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Feature Review

Advances in Meningeal Immunity

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The central nervous system (CNS) is an immunologically specialized tissue protected by a blood–brain barrier. The CNS parenchyma is enveloped by a series of overlapping membranes that are collectively referred to as the meninges. The meninges provide an additional CNS barrier, harbor a diverse array of resident immune cells, and serve as a crucial interface with the periphery. Recent studies have significantly advanced our understanding of meningeal immunity, demonstrating how a complex immune landscape influences CNS functions under steady-state and inflammatory conditions. The location and activation state of meningeal immune cells can profoundly influence CNS homeostasis and contribute to neurological disorders, but these cells are also well equipped to protect the CNS from pathogens. In this review, we discuss advances in our understanding of the meningeal immune repertoire and provide insights into how this CNS barrier operates immunologically under conditions ranging from neurocognition to inflammatory diseases.

The Meninges Support Neuroinflammation

The central nervous system (CNS) is protected by several membranes that are collectively referred to as the **meninges** (see [Glossary](#)). Despite serving as a barrier and interface between the CNS and periphery, little is known about the immune composition of the meninges or how meningeal immunity affects the CNS under steady-state and inflammatory conditions. Recent advances in the field of meningeal immunity have begun to reshape our understanding of this compartment and indicate that it plays an important role in directing and coordinating immune traffic throughout the CNS [1]. Most CNS immune responses begin in the meninges before gaining access to the **parenchyma**, and emerging data indicate that the meninges are far more supportive than the parenchyma in hosting inflammation [2]. Compared with the CNS parenchyma, the meninges are an under-studied compartment and thought mainly to serve as a protective barrier, yet meningeal immunity can profoundly influence CNS homeostasis and even contribute to neurological disorders. Here, we provide a contemporary view of the meninges, focusing first on its exquisite anatomy and then define meningeal immunity under steady-state and inflammatory conditions.

Meningeal Anatomy Is Highly Specialized

Structure and Fluid Movement

The meninges consist of three cellular layers with different properties: the **dura mater**, which is adjacent to the skull, the **arachnoid mater**, and the **pia mater**, which is the layer just above the brain and spinal cord parenchyma (Figure 1). The dura mater in humans is a dense, tough, collagenous membrane [3] that is highly innervated, vascularized, and contains **lymphatics** [4]. The arachnoid mater is a barrier that separates the dura mater from the rest of the CNS. Importantly, cells in the arachnoid mater have **tight junctions** and regulate the transport of molecules, similar to the **blood–brain barrier** [5,6], although studies in mice have demonstrated that the arachnoid mater is permissive to the passage of molecules (up to 40 kDa) applied to the dura mater [7]. Beneath the arachnoid mater lies the **subarachnoid space**,

Highlights

The CNS is protected by layers of cells that are collectively referred to as the meninges. Far from being an inert connective tissue, emerging data indicate that the meninges serve as an interface with the periphery and actively contribute to CNS homeostasis and immunity.

The meningeal layers have a far more diverse immune repertoire than the CNS parenchyma and are innervated, have lymphatic drainage (dura), contain permeable blood vessels (dura), and support robust inflammatory responses.

The meninges serve as a gatekeeper that isolates the brain and spinal cord parenchyma from the periphery. The meninges develop and support immune responses far more readily than the parenchyma, which can both protect and harm the CNS.

The meninges are highly innervated, which can influence meningeal immunity and vascular tone.

Both sterile and infectious challenges can trigger meningeal inflammation, which results in subsequent degradation of the glial limitans and immune infiltration into the CNS parenchyma. This process can give rise to neurological disorders.

Therapeutically, the meninges are far easier to access from the periphery than the CNS parenchyma (especially the dura mater). Modulation of meningeal immunity represents a promising therapeutic target to treat inflammatory neurological disorders and possibly to regulate neural homeostasis.

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through which **cerebrospinal fluid (CSF)** flows. This space contains trabeculae and collagen bundles generated by fibroblast-like cells that connect the arachnoid to the pia mater [8]. The CSF within this space is important for brain buoyancy and is produced by choroid plexus epithelium. CSF flows through the ventricles as well as subarachnoid spaces and eventually effluxes into the blood (reviewed in [9]). Lastly, the pial membrane, which covers the brain and spinal cord parenchyma, is semipermeable to the CSF that flows along penetrating vessels in perivascular spaces (Figure 1) [10]. Together, the arachnoid and pial membranes are referred to as the **leptomeninges**, which are relatively thin when compared with the thick dural membrane.

Within the CNS parenchyma, the surface-associated astrocytes are juxtaposed to the pial membrane, providing yet another protective barrier referred to as the glial limitans. This structure follows penetrating blood vessels in the CNS parenchyma, with astrocytic endfeet forming a dense and highly charged basement membrane that is permeable to some molecules within the CSF (Figure 1) [11]. In rodents, fluorophores (0.8 kDa), fluorescent dextran (3, 10, 70, and 2000 kDa), albumin (45 kDa), and immunoglobulin (150 kDa) injected into the CSF via the cisterna magna are seen in the perivascular spaces along descending pial arteries within a few minutes [11–14]. Once within these spaces, some molecules can gradually enter the brain parenchyma in a size-dependent manner. For example, a 0.8 kDa fluorophore injected into the CSF was observed throughout the brain parenchyma 30 min after injection [13], whereas slightly larger molecules (3–10-kDa dextrans) were only able to penetrate 50–100 μm into the parenchyma [12,14]. Even larger molecules (45 and 70 kDa) were detected up to 35 μm below the **glia limitans** [13,14], and molecules from 150 to 2000 kDa were found to be completely excluded from the parenchyma and remained in the perivascular spaces [11–14]. Based on this size exclusion system, soluble mediators like cytokines (~10–50 kDa) would only have limited ability to enter and affect the parenchyma under steady-state conditions with the CNS barrier system intact. It should be noted, however, that all the data above are based on injection of fluorescent tracers directly into the CSF. The CSF is produced at a rate of 0.35 $\mu\text{l}/\text{min}$ in mice [15]. Injection of additional fluid into CSF above this rate has the potential to cause edema and artefactual intracranial pressures. For example, tracers in the aforementioned studies were injected into the CSF at rates ranging from 0.17 to 2 $\mu\text{l}/\text{min}$. The acute inflammatory response generated by insertion of a needle into the ventricle must also be considered when interpreting these fluorescent tracer studies. Nevertheless, the glial limitans appears to be permissive, in a size-dependent manner, to the passage of at least some molecules. Molecules excluded from parenchymal entry on the basis of size must rely on transport systems. The driving force behind the entry of CSF and small molecules into the parenchyma is unclear. According to the ‘glymphatics’ theory, fluid is actively pushed from the perivascular spaces along penetrating blood vessels into the CNS parenchyma [13]; however, a more recent study called this theory into question, concluding that the movement of fluid is based instead on simple diffusion [14]. Future studies are required to resolve this discrepancy and determine how the glial limitans regulates entry of molecules from the CSF into the parenchyma.

The Meninges Are Highly Vascularized

The CNS is one of the most irrigated organs and is perfused via the external and internal branches of the carotid artery (reviewed in [16]). The external carotid artery gives rise to the anterior, middle and posterior meningeal arteries that irrigate the dura mater and skull. The dural meninges are thus highly perfused by blood vessels, and the vasculature is interwoven with peripheral nerve fibers (Figure 2). Blood to the dura mater is then drained by venous sinuses that empty both dural and cerebral veins (reviewed in [1]). Vessels within the dura mater contain capillary segments and postcapillary venules capable of supporting immune cell traffic. In

Glossary

Arachnoid mater: a meningeal layer that resides just beneath the dura mater that serves as the upper barrier for the subarachnoid space. This barrier is impermeable to most molecules due to the presence of tight junctions between individual cells.

Blood–brain barrier: a specialized barrier that protects the brain and spinal cord parenchyma from the periphery. The barrier is formed at the level of CNS vasculature by endothelial cells connected by tight junctions and astrocytic endfeet that are wrapped around individual vessels. This structure regulates the passage of molecules and cells from the blood into the CNS parenchyma.

Cerebrospinal fluid (CSF): a fluid produced by the choroid plexus that moves through the subarachnoid space and ventricles.

Dura mater: the outermost meningeal layer residing just beneath the bone. It contains fenestrated blood vessels as well as lymphatic vessels.

