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The Meninges: New Therapeutic Targets For Multiple Sclerosis

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Abstract

The CNS is largely comprised of non-regenerating cells, including neurons and myelin-producing oligodendrocytes, which are particularly vulnerable to immune cell mediated damage. To protect the CNS, mechanisms exist that normally restrict the transit of peripheral immune cells into the brain and spinal cord, conferring an “immune specialized” status. Thus, there has been a long-standing debate as to how these restrictions are overcome in several inflammatory diseases of the CNS, including multiple sclerosis (MS). In this review, we highlight the role of the meninges, tissues that surround and protect the CNS and enclose the cerebral spinal fluid, in promoting chronic inflammation that leads to neuronal damage. Although the meninges have traditionally been considered structures that provide physical protection for the brain and spinal cord, new data has established these tissues as sites of active immunity. It has been hypothesized that the meninges are important players in normal immunosurveillance of the CNS but also serve as initial sites of anti-myelin immune responses. The resulting robust meningeal inflammation elicits loss of localized blood barrier integrity and facilitates a large-scale influx of immune cells into the CNS parenchyma. We propose that targeting of the cells and molecules mediating these inflammatory responses within the meninges offers promising therapies for MS that are free from the constraints imposed by the blood brain barrier. Importantly, such therapies may avoid the systemic immunosuppression often associated with the existing treatments.

Introduction

Inflammatory responses are best known for their protective functions after tissue injury. However, they can easily pose a threat to the organism when they persist. In the setting of microbial infections, the physiological functions of inflammation dominate. Pathogenic microbes most readily gain access to the body through sites that interface with the external

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environment, such as the skin, gastrointestinal tract, and respiratory tract. These “barrier sites” are replete with resident innate immune cells such as mast cells, dendritic cells, macrophages and innate lymphoid cells. Microbes express pathogen associated molecular pattern molecules (PAMPs) and can engage pattern recognition receptors (PRRs) displayed on resident immune cells. The ensuing activating signals indicate “danger” to the host and trigger increased expression of a number of immunomodulatory molecules by immune cells including major histocompatibility complex-I and II (MHC Class I and Class II) proteins, adhesion molecules, homing receptors, chemokines, and cytokines. These molecules exert a variety of effects that collectively a) act on the local endothelium and increase vascular permeability, b) direct migration of circulating leukocytes into the affected tissue, c) increase antigen uptake by phagocytes and d) enhance immune cell effector function. Under most circumstances, the response of resident and infiltrating immune cells leads to clearance of the microbe. In the absence of the microbial trigger, inflammation subsides and tissue homeostasis is reestablished. In contrast, chronic inflammation is pathologic and can occur with intractable infections or in autoimmune diseases where the eliciting antigen(s) persist. In autoimmunity, self-antigens are the target of the adaptive and innate immune response and the outcome is tissue destruction. The initiating danger signals that elicit an autoreactive response remain undefined.

This review will focus on the factors that contribute to the chronic inflammation associated with multiple sclerosis (MS), a central nervous system (CNS) demyelinating disease. Inflammation in the CNS is particularly devastating because unlike most peripheral cells, neurons and oligodendrocytes (the myelin producing cells) are largely post-mitotic and unable to regenerate. We will discuss the physiological mechanisms that limit immune cell entry into the CNS. The evidence that the meninges, tissues adjacent to the CNS, are an immunologically active barrier site, much like the gut or the lungs, will also be reviewed. It is hypothesized that a primary role of the meninges is to serve as a first line defense against infections that threaten the CNS. However in MS, there is compelling evidence that meningeal inflammation initiates the events that lead to demyelination. We speculate that meningeal inflammation also influences other inflammatory CNS diseases and that cells in the meninges are potential therapeutic targets, free from the restrictions imposed by the BBB.

Immune specialization in the CNS

The brain and spinal cord are often described as immune-privileged, indicating they are not accessible to the peripheral immune system. However, small numbers of peripherally derived immune cells patrol the CNS during homeostasis. Thus, a new term, immune-specialized, has been applied to encompass the tightly controlled access of the immune system to the CNS (reviewed in [1]). A primary determinant of immune specialization in the CNS is the ability of the blood brain barrier (BBB) to restrict the entry of large molecules and cells. The BBB is a dynamic structure formed by specialized endothelial cells that separates the CNS from systemic circulation. Unique to this vasculature is its interaction with astrocyte foot processes and the presence of tight junctions between the endothelial cells. In addition, the CNS parenchyma lacks resident professional antigen presenting cells (APCs) and instead relies on microglia, which have low resting MHC Class II expression

and minimal phagocytic capacity [2]. High concentrations of anti-inflammatory mediators such as IL-10 and TGF β are also detected within the CNS of healthy individuals [3, 4]. Despite these protective mechanisms, inflammatory damage is a key contributor to many neurologic diseases including stroke, traumatic brain injury, Parkinson's disease, neuromyelitis optica, and multiple sclerosis (MS).

