoimmune, infectious or trauma-induced neuroinflammation are activated in the lymphoid organs outside of the nervous tissue. How CNS antigens are actually delivered to the peripheral lymphoid tissue from the CNS is unclear. In non-CNS tissues there are DCs present that can gather antigens from the local environment and after receiving additional environmental stimulatory signals, they migrate to lymphoid tissues and induce T cell activation. According to Janeway's "stranger" model [20], DCs carry receptors specific for pathogen associated molecular patterns (PAMP), and the recognition of microbes via these receptors enables their migration from tissues to lymph nodes. In addition to carrying antigenic peptides from the tissues, the DCs deliver targeting and differentiating messages to naïve T cells located in the lymph nodes that will direct the migration of effector T cells into the tissue of DC origin. This model was later modified by Matzinger [21], who hypothesized that the crucial event controlling the initiation of immune responses was not the recognition of infections via PAMPs but the recognition of danger signals known as damage associated molecular patterns (DAMPs) by DAMP receptors expressed on DCs. DAMPs are produced from cells stressed, damaged and/or dying in local tissues. It has also been suggested that DAMPs are released as a consequence of PAMP recognition (infectious induction), thereby connecting these two models. This general view is somewhat more complex in the CNS when we consider the fact that DC migration from tissues to the lymphoid organs is through lymphoid vessels and the CNS has no detectable lymphoid drainage or lymphatic vessels. However, by the increased expression of DAMPs in the inflamed CNS (due to trauma or infection), the significance for the absence of lymphatic vessels lessens as perivascular space widens, extracellular fluid mediated drainage accelerates, and the BBB becomes compromised, allowing the infiltration of Th cells and DCs.

In general, CD4⁺ T cells have to be activated in the peripheral lymphoid tissues prior to their migration into the CNS. In this process, CD4⁺ Th cells differentiate into distinct effector subsets that are characterized by their unique cytokine expression and immunoregulatory function [22, Abbas, 1996 #959]. Th1 and Th2 cells produce predominantly IFN- γ and IL-4,

respectively, as autocrine factors necessary for selective lineage commitment (Figure 2). The Th17 subset is probably a distinct Th lineage mediating tissue inflammation (Figure 2). It has been shown that Th17 differentiation is initiated in the presence of TGF- β , IL-6 and IL23, and leads to the activation of signal transduction and activators of transcription-three (STAT3) and ROR- γ and - α (reviewed in [23]). Th17 cells produce IL-17, IL-17F and IL-22, all of which predominantly regulate inflammatory responses. IL17-producing T cells were found in perivascular lesions from patient with MS and there were fewer T17 cells in quiescent MS [24•]. It was also shown that the cytokine IL-21 is highly expressed by mouse Th17 cells [25]. IL-21 potently induces Th17 differentiation and suppresses Treg cell induction. IL-21 deficiency impairs the generation of Th17 cells and results in protection against EAE [26]. Th17 cells undergo at least three steps of development: differentiation, amplification and stabilization in which distinct cytokines play a role [27]. This notion is probably true for other types of T cells. This indicates that the local tissue environment can modify the stabilization of T cell lineages as T cells can sense environmental signals and respond to them. This was recently shown by demonstrating that the intracellular ligand-activated transcription factor aryl hydrocarbon receptor (AHR) is a regulator of both Treg and Th17 cell differentiation in mice [28]. AHR activation by its ligand, 2,3,7,8-tetrachlorodibenzo-p-dioxin, induced functional Treg cells that suppressed EAE. On the other hand, AHR activation by 6-formylindolo[3,2- β]carbazole interfered with Treg cell development, boosted Th17 cell differentiation and increased the severity of EAE in mice. Thus, AHR regulates both Treg and Th17 cell differentiation in a ligand-specific fashion. To understand the local environmental signals that regulate T cell lineage stabilization in the CNS will be critical to develop novel approaches for regulating immune responses in this tissue.

When activated T cells receive re-stimulatory signals, they are stabilized and expand in the CNS. Activated autoimmune T cells further stimulate the recruitment of immune cells, most notably macrophages and DCs into the CNS, and induce a broader inflammatory reaction that may lead to the destruction of the myelin sheet. Throughout this process, activated immune cells will first encounter residential cells of the neurovascular unit and later resident cells of the CNS. In the next paragraphs we will outline some of the environment-sensing molecules and regulatory signals that affect activated CD4⁺ T lymphocytes or DCs in these two compartments and their role in initiation of CNS neuroinflammation.