Glia limitans: a CNS barrier consisting of astrocyte endfeet that wrap parenchymal blood vessels, as well as surface-associated astrocytes that form a structural border beneath the pia mater.

Leptomeninges: this term refers to the arachnoid and pial layers of meninges, excluding the dura mater.

Ly6c⁺ monocyte: a circulating blood monocyte with short lifespan (days) that is associated with inflammatory responses.

Ly6c⁻ monocyte: a circulating blood monocyte with an intermediate lifespan (weeks) that can scan the vasculature and is associated with tissue repair.

Lymphatics: vessels that sample and drain tissue fluid into lymph nodes.

Macrophage: differentiated innate immune cell that participates in inflammatory, phagocytic, antimicrobial, and tissue repair functions depending on its activation state.

Mast cell: an innate immune cell found in barrier and connective tissues (including the dura and pia mater) that releases granules containing histamine, serotonin, and

addition, dural blood vessels appear to be more responsive to mechanical and chemical stimulation than vessels in the underlying cerebrum. Vasoconstriction of these vessels can be caused by increased luminal pressure, as well as neurotransmitters (e.g., noradrenaline) and neuropeptides (e.g., neuropeptide Y). Vasodilation, by contrast, can be induced by electrical stimulation, neuropeptides (e.g., vasointestinal peptide, calcitonin-gene related peptide, substance P), acetylcholine, histamine, and serotonin (Figure 2) [17]. Importantly, dural (unlike pial and cerebral) vessels are fenestrated and open to the passage of relatively large (e.g., 43 kDa) molecules injected into the blood [5]. Moreover, their permeability increases even further upon trigeminal nerve stimulation or histamine release (Figures 1 and 2) [18]. Because of the relative openness of dura vessels, the impermeable arachnoid mater plays a crucial role in preventing entry of blood-derived materials into the CSF.

The internal carotid arteries form an anastomotic ring with other arteries at the basis of the skull, called the circle of Willis (reviewed in [16]). This arterial circle gives rise to the anterior, middle, and posterior cerebral arteries that run along the pial surface of the brain before penetrating the parenchyma to supply blood to the cerebral cortex. Penetrating arterioles become progressively enveloped by astrocytic endfeet after they dive into the parenchyma, which forms part of the blood–brain barrier. Unlike dural blood vessels, the endothelial cells comprising pial vessels are connected by tight junctions and can be divided in two groups: 25% of them have no space between endothelial cells, whereas the other 75% have 2.8 nm-gaps [19]. Despite these small gaps, pial vessels under steady-state conditions are relatively impermeable to molecules applied intravenously, such as ferritin (440 kDa), HRP (44 kDa), microperoxidase (1.8 kDa), sodium fluorescein (0.4 kDa), and ionic lanthanum (0.1 kDa) (reviewed in [20]). Pial vessels can respond to vasoactive compounds, although to a much lower extent than dural vessels (reviewed in [1]).

Meningeal Innervation Is Extensive

The meninges are highly innervated, especially in the dura mater (Figure 2) [21]. Meningeal innervation consists of thousands of sympathetic, parasympathetic, and sensory fibers (some of which are myelinated) that produce noradrenaline, acetylcholine, and neuropeptides, among others [1,22,23]. Sensory fibers within the meninges respond to changes in temperature, pH, and mechanical pressure [24], providing important sensory feedback regarding the state of this compartment. Peripheral nerve fibers project to both the dura and leptomeninges, although the nerve fibers within the pia appear to terminate primarily on blood vessels [25], whereas dural fibers have both vascular and nonvascular targets [26]. Collectively, these fibers innervate meningeal blood vessels, including the middle meningeal artery (MMA) and middle cerebral artery (MCA), as well as the superior sagittal sinus and different resident meningeal cells [23]. Interestingly, projections from the trigeminal ganglion to the MCA extend collaterals to the dura mater and MMA [26], which might represent a mode of communication across the impermeable arachnoid barrier.

Lymphatics ‘Rediscovered’

Lymphatics were first described within the dura mater in the 18th century [27] and again in the 1900s [21,28], but were more recently ‘rediscovered’ in mice [29,30] and humans [4], which renewed interest in the topic. Because blood vessels in the dura mater are fenestrated, like in most peripheral tissues, it is not surprising that a network of dural lymphatic vessels exist to drain tissue fluid from this compartment into lymph nodes. Intracranial lymphatic vessels run along the dural sinuses and the MMA [29,30]. In addition, extracranial lymphatic vessels follow cranial nerves that exit through the cribriform plate and the base of the skull [30,31]. Although it is generally accepted that materials from the CSF and parenchyma can drain into the deep

other molecules during states of allergy and inflammation.

Meninges: the barrier structure consisting of the dura, arachnoid, and pia mater that lines the brain and spinal cord.

MHC II: major histocompatibility complex II; an antigen-presenting molecule found on the surface of antigen-presenting cells (e.g., dendritic cells and macrophages), B cells, some endothelial cells, and stromal cells, as well as thymic epithelial cells. MHC II displays small peptide fragments obtained from extracellular proteins to CD4⁺ T cells.

Monocyte: a circulating population of innate immune cells found in the blood that can differentiate into macrophages after tissue infiltration.

Myelomonocytic cell: a term referring to innate immune cells from the myeloid lineage, including monocytes/macrophages, monocyte-derived dendritic cells, and neutrophils.

Neutrophil: a short-lived circulating innate immune cell found in the blood that responds to tissue damage or infection by releasing inflammatory and antimicrobial molecules.

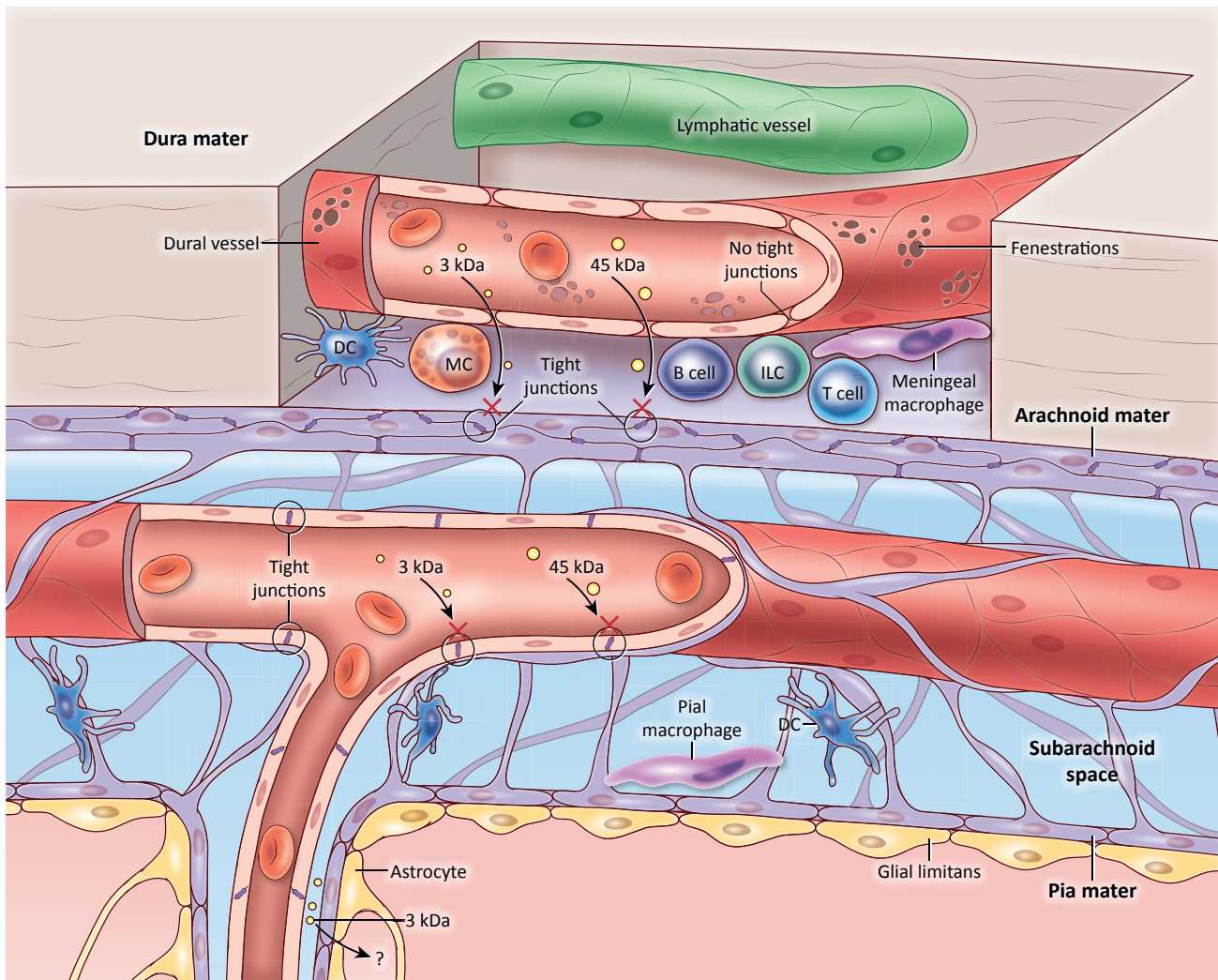
Nociceptive: relates to pain generated by the stimulation of peripheral nerve fibers.

