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REVIEW

Current concepts on communication between the central nervous system and peripheral immunity via lymphatics: what roles do lymphatics play in brain and spinal cord disease pathogenesis?

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Abstract
The central nervous system (CNS) lacks conventional lymphatics within the CNS parenchyma, yet still maintains fluid homeostasis and immunosurveillance. How the CNS communicates with systemic immunity has thus been a topic of interest for scientists in the past century, which has led to several theories of CNS drainage routes. In addition to perineural routes, rediscoveries of lymphatics surrounding the CNS in the meninges revealed an extensive network of lymphatics, which we now know play a significant role in fluid homeostasis and immunosurveillance. These meningeal lymphatic networks exist along the superior sagittal sinus and transverse sinus dorsal to the brain, near the cribriform plate below the olfactory bulbs, at the base of the brain, and surrounding the spinal cord. Inhibition of one or all of these lymphatic networks can reduce CNS autoimmunity in a mouse model of multiple sclerosis (MS), while augmenting these lymphatic networks can improve immunosurveillance, immunotherapy, and clearance in glioblastoma, Alzheimer's disease, traumatic brain injury, and cerebrovascular injury. In this review, we will provide historical context of how CNS drainage contributes to immune surveillance, how more recently published studies fit meningeal lymphatics into the context of CNS homeostasis and neuroinflammation, identify the complex dualities of lymphatic function during neuroinflammation and how therapeutics targeting lymphatic function may be more complicated than currently appreciated, and conclude by identifying some unresolved questions and controversies that may guide future research.

Keywords Meningeal lymphatics · Cribriform plate · Cerebrospinal fluid · Neuroinflammation · Autoimmunity · Efflux · Neuroimmunology

Introduction

The vascular system is comprised of blood vasculature and lymphatic vasculature. Many critical functions are attributed to the lymphatic system, which include maintaining fluid homeostasis and pressure, absorption of fats, waste clearance, and immunosurveillance through immune cell trafficking and antigen drainage (Oliver et al. 2020; Petrova et al. 2020; Cueni et al. 2008). Lymphatic dysfunction causes the swelling of fluid in affected tissues, also known as lymphedema, which in limbs can cause aching, discomfort, restricted range of motion, and recurring infections (Azhar et al. 2020). Unlike limbs, the CNS is encased by a rigid skull, in which case cerebral edema can cause a wide range of neurological symptoms from headaches and nausea to seizures, coma, and death (Stokum et al. 2016; Nehring et al. 2020). In fact, cerebral edema is often the most likely cause of death in ischemic strokes and traumatic brain injury (Stokum et al. 2016). In addition to the lymphatic system being critical for fluid homeostasis, it also plays a role in immunosurveillance through the trafficking of antigens and leukocytes including dendritic cells (Schwager et al. 2019; Hampton and Chtanova 2019; Alitalo 2011).

Recent evidence also suggests lymphatic endothelial cells play a much more significant role in regulating immunity than only through the drainage of antigens and leukocytes. Firstly, lymphatic vessels are able to dynamically respond

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to inflammation by undergoing lymphangiogenesis, and modulation of lymphatic function has thus gained therapeutic interest (Alitalo 2011; Alitalo et al. 2005; Tammela and Alitalo 2010). Additionally, lymphatic endothelial cells, particularly in the lymph nodes, have been shown to directly modulate immunity through antigen processing and presentation, antigen archival, and the expression of tolerogenic ligands that can balance immunosurveillance with tolerance (Santambrogio et al. 2019; Rouhani et al. 2015; Lucas and Tamburini 2019; Humbert et al. 2017; Yeo and Angeli 2017). Single-cell RNA sequencing of lymphatic endothelial cells in both mouse and human lymph nodes reveals heterogenous subpopulations of lymphatic endothelial cells, with each subpopulation specializing in distinct functions such as leukocyte migration, antigen presentation, pathogen interactions, cell–cell interactions, and tolerance including the expression of CD274 (Takeda et al. 2019; Xiang et al. 2020). Interestingly, these large-scale sequencing studies identified these immune-regulating lymphatic endothelial cells after cellular and molecular experiments functionally demonstrated the capability and ability of lymphatic endothelial cells to engage in antigen archival, presentation, and leukocyte crosstalk through ligands such as PDL-1 (Santambrogio et al. 2019; Rouhani et al. 2015; Lucas and Tamburini 2019; Humbert et al. 2017; Yeo and Angeli 2017). Both sequencing and functional experiments demonstrate that lymphatic vessels are able to respond to the microenvironment during inflammation, in which a subset can even engage in immune cell crosstalk and regulation. In this review, we discuss the role of the different CNS efflux routes, consisting of both CNS meningeal lymphatic vessels and perineural routes of drainage, in steady-state and neuroinflammation. This includes evidence over the past years, current theories of CNS drainage routes, the immunological role of these routes during neuroinflammation, and some of the many unresolved questions that remain.

Historical context

The first description of lymphatic vessels dates back to the third century BC by the Greek anatomist Herophilos (reviewed in Ambrose 2006). While most tissues of the body contain tissue parenchyma resident lymphatics, the CNS parenchyma is devoid of such lymphatics (Engelhardt et al. 2017; Louveau, Harris et al. 2015). Evidence for the "immune privileged" CNS began emerging in the early 1920′s in which tissues transplanted into the brain were able to escape immune surveillance (Shirai et al. 1921; Murphy and Sturm 1923). The term "immune privileged" later coined by the Nobel laureate Sir Peter Medawar in the 1950′s was further illustrated through a series of prolonged skin graft survival in the eye and brain (Billingham et al. 1953), suggesting a lack of communication between the brain and systemic immunity, and since then was in part attributed to the lack of conventional lymphatics within the tissue parenchyma (Engelhardt et al. 2017). Nevertheless, meningeal lymphatics surrounding the CNS were first described in the eighteenth century by Paolo Mascagni, and only recently have researchers identified and characterized the functionality of a vast network of lymphatic vessels in the meninges surrounding the CNS using modern lymphatic markers (Da Mesquita et al. 2018) (Fig. 1). We now know that the CNS isn't as immune privileged as once thought, and that there is in fact communication between the CNS and immune system even during steady-state conditions (Louveau, Harris et al. 2015; Harris et al. 2014), likely due to the extensive network of lymphatics in the meninges surrounding the CNS (Engelhardt et al. 2017; Louveau, Harris et al. 2015; Da Mesquita et al. 2018). Furthermore, recent evidence has shown that these drainage pathways play a key immunological role during neuroinflammation, as they contribute to immunosurveillance, immune regulation, and fluid homeostasis (Engelhardt et al. 2017; Louveau, Harris et al. 2015; Da Mesquita et al. 2018). These lymphatics include meningeal lymphatic vessels (mLVs) dorsal to the brain in the superior sagittal sinus, confluence of sinuses where the superior sagittal sinus and transverse sinuses meet, and laterally along the transverse sinuses, the basal mLVs below the brain, and mLVs on the CNS side of the cribriform plate (Engelhardt et al. 2017; Louveau, Harris et al. 2015; Da Mesquita et al. 2018). All of these lymphatics have been recently shown to functionally drain macromolecules and cerebrospinal fluid (CSF) (Louveau, Smirnov et al. 2015; Aspelund et al. 2015), and manipulation of mLVs is sufficient to modulate many CNS diseases including mouse models multiple sclerosis (Louveau et al. 2018; Hsu et al. 2019), stroke (Yanev et al. 2020), traumatic brain injury (Bolte et al. 2020), brain tumors (Ma et al. 2019; Hu et al. 2020; Song et al. 2020), Alzheimer's disease (Da Mesquita et al. 2018), and cerebrovascular injury (Chen et al. 2018) (Fig. 1).

