Microglial cell origin and phenotypes in health and disease

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Abstract | Microglia — resident myeloid-lineage cells in the brain and the spinal cord parenchyma — function in the maintenance of normal tissue homeostasis. Microglia also act as sentinels of infection and injury, and participate in both innate and adaptive immune responses in the central nervous system. Microglia can become activated and/or dysregulated in the context of neurodegenerative disease and cancer, and thereby contribute to disease severity. Here, we discuss recent studies that provide new insights into the origin and phenotypes of microglia in health and disease.

Astrocytes
Glial cells that are found in vertebrate brain and are named for their characteristic star-like shape. These cells provide both mechanical and metabolic support for neurons, thereby regulating the environment in which they function.

Oligodendrocytes
Glial cells that create the myelin sheath that insulates axons and improves the speed and reliability of signal transmission by neurons.

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Microglia are specialized macrophages of the central nervous system (CNS) that are distinguished from other glial cells, such as astrocytes and oligodendrocytes, by their origin, morphology, gene expression pattern and functions[1,2]. Microglia constitute 5–20% of total glial cells in rodents, depending on the specific region of the CNS[3,4]. In contrast to neurons and other glial cells, microglia are of haematopoietic origin and act as primary responding cells for pathogen infection and injury. Microglia exhibit several features that distinguish them from other populations of macrophages, such as their 'ramified' branches that emerge from the cell body and communicate with surrounding neurons and other glial cells. Microglia rapidly respond to infectious and traumatic stimuli and adopt an 'amoeboid' activated phenotype. Activated microglia produce many pro-inflammatory mediators — including cytokines, chemokines, reactive oxygen species (ROS) and nitric oxide — which contribute to the clearance of pathogen infections. However, prolonged or excessive microglial cell activation may result in pathological forms of inflammation that contribute to the progression of neurodegenerative and neoplastic diseases[5,6].

The emerging recognition of the roles of microglia in health and disease has stimulated substantial efforts to more clearly define their origins and the regulatory mechanisms that control their functions. In the last year alone, more than 200 papers were published on the topic of microglia and inflammation. In this Review, we discuss some recent findings that are particularly relevant to understanding the development of microglia and their functions in neurodegenerative disease and cancer, and suggest areas for future investigation.

The origin of microglia

Microglia are classified as macrophages[7], and they express many macrophage-associated markers, such as CD11b, CD14 and EGF-like module-containing mucin-like hormone receptor-like 1 (EMR1; also known as F4/80 in mice)[1]. All microglia appear to express the colony-stimulating factor 1 receptor (CSF1R) and can be marked by transgenic expression of green fluorescent protein (GFP) under the control of *CSF1R* regulatory elements[8]. In addition, microglia are absent in mice deficient for PU.1, a key transcription factor that controls the differentiation of B cells and myeloid cells[9]. Clustering analysis of global transcript levels in microglia derived from the brains of newborn mice and in various haematopoietic and non-haematopoietic cell types indicates a particularly close relationship with bone marrow-derived and thioglycollate-elicited macrophages (FIG. 1a). The set of genes exhibiting near exclusive or preferential expression in microglia is enriched for functional annotations linked to wound and inflammatory responses and chemotaxis, as shown by gene ontology analysis of microarray data. One limitation of this analysis is that the process of isolating microglia from the CNS environment is likely to significantly affect gene expression. Methods to characterize the microglial cell transcriptome under physiological and pathological conditions will be an important goal for the future (BOX 1).

Although the lineage relationship between microglia and macrophages is clear, a major question has been whether or not circulating monocytes and/or myeloid progenitor cells contribute to the steady-state population of microglia in the healthy CNS. The detection of donor-derived microglia in irradiated mice following

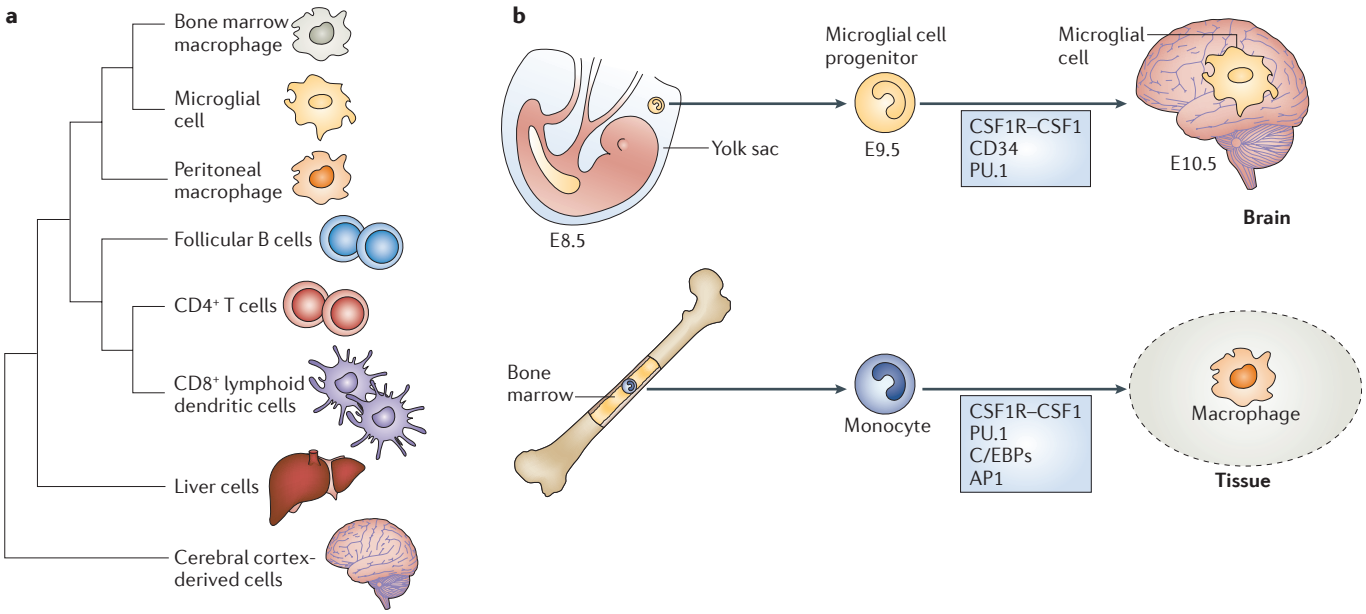

Figure 1 | **Relationship of microglia to myeloid-lineage cells. a** | The figure illustrates the molecular relationships between different primary mouse haematopoietic lineage cells, liver cells, brain cells and microglia isolated from C57BL/6 wild-type mice based on gene expression as determined by genome-wide microarray analysis. Microglia are most closely related to bone marrow-derived and thioglycollate-elicited macrophages, and more distantly related to other haematopoietic and non-haematopoietic cell types. Microarray data for cluster analysis were taken from the BioGPS data set from the Genomics Institute of the Novartis Research Foundation (see the BioGPS website). **b** | The figure shows the developmental relationship between microglia and macrophages. Microglia are derived from primitive haematopoiesis in the fetal yolk sac and take up residence in the brain during early fetal development. Microglia differentiation and proliferation requires colony-stimulating factor 1 (CSF1), the CSF1 receptor (CSF1R), CD34 and the transcription factor PU.1. By contrast, at least some tissue macrophages are derived from haematopoietic stem cells in the bone marrow (definitive haematopoiesis). Macrophage differentiation and proliferation requires CSF1, CSF1R and the transcription factors PU.1, CCAAT/enhancer-binding proteins (C/EBPs) and activator protein 1 (AP1). E8.5, embryonic day 8.5.

bone marrow transfer supported the idea that bone marrow-derived cells could migrate into the CNS and give rise to microglia[10–13]. However, brain irradiation was observed to influence the extent to which these cells entered the CNS in chimeric mice with focal ischaemia[14] or Alzheimer's disease[15]. Furthermore, experiments using the technique of parabiosis (which surgically connects the circulatory systems of two organisms) indicated that very few donor cells enter the CNS of recipient mice in the absence of total body irradiation[16,17]. It was also thought that inflammation in the CNS could alter the blood–brain barrier so that circulating cells could immigrate into the CNS and differentiate as microglia. However, parabiosis experiments failed to support this hypothesis, as donor cell-derived microglia were not detected in recipient mice following injury or the induction of neurodegenerative disease unless the CNS was preconditioned by irradiation[13,15].

