NEUROBIOLOGY

A drain at the base of the brain

A set of lymphatic vessels that wrap around the base of the mouse brain have been shown to drain fluid from the brain into the peripheral lymphatic system, and to exhibit a decline in function with ageing. SEE ARTICLE P.62

TAIJA MÄKINEN

Lymphatic vessels around the body drain excess fluid and protein from tissues and serve as a conduit for trafficking immune cells. The central nervous system (CNS) was long thought to lack lymphatic drainage; fluid and macromolecules were instead thought to be cleared from the CNS by other routes, such as through absorption into the bloodstream, or through channels running along the outside of blood vessels or nerves to reach the lymphatic system outside the CNS[1,2]. A few years ago, lymphatic vessels were discovered in the dura mater[3,4], the outermost of the three meningeal membranes that envelop the CNS. They were reported to provide a route for immune cells trafficking from the CNS and for clearing CNS waste[3,4], and to thus represent potential therapeutic targets for neurological diseases[5,6]. However, exactly how they might enable drainage from the brain has remained unclear, and whether they even have a role in drainage has been questioned[1,2,7].

Ahn *et al.*[8] now demonstrate on page 62 that meningeal lymphatic vessels at the base of the rodent skull (basal mLVs) provide a direct route for the clearance of proteins and other large molecules from the CNS to the peripheral lymphatic system. They further characterize age-related changes in these vessels that impair their drainage function and that might contribute to ageing-associated neurological diseases.

Previous studies have shown that, in mice, mLVs grow in the first month after birth from the base of the skull along blood vessels and nerves[9] into an intricate network that extends to the upper (dorsal) part of the skull[3,4,9]. Ahn *et al.* used fluorescence microscopy to characterize the morphology of the mLVs in the dorsal and basal skull[8], and thus extend previous studies[3,4,9]. They showed that, unlike dorsal mLVs, basal mLVs possess specialized features associated with both uptake and drainage of fluid (Fig. 1). For example, the authors observed that the basal mLVs have tiny, blunt-ended capillary branches where the endothelial cells that make up the vessel walls are joined together loosely by intermittent, 'button-like' junctions, enabling fluid uptake. Basal mLVs, but not dorsal mLVs, also have 'pre-collecting' vessels that drain the capillaries and have valves inside them that enable flow in one direction[3,4,8,9]. The endothelial cells in the pre-collecting vessels are joined by both button-like junctions and continuous 'zipper-like' junctions, suggesting that they have a dual function of taking up and transporting fluid.

