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Opinion

How Do Meningeal Lymphatic Vessels Drain the CNS?

Daniel Raper,[1,2,‡] Antoine Louveau,[1,3,‡] and Jonathan Kipnis[1,3,*]

The many interactions between the nervous and the immune systems, which are active in both physiological and pathological states, have recently become more clearly delineated with the discovery of a meningeal lymphatic system capable of carrying fluid, immune cells, and macromolecules from the central nervous system (CNS) to the draining deep cervical lymph nodes. However, the exact localization of the meningeal lymphatic vasculature and the path of drainage from the cerebrospinal fluid (CSF) to the lymphatics remain poorly understood. Here, we discuss the potential differences between peripheral and CNS lymphatic vessels and examine the purported mechanisms of CNS lymphatic drainage, along with how these may fit into established patterns of CSF flow.

Trends

Functional classic lymphatic vessels exist in the dura, and can function to drain fluid and immune cells from the meninges, parenchyma, and CSF.

The meningeal lymphatic system is necessary for the efficient clearance of brain ISF, and may be a common pathway for removal of wastes initially cleared from brain parenchyma through the glymphatic system of CSF–interstitial fluid (ISF) exchange.

The precise location of meningeal lymphatic vessels within the layers of the meninges needs to be further investigated.

The hemodynamics of the flow and access of the CSF to meningeal lymphatic vessels are still poorly understood.

Function of the Meningeal Lymphatics

The historical view of the immune privilege of the CNS has been challenged over the past 20 years by a body of work demonstrating that immune surveillance of the CNS is an important aspect of its homeostasis as well as response to injury and neurodegenerative conditions [1–9]. The meninges are an essential immunological site that allows CNS immune surveillance to function correctly [6,7,10–12]. In searching for the pathways of immune cell movement throughout the meninges, a system of vessels that run along the perisinusal space has recently been demonstrated [1,13,14]. These vessels have immunohistological and structural characteristics of lymphatic vessels, and are capable of carrying fluid and macromolecules [1,13]. They express all traditional markers of tissue lymphatic endothelial cells, including **Prospero homeobox protein 1** (Prox1), **CD31**, **lymphatic vessel endothelial hyaluronan receptor-1 (**Lyve-1), **Podoplanin**, **vascular endothelial growth factor receptor 3** (VEGFR3), and **chemokine (C-C motif) ligand 21** (CCL21) (see Glossary) [1,13]. Furthermore, functional experiments demonstrated that these meningeal lymphatic vessels carry numerous immune cells under physiological conditions, suggesting their role in normal immune surveillance of the brain [1].

Apart from its role in immune surveillance, a CNS lymphatic system is also likely to have a role in waste clearance from the brain parenchyma. A system of CSF–interstitial fluid (ISF) exchange, called the 'glymphatic' system, may be responsible for clearance of hydrophilic and lipophilic compounds as well as of waste products from the brain parenchyma into the CSF [15–19]. Subsequently, macromolecules and other waste products are assumed to be cleared from the CSF via drainage through the nasal mucosa lymphatics into cervical lymph nodes [9,20], purportedly via the cribriform plate [21,22].

