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Meningeal Lymphatics: From Anatomy to Central Nervous System Immune Surveillance

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At steady state, the CNS parenchyma has few to no lymphocytes and less potent Ag-presentation capability compared with other organs. However, the meninges surrounding the CNS host diverse populations of immune cells that influence how CNS-related immune responses develop. Interstitial and cerebrospinal fluid produced in the CNS is continuously drained, and recent advances have emphasized that this process is largely taking place through the lymphatic system. To what extent this fluid process mobilizes CNS-derived Ags toward meningeal immune cells and subsequently the peripheral immune system through the lymphatic vessel network is a question of significant clinical importance for autoimmunity, tumor immunology, and infectious disease. Recent advances in understanding the role of meningeal lymphatics as a communicator between the brain and peripheral immunity are discussed in this review. The Journal of Immunology, 2020, 204: 286–293.

The CNS is often described as an immune-privileged organ with tolerance to normally rejected stimuli such as tissue grafts. Over the last century the precise meaning of this term has evolved, diverging from its meaning in other tissues with unique immunological regulation, such as the testis and eye (1–4). Immune privilege could be enacted through lenient immune surveillance, through the modulation of the immune response itself or a combination of the two. A foundational experiment by P. B. Medawar disclosed that whereas skin allografts in the brain were not rejected initially, a subsequent peripheral allograft would induce priming and cause rejection of both grafts (5). This led to the long-held conclusion that whereas an immune response can be mounted effectively within the brain parenchyma, immune surveillance must be limited such that Ags can escape immune detection. This model was in line with the consensus that because the brain (unlike almost all other tissues) does not contain lymphatic vessels, the immune system would not have the access to CNS Ags that it would need to develop a response. However, the existence of a functional connection from the CNS to the cervical lymph nodes had been reported using a variety of tracers in animal and cadaver studies, although this was speculated on mainly as a fluid reabsorption process rather than from an immunological perspective (6–10). Studies would go on to show that Ag delivered to the CNS could induce a robust humoral response via the cervical lymph nodes and the spleen (11–13). Notably, the CNS-lymphatic connection has recently been demonstrated in vivo in humans through contrast magnetic resonance imaging (14). Thus, the immune privilege of the CNS cannot be as simple as a lack of lymphatic immune surveillance nor isolation from peripheral immune responses but rather depends on dynamic physiological processes and cellular interactions.

A new challenge in understanding fluid drainage of the brain erupted in 2015, with the publication of studies describing an intracranial lymphatic network in mouse meninges that absorb tracer from the cerebrospinal fluid (15, 16). Analogous structures were found also in post mortem human meninges (15) and were later visualized in vivo by means of magnetic resonance imaging in humans and nonhuman primates (17). A large body of literature around fluid dynamics in the CNS developed prior to these discoveries, and it is essential that we acquire a detailed understanding of the mechanisms that underlie exchange of fluids in the CNS. Furthermore, the immunologic implications of direct intracranial lymph for-
Barriers and pathways for efflux from the nervous system

Two distinct but communicating extracellular fluid compartments are found within the CNS: the cerebrospinal fluid, which occupies the ventricles and subarachnoid space, and the interstitial fluid (ISF), which perfuses the brain parenchyma. There is net fluid production in both compartments (47–51) that ultimately provides both a drainage pathway for waste products and a site for sensing antigenic material within the brain and meninges. Reuptake of ISF occurs through the cerebrospinal fluid presumably directly to the blood and through the lymphatic system. Although the relative contributions of each remains controversial, the role of lymphatic drainage is increasingly appreciated in recent studies (52–54). Physiologically, this reuptake is necessary to balance fluid influx to the CNS from the blood as cerebrospinal fluid and ISF. Immunologically, however, lymphatic drainage of the CNS is a powerful mechanism for remote immune surveillance of the CNS and leaves open questions. To what extent is Ag collected and delivered to the immune system in the process of CNS fluid circulation?

The brain is separated from the periphery by an intricate system of barriers that provide the context for its unique interactions with the immune system. Starting from the cranium, the three meningeal layers surrounding the brain are the dura mater, the arachnoid mater, and the pia mater. Whereas the dura and arachnoid membranes are in contact with one another, the pia is separated from the arachnoid by the subarachnoid space. This space is filled with cerebrospinal fluid and is bridged by the arachnoid trabeculae, a dense network of connective filaments. Underneath the pia mater begins the brain parenchyma with a layer of glial processes known as the glia limitans. Additional CNS structures that are important from a cerebrospinal fluid efflux perspective are cerebral ventricles. The ventricles are cerebrospinal fluid–filled cavities that are connected with each other and the subarachnoid space. They contain the choroid plexus, a unique, well-vascularized organ with a distinct epithelium that secretes cerebrospinal fluid from the selective filtration of blood plasma (47, 48, 50, 55). Once blood vessels cross the arachnoid matter into the brain, they maintain a stricter barrier function than elsewhere in the body, decreasing water conductivity and passive permeability to most solutes (56, 57) to restrict the access of molecules from circulation into surrounding neural tissue and vice versa. For many solutes, the existence of this barrier has shifted focus for removal to distal sites such as the dural venous sinuses and lymphatic networks. For some specific solutes, including amyloid β, however, higher exchange rates are achieved at the vasculature through active transport (58–60), and a decreased rate of removal through all routes is implicated in Alzheimer’s disease (61).