Prior to the re-discovery of meningeal lymphatics, the predominant hypothesis of fluid drainage from the CNS involved perineural pathways along cranial nerves, particularly the olfactory cranial nerves that cross the cribriform plate (Cserr, Harling-Berg et al. 1992; Weller et al. 2010; Kida et al. 1993; Laman and Weller 2013; Mollanji et al. 2001; Bozanovic-Sosic et al. 2001; Pollay 2010) (Fig. 1), also reviewed extensively by Lena Koh, Andrei Zakharov, and Miles Johnston (2005). Notably, several groups were aware of lymphatic vessels residing in the dura dorsal and basal of the brain in which tracers could be found after subarachnoid injection (Andres et al. 1987; Földi et al. 1966; Holtz et al. 1983; Killer et al. 1999), many of which were described well before the recent re-discovery of mLVs in 2015 (Louveau, Smirnov et al. 2015; Aspelund et al. 2015),

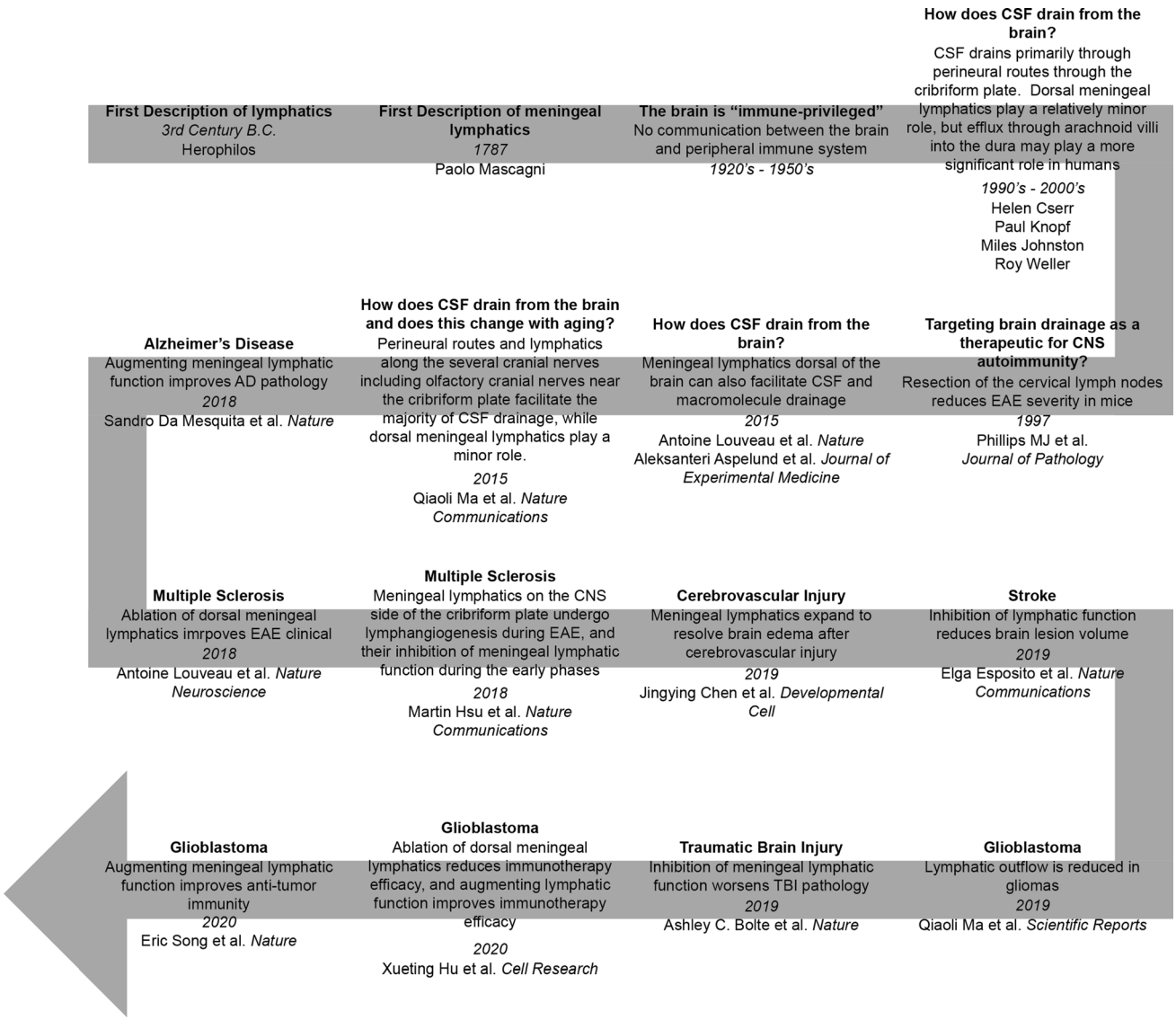

Fig. 1 Brief timeline of CNS-immune communication

yet many macroscopic studies that infused dyes into the CSF to visualize efflux pathways concluded that the dural mLVs played a relatively minor role in fluid drainage (Cserr, Harling-Berg et al. 1992; Koh et al. 2005). It was hypothesized that this was due to the presence of an arachnoid barrier that contains tight junctions to contribute to the blood–CSF barrier (Engelhardt et al. 2017; Rodriguez-Peralta 1957; Brøchner et al. 2015; Hannocks et al. 2018; Castro Dias et al. 2019). These studies revealed that the majority of fluid seemed to flow toward and through the cribriform plate beneath the olfactory bulbs, to presumably be picked up by lymphatics near the cribriform plate or further downstream within the nasal cavity (Cserr, Harling-Berg et al. 1992; Weller et al. 2009; Kida et al. 1993; Laman and Weller 2013; Mollanji et al. 2001; Bozanovic-Sosic et al. 2001; Pollay 2010; Koh et al. 2005) and have been since validated using current methodologies (Norwood et al. 2019; Ma et al. 2017). Tracers infused into the CSF could also be found along other cranial nerves, including the optic nerve and trigeminal nerve (Pollay 2010; Ma et al. 2017). While especially true for rodents which contain a relatively large olfactory bulb to brain volume ratio, drainage predominantly through the cribriform plate was later validated in many non-rodent animal models including cats, dogs, pigs, sheep, non-human primates, and even in post-mortem human samples (Koh et al. 2005). In contrast, the dorsal mLVs seem to play a more significant role in humans due to a higher density of arachnoid villi, structures that are hypothesized to facilitate CSF uptake by the mLVs through the arachnoid barrier (Cserr, Harling-Berg et al. 1992; Weller et al. 2010; Koh

et al. 2005). These historical studies not only described how fluids and tracers may exit the CNS but identified a potential immunological significance for soluble antigen drainage through these pathways (Fig. 1).

Similar to cisterna magna injection of CSF tracers, soluble antigen drainage has been studied using intracranial (I.C.) antigen injection and tracking antigen drainage along with antigen-specific T cell activation in the periphery (Cserr, Harling-Berg et al. 1992; Cserr, DePasqiale et al. 1992; Cserr and Knopf 1992). Such studies were aimed at investigating the immunological significance of CNS drainage through these different routes. Indeed, protein antigens I.C. injected into different CNS parenchymal regions (caudate nucleus, internal capsule, and midbrain) or into the CSF could be found in the cervical lymph nodes (Cserr, Harling-Berg et al. 1992; Cserr, DePasquale et al. 1992; Cserr and Knopf 1992). Functionally, protein antigens draining to the periphery are capable of eliciting adaptive immune responses and may even be more immunogenic than when the same antigens are introduced systemically (Gordon et al. 1992; Harling-Berg et al. 1989; Karman et al. 2004; Ling et al. 2003, 2006; Qing et al. 2000). Several neural cell-specific neoantigen models have also been generated in which expression of immunogenic antigens are restricted to neurons (Sanchez-Ruiz et al. 2008; Scheikl et al. 2012), astrocytes (Cornet et al. 2001), oligodendrocytes (Cao et al. 2006; Saxena et al. 2008), or both oligodendrocytes and Schwann cells (Schildknecht et al. 2009). These models allow researchers to more elegantly test how CNS cell-specific neoantigens are recognized by the immune system, and how these soluble antigens drain from the CNS. Findings using these models suggest that afferent immunity is intact, but efferent immunity may be restricted due to barriers that restrict overt accumulation of antigen-experienced cells in the CNS.

