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# Heterogeneity of CNS myeloid cells and their roles in neurodegeneration

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**The diseased brain hosts a heterogeneous population of myeloid cells, including parenchymal microglia, perivascular cells, meningeal macrophages and blood-borne monocytes. To date, the different types of brain myeloid cells have been discriminated solely on the basis of their localization, morphology and surface epitope expression. However, recent data suggest that resident microglia may be functionally distinct from bone marrow– or blood-derived phagocytes, which invade the CNS under pathological conditions. During the last few years, research on brain myeloid cells has been markedly changed by the advent of new tools in imaging, genetics and immunology. These methodologies have yielded unexpected results, which challenge the traditional view of brain macrophages. On the basis of these new studies, we differentiate brain myeloid subtypes with regard to their origin, function and fate in the brain and illustrate the divergent features of these cells during neurodegeneration.**



## The diversity of myeloid cells in the brain

Myeloid cells in the brain are a diverse group of mononuclear cells that mediate the local immune response in the CNS during development, health and neurodegenerative diseases[1,2]. As such they are critical effectors and regulators of inflammation and the innate immune response, the immediate arm of the immune system. They have a common origin in hematopoietic stem cells and develop along distinct differentiation pathways in response to internal and external signals. The mononuclear phagocyte system of the CNS is in part formed by a subgroup of the white blood cells (leukocytes), originally described as a population of bone marrow–derived myeloid cells that circulate in the blood as monocytes and populate tissues as macrophages in steady state and during inflammation. However, this simplified view has changed markedly in the last years as a result of the discovery of new subtypes of mononuclear phagocytes and their distinct roles in disorders of the CNS, including neurodegenerative diseases.

Monocytes are blood mononuclear cells with a bean-shaped nucleus. They express CD11b, CD11c, CD14 and CD16 in humans, and CD11b and F4/80 in mice (**Table 1**), and they lack B, T and NK cell markers. Recent studies have found considerable heterogeneity of circulating mouse monocytes. On the basis of their differential expression of the chemokine receptors CCR2 and CX3CR1, so-called inflammatory monocytes (Ly-6C$^{hi}$ CCR2$^{+}$ CX3CR1$^{lo}$), which are highly mobile and rapidly recruited to inflamed tissues, can be distinguished from resident monocytes (Ly-6C$^{lo}$ CCR2$^{-}$ CX3CR1$^{hi}$), which are larger in size and are supposed to be important for patrolling along blood vessels (**Table 1** and **Supplementary Glossary**)[3,4]. The Ly-6C$^{hi}$ CCR2$^{+}$ monocytes are able to produce inflammatory molecules, such as tumor necrosis factor (TNF)-α and inducible nitric oxide synthase (iNOS), which is highly reminiscent of monocyte-derived, inflammatory, CD11c-expressing, TNF/iNOS-producing dendritic cells[5]. In fact, disease-specific mobilization and recruitment of CCR2$^{+}$ CD11b$^{+}$ Ly-6C$^{hi}$ monocytes into the inflamed CNS was recently observed in a number of studies[6,7] (**Fig. 1**).

As the key immune effector cells of the CNS, resting/surveillant microglia are distributed throughout the brain and act as sensors of pathological events. The fine cellular processes of microglia are highly motile and continually survey (whence surveillant) the microenvironment, whereas the soma itself is static[8,9]. It was proposed that the high motility of the protrusions has protective functions by scanning the environment for pathological changes or inflammatory stimuli, but recent experiments suggest that microglia also support and monitor synaptic function[10], control synaptogenesis[11] and induce developmental apoptosis of Purkinje cells (at least *in vitro*)[12]. Thus, microglia are important for the development and maintenance of the CNS.

In contrast with the microglia that engraft in the brain during early embryogenesis, it has been postulated that bone marrow–derived microglial progenitors might penetrate the brain even in normal adult mice to replace senescent microglial cells. Moreover, during CNS diseases, mononuclear phagocytes with morphological similarities to endogenous microglia can be derived from bone marrow cells or from circulating monocytes that subsequently become an integral part of the pathology and can be incorporated into the local cellular networks[13–15]. However, conflicting results were published in recent years, which complicate the interpretation of these observations. One of the most important questions in neuroimmunology today is whether functional bone marrow–derived microglia exist and, if so, how they can be targeted to the diseased brain. Answers to this seemingly simple question have tremendous clinical implications for the treatment of brain diseases, such as amyotrophic lateral sclerosis (ALS), Alzheimer's disease and Parkinson's disease. In principle, if the practical hurdles can be overcome, specific myeloid subpopulations,

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**Table 1 Myeloid cell populations present in the CNS and periphery during neurodegenerative disorders**

| Cell type | Localization | Origin | Marker | Function | Turnover | Refs. |
|---|---|---|---|---|---|---|
| Microglia | Parenchymal or juxtavascular | Yolk sac macrophages | $CX_3CR1^{hi}$, Iba-1, F4/80, CD11b, $CD45^{lo}$, $IL\text{-}B_4$ | Local immune surveillance, removal of dead neurons & Aβ peptides, restriction of inflammation | Self renewal, no exchange with blood circulation throughout lifetime | 8,21 |
| Perivascular macrophage | Perivascular | Blood | $CX_3CR1^{hi}$, Iba-1, F4/80, CD11b, $CD45^{hi}$, $IL\text{-}B_4$, $CD45^{lo}$, CD163 | Local immune surveillance, shuttling of Aβ from endothelia outside the brain | High exchange with blood myeloid cells | 48,95 |
| Meningeal macrophage | Meninges | Blood | $CX_3CR1^{hi}$, Iba-1, F4/80, CD11b, $CD45^{hi}$, $IL\text{-}B_4$ | Local immune surveillance | High exchange with blood myeloid cells | 96,97 |
| Choroid plexus macrophage | Choroid plexus | Blood | $CX_3CR1^{hi}$, Iba-1, F4/80, CD11b, $CD45^{hi}$, $IL\text{-}B_4$ | Surveillance of cerebrospinal fluid production | High exchange with blood myeloid cells | 97 |
| Resident monocyte | Blood | Bone marrow | Ly-$6C^{lo}$, $CX_3CR1^{hi}$, CD11b, $CD45^{hi}$, CD115 | Vessel patrolling | Derived from inflammatory monocytes | 4,98 |
| Inflammatory monocyte | Blood | Bone marrow | Ly-$6C^{hi}$, $CX_3CR1^{lo}$, CCR2, CD11b, $CD45^{hi}$, CD115 | Inflammatory response | High turnover from the bone marrow | 4,98 |