Studies using CX_3C-chemokine receptor 1 (*Cx3cr1*)–GFP knock-in mice (in which microglia and peripheral macrophages are GFP-positive, as they express CX_3CR1) detected GFP$^+$ cells in the fetal yolk sac at embryonic day 9.5 (E9.5) and microglial cell migration into the CNS at E10.5. Lineage-tracing experiments showed that macrophage progenitor cells expressing runt-related transcription factor 1 (RUNX1) and CD11b migrated from the yolk sac into the CNS between E8.5 and E9.5 by blood circulation after the establishment of the embryo vascular system[18]. These experiments used embryos derived from the breeding of mice expressing a tamoxifen-inducible *Cre* gene under the control of the endogenous *Runx1* promoter with mice carrying a Cre-inducible *lacZ* allele[19]. A key aspect of this experiment is that RUNX1 expression (and by extension Cre expression) is restricted to the extra-embryonic yolk sac at the time of Cre induction at E7.25 (REF. 19). Although not all microglia were genetically marked in these experiments, nearly all originated from yolk sac cells and were marked before definitive haematopoiesis had begun[18]. The interpretation of these findings is complicated by the fact that a lineage-tracing experiment using the same deletion system to mark cells at nearly the same time (E7.5) resulted in labelling of all the major haematopoietic lineages[19]. Thus, there is an extremely narrow window of time in which to selectively establish the relative contributions of the yolk sac and haematopoietic stem cells to macrophage lineages.

Interestingly, these studies also demonstrated that microglia are dependent on CSF1R but not its classic ligand, CSF1 (also known as M-CSF). Expression of interleukin-34 (IL-34), a newly identified ligand for CSF1R, was increased in the embryonic tissue and the brain, and this suggests that IL-34 might support the maintenance of microglia in the CNS[18]. Overall, these recent findings

Alzheimer's disease
The most common type of neurodegenerative dementia. Patients often have impairments in learning and memory. The neuropathology of the disease includes neuron loss in the cerebral cortex and in some subcortical regions, and the presence of aggregates in the form of senile plaques (which contain amyloid-β) and neurofibrillary tangles (which contain hyperphosphorylated tau).

Blood–brain barrier
A barrier formed by tight junctions between endothelial cells that markedly limits entry to the central nervous system by leukocytes and all large molecules, including to some extent immunoglobulins, cytokines and complement proteins.

Box 1 | **Towards a molecular understanding of microglial cell origin and phenotype**

Despite the importance of microglia in the maintenance of central nervous system (CNS) homeostasis and the pathogenesis of neurodegenerative diseases, our understanding of the molecular mechanisms responsible for their development and function is still incomplete. A major impediment is that microglia are difficult to isolate in large numbers and are likely to be significantly altered by the procedures used to establish primary cultures. Improved methods for microglial cell isolation and maintenance and the generation of cell lines that can be used to model specific microglial cell phenotypes would be highly enabling for many types of studies. A method termed 'translating ribosome affinity purification' (TRAP) was recently reported for global profiling of mRNAs undergoing translation in specific cell types in the CNS *in vivo*[112]. Application of this methodology to microglia would be extremely valuable in establishing bona fide molecular phenotypes in normal and disease states and could be used to validate *in vitro* model systems. In parallel, the use of genome-wide approaches to map the enhancer elements within the microglial cell genome, on the basis of specific histone modifications and DNase hypersensitive sites, would enable the application of computational approaches to identify key transcription factors required for microglial cell identity and thus also aid reprogramming efforts[32].

A surprising barrier to defining the roles of candidate genes is the lack of mouse lines that direct highly effective and microglial cell-specific expression of Cre recombinase. The creation of mice in which Cre recombinase has been inserted into loci that are specifically and highly expressed in microglia would be of great value to the microglial cell and neuroinflammation research communities.

Finally, a major challenge will be to apply induced pluripotent stem cell technology to establish patient-derived microglia that can be used as components of microglia–astrocyte–neuron co-cultures *in vitro*. These types of system would logically follow from successful reprogramming efforts and are likely to be of value in understanding the impact of natural genetic variation on microglial cell function, in defining the mechanisms and consequences of phenotypic polarization, and in modelling neurodegenerative disease mechanisms *in vitro*.

suggest that microglia originate from yolk sac macrophages that migrate into the CNS during early embryogenesis and are independent from cells that arise by definitive haematopoiesis in the bone marrow and from circulating cells, at least in mice (FIG. 1b).

Invasion of circulating monocytes into the CNS is often observed in rodent disease models that damage the blood–brain barrier, such as experimental autoimmune encephalomyelitis (EAE)[20,21]. In EAE, circulating monocytes contribute to disease progression, but do not appear to differentiate into microglia[22]. It is not yet clear whether circulating monocytes migrate into the CNS and contribute to the progression of diseases under pathological conditions in humans.

Another unsolved question is the lifespan and replicative capacity of microglia. Under pathological conditions, increased numbers of microglia are often observed (a state referred to as microgliosis)[5,23,24]. This suggests that microglial cell populations can locally expand in the CNS. Consistent with this, early studies based on [^{3}H]thymidine uptake suggested that microglia have the ability to proliferate[25]. The longevity of microglia and the mechanisms that control their numbers within the CNS are unknown. In addition, it is unclear whether radiation-resistant microglial cell progenitors exist in the CNS parenchyma and what functional differences exist between these potential microglial cell progenitors and mature microglia. In this regard, the myeloid-derived Langerhans cells of the epidermis may provide an instructive cell type for comparison, as they represent a self-renewing macrophage population that takes up residence during late embryogenesis and proliferates extensively *in situ* after birth[26].

Understanding the origins of microglia and the mechanisms by which their precursors enter the CNS is important for deciphering their specialized functions and therapeutically exploiting them. The extent to which the observed differences in gene expression between microglia and macrophages reflect their different origins or distinct tissue environments is still unclear. Because macrophage progenitors are derived from the haematopoietic system and are capable of entering tissues, they have been considered as possible vehicles for the delivery of genes or gene products for therapeutic purposes[27]. However, the recent findings from parabiosis experiments suggest that haematopoietic precursors may not be useful for the treatment of CNS-related diseases in which the integrity of the blood–brain barrier is preserved unless there is a safe and effective means for targeting them to the brain.

Although the origin of microglia has been largely clarified, the key transcription factors and signalling pathways that contribute to the differentiation of progenitor cells into microglia are unknown. Interest in the potential utility of studying patient-derived microglia has been raised by the recently accrued capacity to model neurodegenerative diseases using patient-derived induced pluripotent stem (iPS) cells that can give rise to neurons and astrocytes with disease phenotypes in culture[28–30]. In contrast to neurons and astrocytes, however, it has not yet been possible to generate microglia-like cells from human iPS cells. Some progress has been reported for mouse microglia[31], but the ability to reprogram iPS or other cell types to microglia remains a largely unmet goal. Recent studies of macrophages and other cell types suggest that genome-wide approaches can be used to identify lineage-determining transcription factors, which establish the majority of the enhancer-like elements that regulate cellular identity and function[32]. It is therefore possible that these technologies will be useful for the identification of the key transcription factors and signalling pathways required to specify the microglial cell phenotype (BOX 1).

