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REVIEW

The role of peripheral immune cells in the CNS in steady state and disease

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The CNS is protected by the immune system, including cells that reside directly within the CNS and help to ensure proper neural function, as well as cells that traffic into the CNS with disease. The CNS-resident immune system is comprised mainly of innate immune cells and operates under homeostatic conditions. These myeloid cells in the CNS parenchyma and at CNS–periphery interfaces are highly specialized but also extremely plastic cells that immediately react to any changes in CNS homeostasis and become reactive in the context of neurodegenerative disorders such as Alzheimer's disease or Parkinson's disease. However, when the blood–brain barrier is impaired during CNS diseases such as multiple sclerosis or altered with cerebral ischemia, peripheral adaptive and innate immune cells, including monocytes, neutrophils, T cells and B cells, can enter the CNS, where they execute distinct cell-mediated effects. On the basis of these observations, we assess strategies for targeting peripheral immune cells to reduce CNS disease burden.

Subtle changes in the microenvironment of the CNS either due to local alterations (changes of pH, metabolic disturbances or microbleedings, among others) or peripheral changes in the blood circulation (bacterial or viral infection) or in other organs (impaired function, dysbiosis or inflammation) can have a major impact on CNS function, resulting in changes in cognitive function, mood and behavior. Due to its exceptional importance, the CNS requires a highly specialized and dynamic system of anatomical and functional features to fulfill these vital tasks. Hematopoietic cells play important roles in both homeostasis and disease pathogenesis.

Several recent reviews have comprehensively discussed the structural basis of immune protection of the CNS[1–3]. Here we provide an overview of the progress in our understanding of the fate and function of classical immune cells in the CNS during homeostasis and disease. This information may help us to develop therapeutic strategies that enhance functional recovery after disease and boost normal tasks of the CNS.

CNS immunity during homeostasis

The surface of the adult brain is protected by several layers to ensure its structural integrity. The skull is the covering bone structure of the brain and has two adherent meningeal layers, the dura and the leptomeninges. The latter consist of the arachnoid mater (called so because of its resemblance to spider silk) and the pia mater, and cover the brain and the intracerebral vascular system to the depth of the CNS parenchyma, building up the perivascular (Virchow–Robin) space (**Fig. 1**). Both meningeal compartments have been subjects of considerable interest in recent years because they contain some immune cells. Dural lymphatics were described structurally 50 years ago[4], but their functional relevance remained unclear until recently. In laboratory animals, up to 47% of proteins injected into the brain or cerebrospinal fluid (CSF) were found to pass through lymph via prolongations of the subarachnoid space along cranial and spinal nerves[5]. Recently, dural lymphatic vessels were found to absorb CSF from the subarachnoid space and brain interstitial fluid via the glymphatic system. The dural lymphatic vessels then transport the fluid into deep cervical lymph nodes via foramina at the base of the skull[6], as has been suggested before[7]. Using both lymphatic-cell-reporter mouse strains[6,8] and dissection of mouse meninges[8], conventional lymphatic vessels were found in the dura lining the sinuses and leaving the CNS at the base of the skull. Notably, classical immune cells, such as T and B cells and fractalkine receptor (CX_3CR1)-expressing myeloid cells, were found to be present in the nondiseased lymphatic vessels[8], suggesting that the meningeal lymphatics may participate in the trafficking of immune cells from the meninges during steady state. It is now supposed that the fluid part of the CSF drains back into the bloodstream through arachnoid granulations localized along the sinuses, whereas immune cells and proteins in CSF may drain primarily through dural lymphatic structures to reach the deep cervical lymph nodes. The topography and the structures involved in antigen drainage from the CNS, however, are still a matter of controversy[9].

The relevance of the dural lymphatics and associated immune cells for CNS diseases, such as multiple sclerosis, stroke or neurodegenerative diseases, is still unclear. Furthermore, it is largely unknown what damage-induced signals mediate the immune responses that lead to CNS autoimmune inflammation.

Physically, the CSF bathes the brain and provides some buoyancy for the CNS, as well as a means of transporting cytokines, neurotransmitters and hormones[10]. CSF flows between the arachnoid and the pia maters and is produced as a filtrate of the fenestrated blood vessels in