Similarly, our laboratory generated an oligodendrocyte cell-specific neoantigen expressing transgenic mouse which contained moderate proliferation of antigen-specific T cells in peripheral lymphoid tissue during steady-state conditions without the cerebral microinjury often associated with I.C. antigen injection protocols (Harris et al. 2014). The level of antigen-specific T cell proliferation in this transgenic mouse model was approximately one-third the level observed in identical experiments when the same neoantigen is restricted to the intestinal epithelium. Immediate ex vivo antigen recall experiments on cells isolated from the cervical lymph nodes further revealed that, in addition to proliferating, CNS neoantigen-specific CD8 + T cells had differentiated into cytokine-producing effector T cells; however, these cells were not found in CNS tissues (Harris et al. 2014). These studies reveal that while antigen drainage from CNS tissue is restricted during steady-state conditions, immune surveillance still occurs and is capable of priming immune responses to novel antigen determinants. However, it is not sufficient to induce neoantigen-specific T cell accumulation into the naïve CNS.

It was proposed by Helen Cserr and Paul Knopf that the most likely drainage pathway for soluble antigens from the CNS into the periphery is by exiting the subarachnoid space through arachnoid villi, which protrude into the dural sinuses, particularly in humans (Cserr, Harling-Berg et al. 1992). They among many others also proposed additional routes of drainage along cranial nerves through the cribriform plate and into nasal lymphatics, as highlighted previously with macroscopic CSF tracer studies and extensively reviewed by Miles Johnston's group (Koh et al. 2005). In addition to perineural pathways of drainage, there was also speculation of lymphatic vessels particularly near the cribriform plate and to a much lesser extent the dura dorsal to the brain that may also facilitate drainage, although the precise anatomical location of the cribriform plate lymphatics and whether or not they existed on the CNS side of the cribriform plate were unknown (Weller et al. 1992). Since the re-discovery of meningeal lymphatics near the dural sinuses in 2015 (Louveau, Smirnov et al. 2015; Aspelund et al. 2015), much more emphasis has been placed on their relative contribution to the drainage of fluid and CNS-derived molecules, proteins, antigens, and cells. Consequently, the importance and relative contribution of the perineural routes of drainage along cranial nerves has been questioned by recent studies (Louveau et al. 2018; Melin et al. 2020), further outlined and discussed by other reviews (Engelhardt et al. 2017; Louveau et al. 2016; Engelhardt 2018).

Current theories of CNS drainage

It is becoming increasingly obvious that there is heterogeneity in the development, phenotype, and function between the different CNS lymphatic networks. This suggests that there may also be heterogeneity in their ability to sample CSF, and how they regulate tissue homeostasis. During development, the dorsal mLVs are significantly delayed in their formation relative to other lymphatics (Antila et al. 2017). In mice, many lymphatic vessels develop and express mature lymphatic markers by or near postnatal day 0 (Yang and Oliver 2014), including the basal mLVs and cpLVs (Antila et al. 2017). The exception is the dorsal mLVs which exhibit a significant delay in development, with those in the superior sagittal sinus developing as late as postnatal day 21 (Antila et al. 2017). The significance of a delayed development for the dorsal mLVs remains unknown, although some functional evidence suggests that the dorsal mLVs may be less stable as a consequence. One example is that VEGFC–VEGFR3 is required for lymphangiogenesis during development, but during adulthood most mature lymphatic

vessels become independent of VEGFC–VEGFR3 signaling (Karaman et al. 2018). This is also true for the cpLVs, where inhibition of VEGFR3 signaling during adulthood does not affect baseline levels (Hsu et al. 2019). In contrast, inhibition of VEGFR3 during adulthood causes dorsal mLV regression (Hsu et al. 2019; Antila et al. 2017), suggesting sustained VEGFC–VEGFR3 signaling throughout adulthood is required for the maintenance of these particular lymphatics. The differential regulation of CNS lymphatics has implications for therapeutic targeting of lymphatics to treat CNS diseases, which will be discussed in a later section.

Current lymphatic markers such as Prospero homeobox protein 1(Prox-1), Lymphatic vessel endothelial receptor (Lyve-1), Podoplanin, Vascular Endothelial Growth Factor Receptor 3 (VEGFR3), and CD31 (also known as Platelet Endothelial Cell Adhesion Molecule -PECAM-1) along with advances in microscopy has not only validated the ability of dorsal mLVs to carry macromolecules and CSF but has generated a lot of excitement (Louveau, Smirnov et al. 2015; Aspelund et al. 2015; Blanchette and Daneman 2017; Antila et al. 2017). Since then, many groups have replicated these studies in both steady-state and neuroinflammation (Louveau et al. 2018; Yanev et al. 2020; Bolte et al. 2020; Hu et al. 2020; Song et al. 2020; Da Mesquita et al. 2018; Chen et al. 2019), suggesting that the dorsal mLVs may play a significant role in CNS drainage. Although the dorsal mLVs contribute to the drainage of CSF, their contribution relative to other mLVs residing at the base of the brain (Ahn et al. 2019) and on the CNS side of the cribriform plate remain controversial (Hsu et al. 2019, 2020), which is discussed in more detail in a later section. Further characterization of mLVs surrounding the CNS revealed an extensive network of lymphatics at the base of the brain, which are hypothesized to play a more significant role in drainage than those dorsal to the brain due to their closer proximity to the CSF-filled subarachnoid space and much larger CSF pools (Ahn et al. 2019). Our group has also characterized meningeal lymphatic vessels (cpLVs) on the CNS side of the cribriform plate as playing a significant role in fluid drainage (Hsu et al. 2019). These particular lymphatics reside in a location where the majority of fluid has been shown to flow through in mice, are in close proximity to a relatively large CSF pool similar to the basal mLVs, and seem to have direct access to CSF due to gaps in the E-Cadherin + epithelial cell layer that comprises the arachnoid barrier (Hsu et al. 2020).

