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Microglia – the brain's busy bees

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Abstract

Recent years have seen significant changes in the way scientists view microglia and their role in health and disease. For decades, it was presumed that microglia were stationary, inactive immune cells in the brain, waiting for an immunologic insult to call them into action. In contrast, modern imaging techniques have revealed that microglia are constantly in motion, surveying their environment. Lineage tracing studies have led to the understanding that microglia are part of a larger family of cells, called tissue-resident macrophages, which arise from early yolk sac progenitors during embryogenesis and engraft nearly every organ in the body. Microglia, and all tissue-resident macrophages, rely on signaling through CD115 (the colony stimulating factor 1 receptor) for survival, primarily through the ligand, macrophage colony-stimulating factor. However, it is now understood that some microglia have a specific need for another CD115 ligand, Interleukin-34, which is only shared with Langerhans cells in the skin. In contrast to classical views, recent evidence suggests that the primary functions of microglia may occur during postnatal neurodevelopment and adult homeostasis, as absence or impairment of microglia results in a pathology separate from inflammatory immune function. In summary, these advances suggest that microglia might eventually be utilized or targeted to improve disease outcomes via encouraging or enhancing their health-promoting homeostatic functions.

Introduction

For decades microglia were regarded as the "enemy within" the brain, primarily responding to infiltrating pathogens and injury, and potentially causing disease and damage through inflammation [1,2]. Work in recent years has revealed that microglia spend most of their time as non-inflammatory yet very active participants in the development and homeostasis of the central nervous system (CNS) [3-14]. Essentially, while microglia do respond to immunologic stimuli and can become inflammatory [1,2], their primary role seems to be homeostatic and developmental [3-14]. In addition, microglia are part of a larger family of closely related cells called tissue-resident macrophages [15,16].

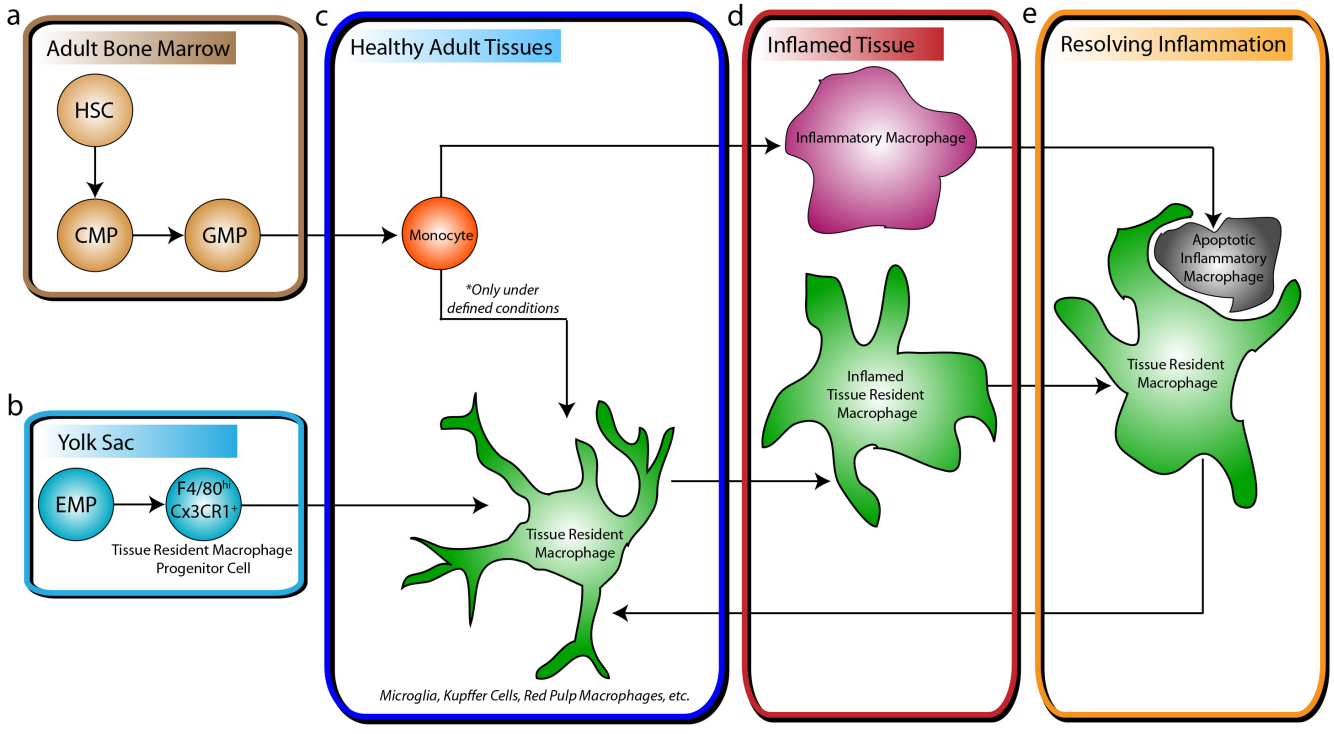
Tissue-resident macrophages arise early in embryogenesis from primitive macrophages in the yolk sac, prior to the development of the bone marrow-derived hematopoietic system [15,16]. Tissue-resident macrophages are a versatile and diverse group of cells, but the significance of this diversity is often overlooked. The existence of multiple, organ-specific terms coined by histopathologists (such as Kupffer cells in the liver, red pulp, metallophilic, and marginal-zone macrophages in the spleen, alveolar macrophages in the lungs, the multinucleated cells termed osteoclasts in the bones, and microglia in the brain) emphasizes this diversity. These different organ-specific macrophage subtypes have been known for over a century, being first described by Elie Metchnikoff (see [17]). Along with this list of uniquely named tissue-resident macrophages, it should be noted that additional types of tissue-resident macrophages are found in nearly every tissue, and they play an essential role in development and homeostasis [3,4,7,18-22].

