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The movers and shapers in immune privilege of the CNS

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Discoveries leading to an improved understanding of immune surveillance of the central nervous system (CNS) have repeatedly provoked dismissal of the existence of immune privilege of the CNS. Recent rediscoveries of lymphatic vessels within the dura mater surrounding the brain, made possible by modern live-cell imaging technologies, have revived this discussion. This review emphasizes the fact that understanding immune privilege of the CNS requires intimate knowledge of its unique anatomy. Endothelial, epithelial and glial brain barriers establish compartments in the CNS that differ strikingly with regard to their accessibility to immune-cell subsets. There is a unique system of lymphatic drainage from the CNS to the peripheral lymph nodes. We summarize current knowledge on the cellular and molecular mechanisms involved in immune-cell trafficking and lymphatic drainage from the CNS, and we take into account differences in rodent and human CNS anatomy.

The term "immune-privileged site" refers to an organ in which experimentally implanted tissue grafts are incapable of provoking immunity leading to graft rejection[1]. The CNS is thus an immune-privileged site, as tissue grafts survive well when implanted into the CNS parenchyma[2,3]. Although some have questioned whether humoral immunity is absent from the CNS parenchyma[4], bacterial and viral antigens introduced directly into the CNS parenchyma do evade systemic immunological recognition (summarized in ref. 5). The CNS parenchyma similarly lacks a potent innate immune response. Neither injection of bacterial products, and thus exposure to pathogen-associated molecular patterns[6], nor experimental induction of cell death, and thus exposure to danger-associated molecular patterns[7,8], in the CNS parenchyma elicits rapid infiltration of myelomonocytic cells as observed during a stereotypic response to such stimuli in peripheral organs.

These observations led to the concept that antigens are sequestered within the CNS and thus are invisible to the immune system. The endothelial blood–brain barrier (BBB) was envisaged to inhibit the efferent arm of the immune system by blocking immune-cell entry into the CNS, whereas the absence of conventional lymphatic vessels in the CNS parenchyma was thought to block the afferent arm of the immune system, including the drainage of CNS antigens into peripheral lymphatic tissues (summarized in ref. 5).

Studies showing that activated circulating T cells can cross the BBB in the absence of neuroinflammation (summarized in ref. 9), that tissue grafts are rejected when transplanted into the cerebral ventricles instead of into the brain parenchyma[10,11], and that cerebrospinal fluid (CSF) drains into deep cervical lymph nodes[12] have repeatedly triggered discussions questioning the immune-privileged status of the CNS.

Studies based on modern *in vivo* imaging technologies have contributed to the call to abandon the 'historic view' or revisit the 'dogmas' regarding the immune-privileged status of the CNS[13,14]. Findings supporting this movement include the rediscovery of an exchange system between CSF and interstitial fluid (ISF), termed the glymphatic system[15], that suggests a means for efficient clearance of soluble compounds from the CNS parenchyma into the CSF[16]. Similarly, rediscovery of lymphatic vessels in the dura mater, which were first described in the 18th century[17], prompted suggestions that some ISF may be able to drain to lymphoid tissues via subarachnoid CSF[14,18].

We propose that an understanding of CNS immune privilege requires an awareness of CNS anatomy, and especially of the localization and function of the brain barriers, which establish compartments in the CNS that differ functionally in their communication with the immune system. In contrast to the CSF-drained ventricular and subarachnoid spaces in the CNS, which have established afferent and efferent connections to the peripheral immune system and provide limited drainage of parenchymal antigens, the CNS parenchyma proper is protected from the immune system. It prohibits immune-cell entry through the glia limitans, it lacks migration of resident microglial cells to draining lymph nodes for the presentation of CNS antigens, and it limits lymphatic drainage to small, soluble parenchymal antigens. Finally, we emphasize the potential differences between human and rodent CNS anatomy, raising the question of how well observations made in mouse and rat models will translate to humans.

Acellular and cellular brain barriers

Three layers of meninges surround the CNS and build superficial barriers between the CSF and the CNS (**Fig. 1**). The outermost layer is the

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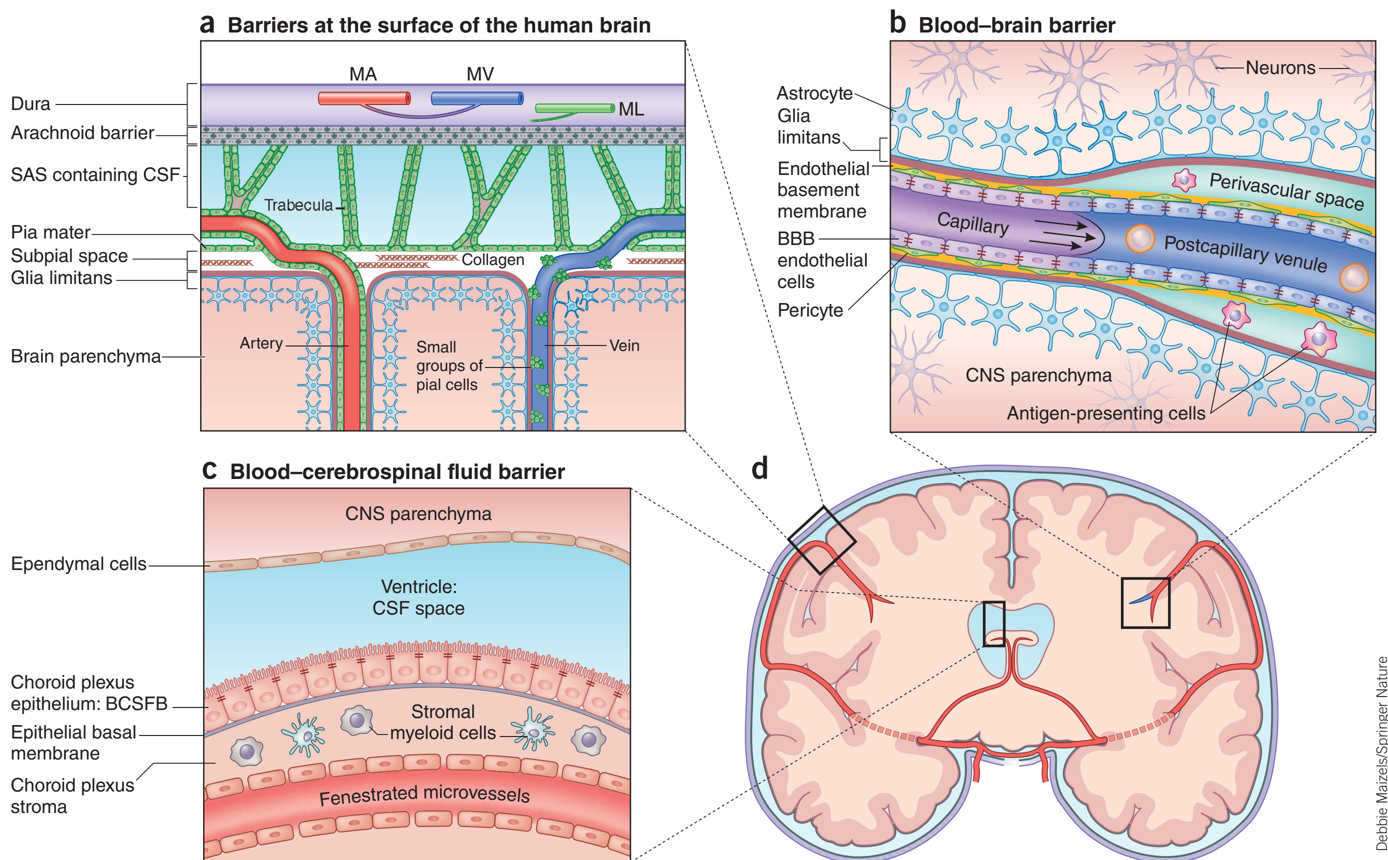


