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INSIGHTS

Charting the meningeal lymphatic network

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A genuine network of lymphatic vessels can be found in the dural layer of the meninges that ensheathe the brain and spinal cord of mammalians. In this issue, Jacob et al. (2022. *J. Exp. Med.* https://doi.org/10.1084/jem.20220035) employ light sheet fluorescence imaging of intact mouse heads to provide a more comprehensive chart of the meningeal lymphatic vasculature and draw a parallel between lymphatic drainage of cerebrospinal fluid in mice and humans.

The mechanisms governing fluid and molecular waste purging from the central nervous system (CNS) continue to be a hot topic of research. Not long ago, a series of pioneering studies showed that lymphatic vessels, specialized vascular structures that drain the interstitium of most tissues, do in fact extend into the meningeal tissue that wraps the entire CNS of mice, rats, primates, and humans (Aspelund et al., 2015; Louveau et al., 2015; Absinta et al., 2017; Jung et al., 2017). Lymphatic capillaries spread along the vicinity of the major arteries and veins of the meninges during postnatal stages and are thought to remain confined to the meningeal outmost dural layer under healthy conditions (Aspelund et al., 2015; Louveau et al., 2015; Antila et al., 2017). Meningeal lymphatic capillaries are composed by endothelial cells expressing characteristic lymphatic lineage markers, including cluster of differentiation 31, lymphatic vessel endothelial hyaluronan receptor 1 (LYVE1), prospero homeobox protein 1, podoplanin, and chemokine (C-C motif) ligand 21, and bound mostly by discontinuous button-like junctions. Capillary lymphatics converge into pre-collecting vessels, which contain specialized valves to prevent lymph backflow but are not enclosed by smooth muscle cells. Meningeal pre-collecting lymphatics then exit the skull through the foramina and fissures, alongside major cranial blood vessels and nerves, and convert into true collecting lymphatics that are wrapped by smooth muscle cells (Antila et al., 2017; Ahn et al., 2019; Jacob et al., 2022). After numerous independent studies, it is now widely accepted that the meningeal lymphatic vessels drain brain cerebrospinal fluid (CSF). Sophisticated experiments have shown that CSF molecular content can easily access the dural stroma, where it can either be internalized by resident phagocytes, follow dural skull channels to reach the bone marrow, or enter the lymphatic vessels to be subsequently drained through extracranial collecting lymphatics into the cervical LNs (cLNs; Louveau et al., 2018; Rustenhoven et al., 2021; Mazzitelli et al., 2022). An unexpected functional crosstalk between meningeal lymphatic outflow and brain glymphatic fluid circulation has also been reported, despite the clear anatomical segregation between meningeal lymphatic vessels and the brain parenchymal perivascular spaces (Da Mesquita et al., 2018). These initial groundbreaking studies have turned a bright spotlight onto the involvement of the meningeal lymphatic system in brain fluid homeostasis and led to numerous new captivating questions about the modulation of neurophysiology and neuropathology by lymphatic drainage.

In this issue of *JEM*, Jacob et al. (2022) start by filling in some important knowledge gaps regarding meningeal lymphatic anatomy by providing an in-depth chart of CSF access to intracranial meningeal lymphatic vasculature, and subsequent drainage into extracranial neck lymphatics, using intact and cleared mouse head preparations. By light sheet fluorescence microscopy (LSFM) imaging of the intact heads at different time-points upon the spinal or the subarachnoid injection of small fluorescent tracers, the authors elegantly show that CSF reaches the brain perivascular spaces, the meningeal dura, and is drained by the meningeal lymphatics into the deep cLNs in a matter of minutes. These observations corroborate previous data in which meningeal dura and deep cLNs were collected and analyzed separately (Da Mesquita et al., 2018; Louveau et al., 2018; Rustenhoven et al., 2021). The researchers take a step further and show that drained CSF content is taken