Overview of multiple sclerosis (MS)

Clinical manifestations

MS is the most common demyelinating disease of the CNS, affecting more than 400,000 individuals in the United States and 2.5 million worldwide [5, 6]. It commonly presents in young adulthood with transient neurological symptoms, which often progress to a variety of more permanent neurological deficits, including muscle weakness and spasms; difficulties with coordination, balance, and movement; fatigue; acute or chronic pain syndromes; and bladder and bowel difficulties [7]. Many patients also experience impairments in cognitive function, including memory loss, decreased mental processing speed, and diminished executive function. These symptoms can occur early in disease and often worsen with time, even if physical symptoms remain stable [8, 9].

Two clinically predominant variants of MS are recognized. Relapsing-remitting (RR) MS affects 85% of MS patients and primary progressive (PP) affects 10% of patients [10, 11]. The former is characterized by transient neurological symptoms with almost complete recovery between relapses, while the latter presents with steadily decreasing neurological function from the time of initial presentation [12]. Nearly 70% of patients with RR-MS will progress to a secondary progressive phase (SP-MS), in which neurological deficits begin to accumulate between relapses [11]. Furthermore, disease courses differ greatly between men and women. Women are nearly three times more likely than men to develop MS [13]. Women are also more likely to present at a younger age and follow a RR-MS course. On the other hand, men are diagnosed later and more frequently exhibit a PP-MS course. The mechanisms behind this sex difference remain unknown, but likely involve interplay between hormones, microbiota, and X chromosome dosage [13–21].

Murine models of MS

Several mouse models are available to study the events associated with CNS demyelinating disease. These include a chemically-induced model in which mice are fed the toxin cuprizone (bis-cyclohexanone oxaldihydrazone) and infection with the CNS-tropic virus, Theilers encephalomyelitis virus (TMEV). However, experimental autoimmune encephalomyelitis (EAE) is the most widely model used. Albeit imperfect, EAE is thought to recapitulate many of the clinical, pathological, and immunologic events observed in human disease [22–25]. EAE typically presents with ascending paralysis, with mice first exhibiting a limp tail and then progressing to hind limb paralysis. Similar to MS, EAE is characterized by loss of focal BBB integrity and CNS-infiltrating adaptive and innate immune cells, leading to subsequent neuron and oligodendrocyte damage. C57BL/6 and SJL mice, which are particularly susceptible to disease, are the most commonly used strains. When immunized with myelin peptides in Complete Freund's Adjuvant (CFA), these mice

develop either a chronic progressive (C57BL/6) or a relapsing-remitting disease course (SJL). Importantly, SJL mice also recapitulate the sex dimorphism of MS with female SJL mice exhibiting more severe disease, increased incidence, and a more consistent relapsing pattern than their male counterparts [20]. While it can be argued that the SJL disease model is more clinically relevant, the C57BL/6 model offers a multitude of transgenic and knockout mice for interrogating the contributions of specific molecules.

Conventional views of immune system dysregulation in MS

The etiology of MS remains unknown, however, it is generally considered to be an autoimmune disease initiated by an underlying dysregulated immune system. Recent genome-wide association studies (GWAS) have provided support for this theory. In the largest such study data from approximately 10,000 patients was evaluated and more than 50 genetic susceptibility loci were identified. The vast majority of these loci mapped to genes with important immune cell functions, most notably those associated with MHC Class II antigen presentation and T helper cell differentiation [26]. It is widely believed that an unidentified environmental risk in genetically susceptible individuals promotes the activation of myelin-specific T cells, which normally reside in the periphery in a tolerant state. If these cells are able to cross the BBB and enter the CNS, they are reactivated by infiltrating and/or resident APCs presenting myelin antigens. Once reactivated, these T cells orchestrate localized inflammation leading to myelin and axonal damage. While this idea has provided a useful framework for understanding how disease is initiated, it fails to account for the fact that the CNS is immune-specialized. Thus, several questions remain unanswered: what causes loss of BBB integrity in MS? and what are the signals that initiate trafficking of peripherally derived cells into an uninfamed CNS?

Immune mediated demyelination in the CNS-evidence for both white and gray matter involvement

Insights into potential mechanisms of disease initiation come from studies on the location of MS lesions. While still a diagnosis of exclusion, MS is most often diagnosed by the detection of lesions disseminated in space and time using magnetic resonance imaging (MRI). Most often, these lesions are observed in areas of highly myelinated white matter and are easily visualized by MRI. White matter is primarily composed of nerve axons and myelinating oligodendrocytes and white matter lesions delineate regional destruction of myelin, oligodendrocytes and nerve axons. Although white matter plaques are considered a hallmark of MS [27], there is increasing evidence that damage to the cortical gray matter also greatly contributes to disease pathogenesis [28–35]. The gray matter is comprised of the less myelinated nerve cell bodies and dendrites, glial cells, and many capillaries. In the brain, the gray matter is concentrated at the surface of the cerebral hemispheres and cerebellum (Figure 1), as well as deep within the diencephalon and brainstem. In contrast, the gray matter of the spinal cord is located at the center and the white matter comprises the peripheral aspects of the cord. Cortical gray matter damage is more difficult to visualize by MRI than white matter lesions, but is commonly observed in both early stage and chronic progressive disease [31, 36]. Notably, a prominent feature of cortical demyelination is overlying inflammation in the meninges [36]. As discussed in more detail below, the meninges interface with the gray matter of the cerebral cortex and their blood vessels

transition from peripheral to cerebral vessels, gaining the impermeable properties exclusive to the BBB. The proximity of the meninges to the CNS parenchyma together with its ability to initiate and sustain robust inflammatory responses has lead investigators to ask the question: Can immune responses within the meninges drive CNS-inflammatory disease?