such as phagocytes from the yolk sac, bone marrow or blood, might be used to deliver neuroprotective or restorative molecules into the CNS to ameliorate the disease.

### Microglia or bone marrow–derived phagocytes: origin matters

The precise origin of microglia during brain development has been a matter of controversy for decades. The first macrophage-like cells with an amoeboid shape were found in the rodent neuroepithelium as early as day 8.5 of embryogenesis[16]. At this early time point, the first immature macrophages can already be detected in the yolk sac[17]. These macrophages may act as precursors of microglial cells[17], which then develop through a non-monocyte pathway. At embryonic day 13.5 (E13.5), when the fetal liver is the primary hematopoietic organ and the main site of hematopoietic stem cell (HSC) expansion and differentiation[18], microglial precursors can be detected in substantial numbers in the ventricular lining of the fourth ventricle[18]. However, whether organs of definitive haematopoiesis, such as the fetal liver, are a substantial source of adult microglia in the brain has been unclear. Notably, a significant increase in the number of $CD11b^+$ $F4/80^+$ microglia can be observed during the early postnatal periods in rodents[17]. It has long been uncertain whether this increase was a result of the proliferation of embryonic microglial precursors, a phenomenon that is frequently observed in the developing brain[19], or whether a new recruitment of monocyte-derived microglial precursors occurs. The latter hypothesis was indirectly supported by the observation that the absence of microglia in mice deficient for the transcription factor PU.1 can be rescued by the injection of wild-type bone marrow cells into newborns, leading to a complete repopulation of the CNS by donor-derived microglia[20].

Finally, a recent study shed light on the mysterious origins of microglia[21]. By inducing Cre recombinase activity through injections of tamoxifen into pregnant mice between days E7.00 and E7.50 after conception, when embryonic haematopoiesis is restricted to the yolk sac, the authors identified immature yolk sac macrophages as the predominant source of microglia. Notably, myeloid progenitors from the blood after birth did not significantly contribute to the pool of adult microglia, which is at odds with the results of previous studies and strongly suggests that the expansion of microglial numbers in the

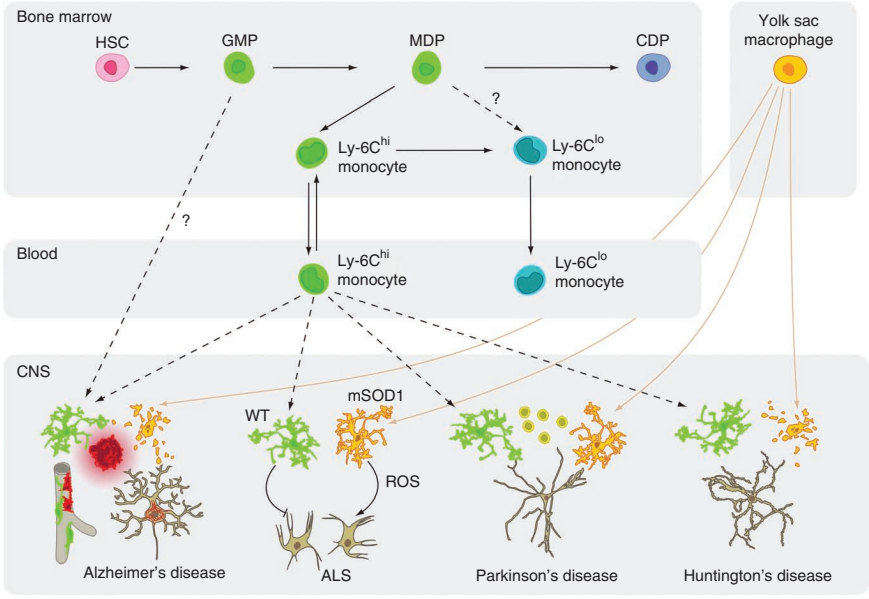


**Figure 1** Myeloid cells in the CNS, their origin and their involvement in neurodegeneration. Microglia in the CNS (orange ramified cells) are predominantly yolk sac–derived from early embryonic days. In neurodegenerative diseases, bone marrow–derived phagocytes can engraft in the brain (green ramified cells). These myeloid cells originate from circulating Ly-$6C^{hi}$ monocytes or from bone marrow–derived progenitors, for example, granulocyte-macrophage progenitors (GMPs) or other progeny of hematopoietic stem cells (HSCs). Peripheral blood monocytes arise from macrophage/dendritic cell progenitors (MDPs) in the marrow, which also generate common dendritic cell progenitors (CDPs). Bone marrow–derived phagocytes, which are recruited into the brain as a result of neurodegenerative conditions, may be functionally distinct from microglia. In models of Alzheimer's disease and Huntington's disease, endogenous microglia degenerate, whereas bone marrow–derived phagocytes, including perivascular macrophages, decrease amyloid burden. In genetic models of ALS, motor neuron death is propagated by endogenous microglia expressing the mutant SOD1 protein and ROS, whereas wild-type (WT) bone marrow–derived phagocytes can alleviate the disease course. Microglia, bone marrow–derived phagocytes and invading lymphocytes (yellow round cells) also partake in the neuroinflammatory condition, which is held to be responsible for dopaminergic neurotoxicity in models of Parkinson's disease.

postnatal period depends on proliferation of the resident microglia population. Thus, the vast majority of adult microglia appeared to be yolk sac derived (from a remarkably restricted time period during early embryogenesis). It remains open whether adult microglia could also be derived in part from the embryonic liver or other hematopoietic organs during embryogenesis, such as aortic-gonadal-mesonephros. One limitation of this seminal work[21] is that only one third of yolk sac macrophages could be labeled genetically.