Figure 1 The acellular and cellular brain barriers. (**a**) Barriers at the surface of the human brain. Dura mater is supplied by meningeal arteries (MA) and veins (MV), and dural microvessels lack a BBB; the dura mater also contains lymphatics (ML). The dura is immediately adjacent to the arachnoid mater, which forms the outer BCSFB of the SAS. Sheet-like trabeculae cross the SAS to the pia mater and to the leptomeningeal arteries and veins. The subpial space contains bundles of collagen. The pia mater coats veins and arteries in the SAS and separates the SAS from the subpial space, and a sheath of pia mater surrounds arteries entering the brain. The glia limitans forms a barrier at the surface of the CNS parenchyma and around blood vessels. (**b**) The blood–brain barrier. The capillary BBB is the site of controlled transport of fluids and solutes into the CNS, whereas extravasation of immune cells into the CNS parenchyma occurs at the level of postcapillary venules, the vascular segments into which blood flows after passing through the capillaries. Highly specialized BBB endothelial cells are coated by a basement membrane formed by the fusion of endothelial and glia limitans basement membranes. Pericytes are embedded in the endothelial basement membrane. CNS microvessels are surrounded by the glia limitans, which is composed of the parenchymal basement membrane and astrocyte end-feet. At the capillary BBB, the endothelial and parenchymal basement mebranes fuse; at the level of the postcapillary venules, the adjacent basement membranes leave a virtual perivascular space in which occasional APCs are embedded. (**c**) The blood–CSF barrier of the choroid plexus. Choroid plexus epithelial cells establish the BCSFB. The choroid plexuses are localized in the ventricles of the brain and produce CSF. The choroid plexus stroma harbors DCs and macrophages and is supplied by a dense microvascular network that does not establish a BBB; instead the epithelial cells of the choroid plexus are connected by unique parallel running tight junctions and express efflux pumps. (**d**) Schematic coronal brain section depicting the localization of the brain barriers.

dura mater, which is approximately 1 mm thick in humans and is composed of dense fibrous tissue[19,20]. Arteries, veins and lymphatics in the dura are separate from the CNS. Dural capillaries are fenestrated and do not establish a BBB. The arachnoid mater is 200 mm thick in humans, is impermeable to fluids and abuts the inner aspect of the dura, forming a border layer of cells joined by tight junctions[19] and expressing efflux pumps, similar to the BBB[21]. The arachnoid mater establishes a barrier between the dura mater and the CSF-drained subarachnoid space (SAS) and is thus a true blood–CSF barrier. A layer of pia mater, one cell thick, coats the surface of the CNS and is permeable to solutes and immune cells but not to erythrocytes[22]. The pia mater is reflected from the surface of the CNS to cover arteries and veins in the SAS and separates the SAS from the CNS and perivascular compartments[22,23]. The surface of the CNS is coated by the glia limitans, consisting of compacted astrocyte foot processes and an overlying parenchymal basement membrane. Fluid and low-molecular-weight tracers pass freely through the glia limitans from the CSF[15], but trafficking of T cells is limited[24].

As arteries enter the surface of the CNS from the SAS, they pass through the subpial space, carrying with them a sheath of pia mater into the CNS. In both human[25] and rodent[26] cerebral cortex, the glia limitans, pia mater and artery wall are compacted so that there is no perivascular (Virchow–Robin) space (**Fig. 1a**). Artifactual spaces due to swelling of astrocyte processes have been misinterpreted as perivascular spaces in the cerebral cortex[27], but electron microscopy studies of well-fixed human and mouse brain tissue reveal no such space[25,26] (**Fig. 1a**). Perivascular spaces, however, are present in the basal ganglia and white matter[28,29]. Veins in the CNS do not have a complete coating of pia mater, but a layer of leptomeningeal cells does cover veins in the SAS[25].

