The anatomical and cellular basis of immune surveillance in the central nervous system

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Abstract | The central nervous system (CNS) comprises the brain, spinal cord, optic nerves and retina, and contains post-mitotic, delicate cells. As the rigid coverings of the CNS render swelling dangerous and destructive, inflammatory reactions must be carefully controlled in CNS tissues. Nevertheless, effector immune responses that protect the host during CNS infection still occur in the CNS. Here, we describe the anatomical and cellular basis of immune surveillance in the CNS, and explain how this shapes the unique immunology of these tissues. The Review focuses principally on insights gained from the study of autoimmune responses in the CNS and to a lesser extent on models of infectious disease. Furthermore, we propose a new model to explain how antigen-specific T cell responses occur in the CNS.

Multiple sclerosis
A neurological disease that is characterized by focal demyelination in the central nervous system with leukocyte infiltration.

Neuromyelitis optica
An autoimmune disease of the central nervous system associated with antibodies to aquaporin 4, the astrocyte water channel.

Deep cervical lymph nodes
(DCLNs). A group of lymph nodes situated around or near the internal jugular vein.

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doi:10.1038/nri3265
Published online
20 August 2012

The central nervous system (CNS) is structurally and functionally unique but, in common with all other tissues, requires effective immune mechanisms to protect against infection. However, failure to control immune responses in the CNS can result in chronic immunopathological disorders, such as multiple sclerosis and neuromyelitis optica. The CNS has long been recognized as a site of 'immune privilege' — that is, antigens administered into CNS parenchymal tissues do not induce the development of adaptive immune responses (BOX 1). Nevertheless, adaptive immune responses can be initiated against CNS antigens, so how do these occur?

One barrier to understanding the immunology of the CNS is the complexity of its tissues. In this Review, we describe the anatomy of the CNS in order to explain how this structural organization shapes the nature of immune responses at this site. We detail the different immune cell populations that are found in the CNS and examine how leukocyte trafficking contributes to immune surveillance in the CNS. Given the immune privilege of the CNS, it is logical that adaptive immune responses against CNS antigens are initiated in the periphery and subsequently propagated to the CNS by circulating memory T cells, which are re-stimulated by antigens within the CNS. Here we offer a novel proposition: that memory T cells constitutively monitor the CNS by trafficking through cerebrospinal fluid (CSF) within the subarachnoid space (SAS), where they encounter a diverse range of antigen-presenting cells (APCs). We discuss recent clinical observations that support this model and its implications for therapy.

Delivery of CNS antigens to lymph nodes

Despite lacking lymphatic vessels, the CNS possesses a soluble route for antigen delivery. The interstitial fluid of the CNS drains via perivascular channels into the CSF, allowing meningeal macrophages and other APCs in the SAS to sample the full range of CNS antigens. Furthermore, CNS-derived soluble antigens are transported in the CSF to the nasal mucosa, where afferent lymphatics to the deep cervical lymph nodes (DCLNs) reside, leading to the accumulation of these antigens in the DCLNs[1] (FIG. 1). Soluble antigens placed in cerebral ventricles induce the generation of detectable antibody-secreting cells in DCLNs, indicating the functional relevance of antigen delivery via the CSF[2]. Thus, by draining interstitial fluid and conveying antigens to lymph nodes, the CSF acts as a functional equivalent of lymph.

However, parenchymal dendritic cells (DCs) that could provide a cellular route for antigen delivery from the CNS to DCLNs have not been observed (BOX 1). The challenge of determining whether APCs leave the CNS and migrate to lymph nodes has remained unmet, despite persistent experimental assault. Some discrepancies have arisen owing to semantics. Indeed, it is clear that myeloid cells with DC-associated markers are

Box 1 | **Immune privilege of the CNS**

- Observations that underpin central nervous system (CNS) immune privilege have been reported serially over approximately 90 years and, over time, the definition has become imprecise. The concept was recently reconsidered, precisely defined and made scientifically coherent, principally in a seminal review[13].
- CNS immune privilege is demonstrated by placing immunogenic material (such as tumour cells, viruses or bacteria) carefully in the brain parenchyma, avoiding the ventricles and meninges and minimizing tissue disruption by injecting small volumes slowly. Using this procedure, immunogenic materials escape immune recognition (that is, they fail to elicit a timely adaptive immune response).
- Two observations constitute the foundation of the concept of CNS immune privilege. The first is the lack of a cell-mediated response to instilled antigens, as discussed above. The second key observation is that a brisk, robust response to intraparenchymal antigens can be elicited by peripheral immunization. This finding implies a mechanism for immune surveillance of the CNS (and a means to initiate the efferent limb of CNS immunity), which is the topic of this Review.
- Immune privilege is not absolute; eventually an immune reaction may be observed.
- The cellular basis of CNS immune privilege lies in the absence of a route to the lymph nodes for the antigen-presenting cells that reside in the parenchyma and perivascular spaces of the healthy CNS.
- Other attributes of the CNS — such as the blood–brain barrier, the lack of MHC molecule expression by CNS parenchymal cells and the anti-inflammatory nature of the CNS tissue environment — help to account for the specialized and largely subdued character of CNS inflammatory reactions, which is extremely important for the preservation of the delicate non-regenerating tissue elements in the CNS. However, these characteristics of the CNS are not directly relevant for immune privilege.

present at various CNS sites, including the parenchyma, where myeloid cell-derived processes are extended into the perivascular space across the glia limitans[3,4]. Furthermore, the choroid plexus, cerebral ventricles and meninges contain cells that phenotypically resemble DCs and might potentially be capable of travelling to DCLNs.

However, there are currently only very limited data to support the possibility that DCs migrate from the CNS to DCLNs. One study showed that intraparenchymal injection of an antigen elicited an immune response, and, following the injection, the antigen could be found in association with intraparenchymal DC-like cells in draining lymph nodes[5]. These experiments have yet to be replicated by others, preventing the generalization of these findings. However, it is useful to keep these data in mind when considering CNS immunity and not to uncritically adhere to dogma. During CNS inflammation in patients with viral encephalitis or multiple sclerosis, as well as in relevant animal models, cells with varied DC phenotypes can be found within the brain parenchyma[6]. Analogous cells described by others have the capacity to stimulate resting T cells *in vitro,* directly *ex vivo* and possibly also *in vivo*[7–9]. Although DCs from the inflamed CNS parenchyma have not been shown to exit into CSF, observations made while studying active multiple sclerosis tissue lesions suggest the possibility that such an event might take place[10].

In particular, active subpial cortical demyelination has occasionally been detected in lesions that are present in tissue sections from patients with multiple sclerosis. In this context, active demyelination is defined by the presence of phagocytic macrophages containing myelin protein inclusions. Some macrophages that contained myelin protein inclusions were localized to the juxtapial meninges and expressed CC-chemokine receptor 7 (CCR7), indicating the capability to enter lymph nodes and potentially to present myelin antigens to naive or memory T cells. However, a crucial unresolved question is whether any APC has the capacity to leave the CNS and transport CNS-derived antigens to draining lymph nodes.

Anatomy of the CNS

Anatomy defines the immunological challenges faced by each organ and specifies the defence mechanisms deployed to meet them. In no case is this principle more salient than in the CNS. Here, we present the anatomical features of the CNS that are relevant for understanding how immune and inflammatory reactions occur at this site. We focus on the brain and spinal cord rather than the eye, which has been recently reviewed[11].

