REVIEW

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The myeloid cells of the central nervous system parenchyma

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A microglial cell is both a glial cell of the central nervous system and a mononuclear phagocyte, which belongs to the haematopoietic system and is involved in inflammatory and immune responses. As such, microglia face a challenging task. The neurons of the central nervous system cannot divide and be replenished, and therefore need to be protected against pathogens, which is a key role of the immune system, but without collateral damage. In addition, after physical injury, neural cells need restorative support, which is provided by inflammatory responses. Excessive or chronic inflammatory responses can, however, be harmful. How microglia balance these demands, and how their behaviour can be modified to ameliorate disorders of the central nervous system, is becoming clear.

The central nervous system (CNS) is now thought to consist of three main cell types: neurons, glial cells and vascular cells. In the CNS parenchyma (the tissue proper, exclusive of cerebrospinal-fluid spaces, blood vessels or meningeal coverings), microglial cells are the only type of cell that is not a neural cell or a vascular cell. Instead, microglial cells are the resident inflammatory cells of the CNS. The study of microglia provides insight into the development of the nervous system, as well as the mechanisms of neural damage and repair.

Progress has been made recently by using genetic models of development and disease, often in combination with novel imaging modalities such as two-photon microscopy. The findings have allowed researchers to begin delineating how the activation of microglia is controlled. These topics are of compelling interest to neuroscientists and other students of neurological disease, now that it is generally acknowledged that all CNS disorders are characterized by microglial-cell activation and that the progression and resolution of many diseases is contingent in part on the activity of microglia.

It is often a challenge to find strategies for modulating the underlying processes of neurodegeneration. However, numerous agents that promote or suppress aspects of inflammation or immunity are used in contemporary clinical practice as effective treatments for a range of conditions that affect virtually every organ system. Because much is known about the safety profiles and molecular mechanisms of these agents, understanding the roles of microglia in neurological diseases promises a translational reward in the near future.

Microglial-cell biology offers immunologists a lens that provides insight into neuroinflammation, neurodegeneration and neural repair. Conversely, for neurobiologists, it is essential to understand microglia in order to comprehend the inflammatory components of CNS processes. To address these topics, we review the ontogeny of microglia, how the activated state(s) of these cells is unleashed, and how some of their effector mechanisms operate in the CNS.

Microglia are the myeloid cells of the CNS parenchyma

The study of microglia is complicated by controversy and nomenclature disputes. It is reassuring to realize that this vigorous discussion about the origins and nature of microglia has been ongoing since the cells were first discovered in the late nineteenth century — and that progress has been made. At the same time, it is sobering to note that the giants who established the techniques and concepts of neuroanatomy — including Camillo Golgi, Franz Nissl, Rudolf Virchow, Santiago Ramón y Cajal and Pío del Río-Hortega — formulated much the same questions as are being asked today.

Cells of the CNS

Early neuroanatomists endeavoured to distinguish the varied types of cell in the CNS. This was a formidable task given the dense intertwined web of cellular processes from which the cell bodies needed to be differentiated. Using Golgi's metal-impregnation techniques for staining tissue as a starting point[1,2], Cajal identified the following cell types in 1913: neurons (which he called the first element)[3]; astrocytes (the second element); and a third element (cells with small, round nuclei). Between 1919 and 1921, del Río-Hortega clarified the composition of the third element, proposing initially that it comprised two distinct populations, one of which, oligodendrocytes[4], were related more closely to astrocytes and therefore belonged to the second element. He completed his tour de force by coining the term 'microglia' for the authentic third-element cells[5], which he suggested could transform themselves into migratory phagocytic cells. These suggestions were compatible with Nissl's[6] earlier description, in 1899, of 'rod cells', which he characterized as reacting to tissue pathology and likened to the leukocytes found in other organs. In summary, microglia were accurately placed in the range of cellular elements in the CNS, as well as accorded their fundamental function as phagocytes of the nervous system, about 90 years ago.

Classification challenges

Classifying mononuclear phagocytes, the category to which microglia belong, has proved a more durable challenge than that of classifying cellular elements of the CNS. Indeed, a lively debate on this topic was published earlier this year[7]. In parallel, mononuclear phagocyte investigators and neuroscientists have been labouring to understand how microglia fit into the overall scheme of mononuclear phagocytes, both during embryonic development and in the adult CNS. This distinction between adult and embryo is important, because it helps to clarify the investigator's approach to the ontogeny, function and maintenance of microglia and their relevance to disease. Each of these processes needs to be defined at the molecular level if research scientists are to achieve

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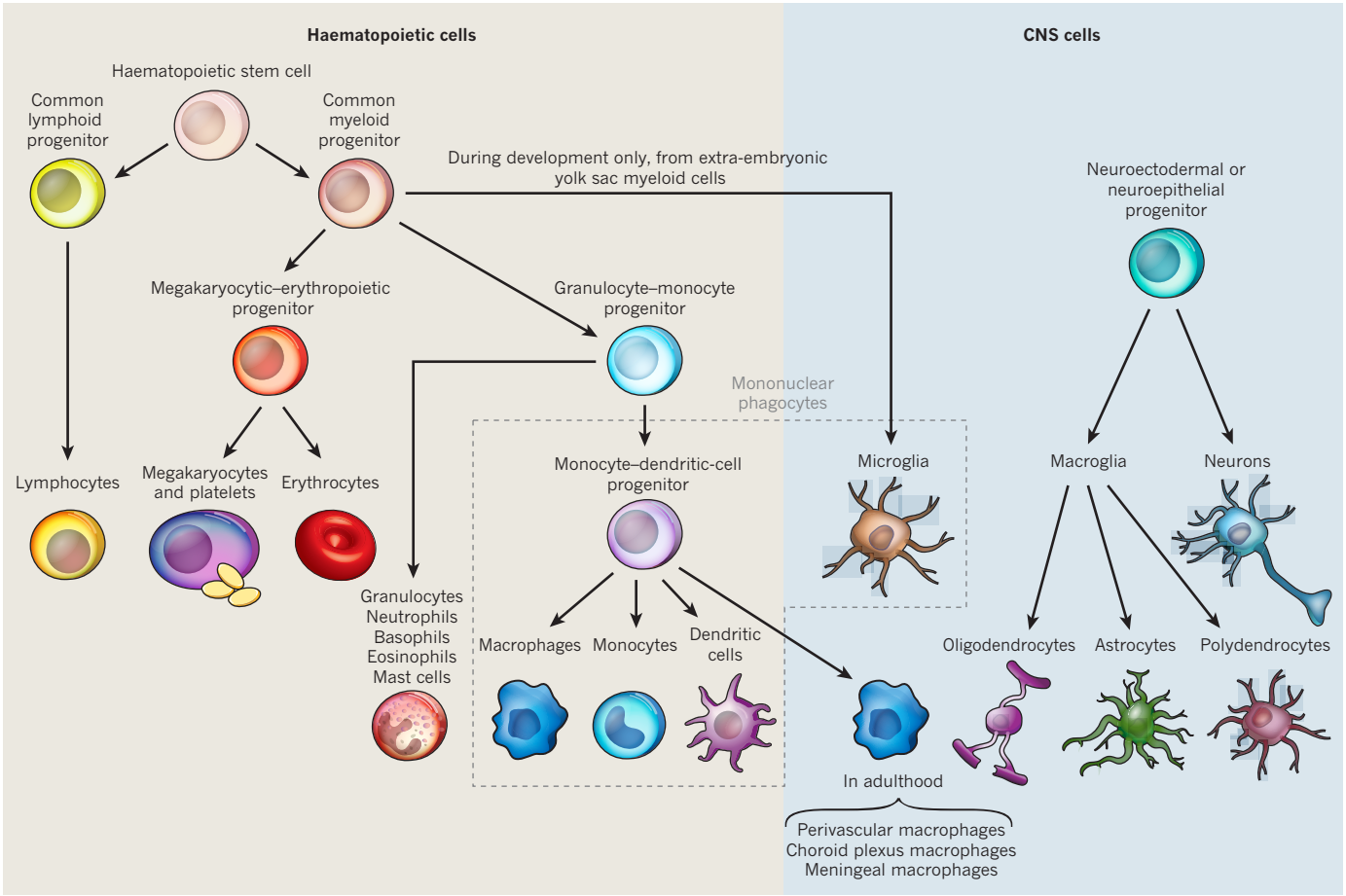