The BBB establishes a barrier between the blood and the CNS parenchyma and consists of two major elements (**Fig. 1b**). A BBB for solutes is formed by specialized capillary endothelial cells with low pinocytotic activity that are joined by complex tight junctions[30]. The BBB that regulates the entry of immune cells into the CNS is in postcapillary venules[31]. To reach the CNS parenchyma from the blood, T cells have to penetrate

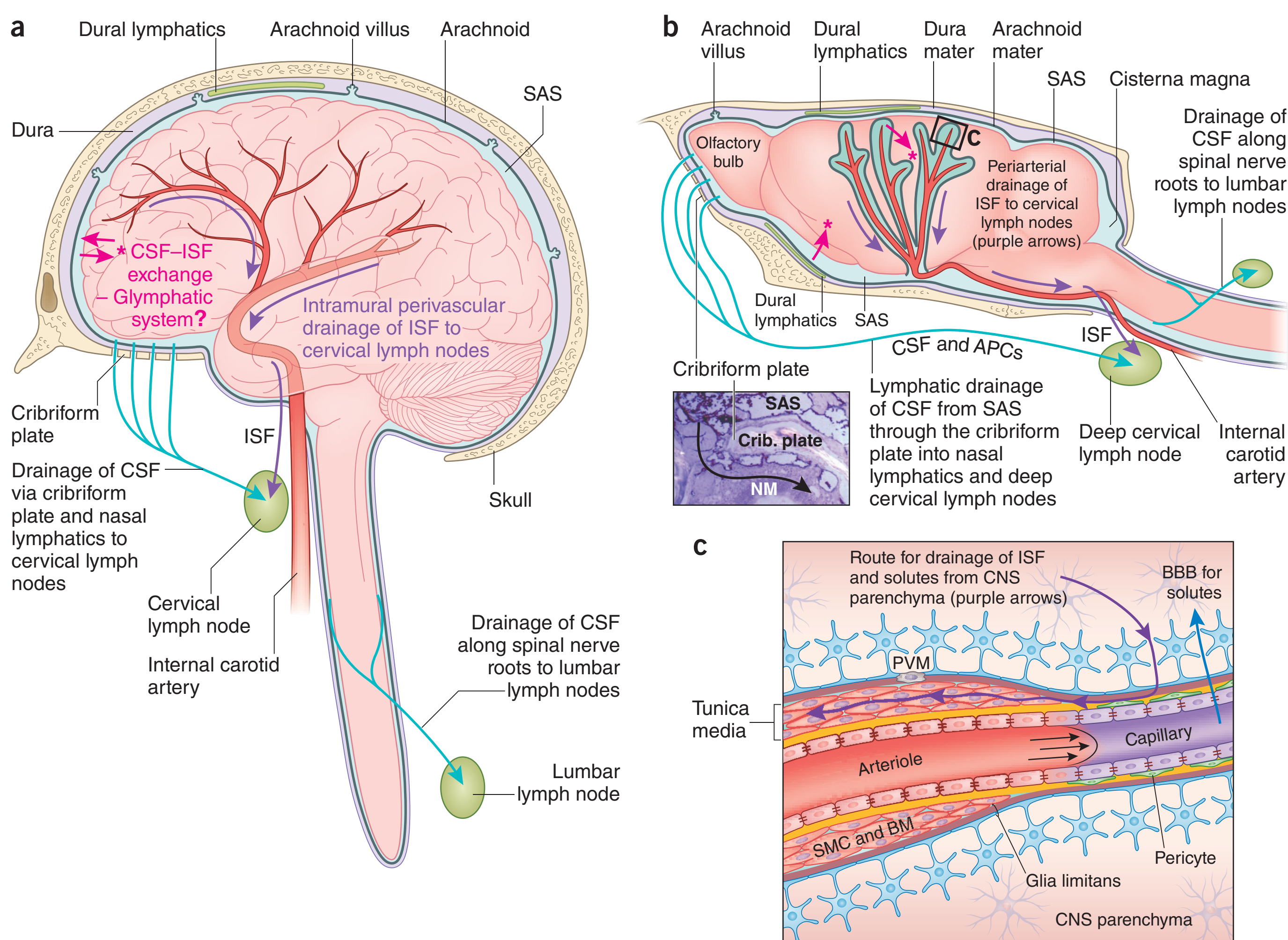


Figure 2 Lymphatic drainage of the CNS in humans and in rodents. (**a**,**b**) Drainage pathways for CSF are very similar in the human (**a**) and rodent (**b**) brains. CSF drains from the SAS through arachnoid villi into the blood of venous sinuses (villi are smaller and less numerous in rodents). The cerebral SAS in rodents is confined mainly to the basal aspects of the brain and to channels around major cerebral arteries (**b**). Lymphatic drainage of CSF to cervical lymph nodes occurs via the cribriform plate and nasal lymphatics, as well as via dural lymphatics and along cranial nerves. The microscopy image in **b** (lower left) shows channels draining black India ink tracer from the SAS via the cribriform (Crib.) plate into lymphatic channels (black arrow) in the nasal mucosa (NM) (reproduced with permission from ref. 37). CSF also drains along spinal nerve roots to lumbar lymph nodes. The black rectangular outline in **b** indicates the region represented in detail in **c**. (**c**) ISF from the brain parenchyma drains along the walls of cerebral capillaries and arteries to cervical lymph nodes in both humans and rodents. The exchange of CSF and ISF occurs at the surface of the brain in both humans and rodents (pink arrows with asterisks in **a**,**b**). PVM, perivascular macrophage; SMC, smooth muscle cells; BM, basement membrane. (The basement membrane surrounds the smooth muscle cells.)

first the vascular endothelium and then the glia limitans[31].

Additionally, solutes and immune cells can pass from the blood into the stroma of the choroid plexuses (**Fig. 1c**). A blood–CSF barrier (BCSFB) located in the choroid plexus epithelium regulates the composition of the CSF[32] and probably the passage of immune cells into the CSF[33]. Immune cells can also directly enter the CSF-drained SAS by extravasation through the walls of subarachnoid veins. It is important to note that the ventricular and subarachnoid CSF spaces do not exhibit the same immune privilege as the CNS[34]. In summary, the major barriers to the entry of immune cells into the CNS are the vascular endothelium, the choroid plexus epithelium, and the glia limitans that invests the surface of the CNS and surrounds blood vessels in the CNS.