In addition, the cerebrovasculature allows minimal entry of peripheral immune cells to the brain parenchyma at steady state compared with other organs. The vascular endothelial cells of the brain exhibit low expression of adhesion molecules, including selectins, VCAM-1, and ICAM-1 at rest, but can upregulate adhesion molecules during inflammation (62, 63). At the resting state, this barrier enforces isolation of the specialized brain-resident immune cell populations from circulating cell types (64). The specialization of CNS immune cells as well as apparent spatial separation of immune surveillance is likely related to the low tolerance of the brain for inflammation and poor regenerative capacity.

Cerebrospinal fluid and ISF circulation

The pia mater, which is the largest interface between ISF and cerebrospinal fluid, is not an absolute barrier, and permeability
to macromolecules is dependent on concentration gradient, size, and solubility in cerebrospinal fluid but is not completely characterized (65–67). Several groups have published compelling evidence that diffusion across this boundary alone does not fully account for the movement of interstitial macromolecules from the parenchyma to the cerebrospinal fluid (53, 68–71). Rather, parenchymal solutes appear to move as a consequence of bulk flow, carried by the movement of the surrounding fluid. This is apparent from the rates of tracer influx and efflux from the parenchyma and the rapid distribution throughout perivascular spaces (52, 71–73).

In traditional models of cerebrospinal fluid flow, the lymphatic collection of cerebrospinal fluid is not sufficient to constitute immune surveillance of the brain as freshly produced cerebrospinal fluid from the choroid plexus would not contain parenchymal Ag, such as myelin proteins. In this model, most of the cerebrospinal fluid is produced at the choroid plexuses, flows through the ventricular system, and exits into the subarachnoid space via the foramen of Magendie and the foramina of Luschka with relatively little crossing into the interstitium of the brain parenchyma (15, 70). However, small fluorescent tracers introduced into the subarachnoid cerebrospinal fluid demonstrate more complex movements along perivascular spaces into the parenchyma (70) and are removed from the CNS through lymph or blood (15, 16, 54, 74). These studies suggest an extensive exchange of cerebrospinal fluid and ISF solutes, which is consistent with studies showing that interstitial metabolites and Ags accumulate in the cerebrospinal fluid (75–77). The formation of brain ISF, whether through cerebrospinal fluid influx, vascular extravasation, or metabolism, is balanced by fluid efflux suggested to occur through multiple routes, including return to cerebrospinal fluid before being taken up as lymph or blood (52, 64, 65). Lymphatic transport is essentially an advective process, and therefore, a fluid source is needed to account for the observed drainage of CNS tracers. Currently, we know very little about how APCs respond to the dynamics of this process to access and extract information from the CNS to maintain equilibrium or instigate inflammation.

The mechanisms driving fluid dynamics in the brain are extremely complex and challenging to measure non-invasively. In mice, a genetic model of disrupted fluid dynamics is the deletion of the aquaporin-4 water channel (AQP4). AQP4 is abundantly expressed on astrocytes, in which it is polarized to the cellular endfeet that form the glia limitans (78–81). AQP4-deficient mice show a decreased influx of cerebrospinal fluid tracers to the parenchyma and impaired clearance of intracortically injected tracer (70, 82) as well as decreased drainage to the draining lymph nodes (75). Apart from these fluid tracing experiments, it is particularly interesting that changes in mounting an active immune response to a CNS-derived Ag occur when ISF movement is disrupted. Previous immunization studies of AQP4-deficient mice have suggested decreased immune surveillance of the CNS, and the knockout mice present with delayed and less severe EAE (83). Although the complete mechanisms underlying these changes are not yet clear, it appears that the dynamics of the brain ISF and cerebrospinal fluid modulate Ag availability from the brain parenchyma.