CSF: an immunologically active body fluid. The brain and spinal cord float in CSF, which provides protective padding for these delicate tissues and contributes to CNS metabolism and homeostasis. The great majority of CSF is produced within the ventricles of the brain by the choroid plexus (FIG. 1), which is composed of networks of capillaries with fenestrated endothelia that are embedded within a stroma. The stroma of the choroid plexus is, in turn, covered by a monolayer of epithelial cells. Choroid plexus epithelial cells generate the CSF from arterial blood through diffusion and active transport. A small portion of CSF is derived by diffusion from CNS interstitial fluid. Propelled by pulsations of the choroid plexus and the action of cilia on ependymal cells that line the ventricles, CSF circulates from the ventricles, which are filled with CSF, through brainstem exit ports to cover the exterior surfaces of the spinal cord, brain stem, cerebellum and cerebral cortex. Direct reabsorption of CSF into blood takes place at arachnoid villi, which are outpouchings of the arachnoid membrane through the dura and into venous sinuses, where the CSF is exposed to venous blood. CSF volumes range from approximately 140 to 200 ml. As daily CSF production averages more than 500 ml, the fluid turns over between three and five times per day.

In addition to being reabsorbed into blood, CSF drains along cranial and spinal nerves to local afferent lymphatics, thereby carrying soluble antigens to the draining lymph nodes (reviewed in REF. 12) (FIG. 1). The best-characterized passage of this type involves the percolation of soluble antigens in CSF to the base of the frontal lobes of the brain, followed by transport along olfactory rootlets to the nasal mucosa and then to the afferent lymphatics of the DCLNs. When a labelled antigen is placed into the cerebral ventricles, approximately half of the amount administered is subsequently found in DCLNs. When placed within CSF around the brain, the antigen is found in DCLNs within 2 hours of injection, and antigen-specific antibody-secreting cells are found in DCLNs within 4 days, confirming that this route of antigen transit[2] is integrated in functional immunological circuitry.

Olfactory rootlets
Afferent input to the olfactory bulb neurons within the central nervous system (CNS) comes from olfactory rootlets, the sensory endings of which lie in the nasal epithelium in the nasal mucosa. These nerves enter the skull across the cribriform plate at the base of the skull below the frontal lobes. Because these rootlets are part of the CNS, they are covered by meninges containing cerebrospinal fluid, which percolates outwards to the nasal mucosa.

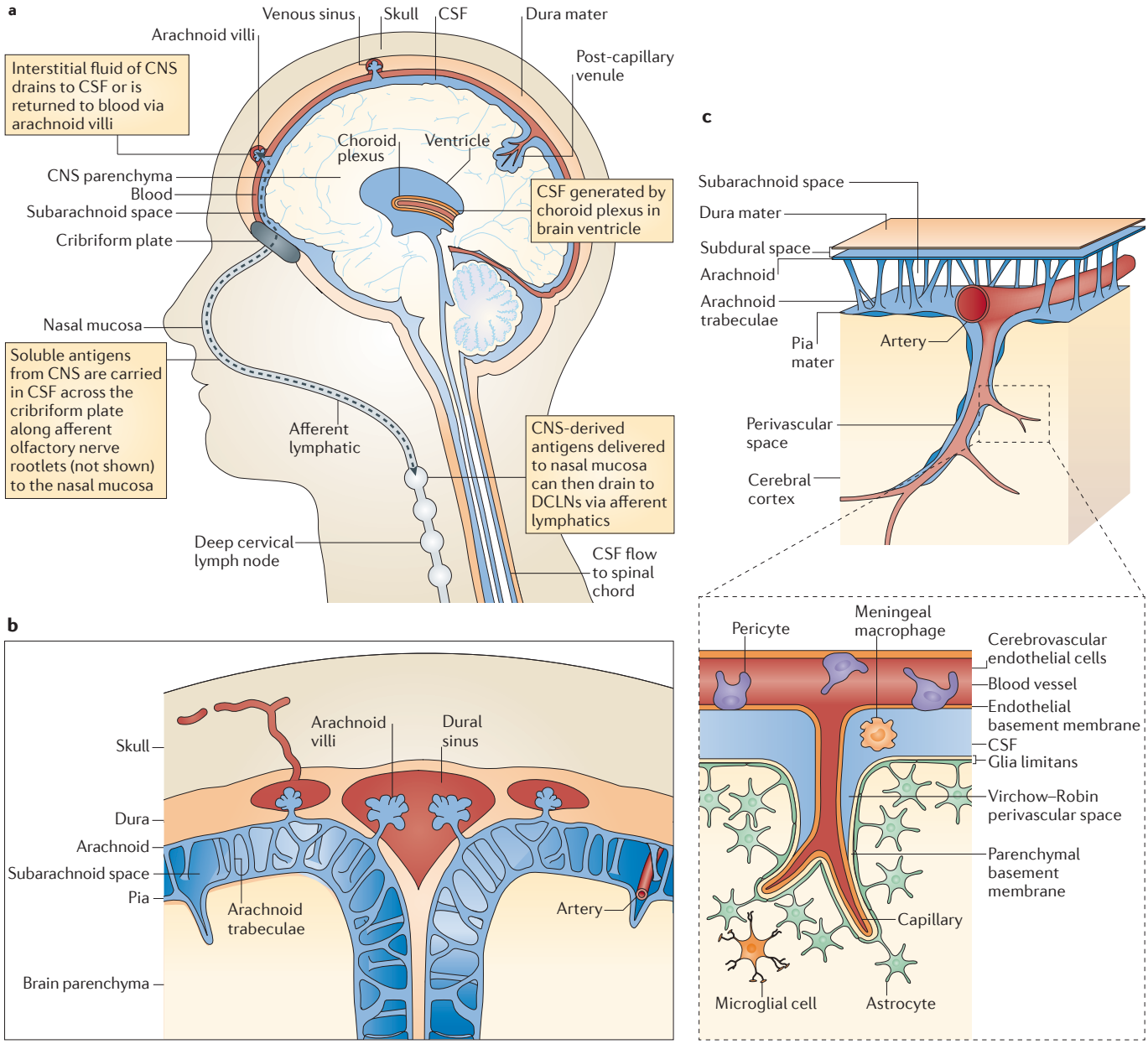

Figure 1 | **CSF-mediated drainage of interstitial fluid and CNS antigens to deep cervical lymph nodes.** **a** | A human head in midline sagittal section, showing revelant anatomical structures (namely the ventricle, choroid plexus, central nervous system (CNS) parenchyma, lymphatics and deep cervical lymph nodes (DCLNs)) in schematic form. **b** | Arachnoid granulations in relation to the subarachnoid space and brain parenchyma. **c** | Subpial vasculature in relation to subarachnoid space and brain parenchyma, indicating the anatomy discussed in the main text. The inset shows the cellular components of cerebral capillaries, the glia limitans and the basement membranes in relation to the perivascular space. CSF, cerebrospinal fluid.

