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Review

# Lymphatics in Neurological Disorders: A Neuro-Lympho-Vascular Component of Multiple Sclerosis and Alzheimer's Disease?

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Lymphatic vasculature drains interstitial fluids, which contain the tissue's waste products, and ensures immune surveillance of the tissues, allowing immune cell recirculation. Until recently, the CNS was considered to be devoid of a conventional lymphatic vasculature. The recent discovery in the meninges of a lymphatic network that drains the CNS calls into question classic models for the drainage of macromolecules and immune cells from the CNS. In the context of neurological disorders, the presence of a lymphatic system draining the CNS potentially offers a new player and a new avenue for therapy. In this review, we will attempt to integrate the known primary functions of the tissue lymphatic vasculature that exists in peripheral organs with the proposed function of meningeal lymphatic vessels in neurological disorders, specifically multiple sclerosis and Alzheimer's disease. We propose that these (and potentially other) neurological afflictions can be viewed as diseases with a neuro-lympho-vascular component and should be therapeutically targeted as such.

## Role of the Lymphatic System in Tissue Maintenance

Most, if not all, of the body's organs are vascularized by blood and lymphatic vessels. Whereas blood vessels provide the tissues with the nutrients needed for their various functions, the lymphatic system ensures tissue homeostasis by recycling interstitial fluid (ISF) (Alitalo, 2011; Kerjaschki, 2014; Wang and Oliver, 2010) and maintaining immune surveillance (Aebischer et al., 2014; Betterman and Harvey, 2016; Card et al., 2014).

### *Maintenance of ISF balance*

The lymphatic vascular network consists of "initial" and "collecting" vessels (Kerjaschki, 2014). Initial lymphatic vessels are composed of a single layer of lymphatic endothelial cells (LECs) with irregular thin basement membrane and are devoid of pericytes and smooth muscle cells (Kazenwadel and Harvey, 2016; Schulte-Merker et al., 2011; Tammela and Alitalo, 2010). Initial lymphatics remain anchored on the extracellular matrix by fibrillin-containing anchoring filaments. Under physiological conditions, pressure gradients induced by the ISF facilitate the uptake of fluid, macromolecules, and cells into the lymphatic capillaries (Leak, 1976; Leak and Burke, 1968). The extracellular matrix glycoprotein Emilin1 is important for generation of the anchoring filaments and for proper lymphatic drainage (Danussi et al., 2008; Pivetta et al., 2016). Muscle contraction and arterial pulsation also promote the initial formation of lymph. The button-like discontinuous expression pattern of cell-junction molecules renders the initial lymphatic vessels permeable to macromolecules (Baluk et al., 2007). Lymph from the initial lymphatics then enters the collecting lymphatics and returns to the blood vasculature via lymphovascular valves in the cervical area (Koltowska et al., 2013; Schulte-Merker et al., 2011; Tammela and Alitalo, 2010). Compared to initial lymphatics, the collecting lymphatic vessels are larger and are surrounded by pericytes and smooth muscle cells whose contractions drive the flow through the lymphatic vessel (von der Weid and Zawieja, 2004; Zawieja et al., 2011). Unidirectionality of flow within collecting lymphatics is ensured by valves that prevent backflow (Vittet, 2014). Multiple molecules have been implicated in the formation and function of lymphatic valves and the maintenance of unidirectional flow (Bazigou et al., 2011; Vittet, 2014). They include Forkhead box protein C2 (FOXC2) (Petrova et al., 2004), Ephrin type-B 2 (Katsuta et al., 2013; Mäkinen et al., 2005), and connexin 43 (Kanady et al., 2011; Sabine et al., 2012), which are important for the initiation of valve formation, and connexin 47 (Kanady et al., 2011; Sabine et al., 2012), fibronectin 1 (Bazigou et al., 2009), GATA2 (Kazenwadel et al., 2015), and integrin alpha-9 (Itga9) (Bazigou et al., 2009), important for valve maturation. The flow within the collecting lymphatics is not only necessary for vessel formation (Sweet et al., 2015) and controlled by the contractility of surrounding smooth muscle cells but also appears to be modulated by the innervation of LECs, intralymphatic fluid pressure, and shear stress (Breslin, 2014; Kunert et al., 2015; Munn, 2015).

Dysfunction of the lymphatic vasculature results in disruption of the ISF balance and induces the formation of local edema or lymphedema (Brouillard et al., 2014). Primary lymphedema is caused by a developmental failure of the lymphatic system, leading to structural and/or functional impairment of the lymphatic vasculature (Brouillard et al., 2014). Several genes are involved in the development of the lymphatic vasculature and are associated with the development of lymphedema. The vascular endothelial growth factor *C*/VEGF receptor-3 (VEGF-C/VEGFR-3) signaling pathway, FOXC2, collagen- and calcium-binding EGF

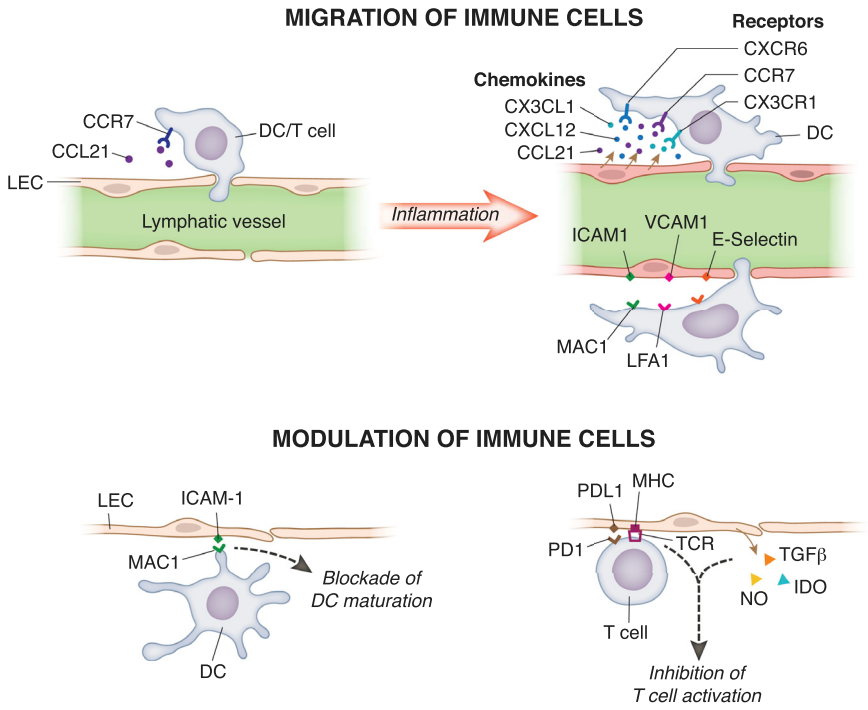


**Figure 1. Modulation of Immune Cell Migration and Function by Lymphatic Endothelial Cells**

Lymphatic endothelial cells (LECs) are able to secrete chemokine (C-C motif) ligand 21 (CCL21) and promote the entry of dendritic cells (DCs) and T cells into the lymphatic vasculature in a chemokine receptor type 7 (CCR7)-dependent manner. Upon an inflammatory stimulus, LECs produce chemokine (C-X3-C motif) ligand 1 (CX3CL1) and chemokine (C-X-C motif) ligand 12 (CXCL12), which bind the chemokine receptors CX3CR1 and CXCR4 on the surface of DCs and further facilitate their migration through the lymphatic vasculature. Adhesion and entry of the immune cells into the lymphatic vasculature are also promoted by the expression of intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and E-selectin by LECs. In the lymph nodes, LECs are also able to inhibit the maturation of DCs through an ICAM-1/macrophage-1 antigen (MAC-1)-dependent mechanism and the activation of T cells by releasing nitric oxide (NO), indoleamine 2,3-dioxygenase (IDO), and transforming growth factor (TGF)-β and direct interaction between major histocompatibility complex (MHC)/T cell receptor (TCR) and programmed death-ligand 1 (PD-L1)/programmed death 1 (PD-1).

domain-containing protein 1 (ccbe1), GATA2, and gap junction gamma 2 (GJC2) are associated with specific forms of primary lymphedema (Brouillard et al., 2014). A secondary and more common form of lymphedema is caused by lymphatic dysfunction resulting from infection (filariasis) or cancer (in the latter case as a direct effect or a secondary effect due to treatment) (Douglass et al., 2016; Newman et al., 2012).