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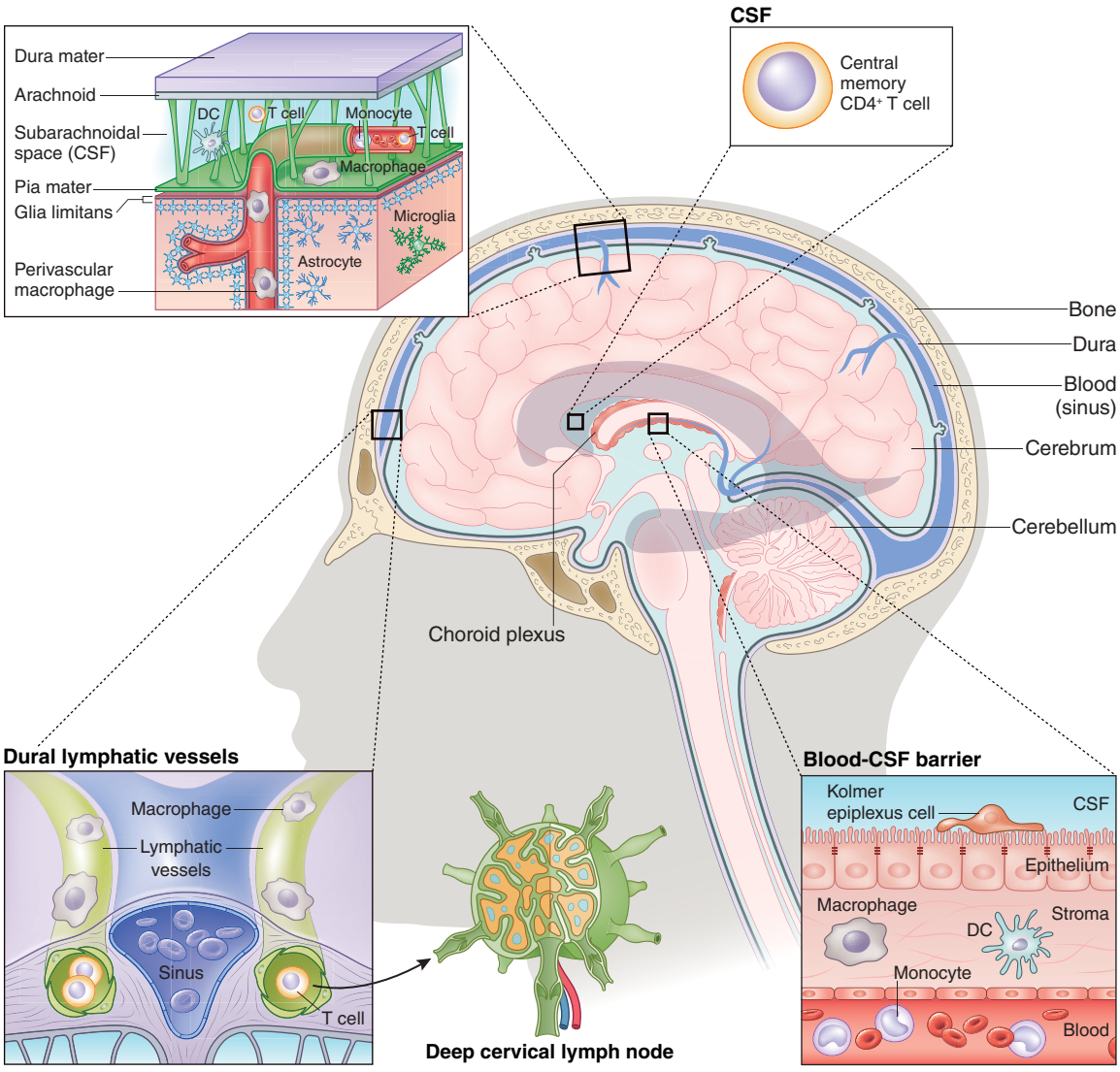

Figure 1 The CNS immune system during homeostasis. Scheme of the nondiseased brain, depicting anatomical structures and cells involved in ensuring tissue integrity. Under steady-state conditions, the brain is covered by the skull and the galea aponeurotica (not shown). Layers of connective tissue sheets, the dura mater and leptomeninges (arachnoid mater and pia mater), are located beneath the skull. CSF is produced in the choroid plexus (which has a blood–CSF barrier), bathes the brain, contains T cells and flows both in the parenchyma and in the subarachnoid area, which comprises arteries and the perivascular space. Whereas the CSF drains back to blood circulation, immune cells and proteins in CSF may be drained primarily through meningeal lymphatic structures to reach deep cervical lymph nodes, prototypical secondary lymphoid organs. Leukocytes (granulocytes, T and B cells) stay within the blood vessels and usually do not enter healthy brain tissue. Thus, the only endogenous immune cells within the CNS are parenchymal (microglia) and nonparenchymal macrophages (perivascular, meningeal and choroid plexus (for example, Kolmer epiplexus cells) macrophages).

the choroid plexus in the ventricles of the brain. From there, it flows through the third ventricle, passing the midbrain through the aqueduct and reaching the outer surface of the brain via the fourth ventricle. Despite its leukopenic qualities, few T lymphocytes positive for CD4, a T cell surface glycoprotein, and even fewer antigen-presenting cells (APCs) or monocytes can be found in CSF. The population of $CD4^+$ T cells present in the CNS comprises very few naïve cells[3]; most CSF T cells are effector-memory T cells, which express receptors that allow homing to inflamed tissues[11]. Effector-memory T cells can exert immediate effector functions without the need for further differentiation. It appears likely that T cells enter the CNS via the fenestrated blood capillaries in the choroid plexus in the ventricles[12].

Despite their pathogenic role during autoimmune inflammation, under steady-state conditions lymphocytes are only sparsely present in the CSF of the subdural meningeal structures. The leptomeninges contain collagen-rich stromal cells and arachnoidal epithelial cells but virtually no cells of the adaptive immune system[13]. Accordingly, human brain tumors involving the meninges, such as classical meningiomas, xanthoastrocytomas and fibrous collagenous tumors, almost always lack T and B cells in neoplastic lesions[14]. In fact, under homeostatic conditions, both human and mouse brain parenchymas are devoid of any lymphocytes, whereas only single lymphocytes are present in the leptomeninges[15]. Given this neuropathological evidence, reports on the contribution of T cells in neurogenesis and cognitive function in mice are unexpected[16]. Similarly, meningeal T cells are thought to be the source of cytokines like interferon (IFN)-γ and interleukin (IL)-4, which modulate cognition and behavior in mice[17,18]. Notably, these studies either used immune-deficient mice or performed transplants of bone marrow cells or T cells. Some groups confirmed the participation of T cells in murine neurogenesis[19], whereas other studies came to the

opposite conclusion[20]. Recent data suggest a more complex role for T cells in adult neurogenesis. A correlation was found between the rate of neurogenesis and the relative proportions of $CD4^+$ and $CD8^+$ subsets of αβT cells[21]. This correlation is driven by genetics, because specific quantitative trait loci contribute to variation in neurogenesis in the hippocampus and to variation in the relative proportion of $CD4^+$ and $CD8^+$ T cells. Further, this genetic correlation seems to be due to natural sequence variants that differentiate inbred strains of laboratory mice[21].