**Routes of fluid drainage from the CNS**

Afferent to the cervical lymph nodes, several lymphatic networks have been proposed to collect both cerebrospinal fluid and interstitial macromolecules. Although the CNS parenchyma does not have a conventional lymphatic drainage system, an extensive lymphatic network has been discovered in the dura mater along the transverse and superior sagittal sinuses (15, 16). True to their acknowledged function, these vessels assist in the drainage of cerebrospinal fluid and facilitate T lymphocyte and DC movement. The vessels run alongside the venous sinuses and exit together with the jugular veins (16). Lymphatic vessels in the meninges are broadly similar to initial lymphatic vessels described in other tissues with subtle exceptions. As with other initial lymphatics, they are not ensheathed by smooth muscle cells and lack lymphatic valves (15, 16, 74, 84). As noted previously, these vessels do not penetrate the brain parenchyma, which subsequently brings us to the question of how dural lymphatic vessels access parenchymal macromolecules (Fig. 1).

The arachnoid mater features tight junctions and is considered to demarcate the boundary between cerebrospinal fluid–filled spaces and dura and thus plays a major role in compartmentalizing the CNS (42, 85, 86). Injection of fluorescent tracer into the cerebrospinal fluid reveals bilateral “hot spots” in the lateral portions of the vessels along the transverse sinuses that appear to be the first sites of cerebrospinal fluid uptake (74). These regions possess increased vessel density and complexity, including short loops and terminal sprouts. Importantly, photodynamic ablation targeting this lymphatic vasculature was found to decrease drainage to the deep cervical lymph nodes of fluorescently labeled OVA, polystyrene beads, and T cells (61, 74). Recently, another study reported that these regions of increased vascular complexity and apparent uptake capacity extend further ventrally along the transverse sinus as it splits into the sigmoid and petrosquamosal sinuses before exiting the cranium (87). Ags have been suggested to move from the cerebrospinal fluid compartment to CNS-draining lymph nodes through perineural routes as well (74, 88), but only ablation of dural lymphatic vessels, and not ablation of nasal networks, resulted in a delay in onset and a decrease in the severity of EAE (74). Furthermore, the impairment of meningeal lymphatic drainage impeded both the influx of cerebrospinal fluid tracer to the perivascular spaces and the brain parenchyma as well as intraparenchymal tracer clearance (61).

Within the cranium, vessels have also been found along the middle meningeal artery, but in contrast to dural meningeal lymphatics, tracer studies do not support their role in the uptake of molecules from the cerebrospinal fluid (74, 84). These potential two networks of lymphatic vasculature were suggested to have different effenter routes at the base of the skull, with the middle meningeal artery–associated vessels appearing to exit along the internal carotid vein (16). Given the lack of cerebrospinal fluid tracer uptake, it has been proposed that these vessels drain the dura (74), but the complete network of collecting lymphatics and their connections to lymph nodes in the neck are not fully understood (54, 87).

Several other routes of cerebrospinal fluid efflux and collection from the CNS have been studied and been suggested to follow a perivascular or perineural exit route from the cranial
cavity into a peripheral lymphatic network (54, 89–92). These pathways generally describe movement through a series of connected interstitia until they reach extracranial lymphatic networks. The exact transition from ISF to lymph, however, is not well demarcated. The most recent studies have rapidly expanded the known extent of intracranial and transcranial lymphatic vasculature, in part because of the identification of lymphatic markers and the development of reporter mice and rats (93–95). Given recent descriptions of meningeal lymphatic vessels sharing many of the vascular and neural foramina, the possibility that lymphatic uptake occurs intracranially in locations other than the hot spots merits further scrutiny (74, 96–98).

Lymph node microenvironment and lymph composition

In mice, tracers from the CNS accumulate mainly in two sets of lymph nodes: the superficial cervical lymph nodes, which are generally thought to drain from the face and the oronasal tissues, and the deep cervical lymph nodes, which show more specific accumulation of tracers introduced into the cerebrospinal fluid (15, 54, 74, 99–101). However, the drainage territories of lymph nodes are not definitively mapped in humans nor model species. Tracers introduced into spinal cerebrospinal fluid drain through lymph nodes that differ from those draining cranial cerebrospinal fluid tracers, and the spine possesses additional lymphatic vasculature (74, 84, 102–104). Connectivity between the different sets of lymph nodes has been described differently in different studies, and the relative involvement of superficial and deep nodes as well as the vascular connections between nodes remain controversial (54, 74). The consequences of different drainage patterns from the CNS require detailed studies. Experiments in the intestinal system have shown that the immune surveillance carried out by different lymph nodes produces differently orchestrated responses in their respective territories (105, 106). In cases of spontaneous EAE in mice, the deep cervical lymph nodes express activation markers prior to the superficial cervical lymph nodes (107). Even in immunization-induced EAE, in which adjuvant and Ag are processed in the periphery, the main site of CNS autoimmune reaction appears to be the deep cervical lymph nodes (74). It is important to note that other laboratory animals, and also
humans, have additional lymph nodes in the head and neck, making the networks more complex. The past several decades have seen periods of immense interest in mapping the drainage territories of individual lymph nodes, although recently, this research field has become smaller, focusing on sentinel nodes for cancer metastasis and distribution of edema (99, 108). In species with more nodes, drainage territories may be further subdivided, or more nodes may occur along a given network if the area that needs to be surveilled is larger.