To what extent may myeloid cells engraft in the adult CNS during neurodegeneration? Circulating leukocytes make a tortuous journey into the CNS, a topic which has been recently reviewed[22]. Important new findings[23–25] related to the most salient hurdle, the blood-brain barrier (BBB), identified pericytes (contractile cells which enwrap capillaries) as being critical for barrier formation and maintenance, and determined that failure of pericyte function is associated with neurodegeneration. Microglia are the only CNS myeloid cells, which reside behind the BBB (**Table 1**).

Initial cell transplantation experiments in rats revealed that perivascular macrophages, but not cells with ramified microglia characteristics, were present in the CNS parenchyma after irradiation and bone marrow transplantation[26]. Similar results were obtained in humans, when women who underwent sex-mismatched bone marrow transplantation were examined for the engraftment of Y chromosome–positive microglial cells[27]. Notably, all of these studies were based on immunohistochemical approaches and therefore lacked the sensitivity of cell transfer experiments with genetically labeled cells. A later study used retroviral transduction of hematopoietic cells with green fluorescent protein (GFP) to examine the long-term fate of myeloid cells in the murine CNS after bone marrow transplantation in an experimental setting including whole body irradiation[19]. This study found GFP-expressing parenchymal microglia deep in the mouse cerebellum, striatum and hippocampus several weeks after transplantation[19]. Despite the differences with the earlier studies mentioned above, the concept of bone marrow–derived phagocytes in the CNS was firmly established. Subsequently, a plethora of publications examined the function and fate of bone marrow–derived mononuclear phagocytes in different neurological disease models using similar experimental procedures. Infiltration of bone marrow–derived phagocytes was found in animal models without obvious BBB damage, such as ALS[28], Alzheimer's disease[29], scrapie[30] and many more[14,31] (**Fig. 1**). However, all of these studies used irradiation of the recipients followed by whole bone marrow transplantation to discriminate between the progeny of donor-derived labeled hematopoietic cells and the resident microglia in the hosts. To elucidate the effect of irradiation on the engraftment of myeloid cell in the CNS, the heads of the recipient mice were protected from the irradiation by shielding[13]. Notably, *de novo* generation of bone marrow–derived phagocytes from the circulation was strongly diminished in the brains of mice that were not irradiated before transplantation[13]. Another study provided complementary data from investigations of the recruitment of peripheral myeloid precursors into the CNS using parabiosis (in which the circulations of mice are joined for a period of time to allow population of the circulation of a parabiotic recipient with labeled cells). In contrast with preliminary findings in an earlier report[32], this study convincingly showed that there were no bone marrow–derived phagocytes cells in the CNS of the GFP-negative partner under any tested conditions, including total-body irradiation of the parabiotic recipient[33].

These findings indicate that the engraftment of bone marrow–derived myeloid cells in the CNS is an extremely rare event, which is strongly influenced by the experimental design (for example, cranial irradiation and intravenous transfer of femoral bone marrow enriched for hematopoietic progenitors and stem cells). Furthermore, these results underscore the fact that endogenous microglia are of yolk sac origin and exhibit a high potential for self-renewal and proliferation. Nevertheless, in our view, bone marrow–derived phagocytes might be capable of exploitation for potential therapeutic application in neurodegenerative settings, although the requirement for cranial irradiation to achieve CNS engraftment might limit its utility.

## Microglia and Alzheimer's disease

Alzheimer's disease is a neurodegenerative disorder whose severity and prevalence have caused an intense research effort to be directed to its understanding and treatment[34]. At present, no established treatment ameliorates the natural history of Alzheimer's disease. The essential neuropathological character of Alzheimer's disease entails the aggregation and accumulation of intracellular and extracellular components[35]. Specifically, the brains of individuals with Alzheimer's disease contain senile plaques composed of extracellular deposits of amyloid peptides (collectively termed Aβ) derived from amyloid precursor protein (APP). In addition, neurons in affected regions contain intracellular aggregates (designated neurofibrillary tangles) comprised of hyperphosphorylated forms of the microtubule-associated protein tau. During the development of Alzheimer's disease, these two processes interact in poisonous succession[36].

Beyond alterations in neuropil and neurons, which are regarded as etiological, neuroinflammation is implicated in Alzheimer's disease from convergent streams of evidence. First, genetic association studies (collated at http://www.alzgene.org/default.asp) have identified susceptibility-linked gene variants in inflammatory pathways, such as complement (complement receptor 1/CR1, complement inhibitor clusterin) and chemokine CXCL8. Reassuringly, the clusterin- and CR1-associated single-nucleotide polymorphisms were identified in a large genome-wide association study and subsequently verified in another study[37,38].

Second, epidemiological investigations consistently document a protective effect for mid-life exposure to nonsteroidal anti-inflammatory agents against later development of Alzheimer's disease[39]. Disappointingly, however, administration of nonsteroidal anti-inflammatory agents had no effect on the progression of established Alzheimer's disease, in several clinical trials[40].