Figure 1 | How microglia are related to haematopoietic cells and CNS cells. Cells of the haematopoietic system and CNS are depicted, with arrows indicating lineage relatedness. Microglia are the only haematopoietic cells found in the parenchyma of the CNS. Note that the derivation of the perivascular, choroid plexus and meningeal macrophages from the monocyte–dendritic-cell progenitor is speculative and the blood cell from which they originate is unknown.

the goal of modulating microglial-cell properties to ameliorate CNS disorders. Furthermore, characterizing microglia and other CNS mononuclear phagocytes in this way is fundamental to understanding CNS inflammation and immunity.

Mononuclear phagocytes

Starting at the later stages of gestation, the cells of the mammalian haematopoietic system — that is, lymphocytes, erythrocytes, megakaryocytes (which produce platelets), granulocytes and mononuclear phagocytes — are renewed continually from bone-marrow haematopoietic stem cells throughout life (Fig. 1). Cells of the mononuclear phagocyte lineage are distributed across the bone marrow, blood and tissues, and include bone-marrow mononuclear phagocyte progenitors, blood monocytes, tissue macrophages and dendritic cells. These cells cannot be distinguished in a straightforward manner by immunohistochemical staining of tissues or immunofluorescence flow cytometry using cells from blood or tissue lysates. Instead, mononuclear phagocytes are classified by a combination of phenotype (cell-surface or enzymatic markers), location (bone marrow, blood or tissue), function and derivation (cell of origin).

Dendritic cells (DCs) are noteworthy because they are uniquely required for initiating adaptive immune responses, by taking up antigenic material in the tissue, gaining access to the local lymph nodes, and presenting antigen efficiently to naive T cells there[8]. The current consensus is that DCs can be found in the spleen, lymph nodes and skin, and at mucosal surfaces, but are unlikely to be present in healthy CNS tissues. Indeed, the cellular basis of CNS immune privilege rests mainly on the absence of dendritic cells in the healthy CNS[9].

During postnatal life, myeloid progenitor cells in the bone marrow give rise to common monocyte–dendritic-cell progenitors, which in turn yield blood monocytes and dendritic-cell progenitors. Dendritic cells arise from this committed progenitor. It remains uncertain whether monocytes differentiate into dendritic cells *in vivo,* although dendritic cells are readily obtained *in vitro* from monocytes by using well-established protocols. Tissue macrophages can be derived from blood monocytes or from circulating mononuclear phagocyte progenitors that are incompletely defined; they are maintained either by local self-renewal or by influx of cells from the circulation.

Microglia are derived from myeloid-lineage progenitors

Controversy about the possible origins of microglia from neuroectodermal cells, instead of myeloid progenitors, persisted well into the 1990s[10,11]. This debate occurred despite the fact that, by 1921, del Río-Hortega had clarified that microglia constituted Cajal's 'third element' of CNS cells. Furthermore, he had proposed that microglia were mesodermal in origin, not ectodermal, a concept that is regarded as correct at present. Perhaps the most convincing data favouring the myeloid origin of microglia came with the demonstration that differentiation of cells in the myeloid lineage, including into macrophages and neutrophils, failed in mice that lacked the transcription factor PU.1 (*Pu.1*$^{-/-}$ mice) and that no microglia were detected in the CNS of these mice[12]. It is worth noting that yolk-sac embryonic stem cells from *Pu.1*$^{-/-}$ mice can yield macrophage-like cells *in vitro* and that at embryonic day (E) 11.5 *Pu.1*$^{-/-}$ mice had typical embryonic phagocytes. These findings suggest that myeloid progenitors from the yolk sac may be a different population to those arising from definitive haematopoiesis in the embryo[13]. Interestingly, embryonic phagocytes appeared in *Pu.1*$^{-/-}$ mice several days after microglial progenitors are found in

wild-type animals, underlining the restricted time frame within which microglial progenitors seed the CNS[14].

Earlier well-designed studies assigned some parenchymal microglial cells to the neuroectodermal lineage for reasons that were logically derived from careful observations, although this interpretation is now regarded as incorrect. In one study[15], myeloid-marker-bearing microglia were meticulously tracked in the CNS anlage of rodents during early embryonic life, but myeloid-marker-bearing cells were not detected later in gestation, leading to the plausible interpretation that this wave of early infiltrating cells underwent programmed cell death. In addition, the researchers transferred bone-marrow cells that had been labelled with a genetic marker to irradiated rodents. Subsequent assessment of the CNS tissues showed that ~10% of parenchymal microglia bore the bone-marrow-derived genetic marker. Taken together, these results were reasonably construed to mean that after the widespread death of early-embryonic microglia, their replacements came from a non-bone-marrow (neuroectodermal) source. With the advantage of hindsight, it seems more probable that the preliminary wave of microglial-cell colonizers downregulated their myeloid markers during maturation in the profoundly immune-inhibitory CNS environment, and then weakly expressed selected indicators of their myeloid provenance only later[16]. A recent report used lineage tracing to show conclusively that adult mouse microglia are derived from primitive myeloid precursors that arise in the extra-embryonic yolk sac in a remarkably restricted time frame, just before E8.0, and invade the embryo after blood vessels form, at E9.0. There seem to be no contributions to microglia from haematopoiesis in the embryo or the adult. Microglia are accordingly distinguished from other tissue macrophages by their properties of local self-renewal and resistance to ionizing radiation[14]. Given these results, it was not surprising that other hypotheses, such as that microglia arose from non-endothelial vessel-associated mesodermal cells, have failed to gain experimental support[8].