In contrast to peripheral organs, no classical dendritic cells (DCs) can be functionally defined in the CNS parenchyma[2]. In the peripheral immune system, the main function of DCs is to process antigenic material and present it to the cell surface of naive T cells via major histocompatibility class (MHC) II (in addition to MHC I) molecules in concert with the costimulatory molecules CD80 and CD86 (ref. 22).

Despite acquiring a few DC surface markers, such as CD11c, MHC II and CD11b, CNS parenchymal cells are not DCs by any reasonable functional definition. In fact, CD11c expression was found on activated microglia[23], bone marrow-derived phagocytes in the murine CNS[24], as well as in human CNS samples[25]. $CD11c^+eYFP^+$ cells within brain parenchyma express several macrophage markers and show typical microglial morphology but are negative for MHC II (ref. 26). However, $CD11c^+$ cells in the meninges and choroid plexus of healthy mice are responsive to a classical DC ligand, FMS-like receptor tyrosine kinase (Flt) 3 (ref. 27). Using comprehensive immunophenotyping, a recent study convincingly demonstrated that parenchymal $CD11c^+$ $eYFP^+$ cells do not display the phenotype of DCs but rather represent a subpopulation of microglia[28], which supports the notion that the CNS is devoid of classical DCs. Along this line, another study demonstrated that virus-specific T cells are able to induce microglial proliferation and to convert microglia into $CD11c^+$ APCs[29].

The question is, "which CNS immune cells provide immune surveillance under homeostasis, represent the first line of defense against invading pathogens and modulate CNS inflammation?" Like other organs of the body, the CNS contains tissue macrophages as prototypical innate immune cells. In addition to parenchymal microglia, this family comprises perivascular macrophages, meningeal macrophages and choroid plexus macrophages, the last of which include Kolmer's epiplexus cells and other choroid plexus macrophages[30]. Despite the fact that all of these macrophage populations originate from prenatal sources, namely the yolk sac and/or fetal liver[31–36], and share numerous myeloid- and macrophage-specific markers (such as Iba-1, F4/80 (also known as EMR1) and CX_3CR1), they have quite diverse, cell-specific functions[37]. Strategically positioned at the CNS barriers, perivascular, meningeal and choroid plexus macrophages may modulate immune cell entry and phenotype during inflammation. The myeloid cells in the CNS-adjoining tissues have thus been implicated in various immunopathological processes, including antigen presentation to circulating lymphocytes[27,38,39]. During homeostasis, perivascular macrophages are believed to sense blood danger signals, including damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs), because of their localization between the laminin-positive endothelial and glial basement membranes[34]. During angiogenesis, they modulate anastomoses in the developing vasculature[40]. Choroid plexus macrophages are believed to surveil CSF production under steady-state conditions[41,42].

Like other tissue macrophages with a prenatal origin[32], microglia and their nonparenchymal family members are extremely long-lived and have no turnover with blood-derived monocytes[43,44]. However, they exhibit considerable self-renewal under homeostasis[45–47]. The longevity and the absence of exchange with blood cells make tissue macrophages in the CNS very vulnerable to inflammatory events in early life[48] and monogenetic disorders with detrimental outcomes, such as colony stimulating factor 1 receptor (CSF-1r) mutations causing hereditary diffuse leukoencephalopathy with spheroids (HDLS) in humans[49] or ubiquitin-specific protease (USP) 18 mutations in mouse[50] and man[51]. Unfortunately, it is still not possible to efficiently target microglia to decrease disease burden without the application of irradiation, cell ablation or peripheral myeloablation, which all induce considerable changes of the CNS milieu[52].

Notably, blood-derived monocytes never leave the endovascular space of the healthy brain to populate the CNS parenchyma, and therefore reports ascribing any effects to these cells might be due either to indirect mechanisms[53] or to their minimal physiological passage through the choroid plexus[34,54]. In sum, the only immune cells that appear to populate the healthy brain and spinal cord parenchyma are long-lived tissue macrophages, including parenchymal microglia, meningeal and perivascular macrophages, and choroid plexus macrophages. Peripheral immune cells are prevented from CNS entry by the presence of the blood–brain barrier (BBB) and a local tissue environment that penalizes bone marrow-derived blood cells[55]. However, inside the CSF, memory T cells possibly monitor the CNS within the subarachnoid and leptomeningeal spaces, where they can encounter the abovementioned macrophages at brain interfaces (**Fig. 1**). The latter could potentially function as antigen-presenting cells upon activation.

Redefining neuroinflammation

Alzheimer's (AD) and Parkinson's disease (PD) are common neurodegenerative disorders that result in a huge socioeconomic burden. At present, there are no disease-modifying treatments for AD or PD, and this is also true for less-common neurodegenerative conditions, such as Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), Creutzfeldt-Jakob disease and others. The neuropathological features of these neurodegenerative disorders include aggregation and accumulation of intracellular and/or extracellular proteins that are associated with neuronal loss in disease-specific regions of the CNS. In addition, proliferation and activation of glial cells, termed 'gliosis', is a well-established hallmark of these diseases. In fact, microglial responses are believed to have disease-modifying functions, which make them attractive for numerous ongoing experimental studies[41,56].