Activated CD4⁺ T lymphocytes and DCs in the perivascular space

In the CNS there are at least two major types of perivascular spaces that surround the blood vessels. The first type is the enlarged perivascular space or Virchow-Robbins space (named after Rudolf Virchow and Charles-Philippe Robin, French anatomist, 1821–1885), which is an extension of the subarachnoid space. These spaces are formed when large surface vessels penetrate into the parenchyma and bring along the pial membrane with them for a short distance. In the Virchow-Robbins space, the glial limitans borders the pial membrane; here the endothelial cells are surrounded first by the pia, then the glial limitans. The space that exists between the pial membrane and the invading vessel is what is classically referred to as the Virchow-Robbins space. As the invading vessels begin to ramify and form microvessels, the pial covering is lost and only the glial limitans then subtends these small vessels. The area surrounding the microvessels is divided into two compartments, created by the basement membranes (BM) of endothelial cells (EBM) and astrocytes (AstrBM) (Figure 2). During immune cell extravasation, which occurs along the post-capillary venules, these two membranes separate, giving rise to the abluminal space (between the endothelial BM and the endothelium) and the perivascular space (between the EBM and AstrBM). Immune cells can temporarily reside in each of these spaces, as if encountering traffic stops during their journey into the parenchyma.

The perivascular space contains plasma substances, lymphocytes, perivascular macrophages, and DCs. Cellular interactions in the perivascular space, particularly the re-stimulation of infiltrating T cells by APCs (such as perivascular macrophages or DCs) seem to be required for the further migration of these cells across the glial limitans [29,30]. In inflammation, transmigration of DCs across the BBB might be regulated by the MIP-1 α chemokine and matrix metalloproteinases (MMPs) [31•]. It has been shown that DCs localize to the proximity of the inflamed microvessel wall in MS lesions [32] and can be found for a longer period of time in the perivascular space. In this space, DC cells provide a regulatory platform on which T cells can interact with each other and further amplify the inflammatory process [33•]. By the up-regulation of CD40 costimulatory molecules on DCs and IL-2 production by the activated T cells, DC platforms contribute to the inflammatory micro environmental niche in which IFN- γ production by antigen-specific T cells is enhanced locally and neuroinflammation is amplified. The perivascular space provides multiple additional environmental signals for immune cells. Some of these environmental signals originate in the extracellular matrix constituting the basement membrane that surrounds the blood vessels. The extracellular matrix has multiple molecular components including proteoglycan matrix components, such as heparan sulfate proteoglycans, and non-proteoglycan matrix components, such as hyaluronic acid, collagen, fibronectin, elastin and laminin [34]. The molecular composition of the extracellular matrix of the vascular and astroglial BM in chronic active and active MS lesions was characterized. Interestingly, a differential expression of specific laminin chains in vascular and astroglial BMs was observed [34]. Additionally, fiber-like depositions of extracellular matrix within the inflammatory cuffs contained laminin isoforms, fibronectin, collagen IV and heparan sulfate proteoglycans in close contact with perivascular infiltrates [34].

Integrin molecules, which are extracellular matrix receptors, are expressed on activated T cells and DCs and might also influence the fate of these cells in the perivascular space. It has been shown that endothelial BM may contain laminin 8 (α 4 β 1 γ 1) and/or 10 (α 5 β 1 γ 1), and the expression of these molecules can be influenced by pro-inflammatory cytokines or angiostatic agents [35]. T cells emigrating into the CNS during EAE first encounter the endothelial BM (containing laminins 8 and/or 10) and then the parenchymal BM (containing laminins 1 and 2). Inflammatory cuffs occur exclusively around endothelial basement membranes containing laminin 8, whereas in the presence of laminin 10 no infiltration was detected. Down-regulation of integrin α 6 on the cerebral endothelium at sites of T cell infiltration plus a high turnover of laminin 8 at these sites suggested two possible roles for laminin 8 in the endothelial BM: one at the level of the endothelial cells resulting in reduced adhesion, thereby increasing penetrability of the monolayer, and the second at the level of the T cells providing direct signals to the transmigrating cells [35].