The double-edged sword of neuro-meningeal inflammation

The meninges are a collection of three thin and fibrous tissues that surround and protect the CNS

The meninges are highly vascularized tissues composed of the outermost dura mater, which lies directly under the calvarium or vertebral column, the middle arachnoid mater, and the innermost pia mater, which lies directly on the CNS parenchyma (Figure 1). The dura mater is a loose collection of fibroelastic cells with interdigitating cell processes, no extracellular collagen, and significant extracellular space [37]. Large dural sinuses exist between the two layers of the dura mater of the brain that drain blood and cerebral spinal fluid (CSF) from the cerebral vessels and ventricles, respectively. The arachnoid mater is named for its web-like appearance and is also a fibrous membrane formed by flattened, closely connected cells with no extracellular collagen [38]. A large number of filaments, called arachnoid trabeculae, extend from the arachnoid mater to blend with tissue of the pia, allowing for drainage of CSF. Notably, there is an anatomical space between the arachnoid and pia mater, denoted the subarachnoid space (SAS), a spongy tissue full of arachnoid trabeculae that encloses the CSF. CSF is produced by the choroid plexus, a single layer of highly vascularized epithelium that lines all four ventricles of the brain. It provides buoyancy for the brain, regulates cerebral blood flow, and drains toxins and metabolic waste from the CNS (Figure 2). The pia mater directly adheres to the CNS parenchyma, following the many sulci and gyri of the human brain [39]. Thus, the pia mater has the largest surface area of the three membranes. Unlike the other layers, the pia is continuous with the perivascular connective tissue sheath of blood vessels of the CNS [37] (Figures 1 and 3). As blood vessels transverse the pia mater, they gain elaborate tight junctions between the endothelial cells and are surrounded by astrocyte foot processes, forming the BBB of cerebral vessels [40] (Figure 3). Until recently, the primary role of the meninges was thought to be physical protection of the CNS; however, the meninges are gaining appreciation as non-lymphoid sites of active immune responses [31, 36, 41].

The meninges are barrier sites of active immunological responses

Similar to the gut and lungs, which protect the body from the external environment, the meninges house a wide variety of immune cells, suggesting they too are barrier sites. Resident cells include macrophages, dendritic cells, mast cells, innate lymphoid cells, fibroblasts, and even stem cells, while neutrophils, T cells, and B cells infiltrate during inflammation ([42–46] and unpublished observations). Together with immune cells, mouse meningeal fibroblasts produce pro-inflammatory mediators such as TNF, IL-1 β , iNOS, and IL-6 in response to inflammatory signals, phagocytose debris, and form networks with meningeal macrophages via gap junctions [46–48]. Many of these cells also express receptors for TNF p55 [49] and IL-1 β [50], demonstrating that they are targets for systemically produced cytokines. Meningeal cells from primary mouse cultures have been

shown to secrete antimicrobial peptides [51] and along with choroid plexus cells, express PRRs; including TLR4, TLR7, and TLR9 [52, 53]. Such diverse cellular responses likely allow for effective protection against microbes. But similar to other barrier sites, the meninges can be sites of chronic inflammation.

The threat of neuro-meningeal inflammation

There is clear evidence in infection settings that inflammation predominating in the meninges can have consequences for neurologic function. In humans, this is well documented in pneumococcal meningitis where the robust meningeal inflammatory response can cause permanent neurological sequelae. For this reason, the paradoxical use of antibiotics together with steroids, to control the inflammation, is often the treatment of choice. There is also evidence in mice that aspects of the meningeal response to *S. pneumoniae* cause more harm than benefit. When infected with *S. pneumoniae*, interferon- γ (IFN γ) knockout (KO) mice exhibit prolonged survival compared to their wildtype (WT) counterparts [54]. Furthermore, with antibiotic treatment the IFN γ KO mice not only survive long term, but also have no neurological sequelae. Classic pro-inflammatory mediators, most predominately TNF, IL-1 β , and reactive oxygen species (ROS), are also implicated in microbial-induced meningeal inflammation [54–56]. ROS are particularly damaging to the CNS given the high oxygen consumption relative to other organs. Collectively, these data suggest that inflammatory responses in the meninges affect the CNS and if robust enough, pose just as much, if not more of a threat, to the individual's health than the infection itself.

Evidence for meningeal inflammation in multiple sclerosis

Meningeal inflammation is associated with MS

There is a striking spatial relationship between meningeal inflammation and gray matter lesions and data from both human and mouse studies support the concept that meningeal inflammation is an unavoidable and perhaps necessary prelude to leukocyte infiltration into the CNS in MS. Using immunohistochemistry to assess post-mortem tissue samples from MS patients, Choi and colleagues observed that high numbers of meningeal lymphocytes correlated with severe disease and death at a younger age [57]. In some patients with chronic MS, a higher density of T cell infiltrates is observed in the meninges when compared to the brain parenchyma [58]. Damage to the glia limitans and subpial demyelination is most often observed in cases of MS with more severe meningeal inflammation [57, 59]. In addition, the meninges are often sites of ectopic lymphoid follicle (organized aggregates of T cells, B cells, antigen-presenting cells, and antigen) formation in chronic-progressive MS. These aggregates are frequently associated with chronic inflammation in other diseases and serve as sites for T cell reactivation and antibody production [41, 60, 61].