It is important to note that cellular responses in AD, PD, HD, ALS, Creutzfeldt-Jakob disease and others are consistent with an innate immunity. Adaptive immune responses involve immune specificity (for antigens) and memory (indicating an accelerated reaction to antigen re-exposure) based on the unique properties of T and B lymphocytes. Notably, only the presence of these and other hematopoietic cells within the CNS may warrant the term 'neuroinflammation' as typically seen in bacterial, viral and autoimmune CNS diseases. Neurodegenerative diseases such as AD should be considered innate immune reactions that can also include the release of cytokines and chemokines (**Fig. 2**). Despite the breakdown of the BBB in later stages of neurodegeneration, the presence of lymphocytes in AD and PD has been reported in some cases[57,58]. Nevertheless, the meaning of the term inflammation has undergone considerable revisions within the last years, and therefore, the concept of neuroinflammation has gradually expanded to also include neurodegenerative diseases. In fact, numerous recent reports have considered AD as a neuroinflammatory condition rather than an innate immune response to a state of neurodegeneration[59,60]. This is clearly not just a semantic problem, since this inaccuracy may lead to a misunderstanding of the pathophysiology of neurodegeneration and may consequently hinder cell-specific therapeutic approaches.

Peripheral immune cells in the CNS

Alzheimer's disease. Immune cells in AD have gained major attention in the recent years. This is mostly due to significant technical advances that

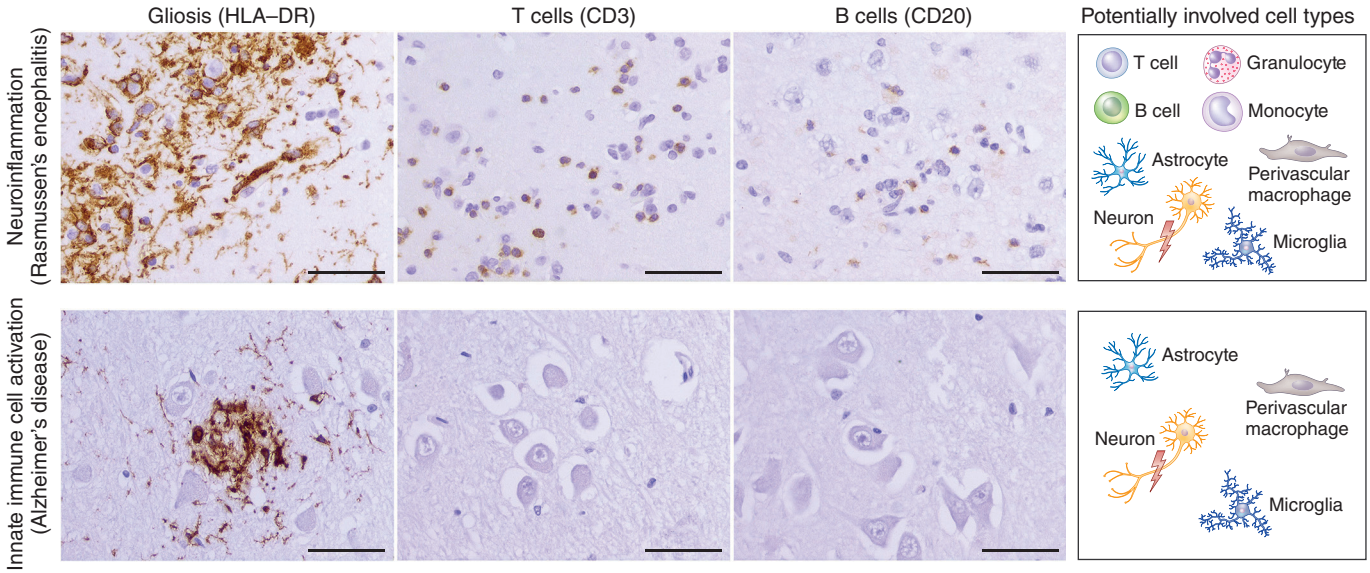

Figure 2 Histopathological characteristics of neuroinflammation versus neurodegeneration in humans. Loss of distinct neuronal cell populations and concomitant gliosis are characteristic histopathological hallmarks of both neuroinflammatory diseases (Rasmussen encephalitis, upper panels) and neurodegenerative conditions (Alzheimer's disease, lower panels). However, the term neuroinflammation should only be used when additional hematopoietic cells, particularly those of the adaptive immune system, such as T or B cells, as well as innate immune cells like blood-borne monocytes and granulocytes, are present. Thus, typical neurodegenerative diseases such as AD, PD, HD, ALS and many more that are usually devoid of adaptive immune cells should be considered innate immune reactions. Scale bar, 50 μm. Immunostainings for human leukocyte antigen–antigen-D-related (HLA-DR) (MHC class II), CD3 and CD20 reveal activated microglia, T cells and B cells, respectively. These images are unique to this review. Human temporal brain sections were examined histologically, after approval by the Faculty of Medicine, University of Freiburg. These histological samples were obtained from daily routine diagnostic examinations from in-house patients at University Hospital Freiburg. The usage of this patient material was approved by the local ethical committee and informed consent was obtained from patients' families. Paraffin sections were dewaxed in xylene and rehydrated by decreasing ethanol concentration. Immunohistochemistry was performed using the CoverplateTM system. Sections were washed in Tris-buffered saline (TBS). After washing with TBS the following primary antibodies were applied: mouse monoclonal α-HLA-DR (cat. no. M0775, Dako, Hamburg, Germany, dilution: 1:400), mouse monoclonal α-CD3 (cat. no. NCL-L-CD3-565, Leica, Wetzlar, Germany, dilution: 1:50), mouse monoclonal α-CD20 (cat. no. M0755, Dako, Hamburg, Germany, dilution: 1:400). Following incubation with the primary antibody, the sections were rinsed in TBS, exposed to a biotinylated secondary antibody for 15 min at room temperature and washed again in TBS.