Finally, neuropathological characterization of Alzheimer's disease tissues revealed the presence of numerous mediators of innate immunity, including complement components and chemokine system elements such as CXCL8 and its receptor CXCR2 (ref. 41). These factors are also present early in the course of several animal Alzheimer's disease models, such as those that induce pathology via overproduction of Aβ, or through transgenic overexpression of mutant tau species that cause frontotemporal dementia[42].

It's important to note that the inflammatory reaction in Alzheimer's disease is consistent with innate immunity, according to a scheme in which immune reactions are characterized as adaptive or innate. Adaptive responses involve immune specificity (for antigen) and immune memory (indicating an accelerated reaction to antigen re-exposure) based on the unique properties of T and B lymphocytes. Innate immunity is responsible for antigen-independent reactions against pathogens or tissue damage, carried out by myeloid cells and mediators such as complement, interferons and inflammatory cytokines. In tissue from individuals with Alzheimer's disease, the inflammatory cellular reaction is comprised of myeloid cells (microglia, monocytes and perivascular macrophages) and astrocytes, arguing against the involvement of adaptive immunity. Given these conclusions, it's important to understand the role(s) of myeloid cell subsets in the disease process.

It has proven to be fiendishly difficult to decipher the role(s) of microglia in Alzheimer's disease–associated neuroinflammation and, in particular, to answer the critical question of whether microglia are neurotoxic or neuroprotective in the pathogenesis of Alzheimer's disease. A great deal of the challenge in unraveling whether microglial effector functions are harmful or beneficial in Alzheimer's disease comes from unanswered questions in microglial biology. Some of these questions have recently been addressed satisfactorily, so it's possible to revisit open questions about microglia and Alzheimer's disease in the light of these new insights.

First, are the microglia the only myeloid cells involved in Alzheimer's disease? This question is important because of potential differences of effector properties for CNS-resident microglia as compared with bone marrow–derived phagocytes. Early studies used several approaches to address the distinct functions of infiltrating versus resident myeloid cells[15,43]. Radiation bone marrow chimerism was used to populate the circulation of Alzheimer's disease model transgenic mice expressing mutant forms of APP (APP32 mice) or APP and presenilin-1 (APP-PS1 mice) with GFP-labeled cells. Numerous myeloid GFP-positive cells were found in the proximity of amyloid plaques in 5-month-old animals[15,43] (**Fig. 1**). These data suggest that these cells were attracted from the bloodstream to plaques. To address whether these bone marrow–derived phagocytes were functionally important for amyloid plaque formation, APP-PS1 mice were crossed with mice expressing an inducible myeloid-specific suicide transgene in which herpesvirus thymidine kinase (HSV-TK) was regulated by CD11b (yielding APP-PS1; CD11b–HSV-TK mice). Intracerebroventricular (ICV) installation of gancyclovir (GCV) for 28 d in 3.5–6.0-month-old mice caused cell death for nearby proliferating CD11b$^+$ cells[15]. Based on inferences from radiation chimerism studies, it was proposed that infiltrating monocytes, but not resident microglia, would be affected. In these studies, ICV GCV–mediated depletion of CD11b$^+$ cells was associated with increased plaque size and number at 6 months, whereas the numbers of myeloid cells associated with each plaque were not changed. The authors proposed that infiltrating monocytes were abundant in tissue from individuals with Alzheimer's disease, differentiated into microglia, and were important for clearing amyloid and limiting its deposition. This hypothesis was innovative given that previous reports suggested that microglia phagocytosed amyloid poorly[44] and that their production of inflammatory cytokines represented a default response to amyloid that they were unable to clear. Notably, these investigators addressed these questions using techniques that allowed the manipulation of the CNS myeloid populations *in vivo*.

Subsequently accelerated CNS tissue pathology and early demise were reported in a mouse model of Alzheimer's disease that was unable to recruit monocytes to the CNS as a result of absence of CCR2, a receptor specific for the monocyte chemoattractant protein family of chemokines[45]. In particular, tg2576 mice expressing a mutant APP transgene were crossed to *Ccr2*$^{-/-}$ mice and had markedly shortened lifespans, along with increased Aβ peptide levels and greatly enhanced deposition of amyloid in cerebral vessels (called congophilic angiopathy). These data appear to be consistent with those derived from bone marrow chimerism studies and suggested that bone marrow–derived phagocytes entered Alzheimer's disease tissues and were often attracted to nascent plaques, and that infiltrated peripheral myeloid cells were much more efficient at amyloid clearance than resident CNS microglia.

The matter rested there for more than 2 years, when additional studies using HSV-TK/GCV to deplete CD11b$^+$ cells from the CNS of Alzheimer's disease model mice were reported[46]. Thus study used a similar, but not identical, Alzheimer's disease model, crossed to CD11b–HSV-TK and treated with ICV-infused GCV. Using a panel of myeloid markers as well as electron microscopy, it was shown that local microglia (and by implication, peripheral myeloid cells bearing these markers) were virtually abolished immediately after a 4-week GCV infusion. There was no effect on plaque size or number in either these initial experiments or in follow-up studies using another, less aggressive Alzheimer's disease model. At present, no unambiguous conclusions can be drawn, except that myeloid cells (whether CNS resident or peripherally derived) seem to be dispensable for amyloid deposition and don't seem to modify the amount or distribution of amyloid in CNS regions depleted of myeloid cells. The differences between the previous[15] and more recent[46] report may lie with the GCV infusion protocol, which may have more completely abrogated myeloid cells in the latter.

We recently identified CCR2-expressing myeloid cells as the population that was preferentially recruited to Aβ deposits[47]. Unexpectedly, brains of individuals with Alzheimer's disease with dysfunctional microglia and devoid of parenchymal bone marrow–derived phagocytes did not show overt changes in plaque pathology and Aβ load. In contrast, restriction of CCR2-deficiency to perivascular myeloid cells markedly impaired Aβ clearance and amplified vascular Aβ deposition, whereas parenchymal plaque deposition remained unaffected. Taken together, these data advocate selective functions of CCR2-expressing myeloid subsets in Alzheimer's disease.