While the above examples highlight the association of meningeal inflammation to chronic disease, until recently, little was known about the cause and effect relationship of meningeal inflammation to CNS damage. That is, does meningeal inflammation initiate CNS damage or is it simply a bystander effect? A significant study by Lucchinetti *et al.* provides the first evidence that addresses this question. Rather than using post-mortem samples, the investigators analyzed brain biopsies from MS patients at initial disease presentation and

determined that meningeal inflammation occurred early in disease and preceded the appearance of classic white matter lesions in many patients [31]. Furthermore, meningeal inflammation was observed most often adjacent to gray matter lesions, cortical demyelination, and regions of BBB damage. Extensive perivascular immune cell inflammation in the meninges early in disease often corresponds to a more severe progressive disease course [31, 41]. The above studies suggest a direct relationship between the magnitude of early meningeal inflammation and the extent of CNS damage and disease outcome.

Studies in EAE help elucidate the role of meningeal inflammation in MS

Most data that directly implicates the meninges in disease pathogenesis has come from rodent studies. Several independent studies demonstrated that myelin-specific T cells migrate to the meninges during the pre-clinical phase of EAE where they are reactivated within the subarachnoid space by antigen presenting cells [43, 62–64]. MHC Class II+ dendritic cells in association with extracellular myelin and MHC Class II+ macrophages have been shown to directly interact with T cells in the meninges [43, 62, 63, 65–67]. Importantly, lymphoid organs and MHC Class II expression by CNS resident cells are dispensable for EAE development, but MHC Class II expression by CD11c+ meningeal vessel-associated DCs is not, further implicating meningeal-derived immune cells in disease [64].

While meningeal inflammation prior to clinical disease onset has been documented in mice, less is known about the role of meningeal inflammation during relapses. To address this question, our laboratory compared the kinetics of immune cell entry into the CNS and meninges over the course of relapsing-remitting EAE in SJL mice (Walker *et al.* manuscript submitted). We observe an influx of CD45+ cells (detected by flow cytometry) in the meninges during preclinical disease (6 and 10 days post immunization [dpi]) that precedes the loss of blood brain barrier integrity and entry of inflammatory cells into the CNS parenchyma. Appreciable numbers of CD45^{hi} cells are not observed in the CNS until peak clinical disease (14 dpi). Inflammatory cell numbers in the meninges and CNS decrease significantly during remission (25 dpi). Surprisingly in relapse, neither overt blood brain barrier permeability nor large scale immune cell accumulation in the CNS was detected. Rather, increases in immune cell numbers and pro-inflammatory mediators in the meninges correlates most closely with clinical relapse (35 dpi). This suggests that meningeal inflammation may regulate the onset of clinical relapses.

The meninges provide access to the uninflamed CNS

The “two-step” model: a new concept to explain peripheral immune cell entry into the CNS

A major unresolved question in MS research is: How do peripherally primed autoreactive T cells first gain access to the uninflamed CNS through the highly restrictive BBB? According to the conventional wisdom, in order to initiate disease, a small number of myelin-specific T cells directly cross the BBB, where they encounter APCs presenting myelin. This leads to T cell reactivation and a localized inflammatory response is established. The ensuing inflammation elicits loss of BBB integrity and large-scale immune cell influx to the CNS.

However, several recent studies have provided data that support a “two-step” model, in which immune cells first transit through the meninges, as part of normal immune surveillance [43, 62, 63, 68, 69]. This model also posits that leukocyte access to the CNS parenchyma occurs only when meningeal inflammation is prolonged and robust.

In the “first wave,” T cells circulate via the bloodstream to the choroid plexus. Although tight junctions between the choroid plexus epithelium cells exist and create the moderately restrictive blood-CSF barrier, the endothelial cell tight junctions and astrocyte foot processes critical to the BBB are absent (Figure 4A). The choroid plexus also harbors an extensive network of fenestrated capillaries which allows for the transit of a small number of T cells from the systemic circulation, across the blood-CSF barrier, and into the CSF [70]. Once in the CSF, T cells circulate through the ventricles and into the subarachnoid space of the meninges (Figure 2). In addition to microbes and toxins, CNS-derived self-antigens, such as myelin, can also access the subarachnoid space through this route and be processed and presented by resident APCs. Unlike other tissues, the CNS does not have a true lymphatic system. Therefore, immune cells within the meninges are the first to interact with draining antigens from the CNS.

The first wave in the “two wave” hypothesis represents the presumed physiological immune surveillance of the CNS (Figures 4A and B). However, the interaction of myelin-specific T cells with myelin-loaded APCs can elicit T cell re-activation and mediator production in the meninges (Figures 4B and C). The resultant recruitment of additional inflammatory cells leads to loss of local BBB integrity (Figure 4D) and a robust non-specific “second wave” of peripheral leukocyte influx into the CNS. It is this large-scale population of infiltrating cells that likely mediate significant demyelination, neuronal damage and lesion formation (Figure 4E).