Structure of the meninges and their relation to the surfaces of the brain. The meninges comprise a series of three membranes that enclose the CNS parenchymal tissues and the CSF. The outermost membrane is the tough fibrous dura, which is beneath the inner surface of the skull. CSF is constrained between the two inner membranes: the arachnoid, which lies under the dura; and the pia, which closely covers the CNS parenchyma. The fluid-filled SAS is criss-crossed by a dense stroma and traversed by arterial vessels that supply the brain and spinal cord. These vessels penetrate the CNS parenchyma and are lined by the arachnoid and pia for several millimetres from the site of parenchymal entry, giving rise to perivascular spaces (termed Virchow–Robin spaces) that are directly continuous with the SAS. Such CNS perivascular spaces are enclosed and delimited by an endothelial basement membrane on the abluminal side of the vessel wall and the glia limitans (specifically the glia limitans perivascularis) on the parenchymal side of the perivascular space (see below and FIG. 1). The external surface of the brain and spinal cord are covered by a network of astrocytic processes along with the parenchymal

Experimental autoimmune encephalomyelitis
(EAE). An experimental model for the human disease multiple sclerosis. Autoimmune disease is induced in experimental animals by immunization with myelin or peptides derived from myelin. The animals develop a paralytic disease with inflammation and demyelination in the brain and spinal cord.

Exosomes
Small lipid-bilayer vesicles that are produced by various cell types and released into the extracellular space following the fusion of multivesicular bodies with the plasma membrane.

basement membrane, collectively designated the glia limitans superficialis, which is continuous with the glia limitans perivascularis. In this sense, the perivascular space lies outside the brain parenchyma.

Interstitial fluid of the brain joins CSF either by permeating from the cortical grey matter towards the surface of the brain along the perivascular spaces of cerebral arterioles to the Virchow–Robin spaces or by flowing along nerve fibre tracts through the white matter centripetally to the cerebral ventricles, the ependymal lining of which lacks barrier properties. Therefore, solutes in parenchymal interstitial fluid contribute to the composition of CSF. These solutes can be transported to DCLNs as noted above, or they can be sampled by the myeloid cells of the SAS, which include meningeal macrophages and perivascular macrophages, all of which are endowed with the capacity for antigen presentation (BOX 2). Neither CSF nor the CNS parenchyma contains naive T cells under physiological circumstances, and classical studies suggest that primary immune responses are not primed in the parenchyma[13]. However, in the highly inflammatory context of ongoing experimental autoimmune encephalomyelitis (EAE), T cells expressing transgenic T cell receptors (TCRs) that recognize myelin epitopes with high affinity are activated in the CNS (not in DCLNs or in the periphery)[9]. This result confirms that APCs do not migrate from the CNS to local lymph nodes or to remote secondary lymphoid tissue in this context. In a different genetic model, T cells with myelin-specific transgenic TCRs and a naive phenotype were detected in the healthy CNS[14]. These cells were tolerized, probably by local exposure to CNS antigens.

Another type of myeloid cell population (termed Kolmer's epiplexus cells[15]) is interdigitated with the choroid plexus epithelium and ventricular ependyma, and these cells could potentially present antigens to lymphocytes in the ventricles. When pathogens have gained access to the CNS parenchyma, this APC network could sample microbial components from interstitial fluid and CSF and promote host defence. However, cellular constituents derived from tissue turnover are also found in the interstitial fluid, and, as such, CSF provides a source of self antigens that might promote the development of autoreactive immune cells. A recent fascinating study showed that oligodendroglial cells — which are unable to recycle membrane components from compact myelin sheaths remote from their somas — pinch off myelin-rich exosomes that are taken up by local microglia[16]. Interestingly, inflammatory stimuli reduce the capacity of microglia to ingest these exosomes. This suggests that inflammation might promote myelin-specific autoimmune responses by enabling myelin-rich exosomes to bypass microglia in the brain parenchyma and be transported along with interstitial fluid to meningeal macrophages, which can then present myelin-derived antigens to myelin-specific T cells in the CSF.

CNS vascular anatomy. The architectural features of CNS microvessels are uniquely suited for their various tasks. Most importantly, neuronal activity in the CNS depends on stringent ionic homeostasis. Therefore, endothelial cells lining CNS capillaries establish the blood–brain barrier (BBB), which strictly controls the movement of solutes across the CNS vasculature[17] (FIG. 1). In addition, CNS microvessels have the capacity to rapidly respond to increases in the electrical activity of neurons by enhancing regional blood flow, an essential property referred to as neurovascular coupling. Brain microvascular endothelial cells are embedded in a unique microenvironment termed the neurovascular unit, which includes an endothelial basement membrane that encloses numerous pericytes, which are essential for vessel maturation[18–20], as well as astrocyte endfeet and neuronal processes. Indeed, cerebral microvascular endothelial cells acquire BBB characteristics (such as intercellular tight junctions, limited macropinocytosis and efficient efflux transporters) as well as rapid responsiveness to neuronal activity through contact-dependent and -independent signals from elements of the neurovascular unit.

The entire abluminal aspect of CNS parenchymal microvessels is also ensheathed by the glia limitans perivascularis, which comprises a parenchymal basement membrane and a layer of astrocytic endfeet. Astrocytes lay down the parenchymal basement membrane, produce

Box 2 | **Immune cells of the healthy CNS**

Parenchymal microglia
Parenchymal microglia arise from a yolk sac progenitor in mice between embryonic day 7 (E7) and E7.5, before definitive haematopoiesis, and enter the brain rudiment at E10.5. They are subsequently maintained by local proliferation and possess abundant proliferative capacity so that microgliosis can occur without blood cell infiltration.

Choroid plexus macrophages, dendritic cells and Kolmer's epiplexus cells
Choroid plexus macrophages and dendritic cells (DCs) reside in the choroid plexus parenchyma (outside the central nervous system (CNS)). They express DC-associated cell-surface markers such as CD11c and are increased in number during CNS inflammation, and they are probably replaced from blood elements, although there is no formal evidence of this. Kolmer's epiplexus cells are localized at the apical side of choroid plexus epithelial cells facing the cerebrospinal fluid (CSF) space and are thought to function as local antigen presenting cells.

Meningeal macrophages
Meningeal macrophages have a morphology and surface phenotype ($CD11^{+}CD45^{hi}F4/80^{+}$) that is typical of tissue macrophages, but their origins and homeostatic maintenance have not been formally addressed. They have antigen-presenting cell functions during experimental autoimmune encephalomyelitis.

Perivascular macrophages
Perivascular macrophages are found both in Virchow–Robin spaces (within a small distance of penetration of subpial vessels) and in the perivascular spaces of the parenchyma. They can have an elongated morphology and can be closely associated with the abluminal endothelial surface. Indeed, detailed confocal microscopy studies showed that the supposed expression of MHC class II molecules by cerebrovascular endothelium was actually attributable to perivascular macrophages. These cells are responsible for re-stimulating lymphocytes with peptide–MHC complexes after extravasation and for licensing these lymphocytes to enter the CNS parenchyma.

Cellular composition of CSF
Of the total cells trafficking through CSF, ~90% are T cells, ~5% are B cells, ~5% are monocytes and <1% are DCs.

Other cell types found in the CNS
Meningeal and perivascular mast cells are also present in the CNS.

Absent from the healthy CNS
Neutrophils and other granulocytes are absent from the healthy CNS, as are resident DCs, except for those found in the choroid plexus.

growth factors for BBB maturation and maintenance, and regulate local water transport (reviewed in REF. 21). At the capillary level, the endothelial and parenchymal basement membranes fuse into a double basement membrane, effectively limiting the perivascular space to a potential space that appears collapsed in most histological preparations but can expand to accommodate oedema fluid or infiltrating cells. At the level of CNS post-capillary venules, the two basement membranes separate, making room for a small perivascular space containing extracellular fluid, where occasional APCs are found. Around CNS arteries and veins, the endothelial and parenchymal basement membranes separate further, extending the perivascular space to accommodate leptomeningeal mesothelial cells and additional APCs. Finally, at the pial surface of the brain and spinal cord, the perivascular spaces open towards the SAS and the endothelial basement membrane accompanies the endothelial layers of the vessels. By contrast, the glia limitans perivascularis separates from the vessels and continues as the glia limitans superficialis, surrounding the entire surface of the brain and spinal cord.