Parenchyma: the soft tissue of the brain and spinal cord that is surrounded by the glial limitans and meninges. The parenchyma houses various resident populations, including neurons, astrocytes, oligodendrocytes, microglia, etc.

Pia mater: the innermost layer of the meninges and lower boundary of the subarachnoid space. The pia mater resides above the glial limitans and brain/spinal cord parenchyma.

Subarachnoid space: a space in the meninges between the arachnoid and pia mater, through which cerebral spinal fluid flows. This space contains a dense network of fibroblast-like stromal cells as well as dendritic cells.

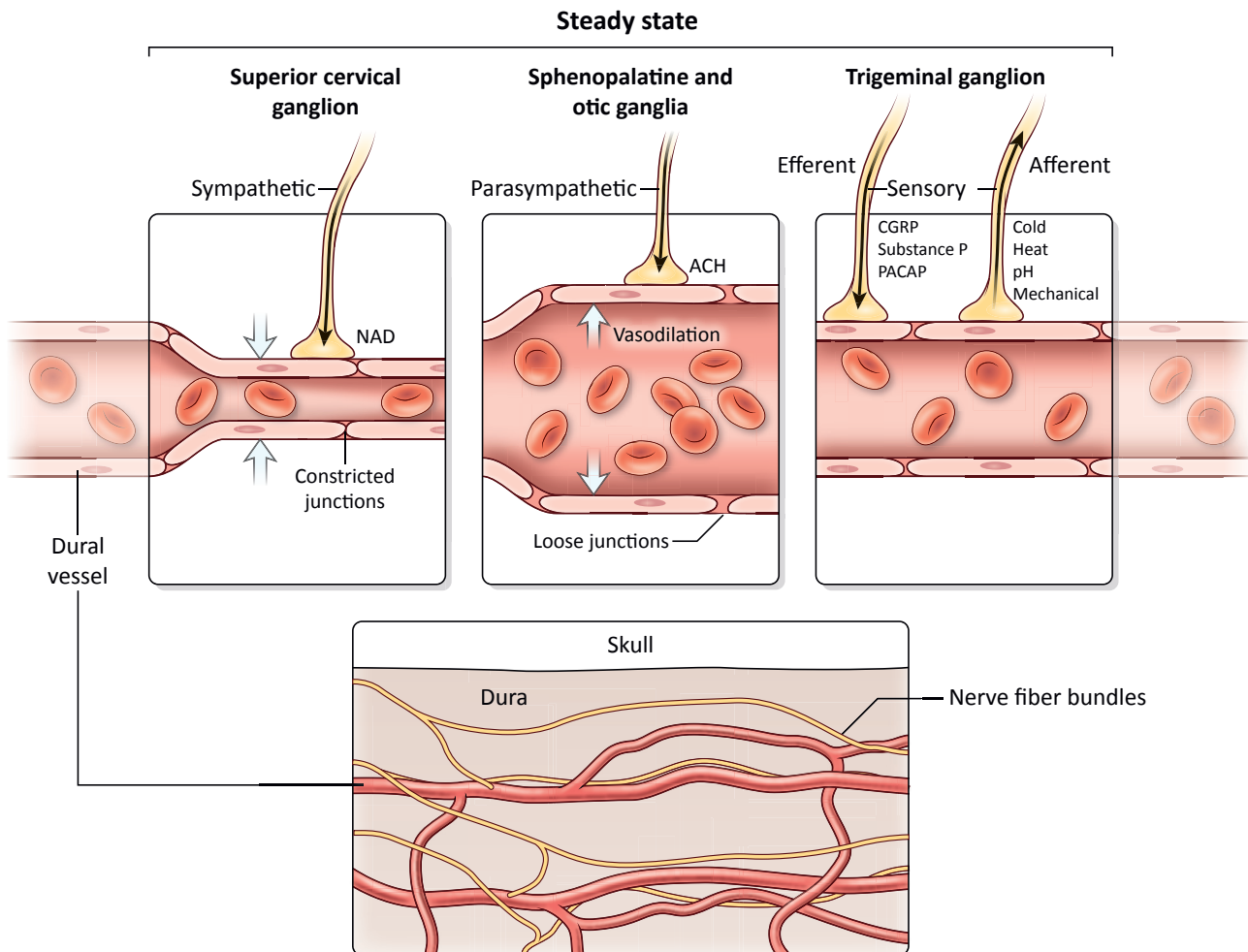
Tight junction: a tight zipper-like connection between two cells designed to limit the passage of molecules between two cells. Tight junctions are found between cells comprising the arachnoid mater as well as vascular endothelial cells comprising pial and parenchymal blood vessels.



Trends in Molecular Medicine

Figure 1. Steady-State Meningeal Anatomy and Immune Composition. The meninges covering the central nervous system (CNS) parenchyma consist of the dura mater (adjacent to the skull which is not shown), arachnoid mater, and pia mater. The dura mater is similar to a peripheral tissue with its lymphatic vessels and fenestrated vasculature that allow entry of large molecules (e.g., 45 kDa) from the blood. The dura mater has a large repertoire of immune sentinels, such as dendritic cells (DC), mast cells (MC), innate lymphoid cells (ILCs), meningeal macrophages, T cells, and B cells. The arachnoid mater represents the first impermeable barrier to the CNS parenchyma, as cells within this layer are joined by tight junctions. Therefore, molecules cannot diffuse freely from the dura mater into the subarachnoid space. The subarachnoid space is filled with cerebrospinal fluid, which removes cells and compounds unless they are attached to the stromal matrix (purple). The brain surface is traversed by nonfenestrated veins and arteries encased in the pial sheaths (not shown). Although not surrounded by astrocytic endfeet, vascular endothelial cells in the pia mater meninges are impermeable and joined by tight junctions. The pia matter also contains immune sentinels, although less numerous than in the dura, such as mast cells (not depicted), macrophages, and dendritic cells. The pia mater and glia limitans (formed by surface-associated astrocytes and astrocytic endfeet) represent the final barrier before the parenchyma. Diffusion of small molecules (3 kDa) is restricted to the superficial layers of the parenchyma, and larger molecules cannot cross this barrier.

cervical lymph nodes (dCLN), the exact route of transport (as assessed by intrathecally injected tracers) is debated, with some suggesting that CSF tracers can traverse the arachnoid mater and enter the dural lymphatics [29,30] and others proposing that these tracers exit instead via perineural routes through foramina in the skull bone [31]. Experimental confounds linked to tracer injection methodology (e.g., disruption of meningeal architecture, inflammation,



Trends in Molecular Medicine

Figure 2. Meningeal Innervation. The dura mater is innervated by a network of peripheral nerve fibers that project onto vascular and nonvascular targets. Upon activation, sympathetic fibers from the superior cervical ganglion release noradrenaline (NAD), which promotes vasoconstriction and restricts permeability. By contrast, parasympathetic fibers from the sphenopalatine and otic ganglion increase vascular permeability and promote vasodilation through the action of acetylcholine (ACH). Finally, branches of the trigeminal ganglion, that also innervate the pia mater (not shown), can both release neuropeptides [e.g., calcitonin gene-related peptide (CGRP), substance P, pituitary adenylate cyclase-activating polypeptide (PACAP)] through efferent fibers and sense **nociceptive** stimuli, such as cold, heat, pH, and mechanical forces through afferent fibers. Myelinated fibers are not shown.

increased intracranial pressure) indicate that less invasive methods are required to investigate routes of CSF egress. Despite the controversy, the rediscovery of the dural lymphatics has triggered a considerable amount of interest in meningeal architecture and immunology.