One recent pertinent observation was that eliminating perivascular macrophages with toxic liposome infusions caused increased accumulation of vascular amyloid[48]. At the same time, one must emphasize that the competence of microglia for efficient phagocytosis of amyloid *in vivo* also remains uncertain[44]. Notably, microglia might mediate amyloid clearance or inhibit amyloidogenesis by means other than phagocytosis, including production of proteolytic enzymes[49,50]. Alternatively, microglia or monocytes might induce the expression of Aβ-degrading enzymes in other CNS cells. Given recent findings of deficient amyloid clearance in individuals with Alzheimer's disease[51], this line of investigation carries considerable urgency. In addition, functions of microglia in the context of Alzheimer's disease immunotherapy, using passive transfer of antibodies to Aβ[52,53], must be deciphered if the mechanism underlying this promising approach is to be unveiled.

During the years after these reports, application of radiation bone marrow chimerism to microglial research has, however, been noted to introduce confounds, including permanent alteration of the BBB, additional changes in the brain and elevated blood levels of hematopoietic stem cells, both of which promote nonphysiological entry of myeloid cells from circulation into the CNS[31,33]. Thus, results using this approach must be interpreted with the understanding that physiological trafficking of myeloid cells from periphery to CNS cannot be addressed using radiation bone marrow chimerism.

At the same time, it has quite recently become clear that it will be essential to distinguish peripheral from CNS-resident myeloid cells to fully comprehend their complementary or antagonistic roles in the pathogenesis of neurodegenerative disease. As noted above, recent data has shown that microglia constitute a distinct myeloid population that self-renews throughout life without contribution from the periphery[21]. Given that monocytes and microglia are fundamentally different, the instincts of earlier investigations[15], to differentiate their functions, must be regarded as prescient. We recently reported[54] a mouse model in which CNS microglia were labeled with Cx3cr1-GFP, whereas blood monocytes capable of entering inflamed tissues were labeled with Ccr2–red fluorescent protein (RFP). In the short-term inflammatory disease model of experimental autoimmune encephalomyelitis, these genetic markers nicely distinguished macrophages derived from infiltrating monocytes

from those macrophages that arose from resident microglia. The development of genetic approaches for permanent differential labeling of circulating monocytes, as contrasted with resident microglia, is currently being researched. Because no promoters clearly distinguish the two myeloid cell types, innovative techniques, including selective peripheral or CNS activation of conditional-inducible labels, might be required.

Second, how are microglia activated in Alzheimer's disease and what are the consequences of their activation? It was recently established that microglia constitute a unique population of myeloid cells[21], which spend adult life encircled by neuronally derived inhibitory components, isolated from plasma proteins behind the BBB[1]. One view holds that microglial activation is equivalent to removal of suppression[55]. Study of Alzheimer's disease model mice harboring microglia deficient for the modulatory chemokine receptor CX3CR1 (ref. 56) lends credence to this view. In the CNS, CX3CR1 is exclusively found on microglia, whereas the ligand CX3CL1 is expressed only by neurons as a transmembrane protein released by stimulus-dependent proteolysis[57]. Therefore, neuronal injury will initially liberate increased amounts of CX3CL1 (ref. 58), whereas subsequent loss of neurons will result in CX3CL1 privation[59].

One study[60] evaluated neuronal cell loss in cortical layer III, using Alzheimer's disease model mice that expressed mutant pathogenic isoforms of APP, PS1 and tau, as well as different fluorescent labels for neurons and microglia, which were either *Cx3cr1*$^{+/-}$ or *Cx3cr1*$^{-/-}$. Monitoring neurons and microglia over 28 d in 4–6-month-old animals using repetitive two-photon imaging, the authors found a loss of 1.8% of layer III neurons in heterozygous, as compared with knockout, mice. Neuron loss preceded amyloid deposition or tau pathological change and was associated with increased motility of *Cx3cr1*$^{+/-}$ microglia toward neurons. The results suggest that CX3CR1 signaling promotes neuronal cell loss in the presence of early-stage Alzheimer's disease–like pathology, but did not distinguish the separate contributions of mutant APP, PS1 and tau. The issue of how Alzheimer's disease–related amyloid pathology was affected by the lack of CX3CR1 was shortly addressed[61]. In genetic mouse models of amyloid pathology, deficiency for CX3CR1 was associated with gene dosage–dependent amelioration of amyloid plaque deposition (**Fig. 2a**,**b**). APP processing to amyloidogenic peptides was unaltered, raising the question of whether catabolism by microglia was affected. Closely compatible results were also reported in a separate study[62]. The inflammatory cytokine IL1β was highly expressed[61], compatible with results from other disease models involving CX3CR1 deficiency[56] and consistent with a role for IL1β in promoting amyloid clearance[63] (**Fig. 2b**).

Complementary findings emerged from examining the role of CX3CR1 in tau pathology[64], using hTau mice in which a human tau genomic transgene (expressing all alternatively spliced isoforms of tau) was expressed on a murine tau-deficient background. In these studies, *Cx3cr1*$^{-/-}$ hTau mice exhibited worsened tau pathology, as judged by tau hyperphosphorylation and tau aggregation (**Fig. 2c**). *In vitro* co-culture and conditioned medium experiments delineated a pathway by which microglia from *Cx3cr1*$^{-/-}$ mice produced elevated levels of IL1β, which activated p38 MAP kinase, leading to hyperphosphorylated tau (**Fig. 2d**). Thus, lack of CX3CR1 signaling, associated with elevation of IL1β, mediates enhanced clearance of amyloid at the expense of heightened tau pathology, possibly coupled to a loss of layer III cortical neurons. In this sense, both CX3CR1 and IL1β are double-edged neuroinflammatory modulators.