Microglia as mononuclear phagocytes

Taking into account the findings described above, in this Review we use the following definitions. Monocytes are mononuclear phagocytes circulating in the blood. Macrophages are phagocytic cells that reside in the tissue. Dendritic cells are mononuclear phagocytes that initiate immune responses by stimulating T cells[17], and are present in the spleen, lymph nodes and skin and at the mucosal surfaces. Microglia are myeloid cells of the CNS parenchyma.

Microglia have the special distinction of being both glia of the CNS and a unique type of mononuclear phagocyte (Fig. 1). In addition to the microglia, which are present in the CNS parenchyma, the CNS (including the brain and spinal cord, as well as their meningeal coverings, the subarachnoid and perivascular spaces and the choroid plexus) contains other mononuclear phagocytes: meningeal macrophages, choroid plexus macrophages, epiplexus cells and perivascular macrophages, each with a distinct location, marker profile and presumed function in host defence (Table 1).

These limited definitions of the mononuclear phagocyte subgroups are offered in the hope that they will be useful until definitive molecular markers for these populations emerge. In this regard, microglia are recognized at present as a subset of tissue macrophages that do not have dendritic-cell characteristics *in vivo*.

Some provisional conclusions can be drawn from this definition of microglia. First, if microglia do not initiate immune responses by activating naive T cells through antigen presentation (as dendritic cells do), then the lack of dendritic cells residing in the CNS parenchyma is a satisfactory cellular basis for the immune privilege of the CNS[9]. Second, it remains important to determine the capacity of microglia to restimulate tissue-invading memory T cells during host defence or immunopathological responses such as those that give rise to multiple sclerosis. Finally, it is incumbent on researchers to apply their understanding of mononuclear phagocyte biology to clarifying the consequences of the unique origins and maintenance of microglia throughout embryonic and adult life. We propose that the capacities of microglia are defined in part by their ontogeny and that monocyte-derived cells that enter the CNS during disease have properties that are distinct from those of microglia, as suggested by studies in animal models[18–20]. If this hypothesis is correct, then exploiting our new understanding of microglial-cell ontogeny[14] will help researchers to decide when to modulate microglial-cell activation, when to block infiltrating monocytes or their progenitors, and what, if any, potential exists for delivering genes or gene products to the CNS, in the context of neurological disease, by loading them onto potential microglial-cell progenitors[21].

Ontogeny of microglia

In summary, microglia are mononuclear phagocytes, and we are now in a position to consider when their progenitors enter the CNS and which route of entry they use[14].

Table 1 | Location and immune function of mononuclear phagocytes in the CNS

Type	Location	Phenotype	Immune function	Outcome of interaction with T cells
Choroid plexus macrophages	Choroid plexus stroma	Dendritic-cell-like: armed with both MHC class II molecules and co-stimulatory molecules, allowing antigen presentation	Present antigen derived from the peripheral circulation to T cells, thereby restimulating memory $CD4^+$ T cells as they traffic into the cerebrospinal fluid	Interact with T cells that are crossing from blood to CSF Host defence against extracellular bacteria Possibly autoimmune responses Facilitation of activation of parenchymal vasculature of the blood–brain barrier
Meningeal macrophages	Associated with the pial vasculature and the stroma of the subarachnoid space	Armed mainly with MHC class II molecules	Present peptide antigens derived from breakdown of CNS cellular components (cellular or pathogen derived) to T cells, thereby restimulating memory $CD4^+$ T cells as they traffic through the cerebrospinal fluid	Interact with CSF T cells in subarachnoid space Host defence against viruses and tumours Possibly autoimmune responses Facilitation of activation of parenchymal vasculature of the blood–brain barrier
Perivascular macrophages	Within the perivascular spaces of the parenchymal and subpial vessels on either the 'vascular' side or the 'parenchymal' side of the glial limitans basement membrane	Armed with both MHC class II molecules and co-stimulatory molecules, allowing antigen presentation	Restimulate effector T cells that have extravasated across activated subpial vessels (which have interendothelial tight junctions) or the parenchymal blood–brain barrier	Interact with T cells in parenchymal perivascular space and facilitate invasion of the parenchyma proper during the effector phases of host defence and autoimmunity
Parenchymal microglia	Within the parenchyma near the neurovascular unit but not directly associated with vessels May form part of the perivascular glial limitans	Express cytosolic more than membrane MHC class II	Local source of potential phagocytes Produce antibacterial effectors through an oxidative burst Produce cytokines	Interact with T cells in the parenchyma proper Much interaction occurs at a distance, through response to T- cell-secreted cytokines such as IFN-γ, and is not antigen specific