Steady-State Meningeal Immunology

T Cells Influence Meningeal Homeostasis

The meninges, under steady-state conditions, are populated by many different immune cell populations (e.g., **macrophages**, dendritic cells, innate lymphoid cells (ILCs), **mast cells**, **neutrophils**, B cells, and T cells) (Figure 1) [1,32–34]. Apart from the choroid plexus, T cells are largely absent from the CNS parenchyma, but can be detected under steady-state conditions in the mice meninges [35], although their exact location (leptomeninges versus dura) and

trafficking patterns are not entirely understood. Parabiotic studies have revealed that ~20% meningeal CD4⁺ T cells are derived from the blood 2 weeks after the vascular systems between the two animals were equilibrated [36]. In addition, surgical resection of the dCLN led to a small accumulation in meningeal CD4⁺ T cells (increased from ~1300 to 1600 T cells) 2 weeks later. Together, these data indicate that CD4⁺ T cells can traffic from the blood into the meninges, where they likely scan the tissue before entering the dCLN. It was also recently discovered using mass cytometry that T cells are enriched in the CNS of mice upon aging [37]. This increase could be linked to the natural process of neurodegeneration that occurs during aging and/or the cumulative exposure to environmental antigens and microbes. Mice, for example, continually inhale materials through their nose, which may influence immune traffic through relatively open barrier structures like the dura mater. As humans encounter far more environmental antigens and microbes than laboratory rodents, it is not surprising that the T cells found under steady-state in the CSF have a central memory phenotype and express homing molecules that allow them to access secondary lymphoid organs [38,39]. This phenotype is consistent with the notion that T cells circulate from the blood into the meninges and then into secondary lymphoid organs.

The importance of T cells in CNS homeostasis emerged in part from the discovery that T cell-deficient mice had an impaired ability to perform in spatial learning and memory tests such as the Morris water maze [40–43]. In the Morris water maze, rodents are trained to find an underwater platform, usually based on the position of external visual cues. While investigating the mechanism underlying this defect, it was revealed that wild type mice tested in the Morris water maze had an increase in meningeal IL-4-producing CD4⁺ T cells after 4 days of training [35]. Reducing the meningeal T cell number, by preventing trafficking from the blood, impaired performance in this assay. Moreover, severe combined immunodeficient (SCID) mice, which lack T and B cells, showed a learning deficit that could be reversed by intraperitoneal injection of wild type, but not IL-4-deficient, CD4⁺ T cells [35]. Depletion of CD4⁺ T cells in adult mice using an antibody also impairs Morris water maze performance, and this was linked to decreased brain-derived neurotrophic factor (BDNF) production and hippocampal neurogenesis [44]. Astrocytes treated with IL-4 *in vitro* express more BDNF, and the hippocampi of mice trained in a Morris water maze have elevated BDNF mRNA that is abrogated in IL-4-deficient mice [35]. It therefore appears that CD4⁺ T cells produce IL-4 when mice are engaged in a spatial learning/memory test that enhances hippocampal BDNF production and neurogenesis; however, it is presently unclear where CD4⁺ T cells release this IL-4, as they are not typically found in the hippocampus or CNS parenchyma. In addition, Jeon and colleagues showed that mice with a restricted CD4⁺ T cell repertoire had impaired hippocampal neurogenesis and cognitive performance on a memory test, despite elevated levels of IL-4 in the CNS [45]. In fact, IL-4 was shown to inhibit proliferation of adult hippocampal neural stem cells in a dose-dependent manner *in vitro*. Thus, the role of CD4⁺ T cell-derived IL-4 in memory and learning remains unclear.

Another question that emerges from these studies pertains to T cell antigen specificity. Because reconstitution of T cell-deficient mice with wild type CD4⁺ T cells from naïve spleens restored Morris water maze performance, Wolf and colleagues concluded that specificity toward CNS antigens is not required for procognitive T cell activity [44]. However, additional studies challenge this view. For example, Radjavi *et al.* demonstrated that mice with resected dCLN had learning deficits when analyzed in the Morris water maze [36]. The absence of dCLN would render mice less capable of sampling CNS antigens. Similar results were obtained in OT-II T cell receptor (TCR) transgenic (tg) mice, in which >90% CD4⁺ T cells are specific to ovalbumin, an irrelevant egg white protein [46]. These data suggest that TCR specificity against

CNS-antigen presentation is important for procognitive CD4⁺ T cell function, like IL-4 production. Consistent with this hypothesis, OT-II TCR-tg mice reconstituted with myelin peptide-specific CD4⁺ T cells perform comparably to wild type mice in the Morris water maze [46]. It is important to note, however, that the steady-state requirement for T cell antigen specificity can be bypassed by exposure to alarmins that promote expansion of IL-4⁺ CD4⁺ T cells in a MyD88-dependent, but TCR-independent manner [47]. In the optic nerve crush model of neuronal injury, it was shown that injection of CD4⁺ T cells from both wild type and OT-II TCR-tg (with a restricted TCR repertoire) mice could promote neuronal regrowth [47]. The authors proposed that this was linked to TCR-independent IL-4 production by CD4⁺ T cells, which then acted directly on the damaged neurons, providing supplementary neurotrophic support [47]. These data suggest that alarmins released from damaged neural tissue can promote TCR-independent cytokine release by CD4⁺ T cells.

Another impaired function seen in T and B cell-deficient SCID mice was linked to social behavior. To test social behavior, the preference of one mouse for another mouse versus an inanimate object is evaluated. Wild type mice, when evaluated this way, spend more time around a mouse, whereas SCID mice fail to do so [48]. Adoptive transfer of T cells can rescue this impaired social behavior, suggesting that the absence of T cells was responsible for the dysfunction. Interestingly, IFN- γ ^{-/-} mice showed a similar deficit in social behavior, leading the investigators to speculate that T cell-derived IFN- γ promotes social behavior. The researchers then specifically deleted the IFN- γ receptor from neurons in the prefrontal cortex and demonstrated that this also impeded social activity. Under steady-state conditions, IFN- γ appears to induce activation of inhibitory circuits in the prefrontal cortex that prevent hyperconnectivity, an aberrancy observed in autism spectrum disorders. An interesting correlation was also observed in this study between social behavior and the IFN- γ -induced transcriptional signature in the brains of rodents that were group-housed versus isolated [48]. These data suggest that the IFN- γ -mediated immune defense against pathogens coevolved with social behavior (a neural function), resulting in a mutually beneficial outcome for both systems. As animals become more social, they are exposed to more pathogens and thus require IFN- γ -mediated immunity, which in turn benefits neural function.

From the studies of procognitive T cells emerge a series of questions that should open new areas of investigation. For example, what signals instruct IL-4-producing CD4⁺ T cells to accumulate in the meninges after rodents conduct the Morris water maze test, and why does IL-4 stimulate hippocampal neurogenesis in some cases but not others? Given the anatomical restrictions in the CNS barrier system (Figure 1), it is important to determine how cytokines within the CSF transmit their signals to neural cells within the CNS parenchyma. A similar question can be asked about IFN- γ -producing T cells that promote social behavior. If cytokines are unable to cross the glial limitans, then it is conceivable that they act on perivascular macrophages, glial limitans astrocytes, and/or pericytes. Perhaps some of these cytokines influence neuronal activity by modifying endothelial/epithelial barriers [49] or regional blood flow. It will also be important in future studies to determine where T cells under steady-state conditions elicit their neuromodulatory activities (e.g., dura mater, subarachnoid space, perivascular spaces, choroid plexus). Knowing where these cells operate will help us better understand the mechanics of neural-immune interactions and how best to manipulate the system therapeutically.

Barrier Macrophages Survey the Meninges

Under steady-state conditions, meningeal and perivascular macrophages are dynamic and constantly sample their environment like microglia [32,50]. Intravital imaging studies have

revealed that CX3CR1^{9fp/+} macrophages are found in a high density in the dura (400 cells/mm²) as well as the pia mater (300 cells/mm²), but not the subarachnoid space (Figure 1), although macrophages were observed in the subarachnoid space using electron microscopy [51]. By contrast, dendritic cells are present in the dura mater (10 cells/mm²), the subarachnoid space (25–30 cells/mm²), and the pia mater (25–30 cells/mm²). Meningeal macrophages do not appear to migrate and remain localized along meningeal blood vessels [52]. It was previously thought that meningeal macrophages had a high turnover and were replaced by blood-derived **monocytes** [53]; however, a recent study indicated that pial and perivascular macrophages are derived from embryonic precursors during development and are long-lived like microglia [54]. Macrophages residing in the choroid plexus are also thought to be derived in part by embryonic precursors, but are replaced over time by circulating monocytes [54]. These data suggest that the myeloid landscape in the dura mater and choroid plexus can be influenced by peripheral immune traffic.