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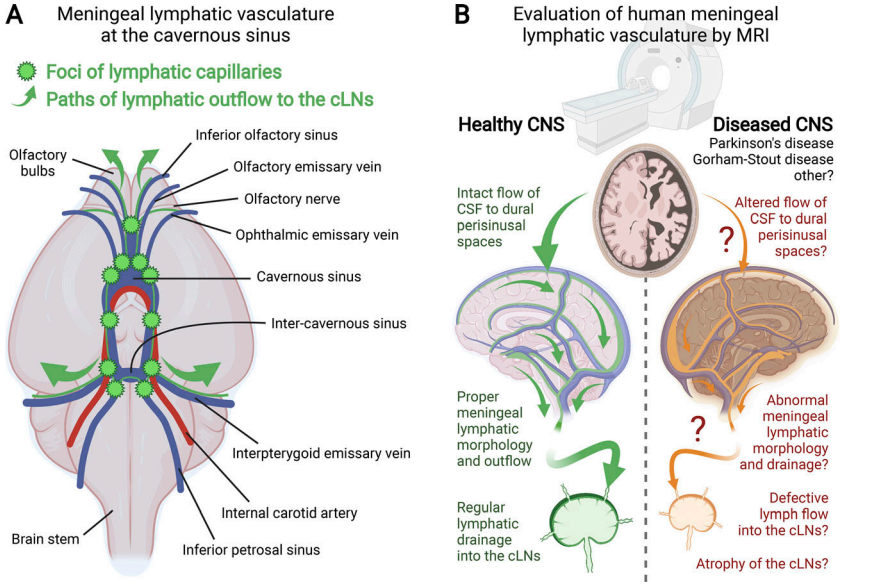

(A) Scheme depicting the different foci of lymphatic capillaries identified along the cavernous sinus and adjoining blood vessels in the murine meninges. (B) Jacob et al. (2022) perfected an MRI technique that combines intravenous injection of gadobutrol, followed by elliptic venography, T1 SPACE, and DANTE sequences, and permits a reliable 3D anatomical reconstruction of the meningeal lymphatic network in human subjects. Future clinical studies should use identical imaging approaches to evaluate meningeal lymphatic function and CSF outflow into the cLNs in diseases other than Parkinson's and Gorham-Stout (Ding et al., 2021; Jacob et al., 2022). Created with BioRender.com.

up by myeloid cells in the deep, mandibular, and accessory mandibular cLNs, but not in the parotid cLNs, indicating that the latter do not drain the CNS. While essential, it will be challenging to perform similar postmortem assessments of CSF outflow into cLNs in humans, mostly due to the increased number of deep and superficial cLNs and their sometimes-remote anatomical localization. These challenges might be bypassed by the implementation of non-invasive magnetic resonance imaging (MRI) techniques, such as the ones used in this issue, that facilitate the longitudinal measurements of lymphatic outflow into the cLNs in humans. Such techniques permit an accurate quantification of contrast agents (e.g., gadobutrol) that end up in the cLNs after being administered directly into CSF and drained by the perisinusal lymphatics (Eide et al., 2018; Eide and Ringstad, 2021).

Combining the iDISCO and LSFM techniques, Jacob et al. (2022) unveil a previously overlooked network of LYVE1$^+$ lymphatic capillaries in the murine mid-anterior skull base along the dural cavernous sinus. Three consecutive and interconnected foci of lymphatics are distinguished along each side of the murine caudal cavernous sinus: at the inferior petrosal sinus, foramen of the interpterygoid emissary veins, and intersection between the cavernous sinus and internal carotid arteries. In the rostral cavernous sinus, there are three additional lymphatic hubs: two at the confluences with the ophthalmic and olfactory emissary veins (in each side of the cavernous sinus), and another at the inferior olfactory sinuses (see panel A of figure). Together with the rest of the lymphatics juxtaposed to the transverse, sigmoid, and petrosquamous sinuses, the cavernous sinus lymphatics form a network of interconnected lymphatics that sample substantial amounts of tracer molecules injected into the CSF. It will be interesting to examine whether these additional lymphatic foci at the dural cavernous sinus share the features of its neighboring dorsolateral meningeal lymphatics, especially in terms of their postnatal development, dependence on vascular endothelial growth factor C signaling, and functional decay with aging (Antila et al., 2017; Da Mesquita et al., 2018, 2021; Ahn et al., 2019).