now allow for full sequencing the human genome. In fact, genome-wide association studies in many thousands of patients resulted in the identification of susceptibility variants in loci harboring innate immune-related genes that are expressed by myeloid cells, including CD33 (Siglec-3)[61–63] and TYROBP (DAP12; ref. 64) in AD, triggering receptor expressed on myeloid cells (TREM) 2 in frontotemporal dementia[65] and in AD, and others. In addition, common variants in TREM1 and TREM2 are associated with increased AD pathology and cognitive decline[66].

How these risk loci affect the functions of innate immune cells inside and outside of the CNS and how they increase AD susceptibility is not well understood. Apparently, the CD33 risk allele leads to increased TREM2 protein expression on myeloid cells, most likely by altering the expression of other innate immune surface receptors[67]. TREM2 is an important receptor that is thought to modulate CNS tissue debris clearance in general[68] but is also involved in the phagocytosis of amyloid plaques[56]. In mouse models of AD, TREM2 deficiency prevents microglia clustering around amyloid plaques, suggesting a major role of the mutated gene for microglia function during disease[69]. Whereas a pathogenic role of TREM2 expression on microglia was elegantly shown for AD in a parabiotic mouse model[70], other studies suggest a pivotal role for TREM2 on peripheral monocytes[71].

Circulating monocytes in mice are either Ly-6C^{hi}CCR2^{+}CX$_3$CR1^{lo} or Ly-6C^{lo}CCR2^{-}CX$_3$CR1^{hi}, whereas in humans they are CD14^{+} and/or CD16^{+} (refs. 72,73). Monocytes can easily be targeted by antibodies, liposomes or cell transfer[52], which makes them attractive candidates for therapeutic approaches in neurodegeneration. However, there is currently considerable doubt that an influx of peripheral immune cells is present at all in AD mouse models or in human AD brains, compared to age-matched controls (**Fig. 3**)[74]. Indeed, engraftment of blood-derived cells in the brains of mouse AD models could only be observed when bone marrow transplantation was combined with head irradiation[75–77] but not when the brain was protected from irradiation[78] or when parabiotic AD models were studied[70]. These results are in agreement with the general observation that, if circulating monocytes engraft in the diseased CNS, they are short-lived and do not permanently integrate into the existing microglia network[46] even when endogenous microglia were depleted beforehand[47]. Interestingly, a pathogenic role of chemokine receptor (CCR) 2 deficiency was observed in transgenic mouse models of AD[78,79], even though microglia do not express CCR2 in health or disease[80], and brain perivascular macrophages renew independently of peripheral monocytes[34].

Several proinflammatory factors produced by CNS macrophages also influence the pathogenesis of AD. Amyloid precursor (APP) and presenilin (PS)1 transgenic mice lacking both IL-23 and IL-12 develop reduced amyloid pathology and less memory impairment without altered APP processing[81]. Furthermore, the NLRP3 (NACHT-, LRR- and PYD-domains-containing protein 3) inflammasome has been implicated in AD pathogenesis since it senses aggregated proteins like amyloid[82]. A subsequent study provided further evidence for the importance of the NLRP3 system in AD, since the absence of NLRP3 in APP-PS1 mice resulted in accelerated amyloid clearance, reduced plaque formation and fewer memory deficits[83].

Two recent reports also suggested a pathogenic role for circulating neutrophils in mouse models of AD and in human AD brains[84,85].