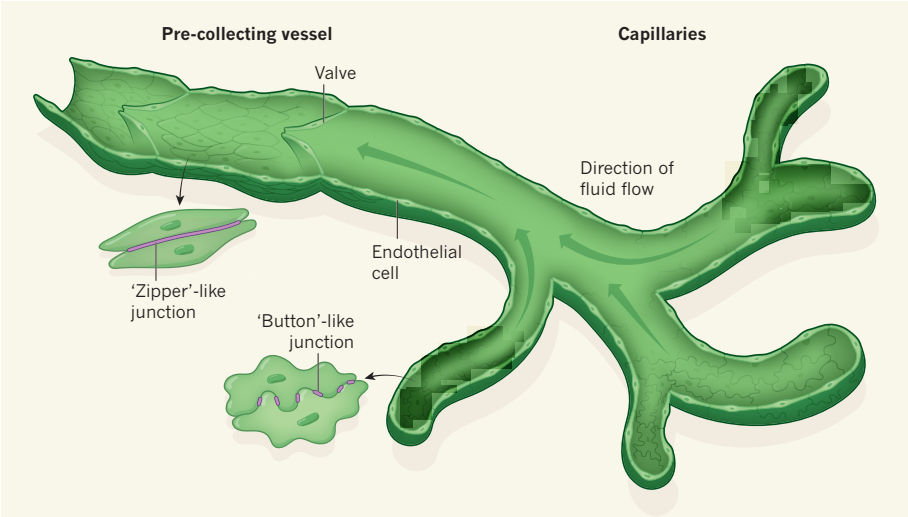

Figure 1 | Structural features of meningeal lymphatic vessels at the base of the skull. Ahn *et al.*[8] showed that lymphatic vessels in the dura mater (the outermost of the three meningeal layers that envelop the brain and spinal cord) at the base of the skull in mice show structural features that are the same as those seen in lymphatic vessels in the periphery[11] that enable them to take up and drain fluid from the central nervous system. Blunt-ended capillary vessels are formed of endothelial cells that are joined together loosely by 'button'-like junctions. By contrast, the endothelial cells that make up the pre-collecting vessels are joined by both button-like junctions and continuous 'zipper'-like junctions, and these parts of the vessels contain valves that allow the flow of fluid in one direction.

It is unclear whether and how cerebrospinal fluid (CSF) — which surrounds the brain and spinal cord and fills cavities in the brain known as ventricles — can cross the arachnoid layer (the middle meningeal membrane) to access the mLVs in the dura mater. Ahn *et al.* found that basal, but not dorsal, mLVs were close enough to the CSF-filled space between the innermost layer of the meninges and the arachnoid layer (known as the subarachnoid space) to be accessible for fluid entry. Through technically challenging analysis of the skull base around the openings where nerves and vessels exit the cranial cavity, the authors showed that the basal mLVs are distinct from the nerves along which CSF drainage was thought to occur[7]. Instead, the basal mLVs drain directly into collecting lymphatic vessels outside the CNS, which have previously been shown to transport molecules from the CSF to lymph nodes in the neck called deep cervical lymph nodes[3–8].

Next, the authors assessed the drainage function of mLVs. They infused contrast agents into the CSF-filled subarachnoid space in rats and tracked these using magnetic resonance imaging as they moved along the lymphatic vessels exiting the base of the skull and into the deep cervical lymph nodes. Similarly, the researchers used microscopy analyses to track a fluorescently labelled tracer that had been infused into the CSF or into the interstitial fluid of the brain tissue in mice. In both cases, they detected the fluorescent tracer inside basal mLVs (both in capillaries and in pre-collecting vessels) and in deep cervical lymph nodes in the neck.

A study last year reported that dorsal mLVs in a few specific areas can take up CSF[6]. However, consistent with another previous study[7], Ahn *et al.* were unable to detect evidence of uptake of the tracer from the CSF to dorsal mLVs. Overall, on the basis of the functional experiments, combined with the anatomical and morphological observations, the authors conclude

that basal mLVs provide the main route for macromolecule uptake and for drainage of CSF and interstitial fluid from the brain directly into the peripheral lymphatic system.

The flow in lymphatic vessels that carry fluid drained from the CSF to lymph nodes in the periphery was previously reported to be slower in older compared with younger mice[7], and such a decline in drainage function might have implications for age-related neurological diseases[1,2,5]. The authors therefore compared basal and dorsal mLVs in young mice (3 months old) with those in aged mice (24–27 months old). Whereas dorsal mLVs in aged mice showed deterioration, basal mLVs in aged mice were enlarged and more numerous. The basal mLVs in older mice also had fewer luminal valves compared with younger mice, and the junctions between the endothelial cells that form the vessel walls in older mice showed signs of disintegration. The authors confirmed that these age-related changes in basal-mLV morphology correlated with reductions in the drainage of macromolecules from the CSF in the aged mice.

A decline in mLV function has been suggested to lead to a build-up of proteins in the brain and to contribute to cognitive deficits and brain pathology in Alzheimer's disease[5]. One way of counteracting age-dependent reductions in drainage function might be to stimulate mLV growth and to increase the diameter of mLVs. The endothelial cells that compose the mLVs in adult mice express the receptor VEGFR3, which is activated by the growth factor VEGF-C, and treating adult mice with VEGF-C induces growth and widening of mLVs[4,9]. The authors used a genetic manipulation to remove VEGFR3 from all lymphatic vessels, including mLVs, in adult mice. This approach revealed that, consistent with previous findings[9], when VEGF-C signalling is lost, dorsal mLVs deteriorate more rapidly than do basal mLVs. However, it remains unclear whether VEGF-C–VEGFR3 signalling is affected in ageing and whether it could be targeted to counteract the observed ageing-associated changes in mLV function.