### *Immune Surveillance*

The lymphatic system not only plays a major role in maintaining ISF balance, but is also a prime player in regulating immune responses and immune surveillance of tissues (Betterman and Harvey, 2016; Card et al., 2014).

Both dendritic cells (DCs) and T cells actively migrate from tissues to draining lymph nodes and are then returned to the blood circulation via the lymphatic vasculature (Teijeira et al., 2013). The chemokine (C-C motif) ligand (CCL)21/chemokine receptor type (CCR)7 pathway is the major chemokine-signaling pathway for the entry of both T cells and DCs from the tissues into the lymphatic vasculature (Debes et al., 2005; Ohl et al., 2004; Saeki et al., 1999; Weber et al., 2013) (Figure 1). The chemokine (C-X-C motif) ligand (CX3CL)1/CX3CR1 (Johnson and Jackson, 2013) and CXCL12/CXCR4 (Kabashima et al., 2007; Torzicky et al., 2012) pathways also appear to play a part in DC migration. Their role, however, seems to be minor compared with that of CCL21/CCR7 and restricted to inflammatory conditions, in which application of tumor necrosis factor and haptens induces the expression of CX3CL1 and CXCL12 by LECs (Johnson and Jackson, 2013; Kabashima et al., 2007) (Figure 1).

The existence of a lymphatic flow appears to be important for immune cell trafficking, and lack of lymphatic drainage greatly decreases the LEC-induced expression of CCL21 (Johnson and Jackson, 2010; Miteva et al., 2010; Tomei et al., 2009). Mice treated with the sphingosine-1-phosphate receptor 1 (S1PR1) analog FTY720 were recently shown to exhibit reduced migration of DCs from the skin to the lymph nodes, suggesting a role for S1P-S1PR1 in the migration of immune cells through the lymphatic vasculature (Czeloth et al., 2005; Gollmann et al., 2008). Immune cell trafficking can also be regulated by other LEC-produced signals, such as Sema3a, which binds to plexin-1 and neuropilin 1 on DCs, to regulate the migration of immune cells across the lymphatic endothelium (Takamatsu et al., 2010). Inflammatory conditions induce LEC upregulation of adhesion molecules such as CLEVER-1/stabilin-1 (Karikoski et al., 2009), intercellular adhesion molecule 1 (ICAM-1) (Rouzaut et al., 2010; Vigl et al., 2011), vascular cell adhesion molecule 1 (VCAM-1) (Malhotra et al., 2012; Vigl et al., 2011), and E-selectin (Johnson et al., 2006; Sawa and Tsuruga, 2008) to facilitate the migration of DCs into the lymphatic vasculature, thereby promoting generation of an immune response (Figure 1). Even though collecting lymphatics (unlike initial lymphatics) are impermeable, especially to immune cells, they evidently express the chemokine CCL27 and are important for the trafficking of CCR10-expressing T cells (Wick et al., 2008). Interestingly, DCs were recently shown to be present within the walls of collecting lymphatic vessels in adipose tissue (Kuan et al., 2015). These DCs help to maintain the contractility of collecting lymphatic vessels while also preventing lymphatic wall thickening and collagen deposition (Ivanov et al., 2016; Kuan et al., 2015). It is not yet known whether the DCs can directly enter the collecting lymphatics under normal or inflammatory conditions.

Recent studies have demonstrated that LECs can interact directly with immune cells and hence shape an immune

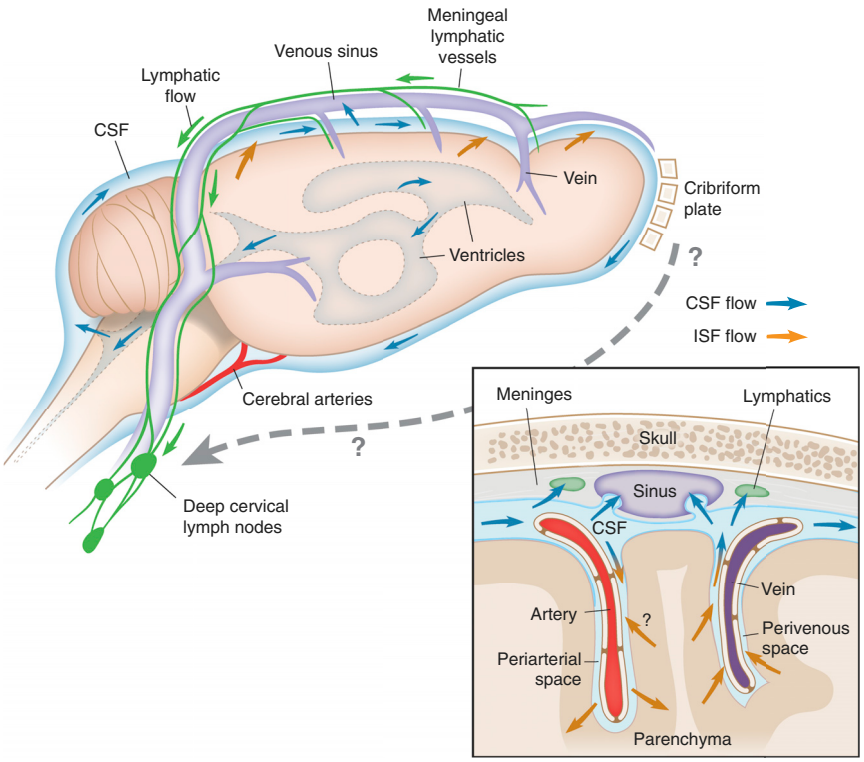


**Figure 2. Pathways of Fluid Circulation and Drainage in the Brain**

The glymphatic system results from the paravascular bulk flow of interstitial fluid (ISF) into the cerebrospinal fluid (CSF) and is, to some extent, responsible for the removal of macromolecules and hydrophilic compounds from the brain parenchyma into the CSF. The circulation of ISF is driven by arterial pulsation along the basement membrane of the brain blood vessels toward the leptomeninges (orange arrows). On the other hand, the CSF recirculates from the brain ventricles to the subarachnoid meningeal space between the pia and the arachnoid mater (blue arrows). The two major routes of drainage of the aqueous content of the CSF are (1) the arachnoid granulations, which drain directly into the major veins (sinuses) in the dura mater, and (2) the olfactory nerves crossing the ethmoid plate into the lymphatic network of the nasal mucosa. Of note, the recently described conventional meningeal lymphatic vasculature that is capable of draining macromolecules and immune cells from the meningeal spaces and the brain parenchyma into the deep cervical lymph nodes (green arrows) prompted the reassessment of the contribution of each route to the drainage of the aqueous CSF content and, specifically, to the removal of macromolecules and immune cells from the CNS.