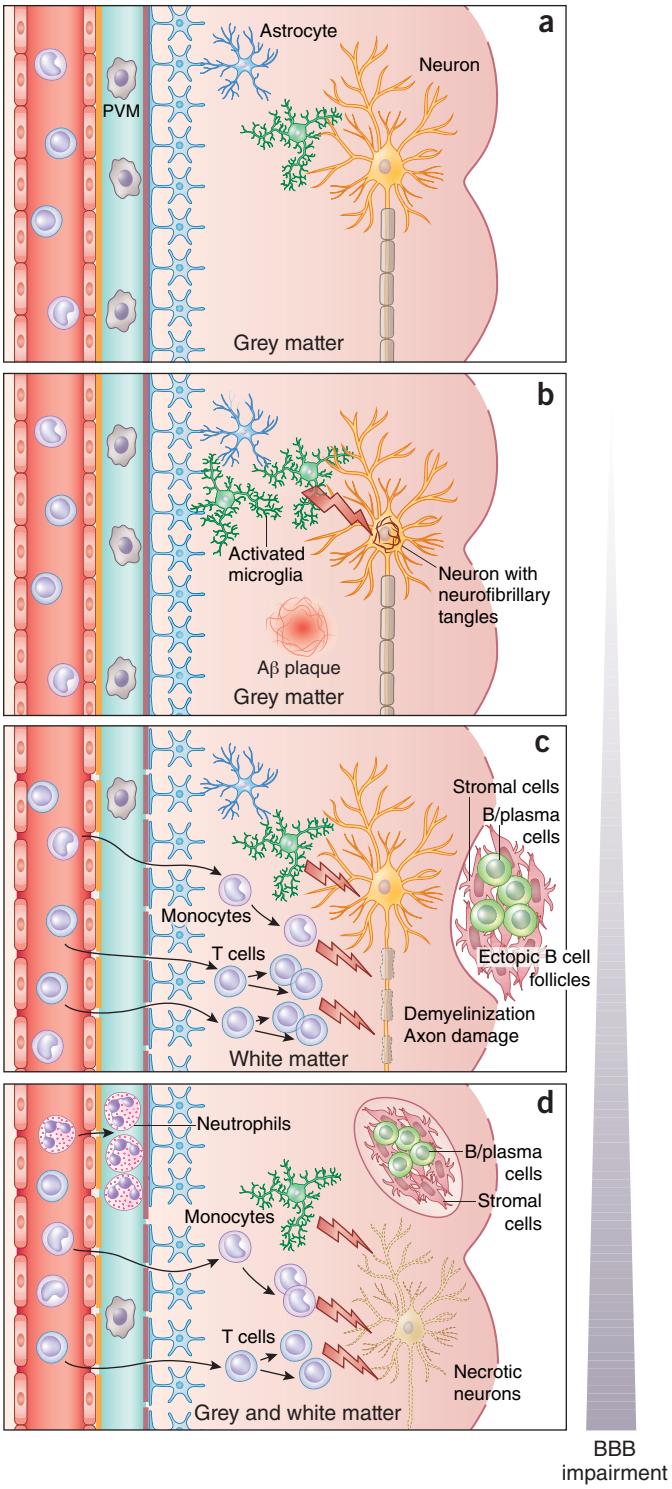

Figure 3 Peripheral immune cells in the CNS immune system during disease. The presence and severity of BBB disruption determines which circulating immune cells can gain access to the diseased brain. (**a**) The BBB is a dynamic structure composed of the endothelial cell layer (cells are closely connected by tight junctions), endothelial basal lamina, perivascular space with perivascular macrophages and the astrocytic end feet from the parenchymal side, all of which prevent blood cell engraftment. (**b**) During neurodegeneration such as in Alzheimer's diseases, activated microglia (and astrocytes) can be found in close proximity to extracellular amyloid (Aβ) plaques and damaged neurons with neurofibrillary tangles. Microglial activation might be the consequence of pathology but may also play a pathogenic role. Aβ is also present in perivascular macrophages, but the BBB is normally unaltered, preventing any influx of hematopoietic cells. (**c**,**d**) In contrast, considerable leakage of the BBB is present in (**c**) MS and (**d**) stroke, allowing hematopoietic cells to enter the CNS. Here, T- and B-cell infiltrates are typical and may persist in the subarachnoid space as 'tertiary follicles' for several months. Circulating monocytes infiltrate the diseased brain, where they execute disease-specific functions that differ from those of endogenous myeloid cells (such as tissue macrophages). The composition of the immune cell infiltrate and its interactions with glial and nonglial cells in the brain determine the extent of tissue damage and consequently the clinical sequelae of disease.

In transgenic mouse models of AD, neutrophil depletion or inhibition of neutrophil trafficking via LFA-1 integrin blockade reduced AD-like neuropathology and improved memory in mice that already showed cognitive dysfunction[80]. These findings shed new light on this innate blood cell type but still await confirmation. Notably, neutrophil invasion is not a neuropathological hallmark of AD in humans or in rodent models.

The majority of the existing literature points to a predominant role for tissue macrophages, namely microglia and perivascular macrophages, in AD pathogenesis. Blood-derived immune cells such as lymphocytes, monocytes or granulocytes may have disease-modulating functions, but the extent to which they contribute to disease pathogenesis remains to be determined.

Multiple sclerosis. Multiple sclerosis (MS) is a frequent autoimmune inflammatory disorder of the CNS leading to irreversible axonal damage and increasing neurological disability. Peripheral immune cells are considered to be causative in MS. The detailed immunopathogenesis of MS and its animal model, experimental autoimmune encephalomyelitis (EAE), have been discussed in several excellent reviews[3,86–88]. In general, the CNS of MS patients is characterized by an invasion of peripheral autoreactive immune cells and a concomitant activation of the innate immune system in the CNS. In mouse models, previously dormant myelin-specific T cells were found to be activated in secondary lymphoid organs, such as the deep cervical lymph nodes, and reactivated by myeloid cells with APC features at CNS interfaces. Subsequently, they act in concert with myeloid cells to cause inflammatory lesions in the white matter. The human disease can be partially recapitulated in the predominantly $CD4^+$ T cell-mediated EAE disease model. Under healthy conditions, the BBB and the blood–CSF barrier separate the CNS from the circulation. In MS and EAE, the BBB is disrupted, which allows peripherally activated T cells and monocytes to gain access to the CNS (**Fig. 3**). In MS, myeloid cells dominate the infiltrate, followed by $CD8^+$ T cells, with lower numbers of $CD4^+$ T cells, B cells and plasma cells[87,89].

The presence of T cells within MS lesions can be detected in early lesions[90]. Autoreactive T cells directed against oligodendrocyte antigens, such as myelin basic protein, myelin oligodendrocyte glycoprotein and proteolipoprotein, can be found in circulating $CD4^+$ T cells[91] and in lymph nodes[92] of MS patients. These autoreactive $CD4^+$ T cells express CCR6 in humans, which has been shown to be essential for T-cell migration to the CNS in EAE models[12]. However, others found enhanced autoimmune inflammation in the absence of CCR6 (ref. 93).