In summary, microglia are CNS-resident macrophages that originate from primitive progenitors in the yolk sac and migrate into the CNS during early embryogenesis. Their proliferation and differentiation is dependent on a set of transcription factors and growth factor receptors (including PU.1 and CSF1R) that overlaps with the set required for the development of tissue macrophages that arise from definitive haematopoiesis in the bone marrow and the fetal liver (FIG. 1b). However, microglia appear to represent a distinct compartment of macrophages that are long-lived and/or locally self-renewing, and are not normally replaced by bone marrow-derived cells. Whether there are distinct microglial cell phenotypes within specific anatomical regions of the brain remains largely unknown, but the observation of CNS region-specific expression of several cell-surface proteins with regulatory functions suggests that this is the case[33].

Modulation of microglial cell phenotypes

Studies of peripheral macrophages have led to the concept of different macrophage activation states, ranging from 'classical' activation (also referred to as M1-type macrophage activation) to so-called 'alternative' activation (also referred to as M2-type macrophage activation). Experimentally, these states are most commonly achieved by treating macrophages *in vitro* with potent polarizing ligands. Toll-like receptor (TLR) agonists — especially the TLR4 ligand lipopolysaccharide (LPS) — and interferon-γ (IFNγ) are typically used to induce a classically activated phenotype, which is relevant to responses to bacterial and viral infection. By contrast, IL-4 and IL-13 are commonly used to induce an alternatively activated phenotype, which is associated with immune responses to parasites and tissue-repair programmes[34].

In general, classically activated macrophages are most commonly associated with disease states that are at least partially driven by low-grade forms of inflammation, exemplified by atherosclerosis and type 2 diabetes mellitus (T2D)[35,36]. Disruption of genes that promote classical activation, such as the gene encoding TLR4, results in amelioration of disease in mouse models. By contrast, alternative activation states are generally associated with protection from diseases in which classical activation is pathogenic. Alternative activation states can be deleterious in other disease states, particularly cancer[37]. The relationship between the macrophage activation phenotypes observed *in vitro* and the actual functional states of macrophages in pathological settings *in vivo* is an ongoing topic of debate, and new technologies for characterizing patterns of gene expression *in vivo* will help to resolve this issue (BOX 1).

Application of the classical or alternative activation concept to microglia is most clear-cut in the case of classical activation and might also be applicable in the case of alternative activation. However, the primary determinants of the steady-state (naive) and deactivated microglial cell phenotypes are less well defined and may be quite different from those that impose a steady-state macrophage phenotype in peripheral tissues. It is not clear whether deactivated microglia return to the same functional state as resting microglia or retain some sort of memory of prior activation. However, there is sufficient evidence in support of the associations between distinct activation states and pathology to consider the regulation of microglial cell phenotype as a potential approach for therapeutic intervention.

Steady-state microglia and homeostasis in the CNS

In the steady state, microglia exhibit a resting phenotype characterized morphologically by extensively ramified processes that perform continuous surveillance of their surroundings in the CNS[38]. Many *in vitro* experiments have demonstrated that microglia can secrete neurotrophic factors, such as insulin-like growth factor 1 (IGF1), brain-derived neurotrophic factor (BDNF), transforming growth factor-β (TGFβ) and nerve growth factor (NGF)[27,39]. Synaptic pruning by microglia has been suggested to be required for normal brain development[40]. In addition, phagocytic functions of microglia have been suggested to support neurogenesis. The majority of neuroblasts that are generated in the subgranular zone of the dentate gyrus undergo apoptosis, and steady-state microglia phagocytose these apoptotic cells, with this activity being most prominent in young (1-month-old) mice[41].

A number of mechanisms have been proposed to maintain a resting phenotype under steady-state conditions (FIG. 2a). First, neurons have been suggested to suppress the activation of microglia through cell–cell contact, as well as secreted factors. For example, signalling by CX_3C-chemokine ligand 1 (CX_3CL1) through its cell-surface receptor CX_3CR1 on microglia restrains microglial cell activity. Mice deficient for CX_3CR1 have a hyperactive microglial cell phenotype and exaggerated neuronal loss in an injury model, a model of Parkinson's disease induced by MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) and a model of amyotrophic lateral sclerosis (superoxide dismutase 1-transgenic mice)[42]. However, in the case of an Alzheimer's disease model (mice with transgenic expression of amyloid precursor protein (APP), presenilin 1 and tau), loss of CX_3CR1 in microglia reduced the death of neurons[43], underscoring the complexity of inflammation states and specific diseases. Signalling induced by the microglial cell receptors CD172, CD200R and CD45 following interaction with the neuronal cell-surface proteins CD47, CD200 and CD22, respectively, has also been reported to inhibit microglial cell activity[44] (FIG. 2a).

Loss of function of the DAP12–TREM2 (triggering receptor expressed on myeloid cells 2) signalling complex, which is primarily expressed by natural killer (NK) and myeloid cells (including macrophages and microglia) results in excessive innate and adaptive immune responses. TREM2 is upregulated in mouse macrophages in response to IL-4 and suppresses TLR signalling[45,46]. In humans, homozygous loss-of-function mutations affecting either DAP12 or TREM2 result in Nasu–Hakola disease[47], which is characterized by frontal dementia and bone cysts. Within the CNS, DAP12 and TREM2 appear to be exclusively expressed by microglia; thus, the development of frontal dementia may primarily reflect an alteration in the resting state of microglia or their excessive responses to mild inflammatory stimuli[48].

Type 2 diabetes mellitus
(T2D). A disorder of glucose homeostasis that is characterized by inappropriately increased blood glucose levels and the resistance of tissues to the action of insulin. Recent studies indicate that inflammation in adipose tissue, liver and muscle contributes to the insulin-resistant state that is characteristic of T2D, and that the anti-diabetic actions of peroxisome proliferator-activated receptor-γ agonists result, in part, from their anti-inflammatory effects in these tissues.

Amyotrophic lateral sclerosis
A neurodegenerative disorder that affects the motor neurons in the brain.

Amyloid precursor protein
(APP). A membrane glycoprotein component of the fast axonal transport machinery, from which amyloid-β is cleaved by proteolytic processing.

Presenilin 1
A transmembrane protease that has an active site in the plane of the membrane and can therefore cleave transmembrane peptides. Mutations of the presenilin 1 gene are associated with early onset Alzheimer's disease.

Tau
A neuronal protein that binds to microtubules, promoting their assembly and stability.

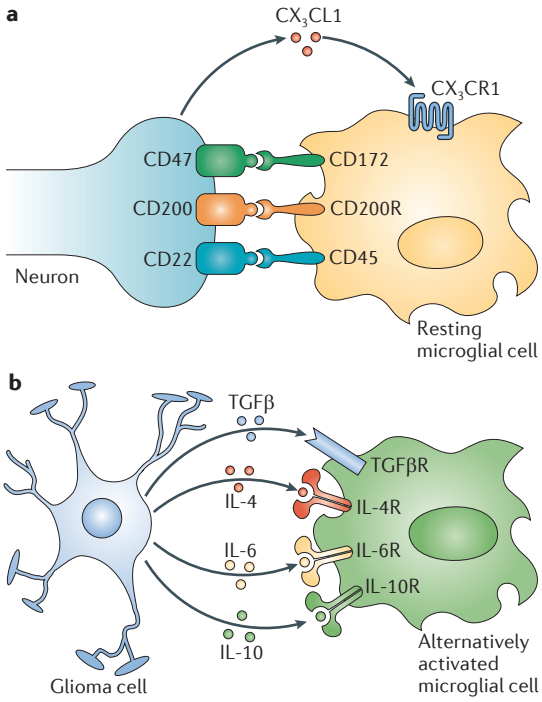

Figure 2 | **Steady-state and alternatively activated microglial cell phenotypes. a** | Under steady-state conditions, microglia exhibit an extensively ramified morphology and a resting phenotype. This phenotype is maintained in part through neuron-derived signals, including CX_3C-chemokine ligand 1 (CX_3CL1), CD47, CD200 and CD22, which act through corresponding receptors expressed by microglia. **b** | Glioma cells secrete factors that induce an M2-like microglial cell phenotype. These factors include transforming growth factor-β (TGFβ), interleukin-4 (IL-4), IL-6 and IL-10.