Some of the environmental factors regulate inflammation by changing those signals that are provided by the perivascular matrix. For example, hyaluronan (HA) molecular forms regulate CD4⁺ T cell functions via a cell surface receptor called CD44. CD44 is a cell-surface glycoprotein that is involved in multiple cell functions, such as cell-cell interactions, cell adhesion and migration, and the binding of different ligands to this molecule might differently influence immune cell function. The expression of this molecule on activated T cells renders the interaction of these cells with HA, an extracellular matrix component. CD44 can also interact with other ligands, such as osteopontin, collagens and MMPs. HA can significantly influence the function of activated CD4⁺ T cells (reviewed in [6]). HA is a non-sulfated glycosaminoglycan distributed widely throughout connective, epithelial and neural tissue, specifically associating with extracellular spaces such as the perivascular space in the CNS. High molecular weight HA (HMW HA) has anti-inflammatory properties [36]; however, in inflammation, hyaluronidase activity is triggered, leading to the formation of low molecular weight HA (LMW HA) that is pro-inflammatory [37]. The state of HA in the local environment

is one mechanism by which effector T cells and DCs can get information about the local inflammatory milieu.

Effector CD4⁺ T lymphocyte and DC regulatory microenvironment in the inflamed brain parenchyma

The accumulation of effector T cells in the brain parenchyma induces the influx of other immune cells, such as DCs, to the inflammatory site, and these cells further influence the inflammatory microenvironment by interacting with each other. The ability to induce or suppress an immune response makes DCs elite antigen-presenting cells capable of finely tuning and directing T cell responses.

DCs are critical contributors to neuroinflammation within the CNS due to their efficient antigen-presenting capabilities and sole ability to induce the activation and differentiation of naïve CD4⁺ T cells [38]. In recent years, DCs have emerged as pivotal players in the development and maintenance of CNS autoimmunity and inflammation. Numerous studies have demonstrated a substantial accumulation of DCs in the brain and spinal cord in response to local inflammation induced by autoimmunity, infection or trauma [39–41]. MS -amongst several autoimmune diseases- had been correlated with aberrant DC function [42]. Cytokines that are known to induce DC differentiation have also been detected in the CNS, including tumor necrosis factor (TNF- α), IL-1 and IL-6 [43,44]. The plasticity of DCs is unusually high, thus due to the micro environmental changes in different phases of CNS inflammation different types of DCs might dominate. For example, plasmacytoid DCs (CD11b⁻CD11c⁺B220⁺CD8a⁺/PDCA⁺) were detected in the CNS of mice with a relapses in EAE, and these cells were relatively inefficient at stimulating CD4⁺ T cells to produce IL-17 [39]. It was also proposed that myeloid DCs might dominate in the CNS in inflammation as these cells are uniquely biased Th17 and not Th1 cell differentiation that correlated with the expression of TGF- β , IL-6 and IL-23 [39].

DCs interact with effector CD4⁺ T cells within the CNS and can inhibit or stimulate T cell responses [45–48]. DCs have been shown to be critical in inducing memory cytotoxic T cell responses in lymphocytic choriomeningitis virus (LCMV) infection [49]. CNS-resident F4/80⁺CD11c⁺CD45^{high} DC cells isolated from the brains of animals experiencing relapsing EAE (R-EAE) or Theiler's murine encephalomyelitis virus-induced demyelinating disease (TMEV-IDD) kept their stimulatory function and presented endogenous myelin proteolipid protein (PLP) antigen and activated naïve PLP_{139–151}-specific T cells *in vitro* [40]. These studies indicate that local CNS APCs, likely DCs, may contribute to the activation of infiltrating naïve T cells in inflamed brain tissue. This is further supported by data showing that in the complete absence of a peripheral lymphoreticular system (i.e. lymph nodes, Peyer's patches, spleen), T cells entering the CNS can recognize target antigens and induce inflammation [30]. It was shown that in *in vitro* organotypic brain slices, naïve antigen-specific T cells can be activated, and this activation depends on the presence of DCs in the culture [50]. Others demonstrated that boosting DC numbers in the brain by systemic injection of Flt-3 leads to a substantial increase in the severity of EAE clinical symptoms [30]. Conversely, inhibition of Flt-3 signaling ameliorates EAE, providing further evidence that DC numbers in the brain correlate with autoimmune responses [51]. These data altogether indicate that DCs can amplify neuroinflammation in the CNS.