In a recent study, nonmicroglial macrophages residing in the CNS were referred to as barrier-associated macrophages [37]: a myeloid population identified previously by expression of the mannose receptor, CD206 [55,56]. Four different subsets of barrier macrophages have been uncovered using mass cytometry, distinguished using the markers CD38, Lyve1, **MHC II**, and CCR2 [37]. The pia mater, perivascular spaces, and choroid plexus are enriched in MHC-II⁺ Lyve⁺ macrophages, a subset not found in the dura mater, which contain MHC-II⁺ Lyve⁻ cells. The dura mater is isolated from the rest of the CNS by the arachnoid membrane and would therefore be expected to have its own specialized immune cell subsets.

While the function of the different barrier macrophage subsets is currently unknown, it is likely that these cells participate in a variety of different functions, such as tissue homeostasis, debris clearance, protection from infections, etc. In addition, these cells appear to be actively maintained in an anti-inflammatory state under homeostasis that requires IL-4 derived from CD4⁺ T cells [35,57]. When mice perform in the Morris water maze test, myeloid cell activation is observed in the meninges, evidenced by increased expression (from 20 to 50%) of the activation marker, CD69, on CD11b⁺ myeloid cells. The reason for this activation is unknown, but is likely due to the stress associated with completing the behavioral test. T cells are known to influence the performance of rodents in cognitive tests, and mice lacking T cells had an increased number of inflammatory (TNF α - and IL-12-producing) myeloid cells in their meninges, which was associated with the behavioral deficit [35]. To follow up on this observation, Derecki and colleagues injected macrophages stimulated *in vitro* with IL-4 directly into the ventricular system of SCID mice, and this partially improved performance in the Morris water maze [57]. Intravenous injection of anti-inflammatory macrophages into SCID mice also improved cognitive performance, which was associated with an anti-inflammatory myeloid cell profile in the meninges. Based on these studies, it is unclear whether anti-inflammatory macrophages act primarily in the periphery or the meninges to influence meningeal homeostasis and cognitive function. The local versus peripheral role of IL-4 also needs to be addressed in this experimental paradigm. It will be interesting in future studies to explore whether meningeal macrophages act as local amplifiers of anti-inflammatory/procognitive cytokines and how IL-4-producing CD4⁺ T cell participate in this process.

Meningeal Inflammation in Disease

Experimental Autoimmune Encephalomyelitis

Multiple sclerosis (MS) is the most common CNS demyelinating disease and affects more than 400 000 individuals in the United States, as well as 2.5 million worldwide [58]. Motor and cognitive deficits accumulate over the course of the disease, as autoreactive lymphocytes and

Box 1. The Meninges Are a Gateway for Immune Cells, Pathogens, and Therapeutics

CNS inflammation has been studied primarily from the perspective of the CNS parenchyma and the blood–brain barrier; however, the meninges surround the brain and spinal cord, representing an important barrier as well as a gateway into the CNS. The meninges, especially the dura mater, can support robust inflammatory responses, which can spread to the CNS parenchyma.

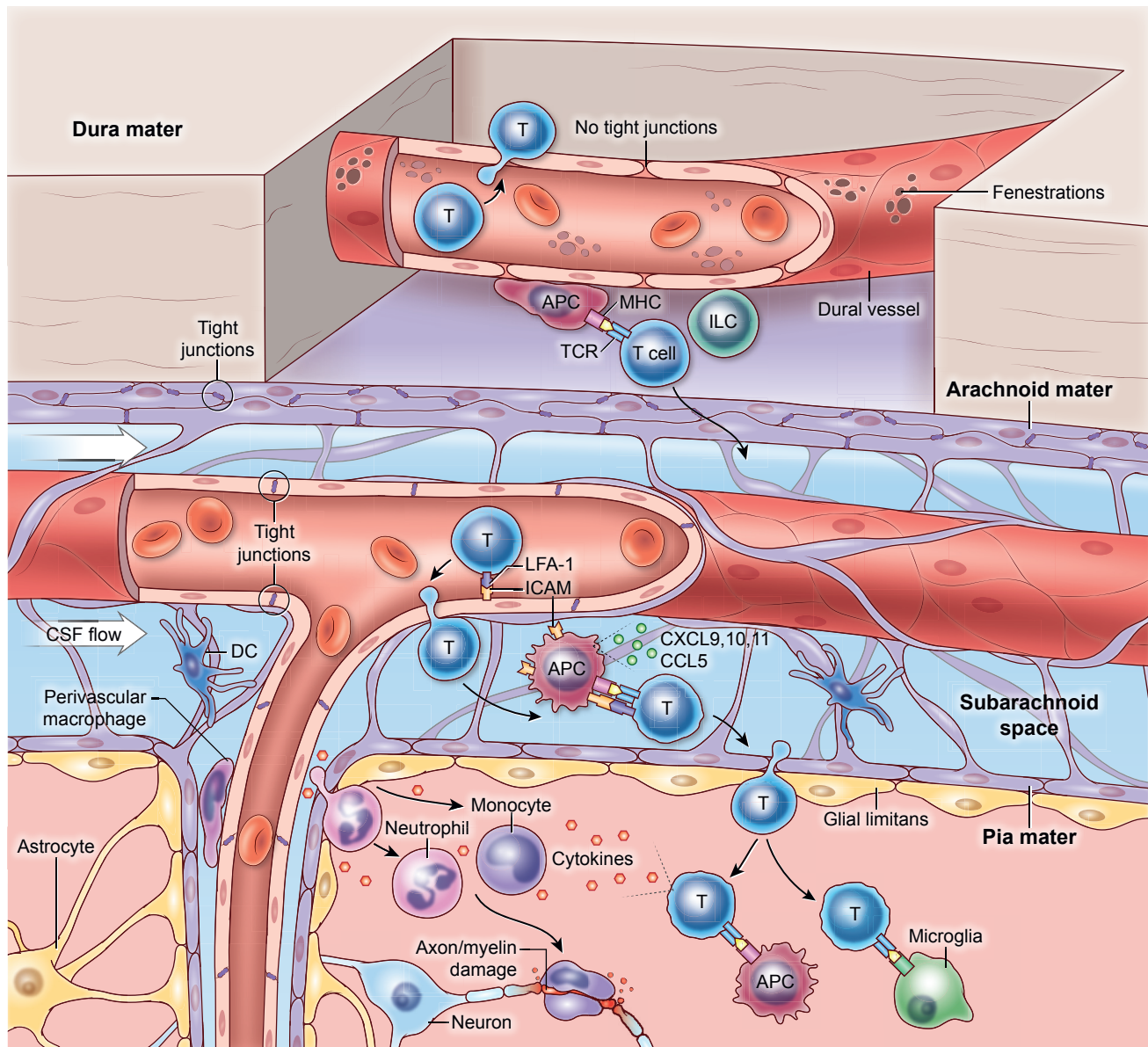
When compared with the CNS parenchyma, the meninges often show earlier and more intense inflammation in response to infectious and noninfectious challenges. Most immune responses develop first in the meninges and perivascular spaces before moving into the parenchyma.

The meninges can serve as a CNS entry point for pathogens. Studies have demonstrated early detection of pathogens (e.g., HIV, Zika virus, *Trypanosoma brucei*) in meninges, and the presence of long-lived tissue resident macrophages can serve as reservoirs for persistent infections (e.g., HIV). Because the meninges support robust inflammatory responses, acute and chronic infection of this barrier compartment can give rise to significant neurological dysfunction.

When characterizing the meningeal contribution to a CNS inflammatory response, it is important to precisely define the meningeal layer(s) involved. For example, the dura mater contains fenestrated vessels and is easy to target therapeutically by administering drugs into the blood. The structures beneath the arachnoid mater are more difficult to target therapeutically due to the CNS barrier systems.

immune infiltrates damage the myelin and axon fibers [58]. The etiology of the disease is unknown, and treatment consists mainly of administering anti-inflammatory drugs. Although most studies have focused on the CNS parenchyma, meningeal inflammation is often seen early on in clinical samples [58] (Box 1). Meningeal tissue from MS patients has a high density of T cells that can be organized in ectopic lymphoid follicles and are associated with damage to the glia limitans, subpial demyelination, and disease severity [58].