How lymphatic vessels in the meninges gain access to the CSF-filled subarachnoid space through the arachnoid barrier remains somewhat controversial. The predominant theory seems to be through arachnoid villi (Cserr, Harling-Berg et al. 1992), highly vascularized structures that protrude from the subarachnoid space into the dural parenchyma (Pollav, 2010; Pardridge 2011). Currently, it seems as if drainage from the CNS primarily occurs through lymphatics; either directly through mLVs or indirectly through perineural routes along cranial nerves to be picked up by downstream peripheral lymphatics such as olfactory cranial nerves and nasal lymphatics, excluding the possibility of a direct CSF-venous connection within arachnoid villi (Ma et al. 2017; Pardridge 2011; Pollay 2010; Mawera and Asala 1996; Johnston et al. 2004). Nevertheless, it is also possible that arachnoid villi facilitate the exchange between CSF, the mLVs, and/or blood circulation (Pardridge 2011; Pollay 2010; Mawera and Asala 1996). This seems to be especially true in humans, who have a higher density of arachnoid villi and a relatively smaller cribriform plate area relative to mice (Pardridge 2011). Several pieces of indirect evidence, mostly through post-mortem examination of arachnoid villi or through ex vivo studies have hypothesized arachnoid villi as the connection between CSF and the dural parenchyma, with direct in vivo evidence remaining elusive (Ma et al. 2017; Pardridge 2011; Pollay 2010; Mawera and Asala 1996; Johnston et al. 2004). Additionally, even within the arachnoid villi, there is the presence of an endothelium with tight junctions separating the CSF compartment from the dura parenchyma (Brøchner et al. 2015; Alksne and Lovings 1972). Nevertheless, studies done by Welch and colleagues (reviewed by Pollay 2010) using light microscopy revealed open-ended vessels when characterized under normal physiological pressures, which disappeared when directional fluid pressures were reversed, suggesting a potential one-way exchange of CSF from the subarachnoid space to the meningeal parenchyma through arachnoid villi. Similar to fluid, particles and proteins could also be perfused through arachnoid villi, suggesting similar pathways of drainage between fluid and proteins within arachnoid villi (Pollay 2010).

In addition to being developmentally heterogenous and having different levels of VEGFR3-dependent maintenance, heterogeneity also seems to exist in the phenotype and function between the different mLV networks. For example, bulk RNA sequencing of the dorsal mLVs shows these particular lymphatics to be phenotypically distinct compared to more conventional lymphatics like those found in the diaphragm or skin (Louveau et al. 2018). Interestingly, genes involved in lymphatic vessel development, proliferation, and structural stiffness seem to be dysregulated in the dorsal mLVs compared to more conventional lymphatics of the diaphragm or skin, suggesting that the dorsal mLVs may be limited in their ability to respond to growth factors or inflammation (Louveau et al. 2018). Functionally, during experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis, cribriform plate mLVs uniquely undergo VEGFR3-dependent lymphangiogenesis to promote drainage, while the dorsal mLVs do not (Hsu et al. 2019). It is possible that the dorsal mLVs require significantly more VEGFC to undergo lymphangiogenesis, as these particular

lymphatics require VEGFC–VEGFR3 signaling to maintain baseline levels. Nevertheless, they have the ability to undergo lymphangiogenesis, as AAV-VEGFC delivery into the cisterna magna is sufficient to induce lymphangiogenesis (Louveau, Smirnov et al. 2015). It is also possible that the different lymphatic networks have different access to CNS-derived molecules such as VEGFC during neuroinflammation. Other differences can be seen not only in terms of how each mLV network responds to neuroinflammation by either undergoing lymphangiogenesis or not, but also in terms of their phenotype and contribution to disease pathology, which will be discussed in a later section.

Due to an increase in the interest of mLVs in the drainage of macromolecules and CSF, the perineural pathways of drainage along cranial nerves have recently been challenged (Louveau et al. 2018; Melin et al. 2020). It is unlikely that we can exclude these pathways as a potential route of drainage due to the extensive evidence in the literature supporting their role in drainage as outlined earlier (Cserr, Harling-Berg et al. 1992; Weller et al. 2010; Kida et al. 1993; Laman and Weller 2013; Mollanji et al. 2001; Bozanovic-Sosic et al. 2001; Pollay 2010; Koh et al. 2005; Norwood et al. 2019; Ma et al. 2017). In addition, several recent studies investigating CSF drainage through mLVs have also reported perineural drainage of tracers (Norwood et al. 2019; Ma et al. 2017), validating this pathway as a route of drainage. While it is currently unknown what the relative contribution of the different lymphatic pathways and perineural pathways are for fluid drainage, several functional experiments suggest that multiple routes of drainage may serve as redundant pathways to ensure proper CSF homeostasis. Sealing the cribriform plate alone is sufficient to increase intracranial pressure and/or reduce tracer clearance (Boulton et al. 1999; Silver et al. 2002; Papaiconomou et al. 2002), suggesting the perineural and/or cribriform plate lymphatics seem to play a measurable role in fluid efflux. In contrast, ablation of the olfactory sensory neurons using intranasal ZnSo4 did not increase intracranial pressure in mice despite reduced outflow to the nasal cavity (Norwood et al. 2019), suggesting that there may be compensatory drainage pathways for the olfactory perineural route. Photoablation of specifically the dorsal mLVs using Visudyne also did not alter intracranial pressure in mice (Bolte et al. 2020), again suggesting compensatory routes of drainage. The differences between these two experiments may also reflect the difficulty in measuring smaller changes in intracranial pressure fluctuations in mice relative to larger mammals (Bolte et al. 2020; Norwood et al. 2019; Boulton et al. 1999; Silver et al. 2002; Papaiconomou et al. 2002), suggesting that in larger vertebrates, the different drainage routes may play a more important role in fluid dynamics. Therefore, it seems that all of these routes, including both mLVs and perineural routes, play a role in some aspect of fluid drainage.

Role of CNS drainage in neuroinflammation

Drainage from the CNS has been hypothesized to play a significant immunological role in CNS pathology (Cserr, Harling-Berg et al. 1992; Weller et al. 2010; Kida et al. 1993). As mentioned previously, CNS-derived antigens can functionally be found in the draining lymph nodes to induce an adaptive immune response (Cserr, Harling-Berg et al. 1992; Laman and Weller 2013; Cserr, Depasquale et al. 1992; Cserr and Knopf 1992; Gordon et al. 1992; Harling-Berg et al. 1989; Karman et al. 2004; Ling et al. 2003, 2006; Qing et al. 2000; Harris et al. 2014). Correlatively, CNS-derived antigens are elevated in both the CSF and the draining lymph nodes of MS patients (Heyligen et al. 1984; Willis et al. 2015; van Zwam, Huizinga, Melief et al. 2009), suggesting antigen drainage may play a role in autoimmunity. Functionally, surgical resection of the draining lymph nodes prior to inducing EAE is sufficient to reduce EAE severity (Louveau et al. 2018; Phillips et al. 1997; van Zwam, Huizinga, Heijmans et al. 2009), validating antigen drainage as contributing to pathology during CNS autoimmunity. Furthermore, the importance of CNS lymphatics in wide array of CNS diseases can be seen in models of multiple sclerosis, stroke, Alzheimer's disease, traumatic brain injury, glioblastoma, and cerebrovascular injury (Louveau et al. 2018; Hsu et al. 2019; Yanev et al. 2020; Bolte et al. 2020; Ma et al. 2019; Hu et al. 2020; Song et al. 2020; Da Mesquita et al. 2018; Chen et al. 2019) (Fig. 1).