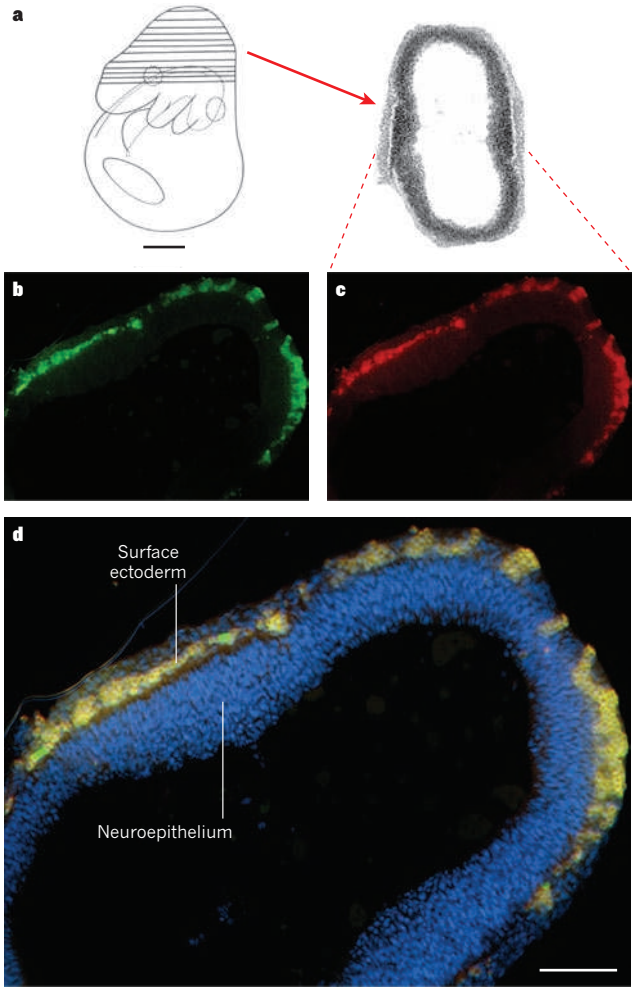

Figure 2 | Microglial precursor cells approach the CNS during embryonic development. To study the origin of microglia in the CNS, we assessed developing CNS tissues for the expression of chemokine receptors that are present at the surface of mononuclear phagocytes. Mice expressing green fluorescent protein (GFP) under control of the *Cx3cr1* promoter (denoted CX_3CR1-GFP^+) and red fluorescent protein (RFP) under control of the *Ccr2* promoter ($CCR2\text{-}RFP^+$) were mated. All mononuclear phagocytes express one or both of these receptors. Tissues from the resultant offspring were analysed *in vitro* at various time points during gestation. Sections were stained with DAPI (4′,6-diamidino-2-phenylindole) to visualize cell nuclei (blue). In addition, confocal fluorescence microscopy images were acquired, and 20-μm *z*-stacks show the developing neuroepithelium. **a**, The embryo is shown in schematic form on the left, with solid lines indicating the plane of the section studied. (Schematic and bright-field micrograph (right) reproduced, with permission, from ref. 98.) **b–d**, Confocal image of a representative section showing the distribution of CX_3CR1-GFP^+ cells (**b**), $CCR2\text{-}RFP^+$ cells (**c**) and a merged image of **b** and **c** (**d**) at E9.5. In the merged image, a dense band of mononuclear phagocytes (yellow) is observed between the neuroepithelium and the surface ectoderm. Distribution of GFP^+ and RFP^+ cells shows that both receptors are expressed by mononuclear phagocytes localized between these tissue layers. Moreover, from these data, it is clear that microglial precursor cells approach the neuroepithelium at a time before the onset of fetal haematopoiesis or vascularization of the developing CNS. Scale bars, 500 μm (**a**) and 100 μm (**d**).

Microglia colonize the embryonic CNS

As noted above, cells bearing myeloid markers are found immediately adjacent to the developing CNS of rodents as early as ~E8.5 (Fig. 2 shows E9.5), and they appear within the parenchymal anlage as early as E10.5 (Fig. 3). The myeloid progenitors of the yolk sac are the source for the embryonic wave of myeloid cells that colonize the developing telencephalon and brain stem, and presumably the spinal cord as well.

It seems most plausible at present that all cells destined to give rise to parenchymal microglia share origins and developmental pathways[22]. Because macrophage-like cells from the yolk sac are functionally distinct from other mononuclear phagocytes, the properties of microglia will probably need to be investigated by focusing specifically on microglia themselves[13]. In the first few days after birth, a marked increase in the number of microglia is observed within rodent CNS tissues[23]. This remarkable expansion of the microglial population arises from *in situ* proliferation. The migration cues for microglial-progenitor-cell transit across vascular beds remain obscure, because microglia are found in the appropriate numbers in the CNS of mice lacking crucial regulators of leukocyte migration[24]: molecules that aid leukocyte interaction with the endothelium such as intercellular adhesion molecule 1 (ICAM1), chemoattractant cytokines (chemokines) such as CCL2, CCL5 and CX_3CL1, and chemokine receptors such as CCR1, CCR2, CCR5 and CX_3CR1.

Questions raised

One problematic issue is immediately apparent from this discussion: are there microglial subpopulations whose attributes are determined in part by their localization within the CNS? At present, the only conclusion that can be made with confidence is that researchers do not yet have the appropriate techniques to uncover the detailed relationships between the haematopoietic cells that enter the various regions of the CNS during development and the microglia found there in adult life.

The definitions of stages along the branched, interrelated myeloid differentiation pathways[7] rely mainly on surface and enzymatic markers. About 50 surface markers characteristic of the mononuclear phagocyte lineage can be readily listed, of which perhaps 35 are thought to be useful for discriminating subsets of macrophages and dendritic cells[8]. In medicine and science, philosophical chasms divide 'lumpers' from 'splitters'[25]. A member of the former camp has noted that the "number of subpopulations of mononuclear phagocytes one can define is an exponential function of the number of markers examined"[7]. This complexity synergizes with differences in temperament, scientific approach and research priority among investigators, limiting progress towards achieving a consensus about subsets of microglia and the possibility that there may be distinct varieties of activated microglia.

Do embryonic microglia carry out developmental functions?

Many other questions remain open. One of these is, what are the key functions of microglia that drive their infiltration of the CNS during development? Numerous important activities have been proposed: directing the invading vasculature[26], removing apoptotic cells (which the developing CNS generates in profusion)[27,28] and synaptic pruning[29]. Elegant histological studies have shown that microglia ingest whole cells (not just cellular processes) and are often, but not always, spatiotemporally concentrated with high numbers of apoptotic cells[30–32]. To interpret these data, it is important to consider that cells other than mononuclear phagocytes participate in the removal of apoptotic cells from the developing CNS[33]. Furthermore, the molecules involved in the recognition and clearance of apoptotic cells are ancient and well conserved throughout evolution, so the expression of these by non-microglial cells is unsurprising[34]. Microglia may help to initiate apoptosis of excess cells generated during development[35,36], although this concept has not been universally accepted[28]. These processes have been difficult to investigate because it is challenging to remove or disable microglia experimentally during development. Recent results showing that colony stimulating factor 1 receptor and, potentially, interleukin 34, are required for the appearance of microglia in the CNS[14] may provide tools for addressing some of these issues.

If it were confirmed that microglia are involved in eliminating excess cells (both by stimulating programmed cell death and removing debris) and trimming away unneeded synaptic connections, this could implicate embryonic microglia in establishing the intricate circuitry of the developing CNS. However, $Pu.1^{-/-}$ mice, which are deficient

in microglia, seem to behave normally until their death from infection at about day 17 after birth despite antibiotic treatment, suggesting that microglial-cell contributions to prenatal neural development are subtle.