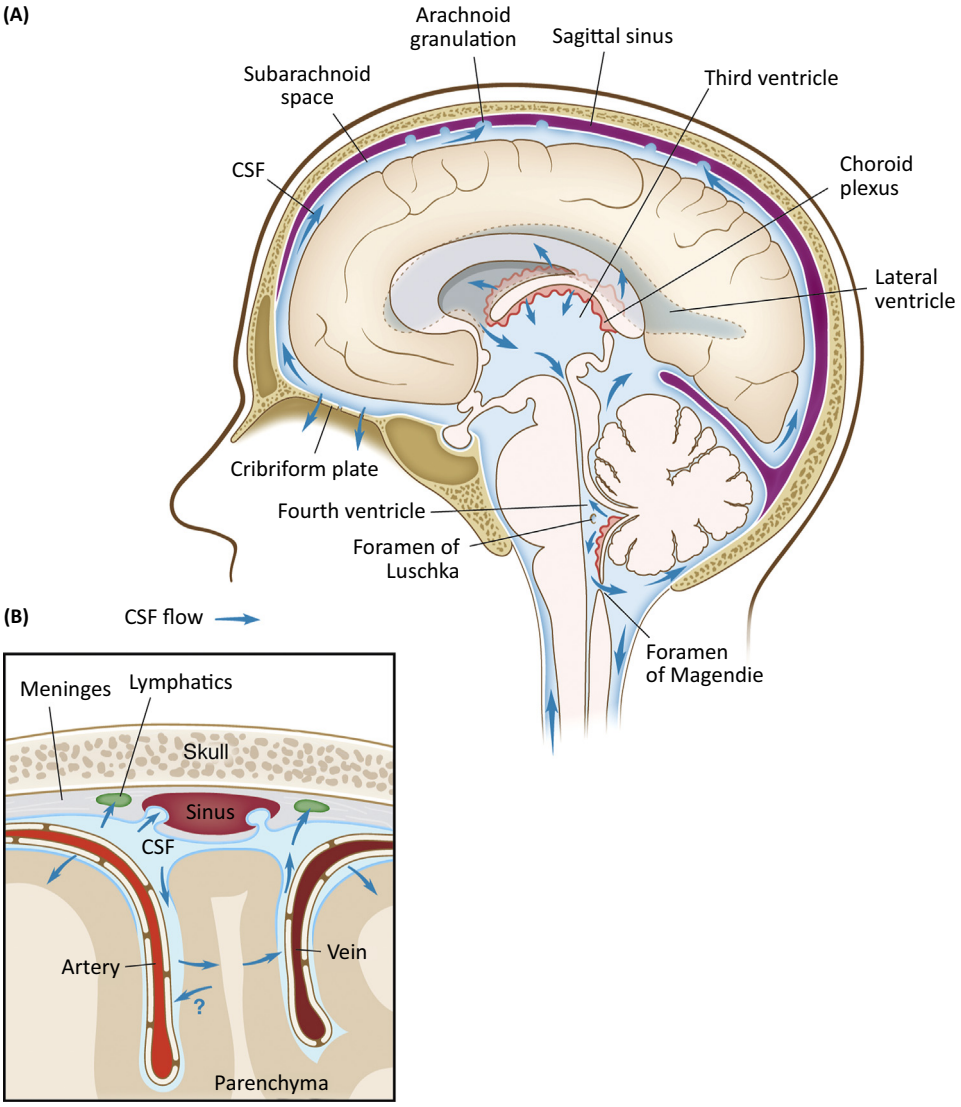
Despite accumulating evidence regarding the various pathways and fluid dynamics of the CNS, several important details in the anatomic and physiologic pathways of lymphatic drainage remain unclear. We highlight these controversies below.

Dynamics of CNS Fluids

CSF flow is a tightly regulated phenomenon with complex fluid dynamics that are as yet incompletely characterized [23–25]. CSF is produced by the choroid plexus, flows through

[1]Center for Brain Immunology and Glia, School of Medicine, University of Virginia, Charlottesville, VA 22908, USA
[2]Department of Neurosurgery, University of Virginia Health System, Charlottesville, VA 22908, USA
[3]Department of Neuroscience, School of Medicine, University of Virginia, Charlottesville, VA 22908, USA
[‡]These authors contributed equally.

*Correspondence: kipnis@virginia.edu (J. Kipnis).



Trends in Neurosciences

Figure 1. Production and Circulation of Cerebrospinal Fluid (CSF) within the Central Nervous System (CNS). Schematic representation of the pattern of intracranial CSF flow. (A) CSF is produced by the choroid plexus of the lateral and fourth ventricles, and flows from the third ventricle to the fourth ventricle through the cerebral aqueduct. After circulating over the hemispheres, CSF absorption into the superior sagittal sinus, transverse sinus, and sigmoid sinuses is via arachnoid granulations, as well as efflux from the CNS along the olfactory nerves through the cribriform plate. (B) Schematic representation of CSF–interstitial (ISF) flow from and to the subarachnoid space. CSF can diffuse in and out of the brain parenchyma along the perivascular space.