Although the relative contribution of the dorsal mLVs relative to other mLVs and perineural routes of drainage for fluid is controversial, their significance in immunity during neuroinflammation is not. Many recent studies have studied the role of mLVs in CNS diseases and highlighted the dorsal mLVs as being critical in contributing to disease pathology (Louveau et al. 2018; Hsu et al. 2019; Yanev et al. 2020; Bolte et al. 2020; Ma et al. 2019; Hu et al. 2020; Song et al. 2020; Da Mesquita et al. 2018; Chen et al. 2019). It is important to note however that many of these studies utilize general manipulation of CNS lymphatic functions through cisterna magna or systemic injection of molecules that augment or inhibit lymphatic function, which affect not just the dorsal mLVs but likely the cribriform plate mLVs, basal mLVs, and downstream lymphatic networks such as those in the draining lymph nodes. Nevertheless, specific ablation of the dorsal mLVs can be accomplished using Visudyne (Louveau et al. 2018; Bolte et al. 2020; Hu et al. 2020; Da Mesquita et al. 2018), a photosensitive dye that is able to reduce the dorsal mLV function in the presence of violet light (Tammela et al. 2011; Kilarski et al. 2014). Notably, photoablation of specifically the dorsal mLVs prior to EAE induction is

sufficient to reduce EAE severity (Louveau et al. 2018). The same can be seen through general lymphatic inhibition through systemic delivery of the VEGFR3 tyrosine kinase inhibitor MAZ51 prior to the onset of EAE clinical scores, which causes both dorsal mLV regression and inhibits lymphangiogenesis by cribriform plate mLVs (Hsu et al. 2019). However, inhibition of both dorsal mLVs and cribriform plate mLVs after EAE onset has no effect (Hsu et al. 2019), suggesting that either the general contribution of lymphatic function is only important for the initiation of EAE, or more likely that the different mLVs may have different and opposing roles in the later stages of EAE, which will be discussed in a later section. Additionally, photoablation of the dorsal mLVs can also reduce intratumor fluid and tumor cell drainage to the cervical lymph nodes (Hu et al. 2020), suggesting that the dorsal mLVs contribute to CNS-derived tumors sampling during neuroinflammation. More generally, augmenting lymphatic function through tumor overexpression of VEGF-C or AAV-VEGFC delivery into the cisterna magna promotes anti-tumor-specific T cell immunity and enhances anti-checkpoint therapy (Song et al. 2020; Da Mesquita et al. 2018), suggesting general augmentation of the dorsal mLV, basal mLV, cribriform plate mLV, and potentially the draining lymph nodes may have therapeutic potential for the treatment of glioblastomas.

Dysfunction in CSF clearance can also be observed in aging and Alzheimer's disease (Da Mesquita et al. 2018; Ma et al. 2017). In mice, macroscopic near-infrared imaging revealed that the majority of tracer injected into the cisterna magna flowed along perineural routes including the olfactory cranial nerve that penetrates the cribriform plate, optic nerve, and trigeminal nerve while the dorsal mLVs play a relatively minor role (Ma et al. 2017). CSF clearance along these routes was also impaired in aged mice (Ma et al. 2017), suggesting that these other routes of drainage may also contribute to age-associated pathology. Although this study contributes a relatively minor role for the dorsal mLVs in CSF clearance, photoablation of specifically the dorsal mLVs exacerbates cognitive decline and pathology in a mouse model of Alzheimer's disease (Da Mesquita et al. 2018). Therefore, although the dorsal mLVs seem to play a relatively minor role in CSF clearance by volume, their dysfunction plays a significant role in the accumulation of toxic proteins and cognitive decline associated with aging and Alzheimer's disease. Cribriform plate mLVs have also been implicated in Alzheimer's disease; in vivo PET imaging of human Alzheimer's disease patients revealed deficits in the clearance of Tau tracers near the cribriform plate (de Leon et al. 2017), suggesting that dysfunction in perineural olfactory cranial nerve drainage and/or cribriform plate mLVs may contribute to Alzheimer's disease (Ethell 2014). Interestingly, an MRI study recently revealed the lack of CSF tracer into the nasal mucosa, suggesting that the perineural route of CSF efflux along olfactory cranial nerves may be less significant in humans (Melin et al. 2020). It is important to note however that the MRI data does reveal some distribution of tracer in the nasal mucosa in some cases, and a large accumulation of tracer in the CNS side of the cribriform plate where cribriform plate mLVs reside (Melin et al. 2020).

Pre-treatment with AAV-VEGFC delivery into the cisterna magna reduces TBI injury through dorsal mLV expansion (Bolte et al. 2020) and likely contributes to expansion of basal mLV and cribriform plate mLV as well. However, pre-inhibition of specifically the dorsal mLVs using Visudyne was sufficient to exacerbate TBI injury, which correlated with significant increases in complement activation within the CNS (Bolte et al. 2020), suggesting that the dorsal mLVs play a significant role in TBI outcome and pathology. Unlike EAE, TBI can in fact induce dorsal mLV expansion (Bolte et al. 2020), suggesting that the dorsal mLVs not only have the capability of undergoing lymphangiogenesis during neuroinflammation, but that they may require a much more direct injury to do so. Another interesting aspect is that elevation in intracranial pressure is sufficient to reduce dorsal mLV function (Bolte et al. 2020), which may suggest that the dorsal mLVs can undergo expansion to compensate. It is unknown what changes, if any, the basal and cribriform plate mLVs may undergo during TBI. However, the expansion of dorsal mLVs up to one-month post-TBI inversely correlates with microbead drainage to the draining lymph nodes (Bolte et al. 2020), suggesting that the lymphangiogenic dorsal mLVs after TBI may be dysfunctional in the drainage of antigens to the draining lymph nodes. Additionally, increased fluid accumulation in CSF reservoirs can also be seen during EAE (Hsu et al. 2020), which as mentioned previously does not induce lymphangiogenesis by dorsal mLVs but does promote functional lymphangiogenesis by cribriform plate mLVs. These data suggest that the dorsal mLVs dysfunction plays an active role in TBI pathology; however, its ability to undergo functional lymphangiogenesis during neuroinflammation seems to be less obvious.

CNS lymphatics also play a role in vascular diseases associated with edema such as cerebrovascular injury (Chen et al. 2019). One of the most striking findings of mLVs during neuroinflammation is their ability to invade the CNS parenchyma to promote blood vessel morphogenesis in two different cerebrovascular injuries in zebrafish (Chen et al. 2019). It is unknown if a similar phenomenon can occur in mammals, as no reports of meningeal lymphatic vessel invasion into the CNS of mammals have been observed under any circumstance so far. Perhaps the closest observation to parenchymal invasion of mLVs is the presence of non-lumenized lymphatic endothelial cells in the leptomeninges beneath the arachnoid barrier and in the pia

mater, which can be found in both zebrafish and mammals, including humans (Shibata-Germanos et al. 2020). These cells, coined Leptomeningeal Lymphatic Endothelial Cells (LLECs) by the authors, are hypothesized to facilitate CSF clearance in collaboration with the mLVs that exist in the dura mater (Shibata-Germanos et al. 2020); however, this is just speculation. Additionally, their existence as individual cells and lack of a proper lumenized vessel structure make LLECs seem unlikely to facilitate fluid drainage. However, they seem to display a remarkable capability of endocytosis, with a greater ability to endocytose amyloid-beta than macrophages (Shibata-Germanos et al. 2020), despite a unique developmental origin from myeloid cells. Consequently, these LLECs that exist in the leptomeninges may be critical for CNS-derived macromolecule clearance and potentially immune regulation through antigen clearance.