Effector T cells in the brain parenchyma can further interact with local cells, including oligodendrocytes, microglia and neurons. Neurons have been neglected as immunoregulatory cells, since it had been believed that neurons do not express class II molecules. However, recent data indicates that while neuronal stem cells derived from embryonic rat forebrain expressed MHC class I and class II molecules at a low level, when these cells differentiated into neurons,

astrocytes, and oligodendrocytes, they lost their MHC expression. However, IFN- γ induced the expression of MHC molecules in both neuronal stem cells and their differentiated lineages *in vitro* [52]. Interestingly, independent of MHC antigens, neurons have been shown to induce the proliferation of activated CD4⁺ T cells through B7-CD28 and TGF- β /TGF- β -receptor signaling pathways *in vitro* [53]. This activation resulted in amplification of T-cell receptor signaling through phosphorylated ZAP-70, IL-2 and IL-9 and led to the conversion of encephalitogenic T cells to CD25⁺ TGF- β 1⁺ CTLA-4⁺ Foxp3⁺ Treg cells, which suppress encephalitogenic T cells and inhibit EAE [53]. Whether neurons are capable of inducing T cell proliferation *in vivo* has yet to be determined.

Aside from interacting with other cells in the CNS parenchyma, such as APCs, immune cells express a milieu of environmental sensing molecules on their surface, enabling them to respond to different signals within the CNS. Activated effector CD4⁺ T cells express numerous cell surface sensors that influence their behavior within different tissues. For example, it has been shown that the expression of tumor necrosis factor receptor family member, death receptor 3 (DR3 or TNFRSF25) is selectively elevated in Th17 cells and the ligand for this receptor, TL1A, promotes the proliferation of effector T cells [54••]. Adoptive transfer of encephalitogenic Th17 cells into TL1A-deficient mice led to ameliorated EAE compared to wild type hosts, indicating that this molecule is critical in maintaining Th17 effector function in CNS autoimmune diseases [54].

Another family of molecules that are expressed on activated T cells and DCs are the semaphorins (Sema) that recognize their receptors, plexin molecules, which are expressed on cells of the nervous system [55]. These molecules were originally discovered to play crucial roles in axon guidance during neuronal development but recently were recognized to be necessary in the regulation of immune responses. For example, it was shown that the production of type I interferons by plasmacytoid DCs is regulated by a receptor, PDC-TREM, which is associated with Plexin- α 1 (PlxnA1) on the pDC cell surface and is preferentially expressed after TLR-stimulation [56]. When TLR signaling is limited, PDC-TREM expression can be induced in the absence of IFN- α production. However, when limited TLR-stimulation is coupled with Sema6D, a ligand for Plexin- α 1, IFN- α is produced. Thus, PDC-TREM and TLR signals cooperate in inducing IFN- α responses in pDCs. Another semaphorin ligand, PlxnA4, was shown to be essential in negatively regulating antigen-specific T cell responses in CNS autoimmune diseases. This was shown using Plexin- α 4-/- mice that exhibited enhanced antigen-specific T cell responses and accelerated clinical scores in EAE [57•]. Similarly, semaphorin 7A (Sema7A; also known as CD108), which is a glycosylphosphatidylinositol-anchored semaphorin, is known to promote axon outgrowth through beta1-integrin receptors. It has also been shown that there is a high expression of Sema7A on activated T cells and is critical for the effector phase of the inflammatory immune response. Sema7A-deficient (Sema7a-/-) mice were defective in cell-mediated immune responses such as contact hypersensitivity and EAE [58•]. This effect was mediated through interaction between Sema7A and alpha1beta1 integrin at the site of inflammation.

These examples illustrate that regulation of immune cell function at the local inflammatory tissue site is critical in the maintenance of the effector phase of immune responses. It is clear from these studies that one of the most powerful methods of regulating local inflammatory responses in the CNS or the overall induction of autoimmune disease may be pharmacological manipulation of DCs, and we will discuss this below.

Pharmacological manipulation of DCs is a new avenue to treat CNS autoimmunity

Since DCs are critical in the development of CNS autoimmune diseases, significant effort has been made in regulating DC function in order to design novel therapies for MS. In the following paragraphs, we will discuss a partial list of pharmacological agents that impede DC function. These drugs largely result in a suppressive DC phenotype, which may lead to new avenues of treatment for CNS autoimmunity (Table 1).