Afferent arm of the neuroimmune system

Lymphatic vessels have a key role as the afferent arm of the immune system for most organs in the body, thereby maintaining fluid homeostasis and acting as pathways for the passage of antigens and antigen-presenting cells (APCs) to regional lymph nodes. The CNS parenchyma has no conventional lymphatic vessels, and although a well-regulated BBB controls the entry of solutes into the CNS, there is still a need to maintain homeostasis and effective afferent pathways to lymph nodes[35,36]. Two extracellular fluids are associated with the CNS: CSF in the ventricles and the SAS[37], and ISF in the CNS parenchyma[38,39]. Both fluids drain to cervical and lumbar lymph nodes, but by separate pathways (**Fig. 2a**). CSF drains along lymphatic vessels that pass through the cribriform plate[37] and the dura mater[14,18], and this pathway allows for trafficking of immune cells[40] (**Fig. 2b**). In contrast, ISF and solutes from the CNS parenchyma drain to lymph nodes along 100–150-nm-thick basement membranes in the walls of cerebral capillaries and arteries[39]. Only 15% of ISF draining from the cerebral hemispheres leaks into the CSF[38]. In contrast to observations regarding the drainage pathways for CSF, no evidence exists showing that immune cells can traffic directly from the CNS parenchyma along the narrow intramural perivascular pathways to lymph nodes[39] (**Fig. 2c**).

CSF is produced by the choroid plexuses in the cerebral ventricles and circulates to the cranial and spinal SAS[32]. The CSF drains from the SAS via two major routes (**Fig. 2a**). CSF drains into the blood of venous sinuses through arachnoid villi and granulations that are large in humans[41] but small and sparse in rodents[21,37]. CSF also drains via nasal[37] and dural lymphatics[14,18]. Channels pass from the SAS through the cribriform plate of the ethmoid bone to join lymphatics in the nasal mucosa in rodents and humans[32,37] (**Fig. 2b**). Drainage of CSF via this route is largely to deep cervical lymph nodes, and the lymphatic channels allow the trafficking of $CD4^+$ T cells[42], monocytes[40] and dendritic cells[43] from CSF to cervical lymph nodes. CSF drainage to cervical lymph nodes via dural lymphatics[14,18] seems to occur mainly at the base of the skull[18]. Cranial and spinal nerve roots also act as pathways for lymphatic drainage of CSF to cervical and lumbar lymph nodes[18,44]. Direct drainage pathways to lymph nodes for APCs in the CSF could be a major reason that CSF spaces are immunologically competent and demonstrate rapid inflammatory responses, the quality of which is distinct from the immunological privilege of the CNS parenchyma[5].

In contrast to CSF drainage along lymphatic channels, ISF and solutes drain rapidly out of the CNS parenchyma along intramural perivascular pathways in the walls of capillaries and arteries to lymph nodes[38,39,45,46]. ISF may also drain from periventricular white matter directly into ventricular CSF, particularly when there is edema of the white matter[32]. Evidence for the intramural perivascular pathway has been obtained in experimental studies using radioactive[38] and fluorescent tracers[39], as well as from observations of cerebral amyloid angiopathy in mice[47] and in humans[48,49]. Small volumes (0.5 μl or less) of fluorescent tracers injected into the cerebral gray matter of the mouse brain drain rapidly (within 5–30 min) from the brain. This drainage occurs along basement membranes in the walls of capillaries and then along basement membranes surrounding smooth muscle cells in the tunica media of arterioles and arteries[39,50] (**Fig. 2c**). Drainage continues along the walls of arteries in the SAS through the base of the skull to cervical lymph nodes[38]. Injection of larger volumes of tracer through large-bore needles commonly results in leakage into the SAS or ventricles, leading to experimental artifacts and thus misinterpretation of the drainage of ISF from the brain parenchyma[39]. During their passage along the intramural perivascular drainage pathways, tracers are taken up by smooth muscle cells and by perivascular macrophages on the outside of arteries in the brain and in the subarachnoid space[39]. In this way, some antigenic material from the brain parenchyma may be delivered to leptomeningeal macrophages for presentation to trafficking lymphocytes.

Intramural perivascular ISF-drainage pathways limit the size of molecules that pass along them, allowing for proteins up to 150 kDa but not for particles 0.2–1 μm in diameter, which instead track outside of arteries adjacent to the glia limitans[39,51,52]. Larger particles, such as proteins aggregated in the form of immune complexes, become entrapped and impair drainage[53]. Age-related impairment of intramural perivascular drainage of ISF and solutes occurs[54], and drainage is impaired when vascular pulsations are reduced[45] or abolished[39] or when arteries are denervated[55]. The observations regarding drainage impairment suggest that the motivating force for the intramural perivascular ISF drainage may be related to arterial pulsations that are impaired when arteries stiffen with age and arteriosclerosis[54,56]. Although this hypothesis is still being tested, mathematical modeling supports a mechanism involving valve-like action in vessel walls[56,57].

Impairment of intramural perivascular drainage with age is particularly noticeable in humans. β-Amyloid (Aβ) peptides derived from the brain act as natural tracers in the mouse and human brains[48,49]. Aβ peptides accumulate with age as insoluble, often brittle, deposits in basement membranes in the walls of capillaries and in the tunica media of cerebral arterioles and arteries in mouse and human brains as cerebral amyloid angiopathy with exactly the same distribution as shown by fluorescent tracers injected into the mouse brain[47–49,58]. In addition to impaired perivascular intramural drainage, binding of Aβ to basement membrane proteins such as laminin[59] may contribute to its accumulation in the vascular wall over time. The presence of Aβ in the walls of arteries in humans ceases at the base of the skull, adjacent to cervical lymph nodes[60,61], as does that of radioactive tracers injected into the rat brain[38]. These observations support the hypothesis that ISF and solutes drain directly from the brain parenchyma by intramural perivascular pathways to cervical lymph nodes in humans and in rodents[38,46].