response (Betterman and Harvey, 2016; Card et al., 2014). LECs express major histocompatibility complex (MHC) I (Cohen et al., 2010; Lund et al., 2012; Nichols et al., 2007) and, presumably, even MHC II (Malhotra et al., 2012; Tewalt et al., 2012) molecules and can directly interact with T cells to induce tolerance. LECs secrete immunosuppressive molecules such as transforming growth factor (TGF)-β (Malhotra et al., 2012), indoleamine 2,3-dioxygenase (IDO) (Nörder et al., 2012), and nitric oxide (Lukacs-Kornek et al., 2011), and exhibit high expression levels of the inhibitory receptor programmed death ligand 1 (PD-L1) together with low levels of the co-stimulatory molecules cluster of differentiation (CD)80 and CD86 to promote immune tolerance (Christiansen et al., 2016; Tewalt et al., 2012). This system has been well described in cancer and could potentially also apply to other systems (Figure 1). Most of those studies are focused on LECs of lymph nodes, although a similar mechanism could presumably operate in tissue-associated LECs. ICAM-1 expressed by tissue LECs during inflammatory conditions has indeed been shown, through its interaction with macrophage-1 antigen (MAC-1), to attenuate the maturation of DCs and diminish the ability of these cells to activate effector T cells (Podgrabinska et al., 2009) (Figure 1).

## The Meningeal Lymphatic System and Cerebrospinal Fluid

Despite some experimental evidence to the contrary (Andres et al., 1987; Foldi et al., 1963), the CNS was long considered to be devoid of a dedicated and conventional lymphatic system. Unusual drainage pathways for the CNS ISF have been proposed over the last several decades (Bakker et al., 2016; Laman and Weller, 2013). Recent advances in imaging, however, led to the conceptualization of a system responsible for the removal of ISF into the cerebrospinal fluid (CSF), mostly around the veins (Iliff et al., 2012; Rennels et al., 1985), but not exclusively (Carare et al., 2008; Schley et al., 2006) (Figure 2). Dubbed the glymphatic system (Jessen et al., 2015; Xie et al., 2013), this clearance pathway arises from the bulk flow of ISF and is responsible for carrying away macromolecules and hydrophilic compounds from the brain parenchyma into the CSF (Iliff et al., 2012; Rangroo Thrane et al., 2013) (Figure 2). This paravascular circulation of ISF seems to be driven by arterial pulsation along the basement membrane of the blood-brain barrier (BBB) toward the leptomeninges and is facilitated not only by smooth muscle cells but also by astrocytes (Iliff et al., 2012; Xie et al., 2013). As it would be described later, this pathway appears particularly important for the removal of amyloid beta (Aβ) from the brain parenchyma.

The CSF is sometimes described as the second ISF of the CNS. Produced by the epithelial cells of the choroid plexus, the CSF recirculates from the brain ventricles to the subarachnoid meningeal space between the pia and the arachnoid mater (Figure 2). Several routes for CSF removal have been proposed over the years. A major part of the aqueous content of the CSF drains through arachnoid granulations, directly into the major veins (sinuses) in the dura mater (Boulton et al., 1996). This system is implicated in the maintenance of intracranial pressure, but is unlikely to serve as the route for the exit of macromolecules or immune cells from the CNS. In patients with hydrocephaly and increased intracranial pressure, the arachnoid granulations are indeed frequently found to be malformed (Jeltema et al., 2014; Sugimoto et al., 2016). A second route for CSF removal is along the olfactory bulb across the ethmoid plate to reach the

lymphatic network associated with the nasal mucosa (Johnston et al., 2004; Kida et al., 1993). This route also appears to be important for removal of the CSF water content, as its surgical blockade results in increased intracranial pressure (Mollanji et al., 2002). Macromolecules (Kida et al., 1993; Nagra et al., 2006) and immune cells (Goldmann et al., 2006; Kaminski et al., 2012) were suggested to leave the CNS through this route.

Two independent studies recently demonstrated the presence of a functioning conventional lymphatic vasculature in the meninges, capable of draining macromolecules and immune cells from the meningeal spaces (Louveau et al., 2015a) and the brain parenchyma (Aspelund et al., 2015) into the deep cervical lymph nodes. This discovery calls for a reassessment of the contribution of each route to the drainage of the water content and the removal of macromolecules and immune cells from the CNS in healthy and diseased brains.

## Disease Relevance

Multiple neurological disorders are associated with inflammatory response and accumulation of toxic debris and molecules in the brain interstitium. Taking into account the function of meningeal lymphatic system, it is plausible that this system may play an important role in initiation and progression of CNS diseases associated with either neuroinflammation or debris accumulation (or both). In the following sections, we will provide insight into the potential role of meningeal lymphatic system in two major neurological disorders, Alzheimer's disease (AD) and multiple sclerosis (MS), and possible implications to other neurological disorders will also be discussed.

## Amyloidosis in the Alzheimer's Brain

AD is the most prevalent form of dementia worldwide (Andrieu et al., 2015) and is distinctively characterized by early and marked cognitive impairment (Andrieu et al., 2015; Ballard et al., 2011). The vast majority (~98%) of AD cases are sporadic (Blennow et al., 2006), and in such cases the etiology of the amyloid pathology is poorly understood (Benilova et al., 2012; Blennow et al., 2006). This is in contrast to familial AD, where rare hereditary dominant mutations in amyloid precursor protein (APP) or in presenilins 1 and 2 drive the uncontrolled formation of Aβ (Hardy and Selkoe, 2002). The foremost risk factor for sporadic AD is age. However, increased risk of this form of AD has also been attributed to diverse genetic abnormalities. One of them is diploidy for apolipoprotein-Eε4 (Apo-Eε4), widely viewed as a major genetic risk factor promoting both early onset of Aβ aggregation and defective Aβ clearance from the brain (Deane et al., 2008; Zlokovic, 2013). Other genetic variants that significantly increase the risk for sporadic AD are Apo-J (or clusterin), phosphatidylinositol-binding clathrin assembly protein (PICALM), complement receptor 1 (CR1), CD33 or Siglec-3, and triggering receptor expressed on myeloid cells 2 (TREM2). All of these proteins, interestingly, have been implicated in different mechanisms of Aβ removal from the brain (Bertram et al., 2008; Guerreiro et al., 2013; Harold et al., 2009; Lambert et al., 2009, 2013; Naj et al., 2011). Despite the ongoing validation of biomarkers, postmortem analysis of the brain is still required for a definitive diagnosis of sporadic AD (Bateman et al., 2012; Fagan et al., 2014). The brain's pathological hallmarks of AD are intracellular neurofibrillary tangles and extracellular amyloid plaques, the latter being a product of the amyloidogenic processing of APP and the resulting deposition of Aβ in the brain parenchyma (Benilova et al., 2012; Hardy and Selkoe, 2002; Ittner and Götz, 2011).

