

The amazing brain drain

In this issue of JEM, Antila et al. (<https://doi.org/10.1084/jem.20170391>) demonstrate that central nervous system lymphatics develop in the mouse meninges during early postnatal periods and display remarkable plasticity in adult periods through manipulation of VEGF-C–VEGFR3 signaling.

The CNS has long been considered an immune-privileged site as tissues grafted into the CNS are not rejected as they are in other tissues (Medawar, 1948; Billingham and Boswell, 1953; Barker and Billingham, 1977). An avalanche of evidence now suggests that this privilege is not absolute, as robust immune responses can occur in the CNS during infections and autoimmune diseases such as multiple sclerosis (MS), as well as other neuroinflammatory diseases. One of the major thoughts as to why there is immune privilege is the lack of CNS parenchymal lymphatics, necessary to drain antigens into the lymph nodes.

Lymphatic vessels (LVs) are critical for maintaining tissue homeostasis by recycling interstitial fluid (ISF), as well as participating in tissue immune responses by draining antigens into lymph nodes and regulating immune cell flux into and out of the tissues (Liao and Padera, 2013). Although there are no lymphatics within the brain parenchyma, there is a complex lymphatic system within the meninges that drains cerebrospinal fluid (CSF) from the meningeal coverings of the brain into the deep cervical lymph nodes (dcLNs). This meningeal lymphatic system was first described two centuries ago by Paolo Mascagni; however, it was largely ignored until the recent characterization of meningeal LVs (mLVs) in the rodent, primate, and human dura (Aspelund et al., 2015; Bucchieri et al., 2015; Louveau et al., 2015; Absinta et al., 2017). These studies, combined with the identification of the glymphatic system whereby the neural ISF is drained into the CSF along the veins, have allowed a deeper understanding of how ISF recycling is regulated in the CNS (Iliff et al., 2012; Asgari et al., 2016).

The characterization of the meningeal lymphatics has thus enabled a

reexamination of how immunity is regulated within the CNS and how this may be important for CNS infections, MS, and other neuroinflammatory diseases. Furthermore, the drainage of ISF molecules has become increasingly of interest especially in the context of Alzheimer's disease (AD) where toxic buildup of amyloid β has been suggested to result from insufficient drainage.

In this issue, Antila et al. first analyze the timing of mLV formation during mouse development and demonstrate that this occurs postnatally, in a spatially coordinated program. The very first mLVs are observed just before birth at the foramen magnum (FM), and starting from postnatal day (P) 0, they extend, following major arteries and venous sinuses until the complete formation of the mLV network at P24. The authors also describe the development of the meningeal lymphatic vascular network in the spinal cord, where it is initiated at P4 from the FM and is completed before P36. mLVs grow on both the dorsal and ventral sides of the spinal cord to develop a complex mLV network. The mLVs develop concomitantly at the cervical and lumbar levels of the spinal cord.

This time course of mouse mLV development provides important physiological information for our understanding of how CNS immunity may be regulated differently during development and adulthood. It is of great interest to determine how the development in mouse correlates to human mLV development. One option is that the development in humans correlates with the same developmental stage as in mouse, and thus the human mLV initiation may occur at the end of the third trimester. Alternatively, mLV development may not be triggered by de-



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velopmental stage but by environmental conditions at birth, such as oxygen content, so it is possible that human mLV development also initiates postnatally. It will also be interesting to determine how the development of the glymphatic and lymphatic systems are coordinated as these two systems appear to cooperate to drain neural ISF, first to the CSF and then to the dcLNs.

The absence of an extended mLV network before birth brings up interesting questions regarding how brain homeostasis is regulated in embryonic periods. First, how is ISF recycled and debris cleared from the CNS before the establishment of the mLV network? There could be either an absence of clearance function or perhaps other mechanisms are in place before the development of the mLVs. Several other mechanisms of CSF drainage have been identified, including via arachnoid granulations and along the olfactory bulb to be drained by the nasal mucosal LVs. Understanding how these different drainage systems are coordinated is critical for understanding how CNS fluid balance is maintained during development and how this can be lost in children with hydrocephalus. Second, is CNS immunity regulated differently before and after the establishment of the mLV network? The lack of mLVs in em-

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byronic development suggests that the brain may be more susceptible to infections and less susceptible to autoimmune disease at these early time points.