The first approach of regulating DC function might be interfering with DC differentiation. This is supported by data demonstrating that DC precursor population and circulating DCs are elevated in patients with multiple sclerosis [42]. DCs are derived from a precursor population of hematopoietic bone marrow progenitor cells that enter the blood or tissue where they further differentiate. Differentiation occurs in response to the proper balance of growth factors and cytokines, such as granulocyte/macrophage colony-stimulating factor (GM-CSF), IL-3 and IL-4. Several pharmacological agents have been shown to interfere with the generation and differentiation of DCs. Some glucocorticoids, which are a class of steroid hormones that suppress cell-mediated and humoral immune responses and which are widely prescribed to suppress autoimmune diseases, have been shown to have effects on DC precursor differentiation. For example, treating human peripheral blood monocytes with the synthetic glucocorticoids dexamethasone, prednisone or methylprednisolone, resulted in partly differentiated DCs [59]. In addition to steroids, the cytokine IFN- β has also been shown to have modulating effects on DC differentiation [60]. Thus far, these studies suggest that treatment with glucocorticoids and certain cytokines would result in non-replenished DC populations and would therefore be unable to continue disease exacerbation.

In the event a precursor cell has properly differentiated into DCs, further development and maturation is required in order to effectively initiate an immune response by these cells. The term 'maturation' is used here to phenotypically describe the DCs that may be capable of priming CD4⁺ T cells [61]. It is not used here as a functional description of their abilities. Expression of co-stimulatory molecules is frequently associated with T cell activation. If DCs lack the proper co-stimulatory molecules, chemokine receptors and antigen presentation machinery, they are effectively unable to migrate to the draining lymph nodes to activate antigen-specific CD4⁺ T cells. Of the glucocorticoids, dexamethasone has been repeatedly shown to have inhibiting effects on DC maturation. Both *in vitro* and *in vivo*, dexamethasone reduces expression of co-stimulatory, adhesion and antigen-presenting molecules [62]. More recent findings indicate that dexamethasone may exert its inhibition of DC maturation by up-regulating glucocorticoid-induced tumor necrosis factor receptor ligand (GITRL) on DCs and GITRL on T cells, resulting in activation of indoleamine 2,3-dioxygenase (IDO) through the noncanonical NF- κ B signaling in DCs [63•].

Apart from interfering with DC differentiation and phenotype, pharmacological agents that disturb antigenic processing and T cell activation ultimately interfere with induction and reactivation of immune responses. This is an especially intriguing aspect of DC immunoregulation to consider for the treatment of autoimmune diseases. One of the widely used agents that impairs antigen presentation and subsequent CD4⁺ T cell activation is dexamethasone. A single injection of dexamethasone into mice was enough to impair antigen-presenting function, and treatment with human DCs *in vitro* significantly reduced CD4⁺ T cell proliferation [62]. This inhibition has been correlated with a decrease in activation of NF- κ B, a nuclear transcription factor responsible for regulating genes involved in immune responses [64]. The immunomodulator glatiramer acetate (GA) impaired DC-induced T cell responses from multiple sclerosis patients [65•]. GA is made up of myelin antigens and given to patients with multiple sclerosis; it induces a Th2 response by competing with inherent immune-

stimulating myelin antigens for binding to DCs' MHC molecules [66]. Another pharmacological agent that inhibits EAE induction via its potential affect on DCs is estrogen, 17 β -estradiol [67]. 17 β -estradiol up-regulates IDO expression in DCs and induces the expansion of regulatory CD4⁺ IL-10-producing T cells [67].

T cell subset differentiation is largely influenced by the cytokine milieu secreted from DCs. DCs alter and shape their cytokine profile based on integrated signals from their environment. For example, many glucocorticoids have demonstrated the ability to shift the DCs' cytokine profile from Th1-promoting to a more down-regulatory profile. These agents include dexamethasone, prednisolone, and corticosterone, and treatment with these chiefly resulted in decreased production of IFN- γ , IL12 and TNF- α and up-regulation of IL-10, IL6 and MCP1 [68,69].

Migration of DCs to the T cell zones of the lymph nodes is continuously occurring, regardless of the nature of the antigen or cytokine milieu within the tissue. Therefore, DC migration to the site of infection and then to the draining lymph nodes is a necessary process that must occur in order to elicit an antigen-specific T cell response. Broad inhibition of DC migration, suppressing migration both to and from inflammatory sites by down-regulating expression of RANTES (CCL5), CCR5 and CCR7, has been shown with a combination therapy of dexamethasone and 1 α 25-dihydroxyvitamin D₃ [64]. Cyclosporin A has been shown to inhibit DC migration more selectively by affecting CCR7 without having any effect on CCR1, 2 or 5 [70]. It was proposed that migration of DCs from the CNS is critical for the initiation of CNS immune responses [71,72]. Therefore, interfering with this migration is critical in order to impede the initiation and reactivation of CNS autoimmunity. Migration of DCs across the BBB has been shown to be mediated by matrix metalloproteinases and chemokines, including MIP-1 α [31]. When MMP inhibitor GM6001 is present, DC migration across an *in vitro* brain microvessel endothelium is significantly reduced [31]. Ultimately, inhibiting or suppressing DC migration, either to or from the site of infection or lymph nodes, would result in decreased T cell activation and reactivation.

