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Clearing the mind: Implications of dural lymphatic vessels for brain function

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Although the peripheral lymphatic system performs such essential physiological functions as interstitial fluid (ISF) and protein homeostasis and immune surveillance, the apparent absence of conventional lymphatic vessels from the central nervous system (CNS) has remained a persistent mystery of neuroscience, particularly in light of neural cells' exquisite sensitivity to the composition of their extracellular environment.

It has long been appreciated that the cerebrospinal fluid (CSF) circulation serves an excretory role, functioning as a sink for metabolic wastes produced within the CNS that cannot be readily eliminated across the blood brain barrier. In a model articulated by Davson[1], and later elaborated by Cserr[2], interstitial solutes in the brain exchange with CSF through local diffusion and ISF bulk flow and are cleared from the cranium with CSF reabsorption. Studies published by Weed in 1914[3] established a model of CSF reabsorption that has persisted largely intact to present day: that CSF and its associated solutes exit the subarachnoid space a) into the dural sinuses via the fine cellular structures of the arachnoid granulations and b) into peripheral lymphatic vessels of the nasal mucosa and neck by their transport along perineural spaces surrounding cranial nerves. Although presumptive primary lymphatic vessels have been previously identified within the dura and implicated in CSF reabsorption[4], Weed's assertion that "the absence of meningeal lymphatics has been proved"[3] has remained a basic tenet of neuroscience.

Two key studies published independently in the last month demonstrate that contrary to this long-held belief, the brain is indeed served by lymphatic vessels associated with the dural sinuses, large venous structures situated in the membrane between the brain surface and the skull.

Both the study by Louveau et al. published in *Nature*[5] and the study of Aspelund et al. published in the *Journal of Experimental Medicine*[6] reported the presence of small sinus-associated vascular structures that express markers of lymphatic endothelial cells, including LYVE1, PROX1 and VEGFR3, but are not labeled with iv tracers, demonstrating that these vessels are not elements of the blood vasculature. While both studies document the association between these putative lymphatic vessels and the superior sagittal and transverse sinuses, the more complete anatomical characterization carried out by Aspelund et al.[6]

demonstrates that these vessels are also associated with the dural middle meningeal arteries (MMAs), and exit the cranium both along veins and arteries associated with these dural vessels.

Functionally, Louveau et al.[5] reported that quantum dots were rapidly cleared from the CSF into these sinus-associated vessels, while Aspelund et al.[6] observed that fluorescently-labeled polyethylene glycol tracers were cleared rapidly from the brain interstitium to the same vessels. From here, both studies reported that these vessels transported the solutes to deep cervical lymph nodes, providing an anatomical link between the brain interstitial and CSF compartments and the periphery. Genetic ablation of the dural lymphatic vasculature by transgenic expression of a soluble VEGF-C/D trap protein slowed the clearance of tracers injected into the brain interstitium and prevented them from reaching the deep cervical lymph nodes, demonstrating that the clearance of solutes from the brain interstitium was dependent upon their movement along these sinus-associated lymphatic vessels[6]. One caveat shared by both studies was their implication that cervical nodes provide an intermediate way-station for fluid traveling from the brain interstitial space to the systemic circulation. While evidently the case for mice, this scheme is inconsistent with the lack of clinical association between meningeal and cerebral infections and cervical adenopathy in humans. As such, the specific anatomic pathways mediating meningeal lymphatic access to the systemic circulation have yet to be fully defined in humans, an avenue of likely fruitful research to come.

These findings both complement and enrich prior studies by our group that have described a brain-wide perivascular pathway that supports the exchange of CSF and ISF. We have found that subarachnoid CSF recirculates into and through the brain interstitium along perivascular spaces surrounding cerebral arteries, while interstitial solutes are cleared from the brain along perivascular channels surrounding large caliber draining veins[7,8]. Because perivascular CSF recirculation and interstitial solute clearance was mediated in part by astroglial intermediaries, and in particular required the astroglial water channel aquaporin-4 (AQP4), this pathway for interstitial solute clearance was designated the ‘glymphatic system’.