Alternatively activated microglia and cancer

At present, there is virtually no evidence that locally produced IL-4 or IL-13 drive an alternative microglial cell activation state analogous to that described for macrophages in lean adipose tissue or other organs. However, alternatively activated microglia may become important in the context of primary and metastatic tumours.

There is substantial clinical and experimental evidence that macrophages promote cancer initiation, malignant progression and the suppression of antitumour immunity[49,50]. Macrophages are thought to contribute to cancer initiation by creating an inflammatory environment that is mutagenic and promotes growth. This biological response is related to the normal functions of classically activated macrophages (which are involved in responses to infection) and includes the production of ROS, which exert antimicrobial effects but also have deleterious effects on host cells. As tumours progress to malignancy, tumour-associated macrophages stimulate angiogenesis and enhance tumour cell migration and invasion. These functions are similar to the functions of alternatively activated macrophages in wound repair, and suggest that tumours communicate with macrophages to induce this maladaptive phenotypic switch. Tumour-associated macrophages also exhibit a reduced ability to present antigens and suppress tumour-specific immunity. At metastatic sites, macrophages appear to prepare the target tissue for the arrival of tumour cells, and different subpopulations of macrophages are thought to promote tumour cell extravasation, survival and subsequent growth.

Within the CNS, ependymal cells, astrocytes and oligodendrocytes may give rise to gliomas. In 1925, it was first reported that microglia were frequently observed in sections of these brain tumours[51]. In this report, microglia were described as amoeboid with phagocytic activities, similar to microglia observed in other pathological conditions. Microglia are often observed surrounding or within glioma tissue, but it is not yet clear whether these cells are entirely derived from the resident microglial cell population. Several lines of evidence suggest that glioma-associated microglia might be different from classically activated microglia and more related to alternatively activated macrophages (FIG. 2b). Interestingly, glioma cells are reported to secrete factors that suppress immune cells, such as IL-10, IL-4, IL-6, TGFβ and prostaglandin E2 (REFS 52–54), and these promote an M2-like phenotype in microglia and/or suppress the M1-like microglial cell phenotype. TGFβ is known to inhibit microglial cell proliferation and production of pro-inflammatory cytokines *in vitro*[55], whereas IL-4, IL-6 and IL-10 produced from glioma cells or glioma stem cells polarize microglia to an M2-like phenotype (FIG. 2). An M2-like polarization of microglia might prevent the production of cytokines required for the support of tumour-specific $CD8^+$ T cells and $CD4^+$ T helper 1 (T_H1) and T_H17 cells, and promote the function of $CD4^+$ regulatory T cells[56–58].

An emerging question is the relationship between glioma-associated microglia and the recently identified population of myeloid-derived suppressor cells (MDSCs), which also have immunosuppressive function. MDSCs inhibit the activation of T cells and NK cells that target the growth of tumours[59,60]. Regardless of their classification as M2-like or MDSC-like, glioma-associated microglia express low levels of MHC class II molecules, which are required to present tumour-specific antigens to T cells[58,61]. As a result, gliomas appear to suppress local tumour-specific T cell responses by compromising the ability of microglia to initiate appropriate T cell responses in the CNS.

Gliomas also modulate other functions of microglia to enable tumour cells to more readily invade normal brain tissues. For example, it was observed that depletion of microglia reduced glioma cell invasion in brain slice cultures *in vitro*[62]. It has recently been proposed that secreted factors from glioma cells stimulate TLRs on microglia. Downstream signalling transmitted through myeloid differentiation primary response protein 88 (MYD88) activates p38 mitogen-activated protein kinase, resulting in induction of the expression of matrix metalloproteinase 14 (MMP14; also known as MT1MMP). MMP14 produced by glioma-associated microglia degrades the matrix proteins in the CNS parenchyma, and this allows tumour cells to migrate through the brain matrix[63]. This

Tumour-associated macrophages
An important component of the tumour microenvironment. These cells differentiate from circulating blood monocytes that have infiltrated tumours. They can have positive or negative effects on tumorigenesis (that is, tumour promotion or immunosurveillance, respectively).

High-mobility group box 1
(HMGB1; also known as amphoterin). A nuclear protein that binds DNA in a non-sequence-specific manner and modulates transcription and chromatin remodelling by bending DNA and facilitating the binding of transcription factors and nucleosomes.

Amyloid-β
A peptide of 39–43 amino acids that is the main constituent of amyloid plaques in the brains of patients with Alzheimer's disease. These plaques are composed of a tangle of regularly ordered fibrillar aggregates called amyloid fibres. Among these heterogeneous peptide molecules, amyloid-β_{1-40} and amyloid-β_{1-42} are the most common isoforms. Amyloid-β_{1-42} is the most fibrillogenic peptide and is thus associated with disease states.

type of matrix remodelling is a normal beneficial function of macrophages in development and wound repair, but is deleterious in the context of malignancy. Thus, activation of classical microglial cell-mediated immunity and/or inhibition of the alternative activation phenotype could be part of a multifaceted therapeutic strategy against glioma. Although less studied, similar sorts of tumour–microglial cell communication may occur between metastatic tumours and microglia to facilitate tumour expansion[45,46].

Features of classically activated microglia

Classically activated microglia initiate T cell responses. Following detection of signs of infection or tissue injury, microglia rapidly convert to an activated state[38,64]. Activated microglia turn on MHC class II expression, which is required for activation of naive T cells, and produce numerous pro-inflammatory cytokines, including cytokines that induce the differentiation of effector T cells[65]. These phenotypic alterations suggest that microglia influence adaptive immune responses, although the functional *in vivo* consequences of the ability of microglia to present antigens to naive T cells are not clearly established (FIG. 3). Generation of mice with microglial cell-specific deletion of key regulatory molecules is required to clearly define the roles of classically activated microglia in adaptive immunity.

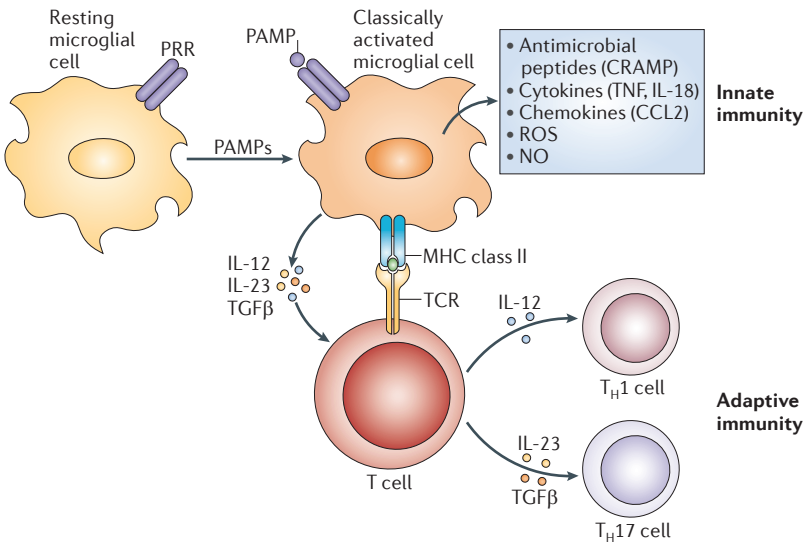

Figure 3 | **Classically activated microglia participate in both innate and adaptive immune responses.** Microglia express pattern recognition receptors (PRRs) that recognize various pathogen-associated molecular patterns (PAMPS) found on bacteria and viruses. Following the recognition of PAMPs by microglia, PRR-mediated signalling induces the production of antimicrobial peptides (such as cathelicidin-related antimicrobial peptide (CRAMP)), cytokines (such as tumour necrosis factor (TNF) and interleukin-1β (IL-1β)), chemokines (such as CC-chemokine ligand 2 (CCL2)), reactive oxygen species (ROS) and nitric oxide (NO). These molecules have key roles in innate immunity and are characteristic features of the classical M1-like microglial cell phenotype. Activated microglia also upregulate the expression of MHC class II molecules to enable them to present antigens to T cells through the T cell receptor (TCR). In addition, activated microglia produce pro-inflammatory cytokines (such as IL-12) to skew $CD4^+$ T cells into T helper 1 (T_H1) cells, or IL-23, IL-6, IL-1β and transforming growth factor-β (TGFβ) to differentiate and activate T_H17 cells. Therefore, classically activated microglia contribute to both innate and adaptive immunity.