Abnormal microglia alter CNS physiology

Nevertheless, minor abnormalities in the microglial-cell input to neural function can combine to have large effects. One example from medicine is Nasu–Hakola disease, a rare autosomal recessive genetic disorder[37,38]. Individuals with this condition have bone cysts and dementia in early adulthood. Its genetic basis lies in homozygous null mutations of the gene encoding TREM2 (triggering receptor expressed on myeloid cells 2). Absence of this cell-surface protein (which is present only on microglia among the cells of the CNS) causes selective functional abnormalities of microglia and of osteoclasts (cells that break down bone and are also derived from the mononuclear phagocyte lineage). Remarkably, a disease phenocopy is observed in individuals lacking the transmembrane adaptor signalling protein DAP12 (refs 38, 39), which transduces signals from TREM2, as well as from other cell-surface components of haematopoietic cells[37]. Mice lacking TREM2 as a result of a targeted gene deletion show some features of the human disorder[40]. Given that a novel loss-of-function mutation in *TREM2* caused early-onset dementia but not bone cysts in one human family[41], disruption of TREM2 is the most persuasive argument that abnormal microglial-cell physiology can disturb homeostasis in the CNS. On the basis of *in vitro* studies using neural cell lines, cell-surface heat-shock protein 60 (HSP60) has been proposed to be a ligand for TREM2 (ref. 42).

Magnetic resonance imaging shows brain abnormalities in individuals with Nasu–Hakola syndrome[39]. A genetic abnormality specific to microglia can also severely perturb behaviour without causing obvious neuroanatomical defects. In particular, mice lacking the homeobox gene *Hoxb8* do not have neuroanatomical defects but have pathological grooming behaviour, in a pattern with similarities to obsessive–compulsive disorder in humans[43]. Because the Hox genes are widely expressed and involved in organ patterning, it was unsurprising to find Hoxb8 expression both in spinal-cord neurons and in brain microglia. The selective deletion of Hoxb8 from spinal-cord neurons by conditional gene targeting caused defective sensory signalling but unexpectedly did not correlate with the obsessive grooming behaviour[44]. Surprisingly, replacement of the haematopoietic cells of these mice with wild-type haematopoietic cells rescued the defect in grooming without altering the sensory abnormalities, thereby dissociating the two phenotypes of sensory impairment and obsessive grooming[44]. Furthermore, selective deletion of *Hoxb8* from haematopoietic cells during development recapitulated the aberrant grooming behaviour in adult mice. Neither T cells nor B cells were required for obsessive grooming in mice lacking haematopoietic-cell *Hoxb8* (ref. 44). The researchers concluded that *Hoxb8* expression in microglia underpins the grooming defect[44]. Interestingly, *Hoxb8* mRNA expression (tracked in otherwise-wild-type mice, by placing a genetic reporter under the control of the *Hoxb8* promoter) was present in about 40% of microglia, and reached that level during the first two weeks of postnatal life.

Although it is risky to make conclusions on the basis of one marker (perhaps *Hoxb8* was simply upregulated during this postnatal period), it is possible that an identifiable subpopulation of microglia regulate the neural substrate in terms of grooming behaviour. The obsessive grooming seen in mice lacking the *Hoxb8* gene in microglia was not associated with marked abnormalities in neural cell populations. Instead, aberrant interactions of microglia with neurons, either at a distance through secreted factors[45] or by direct contact with synapses[46], might be responsible for the behavioural phenotype. Finally, it should also be noted that clinical non-CNS haematopoietic disorders such as lymphoma are associated with severe itching, which could mimic obsessive grooming. Because the bone-marrow chimaerism replaced the entire blood system with *Hoxb8*-sufficient cells, microglia may not provide the full explanation for the phenotype.

Results of microglial–cell responses can be difficult to specify

Microglia show morphological indications of activation in the brains of patients dying as a result of neurodegenerative diseases such as Alzheimer's disease. *In vitro* studies clearly show that cultured microglia can be activated by released amyloidogenic peptides (termed amyloid-β peptides) derived from β-amyloid precursor protein. Proteolytic generation of these peptides is thought to be an early event in the pathogenesis of Alzheimer's disease. The peptides can form toxic soluble oligomers, and they also constitute the fibrils that form amyloid plaques, a major histological hallmark of Alzheimer's disease. The response of microglia to soluble and fibrillar amyloid-β peptides has been studied intensively since it was proposed that phagocytosis of these peptides by microglia could ameliorate the pathogenic cascade that occurs in the brains of individuals with Alzheimer's disease. Conversely, the release of pro-inflammatory mediators by activated microglia could promote Alzheimer's disease pathology. The current understanding of receptor-mediated recognition of aggregated (fibrillar) amyloid-β by microglia[47] is that the signalling leading to phagocytosis and the signalling resulting in production of reactive oxygen and/or nitrogen species might be indistinguishable. Therefore, the acute response of microglia to fibrillar amyloid-β peptides, an endogenous indicator of CNS tissue disturbance, might carry inseparable beneficial and harmful consequences. Investigators have made heroic efforts to deconvolute how microglia respond to fibrillar and soluble amyloid-β peptides, but the results have been challenging to interpret, given their apparent inconsistencies. Complexity is