**Immune processing of brain-derived lymph**

Although lymphatic drainage is needed for fluid and lipid homeostasis and removal of waste, the immunological functions of this elaborate system are of interest in both homeostasis and in disease. Adaptive immune surveillance revolves largely around the lymph nodes, which maintain a substantial subset of the T and B cell repertoire and facilitate cognate–Ag encounters. Because the lymph node is distant from the tissue, contextual information must be transferred to the lymph node along with Ag. Evidence of tissue injury or innate immune activation has to be relayed through the proper processing of Ag and immune cell trafficking via lymphatic vessels. Subsequent migration to the draining cervical lymph nodes is enabled by CCR7 expression; however, the relocation route leading to stimulation of B and T lymphocytes has not been ultimately defined. As discussed previously, continuous CNS immune surveillance is vital for responses to self-antigens, both tolerogenic and inflammatory (109–111). A selection of additional mechanisms has been suggested for Ag sorting and processing peripherally, and the interplay of different mechanisms remains incompletely understood with even less known in the CNS specifically.

In the healthy CNS, as in other tissues, a substantial fraction of Ag drains in the fluid itself rather than carried by APCs. This balance is maintained through a low expression of CCR7 by APCs, low CCL19/21 chemotaxis by the initial lymphatic vessels, and the absence of high levels of cytokine signaling. When an Ag arrives in the lymph node without a cellular chaperone, it is still processed and rarely reaches the blood unimpeded. In the absence of inflammation, this Ag may be used to maintain self-tolerance (112–115). The use of brain-derived Ags for negative selection in the lymph node provides an interesting alternative to negative selection in the thymus, where the promiscuous gene expression creating a library of peripheral epitopes seems to fall short of depleting myelin-reactive T cells (116). Although the endogenous proteome of brain-derived lymph is not well characterized yet, proteins are expected to follow similar patterns to the tracers, which have clearly demonstrated that solutes from the CNS, in healthy animals and in disease contexts, reaches professional APCs and the cervical lymph nodes. Recent studies generally introduce a fluorescent tracer to the cerebrospinal fluid via injection into the cisterna magna or lateral ventricles and have shown drainage to cervical lymphatics as well as active cerebrospinal fluid–ISF exchange (15, 17, 61, 70, 74, 117). Furthermore, because autoreactive T cells are present in autoreactivity as well as healthy subjects (118), the specific processing of Ag and mobilization to draining lymph nodes via lymphatic vessels appears to be crucial to the suppression of CNS autoimmunity.

During inflammation, however, changes in the expression of chemoattractants and migration of APCs through the lymphatic system affect the nature of the immune response. CCR7 upregulation on DCs increases mobility and initial lymphatic CCL21–CCR7 interactions, encouraging DCs to enter the lymphatic vasculature. As discussed, this cellular transport of Ag can result in Ag transfer to node-resident APCs or direct stimulation of lymphocytes. For free Ag, however, changes in the node during inflammation will have an impact as well. The structural characteristics of the node change, along with cellular activities to increase the retention and processing of Ag (119, 120), which suggests that some factors in specialized CNS immunity may originate distally from the inflammatory state of the draining lymph nodes. Studying this further will, however, require definitive understanding of the drainage territories of individual nodes and vascular networks. Similarly, the stromal cells of the meninges have the ability to respond to the inflammatory state and shape the immune cell dynamics of the CNS in ways that we are only beginning to understand (121). Our understanding of the immune response in other peripheral tissues has advanced rapidly in recent years, but there are still many mechanistic details that need to be addressed in the CNS.

**Conclusions**

Immune surveillance of the CNS differs from surveillance of other organs, and (contrary to historical viewpoints) lymphatic collection of Ags from the CNS is an active part of its homeostasis. In general, the priming of immune cells toward brain-derived Ags requires the ability of Ag to move from the parenchyma to the cerebrospinal fluid–filled space and across the arachnoid mater. Limited access through specific lymphatic protrusions (hot spots) and the existence of a sophisticated barrier system can regulate peripheral immune traffic to and from the CNS differently than in other tissues. Nonetheless, memory responses to Ags derived from the brain are comparable to those in peripheral tissues. Understanding what contributes to these unique immune responses and how these mechanisms predispose the brain to autoimmune attacks or protect it may uncover new insights into multiple sclerosis and many other neuroinflammatory conditions.

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