TLR signalling in classically activated microglia. Like macrophages, microglia express many of the receptors that sense pathogen-associated molecular patterns (PAMPs). These pattern-recognition receptors (PRRs) include TLRs, RIG-I-like receptors (RLRs), NOD-like receptors (NLRs) and C-type lectin receptors (BOX 2).

Microglia express all TLRs and act as the primary sensors of PAMPs in the CNS. For example, microglia express TLR4, which recognizes LPS, a component of the cell walls of Gram-negative bacteria. Astrocytes also express TLR4, but at much lower levels compared with microglia, and they lack expression of CD14, which is a component of the high-affinity receptor for LPS[66]. Thus, microglia are more sensitive than astrocytes to TLR4-mediated PAMP detection in the CNS. Interestingly, the microglial cell TLR4 signalling pathway is activated not only during pathogen infection in the CNS but also in the setting of systemic infection. When LPS was injected into the peritoneal cavities of mice, rapid and robust TLR4-induced transcription was observed in the brain[67,68].

In addition to mediating responses to PAMPs, TLRs also recognize endogenous damage-associated molecular patterns (DAMPs). Such danger signals are induced by metabolic products (for example, oxidized low-density lipoprotein (LDL)) and molecules released by dead cells (for example, high-mobility group box 1 (HMGB1) and nucleotides)[48,69,70]. Prolonged activation of TLRs on microglia by danger signals might have important roles in pathological forms of inflammation that contribute to neurodegenerative diseases.

A recent report suggested that activation of non-apoptotic caspase signalling in microglia contributes to their neurotoxic activation[71]. TLR4 triggering by LPS was shown to sequentially activate caspase 8 followed by caspase 3 and caspase 7. This caspase activation did not induce microglial cell apoptosis but rather contributed to the downstream signalling of TLR4 in microglia. Activated caspase 3 cleaves protein kinase Cδ (PKCδ), and cleaved PKCδ modulates nuclear factor-κB (NF-κB) activation and the production of neurotoxic pro-inflammatory mediators such as IL-1β, tumour necrosis factor (TNF) and nitric oxide[71]. Inhibition of this caspase was shown to protect neurons in animal models of Alzheimer's disease and Parkinson's disease.

NLR signalling in classically activated microglia. The fibril form of amyloid-β has been reported to stimulate the activity of the NLRP3 (NOD-, LRR- and pyrin domain-containing 3; also known as NALP3) inflammasome in microglia. Although the mechanism by which NLRP3 specifically recognizes fibrillar amyloid-β is not clear, stimulation with fibrillar amyloid-β promotes the oligomerization of the adaptor protein ASC and the maturation of IL-1β (BOX 2). Lysosome damage following the uptake of amyloid-β by microglia was reported to result in the release from lysosomes of cathepsin B, which is involved in the production of IL-1β in microglia[72]. This process might be important for microglia to produce neurotoxic factors that contribute to Alzheimer's disease pathology[72,73].

Box 2 | **TLR and NLR signalling pathways**

Toll-like receptor (TLR) and NOD-like receptor (NLR) signalling contributes to the activation of microglia in response to pathogen-associated molecular patterns (PAMPs). TLR1, TLR2, TLR4, TLR5 and TLR6 are localized to the plasma membrane and mainly recognize the components of microbial membranes. TLR3, TLR7, TLR8 and TLR9 are localized to the membranes of endocytic compartments (endosomes and lysosomes) and mainly recognize nucleic acids from pathogens[113,114].

Following recognition of PAMPs, TLRs activate downstream signalling cascades, which are dependent on adaptor molecules such as myeloid differentiation primary response protein 88 (MYD88) and TIR domain-containing adaptor protein inducing IFNβ (TRIF). MYD88 is used by all TLRs (except TLR3) and activates nuclear factor-κB (NF-κB) and mitogen-activated protein kinase (MAPK) pathways, which eventually induce the transcription of pro-inflammatory mediators that are characteristic of the M1-like microglial cell phenotype. TRIF transmits signals from TLR3 and TLR4, activates NF-κB and interferon-regulatory factor 3 (IRF3) pathways and leads to the transcription of pro-inflammatory mediators, including type I interferons (IFNs)[115,116].

NLRs are a large family of cytoplasmic sensors that detect various PAMPs[117–119]. Some NLRs form multiprotein complexes termed inflammasomes following triggering by foreign PAMPs or endogenous damage-associated molecular patterns (DAMPs). Activation of inflammasomes leads to the maturation of pro-inflammatory mediators (such as IL-1β), which initiate innate and adaptive immune responses in the cells. NLRP3 (NOD-, LRR- and pyrin domain-containing 3) is a well-characterized inflammasome component that has been implicated in neurodegenerative disease. NLRP3 senses many PAMPs, including bacterial, viral and fungal molecules, as well as DAMPs, including uric acid crystals, extracellular ATP, environmental products (such as silica and asbestos) and amyloid-β peptide[120,121]. The NLRP3 inflammasome is composed of three proteins — NLRP3, the adaptor protein ASC and pro-caspase 1. Following triggering by ligands, NLRP3 oligomerizes and induces the clustering of ASC through its pyrin domain. The caspase recruitment domain (CARD) of ASC interacts with the CARD of pro-caspase 1 to assemble the NLRP3 inflammasome, and this activates caspase 1. Activated caspase 1 subsequently cleaves pro-IL-1β and induces a maturation of IL-1β that is required for secretion from cells.

Non-PRR signalling in classically activated microglia. In addition to PRRs, many other receptors are expressed by microglia, including ion channels and receptors for neurotransmitters[1]. These receptors contribute to microglial cell recognition of DAMPs released from damaged or necrotic cells and are important for the clearance of debris and the initiation of tissue repair after injury in the CNS. Here, we briefly discuss two different types of receptors that are important for the recognition of DAMPs in the CNS: purinergic receptors and receptor for advanced glycation end-products (RAGE)[74].

Microglia express many of the P2 purinoreceptors, which can be further divided into two subgroups. Ionotropic receptors (P2X receptors; P2X1 to P2X7) form ion channels that can be opened mainly by the binding of ATP, and metabotropic receptors (P2Y receptors; P2Y1, P2Y2, P2Y4, P2Y6 and P2Y11 to P2Y14) bind purines or pyrimidines and activate downstream signalling cascades through G proteins. Nucleoside triphosphates (NTPs) can be released from injured cells, bind to either P2X or P2Y receptors and activate the downstream extracellular signal-regulated kinase (ERK) pathway. These signalling events eventually induce the transcription of pro-inflammatory mediators through the transcription factors NF-κB and activator protein 1 (AP1)[75,76]. Among NTPs, ATP mainly activates P2X receptors and induces the transcription of pro-inflammatory mediators. In addition, UDP released from dying neurons triggers microglial cell P2Y6 receptors and induces phagocytosis of the neurons by microglia[77]. Dysregulation of purinergic receptors was reported to have implications in many neurodegenerative diseases, such as amyotrophic lateral sclerosis[78].

RAGE-mediated signalling has important roles in the maintenance of homeostasis but may also promote pathological conditions, such as cancer, T2D and Alzheimer's disease[74]. RAGE was originally identified as a receptor for advanced glycation end-products, but can be activated by DAMPs in the CNS. Necrotic cell death is known to release nuclear proteins, such as HMGB1 and histones. Both are known to amplify the lethal condition of LPS-mediated sepsis in animals[69,70]. RAGE, together with TLR2 and TLR4, is triggered by HMGB1 and activates the transcription of pro-inflammatory genes[74,79]. In the CNS, RAGE is also known to be a receptor for amyloid-β, as discussed below in the context of Alzheimer's disease[80].