Besides clearing CNS macromolecules, mLVs also drain immune cells to lymph nodes[4,6], where immune responses are initiated. Indeed, dorsal mLVs were previously identified on the basis that they contained immune cells[4], and Ahn *et al.* also observed such cells in the basal mLVs. A previous study[6] showed that disrupting dorsal mLVs attenuated inflammatory responses in a mouse model of the neurological disorder multiple sclerosis, indicating that mLVs might have a role in neuroinflammatory diseases. Future experiments should investigate whether, independently of their drainage function, mLVs might also promote immune tolerance (that is, a dampening of immune responses to recognized substances), as do the lymphatic vessels in lymph nodes[10].

We still need a better understanding of the mechanisms that enable entry of fluid to the basal mLVs and of how mLVs cooperate with the other systems that clear waste from the CNS. Nevertheless, the identification of the precise exit routes for fluids leaving the brain is a crucial step towards understanding how waste is cleared from the CNS. This finding might eventually enable the development of therapies that promote CNS drainage to combat pathological processes in neurological diseases. ■

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CLIMATE SCIENCE

Weak sensitivity of cloud water to aerosols

Human activities produce tiny airborne particles called aerosols. The discovery that the average impact of these aerosols on the water content of low-level clouds is minimal will lead to more-reliable models of future climate. SEE ARTICLE P.51

ANNA POSSNER

Ever since humans first harnessed fire, we have emitted microscopic particles called aerosols into the atmosphere. These particles remain suspended in the air and alter the amount of sunlight that reaches Earth's surface. Low-level clouds efficiently cool the planet by reflecting sunlight back into space and are readily exposed to human-made aerosols. Such particles can change the reflectance of clouds, and the associated cooling, by modifying the size of droplets[1] or the amount of water[2] in the clouds. On page 51, Toll *et al.*[3] provide compelling evidence that human-made aerosols cause a weak average decrease in cloud water content compared with unpolluted clouds. This result provides an important constraint on the overall cooling effect of aerosol emissions and reduces one of the key uncertainties in climate science.

Earth's global mean temperature is governed

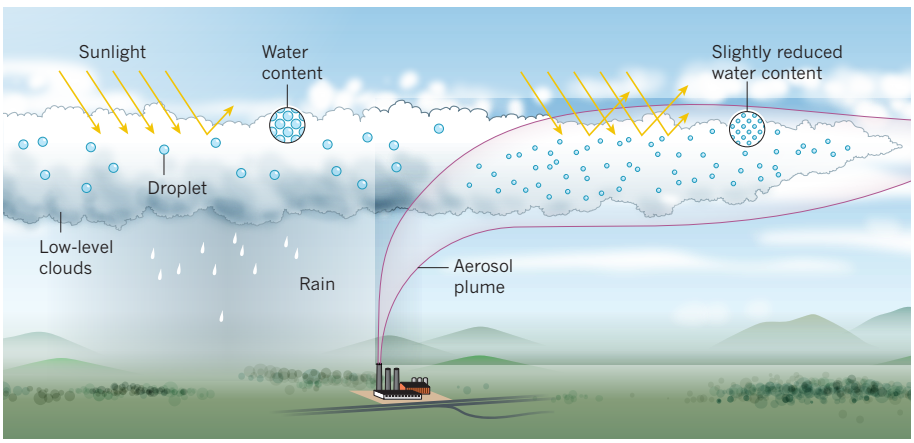

Figure 1 | Impact of human-made aerosols on low-level clouds. Microscopic particles called aerosols are released into the atmosphere by human activity; an aerosol plume from a factory is shown here. Low-level clouds that form in the presence of these particles contain droplets that are smaller and more numerous than usual. As a result, these clouds reflect more sunlight, and have a greater cooling effect on Earth, than do unpolluted clouds. Toll *et al.*[3] show that human-made aerosols also lead to a weak average reduction in cloud water content (and therefore in the frequency of rain) compared with unpolluted clouds. This effect slightly reduces the overall aerosol-induced increase in cloud reflectance.