The results of a number of studies support the “two-step” hypothesis. Particularly compelling is a recent study by Reboldi and colleagues, which also highlights the critical role of the choroid plexus as a gateway for immune cell entry to the CNS [69]. These investigators demonstrate that IL-17-producing, CCR6+ T cells enter the choroid plexus via interactions with CCL20, a chemokine constitutively expressed on choroid plexus epithelial cells. This interaction facilitates T cell migration into the meninges. Although CCR6 KO mice are resistant to EAE, the transfer of naïve CCR6+ CD4+ T cells prior to immunization renders these mice susceptible to EAE. Surprisingly, a large population of CCR6 KO T cells was detected in the CNS of these mice. Presumably CCR6-CCL20 interactions are critical for initiating the “first wave” (entry into the meninges via the choroid plexus), but do not contribute to the “second wave” (entry into the CNS). Thus, the transit of CCR6+ T cells through the meninges was sufficient to initiate local inflammation that allowed migration of CCL6– cells into the CNS. Importantly, the authors also detected elevated levels of CCR6 in the CSF of MS patients and uniform CCL20 expression on human choroid plexuses along with acute inflammatory changes [71]. Choroid plexuses of healthy or amyotrophic lateral sclerosis brains did not show such inflammatory changes. Other molecules implicated in leukocyte entry through the choroid plexus include A2a adenosine receptors (A2AAR) and P-selectin, both of which are expressed on cells in the choroid plexus and subarachnoid space [72–74].

Once T cells enter the meninges via the choroid plexus, how do they initiate the second wave? This is likely achieved by chemokines produced early in the response by the first wave cells, which then induce robust leukocyte migration to the meninges. One chemokine in particular, CXCL10 (IP-10), and its receptor CXCR3, is of interest. CXCL10 levels are elevated in the CSF of MS patients and correlate with the CSF leukocyte count [75]. Furthermore, CXCL10 expression is induced by IFN γ [76], is spatially associated with demyelinating lesions, and correlates with CXCR3 expression [75, 77]. Notably, CXCR3 is expressed by nearly 90% of the CSF invading T cells [75].

It has been proposed that locally expressed mediators within the meninges can diffuse across the pia mater into the CNS parenchyma through the Virchow-Robin spaces, perivascular extensions of subarachnoid space that surround perforating vessels (Figure 3). This mediator diffusion allows for direct cytotoxic actions on oligodendrocytes and neurons as well as indirect activation of microglia [60, 78, 79]. A significant study by Gardner and colleagues tested this hypothesis by injecting the pro-inflammatory cytokines TNF and IFN γ into the subarachnoid space of rats pre-immunized with a subclinical dose of myelin antigen [79]. These rats developed extensive subpial demyelination accompanied by a gradient of cortical microglial activation that was not observed in rats immunized with myelin alone nor in unimmunized control rats injected with cytokines.

Specific and targetable meningeal cell populations

Meningeal mast cells recruit neutrophils to the meninges

Mast cells are perhaps best known for their pathologic role in allergic disease, but have also been implicated in both EAE and MS (for review see [80, 81]). Mast cells were documented in the CNS lesions of MS patients over a century ago [82] and in both MS and EAE, sites of inflammatory demyelination correspond with sites of mast cell accumulation in the brain and spinal cord [83, 84]. Furthermore, levels of histamine and the mast cell specific protease, tryptase, are elevated in the CSF of MS patients [85, 86]. Microarray analyses of MS lesions show that transcripts encoding tryptase, histamine 1 receptor, and Fc ϵ RI are significantly increased in chronic disease [87]. *In vitro*, myelin can directly stimulate mast cell degranulation and mast cell proteases degrade myelin protein [88–92]. The above data strongly suggests mast cell activation is associated with MS/EAE.

Using mast cell deficient mice *Kit*^{W/W^{-v}} mice, our laboratory was the first to demonstrate that meningeal mast cells influence disease initiation and progression in both the chronic C57BL/6 and relapsing-remitting SJL models of disease [93, 94]. *Kit*^{W/W^{-v}} mice have two independent mutations in *Kit*, which encodes c-kit (also known as CD117), the receptor for stem cell factor (SCF). While most cells develop normally in these mice [95], mast cells require sustained signaling for normal development and survival. Thus *Kit*^{W/W^{-v}} mice have a profound mast cell deficiency. While *Kit*^{W/W^{-v}} female mice are protected from severe EAE, selective reconstitution of mast cells to the meninges by injection of bone marrow derived mast cells at the cranial vertex restores disease susceptibility. Notably, mast cells are activated within an hour of active disease induction and secrete several mediators including TNF, IL-1 β , and CXCL2 [96], indicating the role of mast cells in early disease. Meningeal

mast cell-derived TNF initiates an early neutrophil influx to the meninges, which is essential for loss of BBB integrity and leukocyte influx into the CNS [44].

Evidence that mast cells in the meninges modulate T cell effector function

Mast cells have established roles in modulating T cell responses. Through their expression of cytokines and co-stimulatory molecules, these innate cells have been implicated in T helper cell polarization, T cell proliferation, and activation [97–100]. Our recent studies suggest that meningeal mast cells also affect both the numbers and effector function of myelin specific T cells within the meninges. Using an adoptive transfer system, we tracked the entry of myelin specific T cells in the meninges of WT and *Kit*^{W/W-v} mice and observed that in the absence of mast cells, autoreactive T cells do not accumulate in the meninges (unpublished observations).