In summary, soluble antigens drain with the CSF from the SAS to regional lymph nodes via nasal or dural lymphatic vessels. This pathway also allows for immune-cell trafficking. ISF, in contrast, drains from the CNS parenchyma directly to lymph nodes along basement membranes in the walls of capillaries and arteries. This intramural perivascular pathway is too narrow to allow the migration of immune cells directly from the CNS parenchyma to lymph nodes, and this may be a major factor in the immune privilege of the CNS parenchyma.

Interconnection of CSF and ISF

Although ISF and CSF drain to lymph nodes by separate pathways, there is an interconnection between the two fluids, as CSF and ISF are equilibrated[15,62] (**Fig. 2a**,**b**). Low-molecular-weight tracers injected into the CSF mix with the ISF by passing through the glia limitans on the surface of the brain. Furthermore, tracers penetrate into the brain along the outer aspects of arteries[15,26] and enter the brain parenchyma through the perivascular glia limitans[15,62]. It has been proposed that this process, originally termed convective tracer influx[62], should be renamed the 'glymphatic system'[15], because the entry of tracers into the CNS parenchyma from the CSF is regulated by the water channel aquaporin 4 in astroglial end-feet of the glia limitans, and because the CSF–ISF mixture most probably drains to regional lymph nodes. Tracers that have passed from the CSF into the ISF may drain back into the CSF of the SAS or drain along the walls of veins[15], although the anatomical route for drainage to regional lymph nodes along the walls of cerebral veins has not been fully documented.

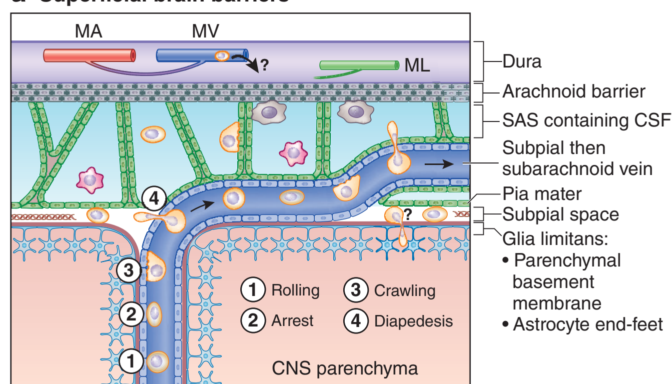
At present the contribution of a glymphatic system to the drainage of antigens from the CNS parenchyma to regional lymph nodes is unclear. A number of observations have challenged the glymphatic concept. The existence of a bulk water flow from arterial to venous paravascular spaces of the CNS, proposed to underlie the glymphatic system, has been questioned[16,36]. Also, efficient clearance of solutes and waste from the CNS parenchyma into the CSF as proposed by the glymphatic concept would include efficient antigen drainage into the CSF and ultimately into regional lymph nodes. Thus, this concept is difficult to reconcile with the immune privilege of the CNS parenchyma.

Efferent arm of the neuroimmune system

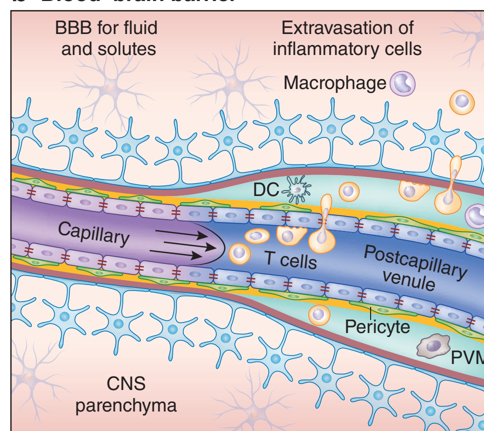
Despite the established lymphatic drainage pathways from the CNS to peripheral lymph nodes, the immune response mounted in the CNS differs from that of peripheral organs. Unique subsets of long-lived APCs help to shape the immune privilege of the CNS. Microglial cells residing in the CNS parenchyma are derived from hematopoietic precursors in the yolk sac[63]. Perivascular and subarachnoid spaces harbor long-lived macrophages that arise from from hematopoietic precursors during embryogenesis[64]. The low turnover of these cells supports the notion that they rarely migrate to the lymph nodes that drain the CNS. This situation is in contrast to that in peripheral tissues, where activated dendritic cells (DCs) migrate via lymphatic vessels to lymph nodes. In the skin and the gut, DCs process additional cues

Figure 3 Efferent immune pathways to the CNS. Three potential immune-cell entry sites into the CNS are described and localized to superficial leptomeningeal vessels, parenchymal vessels and the choroid plexus. (**a**) Immune-cell extravasation across leptomeningeal vessels. Activated T cells (yellow) cross the walls of subpial venules in a multistep cascade in which rolling of T cells is followed by their arrest, crawling against the direction of blood flow, and diapedesis across the vascular wall into the subpial space. Immune cells can additionally be extravasated across the walls of subarachnoid veins directly into the CSF-drained SAS. Extravasated T cells need to recognize their cognate antigen on leptomeningeal macrophages in order to cross the glia limitans; alternatively, they can crawl along the abluminal aspect of blood vessels or get flushed away to eventually reach the CSF-drained ventricles. T cell extravasation across dural blood vessels has not been studied yet. Question marks highlight migration pathways for which direct experimental proof is lacking. (**b**) Immune-cell extravasation across inflamed parenchymal postcapillary venules. The angioarchitecture of the CNS prohibits direct imaging of immune-cell extravasation across parenchymal postcapillary venules; multistep extravasation has been extrapolated from observations of superficial CNS vessels. Activated but not naive T cells can cross this vascular wall and reach perivascular spaces, where they can accumulate. Crossing the glia limitans requires local reactivation of immune cells by recognition of their cognate antigens on perivascular APCs and occurs only during neuroinflammation. (**c**) Immune-cell entry into the CNS via the choroid plexus. The choroid plexus stroma harbors many tissue-resident immune cells (tissue-resident and short-lived macrophages, DCs, and T cells). The precise molecular mechanisms of immune-cell extravasation into the choroid plexus stroma have not been defined. T_H17 cells might cross the epithelial BCSFB established by choroid plexus epithelial cells in a CCR6/CCL20-dependent manner. Adhesion molecules ICAM-1 and VCAM-1 are expressed exclusively on the luminal side of the choroid plexus epithelium and are unlikely to contribute to T cell diapedesis across the BCSFB. T_{CM}, central memory T cell.