In addition to CNS mLVs, lymphatics downstream of the CNS in the draining lymph nodes have been shown to undergo lymphangiogenesis as early as 3 h post-stroke and regulate innate immunity during the early phases of ischemic stroke (Esposito et al. 2019). Interestingly, the ability of lymphatic endothelial cells to undergo expansion so quickly, 3 h after ischemic stroke, seems to be unique to the superficial lymph nodes. This is surprising considering lymphangiogenesis of cribriform plate lymphatics during neuroinflammation seems to require several days. The proliferation marker Ki67 was also observed in several Lyve-1^- non-lymphatic endothelial cells, and Lyve-1 itself is also expressed by a subset of macrophages. Thus, future studies assessing and validating the kinetics of lymphangiogenesis in both the superficial and deep cervical lymph nodes during different models of neuroinflammation are needed. Of the CNS lymphatics, the dorsal mLVs have only been shown to undergo lymphangiogenesis during TBI, in which dorsal mLVs seem to undergo lymphangiogenesis one-week post-TBI (Bolte et al. 2020). The functionality of lymphangiogenic dorsal mLVs after TBI is unknown as it inversely correlates with bead drainage to the draining lymph nodes. Functional lymphangiogenesis by cribriform plate mLVs can be observed in several models of neuroinflammation including EAE (Hsu et al. 2019, 2020), CNS *Mtb* infection, and stroke (unpublished), which also seems to require at least a week after neuroinflammation (Louveau et al. 2018; Hsu et al. 2019). Nevertheless, lymphangiogenesis in the draining lymph nodes correlates with early macrophage activation, and intranasal administration of the VEGFR3 tyrosine kinase inhibitor MAZ51 can not only inhibit lymph node lymphangiogenesis but also reduce inflammation and brain infarct volume (Esposito et al. 2019). Noteworthy is the intranasal administration of MAZ51, which presumably also targets the cribriform plate mLVs as well as downstream lymphatics in the draining lymph nodes. It is unknown if the dorsal or basal mLVs are also affected by intranasal administration of MAZ51; however, some evidence suggests that macromolecules can perhaps indirectly affect the dorsal and basal mLVs after infiltration into the CNS via glymphatic transport after intranasal delivery (Kumar et al. 2018; Lochhead et al. 2012; Lochhead et al. 2015; Pizzo et al. 2018). These data suggest that during CNS diseases, both local CNS mLVs as well as downstream lymphatics as far as the draining lymph nodes may also be modulated by inflammation and contribute to disease pathology.

Leukocyte entry into the CNS during EAE and stroke has been shown to occur through the blood–CSF barrier of the choroid plexus (Clarkson et al. 2014; Engelhardt et al. 2001; Ge et al. 2017; Llovera et al. 2017; Schiefenhövel et al. 2017); however, the pathway that emigrating leukocytes take to the CNS mLVs is unknown. Data by our laboratory has shown that CNS-derived cells and antigens can in fact be found within cribriform plate mLVs during EAE (Hsu et al. 2019, 2020), suggesting that at least a subset of emigrating leukocytes use mLVs as an exit route during neuroinflammation. Tracking of infiltrating $CD11c^+$ dendritic cells over time during EAE reveal infiltration into the choroid plexus followed by migration to the olfactory bulbs near cribriform plate mLVs along the rostral migratory stream, and subsequently the draining lymph nodes (Clarkson et al. 2015; Schiefenhövel et al. 2017). While our laboratory and others have observed the choroid plexus as one of the primary sites of dendritic cell infiltration during EAE (Clarkson et al. 2015; Schiefenhövel et al. 2017; Engelhardt et al. 2001), It is also likely that other routes also play a role including the blood–brain barrier and the blood–CSF barrier in the meninges (Engelhardt et al. 2017; Engelhardt 2018). Nevertheless, inhibition of dendritic cell efflux and anti-CNS specific T cell responses in the draining lymph nodes can be accomplished by administering the mononuclear cell-sequestering drug fingolimod along the rostral migratory stream (Mohammad et al. 2014), suggesting that migration from the choroid plexus along the rostral migratory stream toward the olfactory bulbs is a significant route of immune cell trafficking during neuroinflammation. Of note, the rostral migratory stream is also used by neural progenitor cells from the subventricular zone to populate the olfactory bulb. Once in the olfactory bulb, it is possible that leukocytes can migrate perineurally along cranial nerves including the olfactory cranial nerve through the cribriform plate and into lymphatics in the nasal mucosa (Hsu et al. 2019, 2020), or along other perineural routes such as along the optic and trigeminal nerves. There is also an extensive network of mLVs on the CNS side of the cribriform plate, which seem to have direct access to the subarachnoid space due to gaps in the arachnoid barrier at this location (Norwood et al. 2019; Hsu et al. 2020). Indeed, CD11c + dendritic cells can be found traversing the olfactory cranial nerve, optic nerve, cribriform plate mLVs, and

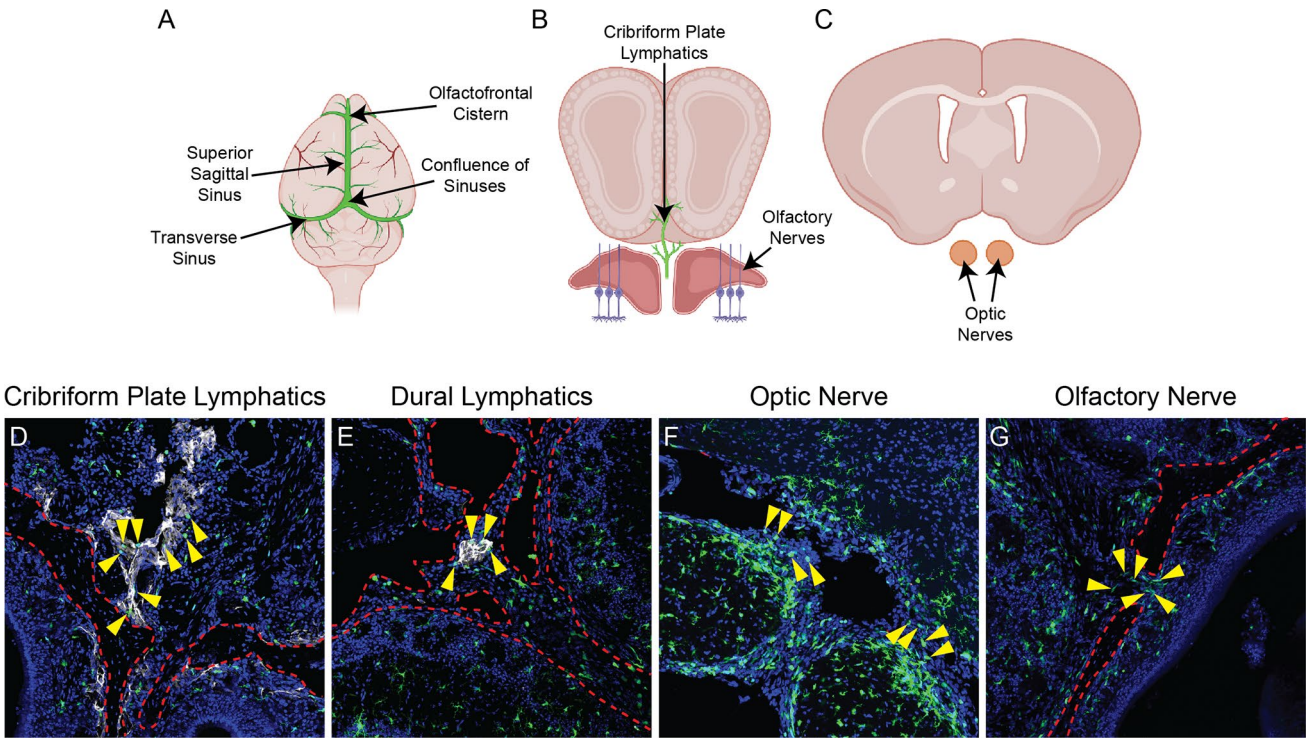

Fig. 2 Identification of CD11c + cells along cranial nerves and mLVs. **a–c:** Visual representations of the dorsal meningeal lymphatics that traverse the superior sagittal sinus, confluence of sinuses, and transverse sinuses **a**, cribriform plate lymphatics that reside between the olfactory bulbs on the CNS side of the cribriform plate **b**, olfactory nerves **b**, and optic nerves **c** were generated using Biorender.com. **d–g** EAE was induced in CD11c-eYFP transgenic reporter mice, and tissues analyzed at EAE score 3.0. The whole heads were processed for coronal sections after decalcification of the bones and visualized by confocal microscopy. CD11c-eYFP$^+$ cells can be observed in and near lymphatics on the CNS side of the cribriform plate **d** and lymphatics in the dura dorsal to the brain **e**. Additionally, CD11c-eYFP$^+$ cells can also be seen along the optic nerves near the optic chiasm **f** as well as along olfactory cranial nerves **g**. The cribriform plate **d, g** or meninges **e** are outlined in red, and yellow arrowheads indicating CD11c-eYFP$^+$ cells

dorsal mLVs during EAE (Fig. 2). However, direct evidence of CNS-derived leukocytes traversing from the CNS parenchyma to the subarachnoid space and mLVs remains elusive, particularly for the dorsal mLVs in which an uninterrupted arachnoid barrier containing tight junctions separates the subarachnoid space from the dura mater and limits the paracellular diffusion of macromolecules and cells (Engelhardt et al. 2017; Rodriguez-Peralta 1957; Brøchner et al. 2015; Hannocks et al. 2018; Castro Dias et al. 2019).