In EAE, T helper type 1 (T_H1) and T_H17 cells are the main $CD4^+$ T-cell subsets implicated in disease[94]. The relevance of the T_H1-to-T_H17 cell ratio in MS is currently under debate: conflicting data described the dominance of one T-cell subtype over the other at defined stages of disease[95,96]. Notably, the functions of T_H17 cells in humans and mice seem to be different. T_H17-derived granulocyte-macrophage colony-stimulating factor (GM-CSF) production contributes to chronic inflammation in EAE (ref. 97), whereas T_H1 lymphocytes and other cell types are the producers of this cytokine in humans[98]. Interestingly, therapeutic approaches against T_H17 cytokines, such as IL-12 and IL-23, failed in clinical trials of MS patients[99]. However, blocking the adhesion

molecule VLA-4 with the monoclonal antibody natalizumab strongly impairs the migration of autoreactive lymphocytes to the CNS, which rapidly decreases CNS inflammation and improves the clinical course in MS, arguing for a pathogenic role for T cells in this disease[100].

As described above, $CD8^+$ T cells are very commonly found in MS lesions. In accordance with their supposed role in disease pathogenesis, autoreactive $CD8^+$ cells are activated by epitope spreading through MHC class I and presented by specific myeloid cells that express CD11c (ref. 101). In active MS lesions, $CD8^+$ T cells were detected that exhibit features of mucosa-associated invariant T cells and produce IL-17 (ref. 102). However, the precise roles of $CD8^+$ T cells in MS and EAE are not entirely clear and require further investigation.

Autoreactive clonally expanding B cells are a typical feature of MS that can be found in the parenchyma, the meninges and the CSF. The frequency of antibody-secreting plasma cells is increased in patients with primary or secondary progressive MS[103]. B-cell clusters, together with T cells, plasma cells and stromal follicular dendritic cells, are the main component of distinct meningeal follicles called tertiary lymphoid structures, which are indicative of chronic inflammation in some patients with secondary progressive disease[104]. By contrast, primary progressive MS shows a more diffuse meningeal infiltration linked to cortical pathology[105]. Meningeal tertiary lymphoid structures can also be induced in mice by chronic activation of T_H17 $CD4^+$ T cells[106] and by the additional action of the proinflammatory cytokine lymphotoxin on T cells[107].

In addition to lymphocytes, several myeloid subsets are involved in CNS neuroinflammation[39,108]. These encompass microglia in the CNS parenchyma, antigen-presenting $CD11c^+$ cells at the CNS interfaces and infiltrating monocytes (**Fig. 3**). Myeloid cells are thought to be proximate effectors in the inflammatory cascade leading to CNS damage, as they can act as phagocytes and are the main producers of cytotoxic factors and reactive oxygen species[109]. Indeed, depletion of myeloid cells in the CNS by a transgenic CD11b herpes simplex virus type 1 (HSV-1) thymidine kinase approach mitigated EAE[110]. The absence of transforming growth factor (TGF)-β-activated kinase 1 (TAK1) in long-lived CX_3CR1^+ tissue macrophages of the CNS abolished demyelinating inflammation and axonal damage[43].

A transgenic approach allowed a recent, elegant study of the differential functions of monocytes versus microglia during CNS inflammation[111]. $CCR2^+$ monocytes exhibited a proinflammatory and cytotoxic gene signature in EAE. They were localized at the nodes of Ranvier in oligodendrocytes and initiated demyelination, whereas microglia appeared to clear debris, which is essential for remyelination[111]. The pathogenic role of circulating Ly-$6C^{hi}CCR2^+$ inflammatory monocytes has been described by King *et al.*[112] and by Mildner and colleagues[44]. Notably, the GM-CSF receptor on $CCR2^+$ monocytes drives inflammation in EAE through receptor activation by T-cell-produced GM-CSF[113].

Strategically placed between the bloodstream, the CNS parenchyma and the CSF, meningeal and perivascular phagocytes can monitor these interfaces and influence the communication between the periphery and the CNS. Perivascular and meningeal macrophages are believed to act as APCs in both rodents and humans because they express major histocompatibility complex (MHC) class II molecules, which implies that they are involved in antigen uptake, processing and presentation to T cells[34,38].