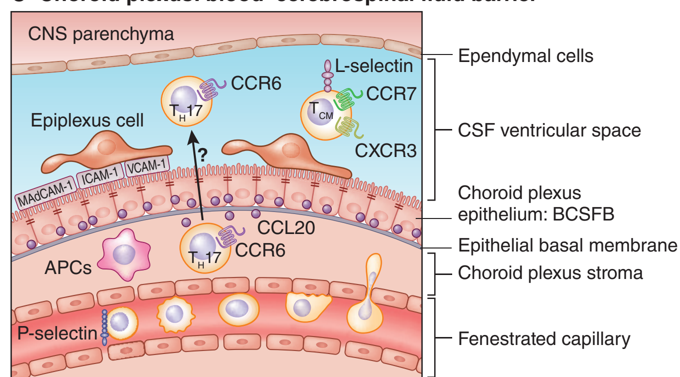
a Superficial brain barriers



b Blood–brain barrier



c Choroid plexus: blood–cerebrospinal fluid barrier



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from the local environment during antigen presentation; for example, vitamins D and A allow for the imprinting of tissue-specific migration programs that include the expression of unique combinations of adhesion molecules and chemokine receptors, respectively, on effector T cells[65]. Although a CNS-specific T cell trafficking program has not yet been established, activation of CNS-specific T cells in cervical lymph nodes seems to have a direct role in mediating the neuroinflammation observed in experimental autoimmune encephalomyelitis (EAE), an animal model for multiple sclerosis. In this model, the removal of lymph nodes draining the CNS reduces the severity of the inflammation in the cerebral hemispheres and spinal cord in EAE[66,67]. These experiments support the notion of export of tissue-specific cues and imprinting of different trafficking programs in lymph nodes draining the CNS. Other studies in the context of EAE, however, have suggested that autoaggressive T cells that are able to enter the CNS need to be 'licensed' in the lung[68] or in the gut, with strong influence from the gut micobiota[69]. Thus, it remains unknown whether there are T cells that specifically home to the CNS and, if so, where they develop this capacity.

Immune-cell entry into the CNS

Although there are no tissue-resident T cells or B cells in the CNS parenchyma of healthy young individuals, immune cells of the adaptive immune system are found in the CSF of healthy individuals. Compared with that in other body fluids, such as synovial fluid, the number of immune cells in the CSF of humans is very low, and the composition of the cellular subsets is unique, with a very low fraction of innate immune cells and a high fraction of effector or effector memory and central memory T cells[70]. This finding is in accordance with observations in rodents showing that immune-cell passage across the brain barriers in the absence of neuroinflammation is rare and limited to activated $CD4^+$ and $CD8^+$ T cells (summarized in ref. 34). Encephalitogenic $CD4^+$ T cells have been observed to use different routes to enter the CNS. They can cross subpial venules or subarachnoid veins to reach the subpial space or the SAS, respectively[71]. Alternatively, they can migrate across spinal cord subpial venules reaching the perivascular spaces[72], or they can reach leptomeningeal spaces via the choroid plexus[73] (**Fig. 3a–c**). The chemokine CXCL12 seems to ensure the entrapment of activated T cells in perivascular or CSF-drained spaces[74].

Activated $CD4^+$ T cells cross the endothelial BBB independent of their antigen specificity[75,76]. Unless they recognize their cognate antigen on perivascular[77] or leptomeningeal[78] APCs, they will not cross the glia limitans and will instead undergo apoptosis or eventually leave the CNS, probably by the CSF-drainage pathways. Recognition of their cognate antigen on perivascular or leptomeningeal APCs is necessary and sufficient to induce local T cell activation in the CNS perivascular spaces around postcapillary venules[77] or in the leptomeningeal spaces[79],

respectively (**Fig. 3a**,**b**). The subsequent release of proinflammatory cytokines alters the barrier characteristics of the endothelial BBB and triggers the upregulation or induction of trafficking molecules, allowing for the recruitment of additional immune-cell subsets, including myeloid cells, across the endothelial brain barriers (summarized in refs. 34,80). The local release of tumor necrosis factor in the perivascular and leptomeningeal spaces triggers the production of metalloproteinases MMP-9 and MMP-2 in recruited myeloid cells, allowing for selective cleavage of dystroglycan at the membrane of astrocyte foot-processes, the establishment of chemotactic gradients, and subsequent infiltration of immune cells across the glia limitans into the CNS parenchyma[81,82]. Clinical disease in individuals with EAE starts when the immune cells cross this inner CNS barrier, which underscores the notion that the entry of immune cells into the CNS parenchyma is incompatible with CNS homeostasis[81].

The choroid plexus contributes to CNS immunity[83,84]. Choroid plexus epithelial cells forming the BCSFB express the cell-adhesion molecules ICAM-1 and VCAM-1 (ref. 85) and have been implicated in mediation of immune-cell migration across the BCSFB into the CNS[86] (**Fig. 3c**). However, ICAM-1 and VCAM-1 are located exclusively on the apical surface of choroid plexus epithelial cells[87] and thus are 'invisible' to immune cells in the choroid plexus stroma. The chemokine CCL20 and the cell-surface proteoglycan syndecan-1 expressed by choroid plexus epithelial cells were shown to mediate the migration of CCR6$^+$ IL-17-producing helper T cells (T_H17 cells) into the CNS via the choroid plexus[73,88]. However, the precise cellular and molecular cues that guide immune cells from the choroid plexus stroma into the CNS remain to be demonstrated.