Crosstalk between activated microglia and astrocytes. Emerging evidence suggests that crosstalk between classically activated microglia and astrocytes can result in the amplification of inflammatory responses, and that this contributes to the production of neurotoxic factors. For example, although microglia are much more responsive than astrocytes to LPS, LPS-induced secretion of factors such as IL-1β and TNF by microglia can result in potent induction of pro-inflammatory gene expression and CSF1 production by astrocytes. Astrocyte-derived pro-inflammatory factors can in turn feed back on microglia to promote further microglial cell activation and microgliosis, thereby establishing a positive feedback loop (FIG. 4). Consistent with this, co-cultures of microglia and astrocytes stimulated with LPS produce significantly more neurotoxic factors than either cell type alone[66]. The functional significance of microglial cell–astrocyte crosstalk in the amplification of inflammatory responses and neurodegeneration *in vivo* remains to be defined.

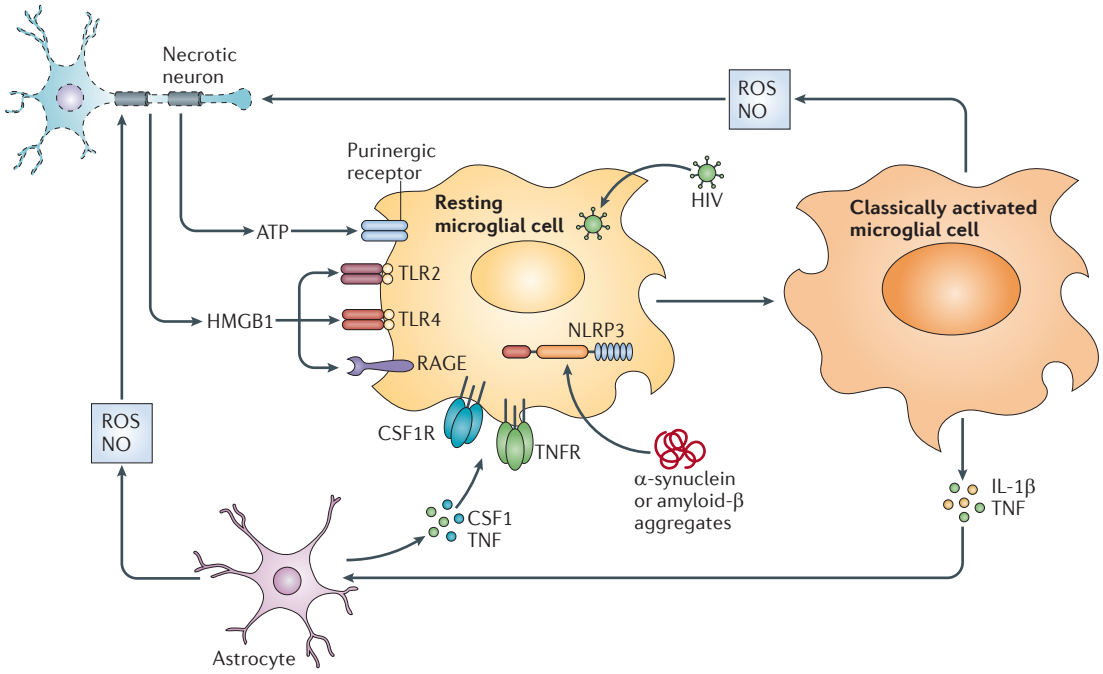

Figure 4 | **Classically activated microglia in neurodegenerative disease.** Various disease-associated factors can activate microglia through pattern-recognition and purinergic receptors to establish an M1-like microglial cell phenotype. Such factors include HIV infection, damage-associated molecular patterns (DAMPs; such as high-mobility group box 1 protein (HMGB1), histones and ATP), as well as neurodegenerative disease-specific protein aggregates (such as α-synuclein or amyloid-β aggregates). Pro-inflammatory mediators produced by classically activated microglia activate astrocytes, and the products released by activated microglia and astrocytes may exert neurotoxic effects. Activated astrocytes also release cytokines — including colony-stimulating factor 1 (CSF1) and tumour necrosis factor (TNF) — that further induce the activation and proliferation of microglia. Communication between microglia and astrocytes may therefore amplify pro-inflammatory signals initially sensed by microglia and contribute to the pathology of neurodegenerative disease. IL-1β, interleukin-1β; NLRP3, NOD-, LRR- and pyrin domain-containing 3; NO, nitric oxide; RAGE, receptor for advanced glycation end-products; ROS, reactive oxygen species; TLR, Toll-like receptor.

Microglia in neurodegenerative diseases

Chronic inflammatory diseases such as atherosclerosis and T2D are typically associated with classically activated macrophages[36,81]. These diseases evolve over decades and are characterized by a persistent low-grade inflammatory component rather than the high-magnitude, self-limited responses associated with infection and injury. M1 macrophages drive the pathogenesis of both types of disease through common mechanisms (pro-inflammatory mediators are involved in each case), as well as through disease-specific mechanisms (for example, the contribution of matrix metalloproteinases to plaque rupture in the case of atherosclerosis). Of note, studies using salicylates to selectively reduce the inflammatory component of T2D in mice and humans have demonstrated therapeutic efficacy[82]. These findings provide one of the first direct lines of evidence that anti-inflammatory therapy in a chronic disease in which inflammation is thought to be an amplifier, rather than an initiator, of pathology is clinically beneficial. The extent to which chronic forms of inflammation contribute to the pathogenesis of the many chronic neurodegenerative diseases and whether inhibition of inflammation in any of these disease states would be beneficial represent important unresolved questions.

The past several years have witnessed a marked expansion of studies on the role of microglia in neurodegenerative diseases, and this topic has been reviewed extensively[6,44,83]. As microglia may exert both protective and pathogenic functions in the CNS, one cannot simply conclude that inhibition of microglial cell-mediated inflammation will result in a beneficial therapeutic outcome. In accordance with this, there have been no successful clinical trials demonstrating a therapeutic benefit of an anti-inflammatory therapy directed at the innate immune function of microglia. It will therefore be essential to systematically identify the mechanisms of microglial cell activation in different neurodegenerative disease states and the components of the microglial cell response that contribute to disease progression in order to define appropriate therapeutic targets. Here, we briefly discuss examples of recent studies on mechanisms that may contribute to the pathologies of Parkinson's disease, Alzheimer's disease, multiple sclerosis and HIV-associated dementia, mainly through the dysregulation of M1-like microglial cell activation (FIG. 4).

Parkinson's disease. The clinical features of Parkinson's disease are characterized by motor symptoms that include bradykinesia, tremor, rigidity and postural instability. The pathological hallmarks of disease are

the loss of dopaminergic neurons in the substantia nigra and the accumulation of protein aggregates of α-synuclein[84]. In addition, signs of inflammation, such as microglial cell activation and astrogliosis, as well as increased levels of pro-inflammatory mediators in the serum and cerebral spinal fluid, are often observed in patients with Parkinson's disease and in animal models of the disease[85].

Death of dopaminergic neurons activates microglia, potentially through the combined activation of PRRs and purinergic receptors, and activation of microglia might contribute to the progression of Parkinson's disease. Conversely, activation of microglia by LPS is sufficient to induce the death of dopaminergic neurons: injection of LPS into the intraperitoneal cavities of animals or the substantia nigra in the brain induces drastic microglial cell inflammatory responses followed by the death of dopaminergic neurons[86]. In addition, conditioned media from TLR4-activated microglia specifically induced the death of dopaminergic neurons *in vitro*[66]. Therefore, classical activation of microglia through TLR4 results in the production of neurotoxic factors.