Stroke. Although stroke is an acute cardiovascular disease, it is associated with a pronounced neuroinflammatory response. More than 80% of all strokes are ischemic; the rest are hemorrhagic. Stroke is followed by a complex interplay between the nervous and the immune systems, which has been the subject of several excellent review articles[114–116]. Innate immune activation of microglia and macrophages occurs within 24 h after cerebral ischemia, followed by the accumulation of neutrophils[117]. The recruitment of neutrophils (**Fig. 3**) distinguishes stroke from the neurodegenerative and autoimmune disorders described above and has been a major target for therapeutic interventions. Unfortunately, clinical trials of anti-inflammatory drugs and blockade of leukocyte adhesion molecules (enlimomab, ASTIN, LeukArrest) in stroke patients have been unsuccessful despite promising preclinical evidence (reviewed in ref. 118). It is noteworthy that neutrophils do not actually enter the brain parenchyma to cause damage after cerebral ischemia; they are restricted to luminal surfaces or perivascular spaces of cerebral vessels[119]. Cerebral ischemia results in the dysfunction and death of neurons and glia, particularly oligodendrocytes[120]. The injured neural cells release DAMPs, which activate the innate and adaptive immune systems. Proliferating microglia are boosted by monocytes and macrophages (**Fig. 3**) that are recruited from the meninges, choroid plexus, perivascular regions[121] and the bloodstream[122]. Using parabiotic mice[123], microglial expansion after stroke was shown to depend on the proliferation of CNS-resident cells. Moreover, microglia and peripherally derived monocytes and macrophages exert different functions after stroke with regard to phagocytosis and the release of proinflammatory mediators[123–125]. Whereas the ablation of proliferating microglia appears to worsen stroke outcomes[126], mice deficient in $CCR2^+Ly6C^{hi}$ monocytes are protected from stroke[127]. The innate immune cells sense DAMPs via pattern-recognition receptors, such as Toll-like receptors, which in turn activate nuclear factor-κB, type I interferons and other signaling cascades. This sets off the activation of the adaptive immune system. T lymphocytes (**Fig. 3**) are recruited from cerebral venules to the ischemic tissue and exert detrimental effects independent of antigen recognition[128,129]. A particular subtype of T lymphocytes, called γδT cells, is specifically attracted to the ischemic brain by bone marrow-derived macrophages that produce IL-23 (ref. 130). γδT cells secrete IL-17, attract peripheral myeloid cells and contribute to neuronal apoptosis in the penumbra during the delayed phase of the ischemia–reperfusion injury[130,131]. Most γδT cells do not enter the brain parenchyma after cerebral ischemia but accumulate in the leptomeninges, where they act as gatekeepers to control the trafficking of monocytes and neutrophils[132]. The detrimental effects of T effector cells in stroke are counteracted by $CD4^+CD25^+Foxp3^+$ regulatory T (T_{reg}) cells, which are anti-inflammatory and antagonize the production of TNF-α and IFN-γ by lymphocytes and microglia via the secretion of IL-10 (ref. 133). Notably, T_{reg} cells confer neuroprotection without entering the ischemic brain[134]. On the other hand, there is also evidence to suggest that T_{reg} cells worsen stroke outcome by inducing microvascular dysfunction[135]. Recently, a novel gut–brain axis involving IL-17^+ γδT cells was recognized[132]. As a result of microbial dysbiosis, dendritic cells induce T_{reg} cells in the mesenteric lymph nodes. These T_{reg} cells home to the gut and suppress IL-17^+ γδT-cell differentiation. Following cerebral ischemia, the trafficking of these γδT cells from the intestine to the meninges is reduced, which decreases the expression of chemokines and the recruitment of leukocytes, thereby improving stroke outcome[132]. In contrast to T-cell infiltration, the infiltration of B cells (**Fig. 3**) into the ischemic brain is much delayed and takes several weeks[136]. Only a subpopulation of IL-10-producing regulatory B lymphocytes accumulate within 1–2 d after stroke; they inhibit the production of proinflammatory cytokines by peripheral T cells and inhibit the infiltration of T cells into the ischemic brain[137]. The initial activation of B cells probably occurs in the deep cervical lymph nodes, palatine tonsils and other secondary lymphoid tissues, where brain-derived antigens accumulate in stroke patients[138]. The exit routes of CNS antigens might involve transport by macrophages via the dural lymphatic network and the CSF[6,8]. Autoreactive T-helper cells and B cells that have escaped negative selection then become activated, and the latter secrete autoantibodies, which may result in long-term impairment of cognitive function after stroke (reviewed in ref. 139). In a process that is still poorly characterized, ectopic B-cell follicles (**Fig. 3**), composed of B cells and plasma cells (less

than 10%), T cells and CD11c$^+$ cells, develop in the infarct core starting 2 weeks after stroke[136]. These are reminiscent of the detrimental autoreactive B-cell responses following spinal cord injury[140] and the meningeal ectopic B-cell follicles found in EAE and MS[141,142].

The autoreactive immune responses after stroke are limited by a secondary immunodeficiency syndrome[143], which follows acute CNS injuries like stroke, traumatic brain or spinal cord injury and which increases susceptibility to infections (reviewed in ref. 144). In the case of cerebral ischemia, long-lasting lymphopenia and defective IFN-γ responses promote spontaneous bacteremia and pneumonia[145]. Recent evidence suggests that stroke results in the dysfunction of the gut barrier with subsequent dissemination of orally inoculated bacteria to peripheral tissues[146]. The CNS can sense systemic inflammation via neural (autonomic nervous system) and humoral (cytokines, hypothalamic–pituitary–adrenal axis) pathways (reviewed in ref. 114). Notably, hepatic invariant natural killer T cells are involved in the cross-talk between the nervous and immune systems after stroke via noradrenergic signaling and are critical in the defense against infections[147]. Cholinergic pathways suppress pulmonary innate immunity and predispose patients to pneumonia after stroke[148]. Thus, stroke is followed by a complex interplay between the CNS and the immune system, which represents an excellent target for future immunomodulatory therapies.

Future directions

The CNS is protected from external influences and intruders via the BBB. The entry and exit gates of the CNS, such as the choroid plexus, meningeal lining and perivascular spaces, are safeguarded by innate immune cells. In the case of neurodegenerative diseases, microglia and other brain macrophages are activated. However, these long-lived cells may eventually become functionally exhausted, suggesting that peripheral myeloid cells may be targeted.

In neuroinflammatory diseases like MS or stroke, the BBB is disrupted and peripheral immune cells gain access to the brain parenchyma. The resident innate immune cells of the CNS are now exposed to a new environment. They encounter innate immune cells that have been primed in the periphery, and how and to what extent the different myeloid cell population's cross-talk and contribute to disease is a subject of ongoing research. Adaptive immunity plays a central role in neuroinflammatory diseases. The recognition of a lymphatic drainage system of the CNS has drawn attention to the meninges and the choroid plexus. However, we still need to better understand the origin, function and fate of lymphocytes and macrophages at the borders of the CNS if we want to exploit this knowledge for the development of novel therapies for MS and stroke. Recent advances in transgenic technologies, genomics and bioinformatics will be of invaluable help in this quest.

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