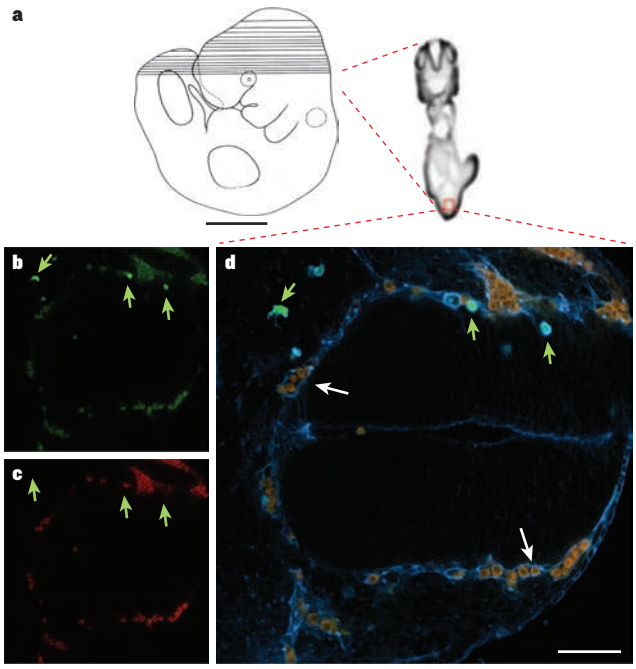

Figure 3 | Microglia colonize the CNS during embryonic development. The study was carried out as described in Fig. 2, except that axial sections of embryos were stained with isolectin IB4 (blue) to visualize the vasculature and microglia. **a**, The embryo is shown in schematic form with solid lines indicating the planes of the sections studied (left) and an image of the plane depicted in **b**–**d** (right) with a square indicating the selected section (the developing spinal cord). (Reproduced, with permission, from ref. 98.) **b**–**d**, Distribution of $CX_3CR1\text{-}GFP^+$ cells (**b**), $CCR2\text{-}RFP^+$ cells (**c**) and a merged image of **b** and **c** (**d**) at E10.5. $CX_3CR1\text{-}GFP$ (**b**) but not CCR2-RFP (**c**) is expressed by developing microglia. In the merged image (**d**), newly migrated amoeboid microglia (green arrows) appear $CX_3CR1\text{-}GFP^{hi}$, $IB4^+$ and $CCR2\text{-}RFP^-$. Mononuclear phagocytes remaining in the vasculature (white arrows) have a $CX_3CR1\text{-}GFP^{low}CCR2\text{-}RFP^+$ phenotype. From these data, it is clear that amoeboid microglia enter the developing CNS (parenchymal anlage) around E10.5, coincident with vascularization. Scale bars, 1 mm (**a**) and 100 μm (**d**).

inherent in the biology: signalling downstream of the recognition of fibrillar amyloid-β peptides by microglia involves an aggregate comprising various combinations of nine transmembrane and endosomal proteins, including class A and B scavenger receptors, $\alpha_6\beta_1$-integrin, CD14, CD47, Toll-like receptor 2 (TLR2), TLR4, TLR6 and TLR9 (ref. 48). In addition, to some extent, inconsistent data arise from using a variety of extensively manipulated *in vivo* genetic models. The issues have been incisively discussed recently[48] in a review that also underlined the importance of understanding whether microglial-cell activation is essential for the success of passive and/or active immunotherapy for individuals with Alzheimer's disease. Finally, data from these varied experiments highlight the different capacities of resident microglia and infiltrating blood-derived macrophages for clearing amyloid plaques[18,19].

Microglial–cell homeostasis in the adult CNS

In the adult CNS, more than 10% of all cells are microglia, varying from about 5% to nearly 20% depending on the location in the neuraxis. In the healthy CNS, microglia have a distinctive distribution, with radial, non-overlapping processes, a small cell soma and each cell appearing to occupy its own domain.

Surveillant not 'resting' microglia

Microglia of the healthy CNS have, for decades, been termed 'resting' to discriminate their morphology from the microglia of the injured or diseased CNS. Two-photon imaging of the cerebral cortex of live mice[49] demonstrated vividly that microglia continually palpate the environment with their processes, and it has been estimated[50] that the entire volume of the brain is examined by these endlessly busy cells approximately every four to five hours. The term 'surveillant' has been advanced to describe how microglia continuously monitor the healthy neural tissue. Although we salute the elegance and beauty of these landmark imaging studies, it must be recognized that the information comes exclusively from the outer layers of the cerebral cortex, that the densities and microenvironments of microglia differ throughout the neuraxis, and that the surveillant behaviour of microglia might also vary depending on their location within the CNS. In this regard, recent reports of the feat of sophisticated two-photon imaging of mouse spinal-cord microglia are most welcome[51,52].

Microgliosis indicates increased microglia numbers

In CNS pathology, an increase in the number of microglia is almost always observed. Given that many clinical CNS disorders are associated with disrupted function of the blood–brain barrier, allowing the entry of cells from the bloodstream, it has been unclear to what extent microglia proliferate *in situ* after injury and acquire the appearance of macrophages, and conversely, how many monocytes enter the CNS from the bloodstream and subsequently adopt microglial-cell morphology[53]. This question has proven surprisingly contentious[16,54] and is particularly difficult to settle definitively because no known cell-surface or enzymatic markers differentiate the activated macrophage-like progeny of microglia from those of blood monocytes. Furthermore, the technique that is most widely used for these studies is radiation bone-marrow chimaerism, in which animals are sublethally irradiated and then their bone marrow is replaced with genetically labelled haematopoietic stem cells; microglia remain because they are resistant to radiation. This method is fraught with unavoidable confounding factors[54–56]. At present, it seems most plausible that the number of microglia increases through proliferation *in situ* in pathological states[55] in which the blood–brain barrier has not been grossly disrupted, in such a way as to allow entry of circulating cells to the CNS. One well-studied example comes from transection of the facial nerve in the cheek, resulting in brisk accumulation of microglia around the axotomized ipsilateral brainstem nucleus of the facial nerve[57]. In previous experiments, results from using bone-marrow chimaerism suggested that microgliosis (accumulation of an abundance of microglia in a focal site of injury) stemmed from large-scale infiltration of blood monocytes that had differentiated into microglia. However, it has recently become clear that several artefacts complicate the interpretation of these studies. In particular, a course of cranial irradiation permanently alters the cerebral vasculature; the transfer of bone-marrow cells (taken by femoral flushing) introduces non-physiological levels of haematopoietic stem cells into the circulation; and damage to the CNS is also required to facilitate CNS invasion by circulating monocytes[54–56]. Given these findings, it seems likely that the complement of microglia in the CNS parenchyma is established during the early-embryonic period and is self-renewing thereafter[54,55].

Removal of inhibition as the first step in microglial–cell activation

Microglia in the diseased or damaged CNS are termed 'activated' and are easily distinguished by conventional tissue histology because they bear little morphological resemblance to those found in the healthy brain.

From morphology to molecules

On the basis of their extensively branched processes, surveillant microglia in CNS tissue sections from healthy animals are designated 'ramified'. The morphology of 'activated' microglia includes enlargement of the soma, retraction and shortening of processes and increased expression of myeloid cell markers. Mechanistic information about this morphological transformation by microglia has been obtained: using two-photon imaging as an analytical tool, it was shown[49] that microglia initially extend processes in response to a focal laser lesion and that ATP (either released by damaged cells or in a regulated manner by astrocytes) governs extension of the processes, which rapidly wall off small destructive lesions. The purinoceptor that underlies this response is $P2Y_{12}$ (ref. 58). Subsequent morphological changes were found to be associated with downregulation of expression of $P2Y_{12}$, conversion of ATP to adenosine by the microglial-cell ectoenzymes CD73 and CD39, and increased expression of adenosine A2A receptor[59], which mediated process retraction.