Intravital microscopy provides translational insights

Epifluorescence and two-photon intravital microscopy have provided insights into the vascular system of mouse and rat CNS and have expanded scientific understanding of the cellular and molecular mechanisms mediating immune-cell migration into the CNS[79,89–91]. Computational modeling has extended such understanding beyond what can be achieved by direct observation[92]. Recently, fluorescent vascular imaging has found use in the neurosurgical setting, allowing intravital visualization of blood vessels in the human brain and spinal cord, and thus providing one of the first tools for obtaining insights into the vascular system of the human CNS[93–95]. The drawback of this technology, however, is that one sees only what one stains for. The current lack of technologies allowing for specific visualization of the above-mentioned brain barriers within the CNS, such as vascular basement membranes, the pia mater and the arachnoid mater, make a reliable microscopic navigation across the different CNS spaces difficult. Thus, the question is to what extent imaging studies can be translated to human CNS immune surveillance.

Translation of data from rodents to humans requires the consideration of several features of rodent and human brain anatomy. Weighing 1,200–1,250 g, the human brain is considerably larger than the mouse brain, and the layers of dura and arachnoid mater are much thicker in humans than in the mouse. As a consequence of their size and of the volume of the CSF spaces, trafficking distances are much greater in human brains than in rodent brains, so that any trafficking of immune cells from the choroid plexus, for example, to the surface of the cerebral cortex, as described in the mouse, might be less feasible in humans. The complexity and thickness of the leptomeningeal layers coating the brain[25] and spinal cord[96] are much greater in humans than in rodents. These anatomical differences suggest that the ability of immune cells to penetrate the surface layers of the brain and spinal cord and thus induce intraparenchymal inflammation may be much less substantial in humans than in rodents[24]. At the same time, pial and cortical (gray matter) brain angioarchitecture is very similar in mice and humans (**Fig. 4a**–**f**). Arteries arising from the subarachnoid space feed pial arteries that penetrate the cortex, perpendicular to the surface, to supply the capillary network with a high density of honeycomb-like loops[90]. The capillary network drains into postcapillary venules, which subsequently drain into penetrating venules that are also perpendicular to the surface. A pial venular network forms from which blood eventually flows into major draining veins in the subarachnoid space. The dimensions of the capillary network and postcapillary venules are similar in humans and mice, leading to identical hemodynamic conditions for circulating immune cells at the endothelial lining. The vessels visualized on the dorsal aspect of mouse and human spinal cords are of pial and medullary (white matter) origin (**Fig. 4g**–**l**). Whereas in the mouse

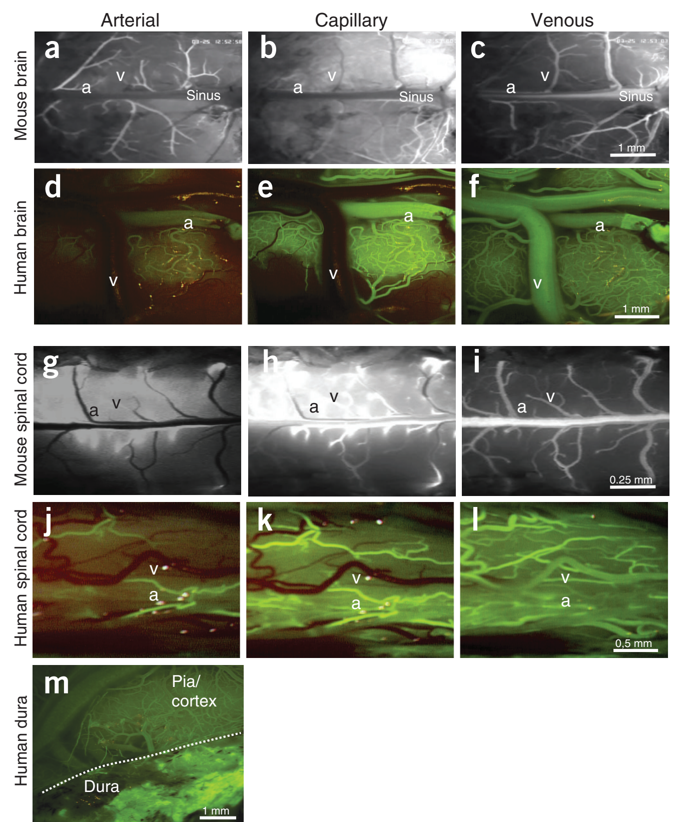

Figure 4 Intravital fluorescence videomicroscopy of mouse and human CNS vasculature. (**a**–**f**) Intravital fluorescence videomicroscopy images of murine pial and cortical brain vasculature (**a**–**c**, through cranial-window preparation) and of human pial and cortical brain vasculature (**d**–**f**, during neurosurgical procedure). (**g**–**l**) Intravital fluorescence videomicroscopy images of murine pial and medullary spine vasculature (**g**–**i**, through spinal-window preparation) and of human pial and medullary spine vasculature (**j**–**l**, during neurosurgical procedure). (**m**) Intravital fluorescence videomicroscopy image of human pial/cortical brain vasculature and blood vessels within the inner layer of the dura mater (facing the subdural space; here reflected for a neurosurgical procedure). Note the difference in extravasation of the fluorescent marker; the pia and cortex show no leakage, whereas the dura shows massive leakage of green fluorescent tracer. (**a**–**c**, **g**–**i**) Injection of fluorescently labeled dextran. (**d**–**f**, **j**–**m**) Injection of fluorescent tracer. Images of mouse and human CNS vasculature depict analogous regions of the brain. a, major supplying pial artery in subarachnoid space; v, major draining pial vein. Scale bars apply to all images in the respective row.

the capillary network is hidden in the depths, in humans the capillary network is close to the dorsal surface of the spinal cord. Structures that can be visualized on the posterior surface of the mouse spinal cord include short postcapillary and perforating veins, pial-draining venules and one large subarachnoid midline vein. In humans, the postcapillary venules and pial veins tend to be individually organized, and the major draining vein is less dominant and shows more variations than that in the mouse.