The cul-de-sac architecture of the CNS perivascular spaces promotes a unidirectional flux of interstitial fluid from the parenchyma along the perivascular spaces towards the SAS, and this is driven by the arterial pulse pressure. This pathway of interstitial fluid flow leads, ultimately, to DCLNs via the CSF, compensating for the lack of lymphatic vessels to mediate the drainage of interstitial fluid in the CNS.

In the SAS, which surrounds all of the surfaces of the brain and spinal cord, meningeal blood vessels lack ensheathment by a glia limitans perivascularis[22]. However, the SAS is sealed off from the parenchymal surface by the glia limitans superficialis. Interestingly, although microvessels in the SAS exhibit BBB properties, there are subtle differences between parenchymal and meningeal microvessels. For example, P-selectin is stored in the Weibel–Palade bodies of meningeal but not parenchymal microvascular endothelial cells[23,24].

In summary, the CNS poses a special case of regional anatomy, one that lacks resident DCs and lymphatic drainage, but possesses a diverse range of fixed APCs that facilitate immune surveillance. The CSF carries out the crucial function of interstitial fluid drainage and also serves as the equivalent of lymph in the CNS, as it drains to DCLNs across the cribriform plate.

Immune cell populations of the CNS

The healthy CNS parenchyma contains only one type of immune cell — the parenchymal microglial cell, a highly specialized tissue macrophage. Microglia originate from a yolk sac progenitor distinct from that which yields monocytes or macrophages[25], and they are maintained throughout life without reconstitution from the bone marrow[26,27]. Within the CNS parenchyma, microglia are confined in an immunosuppressive environment and shielded from plasma proteins[28,29]. The meninges and the choroid plexus contain meningeal macrophages and choroid plexus macrophages, respectively, and the perivascular spaces of the CNS parenchyma are colonized by perivascular macrophages. In addition, mast cells are associated with the meninges and perivascular spaces. Aside from microglia, which are renewed by local proliferation, the other myeloid populations in the CNS are maintained by replacement from blood monocytes[26,27,30]. CSF mainly contains a trafficking population of memory T cells, as well as a few B cells and monocytes. No other immune cells (such as neutrophils or other granulocytes, innate lymphocytes or resident DCs) have been rigorously documented in the healthy CNS (BOX 2).

Innate APC populations in the CNS. Myeloid cells of the CNS can be divided into two main populations. One population comprises epiplexus cells of the choroid plexus and meningeal, perivascular and ventricular macrophages (apart from the juxtavascular parenchymal $CD11c^+$ cells recently described[3]). These cells are exterior to the parenchyma, but they sample its contents by virtue of their presence in the CSF flow pathways. Population homeostasis is achieved by replacement from blood-borne cells, which are most likely to be monocytes. During CNS inflammation, the numbers of myeloid cells increase as a result of infiltration from the blood and the proliferation of the resident microglial cells (see below). All of these different myeloid cells express, to a variable extent, the attributes required for antigen presentation, including MHC class II, co-stimulatory and adhesion molecules. Under highly inflammatory conditions, CNS myeloid cells can upregulate APC- and DC-associated markers — including CD11c, MHC class II and co-stimulatory molecules — and can elicit antigen-driven proliferative responses from resting $CD4^+$ T cells[8,9].

Parenchymal microglia, the second population, are unique among CNS myeloid cells, having entered the CNS in early embryonic life[25]. This population is maintained or expanded by the proliferation of local progenitors[27,31] and is confined behind the BBB, which is formed shortly after birth[25,29]. In explant cultures, microglia express accessory molecules, such as MHC class II, CD40 and other co-stimulatory molecules, particularly following incubation with certain pro-inflammatory cytokines, including interferon-γ (IFNγ). *In vivo,* however, microglia express low levels of the accessory molecules required for efficient antigen presentation and have weak antigen-presenting activity[28,29,32].

CSF also contains a small proportion of monocytes (which constitute ~5% of all CSF cells). About 75% of monocytes in the CSF express CCR5, as compared with 10–20% of monocytes in the blood, suggesting that CSF monocytes are in an activated state[33]. Plasmacytoid and myeloid DCs have also been detected in the CSF of individuals with neuroinflammation (primarily that caused by infection), although these cells represent only a small proportion of the leukocytes in the CSF[34,35] and their functional significance is uncertain.

T cells in CSF. The CSF of healthy individuals contains 1,000–3,000 cells per ml, so that at any time 150,000–750,000 cells are present in the CSF. The cellular composition of CSF is overwhelmingly mononuclear and

Pericytes
Cells embedded in the vascular basement membrane of microvessels that are related to mesenchymal stem cells. They make close cellular contact with endothelial cells, and this interaction is essential for the maintenance of vessel function, as well as for the regulation of angiogenesis and vascular remodelling.

Astrocyte
A type of glial cell that is found in the vertebrate brain and is named on the basis of its characteristic star-like shape. These cells provide both mechanical and metabolic support for neurons, thereby regulating the environment in which neurons function.

Leptomeningeal mesothelial cells
Mesothelial cells constitute the membranes lining body cavities, in this case the arachnoid and pia. These flattened polygonal cells also line the trabecular network in the subarachnoid space. They are capable of solute uptake and can present antigen to $CD8^+$ T cells in an MHC class I-restricted fashion.

Weibel–Palade bodies
Specialized secretory vesicles that are present in resting endothelial cells and sequester molecules such as P-selectin and chemokines that facilitate leukocyte–endothelial cell interaction.

Cribriform plate
A portion of the ethmoid bone that separates the nasal cavity from the brain. The paper-thin cribriform plate is perforated by numerous openings that admit the olfactory rootlets.

lymphocytic, with ≥90% of the cells being T cells. The ratio of $CD4^+$ T cells to $CD8^+$ T cells is about 3.5 to 1 (REFS 36–39). In this regard, CSF is atypical by comparison to other sterile bodily fluids, such as synovial or pleural fluid, both of which contain 10–100-fold more cells than CSF and have a proportion of T cells in the range of 25% or less of the total cells[40,41].

The CSF population of $CD4^+$ T cells contains very few naive cells. CSF $CD4^+$ memory T cells have characteristics both of central memory T cells (T_{CM} cells) and effector memory T cells (T_{EM} cells)[42], as they have a $CD45RA^-CD27^+CD62L^{hi}CCR7^+CXCR3^+$ α4-integrin-positive phenotype[36,43]. These $CD4^+$ T cells of the CSF are comparable with the corresponding $CD4^+$ memory T cell populations in the blood, in that subpopulations express different sets of homing receptors, such as α4β7 integrin and CCR9 or cutaneous leukocyte antigen and CCR4, which promote trafficking to the gut and skin, respectively[44]. They can also express receptors such as CCR5 that promote homing to inflamed tissues[36,43]. Approximately 40% of CSF T cells express the recent-activation antigen CD69, whereas this molecule is expressed by <5% of T cells in blood[36]. It is unclear whether recent activation facilitates T cell entry into the CSF of healthy individuals, or whether cells recovered by lumbar puncture from the SAS had been activated *in situ*.