Mutations or overexpression of the α-synuclein gene are causes of familial Parkinson's disease, and accumulation of α-synuclein is toxic to dopaminergic neurons. α-synuclein also triggers the activation of microglia through PRRs[87]. These findings provide a plausible mechanism by which a disease-specific factor initially derived from neurons can act to induce a programme of classical microglial cell activation. In contrast to infection or injury, which ultimately resolve, chronic stimulation by overproduced or aggregated α-synuclein would potentially result in a chronic, and hence pathological, form of microglial cell-mediated inflammation.

Alzheimer's disease. Alzheimer's disease is one of the most common age-dependent neurodegenerative diseases. The clinical features of Alzheimer's disease include loss of memory, progressive impairment of cognition and various behavioural and neuropsychiatric disturbances. The pathology is characterized by the accumulation of extracellular amyloid-β plaques that comprise aggregated, cleaved products of APP, and intracellular neurofibrillary tangles that are composed of hyperphosphorylated forms of the microtubule-binding protein tau[33,47].

Senile plaques containing the amino-terminal APP cleavage products amyloid-β_{1-42} and/or amyloid-β_{1-40} are generated from APP by β-secretase and γ-secretase. Mutations in the APP, β-secretase and γ-secretase genes are causes of familial Alzheimer's disease.

In addition to the neuron-autonomous toxicity of amyloid-β, oligomerized or aggregated pathological forms of amyloid-β are known to activate microglia though many receptors, including TLRs, NLRP3 and RAGE[72,80,88]. Following the recognition of amyloid-β, cells induce the production of pro-inflammatory mediators, which are known to be neurotoxic[5]. Recently, polymorphisms in RAGE were reported to have an association with the risk of Alzheimer's disease[89,90]. Two-photon *in vivo* imaging of neuron loss in the intact brains of living mice with Alzheimer's disease (mice with transgenic expression of APP, presenilin 1 and tau) revealed an involvement of microglia in neuron elimination[43]. Surprisingly, deletion of *Cx3cr1* prevented this neuron loss, as previously noted.

However, microglia might also have neuroprotective roles in Alzheimer's disease pathology. Many reports have suggested that microglial cell-mediated phagocytosis of amyloid-β is essential for the clearance of plaques[91,92]. Other important genetic factors, such as polymorphisms in the genes encoding apolipoprotein E and tau, were also reported to be involved in the activation of microglia and are reviewed elsewhere[91,93].

Systemic or focal infection is also known to increase the risk of Alzheimer's disease. Loss of memory is frequently observed after sepsis, and periodontitis is reported to be another risk factor for the disease[94,95]. It is now well accepted that T2D is a risk factor for Alzheimer's disease. T2D is characterized by low levels of inflammation in adipose tissue, liver and other insulin target tissues[35,36]. The development of insulin resistance, a key feature of T2D, is associated with a loss of alternatively activated macrophages in these tissues and a marked increase in classically activated macrophages. These macrophages produce numerous pro-inflammatory cytokines and chemokines that establish an insulin-resistant state. How this pro-inflammatory insulin-resistant state increases the risk of Alzheimer's disease is not yet well understood[96,97]. It will be of interest to directly evaluate whether peripheral insulin resistance is causally associated with altered microglial cell activation states.

Finally, recent genome-wide association studies have identified many new loci that are associated with the risk of Alzheimer's disease, including *CD33*, *CD2AP* (CD2-associated protein), *ABCA7* (ATP-binding cassette, subfamily A, member 7) and *MS4A* (membrane-spanning 4-domains, subfamily A), as well as complement receptor genes[98–100]. These molecules are expressed in microglia and may mediate immunological roles in these cells[40]. However, the mechanisms by which microglial cell expression of these molecules might contribute to Alzheimer's pathology remain to be determined.

Overall, Alzheimer's disease provides another example of a disease in which a pathogenic neuron-derived factor, in this case amyloid-β, can act as a chronic inducer of microglial cell activation through PRRs. The extent to which this microglial cell response is specific to Alzheimer's disease and results in pathological inflammation remains to be clarified. Remarkably, depletion of microglia for up to 4 weeks in mouse models of Alzheimer's disease had virtually no measurable impact on the formation or maintenance of amyloid plaques or on neuritic dystrophy[101]. Although these results most logically suggest that microglia are not involved in amyloid metabolism, they could also reflect a balanced elimination of protective and pathogenic mechanisms, a possibility that could be addressed by microglial cell-specific deletion of putative protective and pathogenic genes.

Substantia nigra
A structure located in the midbrain that is important in reward behaviour, addiction and movement. Parkinson's disease is caused by the death of dopaminergic neurons in the substantia nigra.

α-synuclein
A neuronal protein of unknown function that is detected mainly in presynaptic terminals. It can aggregate to form insoluble fibrils known as Lewy bodies, which are observed in pathological conditions such as Parkinson's disease.

Astrogliosis
An increase in the number of astrocytes owing to proliferation at sites of damage in the central nervous system.

Neurofibrillary tangles
Pathological protein aggregates found in the neurons of patients with Alzheimer's disease. Tangles are formed through hyperphosphorylation of the microtubule-associated protein tau, causing it to aggregate in an insoluble form.

Apolipoprotein E
A key protein constituent of certain lipoproteins and a ligand for hepatic receptors.

HIV-associated neurocognitive disorder. HIV primarily infects $CD4^+$ T cells, monocytes and macrophages and downregulates host immune responses. HIV infection thereby causes systemic immunodeficiency, opportunistic infection and cancers. HIV infection can also cause neurological disorders, such as dementia and other neuropsychiatric disorders, collectively termed HIV-associated neurocognitive disorder (HAND)[102,103].

HIV virions or HIV-infected cells are able to cross the blood–brain barrier and enter the CNS, where they infect brain parenchyma-resident cells, such as microglia[104]. In the CNS, viral replication mainly takes place in myeloid-derived cells, including microglia and monocyte-derived macrophages. HIV infection activates an innate immune response in these cells, and this results in subsequent production of neurotoxic factors that are suggested to contribute to HAND pathology[102,105,106].

Recent progress in the application of antiretroviral therapy has made it possible to dramatically lower the rates of viral replication and restore normal immune function. However, there are substantial numbers of patients who are still affected by mild or asymptomatic neurocognitive disorders after apparently successful antiretroviral therapy, suggesting that HIV infection might induce persistent inflammation in the CNS mediated by chronically activated microglia and other immune cells[107]. Consistent with this, persistent neuroinflammation is observed at autopsy in the brains of treated patients, with microglia and macrophages expressing high levels of CD14, CD16, CD68 and MHC class II molecules[106]. In addition, the cerebrospinal fluid in patients with HAND has been reported to contain elevated levels of pro-inflammatory mediators, including CC-chemokine ligand 2 (CCL2), β2-microglobulin, arachidonic acid metabolites and markers for oxidative stress[108]. HAND may thus represent a condition in which microglial cell-driven inflammation is the primary cause of neurodegenerative disease.

Multiple sclerosis. Multiple sclerosis is a complicated heterogeneous autoimmune disease that primarily affects the myelin in the CNS. Autoantigen-specific T_H17 and T_H1 cells and B cells have major pathological roles. However, microglia are known to be an important cell type for the onset of EAE, a mouse model of multiple sclerosis[11]. Studies of the EAE model indicate that microglia can contribute to disease initiation by presenting antigens to naive T cells, as well as by secreting cytokines, such as IL-6, IL-23, IL-1β and TGFβ, that are required for the differentiation and activation of T_H17 cells. In addition, a recent publication suggests that circulating monocytes can migrate into the CNS and contribute to the progression of the disease[15].