Another means for activating microglia is to impose inflammatory stimuli from 'outside', that is, from the systemic circulation[59]. The pathways for microglial activation during systemic infection include diffusion of cytokines across the nonbarrier endothelium of the circumventricular organs[65,66], as well as stimulation of cerebrovascular endothelium by cytokine exposure, causing the endothelial cells to secrete cytokines abluminally, where they impinge on the responsive parenchymal elements: microglia and astrocytes. These provocative preliminary results were recently succeeded by evaluation of individuals with Alzheimer's disease, with the finding that transient common infections such as upper respiratory infections or urinary tract infections can produce permanent worsening of ongoing neurodegenerative disease[67,68].

Finally, it's important to consider that most Alzheimer's disease models rely on expression of mutant genes, which were identified in individuals with rare genetic variants that cause familial forms of Alzheimer's disease, a disease that is typically sporadic[69]. However, as



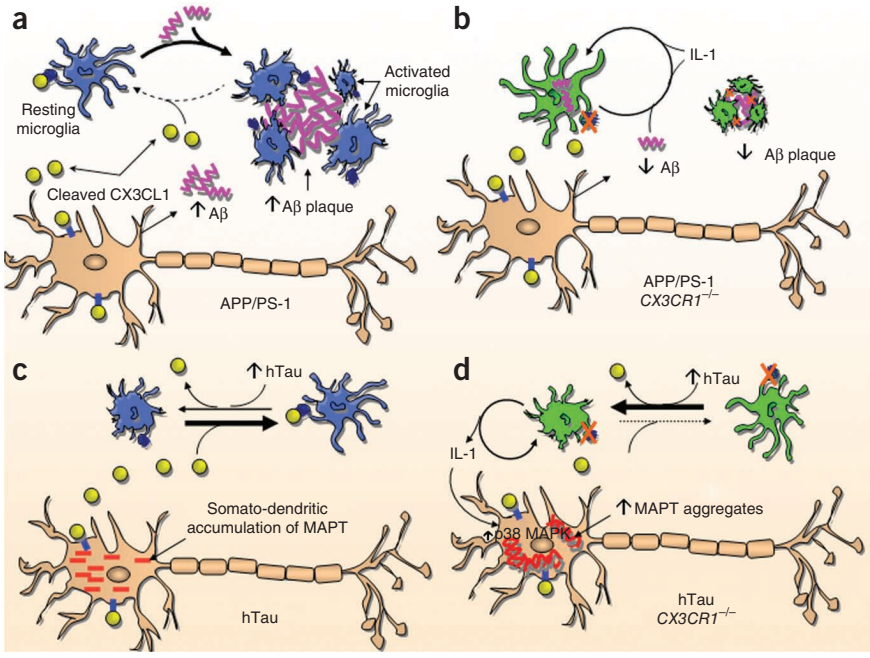


**Figure 2** Loss of CX3CR1 signaling ameliorates amyloid deposition but worsens tau pathology. (**a**) In APP/PS-1 mice, which retain CX3CR1, increased levels of Aβ (purple jagged lines) accumulate in plaques surrounded by activated microglia (blue), whose response is modulated by neuronally derived cleaved CX3CL1 (yellow balls) signaling to microglial receptor CX3CR1. (**b**) APP/PS-1 mice, which lack CX3CR1, show elevated tissue levels of IL-1, which is associated with enhanced microglial activation (green), improved amyloid clearance, and decreased plaque size and number. (**c**) Transgenic mice expressing human tau (hTau) with intact CX3CR1 signaling accumulate hyperphosphorylated tau (red lines) in neuronal somata and dendrites, along with modest microglial activation (blue). MAPT, microtubule-associated protein tau. The straight red lines represent the physiological form of tau, whereas the tangles indicate pathological aggregates of tau. Both are composed of MAPT. (**d**) CX3CR1-deficient hTau mice show highly activated microglia (green), which produce large amounts of IL-1, leading to a neuronal response via the IL-1 type 1 receptor, culminating in activated p38 MAP kinase (MAPK) and in tau aggregates (red).

noted below, expression of mutant genes is often not limited to the neuronal target cells of the disease process, and non–cell autonomous disease mechanisms are being increasingly recognized. One study of mice expressing a mutant form of PS-1 uncovered a pathogenic pathway by which microglia were stimulated by the presence of the transgene product to produce secreted factors that inhibited neurogenesis in the hippocampal dentate gyrus[70]. These types of studies carry the potential to reveal unexpected cell-cell interactions and also to identify molecular targets for therapeutic intervention.