It appears likely that T cells enter the CSF, and thereby gain access to the SAS, mainly across the choroid plexus, particularly in healthy individuals and early in neuroinflammatory disease[43] (FIG. 2). This hypothesis is supported by the equivalence of the ventricular and lumbar CSF $CD4^+$ T cell populations in humans without acute meningeal infection[45]. T cell trafficking to the CNS in mice was altered by deletion of *Ccr6*. However, the effects of CCR6 deficiency on neuroinflammatory disease occurrence were complicated owing to the use of CCR6 both by pathogenic T helper 17 (T_H17) cells and by inhibitory regulatory T (T_{Reg}) cells. In one study, trafficking CCR6-deficient T_H17 cells accumulated in the choroid plexus and disease initiation was impaired[46] (see below). In another study, CCR6-deficient T_{Reg} cells showed decreased entry into the CNS and disease was worsened[47]. Although quantitative studies have not been performed, it is evident that T cells can leave the CSF of rodents via the cribriform plate and enter DCLNs, thereby demonstrating functional attributes of T_{CM} cells (that is, homing to lymph nodes)[48]. The functional significance of lymphocyte trafficking to the DCLNs has been documented through studies of spontaneous autoimmunity in transgenic mice[49]. In particular, in a mouse model of EAE, T cells that express transgenic TCRs specific for a CNS antigen first become activated in DCLNs[49].

In summary, T cells follow the pathways of CSF production and flow during physiological trafficking and early in autoimmune neuroinflammatory disease. To date, CSF seems to be the sole CNS location where $CD4^+$ T cells are readily identified in the healthy brain[35,39]. As autoimmune neuroinflammatory disease progresses in rodents, T cell entry into the SAS across subpial vessels becomes more pronounced, as was first shown by using two-photon imaging to study an adoptive transfer model of EAE. With disease evolution and the inflammation of deeper tissue vessels, entry across the parenchymal BBB dominates the pathological picture.

T cell and APC interaction in the inflamed CNS

T cell–APC interactions in the CNS support the essential function of re-stimulating memory T cells during inflammation. Many of the findings underlying this statement come from studying autoimmunity to myelin, as exemplified by the study of EAE, a mouse model of neuroinflammation. The disease is usually generated by peripheral injection of peptides from myelin proteins, using complete Freund's adjuvant and pertussis toxin according to standard protocols[50]. This same immunization protocol induces antigen-specific T cells in lymph nodes that drain the site of immunization. These T cells can be cultured *in vitro* with cytokines and adoptively transferred to a naive recipient, thereby enabling the direct study of effector mechanisms. Adoptively transferred T cells need several days to accumulate in the CNS. Careful tracking of labelled cells during this prodromal period showed, surprisingly, that these cells reside in pulmonary bronchus-associated lymphoid tissue (BALT), where they become 'licensed' to enter the CNS. This transient dwelling place is functionally significant, as cells transferred to neonates can reside in BALT and be stimulated as much as 3 months later with intra-tracheal antigen to mediate EAE[51].

Mice with EAE demonstrate CNS inflammatory infiltrates and show signs of motor weakness similar to those observed in patients with multiple sclerosis. It has been evident for some years that autoimmune T cells from the periphery require re-stimulation in the CNS to survive and to carry out the programme of cytokine production that culminates in disease[52–56]. Myeloid cells that mediate the re-stimulation of T cells in the CNS and promote T cell survival express the co-stimulatory molecules CD80 and CD86 (REF. 52), and $CD11c^+$ APCs can suffice for re-stimulation[54]. More recently, it was shown that the re-stimulatory encounter of T cells with antigen-laden myeloid cells occurs during the prodromal phase before EAE disease onset in the SAS[57].

To better understand these events, two-photon imaging was used to track the interaction of myeloid cells with adoptively transferred antigen-specific $CD4^+$ T cells[58]. These experiments showed that the T cells initially crawled along the luminal surfaces of inflamed subpial microvessels, presumably in search of exit points containing the correct pattern of immobilized chemokines and endothelial adhesion molecules[59,60] (FIG. 2). Following extravasation, these T cells crawled along the abluminal surfaces of subpial vessels within the SAS and, after encountering antigen-loaded myeloid cells, acquired competence to invade the parenchyma to mediate disease onset. The most striking demonstration of this T cell–APC encounter was produced by transferring

Central memory T cells
(T_{CM} cells). Antigen-experienced $CD4^+$ or $CD8^+$ T cells that lack immediate effector functions but are able to mediate rapid recall responses. These cells also rapidly develop the phenotype and function of effector memory T cells after re-stimulation with antigen. T_{CM} cells possess the migratory properties of naive and memory T cells and therefore can circulate through tissues as well as secondary lymphoid organs.

Effector memory T cells
(T_{EM} cells). Terminally differentiated T cells that lack lymph-node-homing receptors but express receptors that enable homing to inflamed tissues. T_{EM} cells can exert immediate effector functions without the need for further differentiation.