In summary, although classical microglial cell activation enables adaptive responses to infection and injury, it also drives low-grade inflammatory responses during neurodegenerative diseases through the production of neurotoxic factors. Although different neurodegenerative diseases are associated with different 'inducers' of microglial cell activation (such as α-synuclein or amyloid-β), they are all detected by TLRs and other PRRs. As these receptors all couple to signalling and transcription factor pathways involved in pro-inflammatory gene expression (for example, the NF-κB pathway), the neurotoxic inflammatory response may share common mechanisms. However, it is also possible that microglia have distinctive functions in specific neurodegenerative diseases, depending on anatomical region, disease-specific secretion of neurotoxic or neurotrophic factors, communication with the adaptive immune system and the importance of phagocytic activity. An important goal of ongoing investigation is to determine whether pathological forms of inflammation are generic or disease-specific, as this knowledge will influence the development of therapeutic approaches.

Resolution of microglial activation

Mechanisms that regulate the transition of microglia from the activated state associated with acute inflammation to phenotypes associated with tissue repair, and ultimately to phenotypes associated with normal CNS homeostasis, are poorly understood and are currently being investigated. Defects in this transition may contribute to pathogenic forms of inflammation and neurodegenerative diseases.

It is likely that multiple factors, including TGFβ and IL-10, contribute to the restoration of the resting microglial cell phenotype. In addition to these cytokines, small molecules with endocrine, paracrine and autocrine functions that serve as ligands for various cell-surface and nuclear receptors — including steroid hormones and fatty acid metabolites — may regulate the resolution of microglial cell-mediated inflammation.

Members of the nuclear receptor superfamily of transcription factors have well-established roles in the regulation of macrophage phenotypes and have more recently become the focus of investigation in microglia[83]. Potent and selective synthetic ligands have been developed for most of the ligand-dependent nuclear receptors, and many of these can cross the blood–brain barrier and act directly in the CNS. Administration of synthetic ligands for the glucocorticoid receptor, oestrogen receptor-α (ERα), peroxisome proliferator-activated receptor-γ (PPARγ), liver X receptor-α (LXRα) and LXRβ have been reported to reduce disease severity in animal models for neurodegenerative diseases such as multiple sclerosis, Parkinson's disease and Alzheimer's disease[83] (FIG. 5). The extent to which these effects are due to actions in microglia, and whether they reflect normal biological roles of the target receptors, has not been established.

We recently reported that signalling through ERβ may contribute to the maintenance of CNS homeostasis and the resolution of microglial cell-mediated inflammation[109]. Synthetic ligands for ERβ were found to shut off TLR4-mediated inflammation in microglia *in vitro*, and their administration *in vivo* greatly reduced disease severity in a mouse model of multiple sclerosis. Those data led us to identify 5-androstene-3β,17β-diol (Δ5-ADIOL) — which was previously shown to be synthesized in microglia from its precursor, dehydroepiandrosterone (DHEA)[110] — as an endogenous steroid hormone that regulates ERβ activity[111]. The conversion of DHEA into Δ5-ADIOL is mediated by a

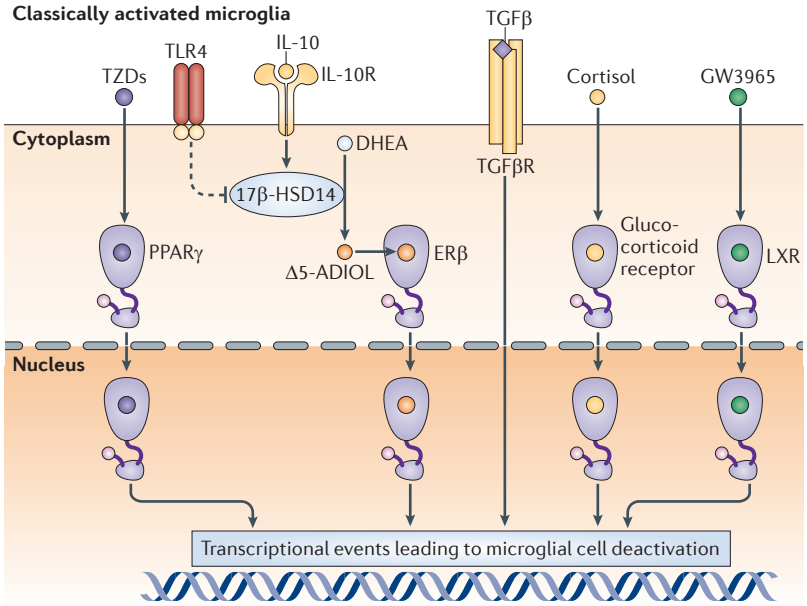

Figure 5 | **Deactivation of classically activated microglia.** The mechanisms that control the resolution of the M1-like microglial cell activation state are poorly understood. Anti-inflammatory cytokines — including transforming growth factor-β (TGFβ) and interleukin-10 (IL-10) — probably have important roles, but how the timing and magnitude of their expression is regulated is not known. Several nuclear receptor ligands suppress the M1-like activation state in microglia. These include cortisol (which acts via the glucocorticoid receptor), thiazolidinediones (TZDs; which act through peroxisome proliferator-activated receptor-γ (PPARγ)) and GW3965 (which acts through liver X receptors (LXRs)). IL-10 induces the expression of 17β-hydroxysteroid dehydrogenase type 14 (17β-HSD14), which mediates the conversion of dehydroepiandrosterone (DHEA) to 5-androstene-3β,17β-diol (Δ5-ADIOL), an anti-inflammatory ligand of oestrogen receptor-β (ERβ). 17β-HSD14 is suppressed by Toll-like receptor 4 (TLR4) signalling, suggesting that this pathway may also contribute to the steady-state microglial cell phenotype and be shut off during the transition from the steady-state to the M1-like microglial cell phenotype.

group of 17β-hydroxysteroid dehydrogenases (17β-HSDs), and one of them, 17β-HSD type 14, is highly expressed by microglia[109]. Loss of function of this receptor–ligand system increased and prolonged TLR4-induced microglial cell activation. Of note, the expression of 17β-HSD type 14 is controlled by inflammatory stimuli; TLR4-mediated signalling downregulates its expression, whereas the anti-inflammatory cytokine IL-10 upregulates its expression[109]. These findings suggest the possibility that this pathway is switched off when cells are stimulated through a PRR such as TLR4 but is subsequently switched on again by IL-10 during the resolution phase of inflammation to restore the levels of Δ5-ADIOL, which is negative regulator for microglia[109] (FIG. 5).

Overall, the molecular mechanisms that control the resolution of microglial cell-mediated inflammation remain one of the least-well-understood aspects of microglial cell biology. Based on their established functions in macrophages, nuclear receptors such as ERβ, LXRs and PPARs may have important roles in integrating the actions of pro- and anti-inflammatory signalling molecules and regulating the resolution phase of inflammatory responses.

Conclusion

The past few years have witnessed considerable progress in the understanding of the origin and functions of microglia. The association of different microglial cell activation states with CNS tumours and neurodegenerative diseases are now reasonably well established at a descriptive level. However, cause–effect relationships between specific activation states and specific pathological processes remain less well defined. It remains unclear, for example, whether pathological mediators produced by microglia in the context of Alzheimer's disease are the same or different from those produced in the context of Parkinson's disease. Importantly, the development of effective therapeutic approaches that target microglia in particular or neuroinflammation in general remains an elusive goal. Answers to some of the many open questions would help to advance therapeutic efforts. In particular, it is not yet clear what combinations of lineage-determining and signal-dependent transcription factors establish microglial cell identity and phenotype. In addition, we do not have a clear picture of the global gene expression programme of microglia *in vivo* in normal or diseased states. Without this knowledge, it will be difficult to generate microglial cell-like cells from precursors through reprogramming methods. The generation of microglial cells from iPS cells derived from healthy individuals and patients could facilitate further *in vitro* analyses and potentiate the development of microglial cell-based therapies. In addition, the development of tools to effectively perform microglial cell-specific genetic gain- and loss-of-function experiments in mouse models will help to define protective or pathogenic roles of specific genes. Progress along these lines will facilitate the next phase of discovery focused on disease-specific forms of inflammation, mechanisms of resolution and the potential to modulate microglial cell phenotypes for therapeutic purposes.

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