Unresolved questions

The extensive network of lymphatic vessels residing in the meninges surrounding the CNS seem to all play a role in some aspect of drainage. Nevertheless, many questions about their relative access, phenotype, and heterogeneity remain. The arachnoid barrier is a less studied blood–CSF barrier (BCSFB) comprised of epithelial cells that separate the CSF-filled subarachnoid space from fenestrated blood and lymphatic vessels in the dura (Engelhardt et al. 2017; Rodriguez-Peralta 1957; Brøchner et al. 2015; Hannocks et al. 2018; Castro Dias et al. 2019). These epithelial cells that make up the arachnoid blood–CSF barrier contain tight junctions including Claudin-11 that limits the paracellular permeability macromolecules (Brøchner et al. 2015; Alksne and Lovings 1972). While several studies have shown CSF tracers being able to access these dorsal mLVs above the dural sinuses, one major caveat is the artificial disruption of the arachnoid BCSFB by cisterna magna injection. Therefore, how CSF and CSF-containing molecules are able to traverse the arachnoid blood–CSF barrier is currently unknown. The predominant hypothesis is through arachnoid villi (Cserr, Harling-Berg et al. 1992; Pollay 2010), which as mentioned previously lacks direct in vivo evidence. Indeed, a recently developed macroscopic in vivo imaging technique using near-infrared imaging validated previous macroscopic tracer studies showing the mLVs dorsal to the brain playing a relatively minor role in CSF drainage, while perineural routes along cranial nerves and lymphatics seemed to facilitate most of the fluid drainage (Ma et al. 2017). This study among several others also concluded that drainage occurred

primarily through lymphatics, and not through direct venous connections through arachnoid villi (Norwood et al. 2019; Ma et al. 2017; Pardridge 2011; Pollay 2010; Mawera and Asala 1996; Johnston et al. 2004).

In humans, it is hypothesized that the majority of fluid drainage occurs through the dorsal mLVs (Cserr, Harling-Berg et al. 1992; Pollay 2010). Recent evidence has suggested CSF access in the dura parenchyma through noninvasive imaging in humans, independent of disruption of the arachnoid barrier surrounding the brain through intrathecal injection (Ringstad and Eide 2020). Additionally, MRI imaging after intrathecal administration of gadobutrol revealed a lack of tracer in the nasal mucosa despite a strong enrichment of tracer in CSF spaces surrounding the cribriform plate (Melin et al. 2020), suggesting that in humans drainage through cribriform plate mLVs and other mLVs may play a stronger role than perineural routes along the olfactory cranial nerves. This in vivo MRI study contradicts another in vivo PET study identifying an enrichment of the tau tracer THK5117 in the nasal turbinate (de Leon et al. 2017), which is reduced in Alzheimer's disease patients. Taken together, these data suggest that the mLVs are able to access CSF despite the presence of an arachnoid barrier, with indirect evidence suggesting arachnoid villi as being responsible for this access. Direct evidence for this still remains elusive, and other mechanisms of CSF sampling independent of arachnoid villi such as perineurally along olfactory cranial nerves through the cribriform plate are still in debate. It is also likely that different lymphatic networks have different levels of access to CSF, with some networks potentially having direct access through holes in the arachnoid barrier.

Recent evidence suggests that the basal mLV and cribriform plate mLV may have greater access to the subarachnoid space than the dorsal mLVs. Basal mLVs were shown to have increased access to CSF by residing in an optimal location for CSF drainage: they anatomically have greater access to a CSF reservoir at the base of the brain and reside in closer proximity to the subarachnoid space than the dural mLVs (Ahm et al. 2019). Furthermore, sagittal sections of a developing mouse head revealed a continuous and uninterrupted Claudin-11^{+} arachnoid barrier separating the subarachnoid space from the dura, with the exception of the cribriform plate which lacked Claudin-11 expression (Brøchner et al. 2015). The authors thus described the cribriform plate as containing a "hole" that may facilitate direct exchange of fluid (Brøchner et al. 2015). Our laboratory confirmed this hypothesis by directly visualizing the E-Cadherin^{+} epithelial cell layer that make up the arachnoid barrier near the dorsal mLVs, basal mLVs, and cribriform plate mLVs. The dorsal mLVs are separated from the subarachnoid space by an uninterrupted arachnoid barrier, while the basal mLVs resided in much closer proximity, and cribriform plate mLVs having direct access to the subarachnoid space due to a lack of E-Cadherin^{+} epithelial cell layer (Hsu et al. 2020). Future studies are needed to elucidate how the dorsal mLVs gain access to the CSF-filled subarachnoid space, what role arachnoid villi play in this process, and what the relative access of each lymphatic network is to the subarachnoid space.

It is likely that the dorsal mLVs, basal mLVs, and cribriform plate mLVs are all connected, but the exact site of CSF uptake, mechanism of uptake, and which lymph nodes each mLV drains to remains elusive. There seem to be connections between the dorsal mLVs and basal mLVs laterally along the transverse sinuses (Aspelund et al. 2015; Antila et al. 2017). Additionally, it is likely that the dorsal mLVs and cribriform plate mLVs connect between the olfactory bulbs, as cribriform plate mLVs can be seen protruding upward toward the dorsal mLVs in between the olfactory bulbs in serial coronal sections (data not shown). Preliminary data by other groups have identified "hot spots" of CSF uptake along the transverse sinuses by the dorsal mLVs (Louveau et al. 2018; Bolte et al. 2020) and basal mLVs (Ahn et al. 2019) however, it is currently unknown how this occurs and whether or not these are the only sites of CSF access by mLVs. Our laboratory among others has identified cribriform plate mLVs as having direct access to the CSF-filled subarachnoid space due to holes in the arachnoid barrier in this region (Brøchner et al. 2015; Norwood et al. 2019; Hsu et al. 2020), suggesting there may be multiple sites of CSF access. If so, the questions of why the CNS would have multiple compartments of CNS sampling, do they drain to different lymph nodes to potentially generate unique immune responses, and what does this mean for systemic immunity are all important questions for the future. Future studies such as light sheet microscopy of whole-head samples undergoing tissue-clearance in a transgenic lymphatic reporter mouse may shed light on the full extent of CNS lymphatics, their relationships and connections to each other, and if there are any differences in which lymph nodes they drain to. Studying the roles of superficial versus deep cervical lymph nodes may also give some insight into heterogeneity in their contributes to CNS immunity; CNS-derived antigen and fluid can be found in both superficial and deep cervical lymph nodes; however, there seems to be heterogeneity in their kinetics and which CNS-derived antigens they have access to (van Zwam, Huizinga, Melief et al. 2009; van Zwam, Huizinga, Heijmans et al. 2009). This perhaps reflects unique access to different CNS compartments.