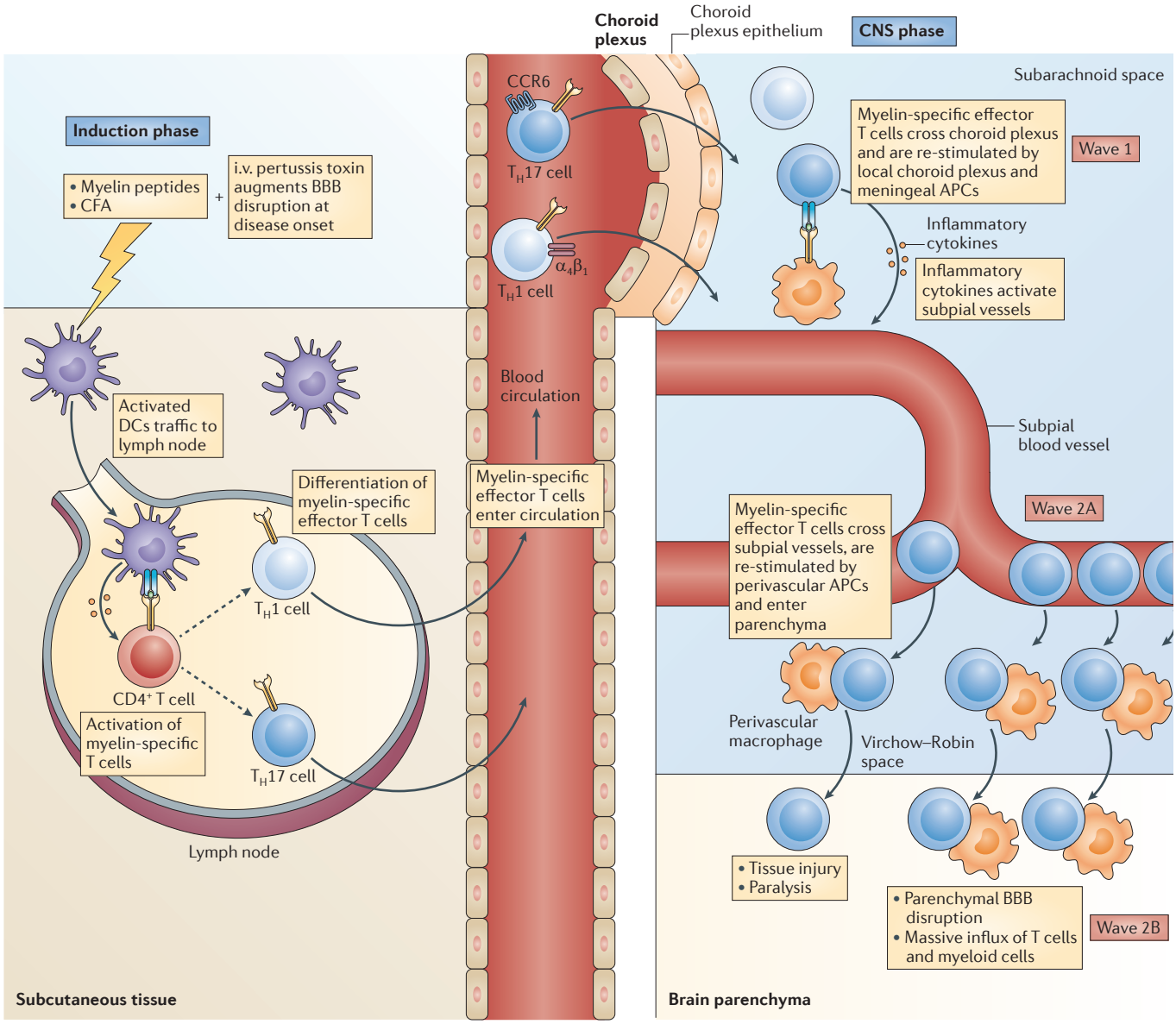

Figure 2 | **Pathogenic cascade in the periphery and CNS during experimental autoimmune encephalomyelitis.** During the induction of experimental autoimmune encephalomyelitis (EAE), immune events that occur in the periphery (left panel) comprise subcutaneous and intravenous (i.v.) injection of emulsified antigen with immune stimuli (such as mycobacterial extracts and pertussis toxin), culminating in the generation of antigen-specific, polarized T helper (T_H) cells, which enter peripheral blood. These cells traffic widely throughout the body. Those that cross the choroid plexus into cerebrospinal fluid (CSF) (Wave 1) can be re-stimulated by local antigen-presenting cells (APCs), leading to focal cytokine production. This cytokine flux activates the subpial vasculature to become permissive for further circulating T cells to enter the subarachnoid space (Wave 2A). In the subarachnoid space, these T cells can be re-stimulated by perivascular and meningeal APCs to enter the parenchyma directly and initiate tissue injury, resulting in behavioural changes. Around the same time, the parenchymal blood–brain barrier (BBB) is disrupted following exposure to pro-inflammatory cytokines, and there is an initial crossing of T cells and myeloid cells, succeeded by a huge influx of effector lymphocytes and myeloid cells (wave 2B), an event that coincides with peak neurological deficits in mice with EAE. CCR, CC-chemokine receptor; CFA, complete Freund's adjuvant; CNS, central nervous system; DC, dendritic cell.

activated myelin-specific and ovalbumin-specific T cells together. Ovalbumin-specific cells remained in the SAS, whereas myelin-specific T cells readily invaded the parenchyma. However, when ovalbumin-pulsed APCs were also placed in the SAS, ovalbumin-specific T cells entered the parenchyma[58].

Although the full molecular details of these interactions are unknown, these data suggest that antigen-specific interactions between T cells and APCs within the SAS are essential for the development of EAE. The APCs involved in these studies are meningeal and perivascular macrophages[54,57], but the roles of microglia are still

uncertain. The severity of disease in mice with EAE was reduced when microglia were selectively depleted before disease induction[61]. However, the underlying mechanisms by which the depletion of microglia reduced EAE severity and the APC functions of microglia in EAE have not yet been resolved.

Importantly, the sequence of events involved in the development of autoimmune disease in the CNS can also be seen in models of CNS infection. For example, intracerebral inoculation with different strains of mouse hepatitis virus has been used to characterize the immune components required for host defence[62]. One consistent finding is that $CD8^+$ T cell priming occurs in DCLNs; strains of viruses that replicate poorly in DCLNs are more virulent, presumably owing to their failure to prime effector $CD8^+$ T cell responses[63,64]. Studies using lymphocytic choriomeningitis virus have shown that $CD8^+$ T cells with virus-specific transgenic TCRs recognize their cognate antigen initially in the SAS[65], in an analogous manner to the autoreactive $CD4^+$ T cells in EAE[57].

CNS immune surveillance and trafficking

As mentioned already, the CNS was long believed to be an immune-privileged site that was inaccessible to T cells. However, more recent data have indicated that memory T cells are present in the CNS, and these cells are likely to be important for immune surveillance of the CNS. Although the best-characterized memory T cell population in the healthy brain is found in the CSF, $CD8^+CD103^+$ memory T cells exhibit long-term persistence in the parenchyma after viral clearance and in the absence of viral antigenic stimulation[66]. Their functional significance remains to be clarified.

Memory T cells that enter the CSF follow a complex trafficking pattern. First they migrate from the blood across a fenestrated endothelium into the choroid plexus stroma, then they travel through the stroma from the site of extravasation to the basolateral surface of the choroid plexus epithelial border, and finally they cross the choroid plexus epithelium to enter the CSF[43]. It seems plausible that a unique array of adhesion molecules, chemokines and chemokine receptors will be implicated in this trafficking.

The goal of immune surveillance is to determine whether immune effector responses are required to address the presence of a pathogen or the occurrence of tissue injury. The following discussion focuses on the trafficking mechanisms that support surveillance. Many of the molecular cues for effector immune cell trafficking have been reviewed elsewhere[101]. Regional variation in trafficking mechanisms has also been noted (for example, between the cortex and the white matter or between the brain and the spinal cord). Notably, regional differences in cytokine production (for example, where and when IFNγ is produced) also exert salient effects, as do the relative proportions of T_H1 and T_H17 cells present[67–69].

P-selectin and trafficking to the CNS. Initial studies showed that, following intraperitoneal injection, activated wild-type lymphocytes accumulate in the meninges in a P-selectin-dependent manner[70]. Later, using samples obtained from autopsies of individuals who had died without suffering any neurological disease, it was shown that the choroid plexus of such individuals expressed P-selectin on the luminal side of stromal vessels. Moreover, a subpopulation of $CD4^+CD45RO^+$ T cells in the blood of healthy subjects showed binding activity for P-selectin, suggesting that in the healthy human CNS, a population of activated or memory $CD4^+$ T cells is able to constitutively traffic across P-selectin-expressing vasculature to gain access to the choroid plexus stroma[36]. Taken together, these observations suggest that P-selectin expression in the choroid plexus stromal vessels and in meningeal vessels (which also showed P-selectin immunoreactivity) promotes lymphocyte trafficking into the CSF and SAS, respectively.

Role of α4 integrin in CNS trafficking. Clinical studies in patients with multiple sclerosis receiving natalizumab (an α4-integrin-specific blocking antibody) have suggested that α4 integrin also contributes to lymphocyte trafficking to the brain. Moreover, preclinical studies had indicated that α4β1 integrin and α4β7 integrin are involved in leukocyte trafficking to various inflamed tissues, including the brain, skin and gut[71–73]. Therefore, it was speculated that the efficacy of α4 integrin blockade in suppressing CNS inflammation arises mainly from the inhibition of lymphocyte transmigration across the BBB, a process that depends on the ability of lymphocyte-expressed α4β1 integrin to interact with vascular cell adhesion molecule 1 (VCAM1) or the fibronectin CS1 epitope on the endothelial cells lining the BBB[74]. In support of this, cells from patients with multiple sclerosis receiving natalizumab failed to cross a model BBB *in vitro,* regardless of whether shear forces were applied or not[75,76]. Furthermore, natalizumab was shown to inhibit the firm adhesion of adoptively transferred human T cells to inflamed CNS microvessels in mice with acute EAE[77]. In natalizumab-treated patients with multiple sclerosis, the mean leukocyte count in the CSF was less than 1,000 cells per ml. Notably, this was lower than the number of leukocytes found in the CSF of healthy controls and markedly lower than the leukocyte numbers seen in untreated patients with multiple sclerosis[38]. The treatment selectively suppressed the accumulation of $CD4^+$ T cells in the CSF, as the ratio of $CD4^+$ T cells to $CD8^+$ T cells in the CSF fell from 3.5 to 1 down to 0.5 to 1 by 1 month after natalizumab infusion[37]. It remains uncertain which stage of lymphocyte migration from the blood to the CSF is affected by natalizumab.