Glossary

Blood–brain barrier (BBB): this essential component of the immunological privilege of the CNS comprises tight junctions of cerebral endothelial cells, along with astrocyte foot processes and pericytes.

Chemokine (C-C motif) ligand 21 (CCL21): a cytokine of the CC chemokine family that functions as an immune cell chemoattractant protein.

CD31: an endothelial marker found on the surface of platelets, monocytes, neutrophils, some T cells, and endothelial cells, among other cell types throughout the body; also known as platelet endothelial cell adhesion molecule (PECAM-1).

Lymphatic vessel endothelial hyaluronan receptor-1 (Lyve-1): a membrane glycoprotein that is a cell surface receptor on lymphatic endothelial cells; also known as extracellular link domain containing 1 (XLKD1).

Podoplanin: a type 1 membrane glycoprotein of uncertain function that is found in lung alveolar cells, kidney podocytes, and lymphatic endothelial cells.

Prospero homeobox protein 1 (Prox1): a transcription factor involved in developmental processes in several organs, including development of the lymphatic system.

Vascular endothelial growth factor receptor 3 (VEGFR3): a tyrosine-protein kinase cell surface receptor for VEGFc and VEGFd that has been implicated in lymphangiogenesis.

the lateral and third ventricles, and exits through the foramina of Luschke and Magendie, to reach the subarachnoid space over the convexities (Figure 1). CSF leaves the intracranial circulation by draining into the dural venous sinuses through arachnoid granulations, which contain valves that prevent the backflow of blood or CSF back into the CSF compartment [26–29] (Figure 1). Granulations have been found in the subarachnoid space between the arachnoid and the dura mater, and were assumed to demonstrate a similar pulsatile flow to CSF in larger intracranial compartments [26–29]. CSF is also absorbed in the paraneural sheaths of cranial and spinal nerves and drains into lymphatic vessels that run close to these structures

[21,30], but the contribution of each route for the drainage of CSF needs to be reassessed in the light of newly discovered paths [1,13].

Intracranial CSF flow is linked to parenchymal ISF circulation, and the relation is governed by bulk flow forces, arterial and venous pressures, and intracranial parenchymal pressure [15–18]. First described during the 1980s and recently reborn, a CSF-ISF exchange, now termed a 'glymphatic' system [15,16,31], has been proposed as a mechanism for the removal of macromolecules from the brain parenchyma into the CSF. This system relies on the influx of CSF into the brain parenchyma through periarterial spaces and efflux, along with both hydrophilic and lipophilic compounds, through the paravenous spaces back into the subarachnoid space [15]. The efficiency of the system relies on arteriole pulsatility and CSF pressure, and appears to be dependent (at least partially) on the water channel AQP4 [15,17,18,32]. Interestingly, this system appears to be more efficient during sleep [18]. Whether ISF flow from the brain parenchyma (into CSF) is along periarterial or perivenular spaces remains a matter of debate [33,34] and needs to be better understood. The meningeal lymphatic system has emerged as a new player in CSF flow, and the relation between the meningeal lymphatic system and the other paths previously described needs to be addressed.

Macromolecules, such as amyloid beta (Aβ), are supposedly cleared from the CNS parenchyma via different mechanisms, such as phagocytosis and proteolytic degradation by mononuclear phagocytes [35–37] and vascular smooth muscle cells [38], transcytosis across the **blood–brain barrier** (BBB) [39–41] and via ISF bulk flow [18,42,43]. Previous studies have demonstrated that around 85% of Aβ is eliminated from the brain by transvascular clearance under normal physiological conditions in mice, while a smaller percentage is cleared by the other routes, notably ISF bulk flow [44,45]. The latter is of particular importance since Aβ has been shown in both mice and humans [46,47] to accumulate along the path of ISF flow, suggesting a potential dysfunction of these routes in the pathology of Alzheimer's disease. Given that mice lacking meningeal lymphatic vessels show dysfunction in their ability to clear parenchymal macromolecules [13], studies will be required to address the anatomical and functional relation between ISF flow and meningeal lymphatic drainage.