We have evidence that mast cell-T cell interactions in the meninges can alter the effector function of myelin-specific T cells. Adoptively transferred myelin-specific T cells that transit through the meninges can interact with resident mast cells and through the actions of mast cell-derived IL-1 β , acquire the ability to produce GM-CSF. GM-CSF is a cytokine that has recently been shown to be necessary and sufficient to confer encephalitogenicity to T cells [101, 102]. Furthermore, mast cells co-cultured with previously primed myelin-specific T cells, induce GM-CSF production by T cells (unpublished observation). Thus, meningeal mast cells appear to greatly amplify the autoreactive T cell response, not only by recruiting T cells to these sites but also by enhancing their ability to cause disease.

Innate lymphoid cells, implicated in ectopic lymphoid follicle formation and memory T cell survival, are residents of the meninges

ILCs are a newly defined heterogeneous group of cells that have properties of both innate and adaptive immune cells (reviewed in [103]). They share functional characteristics with conventional T cells but lack T cell receptors and can respond vigorously to a wide variety of signals. ILCs are classified into three groups (Groups 1, 2, 3) based on their cytokine-producing potential and the expressed lineage specific transcription factors (reviewed in [104]). They are numerous within barrier sites, such as the gut and lungs where their functions have been best described [105–108]. We recently discovered that Group 3 ILCs reside in the meninges (unpublished observations), another indication that the meninges are analogous to other barrier tissues. In addition, ILCs have been implicated in several chronic diseases, including inflammatory bowel disease and airway inflammation [105, 106, 108]. Their previously defined activities make it likely they contribute to chronic CNS disease as well. Group 3 ILCs are defined, in part, by their ability to express IL-17, IFN γ , and GM-CSF, cytokines with pathogenic actions in EAE and MS (reviewed in [103, 104]). These cells also express OX40 and CD30, two molecules that have established roles in maintaining the survival of memory T cell populations [109, 110]. A ckit⁺ subset of Group 3 ILCs promotes the formation of ectopic lymphoid follicles (ELFs), structures observed in the meninges proximal to inflamed vasculature and associated with chronic progressive MS [60, 61, 111–114]. These follicles likely serve as sites for the sequestration and presentation of autoantigen, as well as for intrathecal (subarachnoid) antibody production, another distinguishing feature of MS [115]. Studies to assess the role of ILCs in EAE are ongoing in

our laboratory and are made increasingly relevant by the reports that a drug in clinical trials, daclizimab, targets ILCs and is an effective treatment for MS [116].

Current Therapies

Treatment approaches differ for different patients

Despite the advancements in understanding the disease process, therapy options are still imperfect. Human studies have proven difficult for studying drug efficacy given the heterogeneity of the disease. Furthermore, MS is a multi-stage disease that requires different therapeutic approaches at different times. During a relapse, patients are often treated for symptomatic relief, most commonly with steroids. Thus, the need for disease modifying therapies (DMT) is still substantial. While the goal of any treatment is to modify the disease course, both in the short and long term, a curative therapy that can stop and reverse CNS damage is the ultimate goal

Current DMT approved for MS

Currently, MS therapy relies on six primary DMTs (Table 1). Natalizumab (Tysabri) a monoclonal antibody to $\alpha 4$ -integrin, is an FDA approved therapy that can claim direct translation from studies of EAE. This drug blocks interactions with VCAM-1 (vascular cell adhesion molecule-1) on endothelial cells and ultimately prevents leukocyte entry into the CNS [117]. While proven effective in the clinic, serious concerns about the safety profile of natalizumab arose when a small subset of patients developed progressive multifocal leukoencephalopathy (PML) caused by infection with human polyomavirus John-Cunningham (JC) virus [118, 119]. It has been hypothesized that blocking lymphocyte entry to the CNS prevents normal immune surveillance mechanisms, allowing virus reactivation in a subset of patients, an idea consistent with the two-step model [120]. Post-marketing studies of natalizumab-treated patients identified risk factors in the MS population for developing PML (therapy longer than 2 years, history of immunosuppressant therapy, and JC-antibody positivity) that has allowed for cautious, selective, and effective use of the drug [121].

Other disease-modifying MS therapies include the interferons (Betaseron, Avonex, Rebif) and glatiramer acetate (Copaxone), both of which have high safety profiles and moderate effectiveness [122]. In the past few years, the first oral therapies have been introduced to the market: fingolimod, teriflunomide, and dimethyl fumarate. Fingolimod (Gilenya) is a sphingosine-1-phosphate analogue. When S1P receptors are activated, they induce the egress of T cells from the blood to the secondary lymphoid organs [123]. Fingolimod activates this signaling cascade and sequesters T cells in the secondary lymphoid organs, thus dramatically reducing the number of circulating T cells that could potentially cross into the CSF and/or CNS parenchyma [124]. Teriflunomide (Aubagio) inhibits dihydroorotate dehydrogenase (DHORDH), a critical enzyme for the production of pyrimidine ribonucleotide and thereby prevents effective clonal expansion of lymphocytes and dampens the autoimmune response [125, 126]. Interestingly, teriflunomide also acts as a tyrosine kinase inhibitor [127]. Given the tyrosine kinase activity of c-kit, Teriflunomide's effectiveness may be due, at least in part, to its ability to modulate mast cell and/or c-kit+

ILC functions. Dimethyl fumarate (Tecfidera) targets the harmful production of reactive oxygen species and nitric oxide that damage axonal transport and therefore, the neurons [128].