It is evident that there are a number of pharmacological agents that interfere with different aspects of DC immunity. Most of the above mentioned agents have multiple effects on DCs. The challenge that remains is to find agents that interfere more specifically with different DC functions and are able to reach the CNS. A better understanding of DC biology will help achieve this important goal and facilitate new therapies in autoimmune CNS diseases.

Conclusion

In the last few years we have witnessed the generation of a large body of knowledge regarding the environmental regulators that regulate immune responses in the CNS. Here we discussed some of the critical elements in the CNS that contribute to the special anti- or pro-inflammatory environment in this tissue. These data show that immune cell migration and survival in the perivascular space and CNS parenchyma are highly influenced by environmental factors. We have summarized evidence that DCs are critical contributors to the initiation and regulation of inflammatory diseases in the CNS. Elucidating mechanisms of pharmacological manipulations of immune cells in the CNS will lead to novel immune therapies for controlling CNS inflammatory diseases by blocking DC traffic and function.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest

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Figure 1. Some of the critical physiological, anatomical features and molecules that contribute to the regulation of immune responses in the non-inflamed CNS.

Figure 2. Path of immune cells through the BBB and neurovascular unit in their journey into the CNS parenchyma in CNS inflammation. Cerebral microvascular endothelium, together with astrocytes, neurons, and the basement membranes of endothelial cells (EBM) or astrocytes (ABM), constitute a "neurovascular unit" that is essential for the health and function of the CNS.

Table 1

Strategies to interfere with DCs in autoimmune diseases (references are in the text).

Level of DC Interference And Drug		General Mechanism of Action
Differentiation	Glucocorticoids	Inhibits differentiation of monocyte and/or bone marrow-derived precursors into DCs.
	Corticosteroids	
	Methylprednisolone	
	Cytokines	
	Interferon- β	
	Immunomodulatory	
	1 α ,25dihydroxyvitaminD ₃	
	Estrogen Receptor Modulators	
	Raloxifene	
	Tamoxifen	
Phenotype	Glucocorticoids	Inhibits maturation by preventing expression of costimulatory molecules.
	Dexamethasone	
	Corticosterone	
	Prednisolone	
	Immunomodulators	
Antigen Uptake and Presentation	1 α ,25dihydroxyvitaminD ₃	
	Glucocorticoids	Inhibition includes impairing macropinocytosis and mannose receptor-mediated endocytosis in BMDCs. There is also a reduced capacity to activate alloreactive T cells and induce a Th1 response, often times inducing a Th2 or Treg-like CD4+ T cell response. Decreased ability to stimulate Th1 cells may be attributed to some agents binding MHC molecules, thus inhibiting binding of self-antigen, a decrease in nuclear translocation of NF- κ B, the up-regulation of IDO or by inducing release of PGE2 and MHCII expression.
	Dexamethasone	
	Immunomodulators	
	Glatiramer acetate	
Cytokine Production	1 α ,25dihydroxyvitaminD ₃	
	Glucocorticoids	Provide signals that impair the DCs' Th1-mediated response and instead develop a Th2 or Treg-mediated response. This is often done by impairing production of Th1-favoring cytokines, IL-6, IL-12 and TNF- α , and increasing production of Th2 or Treg-mediating cytokines, IL-10, IL-5, IL-13 and TGF- β .
	Dexamethasone	
	Corticosterone	
	Prednisolone	
	Immunosuppressants	
	Cyclosporin A	
	Mycophenolate mofetil	
	Tacrolimus (FK506)	
	Immunomodulators	
Migration	1 α ,25dihydroxyvitaminD ₃	
	Glucocorticoids	Migration may be prevented by increasing expression of monocyte-chemoattractant protein-1 (MCP-1), blocking CCR7, blocking migratory molecules with monoclonal antibodies or with other specific inhibitors.
	Dexamethasone	
	Anti-CD31 MoAb	
	GM6001	