### ALS and microglia

ALS is an adult-onset, progressive neurodegenerative disorder that specifically affects the upper and lower motor neurons, leading to atrophy of skeletal muscle, spasticity, pareses and subsequently to death in 4–6 years. 20% of familial ALS cases are caused by a mutation in the ubiquitously expressed gene *SOD1*, which encodes the free radical–scavenging metalloenzyme copper, zinc superoxide dismutase (SOD). The fact that microglia are actively involved in this disease was shown in SOD mutant mice[20]. In this study, mice with ALS symptoms caused by the expression of mutant *SOD1* gene (*SOD1G93A*) were bred to transcription factor PU.1–deficient mice, which are characterized by a lack of myeloid cells[71]. Because both *PU.1*$^{-/-}$ (also known as *SPI1*) and *PU.1*$^{-/-}$*; SOD1G93A* mice die shortly after birth[20,71], intraperitoneal bone marrow transfer into newborns was performed. *PU.1*$^{-/-}$*; SOD1G93A* newborns received either wild-type or SOD1G93A bone marrow cells, and survival and motor neuron loss were both analyzed. Notably, transplanted *PU.1*$^{-/-}$*; SOD1G93A* mice showed full reconstitution of the CNS with donor-derived myeloid cells, although recipient mice did not receive prior irradiation. Notably, *PU.1*$^{-/-}$*; SOD1G93A* pups that were reconstituted with wild-type bone marrow showed a substantially longer survival and decreased motor neuron loss, indicating a pathology-promoting role of SOD1G93A microglial cells[20]. Similar results were obtained by using a different approach: inactivation of mutant SODG37R specifically in CD11b$^{+}$ microglia extended the survival of mice significantly, particularly during the late phase of disease[72]. Indeed, the neurotoxic nature of mutant SOD1-expressing microglia was shown directly and additional activation of microglial cells in ALS mice by macrophage-colony stimulating factor treatment resulted in exacerbated symptoms[73]. This treatment led to increased proliferation and an altered morphology of microglial cells, which led to enhanced expression of pro-inflammatory cytokines, such as IL1β and TNF-α[73]. Notably, and in contrast with the long-term reconstitution experiments in neonates[20], bone marrow transplantation of wild-type bone marrow cells in adult 6-week-old SOD1G93A mice[28] or even allogeneic bone marrow transplantation in individuals with sporadic ALS did not result in any beneficial outcome[74]. This discrepancy could be a result of inefficient engraftment of the CNS by bone marrow–derived elements in adults, as was shown previously[33], which could lead to an insufficient replacement of mutant SOD1–expressing microglia by wild-type bone marrow–derived cells.

Overall, the experimental results obtained so far point to a disease-promoting role of mutant SOD1–expressing microglia in familial ALS. Thus, therapeutic application of wild-type or gene-modified microglial precursors, equipped with a high capacity to infiltrate the brain, should be combined with a preconditioning regime to facilitate engraftment in the brain.

### Microglia in Huntington's disease

Huntington's disease is a monogenic autosomal-dominant neurodegenerative condition that is caused by an increased CAG repeat length in exon one of the gene encoding huntingtin. CAG encodes glutamine and a polyglutamine tract in excess of 39 (with healthy individuals showing between 6 and 26) is invariably associated with Huntington's disease. Symptoms and signs of Huntington's disease include choreic movements, cognitive impairment, personality change and weight loss. Both neuropathology and imaging studies have demonstrated profound striatal atrophy, along with predominant loss of medium spiny neurons.

Once the gene defect was established, mouse models of Huntington's disease were generated, beginning with transgenic overexpression of greatly expanded CAG repeats and progressively becoming more refined, through the use of knock-in gene targeting, to place the mutant *huntingtin* gene, carrying repeat numbers typical of human disease, under the control of the endogenous mouse locus. Because the mutant gene product is widely expressed, it's relevant to consider whether neurotoxicity in Huntington's disease is cell-autonomous or requires interactions among CNS cells types, with mutant huntingtin causing different patterns of dysfunction in the varied cells, whose pathological interactions culminate in the Huntington's disease phenotype. In a recent study, highly selective expression of mutant huntingtin in medium spiny striatal neurons of transgenic mice did not cause motor signs or striatal cell loss, arguing forcefully that neurodegeneration in Huntington's disease is not cell-autonomous with regard to neurons[75]. Evidence from an unexpected quarter implicated microglia in Huntington's disease pathology. Expanded CAG repeats cause cell death in yeast, and genetic suppressors of this cytotoxic effect were identified in the highly conserved tryptophan catabolic pathway[76].

Metabolic derivatives of tryptophan include both neurotoxic moieties, such as 3-hydroxykynurenine and quinolinic acid, as well as the neuroprotectant L-kynurenine, produced in a branched catabolic pathway involving both astrocytes and microglia. The neurotoxic effects of 3-hydroxykynurenine and quinolinic acid involve their serving as mimics of excitotoxic neurotransmitters and increasing the production of reactive oxygen species (ROS). The enzyme kynurenine 3-monooxygenase (KMO), expressed virtually only in microglia among CNS cells, lies at a critical branch point favoring production of the tryptophan-derived neurotoxins and shows increased expression in Huntington's disease, as well as in mouse models of Huntington's disease[77]. Genetic or pharmacological inhibition of KMO ameliorated Huntington's disease–like pathology in mice[76]. It has been proposed that microglia respond to the presence of mutant huntingtin with elevated expression of KMO, leading to neurotoxicity. Arguing in favor of altered myeloid cell function in individuals with Huntington's disease, the circulating monocytes of affected individuals were shown to overexpress inflammatory cytokines[78]. These results outline a previously unknown pathway by which microglia are implicated in neurodegeneration; in particular, if a ubiquitous mutant gene causes a loss of specific neuronal cell populations, it is important to consider the effects of the mutant gene product on cells that interact with the target population. The principle of non–cell autonomous neurodegeneration, even where only highly selected neuronal populations are affected, has been neatly demonstrated in several mouse models that rely on transgenic expression of a mutant gene responsible for relatively rare genetically determined cases of disorders, such as ALS and Alzheimer's disease[79].

### Parkinson's disease and microglia

Parkinson's disease is the second most common neurodegenerative disorder after Alzheimer's disease with an age-related increase in incidence. It is characterized by motor symptoms, such as tremor, rigidity, postural instability and bradykinesia. The disease course is often complicated by behavioral and psychiatric symptoms and by cognitive

impairment. The pathological hallmarks of Parkinson's disease are the loss of dopaminergic neurons and the presence of eosinophilic inclusions called Lewy bodies and dystrophic neurites in the substantia nigra pars compacta in the midbrain. However, neuronal loss is not confined to the substantia nigra or the dopaminergic system, but also occurs in other brain regions. Parkinson's disease is characterized by the accumulation of reactive MHC class II–positive microglia in the substantia nigra[80,81]. Positron emission tomography studies using the [11C](R)-PK11195 marker of peripheral benzodiazepine binding sites revealed microglial activation in pons, basal ganglia, and frontal and temporal cortical regions of individuals with Parkinson's disease, starting early in the disease process without significant longitudinal changes[82]. Notably, postmortem samples of individuals with Parkinson's disease also showed infiltration of $CD4^+$ and $CD8^+$ T cells in the substantia nigra, suggesting a pathogenic role of neuroinflammation in Parkinson's disease[81]. Nevertheless, the contribution of microglia to the pathogenesis of Parkinson's disease is far from clear.