The present studies describing sinus-associated lymphatic vessels and our prior anatomical description of the glymphatic pathway may connect to comprise one anatomically-continuous and functionally-seamless system. In our studies, we repeatedly observed that tracers and proteins injected into the brain parenchyma are cleared along specific anatomical routes – following white matter tracts and large caliber draining veins that emptied into sinus-associated cisternal compartments[7,9,10]. For example, tracers injected into the cortex and striatum drained in part along the medial internal cerebral veins, and ultimately into the internal jugular circulation.

As such, it appears that the drainage of interstitial solutes along the peri-venous spaces defined as part of the glymphatic pathway provide these solutes access to the sinus-associated lymphatics, either directly as these large veins merge to form the dural sinuses, or indirectly via the cisternal CSF compartments associated with these structures. In this sense, it may be appropriate to regard these two components, perivascular pathways within the CNS parenchyma and the extra-axial meningeal lymphatic vessels as serial elements of a

wider functional system. One piece supports the clearance of solutes from the brain to the CSF, while the other propels that solute-laden CSF on to the systemic vascular system.

The interaction between the perivascular glymphatic pathway and the sinus-associated lymphatic vessels may also play a role in immune surveillance of the CNS by peripheral immune cells. Louveau et al.[5] reported that T-lymphocytes were strongly associated with sinus-associated lymphatic vessels. Ligation of the collecting vessels draining to the deep cervical lymph nodes resulted in the distension of the dural lymphatic vessels and the accumulation of T-lymphocytes, suggesting that these vessels provide a pathway for the movement of peripheral immune cells out of the cranium. The clearance of interstitial solutes, presumably along perivascular pathways, to peripheral lymph nodes outside the brain parenchyma suggests that this pathway may play a role in antigen presentation and immune surveillance of the CNS, even while maintaining the relative ‘immune privilege’ of the brain parenchyma itself.

Although these two studies describing dura-associated lymphatic vessels represent an important step towards understanding the basic biological function of the brain, important caveats remain. Functionally, it is not clear what the relative contributions of these lymphatic pathways are to the clearance of interstitial or CSF solutes, relative to classical efflux pathways including the dural arachnoid granulations and perineural sheathes. Do these different clearance pathways subserve functionally distinct roles, for instance solute clearance versus immune surveillance? How are these efflux pathways modulated by physiological variation? Even their diurnal variation seems significant: Glymphatic function, and thus interstitial protein waste clearance, is primarily active during sleep[10]. Does interstitial solute clearance and immune cell trafficking exhibit analogous diurnal variation?

The roles of these serially-active efflux pathways in both brain interstitial solute clearance and immune cell trafficking suggests their involvement in diseases as diverse as the proteinopathies and immune demyelination. Amyloid clearance in Alzheimer’s disease, extracellular synuclein clearance in Parkinson’s, Lewy body disease and multisystem atrophy, and indeed the progression of all prion-like proteinopathies, may be influenced by the functional competence of these pathways. Indeed, their functional compromise may suppress not only solute clearance, but also fluid efflux itself, as might be predicted in conditions as syndromically diverse as primary intracranial hypertension and glaucoma. In a similar vein, immune surveillance of agents leaving the brain may comprise a previously unrecognized strategy for mobilizing both immune cells against foreign antigens that invade brain before being effectively recognized. Such a mechanism might provoke rethinking of the mechanisms of both T cell activation and trafficking in multiple sclerosis, as well as of the strategies by which those processes may be therapeutically targeted. It is important, of course, to remember that functional studies of both the glymphatic and meningeal lymphatic systems have thus far been limited to animal models. Thus, while the report by Louveau et al.[5] provided evidence for sinus-associated lymphatic structures in human tissue, the specific anatomy and relative functional importance of these clearance systems in human disease have yet to be evaluated. Fair to say that these issues will provide a wealth of new insight in the coming years, as the causal relationships between the brain’s fluid dynamics and maladies become both better understood and therapeutically exploited.

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