Future therapies

Therapies currently in clinical trials

Several therapies are currently under review for their use in multiple sclerosis (Table 1). These include alemtuzumab, laquinimod, ocrelizumab, and daclizumab. Alemtuzumab is a monoclonal antibody directed to CD52, a glycoprotein on the surface of mature lymphocytes. Alemtuzumab works by depleting all lymphocytes and thus, while effective at reducing relapse rate, will likely result in compromised immunity [129]. Laquinimod's mechanism of action is not well understood but studies suggest that it increases the production of anti-inflammatory cytokines by T cells and decreases leukocyte migration into the CNS [130]. Ocrelizumab is a monoclonal antibody against CD20 and works by depleting B cells [131]. Daclizumab is a monoclonal antibody against CD25, the alpha chain of the IL-2 receptor. Surprisingly, early mechanistic studies demonstrated little impact of daclizumab on activated T cells. More recent studies indicate that daclizumab inhibits early ILC differentiation from CD34+ hematopoietic progenitor cells and induces a phenotypic switch of c-kit+ ILCs to CD56^{bright} NK cells [116].

Future targets for therapy development

There is substantial evidence supporting the concept that the choroid plexus and meninges are a gateway to the uninflamed brain. Should the “two-step” model of immune cell entry into the CNS be further validated in humans, strategies to prevent the meningeal “first wave” response from initiating the “second wave” response in the CNS would be ideal. However, because most patients will not present with symptoms until the second wave is well underway, this may represent a clinical impossibility. Our finding that inflammation in the meninges exacerbates clinical relapses in EAE provides hope that meningeal-targeted therapies may be effective for controlling established disease. Such localized therapies would accomplish two important goals: they would be free from the constraints imposed by the BBB and would also avoid the complications of generalized immunosuppression resulting from more conventional systemic drug delivery regimes.

Although the presumed actions of many of the currently used drugs are within the CNS, given the relatively permeable vasculature of the meninges, we speculate that some of these drugs are actually modulating inflammatory responses in the meninges. That said, delivering directed and localized meninges-targeted therapies provides considerable challenges. A subdural transmeningeal delivery system was recently pioneered for the delivery of drugs to the brain for the treatment of drug-resistant seizures [132, 133], indicating that ideas about the meninges-CNS connections are gaining traction. While we do not envision implanting transmeningeal delivery systems in humans, there may be situations where administration of DMTs by subdural injections would be warranted, particularly to patients experiencing severe clinical relapses. In addition to the subdural route and perhaps more clinically relevant, direct access to the meninges and CNS may be achieved intranasally. The olfactory

neurons extend through the many small foramen of the cribiform plate in the base of the skull and terminate in the olfactory epithelium, where the dendrites penetrate the mucosal epithelium. This direct route from the nasal mucosa to the brain not only bypasses the BBB, but also directly reaches the dura mater and SAS [134]. Recent evidence has demonstrated this connection is a viable route for drug delivery [135–138]. A select few studies have shown that this route may be therapeutically beneficial for the treatment of EAE/MS [135, 139, 140]. One study in particular showed that intranasal administration of glatimer acetate on the first day of clinical signs ameliorated EAE signs more so than subcutaneous administration did [135]. Thus, this drug delivery route directly targeting the meninges and CNS may be beneficial for DMTs already in use.

There are a number of potential meninges-directed targets; including those that interfere with c-kit or IL-2 signaling; or inhibit mast cell, innate lymphoid cell, or memory T cell activation (Table 2). For example, imatinib (Gleevec) is a potent inhibitor of tyrosine kinase signaling that acts on c-kit and is used to inhibit several cancers, most prominently chronic myelogenous leukemia [141]. However, it has also been shown to modulate EAE [142]. Although its specific cellular targets in MS therapy remain undefined, the potential for this or related drugs to inhibit both mast cells and c-kit+ Group 3 ILCs may prove beneficial in MS. Daclizimab, an antibody to the low affinity IL-2R subunit that targets circulating ILCs, is another drug that could have greater efficacy when delivered directly to the meninges. Blockade of CCL20/CCR6 and A2AAR (adenosine receptor) signaling or P-selectin interactions in the choroid plexus holds promise; as does interference with CXCL10/CXCR3 interactions, which may prevent the recruitment of the “second-wave” cells into the CNS parenchyma. CD30 and OX40, which are constitutively expressed on Group 3 ILCs in the meninges (Hatfield and Brown, manuscript in preparation), are also potential therapeutic targets. Antagonists to CD30 and OX40 are currently under development for use in cancer patients, but there is certainly rationale for exploring their ability to limit survival of autoreactive memory T cells in EAE/MS [109, 143]. Kv1.3, a voltage-gated potassium channel and a functional marker for activated effector memory T cells, is another attractive candidate for targeting memory cells that reside in the meninges [144, 145].

In summary, given the new insights into the pathologic influence of meningeal inflammation on CNS disease, the future of disease-modifying therapies for MS and other inflammatory CNS diseases is promising. Moving forward, a greater effort should be made to develop new methods to assess meningeal inflammation in humans, define more meningeal- specific molecular targets, and discover new methods of drug delivery that can provide directed therapies to the meninges.