Increasing aggregation of diffusible Aβ peptides from the ISF and the CSF into toxic oligomeric intermediates and their accumulation in the brain parenchyma (Hong et al., 2011; Iliff et al., 2012) are believed to be precipitating factors for different neuroinflammatory abnormalities (Guillot-Sestier et al., 2015; Hong et al., 2016; Matarin et al., 2015), such as the formation of neurofibrillary tangles (Ittner and Götz, 2011) and the pronounced neuronal dysfunction (Palop et al., 2007; Sun et al., 2009; Walsh et al., 2002) in the AD brain. The finding that patients with sporadic AD exhibit impaired Aβ clearance without any obvious changes in de novo production of Aβ (Mawuenyega et al., 2010; Potter et al., 2013) highlights the critical importance of Aβ clearance mechanisms and their potential impairment in leading to the formation of amyloid plaques and the aggravation of cognitive deficits (Bard et al., 2000; Iliff et al., 2012; Sagare et al., 2007). Altogether, the impaired clearance of Aβ combined with the rapid aggregation of these peptides, especially of $A\beta_{1-42}$ (Suzuki et al., 1994), may lead to serious vascular clogging as well as damage to the cells responsible for fluid drainage and macromolecule clearance from the brain. The ultimate result is severe amyloidosis and overall brain cell dysfunction. Removal of Aβ from the CNS takes place via various processes, including phagocytosis, proteolytic degradation by mononuclear and vascular smooth muscle cells, transcytosis across the BBB, and through the glymphatic system (Tarasoff-Conway et al., 2015). The following sections provide an overview of the mechanisms of Aβ excretion and clearance from the brain described to date, focusing on transcytosis and glymphatic drainage (Figure 3). An understanding of these mechanisms might offer an insight into the possible contribution of the recently discovered meningeal lymphatic vessels (Louveau et al., 2015a) to Aβ clearance from the CNS (Figure 3).

## Aβ at the Neurovascular Unit

The BBB is a critical route for ISF drainage and the exchange of molecules between the brain interstitium and the blood. The barrier is formed by endothelial cells that are bound together by tight junctions and enclosed by an acellular basement membrane, together forming a selectively permeable interface (Brightman and Reese, 1969; Zlokovic, 2008). The BBB endothelium, together with pericytes and smooth muscle cells, astrocytic endfeet, and branches of circulating surveying microglia, forms the "neurovascular unit" (Armulik et al., 2010; Begley and Brightman, 2003; Zlokovic, 2008). Molecular transport across the neurovascular unit depends on the weight, size, and hydrophobicity of a given molecule (Wong et al., 2013). The Aβ monomers, particularly $A\beta_{1-42}$, present a highly hydrophobic carboxyl terminus (Li et al., 2010), which favors their aggregation into low molecular weight oligomers and subsequently, by interacting with adjacent Aβ oligomers, into high molecular weight fibrillar oligomers (Wu et al., 2010). Although Aβ peptides can easily cross through glial endfeet, their exchange between the ISF and blood at the BBB can be achieved only through specialized

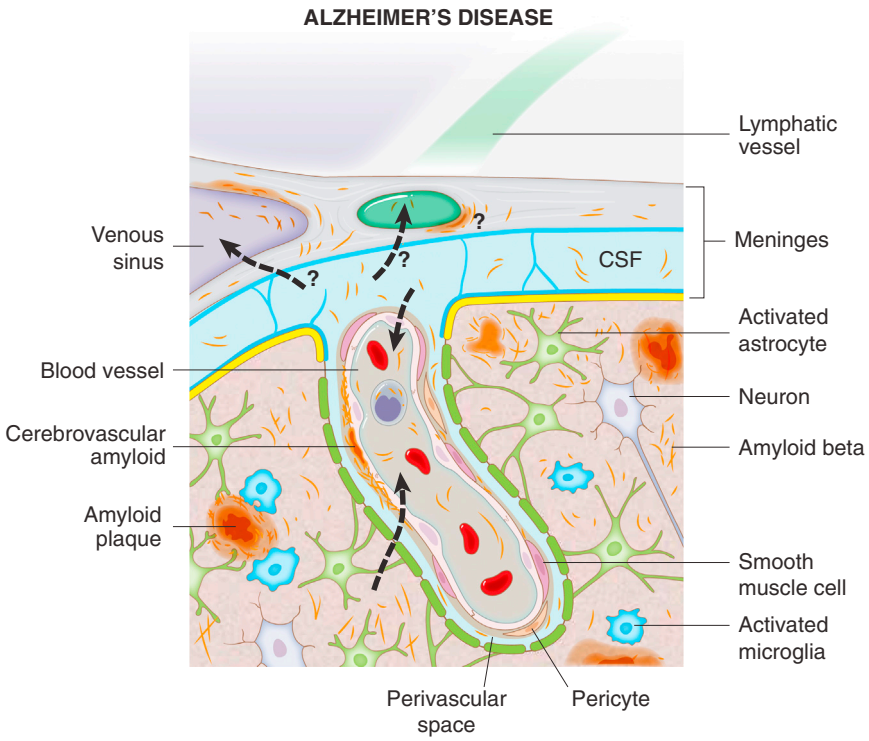


**Figure 3. Hypothetical Role of the Meningeal Lymphatic System in Amyloid Beta Drainage from the Brain Fluids**

The increased accumulation of amyloid beta (Aβ) peptides and consequent formation of extracellular amyloid plaques in the brain parenchyma in Alzheimer's disease (AD) lead to the buildup of an inflammatory milieu that results in glial activation, which becomes inefficient in clearing extracellular amyloid, and neuronal dysfunction. The mechanisms that promote the efflux of Aβ from the brain parenchyma into the blood circulation at the neurovascular unit, as well as the production of proteins that scavenge Aβ from the CSF, become progressively obsolete with the worsening of vascular amyloid deposition (dashed arrows). Whether the function of the meningeal vasculature, and of the lymphatic vessels in particular, is also affected by the high levels of Aβ peptides present in the brain fluids in AD remains unanswered (dashed arrows). Moreover, the ability of the lymphatic vessels to modulate the levels of Aβ, either by draining the subarachnoid CSF or by indirectly modulating the levels of Aβ in the ISF and its paravascular clearance through the glymphatic pathway, is also an important topic that warrants due attention.

transporters that the endothelial cells express (Wong et al., 2013; Zlokovic, 2004). The progressive accumulation of Aβ in the brain vasculature, however, often culminates in cerebral amyloid angiopathy (CAA), a pathological feature that correlates well with the extent of cognitive impairment in AD (Greenberg et al., 2004; Thal et al., 2008). Amyloid deposits, in the form of ring-like structures wrapped around the brain's blood vessels in a way that prevents contact between the astrocytic endfeet and the endothelial cell wall, compromise the functioning of both the astrocytes and the vascular smooth muscle cells and impair the cerebral blood flow (Kimbrough et al., 2015). Therefore, the high prevalence of CAA in AD patients evidently results from the combination of a decreased efflux and an increased influx of Aβ peptides across the BBB (Zlokovic, 2004).

Efflux of Aβ from the ISF into the blood is largely influenced by the expression of p-glycoprotein (Pgp) and low-density lipoprotein receptor-related protein 1 (LRP1) by the vascular endothelial cells (Erickson et al., 2012; Hartz et al., 2016; Shibata et al., 2000). Pgp is less efficient than LRP1 in promoting Aβ excretion; it is possible (though unconfirmed) that Pgp modulates the abluminal efflux of Aβ by boosting the interaction between Apo-E and Aβ in the vicinity of the blood vessels, thereby indirectly promoting the excretion of Apo-E/Aβ complexes through binding to LRP1 (Akanuma et al., 2008; Fitz et al., 2012; Lam et al., 2001). Likewise, the rate of Aβ transport by LRP1 is largely modulated by its ligands, Apo-E and α2-macroglobulin (Blacker et al., 1997; Corder et al., 1993; Liao et al., 1998). Human Apo-Eε2 or Apo-Eε3 proteins, in contrast to Apo-Eε4, show a relatively high affinity for LRP1 and are therefore more efficient in promoting Aβ excretion through the BBB (Bell et al., 2012; Deane et al., 2008). Thus, decreased LRP1 expression in the vascular endothelial cells and pericytes is associated with a marked decrease in the Apo-E-dependent removal of soluble Aβ (Bell et al., 2012; Deane et al., 2004, 2008; Liu et al., 2007). Reduced LRP1 levels in the brain endothelium are indeed observed in aging rodents and are associated with the impaired Aβ clearance and cerebrovascular accumulation of these toxic peptides observed in AD patients (Shibata et al., 2000). In addition, the PICALM protein, whose genetic variants are associated with a greater risk for sporadic AD, was recently shown to be capable of regulating Aβ efflux through the BBB (Zhao et al., 2015). Reduced expression of PICALM by endothelial cells, observed both in AD mice and in patients with AD, was found to correlate with increased brain Aβ pathology and cognitive impairment. Specifically, PICALM deficiency diminishes Aβ clearance across the mouse BBB by modulating the PICALM/clathrin-dependent internalization of LRP1-bound Aβ, as well as by guiding Aβ trafficking to the endocytosis-regulator proteins Rab5 and Rab11, thus leading to Aβ endothelial transcytosis and clearance (Zhao et al., 2015).