Transvascular clearance of Aβ has been shown to occur within the brain parenchyma [37,44,45,48]. This clearing is mediated by receptor-dependent transcytosis, mainly by the low-density receptor-related protein (LRP1) transporter [39,44,45,48]. The ApoE family of molecules has been shown to participate in the transvascular clearance of Aβ [49,50]. Indeed, Aβ binding to apoE2 or apoE3 is cleared rapidly through LRP1, whereas Aβ bound to apoE4, a marker of genetic susceptibility for AD, is only slowly cleared via very low-density lipoprotein receptor (VLDLR)-mediated internalization and transcytosis [49,50]. Similar phenomena may occur across the lymphatic endothelial cells, which express the scavenger receptor SRB1 [51], known to bind fibrillar Aβ [52]. Unraveling the mechanism of macromolecule clearance from the CNS parenchyma by lymphatic endothelial cells is the next step in understanding this complex interaction.

One can hypothesize that the meningeal lymphatic system is not implicated in the drainage of the water content of the CSF, since no change in intracranial pressure was reported in mice lacking meningeal lymphatic vasculature [13]. Similarly, the multiple paths of drainage of CSF might have different functions; the cribriform plate path along with the arachnoid granulations could serve as valves for the maintenance of CSF pressure, while the meningeal lymphatic vasculature would have a more immunological function, that is, drainage of macromolecules and immune cells into the cervical lymph nodes to maintain brain immune surveillance. More studies investigating the interaction between the different drainage paths are necessary to demonstrate the selectivity and functional relevance of each of these paths for the maintenance of brain function.

Meningeal Lymphatic Drainage: Anatomical Considerations

Where do CNS lymphatic vessels run? It has been demonstrated that lymphatic vessels are associated with sinuses [1] and Aspelund *et al.* also demonstrated lymphatic vessels at the base of the skull and along the dural middle meningeal artery [13]. The middle meningeal arteries in humans run on the outer surface of the dura and, therefore, are not part of the CNS. Due to the diminutive size of mouse meninges, it has not been possible to definitely separate the meningeal layers and identify the precise location of meningeal lymphatic vessels. Three possibilities exist: lymphatic vessels may run within dural leaflets, on the inside surface of the dura, or in the subarachnoid space with the cortical veins (Figure 2).

The precise localization of the meningeal lymphatic vessels is of primary importance to understand how they are able to drain tracers and cells from the CSF [1,13]. If the meningeal lymphatics are localized within the subarachnoid space, they would be 'bathed' in the CSF, which, therefore, could easily diffuse into the meningeal lymphatic vasculature. However, if the meningeal lymphatic vessels are indeed localized within the dural leaflets or on the inside surface of the dura, then they are physically separated from the subarachnoid space. The question is then how can tracer, injected into the CSF, the lateral ventricle, or the brain parenchyma, reach the meningeal lymphatic vessels? Studies have suggested that CSF is transported across the arachnoid membrane to the dura [53–55], which would explain the accumulation of CSF tracers in dural-localized lymphatic vessels. Furthermore, expression of transporters by arachnoid cells [56] could also allow the transfer of tracer from the CSF into the dura. Another intriguing possibility would be an arachnoid granulation-like structure that may mediate drainage of CSF into dural lymphatic vasculature across the arachnoid and inner layer of the dura. Further anatomical studies characterizing the meningeal lymphatics are required to better understand the drainage of the CSF by the meningeal lymphatic system.

Meningeal Lymphatic Drainage: Environmental Considerations

In peripheral tissues, macromolecules are able to diffuse into lymphatic vessels from the ISF through permeable endothelial cell junctions [57]. Specialized features of lymphatic vessels in the periphery, including a lack of pericytes and a discontinuous basement membrane, allow cells and molecules to enter. There are different types of lymphatic vessel, including initial and collecting vessels, the latter of which contain bi-leaflet valves to prevent backflow of lymph [58–60]. Lymph is pushed anterograde within lymphatic vessels by the action of the surrounding smooth muscle cells of the collecting vessels and arterial pulsations [57]. Meningeal lymphatics share features of initial vessels (lack of valves and surrounding smooth muscle cells) [1] except at the base of the skull, where potential lymphatic valves have been detected [13], suggesting a transition from initial to collecting vessels as these vessels are exiting the CNS.