At the far end of the range of morphological activation, microglia are termed 'amoeboid', indicating rounded cells with sparse processes and capable of phagocytosis. Interestingly, this morphological transformation recapitulates in reverse that which transpires during development, with amoeboid microglial progenitor cells entering the CNS and slowly adopting the ramified morphology of mature microglia.

The concept that activation of microglia occurs along a linear range, as suggested by the descriptive morphology of the cells in a variety of diseases and models, has been discarded[60]. Current concepts of activation arise, in part, from research into monocyte and macrophage biology, for which it has been elegantly and forcefully demonstrated that differing stimuli cause differentiation along distinct pathways of gene expression. Macrophages that have been exposed to different stimuli are designated M1, M2a, M2b and M2c macrophages, depending on the stimulus and the context. Functional significance is attributed to these varied states of activation, with, for example, M1 macrophages thought to specialize in pathogen elimination and the various categories of M2 macrophage possibly involved in tissue remodelling and repair[7,61]. M1 macrophages and the varied M2 subtypes can frequently appear similar but are distinguished from each other by defined profiles of stimulus-induced gene expression and effector function, lending credence to the categorization. Consistent with this concept, morphologically identical microglia in varied models of CNS disease show widely divergent patterns of gene expression.

It has not, thus far, been possible to develop a coherent account of the different activation profiles of microglia in a way that points researchers towards a usable terminology to differentiate varied microglial activation programs (which might be termed 'Mi1', 'Mi2' and so on). Nevertheless, it is considered highly likely that microglia behave similarly to macrophages, by polarizing their activation states to achieve effector responses that are appropriate for varying challenges to the CNS[16]. One striking example is termed 'stress conditioning': in this

process, a precisely timed and calibrated peripheral challenge to the organism induces a response from microglia that lessens the impact of a subsequent CNS insult. Examples of such a peripheral challenge are endotoxin injection or altered body temperature, whereas the most-studied CNS insults include seizure and stroke[62]. Microglia have been proposed as passive targets of neuroprotective preconditioning, with many experiments showing dampened inflammatory responses. However, genetic ablation studies have shown that endotoxin-induced protection against seizures is eliminated in the absence of microglia, suggesting that microglia have an active role in this form of 'beneficial' inflammatory response[63].

The varied products and activities of activated microglia have been catalogued and discussed over the past few decades[16,64–67]. Much of this work was carried out using *in vitro* cultures, which have disadvantages, including that serum (derived from plasma to which microglia in the healthy brain are never exposed) is commonly used[16]. Detailed gene-expression analysis[68] confirmed a long-standing wariness about extrapolating from such cultures to the *in vivo* situation. The investigators showed, fascinatingly, that microglial-cell cultures that had been stimulated with cytokines and bacterial cell-wall components expressed genes in a pattern that was more similar to that of macrophages from the abdominal cavity (challenged with the same stimuli) than to those of brain microglia (isolated from animals into which the same cocktail of cytokines and bacterial components had been injected into the CNS). The *coup de grâce* came from the demonstration that macrophages that infiltrated the brain after these injections expressed genes more akin to those expressed by brain microglia than those induced in cultured macrophages. The salient conclusion from the study[68] was that the CNS environment contributed more to gene expression by mononuclear phagocytes than did the stage of lineage differentiation. As a generalization, the study of microglial-cell cultures indicates what these cells can do but might not accurately reflect their activities *in vivo*.

On activation, microglia can produce numerous protein mediators, including those categorized as cytokines (both pro-inflammatory and anti-inflammatory cytokines), growth factors, chemokines and neurotrophins. As mononuclear phagocytes, they can also generate reactive oxygen and nitrogen species through enzymes involved in the oxidative burst mechanism and inducible nitric oxide synthase. Microglia can be phagocytic. In addition, they can express molecules that are associated with an ability to stimulate T cells with antigen (a feature that is typical of antigen-presenting cells).

Repressed microglia of the healthy CNS

Researchers spent decades before conclusively defining microglia as myeloid cells from the haematopoietic lineage. One reason for the prolonged controversy over this topic was that microglia do not express many of the markers of mature myeloid cells in other organs. It has been suggested that microglia are restrained by numerous microenvironmental inhibitory influences , many of which are produced by neurons[16,69] (Fig. 4). Neuron–microglial-cell inhibitory signalling is mediated by the following interactions: CD200–CD200 receptor (CD200R)[70], CD22–CD45 (also known as PTPRC)[71], CD172A (also known as SIRP-α)–CD47 (refs 71, 72) and CX_3CL1–CX_3CR1 (refs 73, 74). Some of these signals (CD22–CD45 and CD200–CD200R) require cell–cell contact. Signalling from neuronal CX_3CL1 to microglial-cell CX_3CR1, and from neuronal ICAM5 to microglial-cell LFA1 (lymphocyte function associated antigen 1; also known as CD11a–CD18 and $\alpha_L\beta_2$-integrin), can occur through contact between cell-surface receptors and cell-surface-bound ligands, or at a distance after the generation of the soluble ligands CX_3CL1 or ICAM5, respectively[75–77]. In the healthy CNS, two-photon imaging shows that microglia 'inspect' neuronal synapses *in vivo* in the cerebral cortex of adult rodents[46]. It is not clear whether the subcellular distribution of microglial-cell CD200R and neuronal CD200 or other contact-dependent components would accommodate contacts in the manner demonstrated in this study.