The luminal to abluminal influx of Aβ at the BBB endothelium in AD patients is mediated to a greater extent by the increased expression of the immunoglobulin superfamily receptor for advanced glycation end products (RAGE) (Deane et al., 2003; Zlokovic et al., 1996) and, to a lesser extent, by LRP2 (megalin) (Zlokovic et al., 1996). Under physiological conditions, the mechanism of Aβ influx via LRP2 is minimized by its saturation with Apo-J (Shayo et al., 1997; Zlokovic et al., 1996). In contrast, RAGE binds soluble Aβ in the nanomolar range and can mediate the rapid transport of unbound Aβ into the brain, resulting in a significant increase in cerebrovascular and brain parenchymal Aβ if the RAGE-mediated influx is not counterbalanced by efflux mechanisms from the CNS (Deane et al., 2003; Mackic et al., 1998). With the objective of decreasing the levels of circulating

Aβ peptides and thus preventing interaction between Aβ and RAGE, different strategies have been developed, such as treatment with soluble circulating LRP1 or with anti-Aβ antibodies, resulting in significant improvement in terms of reducing the degree of brain amyloidosis in AD (Bard et al., 2000; Pepys et al., 2002; Sagare et al., 2007).

Besides endothelial cells, pericytes—which, as mentioned earlier, are crucial in maintaining BBB cytoarchitecture, integrity, and overall function (Armulik et al., 2010)—also play a central role in the progression of vascular damage in AD. They do this by modulating BBB permeability and hence the degree of Aβ pathology (Sagare et al., 2013). Loss of pericytes in a mouse model of AD increases Aβ retention in the brain and accelerates CAA by diminishing the clearance of toxic Aβ peptides from brain ISF prior to their deposition in plaques (Sagare et al., 2013). Moreover, activation of cyclophilin A and the downstream matrix metalloproteinase 9 pathway in capillary pericytes results in an age-dependent progressive BBB breakdown (Bell et al., 2012). Curiously, this pathway precedes neuronal dysfunction and can be suppressed by expression of human Apo-Eε2 or Apo-Eε3 (Bell et al., 2012; Deane et al., 2008).

Astrocytic dysfunction is observed in different AD models (Furman et al., 2012; Orre et al., 2014) and is also associated with changes in Aβ clearance at the neurovascular unit (Iliff et al., 2012; Wyss-Coray et al., 1997). Astrogliosis resulting from exacerbated neuroinflammation, the accumulation of Aβ, and the attempt to degrade these toxic peptides (Wyss-Coray et al., 2003) leads to reduced expression of both BBB and neuronal support genes (Orre et al., 2014). Moreover, impaired astroglial function at the neurovascular unit leads to decreased paravascular clearance of solutes from the brain interstitium (this pathway is further explored below), namely of Aβ from the ISF (Iliff et al., 2012). Furthermore, increased production of the cytokine TGF-β by astrocytes in a mouse model of AD leads to a significant decrease in parenchymal Aβ accumulation and plaque formation, but also an increased degree of CAA (Wyss-Coray et al., 2001). This effect of TGF-β seems, moreover, to be mediated by increased Aβ phagocytosis by microglia in the brain parenchyma, but decreased phagocytosis by macrophages and microglia in the vicinity of the BBB vasculature (Weiss et al., 2011; Wyss-Coray et al., 1997). This last observation also highlights the importance of myeloid phagocytic cells in AD pathogenesis. Different studies reported that monocytes are recruited to the lumen of small veins that present amyloid deposits in the brain of AD transgenic mice and effectively engulf Aβ peptides, modulating the progression of amyloid pathology (Jay et al., 2015; Michaud et al., 2013). This process is modulated by the expression of scavenger receptor class B type I (SR-BI) by the astrocytes and smooth muscle cells that compose the neurovascular unit, which was shown to promote the adhesion of perivascular macrophages and the clearance of amyloid deposited in the brain vasculature in a mouse model of AD (Thanopoulou et al., 2010). In fact, some of the newly described polymorphisms that are associated with an increased risk for AD affect molecules that have the ability to modulate the phagocytic function of recruited monocytes/macrophages, namely CD33 and TREM2 (Griciuc et al., 2013; Guerreiro et al., 2013; Naj et al., 2011; Tanzi, 2015). However, regarding the altered expression of TREM2 in particular, more studies are needed in order to fully understand its consequences on mononuclear cell recruitment, neurovascular dysfunction, and brain pathology in AD (Jay et al., 2015; Tanzi, 2015; Wang et al., 2015).

## Choroid Plexus in AD

The choroid plexus is a highly vascularized tissue that is present in all brain ventricles, and whose primary function is the production and renewal of the main constituents of the CSF (Johanson et al., 2004; Liddelow et al., 2012). The inner stroma of the choroid plexus is richly irrigated by fenestrated capillaries (Wolburg and Paulus, 2010), allowing the free passage of circulating molecules and immune cells into this compartment. Under healthy conditions, however, the blood-CSF barrier (BCSFB) formed by the choroid plexus epithelial cells restricts immune cell entry into the CSF and the brain parenchyma (Baruch and Schwartz, 2013; Engelhardt et al., 2001; Hasegawa-Ishii et al., 2013). AD patients exhibit major alterations in choroid plexus structure and morphology, as well as in epithelial cell function, attributable mainly to the increase in toxic Aβ peptides (Brkic et al., 2015; Johanson et al., 2004; Vargas et al., 2010). In addition, the ability of epithelial cells to express the transporters that participate in Aβ efflux, as well as to secrete Aβ-scavenging proteins into the CSF and their corresponding receptors, decreases with age and is compromised in different in vitro and in vivo models of AD (Antequera et al., 2009; Crossgrove et al., 2005; Zlokovic et al., 1996). One of the receptors that participates in the removal of Aβ from the CSF is LRP2, which interacts with different Aβ-carrier proteins such as albumin, transthyretin, and Apo-J (Zlokovic et al., 1996) through a process modulated by insulin-like growth factor 1 (IGF-1) (Carro et al., 2002, 2005). Moreover, many of the transporters involved in Aβ exchange at the BBB, such as LRP1, Pgp, and RAGE, are also present at the BCSFB (Marques et al., 2013). Curiously, in AD the LRP1 and Pgp levels at the BCSFB are increased, plausibly as a compensatory mechanism for the observed decrease in Aβ efflux through the same transporters at the BBB (Pascale et al., 2011). The overall clearance of Aβ from the ventricular CSF occurs much more rapidly than its clearance rate at the arachnoid villi, further highlighting the importance of the expression of Aβ transporters and Aβ-scavenging molecules at the BCSFB (Erickson and Banks, 2013; Marques et al., 2013).