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Meninges Quick Facts

What are the meninges?

- a collection of three highly vascularized and fibrous peripheral tissues that surround and protect the CNS
- a non-lymphoid site of active immunological responses
- an immune “barrier site”

What is the cellular composition of the meninges?

- large cellular variety - including fibroblasts, macrophages, dendritic cells, mast cells, lymphocytes, and stem cells
- these cell populations represent potential therapeutic targets free of the restrictive BBB

What is the physiologic role of the meninges?

- to serve as a first line defense against infections and toxins that threaten the CNS
- to enclose the CSF and provide buoyancy for the brain

Are the meninges involved in pathophysiology?

- yes, similar to other “barrier sites,” the meninges are susceptible to chronic inflammation and infections
- in MS, meningeal inflammation occurs early, preceding white matter damage and may drive clinical relapses
- harmful mediators produced in the meninges can likely diffuse across the pia and damage the adjacent gray matter

Figure 1. The anatomical relationship between the meninges, cerebral blood vessels, and CNS parenchyma

The meninges are composed of three layers: the dura mater, arachnoid mater, and pia mater. These tissues surround the brain and spinal cord and house the cerebrospinal fluid (CSF) located within the subarachnoid space. CSF provides buoyancy to the brain, regulates cerebral blood flow but also serves as a passageway from the peripheral blood circulation into the meninges. CSF is produced by the choroid plexus, which lines the ventricles of the brain. In the brain, the gray matter, containing the less myelinated nerve cell bodies, is located directly adjacent to the meninges with in direct contact with the pia mater. The white matter contains the highly myelinated nerve axons of and is more centrally located. In the spinal cord, the gray matter is located at the center and the white matter comprises the lateral aspects of the cord (not depicted). Pia-lined blood vessels form the blood-brain barrier as they transition into the CNS parenchyma.

Figure 2. CSF flows from the ventricles to the subarachnoid space of the meninges

The choroid plexus is a specialized epithelium lining all four ventricles of the brain (third and fourth ventricles depicted above) that produces cerebrospinal fluid (CSF). The CSF freely flows from the ventricles to the subarachnoid space of the meninges before resorption via the arachnoid villi into the dural sinuses.

Figure 3. Damage by the “second-wave” cells is hypothesized to be executed by pro-inflammatory mediator diffusion from the meninges into the CNS parenchyma. Infiltrating and resident meningeal cells are activated to produce harmful mediators (i.e. $\text{IFN}\gamma$, TNF, and likely GM-CSF, IL-17, etc.). These mediators diffuse into the Virchow-Robin spaces and across the pia mater to damage neurons and oligodendrocytes directly or by activating resident microglia and infiltrating CNS cells.

Figure 4. The “two-step” hypothesis

Proposed sequence of events that mediate T cell access to the uninflamed CNS. The first wave depicts the presumed physiological immune surveillance of the CNS by T cells, while the second wave occurs only if autoreactive T cells interact with their cognate antigen.

Table 1

Therapy	Drug Class	Known Mechanism(s) of Action
<i>Current DMTs</i>		
Natalizumab (Tysabri)	Anti- α 4 integrin monoclonal antibody	Blocks immune cell interactions with endothelium and prevents leukocyte entry into the CNS
Interferons (Betaseron, Avonex, Rebif)	Type 1 interferons	Suppresses antigen-specific T cell proliferation and favors anti-inflammatory cytokine production Increases production of nerve growth factor and improved neuron survival
Glatiramer acetate (Copaxone)	Synthetic polymer	Decoy (mimic) of myelin peptides and competes for MHC Class II binding on APCs Shifts T cell cytokine response from pro-inflammatory to anti-inflammatory
Fingolimod (Gilenya)	Sphingosine-1-phosphate analog	Sequesters T cells in secondary lymphoid organs
Teriflunomide (Aubagio)	Synthetic small molecule	Inhibits DHORDH and protein tyrosine kinases Decreases T cell proliferation
Dimethyl fumarate (Tecfidera)	Methyl ester of fumaric acid	Targets reactive oxygen species (ROS) production
<i>DMTs in clinical trials</i>		
Alemtuzumab	Anti-CD52 monoclonal antibody	Depletes CD52+ (mature) lymphocytes
Laquinimod	Synthetic small molecule	Increases anti-inflammatory cytokine production Decreases leukocyte migration
Ocrelizumab	Anti-CD20 monoclonal antibody	Depletes B cells
Daclizumab	Anti-CD25 monoclonal antibody	Blocks IL-2 receptor signaling Inhibits ILC differentiation

Table 2

Potential therapeutic targets in the meninges

Target	Desired Outcome(s)
mast cells (c-kit)	Decrease meningeal mast cell numbers to inhibit chemokine gradient for leukocytes
ILCs (CD30, OX40, c-kit)	Prevent ILC maintenance of meningeal memory T cells and/or ILC cytokine production
choroid plexus (CCL20, AAR2A, P-selectin)	Prevent effective leukocyte entry into the CSF/subarachnoid space
CXCL10/CXCR3	Prevent chemotactic signals from the meningeal “first-wave” leukocytes