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In Focus

New Brain Lymphatic Vessels Drain Old Concepts

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The brain has traditionally been considered an immune privileged organ, in part due to the lack of evidence for lymphatic vasculature (Galea et al., 2007). Two centuries ago, the existence of lymphatic vessels on the surface of the human brain was proposed but it has since been widely dismissed (Lukic et al., 2003). Therefore, while T cells leave all other organs via the lymphatic system to reach nearby lymph nodes, the prevailing view has been that infiltrated T cells exit the brain via venous blood circulation, circumventing the lymph nodes (Ransohoff and Engelhardt, 2012). The recent evidence for the existence of a lymphatic vascular system in the brain's meninges provided independently by two labs, first by the Kipnis lab (Louveau et al., 2015) and then by the Alitalo lab (Aspelund et al., 2015), challenges this view and raises the intriguing possibility of an alternative gateway for T cells to egress the brain.

The key for identifying these meningeal lymphatic vessels by both labs was the use of a whole-mount preparation where the meninges were fixed while still attached to the skull, thus enabling the preservation of fine meningeal vessels. Kipnis' lab identified a rather simple network of narrow vessels with a distinct lumen that ran along the blood vessels in the superior sagittal and transverse sinuses on the top of the brain and importantly, expressed multiple molecular hallmarks of lymphatic endothelial cells including lymphatic vessel endothelial hyaluronan receptor 1 (Lyve-1) and vascular endothelial growth factor receptor 3 (VEGFR3). Alitalo's lab additionally described that this lymphatic network becomes more extensive at the base of the brain, where it contains lymphatic valves, and exits the skull alongside the cranial nerves. The anatomical and cellular classification of these meningeal vessels was strengthened by complimentary experiments from both labs demonstrating functional implications of VEGFR3. Injections of a VEGFR3 ligand, VEGF-c, into the cerebrospinal fluid (CSF) resulted in an increased diameter of the meningeal lymphatic vessels. Conversely, mice with impaired VEGFR3-VEGF-c signaling showed a complete absence of meningeal lymphatic vessels indicating that these vessels, like peripheral lymphatic vessels, are functionally regulated by VEGFR3.

An essential functional characteristic of lymphatic vasculature is permissiveness to fluid and drainage to lymph nodes. In line with this, dyes injected into the CSF were detected both in the lumen of Lyve-1-expressing vessels and in the deep cervical lymph nodes (dcLN), whereas dyes administered in the blood were not. Ligation of the lymphatic

vessels above the dcLN completely abolished drainage of dye to these lymph nodes and additionally, increased the diameter of the meningeal lymphatic vessels, indicating that these vessels drain to the dcLN. The Alitalo lab further demonstrated that dye injected into the brain parenchyma was absorbed by lymphatic vessels and preferentially drained into the ipsilateral dcLN from the base of the brain. Surprisingly, Kipnis' lab excluded the best-known route of drainage into the dcLN, via the nasal cavity's cribriform plate and the nasal mucosa (Kida et al., 1995), as they failed to see dcLN drainage of dye injected into the nasal mucosa. In contrast, the Alitalo lab's elaborate imaging studies indicated that lymphatic vessels indeed pass through the skull in the meningeal lining of the cribriform plate and into the nasal mucosa, corroborating the nasal drainage route as one of many possible passages to the dcLN. Hence, future research will be required to map out the extent of this newly discovered lymphatic system and its relationship to the rest of the lymphatic circuitry in the body. This may be particularly important given that disrupting drainage of excess fluids in the peripheral lymphatic system leads to peripheral lymphedema which affects millions of people worldwide. Interestingly, blocking drainage to the dcLN has been shown to exacerbate ischemic-evoked edema in rats (Si et al., 2006), raising the question whether the meningeal lymphatic system plays a critical role in reducing brain edema. This possibility should be considered in future scientific discussions both in basic and clinical sciences.

One intriguing question that remains is whether the lymphatic vessels act as a cellular afferent route out of the brain, allowing T cells to circulate through the meninges and to the dcLN. In their study, Kipnis' lab observed a ten-fold higher density of T cells that aligned within the lumen of Lyve-1-expressing vessels compared to that of blood vessels. Importantly, removal of the dcLN increased the density of T cells in the meninges, indicating that drainage to the dcLN might be crucial for T cells to egress. While these data suggest that meningeal lymphatic vessels contain T cells, more experiments are necessary to demonstrate whether T cells indeed are circulating via these vessels. Interestingly, metastasis from primary brain tumors (e.g., gliomas) has been found in the dcLN (Mondin et al., 2010), supporting the notion of a direct cellular route. It would be very interesting to investigate the flow of T cells in meningeal lymphatic vessels during infection and inflammation and to determine fluctuation in number of T cells drawn to (and out of) the brain parenchyma which potentially would change our understanding of T cell circulation in the brain.

The functional implications of this newly discovered lymphatic network in both health and disease are broad and raise many exciting questions. Of particular interest would be how this newly discovered meningeal lymphatic system relates to the recently described glymphatic

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system, which potentiates waste clearance from the brain by facilitating the flux of CSF across the brain parenchyma (Iliff et al., 2012). It will be interesting to investigate if and how pathological agents, such as amyloid β are transported between the glymphatic and lymphatic systems. For example, would removal of amyloid β and other misfolded proteins from the brain parenchyma be reduced in mice with an impaired meningeal lymphatic system (e.g., following removal of the dLN or in VEGFR3-deficient mice)? Do meningeal lymphatic defects correlate with or cause enhanced risk of patients developing neurodegenerative diseases such as Alzheimer's and Parkinson's disease?

Arguably the most appealing aspect of the existence of meningeal lymphatic vessels is the insight it provides about the circulation of T cells in the brain. Immune cells and immune-related molecules are increasingly recognized to play crucial roles in brain wiring in both development and disease. Interestingly, Kipnis' lab has previously shown that removal of dLN can induce learning and memory impairments in mice (Radjavi et al., 2014), suggesting that disrupting T cell circulation has gross behavioral consequences. Mapping the cellular lymphatic route and understanding its potential role in behavioral paradigms such as learning and memory, sleep, and stress will advance our understanding of communication between the central nervous system and the immune system. Furthermore, elucidating a potential path for T cell circulation could potentially unveil new therapeutic targets for interventions in neurodegenerative diseases and neurological disorders, including those considered to be triggered by inflammatory responses, such as multiple sclerosis.

These exciting new findings have reopened the discussion about to what degree the brain is immune privileged by revealing a potential lymphatic gateway for T cells to egress the brain.

Conflicts of Interest

None.

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