Recent studies show that the choroid plexus also engages in processes of immune cell recruitment and neuroinflammation both in mouse models of healthy aging and AD (Baruch et al., 2013, 2014, 2015). Increased secretion of immune-related molecules by the cells (epithelial and stromal) that comprise the choroid plexus, namely CCL11/eotaxin and type I interferons, is viewed as a critical turning point for deficits in brain cell function and plasticity and for alterations in glial activation and amyloid pathology (Baruch et al., 2013, 2014; Kunis et al., 2013; Mesquita et al., 2015; Villeda et al., 2011).

Particularly in AD, the decreased expression of interferon-γ (IFN-γ) and the regulatory T cell-driven immunosuppression at the choroid plexus seem to be closely associated with the rapid progression of brain parenchymal amyloidosis and memory impairment (Baruch et al., 2015; Mesquita et al., 2015). Interestingly, blockade of the PD-1 immune checkpoint was able to

restore IFN-γ signaling in the choroid plexus of AD mice and promote the CCL2-dependent recruitment of myeloid cells, a mechanism that was proposed to facilitate the cleansing of Aβ from the brain (Baruch et al., 2016).

Recent data have shown, however, that the BCSFB is not a preferential site of immune cell entry into the CSF (Schläger et al., 2016). It is therefore still debatable whether the neuroinflammatory choroid plexus changes in AD can modulate amyloidosis through the recruitment and entry of Aβ-cleansing myeloid phagocytes into the brain, or if this is achieved by the increased secretion of Aβ-scavenging molecules into the CSF, increased drainage of Aβ from the ISF into the ventricular CSF, and increased excretion through either the BCSFB or the recently described meningeal lymphatics (Louveau et al., 2015a) (Figure 3).

## Glymphatics and Paravascular Aβ Clearance

The glymphatic-mediated clearance of ISF, described earlier, is highly relevant to AD because, as mentioned above, the marked deposition of Aβ in the AD brain vasculature is believed to be a result of its impaired clearance. Failure of the paravascular ISF bulk-flow pathways, together with the consequent progressive damage to the BBB, is reportedly associated with the increased degree of CAA observed in AD patients and animal models (Hawkes et al., 2014; Kimbrough et al., 2015; Weller et al., 2008). Aging, the main risk factor for sporadic AD, affects the functioning of paravascular ISF bulk-flow mechanisms and leads to CAA, mainly owing to age-related morphological changes in the vessels and specifically to arterial degeneration (Hawkes et al., 2011, 2013; Kress et al., 2014). Moreover, Apo-Eε4, the major genetic risk factor for sporadic AD, can also affect not only the integrity of the neurovasculature but also the paravascular drainage of Aβ (Bell et al., 2012; Chakrabarty et al., 2015), both probably associated with the deleterious effects of Apo-Eε4 on pericyte and glial function (Bell et al., 2012; Chakrabarty et al., 2015). Nevertheless, it is now widely accepted that the proper functioning of smooth muscle cells, pericytes, and astrocytes is of great importance for the maintenance of ISF perivascular drainage, and particularly for the removal of Aβ through the glymphatic pathway (Iliff et al., 2012; Kimbrough et al., 2015). Astrocytic expression of aquaporin 4 (AQP4) is essential for the proper flow of CSF through the interstitium, and the most striking effects on the glymphatic pathway were obtained by the modulation of AQP4 levels (Iliff et al., 2012); following intracerebral injection, the clearance of interstitial $A\beta_{1\text{-}40}$ through the glymphatic pathway was reduced by ≈55% in Aqp4 null mice relative to wild-type controls. This observation suggested that more than half of the soluble Aβ present in the ISF is cleared through the glymphatic pathway rather than through BBB transport mechanisms (Iliff et al., 2012). On the other hand, injection of $A\beta_{1\text{-}40}$ into the cisterna magna resulted in a significant accumulation of these peptides in the interstitium, which was probably indicative of CSF/Aβ recirculation through the glymphatic pathway (Iliff et al., 2012). Strikingly, Aβ drainage through the glymphatic pathway is more efficient during sleep (Xie et al., 2013). Sleep is accompanied by an increase in perivascular space, thereby boosting the drainage of Aβ from the brain parenchyma (Xie et al., 2013). During sleep, about 40% of the Aβ present in the ISF was found to be cleared into the CSF, and it was postulated that the rest of the interstitial Aβ is flushed out into the paravascular spaces and cleared by the BBB, which might also be more efficient at draining brain metabolites in the sleeping rather than in the awake state (He et al., 2014; Xie et al., 2013). This response might be mediated by changes in norepinephrine signaling in the brain during sleep or even by changes in astrocytic AQP4 (Iliff et al., 2012; O'Donnell et al., 2015; Xie et al., 2013). Constitutive loss of AQP4 in APP/PS1 transgenic mice was recently shown to aggravate cognitive deficits and to lead to an increase in Aβ accumulation and in CAA as well as a decrease in hippocampal and cortical synaptic proteins and brain-derived neurotrophic factor (Xu et al., 2015). This worsened phenotype is probably attributable to impaired glymphatic clearance of amyloid in this AD mouse model. However, despite the well-described dysfunction of astrocytes in the AD brain (Abramov et al., 2004; Furman et al., 2012; Medeiros and LaFerla, 2013; Orre et al., 2014) and the effects of an impaired glymphatic pathway in the aged brain (Kress et al., 2014) and in Tau pathology exacerbation in a traumatic brain injury model (Iliff et al., 2014), there is still no reported study, either in transgenic AD models or in AD patients, that focuses on the role of the glymphatic system in the development of amyloid brain pathology and cognitive impairment. Further investigation will also be needed to determine whether other drainage pathways, such as the meningeal lymphatics, play a role in this process of glymphatic modulation of the ISF-to-CSF bulk flow of Aβ.

## Meningeal Lymphatics and AD: A Hypothesis

The meningeal lymphatic vasculature might play a central role in AD. Lack of meningeal lymphatics in transgenic mice indeed delays the elimination of macromolecules injected into the brain parenchyma (Aspelund et al., 2015), pointing to a role for the meningeal lymphatic vasculature in the paravascular removal of macromolecules. Conceivably, dysfunction of the CNS lymphatic system might even initiate or favor the paravascular accumulation of Aβ observed in CAA (Figure 3). Of note, although physically separated from the meningeal lymphatic vessels, the brain vasculature is bathed in ISF, which in turn is in direct communication with the CSF (Iliff et al., 2012). This linkage provided by the ISF-CSF nexus could potentially be modulated by the CNS lymphatic vasculature, which would underlie both the neurovascular changes and brain amyloid pathology observed in AD. Additionally, taking the role of the glymphatic pathway in Aβ paravascular clearance and the recent reports of impaired glymphatic function in a mouse model of AD (Iliff et al., 2012; Peng et al., 2016), it will be important to investigate the extent to which a dysfunctional lymphatic system can underlie the glymphatic impairment in AD. Further studies are needed in order to assess the role of the meningeal lymphatic vasculature in Aβ clearance and in AD pathology as a whole.

As described above, several receptors can bind Aβ and participate in its removal and degradation from the brain parenchyma. Interestingly, LECs express multiple scavenging receptors, namely SR-B1, (Lim et al., 2013) which binds fibrillar Aβ and affects its clearance by myeloid cells (Thanopoulou et al., 2010). Transvascular clearance of Aβ through the lymphatic endothelium might be a mechanism that participates in the removal of Aβ from the CSF. Whether other important Aβ receptors, such as RAGE or LRP1, are also expressed by meningeal LECs is unknown and needs to be further investigated. Moreover,

studies are needed in order to understand the consequences of increased Aβ accumulation for the molecular profile and function of LECs. Accumulation of Aβ in the CSF of AD patients during early phases of the disease (Bateman et al., 2012; Hong et al., 2011) might indeed induce dysfunction of the lymphatic vasculature and trigger a vicious cycle leading to the intraparenchymal aggregation of Aβ.