The most commonly studied animal model of MS is experimental autoimmune encephalitis (EAE), which can be induced by adoptive transfer of autoreactive T cells (passive EAE) or immunization with myelin antigens (active EAE). Imaging of the spinal cord meninges in rats and mice during passive EAE has revealed the presence of autoreactive CD4⁺ T cells in the dura mater [59] and leptomeninges [60,61] before the onset of disease (Figure 3). Activated T cells are seen rolling in the vasculature of peripheral organs a few days after injection [60]. This is likely due to systemic activation of blood vessels, as activated CD4⁺ T cells reactivated in the periphery promote release of inflammatory cytokines into the blood. However, crawling and extravasation are exclusively seen in the CNS [60]. Intravital imaging of rats at the peak of the disease has revealed that autoreactive CD4⁺ T cells are recruited into the leptomeninges by chemoattractants (CXCL9, CXCL10, CCL5) and interact with pial macrophages through adhesion molecules and cognate antigen recognition [60–62]. The use of calcium reporters as a surrogate for T cell signaling has demonstrated that interactions with antigen-presenting cells (APCs) in the leptomeninges promote autoreactive T cell activation [62]. It is likely that local APCs (i.e., macrophages and dendritic cells) are initially loaded with myelin antigens, acquired from myelinated peripheral nerve fibers that traverse the meninges, and obtain more myelin debris as the disease progresses and oligodendrocytes are damaged within the CNS parenchyma. Importantly, meningeal reactivation of CD4⁺ T cells is required for parenchymal invasion of immune infiltrates, which triggers CNS damage [60,63–71]. The mechanism allowing immune cells to traffic from the meninges into the parenchyma is unclear, but it is known that inflammatory mediators [reactive oxygen species (ROS), TNF- α , IFN- γ] produced in the meninges can potentiate glia limitans breakdown and microglial activation in different inflammatory contexts [7,72,73]. During chronic EAE, meningeal infiltrates and inflammatory mediators decrease during the remission phase and are the best predictors of clinical relapses [58].



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Figure 3. Meningeal and Parenchymal Inflammation during EAE. Experimental autoimmune encephalitis (EAE) is triggered by autoreactive T cells that first invade the meninges. They extravasate from inflamed dural vessels and encounter infiltrating antigen-presenting cells (APCs) [e.g., macrophages and dendritic cells (DC)]. They are reactivated within immune clusters that contain innate lymphocytes (ILCs). Those ILCs reside in the meninges and foster APC-T cell interactions, as well as inflammation, by triggering cytokine and matrix metalloproteinase release (see details in the text). T cells also extravasate from pial vessels or possibly cross the arachnoid mater if it has been damaged by inflammation. In the leptomeninges, autoreactive T cells are attracted by APCs that release chemokines such as CCL5, CXCL9, CXCL10, and CXCL11. Adhesion molecules, such as intercellular adhesion molecule 1 (ICAM), are upregulated on APCs and mediate interactions with lymphocyte function-associated antigen 1 (LFA-1) on infiltrating T cells. These interactions prevent T cell detachment into the convective flow of the cerebrospinal fluid (CSF). Following reactivation by APCs, T cells likely cross the glia limitans at the surface of the brain as well as from perivascular spaces (not shown). Within the parenchyma, T cells encounter additional APCs and microglia that promote release of inflammatory molecules and chemoattractants that recruit pathogenic monocytes and neutrophils from the blood. This causes myelin and axonal damage, ultimately causing neurological dysfunction. MHC, Major histocompatibility complex; TCR, T cell receptor.

In addition to APCs, the meninges also contain ILCs and mast cells that can promote the development of EAE. For example, ILC deficiency was shown to reduce the incidence of EAE [59,74]. During disease development, ILCs activated in a T-bet-dependent manner cluster with APCs as well as autoreactive CD4⁺ T cells in the dura mater and are required to amplify expression of chemokines, cytokines, and metalloproteases locally; this enables immune infiltration of the spinal cord parenchyma and subsequent disease [59]. Mast cells also reside in the meninges and are activated early during EAE. These cells participate in neutrophil recruitment [75] and granulocyte/macrophage-colony-stimulating factor production by T cells through local release of TNF- α and IL-1, respectively [76–81]. Mice genetically deficient in mast cells are protected from EAE, and disease can be restored by injection of mast cells into the meninges (reviewed in [58]). These studies demonstrate that innate immune cells residing in the meninges can influence the development and pathogenicity of a parenchymal autoimmune disease.

The meninges also contain fibroblast-like stromal cells that play an important role in supporting immune responses during EAE. Tertiary lymphoid structures are induced by inflammation and are observed in the meninges of MS patients as well as some mice with EAE [82]. Pikor and colleagues demonstrated during EAE that meningeal tertiary lymphoid structures are comprised of a dense network of gp38⁺CD31⁻ stromal cells (also referred to as fibroblastic-reticular-like cells) that can attract different immune cell populations (e.g., APCs, T cells, and B cells) into an organized conglomerate. These cells increase in number (from 6000 to as many as 15 000 cells) during EAE, and rather than simply provide structural support, they are highly responsive to inflammatory stimuli. For example, meningeal stromal cells respond to IL-17 and lymphotoxin by T and B cell chemoattractants, as well as extracellular matrix components such as collagen and fibronectin. Importantly, meningeal tertiary lymphoid structures promote subpial demyelination and astrogliosis, indicating that these structures are pathogenic during a CNS autoimmune disease [82].

The Meninges Are Inflamed during Stroke

Stroke is the fourth most common cause of death in the United States [83]. It is usually triggered by a clog in a parenchymal artery, leading to hypoxia and cell death in the surrounding CNS tissue. Even after reperfusion, cell damage and immune infiltrates cause vascular leakage and edema, which can be fatal or lead to long-term cognitive impairment [83]. Middle cerebral artery occlusion (MCAO) followed by reperfusion is a commonly studied rodent model for stroke. This model recapitulates the inflammatory response in humans, with pathogenic neutrophils and monocytes infiltrating the CNS in response to the ischemic event ([84–87] and reviewed in [88]).

Even though strokes primarily damage the CNS parenchyma, activation and immune infiltration of the meninges often precedes the parenchymal response [87,89]. Mast cells reside in the dura mater and can be activated by release of alarmins from damaged cells or in response to meningeal nerve activity. Notably, mice deficient in mast cells are partially resistant to stroke pathology [86]. These mice have decreased myeloid cell recruitment into the brain parenchyma 3 days after a transient MCAO, which was associated with reduced brain swelling and infarct size [86]. Intravenously or intracranially injected mast cells engraft the meninges for weeks in this model, and engraftment by wild type but not IL-6-deficient mast cells promoted neutrophil and monocyte recruitment into the brain parenchyma [86]. These data indicate that meningeal mast cells have the potential to amplify parenchymal inflammation and enhance brain damage following a transient MCAO.

In addition to mast cells, several studies have shown T cells can also contribute to stroke pathology. CD4⁺, CD8⁺, and $\gamma\delta$ T cells all accumulate in the brain parenchyma following a transient MCAO [85–87,90]. Recombination activating gene 1 (RAG-1) knockout mice, which