Antila et al. (2017) further identify the molecular signaling pathway that drives mLV development. They demonstrate that mLVs are lost in *Vegfc* or *Vegfr3* conditional knockout mice, or by using a VEGF-C/D trap. Additionally, there was no reduction of mLVs in *Vegfd* knockout mice, validating that the mLVs are regulated through VEGF-C–VEGFR3 signaling. Furthermore, using the *Vegfc*^{LacZ/+} mice and β -gal staining, the authors were able to localize VEGF-C expression to cells covering the large blood vessels, suggesting that vascular smooth muscle cells (vSMCs) drive the formation of the mLV network. Therefore, a critical question is how VEGF-C expression is regulated within the vSMCs to determine the timing and location of mLV formation. Interestingly, there are vSMCs covering large vessels throughout the meninges and within the brain parenchyma, yet the meningeal lymphatic network is limited to specific stereotyped locations. Is there something unique about specific meningeal vSMCs that drives the highly localized formation of mLVs? Or is VEGF-C expressed by all vSMCs, and opposing signals that limit mLV formation are expressed in the CNS parenchyma and other regions?

The most surprising finding in this study was that the mLVs display remarkable plasticity throughout life. Antila et al. (2017) show that inhibition of VEGF-C–VEGFR3 signaling in adults through deletion of *Vegfr3* or administration of a VEGF-C/D trap led to an astonishing regression of the mLVs, whereas viral delivery of VEGF-C led to a significant increase in mLVs, together demonstrating that ongoing VEGF-C–VEGFR3 signaling is required for maintenance of the mLV network. The critical question is whether these alterations are specific to genetic manipulations or whether plasticity occurs in humans because of local signals that modulate the mLV network in response to fluctuations in fluid homeostasis, toxic protein buildup, infection, or inflammation.

Interestingly, Antila et al. (2017) observe that 2-yr-old mice display enlarged mLVs and sparse lymphatic structures with fewer valves, suggesting that mLV alterations can occur throughout life. It would be very interesting to determine whether human mLVs regress as observed in aging mice and whether this might be associated with ventricle enlargement in the elderly caused by buildup of CSF (LeMay, 1984). Future work identifying how the VEGF-C–VEGFR3 pathway is regulated in the meninges will allow us to understand how the mLV network is dynamically regulated throughout life.

Antila et al. (2017) also demonstrate that Sunitinib, a multi-target receptor tyrosine kinase inhibitor developed to inhibit tumor angiogenesis, induces regression of mLVs that is reversible after the end of the treatment. The same treatment protocol did not lead to observable changes in intestinal LVs, suggesting that mLVs are more sensitive to VEGF-C–VEGFR3 inhibition (Nurmi et al., 2015). It is intriguing that mLVs are more plastic than peripheral LVs, and it is of extraordinary interest whether this plasticity is important for the function of the neural tissue. Sunitinib is an FDA-approved drug that has been used in humans, raising the question of whether this therapeutic leads to the regression of mLVs in these patients and how this affects their neural function and immunity (Lee et al., 2015). It is quite possible that this therapy could alter CNS fluid homeostasis, elimination of molecules such as amyloid β , and susceptibility to CNS infections and autoimmunity.

The identification of the plasticity of mLVs has significant clinical applications, as modulation of this network could influence the course and treatment of many different diseases. For instance, a build-up of amyloid β is a critical component of AD, and thus increasing the mLV network may increase drainage of this toxic protein and thus limit the progression of the disease. Increasing mLVs may also lead to increased CSF drainage to ease the intracranial pressure buildup in patients after stroke,

brain traumas, and hydrocephalus. Furthermore, a larger mLV network may increase the immune surveillance of the CNS and thus aid in the defense against CNS infections. Conversely, targeted regression of mLVs in patients with CNS autoimmune disorders, such as MS and neuromyelitis optica, may limit antigen drainage into the dLNs, thus limiting the neuroinflammatory response.

The identification of the plasticity of the mLV network underlines the importance of understanding how mLVs regulate CNS fluid homeostasis and immunity, how this regulation is important for brain function, and the role that the mLV network plays in regulating the onset and progression of different diseases. This knowledge will be critical to understand how mLV plasticity can be manipulated to treat a wide array of neurological diseases.

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