The meningeal lymphatic vasculature represents a potential therapeutic target to facilitate the removal of macromolecules from the CNS and to regulate its immune responses. Similar to peripheral lymphatics, meningeal lymphatic network expresses VEGFR-3. Treatment with VEGF-C promotes the growth and proliferation of LECs and increases the lymphatic drainage from peripheral tissues (D'Alessio et al., 2014; Kajiya et al., 2009; Xu et al., 2010). It would therefore be interesting to examine the effects of VEGF-C treatment on the parenchymal aggregation of Aβ, neuronal function, and its associated behaviors that arise in the course of AD.

## Meningeal Immunity in MS/Experimental Autoimmune Encephalomyelitis

As mentioned above, the lymphatic vasculature is responsible not only for removal of debris but also regulation of tissue immune surveillance. Therefore, speculating on a possible role of meningeal lymphatic vessels in neuroinflammatory disease is tempting.

MS is an autoimmune neurological disease in which the immune system targets and damages myelin sheaths, leading to neuronal dysfunction and associated devastating motor and cognitive impairments (Compston and Coles, 2002, 2008; Liblau et al., 2013; Weiner, 2004). MS affects ≈2.5 million people worldwide. Its etiology remains unknown, but both genetic predisposition and environmental factors have been implicated in its development. Indeed, geographic locations toward the equator and certain infections appear to have some influence (Farez et al., 2015; Goodin, 2014). Nonetheless, the factor initiating the autoimmune attack on the brain has yet to be identified. Therefore, understanding how immune responses to brain antigens are generated is one of the major challenges in MS research.

## Drainage of Antigens from the CNS

The "immune privilege" that was traditionally attributed to the CNS was thought to be due in part to its lack of lymphatic drainage (Louveau et al., 2015b). However, the finding that fluorescent tracers and macromolecules injected into the brain parenchyma or the CSF reach the cervical lymph nodes (Kida et al., 1993; Laman and Weller, 2013; Rennels et al., 1990) suggested that a system for drainage of CNS macromolecules exists. Not only do CNS antigens drain into local lymph nodes, but the drainage efficiency appears similar to peripheral tissue (Harris et al., 2014). Moreover, the observed presence of autoreactive T cells in the blood of both healthy volunteers and MS patients indicates that the mere presence of these T cells is not by itself sufficient to trigger MS initiation (MeinL et al., 1997; Pette et al., 1990). Given the normal drainage of CNS antigens to the cervical lymph nodes, it seems reasonable to postulate that in healthy patients there are mechanisms preventing the activation of autoreactive T cells (or, vice versa, mechanisms that activate autoreactive T cells are present in MS patients).

Massive acutely induced death of oligodendrocytes (in transgenic mouse model expressing diphtheria toxin receptor on oligodendrocytes) was recently shown to lead to the generation of autoreactive T cells against the immunodominant epitope of the myelin-oligodendrocyte glycoprotein (MOG) (Traka et al., 2010, 2016). This epitope is usually used as an exogenous peptide to induce experimental autoimmune encephalomyelitis (EAE) in C57BL/6 mice (Croxford et al., 2011; Slavin et al., 1998). That study suggested that the drainage of oligodendrocytic remnants, either directly or via migration of meningeal DCs into the cervical lymph nodes, was sufficient to induce an immune response against CNS self-antigens, demonstrating the importance of drainage from the brain for the generation of a CNS-specific immune response. Such a response, however, was not observed at earlier time points using a similar model (Locatelli et al., 2012), suggesting that a particular threshold of antigen needs to be met in order to ensue autoimmune T cell response to CNS self-antigens. Drainage of CNS antigens by itself, however, is not sufficient to induce an autoimmune attack, as the injection of MOG peptide into the CSF prior to EAE induction actually has a protective effect (Harling-Berg et al., 1991). Drainage of CNS antigens in the context of inflammation or a wider degeneration, such as that seen during massive oligodendrocyte death (Traka et al., 2010, 2016) or after parenchymal cryolesion (Lake et al., 1999), seems to be needed in order to induce the generation of autoreactive T cells. The amount of the draining CNS antigen might also be a relevant factor in the generation of an autoimmune response. Overall, drainage of CNS antigens and of their associated tissue-specific danger signals is necessary for the induction of brain autoreactive T cells, further emphasizing the importance of lymphatic drainage from the brain as an important prerequisite for the development of immune responses.

## Deep Cervical Lymph Nodes and Autoreactive T Cell Activation

The contribution of cervical lymph nodes, and particularly of deep cervical lymph nodes, to EAE development was demonstrated by several groups using a protocol of deep cervical lymph node excision (Furtado et al., 2008; Phillips et al., 1997; van Zwam et al., 2009). Given the presence of CNS antigens observed in deep cervical lymph nodes in EAE or after stroke (Urra et al., 2014; de Vos et al., 2002), it can be argued that these lymph nodes represent the site of activation of CNS-specific T cells. Surgical excision of the deep cervical lymph nodes ameliorates EAE disease severity, even though the antigen is injected peripherally and is therefore present in multiple lymph nodes (Phillips et al., 1997; van Zwam et al., 2009). However, EAE could be induced in mice lacking lymph nodes (Map3k14$^{aly}$ and *LTbR*$^{-/-}$ mice), suggesting that secondary lymphoid organs are not necessary for initiation of the disease (Greter et al., 2009). The presence of non-classical lymphoid organs has been reported in certain tissues, notably in the fat (Bénézech et al., 2015; Elewa et al., 2014), and might participate in tissue-specific immune responses (Bénézech et al., 2015). It is possible that the presence of such a system, or other organs (Greter et al., 2009), could take over the role of secondary lymphoid organs in those strains of mice that lack them.

In order to migrate to the CNS, autoreactive T cells need to acquire a particular migratory phenotype, notably the expression

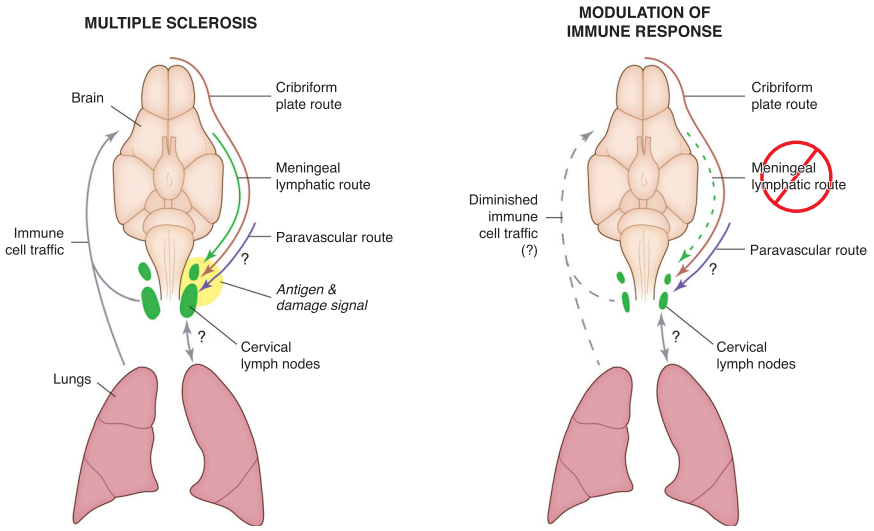


**Figure 4. Potential Involvement of the Meningeal Lymphatic Route in the CNS Autoimmune Response**

Studies suggest that the cervical lymph nodes house macromolecules that are drained from the CNS through different pathways, namely the lymphatic network associated with the nasal mucosa (cribriform plate route, brown arrow) and the recently characterized meningeal lymphatic route (green arrow), and possibly the paravascular route (purple arrow). The cervical lymph nodes, along with the lungs, are closely involved in immune cell traffic and homing into the CNS (gray arrows) in autoimmune diseases, such as multiple sclerosis, and participate in the generation of encephalitogenic immune cells. Whether the modulation of the lymphatic drainage might attenuate antigen drainage (and associated damage signals) into the cervical lymph nodes and epitope spreading, hence decreasing the autoimmune response, and prevent the occurrence of relapses in multiple sclerosis is an important subject that needs to be addressed in the future.