Direct evidence for a neurotoxic function of microglia comes from animal models of Parkinson's disease. Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induces parkinsonian-like symptoms in humans, primates and mice after oxidation to the dopaminergic neurotoxin $MPP^+$ in the brain. As a result of mitochondrial complex I inhibition, neuronal metabolism is compromised and ROS accumulate. Alpha-synuclein, a protein that is mutated in rare familial forms of Parkinson's disease[83], is misfolded and forms aggregates, as is the case in the Lewy bodies of Parkinson's disease. Notably, the cytotoxicity induced by misfolded alpha-synuclein appears to be non–cell autonomous and to involve myeloid cells. Thus, normal and misfolded alpha-synuclein are secreted from dopaminergic neurons and phagocytosed by microglia, which activate NADPH oxidase and produce ROS[84]. Along these lines, mice deficient in inducible nitric oxide synthase or defective in NADPH oxidase exhibit less neuronal loss in the MPTP model of Parkinson's disease[85,86]. In rats, 6-hydroxydopamine administration leads to parkinsonian-like symptoms as a result of selective uptake of the toxin into dopaminergic neurons and subsequent cell death via the generation of ROS. The presence of activated microglia is well documented in the brains of 6-hydroxydopamine–lesioned rats, and pharmacological neutralization of the pro-inflammatory cytokine soluble TNF significantly reduces dopaminergic cell death, suggesting a pathogenic role of inflammation in neurodegeneration[87]. A recent study indicated that the chemokine receptor CX3CR1 is involved in controlling microglial neurotoxicity[56]. Thus, mice deficient in CX3CR1 show increased microglial activation and enhanced dopaminergic cell loss in the substantia nigra after systemic administration of LPS. Overall, inflammation may have a sensitizing function in nigrostriatal pathway degeneration, which is consistent with the epidemiological finding of decreased incidence of Parkinson's disease in chronic users of the nonsteroidal anti-inflammatory drug ibuprofen[88], and the delayed occurrence of postencephalitic parkinsonism after viral infection[89].

Still, the heterogeneity of CNS myeloid cells deserves more attention in future research on Parkinson's disease, in particular when neurotoxin-based animal models are used. Some myeloid subpopulations, perhaps even microglia, may eventually change from culprits to victims. In GFP bone marrow chimeric mice, MPTP intoxication results in substantial engraftment of bone marrow–derived phagocytes in the substantia nigra, striatum and hippocampus[90]. Notably, the vast majority of bone marrow–derived phagocytes (>90%) express inducible nitric oxide synthase, thereby amplifying the deleterious NO production[90]. Selective engraftment of bone marrow–derived-phagocytes in brain regions with marked dopaminergic innervation can also be observed in the chronic MPTP model[91]. On the basis of the very low expression of glial cell line–derived neurotrophic factor (GDNF) in bone marrow–derived phagocytes, the authors conclude that the function of these cells may be rather detrimental[91]. However, bone marrow transplantation in mice can reduce neuronal degeneration in the substantia nigra and improve motor function, even when performed after MPTP administration[92]. Besides the engraftment of myeloid cells in affected brain areas, this may be a result of the immunosuppressive effects on T lymphocytes, which accumulate to a considerable degree in the substantia nigra of MPTP-treated animals[81]. It will be important to examine the interactions of T cells with polarized microglia, macrophages and monocytes in the brain. Finally, recent experimental evidence suggests that macrophages can be genetically engineered to deliver GDNF to the brain and provide neuroprotection in the MPTP model of Parkinson's disease[93]. Thus, specific subsets of myeloid cells may eventually open new avenues for the treatment of chronic progressive neurodegenerative disorders such as Parkinson's disease.

## Summary and conclusions

Microglia are unique myeloid cells that are found only in the CNS parenchyma. Their modes of activation and response patterns on stimulation by neural injury or systemic inflammation are only now coming into view, with definitive understanding of their provenance and their mechanisms of population maintenance. New insights into microglial biology will enable characterization of their functions in neurodegenerative disease.

Aside from extremely rare genetic disorders[94], none of the neurodegenerative diseases appear to be solely caused by microglial dysfunction. Nevertheless, probing microglial responses to identify therapeutic targets for disease modulation remains a vibrant field of investigation. Examination of disease models in correlation with patient material has clarified that the role(s) of microglia in different neurodegenerations appears to vary. In most cases, attention is focused on pathogenic functions of microglia, which are plausible targets for inhibition. Pathogenic properties of microglia include producing toxic inflammatory cytokines, reactive oxygen and nitrogen species and proteolytic enzymes. Stimuli for microglia are typified by impairment of neuronal function and systemic inflammatory mediators. However, in some cases, as in Huntington's disease and many mouse models of neurodegenerative disease, microglia expression of mutant proteins is a stimulus for altered function. In the healthy brain, microglia serve a bewildering array of neuroprotective functions, including debris phagocytosis and clearance, elaboration of growth factors and possibly maintenance of synapses. Thus, it is just as important to consider means of biasing microglia to protective functions as it is to suppress their deleterious activities.

*Note: Supplementary information is available on the Nature Neuroscience website.*

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