The authors also explore other known alternative pathways of CSF lymphatic outflow in mice. They detected CSF-derived tracers in lymphatic capillaries of the nasal mucosa but were unable to find a direct anatomical connection between the extracranial nasal and the intracranial meningeal lymphatic systems, suggesting that these represent two distinct pathways of lymphatic drainage into the cLNs. More work is needed to fully understand how CSF leaks into the nasal tissue and gains access to the extracranial nasal lymphatic vessels in mice. It will also be necessary to elucidate whether the phenomenon of CSF leakage into the nasal mucosa observed in mice is indeed negligible in humans, as recently proposed (Eide and Ringstad, 2021).

Besides providing an exhaustive 3D characterization of the murine meningeal lymphatics, this issue also focuses on charting meningeal lymphatic drainage in a total of 11 human patients using MRI that combined elliptic venography, T1 SPACE (variable flip angle turbo spin echo), and DANTE (delay alternating with nutation for tailored excitation) sequences. Interestingly, Jacob et al. (2022) show that mice and humans present a considerable overlap in terms of meningeal lymphatic anatomy. Pathways of perisinusal lymphatic drainage were also detected in the dorsal and basal regions of the human dura, including at the cavernous sinus, where the entire meningeal lymphatic network seems to converge to promote the bulk of CSF outflow. Analysis of the different disease cases revealed a severe perturbation of meningeal lymphatics in a patient with Gorham-Stout disease, where the skull osseous matrix was invaded by abnormally expanded dural lymphatic vasculature. The authors also speculate about the potential implications of a decreased meningeal lymphatic volume in female patients, especially taking into consideration the increased prevalence of idiopathic intracranial hypertension and multiple sclerosis in women. However, the small sample size, and lack of sex- and age-matched "control" patients (e.g., devoid of CNS pathologies), undermines any type of interpretation regarding a potential sex-dependent effect on meningeal lymphatics in humans. Future clinical studies with increased sample sizes and appropriate controls should make use of the MRI technique developed by Jacob et al. (2022) to reliably discriminate between the effects of sex, age, and disease on meningeal lymphatic architecture and drainage.

By diving deeper into the anatomical complexity of the mammalian meningeal

lymphatic vasculature, and its connections with both the brain glymphatic and the peripheral lymphatic systems, this issue is a good reminder of how little we still know about CNS lymphatic drainage. A growing body of evidence is pointing toward meningeal lymphatic dysfunction as an important pathophysiological element in different models of neurological diseases, including Alzheimer's, Parkinson's, and multiple sclerosis (Da Mesquita et al., 2018, 2021; Louveau et al., 2018; Ding et al., 2021). Yet, we have very limited knowledge about the factors modulating meningeal lymphatic drainage and whether these are disease-specific in nature. We must build upon the non-invasive exquisite MRI techniques refined by Jacob et al. (2022) and other groups to systematically inquire about the status of meningeal lymphatic function in human patients (Absinta et al., 2017; Ding et al., 2021). Longitudinal measurements of meningeal lymphatic drainage by MRI might serve as reliable diagnostic and/or prognostic tools in distinctive diseases that affect the CNS, even beyond idiopathic Parkinson's and Gorham-Stout (see panel B of figure). We are no longer navigating uncharted waters when it comes to meningeal lymphatic anatomy and function. With more investment in translational studies, we will become closer to having a more comprehensive map of human CNS lymphatic drainage in health and disease.

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