of $\alpha4\beta1$ integrin, LFA-1, and others, which would facilitate their extravasation across the brain and meningeal blood vessels (Flügel et al., 2001; Schläger et al., 2016; Yednock et al., 1992). The acquisition of that phenotype is of primary importance since some of the current treatments for MS are targeting the integrins to prevent migration/entry of the T cells into the CNS (Steinman, 2005; Steinman and Zamvil, 2005). The lung was recently proposed as the major site where encephalitogenic T cells are reprogrammed to upregulate their CNS migratory profile for efficient entry into the CNS (Odoardi et al., 2012). Other studies have demonstrated the importance of the secondary lymphoid organs for the activation and acquisition of an encephalitogenic phenotype for T cells (Flügel et al., 2001; Furtado et al., 2008). Expression of the CCR6 by encephalitogenic T cells might be important for their migration across the choroid plexus and CNS blood vessels (Arima et al., 2012; Reboldi et al., 2009). Determining whether the lungs or the cervical lymph nodes are the major site for T cell activation and reprogramming is not, however, the critical issue. Rather, understanding the contribution of each site and the specific conditions that drive this reprogramming will be a major step in our ability to prevent the activation and migration of encephalitogenic T cells in the CNS.

What would render one lymphoid organ more likely than another to generate a particular T cell phenotype? From the tissue it drains, each lymph node receives signals either through direct drainage of tissue antigens or through migrating DCs. Cervical lymph nodes drain the CNS and would thus appear to be a likely key site for the activation and reprogramming of autoreactive T cells. Understanding how the CNS antigens drain into the cervical lymph nodes is therefore of primary importance.

## Role of Meninges in MS

The meninges covering the CNS serve as its immune compartment (Kipnis et al., 2012; Ransohoff and Engelhardt, 2012). In patients with MS, inflammation is often observed in the meninges before it is detected in the parenchyma (Popescu and Lucchinetti, 2012; Howell et al., 2011). In animals with EAE, encephalitogenic T cells enter the CNS through the meningeal blood vessels (Bartholomäus et al., 2009; Walker-Caulfield et al., 2015), where they communicate with the meningeal macrophages (Bartholomäus et al., 2009). These observations suggest that the meninges serve as a key checkpoint for encephalitogenic T cells, where they supposedly acquire the phenotype that allows them to infiltrate the parenchyma.

In addition to functioning as an environment for the transit of pathogenic immune cells on their way to the brain parenchyma, the meninges also serve as sites for the generation of an immune response. Both in MS patients and in EAE models, the meninges have been found to house tertiary lymphoid organs, notably containing germinal centers for B cells (Kuerten et al., 2012; Magliozzi et al., 2007; Peters et al., 2011; Walker-Caulfield et al., 2015). Tertiary lymphoid organs are characterized by the presence of a lymphatic vasculature that enables functional drainage. Podoplanin, a protein that plays a major role in initiating the formation of tertiary lymphoid organs (Buckley et al., 2015; Peters et al., 2011), is expressed in multiple cell types in the meninges, including T cells, LECs, and meningeal stromal cells (Kaji et al., 2012; Kalof and Cooper, 2009; Peters et al., 2015). It will therefore be of interest to investigate the functional role that the meningeal lymphatic vasculature might play in the generation of tertiary lymphoid organs.

Studies using B cell receptor sequencing and lineage tracing suggest that B cells recirculate between the brain parenchyma, the CSF, and the cervical lymph nodes (Palanichamy et al., 2014; Stern et al., 2014). Further studies will be needed in order to analyze the path used by B cells to reach the cervical lymph nodes and to investigate the potential role of the meningeal lymphatic system in this process.

## Potential Implications of the Lymphatic Vascular System in MS

While the role of the meningeal lymphatic vasculature in generating and maintaining immune responses in the CNS remains unknown, its study offers new opportunities for understanding and modulating immune responses in the brain (Figure 4).

Meningeal lymphatics drain CNS macromolecules into the cervical lymph nodes. It seems reasonable to assume that modulation of CNS drainage through the meningeal lymphatic vasculature would reduce the quantity of CNS antigens draining into the cervical lymph nodes, and that this reduction might weaken initiation of the autoimmune response. In MS patients and in some EAE models, relapsing is caused by epitope spreading (Compston and Coles, 2002; Miller et al., 1995; Steinman, 2014; Voskuhl et al., 1996). Although dendritic cells within the CNS have been shown to play a key role in this epitope spreading (Miller et al., 2007), it is plausible to assume that modulation of the lymphatic drainage (of a free antigen and also of DCs loaded with CNS antigens) might attenuate such relapses or even prevent their occurrence.

In addition to their possible modulation via macromolecular drainage, immune responses might directly affect the disease progression via recirculation of immune cells into the cervical lymph nodes, a process that is also mediated through meningeal lymphatic vessels. Interestingly, the presence of lymphatic capillaries in the brain and spinal cord parenchyma of MS patients was suggested in 1979 (Prineas, 1979), but those findings were never verified or even followed up. Clearly, however, further study is needed to confirm the presence and functionality of such putative intraparenchymal lymphatic capillaries that might feed back into the main meningeal lymphatic vessels. Inflammation is known to induce expansion of the local lymphatic vasculature in peripheral tissues (Kim et al., 2014). It would therefore be interesting to study the possible contribution of meningeal lymphatics to the formation of intraparenchymal lymphatic capillaries in MS, and the role of these presumptive newly formed vessels in the drainage of CNS antigens and immune cells.

## Conclusions

Most, if not all, neurological disorders appear to be multifactorial and involve multiple systems. For example, neuronal, immune, and vascular systems are playing major roles in both MS and AD. In MS, the involvement of the immune system is evident and remains the primary target of all available treatments to date (Fernandez et al., 2016). In AD, the involvement of the immune system was recently emphasized by genome-wide association studies linking immune genes to familiar AD susceptibility (Jiang et al., 2016; Yokoyama et al., 2016). Moreover, recent work suggests the involvement of complement system and microglia in early stages of AD development, before any overt brain amyloid pathology (Hong et al., 2016), and the role of T cells in later stages (Baruch et al., 2016). The vascular system plays a major role in both diseases also: in MS, it is the entry point for the autoimmune T cells to the CNS, and in AD, the vascular system is a major component for the clearance of Aβ.

The newly discovered meningeal lymphatic system is a potential new player in these complex neurological diseases, since it could play a central role in the generation and maintenance of the immune responses as well as recycling of ISF and removal of molecular waste.

Therefore, it is plausible to suggest that MS and AD may have a neuro-lympho-vascular component. Such an approach to these diseases might reveal new potential therapeutic perspectives. While the brain parenchyma remains a "hard to access" organ, the meninges and its associated lymphatic vasculature are more easily reachable and drug targetable. Therefore, if (or when) a direct involvement of meningeal lymphatic vasculature is demonstrated for any neurological disorder, modulation or modification of their function may represent the next generation of treatment modalities for these diseases.

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