These similarities between rodents and humans apply also to BBB characteristics of blood vessels in the brain and spinal cord. The use of low-molecular-weight fluorescent tracers in humans has confirmed BBB properties of the CNS blood vessels under physiological conditions, as described in rodents (**Fig. 4a**–**l**). Changes in BBB properties occur during CNS pathologies in humans and provide a diagnostic means to identify brain tumor tissue and delineate it from unaffected brain tissue[97]. Future studies may take advantage of human *in vivo* imaging to provide further evidence of BBB properties in the context of CNS inflammation, hemorrhage or trauma.

In vivo imaging in humans has clearly demonstrated a lack of BBB properties in dural blood vessels (**Fig. 4m**), highlighting the fact that the blood supply to the dura mater is anatomically and functionally distinct from that of the CNS. The dura entertains afferent and efferent connections with the immune system, including dural lymphatics, allowing for rapid activation of immune cell recruitment. Functional differences between leptomeningeal and CNS parenchymal blood vessels may be less obvious in imaging studies of the rodent CNS. The rodent dura mater is very thin, consisting of a few cell layers and a fine connective-tissue-like vascular system that parallels the brain or spinal cord surface. Despite these minor architectural differences, the anatomy and functionality of the CNS microvasculature are similar in rodents and humans. Nevertheless, there are some pitfalls related to the interpretation of results from epifluorescence and two-photon imaging of the rodent CNS that scientists must consider when translating experimental observations into predictions about human pathophysiology. Given the differences in the thickness of the leptomeninges and the size of the leptomeningeal spaces, observations of immune-cell trafficking through the superficial barriers and leptomeningeal spaces should be interpreted with caution and may not be directly translatable from rodents to humans. Furthermore, intravital microscopic observations are often limited to the surface of the brain, because at that level the entire vascular network is organized along the *x*- and *y*-axes. This focal plane of imaging allows for the visualization of pre- and postcapillary vessels over long distances, as well as analysis of immune-cell interactions along the vascular wall and of the cells' extravasation. However, as outlined above, these are the vessels of the cortical surface and the SAS, and thus might not be representative of the intraparenchymal situation. The same holds true for the spinal cord, in which the white matter capillaries are hidden in the depths of the structure. Although modern two-photon imaging techniques provide enough capability for penetration into CNS tissue, they are often not exploited because the blood vessels would run perpendicular to the *xy*-plane, resulting in cross-sectional cuts of the penetrating pre- and postcapillary blood vessels, which are less instructive in microscopy videos. In addition, three-dimensional reconstructions of these vessels may make real-time imaging with adequate temporal resolution difficult. Finally, many researchers leave the dura mater in place when preparing their cranial-window or spinal-window preparations so as to minimize direct trauma to the surface of the CNS parenchyma. This procedural process carries the risk that the intravital microscopic observations will focus on dural and subarachnoid vessels, and thus that the resulting observations will not be representative of the immune-privileged CNS parenchyma in light of the immunocompetence of the dura mater and the SAS. Given the overall similarity between the anatomy and functionality of the rodent and human brain barriers and CNS microvasculature, correct interpretation of intravital microscopy studies in rodents promises to provide important insights into the efferent and afferent neuroimmune pathways of the CNS in humans.

Conclusions and outlook

The CNS parenchyma is characterized by its paucity of adaptive and innate immune responses compared with those of peripheral tissues. Thus, there is no need to revisit the concept of CNS immune privilege in its original experimental definition[1]. The CNS does, however, harbor efferent and afferent connections to the immune system, which prompts a reassessment of the precise mechanisms underlying the immune privilege of the CNS. We propose that endothelial and epithelial brain barriers establish compartments in the CNS that differ in their accessibility to the afferent and efferent arms of the immune system. The brain barriers thus provide an anatomical basis for CNS immune surveillance in the CSF-filled ventricular space and SAS without the risk of disturbing homeostasis in the CNS parenchyma that is entirely surrounded by the glia limitans. Adaptive immune cells carry out immune surveillance of the CNS (addressed by Klein *et al.*[98] in their Review in this issue). Their entry into the CNS is tightly controlled by the BBB and is restricted to specific subsets of T and B cells. Under physiological conditions, their dissemination in the CNS is limited to the CSF-filled ventricular spaces and SAS, which drain to deep cervical lymph nodes. At the same time, the CNS parenchyma that harbors the CNS neurons requires tissue homeostasis. It is completely devoid of adaptive immune cells, which are prevented from reaching this part of the CNS by the glia limitans that provides a tissue barrier around the entire CNS parenchyma. Lymphatic drainage of ISF from the CNS parenchyma along basement membranes in the walls of cerebral capillaries and arteries lacks the capacity for the trafficking of APCs, and is thus distinct from the drainage of CSF. It needs to be emphasized that the dural lymphatics recently shown to drain CSF into deep cervical lymph nodes have no direct connection with the CNS parenchyma and are separated from the CNS parenchyma by two tissue barriers, namely, the glia limitans and the arachnoid BCSFB.

Modern *in vivo* imaging technologies combined with transgenic mouse models designed to express fluorescent proteins in a tissue-specific manner have opened innovative avenues to study the efferent and afferent pathways of immune surveillance of the CNS, and thus have allowed scientists to demonstrate the truth in the suggestion that immune privilege of the CNS is hiding in plain sight[99]. Although seeing is believing, the intrinsic dilemma in applying these technologies is that they may neglect the components that are not visible to the eye. Although second-harmonic signals allow for the visualization of collagen and barriers outside of the CNS parenchyma, more sophisticated animal models that allow the specific visualization of the different cellular and acellular brain barriers will be essential in order for the mechanisms underlying immune surveillance of the CNS to be fully understood. Consideration of the differences in anatomy between rodent and human brains and spinal cords, combined with recent advances in the *in vivo* imaging of blood vessels in the human CNS during neurosurgery, will bridge the gap by enabling observations made in the rodent CNS to be translated to humans. These studies will pave the way to understanding the cellular and molecular principles underlying immune privilege of the CNS, which is critical when designing therapies that inhibit or improve the efferent or afferent arms of immune surveillance of the CNS in neurological disorders such as multiple sclerosis, stroke and Alzheimer's disease.

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The authors declare no competing financial interests.

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