The treatment of patients with multiple sclerosis with natalizumab appeared to be highly efficacious in Phase III clinical trials, without evidence for generalized immunosuppression[78]. However, progressive multifocal leukoencephalopathy (PML) soon emerged as a dangerous complication. This side effect was unexpected, as PML had previously been observed only in those with profound deficits in cellular immunity, for example in patients with AIDS or in immunosuppressed transplant recipients. PML is caused by the accelerated replication of JC polyomavirus (which is present in ~55% of the adult population but normally causes only an innocuous

infection). This accelerated replication leads to the invasion of the CNS by the virus and the subsequent lytic infection of oligodendroglial cells[79].

Because the host defence of other organs was not similarly compromised by natalizumab, we initially speculated that impaired immune surveillance of the CNS — owing to the depletion of T cells from the CSF — could contribute to natalizumab-associated PML development[80]. However, a subsequent study found that the CSF leukocyte count was not decreased in a patient who developed PML after treatment with efalizumab, a neutralizing antibody specific for β2 integrin[81]. Although the range of CSF leukocyte counts associated with active PML is unknown, the CSF of this patient had a normal cell count of 5,000 cells per ml at the time of diagnosis (at least 3 months after onset). The most impressive immunological aberration found in the patient was that circulating $CD4^+$ and $CD8^+$ T cells had a non-activated phenotype. This defect was rapidly reversed after efalizumab treatment was stopped and reversed, suggesting that the dominant effect of the drug was to prevent the stabilization of immune synapses that are required for viral antigen recognition. The restoration of peripheral immune competence in this patient was rapidly followed by a massive accumulation of lymphocytes in the CSF. The role of β2 integrins in lymphocyte trafficking to the SAS in humans therefore remains unresolved.

Chemokine receptors involved in CNS trafficking. Chemokines and their receptors collaborate with adhesion molecules to orchestrate leukocyte trafficking and also coordinate many developmental and physiological events in the CNS (reviewed in REF. 82). Studies of the function of CCR6 defined a role for this receptor in promoting T cell migration from the blood to the CSF[46]. CCR6-deficient mice were refractory to EAE following active EAE-inducing immunization. However, CCR6-deficient T cells were efficiently primed *in vivo*, and their effector functions were unaffected. Following adoptive transfer to wild-type hosts, primed CCR6-deficient T cells were unable to drive EAE disease, and subsequent studies showed that the transferred T cells were trapped in the choroid plexus. Remarkably though, a small aliquot of co-transferred primed wild-type T cells restored the capacity of CCR6-deficient T cells to mediate disease.

Furthermore, when wild-type T cells were transferred to CCR6-deficient recipients, CCR6-deficient host cells could subsequently be found in the brain parenchyma[46]. This suggests that the development of inflammation in the meninges leads to the release of factors that activate the BBB, thereby enabling CCR6-independent lymphocyte trafficking. It was concluded that this model of EAE involves two T cell entry events. In the first, T cells cross the choroid plexus to reach the meninges, where they are re-stimulated by local myeloid APCs. The secondary amplifying events involve amplification of the meningeal infiltrate, invasion of the parenchyma from the meninges and finally activation of the BBB by factors from the inflamed meninges. These secondary events are tightly associated with disease onset and peak severity[57,58] (FIG. 2).

A detailed account of early events at the onset of EAE suggested that cytokine diffusion from the meninges activates parenchymal vessels[83]. The most convincing line of evidence was that the middle cerebellar peduncle, a white matter tract that extends to the pial surface, contained activated blood vessels expressing adhesion molecules before EAE onset, whereas the superior cerebellar peduncle, an adjacent white matter tract that does not reach the pial surface, had quiescent blood vessels[83]. Taken together, these studies identified the SAS as the main site of CNS immune surveillance and showed that P-selectin, α4 integrins and CCR6 promote lymphocyte migration into the CSF. However, a significant fraction but still a minority of CSF T cells in healthy humans express CCR6, suggesting that other molecules can also support this trafficking.

Architecture of immune-mediated CNS lesions

If lymphocyte reactivation occurs in the SAS adjacent to the pial surface of the CNS parenchyma, then it seems likely that initial pathological changes would be observed in nearby structures. Studies of EAE have repeatedly shown that this is the case — lesions in rodents are typified by spinal cord pathology and large subpial inflammatory foci[84]. Subsequent studies suggested that T cell reactivation occurs in the SAS in proximity to such lesions[85,86]. However, these observations do not provide a stringent test of the hypothesis, as myelin-rich white matter is the major site of autoimmune demyelination and the white matter of the spinal cord constitutes its external subpial aspect.

More recently, the cerebral cortex has become a renewed focus of investigation for multiple sclerosis researchers[87,88]. The cerebral cortex contains less myelin than the white matter, explaining the difficulty in detecting demyelinated lesions by standard staining techniques. Furthermore, conventional magnetic resonance imaging (MRI) is insensitive to subpial demyelinated lesions of the brain, accounting for the relative lack of information about disease extent in this crucial region in past years. Autopsy investigations have revealed large subpial demyelinated lesions in the cerebral cortex of recently deceased patients with multiple sclerosis[87,88] and showed, remarkably, that the cortex had often lost more myelin, as a percentage of the total myelin, than the white matter[89]. Subsequent studies using non-human primates revealed large subpial EAE lesions comparable with those observed in the autopsies described above[90].

However, the demyelinated cortical lesions seen in the autopsy material from patients with multiple sclerosis contained few inflammatory cells. This surprising finding could not be explained purely by the long history of disease before death, as such inflammatory cells were readily detected in white matter lesions in the same tissues[87]. These reports raised the possibility that the pathogenesis of cortical subpial lesions in patients with multiple sclerosis was mechanistically distinct from that of white matter lesions in these patients, and perhaps also from that of the lesions seen in animal models of EAE. Indeed, lesions that affected the border zones between white and grey matter (termed leukocortical

Oligodendroglial cells
(Also known as oligodendrocytes). Glial cells that create and maintain the myelin sheath that nourishes and insulates axons as well as improving the speed and reliability of signal transmission by neurons.

lesions) contained more inflammatory cells in the white matter than in the grey matter of individual lesions[87]. Therefore, it remained uncertain whether APC-mediated T cell re-stimulation in the SAS could potentially be relevant for the pathogenesis of subpial lesions in multiple sclerosis.