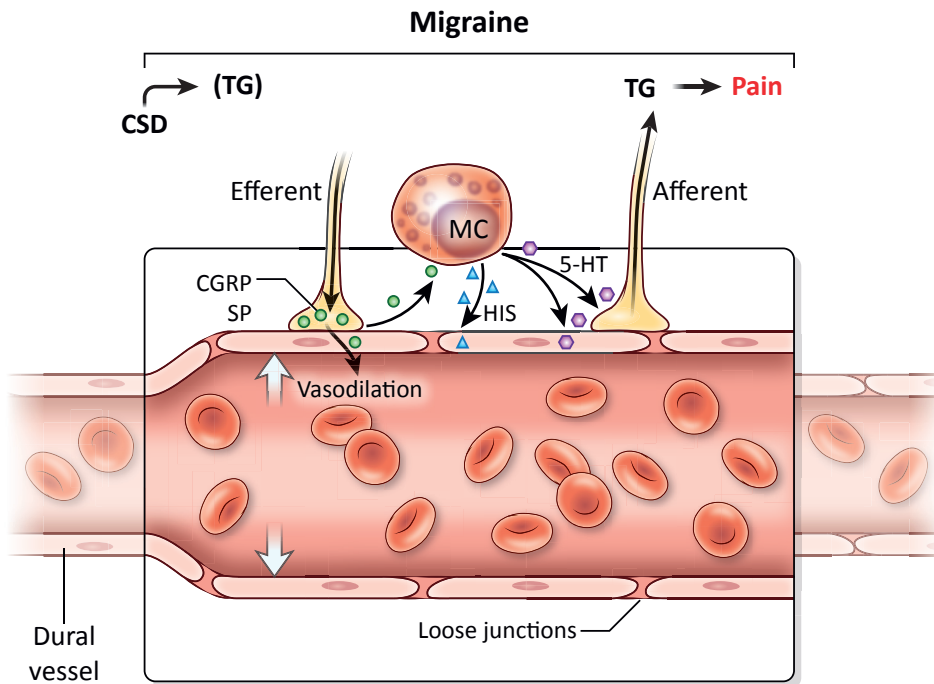
are deficient in T and B cells, as well as mice treated with FTY720 (a drug that prevents T cell recirculation) reduced brain damage following stroke, and this was dependent in part on IL-17 [90]. The majority of IL-17⁺ cells found in the CNS 3 days following an MCAO were $\gamma\delta$ T cells, not CD4⁺ T cells [85,90], and these cells localized primarily to the pia mater and choroid plexus [87]. IL-17- and IL-23-deficient mice have also been shown to have reduced expression of IL-1 and matrix metalloproteinases (MMPs) 3 days after MCAO, but normal myeloid cell recruitment into the CNS [90]. However, another study in a murine model of stroke showed that neutrophil infiltration was significantly reduced after administration of anti-IL-17 or in IL-17R-deficient mice [85]. Because neutrophils are important contributors to the pathophysiology of stroke (reviewed in [88]), and IL-17 is known to promote neutrophil recruitment [85], it is reasonable to postulate that neutrophils contribute in part to IL-17-mediated pathology following MCAO. IL-17 might also exert its pathogenic effects via neutrophil-independent mechanisms.

One potential source of $\gamma\delta$ T cells following stroke is the gut, an environment that is influenced by intestine microbiota. A recent study showed that IL-17⁺ $\gamma\delta$ T cells increase in the dura mater (from 30 to 50 cells) 1 day after MCAO [87]. To investigate the role of microbiota in shaping this response, intestinal dysbiosis was induced with antibiotics in the context of stroke [87]. Interestingly, dysbiosis reduced infarct size and neutrophil infiltration into the parenchyma, but not the meninges. This protective response was associated with an increased ratio of regulatory T cells to IL-17⁺ $\gamma\delta$ T cells in the intestines, and a reduced number of IL-17⁺ $\gamma\delta$ T cells in the meninges. In concert, these data indicate that IL-17/IL-23 axis is activated early in the CNS after stroke, and this response contributes to brain pathology. Interference with this response either with anti-IL-17 or alterations in intestinal immunity might offer a means to reduce pathology and improve outcomes after a stroke.

Migraines Are Associated with Meningeal Inflammation

Although migraines affect more than 15% of the population worldwide, the etiology and underlying mechanisms of this disorder are not entirely understood [91]. Migraine with aura is the most commonly studied subtype (~30% of cases) [92]. During this type of migraine, pain is preceded by partial visual loss and sensory disturbances. There is also an intense parenchymal depolarization (also known as ‘cortical spreading depression’ or CSD), which is followed by activation of the trigeminal ganglion and meningeal afferent fibers (Figure 4) [93]. By stimulating different peripheral nerve fibers in the CNS of awake humans through craniotomies, it was shown that dural efferents projecting from meningeal arteries to the trigeminal ganglion are the most potent triggers of pain [94]. Another study in humans that involved mechanical stimulation of the meninges showed that the pia mater and small cerebral vessels are also sensitive to pain [95], which broadens the possible contributors to migraines.

The natural triggers of meningeal nerve activation responsible for pain during migraines are unclear. For many years, it was thought that vasodilation of dural vessels was responsible for meningeal nerve activation and pain sensation (Figure 4). This conclusion was based on experimental models of migraine, which showed nitric oxide donors, CGRP, and mast cell-derived molecules (e.g., histamine, serotonin) all promote vasodilation [17,96–101]. However, this theory was challenged more recently by studies of naturally occurring migraines in patients, which uncovered no evidence of vasodilation in dural blood vessels [100]. Apart from vasodilation, intracranial infusion of factors (e.g., nitroglycerin, acetylcholine, CGRP) known to promote migraines in experimental models also promote mast cell degranulation [91]. Meningeal sensory efferents can respond directly to mast cell-derived products (e.g., serotonin) independently of vasodilation (Figure 4) [102,103]. Thus, it is unclear to what degree (if any) vasodilation plays in the development of migraines.



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Figure 4. Physiopathology of Migraines. The brain parenchyma does not have nociceptors, and painful stimuli are generally triggered by activation of dural sensory fibers. Among nociceptive stimuli, the most commonly studied form of migraine is triggered by an intense and fast depolarizing wave in the parenchyma of unknown etiology (referred to as a cortical spreading depression or CSD). This promotes activation of fibers emanating from the trigeminal ganglion (TG) that project onto dural blood vessels. Neuropeptides, such as calcitonin gene-related peptide (CGRP) and substance P (SP), are released on blood vessels and mast cells (MC), with the latter producing serotonin (5-HT) and histamine (HIS). This causes vasodilation of dural blood vessels and directly activates efferent fibers, triggering the sensation of pain.

Another potentially important component of migraines is innate immune cells. Using intravital imaging, a recent study demonstrated in mice that CSD is quickly sensed by meningeal immune cells [52]. Researchers followed the dynamics of meningeal macrophages and dendritic cells *in vivo* after experimental CSD. Pial macrophages responded first by immediately retracting their processes. This was followed within minutes by dendritic cells in the pia, subarachnoid space, and dura becoming immotile. Finally, after 20 minutes, dural macrophages also retracted their processes. These cellular changes were linked to immune activation, as injection of lipopolysaccharide (a bacterial product) triggered a similar response. Notably, ~30–40% of macrophages and dendritic cells were juxtaposed to TRPV1⁺ nerve fibers in the dura mater and may therefore sense and amplify their activation. The link between CSD (a parenchymal phenomenon) and widespread meningeal disturbances may rely on the collateral branches of pial nerve fibers that project into the dura [26,92,104]. Collectively, these data suggest resident meningeal immune cells (e.g., macrophages, dendritic cells, and mast cells) respond to CSD and may amplify neurogenic inflammation, resulting in additional pain sensations.

Sterile Injury Triggers Meningeal Inflammation

Traumatic brain injury (TBI) affects more than 2 million people in the United States every year, and there are currently no highly effective treatments available [105]. Meningeal inflammation and vascular leakage are common after TBI [7,106] and are associated with immune infiltration

and cell death that can ultimately damage the underlying parenchyma. The meninges are damaged in up to 50% of patients with mild traumatic brain injuries (mTBI), which promotes recruitment of peripheral immune cells that respond to the damage [7,107] as well as activation of dural mast cells that release histamine and other inflammatory mediators [108,109]. By studying a murine model of meningeal injury that mirrors some of the pathology observed in mTBI patients, it was revealed that meningeal macrophages are highly sensitive to mechanical forces and die almost immediately (within 5–10 min) after mTBI [7]. These cells release their cellular contents into the meninges and likely serve as an early source of pathogenic ROS in this model. This ROS then causes additional damage in the meninges as well the glial limitans and underlying parenchyma. Concurrently, ATP and chemokines are released in the meninges, promoting neutrophil recruitment [7,107]. This mechanism of neutrophil recruitment was also observed in a model of sterile liver injury [110]. Notably, interference with meningeal neutrophil recruitment by blocking the P2X7 receptor (an ATP sensor) increased cell death in the meninges, suggesting that neutrophils are beneficial in this compartment following mTBI [7]. The mechanism underlying this benefit is currently unknown, but might involve facilitation of dead cell clearance and/or recruitment of wound-healing macrophages. A similar beneficial role for neutrophils was observed following spinal cord injury (SCI) [111].