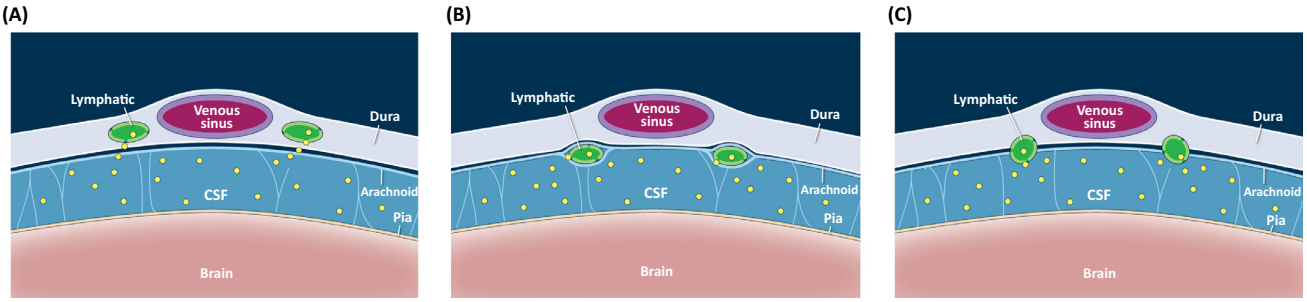


Figure 2. Possible Localizations of Central Nervous System (CNS) Lymphatic Vessels within the Meninges. (A) Lymphatic vessels are located within the dura layers in close apposition to the venous sinuses. (B) Lymphatics are located at the interface between the dura and the arachnoid layers. (C) Lymphatics are exposed to the subarachnoid space. Macromolecules (yellow) are seen within the subarachnoid space and within the lymphatic vessels into which they are draining.

Contrary to the peripheral tissue, the meningeal lymphatics are likely to be exposed to an environment with different physical properties that might affect the development and behavior of the vessels. The meningeal lymphatic vasculature has less tissue coverage and smaller diameter than peripheral lymphatics [1], suggesting that physical environmental pressure or other factors might inhibit the sprouting and enlargement of the meningeal lymphatic vasculature. Furthermore, a single injection of recombinant VEGFc is able to induce lymphatic vasculature sprouting and proliferation in peripheral organs [61,62], whereas, in the meninges, only a small increase in diameter is observed [1]. This observation reinforces the hypothesis that the meningeal environment might be limiting the expansion of the meningeal vasculature due to CSF flow dynamics. One could wonder whether the small diameter of the meningeal lymphatic vasculature limits the amount or quality of antigen being drained into the cervical lymph nodes and participating in the immune privilege of the brain. Further studies on the development and plasticity of the meningeal lymphatic vasculature will be necessary to appreciate the function of these vessels in the drainage of macromolecules and cells from the CNS and understand their role in the immune privilege of the brain.

Outstanding Questions

How can meningeal lymphatic vessels absorb macromolecules and immune cells from the CSF if they are located within the dura? What are the forces that govern this absorption and how to they relate to physiological changes in CSF hemodynamic forces?

Where precisely do meningeal lymphatic vessels run? Are there equivalent structures to somatic precollecting and collecting lymphatic vessels at different locations within the intracranial space?

Concluding Remarks

The emergence of growing consensus around the role of meningeal immunity in CNS surveillance in physiological states has generated several important discoveries that have helped to characterize the specialized CNS immune system. One of these is the presence of meningeal lymphatic vessels capable of carrying fluid, immune cells, and macromolecules from within the CNS and CSF. Although they help explain how the CNS and peripheral immune systems may be linked, these finding raise several questions that remain to be fully elucidated (see Outstanding Questions). Meningeal lymphatic vessels may provide a key component of the mechanism for the immune response to CNS insult from traumatic injury or stroke. By contrast, meningeal lymphatic dysfunction may manifest in a variety of different ways, with implications for several neurological diseases and neurodegenerative conditions.

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