One concern regarding the interpretation of the multiple sclerosis autopsy studies relates to the lengthy disease duration before tissue examination. In addition, experimental observations have indicated that demyelinating cortical inflammation can be remarkably transient[91]. Although MRI is not sensitive for detecting focal subpial lesions in patients with multiple sclerosis, it has nonetheless indicated cortical injury in the form of tissue atrophy early during the course of multiple sclerosis[92]. To try to understand what type of tissue pathology early in the multiple sclerosis disease course led to tissue atrophy, we studied biopsy material. In this regard, biopsies to test for suspected tumours or infections have occasionally diagnosed clinical cases of cerebral white matter inflammatory demyelination, and the majority of such cases are eventually found to exhibit typical signs and symptoms of multiple sclerosis[93,94].

To examine the pathological characteristics of subpial demyelination early in multiple sclerosis, we studied the cortical tissue obtained during stereotactic white matter biopsies of 138 patients, as such procedures involve passing a biopsy instrument through the cortex, a small core of which is removed *en passant*[10]. The majority of these cortical tissues were spatially removed from the white matter biopsy target, so they represented a 1 mm random sample of cerebral cortex from early-stage multiple sclerosis cases with a median time from symptom onset to biopsy of about 30 days. We observed demyelinating lesions in nearly 40% of cases, with subpial demyelinating lesions being present in slightly more than 10% of cases[10]. These cortical demyelinating lesions had an inflammatory character, typified by perivascular and parenchymal lymphocyte infiltrates that included $CD4^+$ and $CD8^+$ T cells as

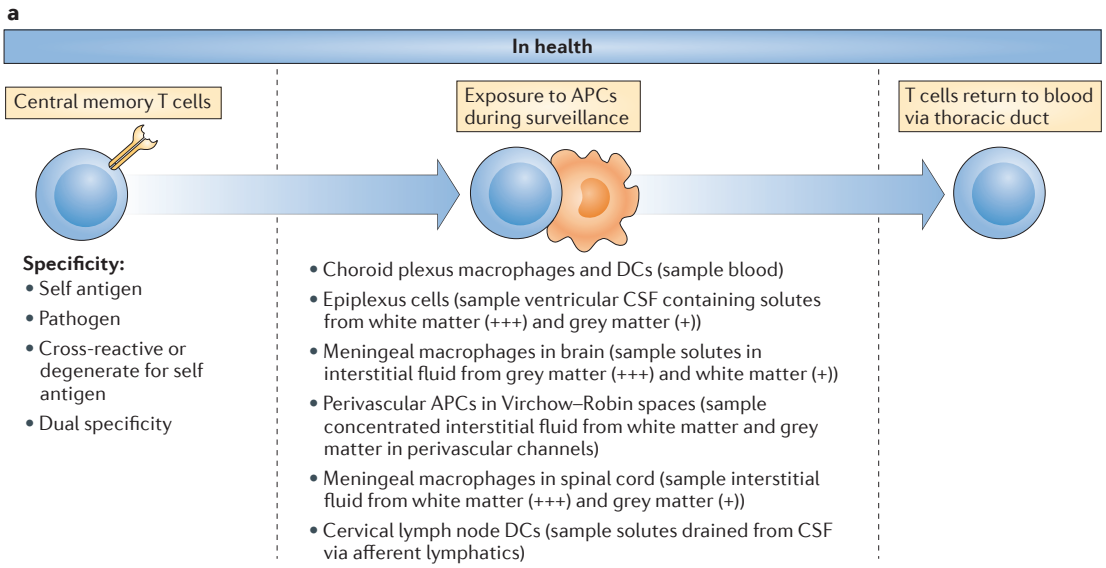

Figure 3 | **Immune surveillance of the central nervous system: T cell–APC interactions and checkpoints for autoimmunity. a** | Memory T cells of varied T cell receptor (TCR) specificities cross into the cerebrospinal fluid (CSF) in a manner that is independent of their antigen specifity (left panel). In the CSF, they are exposed to a diversity of antigen-presenting cells (APCs), which display different arrays of central nervous system (CNS) antigens according to their location (centre panel). If no antigen (self- or pathogen-derived) is recognized by the T cell, it exits the subarachnoid space along with the CSF, crossing the cribriform plate in channels along the entering olfactory afferents (not shown) and draining to the nasal mucosa. From here, T cells use afferent lymphatics to access deep cervical lymph nodes (DCLNs) and ultimately re-enter the blood circulation from the thoracic duct. **b** | The threshold for T cell-mediated autoimmunity to myelin or other self antigens can be lowered in varied ways, which are here termed checkpoints for disease liability. DC, dendritic cell; EBV, Epstein–Barr virus.

well as scattered perivascular accumulations of $CD19^+$ B cells. Demyelination appeared to be mediated by macrophages, which were frequently found to contain myelin debris, as seen in white matter lesions of patients with multiple sclerosis[10]. The meninges were included in over 40 of these 138 biopsy samples, and we detected both diffuse and focal (perivascular) inflammatory leukocyte aggregates in the meninges. Furthermore, there was a striking positive correlation between the presence of meningeal inflammatory foci and subpial demyelination[10]. These observations reinforce the hypothesis that lymphocyte re-stimulation in the SAS may be pertinent for the pathogenesis of some multiple sclerosis lesions, and they support the concept that immune surveillance of the CNS is carried out by CSF T cells (FIG. 3).

Implications for therapy

The goal of obtaining insights into CNS immune mechanisms is to address the inflammatory or immune components of CNS diseases. Our understanding of immune privilege implies that adaptive immune responses that occur in the CNS are initially generated in the periphery, and it seems likely that these responses may also be sustained peripherally[95]. Therefore, many immunotherapies that are targeted against adaptive immune processes in the CNS can be administered systemically, as in the cases of the multiple sclerosis therapies IFNβ and glatiramer acetate. When leukocyte trafficking to the CNS is targeted, researchers will need to consider in terms of efficacy and safety whether transmigration across the BBB to the parenchyma or across the blood–CSF barrier to the SAS is the event of interest[81,96,97]. In addition, it is now important to address the functions of CNS-resident leukocyte aggregates, which seem to be autonomous and long-lasting[10,98–100]. The regulatory determinants and pathological relevance of these tissue components will constitute key issues in neuroinflammation research.

Summary and perspectives

The CNS lacks APCs capable of transporting antigens to lymph nodes and, consequently, CNS immune responses are primed in the periphery. The ability of peripherally primed cells to detect CNS antigens indicates that immune surveillance occurs in the CNS, and we assign this function to CSF T cells. Furthermore, we propose that T_{CM} cells represent the cellular basis of immune surveillance of the CNS, and that myeloid cells found in the meninges and choroid plexus, as well as perivascular macrophages, have crucial APC functions for this process. Immune-mediated recognition of pathogen-derived or self antigens occurs first in the SAS. Given its cellular composition and its importance for the drainage of antigens and other solutes to DCLNs, the CSF mediates lymph-like functions for the CNS. The adhesion molecules, chemokines and chemokine receptors that determine lymphocyte trafficking to the CSF compartment have been partially deciphered. Further characterization of the functional significance of these molecules to CSF lymphocytes will help us to determine their usefulness as therapeutic targets for CNS inflammatory diseases.

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Acknowledgements
Research in the Ransohoff laboratory is supported by the US National Institutes of Health, the National Multiple Sclerosis Society, the Alzheimer's Association, the Williams Family Foundation for MS Research and the Walker family. Research in the Engelhardt laboratory is supported by the Swiss National Science Foundation, the Swiss Multiple Sclerosis Society and the European Union. R.M.R. and B.E. thank their laboratory colleagues for their talent and commitment and are grateful to patients who participate in research programmes.

Competing interests statement
The authors declare no competing financial interests.

FURTHER INFORMATION
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