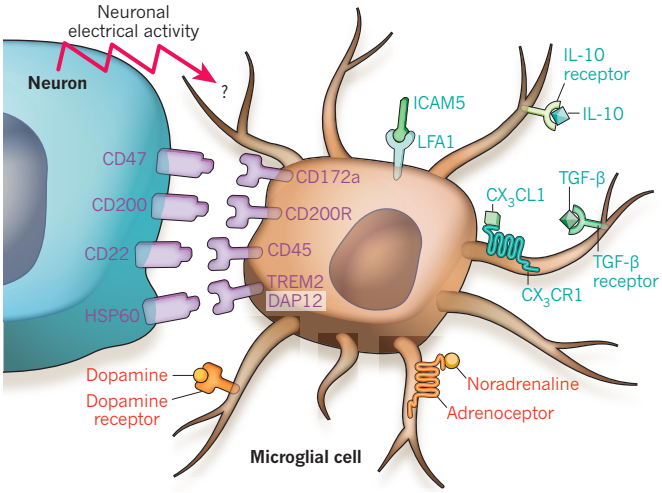

Figure 4 | Neuronal inhibitory influences on parenchymal microglia. Neurons inhibit microglia in several ways. Contact-dependent inhibitory influences are shown in purple. Soluble inhibitory cytokines or adhesion molecules and their receptors are shown in blue, and soluble neurotransmission-associated inhibitors and their receptors are shown in red. For further information, see the following references: contact-dependent inhibition, through CD200–CD200 receptor (CD200R)[70,99], HSP60–TREM2–DAP12 (refs 39–42, 100, 101), CD22–CD45 (ref. 102) and CD47–CD172A[71,72]; cytokine- or soluble adhesion-molecule-mediated inhibition, through soluble ICAM5 (intercellular adhesion molecule 5)–LFA1 (lymphocyte function associated antigen 1)[77], CX_3CL1–CX_3CR1 (refs 80, 103), interleukin 10 (IL-10)–IL-10 receptor[104] and transforming growth factor-β (TGF-β)–TGF-β receptor[104,105]; neurotransmission-associated inhibition through dopamine–dopamine receptor (D1, D2, D3 and D4 subtypes)[106], and noradrenaline–adrenoceptor[106]; and neuronal electrical-activity-mediated inhibition[107].

Unleashing microglia by removing inhibition

An interesting corollary of this precept that neuronal inputs restrain microglia is that microglial-cell activation may be equivalent, to some extent, to removing inhibition. This hypothesis has the attractive feature of introducing a teleological explanation for the abrupt response of microglia to neuronal injury: specifically, that microglia sense damage when the neuronal expression of inhibitors is silenced. Consistent with this idea, microglia are unusual among all mononuclear phagocytes in that they express ion channels, neurotransmitter receptors and an atypically rich range of purinoceptors[69,78,79].

The relevance of CX_3CR1 regulation of microglial-cell activation *in vivo* was demonstrated by studying toxin-induced neuron injury and genetic models of neurodegeneration[80]. In these models, *Cx3cr1*$^{-/-}$ mice showed exacerbated neuron loss compared with *Cx3cr1*$^{+/+}$ mice. This result could be attributed to altered microglial-cell activation, because, among CNS cells, only microglia express CX_3CR1 *in vivo*[80]. In the context of differing forms of CNS challenge, lack of CX_3CR1 might also have a protective role[81,82] or no role[83]. Therefore, the most reliable summary of these results is that CX_3CR1 signalling often modulates microglial-cell responses to CNS injury.

In addition to their inhibitory inputs from neurons, microglia differ from all other mononuclear phagocytes in that they reside, after the first few weeks of life, behind the blood–brain barrier, shielded from plasma proteins[84]. CNS trauma, infection, inflammatory and neoplastic diseases are all accompanied by a loss of blood–brain barrier integrity, and it has been considered axiomatic that exposure to plasma proteins activates microglia. This concept was rendered more specific and able to be molecularly dissected with the demonstration that plasma fibrinogen[85] is a key ingredient in the stimulation of microglia by proteins that extravasate across the disrupted blood–brain barrier. Fibrinogen, which binds to platelet integrins, also engages MAC1 (also known as CD11b–CD18 and $\alpha_M\beta_2$-integrin), an integrin found on activated microglia.

When the domain of fibrinogen that interacts with MAC1 was identified, it was found that the interaction between the fibrinogen and microglial MAC1 can be blocked without suppressing fibrinogen's function in haemostasis. Microglia stimulated by fibrinogen had an increased phagocytic capacity, which was mediated through AKT-dependent and Rho-dependent pathways[86].

Microglia and adaptive immunity

So far, we have focused on the innate inflammatory responses of microglia. The adaptive arm of the immune response involves interactions between mononuclear phagocytes and lymphocytes and is responsible for immunological memory. The CNS parenchyma is an immunoprivileged site[14] because the only mono-nuclear phagocytes that are present — microglia — cannot assume the functions of dendritic cells, which ingest antigen, exit the tissue and enter the draining lymph nodes, where they stimulate naive T cells. Immune responses do, however, occur in the CNS, raising questions about the roles of microglia in processes such as host defence, tumour rejection and immunopathology (Table 1), as typified in individuals with multiple sclerosis.

The CNS parenchyma is inimical to T cells, which do not survive there unless restimulated by the antigen that they recognized initially in the peripheral lymphoid organs[87]. Furthermore, mononuclear phagocytes do not efficiently invade the parenchyma without the cytokine signals secreted by T cells, following this type of restimulation[88]. The restimulation of T cells by mononuclear phagocytes of the CNS has been elegantly dissected *in vivo* using genetic and imaging tools[89–94] and *in vitro* using co-culture systems[95]. The import of these accumulated observations is that the non-parenchymal mononuclear phagocytes of the CNS — those found in the choroid plexus, meninges and perivascular spaces — are responsible for cell restimulation. Despite their competence as antigen-presenting cells and their expression of dendritic-cell markers, CNS myeloid cells do not seem to have the capacity to leave the CNS and transport antigen to the lymph nodes, where immune recognition by unprimed T cells could be initiated — and this is the case even in conditions of ongoing, severe local inflammation[96]. Involvement of the parenchymal microglia seems limited to an undefined role in worsening disease severity, as has been shown in animals with experimental autoimmune encephalomyelitis, a model of immune-mediated brain inflammation[97].

Perspective

Microglia are unique mononuclear phagocytes, which are localized in the unique environment of the brain parenchyma. Understanding the properties of these cells is a challenge to biomedical scientists, who must devise strategies to study the cells *in vivo* and cope with the absence of markers that unequivocally distinguish them from haematogenous, infiltrating macrophages, which can have identical morphologies[53]. Therefore, researchers lack knowledge about the triggers and suppressors of the phenotype(s) of activated microglia. There have been recent gains in our understanding of how and when microglia enter the CNS parenchyma. A crucial line of further research asks whether and how the responses of infiltrating and resident myeloid cells might differ upon activation. Solving these mysteries will require a deeper overall understanding of how microglia interact with their environment (both within the healthy CNS parenchyma and as altered by neurodegeneration, trauma, ischaemia, infection or infiltrating immune cells). Furthermore, given the intricate anatomical specialization of the CNS, researchers will also need more profound insights into the ways in which local microenvironmental cues regulate microglial-cell behaviour.

The rewards for solving some of these conundrums — for example, a means to ameliorate neurodegeneration, to accelerate neural repair or to even deliver genetic or protein cargo to the parenchyma — could eclipse the effort expended. We await the day when researchers' capacity to modulate microglial-cell behaviour is equivalent to even a tiny fraction of that achieved effortlessly by the intact CNS. ■

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