As discussed earlier, differences in mLV phenotype and heterogeneity have broad implications for their immunological role during neuroinflammation. Because of the heterogeneity in mLV development, phenotype, and how they respond to neuroinflammation, general manipulation of CNS lymphatic function may not be specific enough for therapeutic treatment of CNS diseases. As mentioned

previously during glioblastoma, augmentation of lymphatic function promotes tumor-specific immunity (Hu et al. 2020; Song et al. 2020). However, in systemic cancers inhibition of lymphatic function is beneficial to reduce tumor metastasis (Jiang et al. 2019; Padera et al. 2016), suggesting again potentially dual roles of lymphatic function during disease. CNS lymphatic function also declines with age (Da Mesquita et al. 2018; Ma et al. 2017), and augmenting lymphatic function reduces cognitive decline in Alzheimer's disease (Da Mesquita et al. 2018), suggesting improving lymphatic function may be beneficial for preventing age-related neurological diseases. Interestingly, immune cell dysfunction also deteriorates with age, known as immunosenescence (Aiello et al. 2019), and usually is accompanied by increased frequency and severity of diseases such as chronic inflammation, cancer, infections, and autoimmunity, and is believed to be shaped by the number of exposures to antigens that drive memory T cell expansion that consequently reduce naïve T and B cells over time (Aiello et al. 2019). It is unknown if both lymphatic dysfunction and immunosenescence are related; however, a decrease in lymphatic dependent immunosurveillance may partially explain the decreased ability to respond to new antigens with age. In contrast however, augmenting lymphatic function to treat Alzheimer's for example may need to be balanced with the risk expediting immunosenescence through unnecessary memory cell priming, as well as balancing the many diseases associated with immunosenescence such as autoimmunity and cancer (Hakim et al. 2007; Haq et al. 2014) that may benefit from decreased lymphatic function (Jiang et al. 2019; Padera et al. 2016). It is also likely that specific intervention of different mLV networks may also be an important consideration for the treatment of CNS diseases. Ablation of specifically the dorsal mLVs is sufficient to reduce EAE severity (Louveau et al. 2018), suggesting that these particular mLVs may contribute to disease pathology in instances of CNS diseases. In contrast, the cribriform plate mLVs seem to be able to more dynamically respond to neuroinflammation by undergoing lymphangiogenesis and altering their phenotype to regulate leukocyte trafficking and tolerance (Hsu et al. 2020), suggesting that cribriform plate mLVs may be beneficial during the more chronic stages of neuroinflammation in the same disease. Additionally, neuroinflammation can also affect lymphatic networks outside of the CNS, such as those in the lymph nodes. During stroke for example, lymphatic vessels in the cervical lymph nodes undergo expansion, and their inhibition reduces infarct size by reducing innate immunity (Esposito et al. 2019). Future studies are needed to elucidate how to tweak lymphatic function while walking the fine line between their functions of balancing immunosurveillance and tolerance, which can change depending on many contexts such as the disease, stage of the disease, and age.

Additionally, much less is known about how emigrating CNS-derived leukocytes can migrate into the different mLV and perineural routes. During EAE, dendritic cells are required not only for local antigen recognition by myelin-specific T cells, but their phenotype, distribution, and ability to migrate from CNS tissues seem to be rate-limiting factors in both the induction and effector phases of the disease (Zozulya et al. 2010). Because dendritic cells balance both autoimmunity and tolerance, their ability to infiltrate and exit the CNS along with how their phenotype is altered by the local microenvironment is essential for disease pathology. As mentioned previously, migration from the choroid plexus along the rostral migratory stream toward the cribriform plate seems to play a significant role in dendritic cell efflux at least in EAE (Hsu et al. 2019, 2020; Clarkson et al. 2015; Engelhardt et al. 2001; Schiefenhövel et al. 2017). In fact, inhibition of cell trafficking along the rostral migratory stream reduces dendritic cell drainage and T cell priming in the draining lymph nodes, consequently reducing EAE severity (Mohammad et al. 2014). This evidence suggests that inhibition of leukocyte trafficking within the CNS toward the cribriform plate, specifically along the rostral migratory stream, is sufficient in dampening anti-CNS-immune responses. Similar to macromolecule drainage from the subarachnoid space, it is unknown how cells from the CSF and/or the CNS parenchyma are able to enter mLVs across the arachnoid barrier. It is also unknown during neuroinflammation what fraction of cells in the CSF came from the CNS parenchyma, other than it is possible to find a subset of CNS-derived cells within cribriform plate mLVs during neuroinflammation (Hsu et al. 2020). Nevertheless, a significant amount of CNS-derived antigens can be found in the CSF and draining lymph nodes during neuroinflammation (Heyligen et al. 1984; Willis et al. 2015; van Zwam, Huizinga, Melief et al. 2009; van Zwam, Huizinga, Heijmans et al. 2009), suggesting a clear pathway for CNS-CSF exchange of macromolecules and proteins. One likely pathway is through the glymphatic system, described in much more detail in other reviews (Jessen et al. 2015; Mestre et al. 2020), although to what extent they are able to facilitate leukocyte efflux is less obvious. Additionally, while there seems to be a functional connection between the interstitial solute-draining glymphatic and meningeal lymphatic system as interstitial solutes can be found within meningeal lymphatics, (Aspelund et al. 2015), the extent of this connection, where, and how interstitial solutes and fluid are exchanged between the two remain unsolved (Louveau et al. 2017). Consequently, future studies are needed to assess the relative drainage of leukocytes through these different pathways, and consequently what roles they may have in immunity.

All of these questions have important implications for designing therapeutic strategies to target lymphatic function for the treatment of CNS diseases. Differences in lymphatic vessel access to different CNS compartments will likely influence their relative effect in different CNS diseases as well as drug access and effectiveness. Future experiments should not only focus at the level of uptake at the CNS mLV level, but also downstream. Assays that characterize lymph contents may reveal novel information to help evaluate diseases and treatment conditions unique to each disease, as lymph fluids are rich sources of tissue-specific biomarkers during inflammation (Heyligen et al. 1984; Willis et al. 2015; van Zwam, Huizinga, Melief et al. 2009; Broggi et al. 2019). Additionally, advancements in assays that generate both large amounts of quantitative and qualitative data such as mass spectrometry and single-cell RNA sequencing may provide greater resolution to some of the discussed questions. Mass spectrometry for example of different lymph compartments including the ISF, CSF, within different lymphatic networks, downstream within draining lymphatic vessels, and draining lymph nodes may reveal unique changes in the content of the lymph as they flow from one compartment to another. Additionally, single-cell RNA sequencing of the different CNS lymphatic networks may generate well-defined subtypes of lymphatic endothelial cells that shed light on cell heterogeneity, identify potentially novel functions, and even reveal potentially novel therapeutic strategies. Indeed, this has been true for lymphatic endothelial cells of the lymph nodes, where several subsets of lymphatic endothelial cells have been characterized with novel functions including antigen processing and presentation (Xiang et al. 2020) and preferential cell binding and crosstalk with different subsets of leukocytes (Takeda et al. 2019). Single-cell RNA sequencing of the cribriform plate mLVs also reveals similar functions of these particular lymphatic endothelial cells to engage in dendritic cell and CD4 T cell binding, crosstalk, and regulation through antigen processing/presentation and tolerance (Hsu et al. 2020), suggesting that mLVs may play a more significant and direct immunological role than currently hypothesized. Therefore, future experiments are needed to fully elucidate the many functions of lymphatic drainage during neuroinflammation, which will be critical in designing therapies with specific targets and kinetics that can uniquely shape different aspects of disease.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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