The meninges are also important to consider after more anatomically disruptive CNS injuries, such as SCI. In addition to neutrophils, both monocytes and ILCs were shown to participate in the SCI inflammatory response. For example, **Ly6C⁺ monocytes** infiltrate the surrounding meninges within a day of SCI in a CCL2-dependent manner and then invade the parenchymal injury site [112]. These cells are followed by **Ly6C⁻ monocytes** that were postulated to traffic through the choroid plexus and central canal, promoting wound-healing and functional recovery [112]. Interference with monocyte recruitment and differentiation impedes the process of wound-healing following SCI. ILCs may also aid the recovery process following SCI. ILCs are typically found in the meninges, but not the CNS parenchyma under steady-state conditions [59]. To examine the role of these cells, Gadani and colleagues showed that 1 day after SCI, ILC2s in the meninges surrounding the brain produced IL-5 and IL-13 in response to the alarmin IL-33 [113]. Notably, injection of ~5000 wild type, lung-derived ILC2s into the meninges of IL-33R knockout mice promoted partial recovery from SCI. How these cells enhance recovery after SCI is presently unclear, but we speculate that the mechanism might involve release of factors that promote wound-healing macrophages (e.g., IL-13) and/or tissue repair (e.g., VEGF).

Microbes Enter the Meninges and Induce Inflammation

The meninges serve as an entry point for many neurotropic pathogens, which can trigger inflammation, pathology, and neurological dysfunction. For example, following Zika virus infection, inflammation and viral antigens can be found in the meninges of neonates, even without parenchymal infection [114–116]. In addition, phylogenetic studies have revealed that HIV can transit through the meninges before reaching the brain parenchyma [117]. The meninges can also promote the spread of HIV virions from the brain back into the periphery [117]. Thus, the meningeal compartment can serve as a gateway for immune cells and pathogens into and out of the CNS.

One example of a pathogen-induced disease in humans that has a meningeal component is African trypanosomiasis, which is also known as sleeping sickness (reviewed in [118]). This disease is caused by infection with the insect-borne parasite *Trypanosoma brucei*. After transmission of the parasite from flies, it enters the blood before invading the CNS. Early experiments in *T. brucei*-infected primates demonstrated the presence of parasites and immune infiltrates (monocytes, lymphocytes) in the choroid plexus as well as the meninges

at the base of the skull [119]. A similar pattern was observed in *T. Brucei*-infected rodents, where inflammation was first observed in the choroid plexus and basal meninges before the parenchyma [120,121]. The parasite then moves into the space between the pia mater and glia limitans, which represents the final step before invasion of the parenchyma (although parenchymal invasion does not always occur) [122,123]. To monitor the early meningeal inflammatory response to this parasite, intravital imaging has been performed through a thinned skull window in mice [123]. Using this approach, it was revealed that T cells and APCs arrived in the meninges within the first days of infection. Further accumulation of T cells and parasites was observed over the following weeks, primarily in the dura mater. The movement of parasites along the extracellular matrix of the dura mater was restricted over time, and it is tempting to speculate that the composition of the extracellular matrix changes after infection as a mechanism to limit microbial spread. Indeed, inflammation can promote reorganization and expansion of the meningeal stromal network, as demonstrated during EAE and following a *Toxoplasma gondii* or coronavirus infection [82,124,125]. Confining a pathogen to the meninges (especially the dura mater) is likely a crucial step toward preventing neurological disorders associated with invasion of the brain and spinal cord parenchyma.

Lymphocytic choriomeningitis virus (LCMV) is another pathogen that can invade the meninges, causing meningitis in humans, primates, and rodents [126]. Following intracerebral inoculation, LCMV replicates primarily in resident cells of the meninges (e.g., fibroblast-like stromal cells, meningeal macrophages, and dendritic cells) [127]. The virus also spreads into the periphery where it induces a strong CD8⁺ T cell response in secondary lymphoid tissues. These cells infiltrate the meninges at ~5–6 days postinfection, causing immune-mediated pathology and fatal brain edema (reviewed in [126,128]). Depletion of CD8⁺ T cells completely prevents fatal CNS disease in this model, but the mechanism underlying fatal immunopathology is atypical [127]. Mice lacking classical effector pathways used by antiviral CD8⁺ T cells (e.g., FasL, granzyme, perforin, TNF- α , IFN- γ , and degranulation machinery) all succumb to LCMV meningitis, suggesting an alternative mechanism of CD8⁺ T cell-induced pathology [127]. Antiviral CD8⁺ T cells are strongly reactivated via antigen-dependent and independent mechanisms upon arrival in the LCMV-infected meninges and can even divide *in situ* [129]. These interactions promote a significant amplification of the meningeal inflammatory response. For example, antiviral CD8⁺ T cells were shown to produce CCL3, 4, and 5, directly promoting massive recruitment of **myelomonocytic cells** from the blood in mice [127]. Intravital imaging studies of the meninges at the peak of disease revealed myelomonocytic cells synchronously extravasating across meningeal blood vessels, causing significant vascular pathology. Depletion of these innate immune cells promoted survival from meningitis, demonstrating that CD8⁺ T cell-mediated disease can depend in part on injurious vascular recruitment of myelomonocytic cells into the meninges.

Concluding Remarks

The elegantly structured meninges, which in many ways resemble a peripheral tissue, contain a vast network of immune cells that are not found in the CNS parenchyma. This layered compartment contains peripheral nerves, fenestrated and nonfenestrated blood vessels, lymphatic drainage, a complex stromal matrix, dendritic cells, long-lived myeloid cells, and a steady flow of CSF. These features, among others, make the meninges a unique and complex microenvironment worthy of future investigation (see Outstanding Questions). The meninges also appear to influence CNS functions such as cognition and behavior under steady-state conditions, and the immune system is an active participant in these processes. Use of cutting-edge approaches like intravital imaging and meningeal whole mount immunohistochemistry has shed new light on the meninges, revealing that CNS immunity is often orchestrated by the

Outstanding Questions

What is the complete immune landscape in the meninges under steady-state conditions? The meninges contain many immune cell types (e.g., ILCs, mast cells, macrophages, T cells, B cells) not found in the parenchyma. A more unbiased survey (e.g., single cell RNA-seq) of meningeal immune inhabitants without parenchymal contamination is required to fully understand the true capacity of this compartment. It is also important to map out the precise location of these immune cells within the meninges (e.g., dura, arachnoid, subarachnoid space, pia).

To what extent does meningeal immunity influence CNS cognition/behavior, and how do molecules produced in the meninges gain access or transmit information to the CNS parenchyma? Meningeal T cells and the cytokines they produce have been shown to influence cognition and social behavior in mice, and associative studies suggest that the same might be true in humans. However, it is unclear how molecules produced in dura mater or subarachnoid space cross barriers like the arachnoid mater and glial limitans to influence cells residing in the parenchyma. It is also not clear to what degree different innate immune cells residing in the meninges influence cognition and social behavior.

How do peripheral nerves contribute to meningeal homeostasis and inflammation? Peripheral nerves project to vascular (both in the dura and pia mater) and nonvascular meningeal cells. The nerves can influence vascular tone, activate or inhibit resident immune cells in the meninges, and provide sensory feedback regarding the state of the meninges, fostering the sensation of pain in some situations (e.g., migraines). This bidirectional line of communication needs to be fully interrogated in the context of meningeal immunity, both under steady and inflammatory conditions.

What are the key immunological differences among the distinct meningeal layers? From the pia to the dura mater, there are differences in immune diversity, vascular leakiness, innervation, and inflammability, but the relative

meninges and that meningeal inflammation usually precedes neurological dysfunction during both infectious and noninfectious conditions. Resident innate immune sentinels are strategically positioned throughout the meninges to sense damage as well as respond to and sequester pathogens before they reach the parenchyma. These cells can efficiently recruit leukocytes from the blood and sometimes endow them with the capacity to enter the parenchyma. In general, meningeal immune responses can be beneficial or cause great harm depending on the context. Therapeutically, it has been challenging to access the CNS due to the blood–brain barrier, but the meninges should be considered as an alternative therapeutic target from the standpoint of CNS immune modulation. By controlling meningeal immunity, it should be possible to shape inflammatory responses in the underlying CNS parenchyma.

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impact that these different layers have on parenchymal inflammation and function is unclear.

What factors control the spread of inflammation from the meninges to the parenchyma? The dura mater contains fenestrated blood vessels and lymphatic drainage, resembling a peripheral tissue. This compartment readily supports inflammatory responses, but what factors influence transmittance across the arachnoid barrier is unknown. A similar question should be asked about the factors that promote movement of inflammatory responses from the subarachnoid and perivascular spaces across the glial limitans and into the CNS parenchyma.

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