Microglia, the tissue-resident macrophages of the CNS, play key roles in the regulation of neuronal synapses [4,5,11,12], neurogenesis [7,9,23], clearance of apoptotic cells and debris [13,19,24], and trophic factor production in the brain [8,23]. Failure of microglial homeostatic functions can result in CNS pathology, and this understanding has led to the realization that dysfunction of microglia may contribute to many diseases in which they were not previously implicated. In this review, we will discuss the origins of microglia and their relationship to other tissue-resident macrophages, the growth factors essential for their development, and their homeostatic role in the CNS.

Origins, function, and relationship to other macrophages

Immunology, focused on host-defense mechanisms, most strongly recognizes macrophages as an infiltrating cell at a site of injury or inflammation, whose role is to phagocytose debris and pathogens, initially secrete inflammatory cytokines [18], phagocytose apoptotic immune cells (primarily granulocytes) [25,26] and secrete anti-inflammatory cytokines upon resolution of the inflammatory reaction [27]. These inflammatory macrophages are elicited from circulating monocytes (Figure 1), and at the conclusion of inflammation, they typically do not persist in the inflamed tissue, instead either undergoing apoptosis or emigrating to draining lymph nodes [28,29]. In contrast, tissue-resident macrophages, which are already present at the beginning of inflammation, will respond similarly to inflammatory macrophages but remain after the conclusion of inflammation, and are critical for tissue repair and return to normal function [18,30,31]. Tissue-resident macrophages secrete growth factors and continue to phagocytose debris and apoptotic cells, likely aiding in tissue repair and regeneration. Importantly,

Figure 1. Development, relationship, and interaction of microglia with other macrophages



a) Adult hematopoietic stem cells (HSC) give rise to common myeloid progenitors (CMP), which give rise to granulocyte-monocyte progenitor cells (GMP). b) Early in embryonic development, erythroid/myeloid progenitors (EMP) differentiate into primitive macrophage progenitors in the yolk sac, which are F4/80^{hi}CX3CR1^{+}, and give rise to tissue-resident macrophages in many tissues. c) In adult tissues, monocytes are derived and constantly repopulated from the hematopoietic system located in the bone marrow. In contrast, tissue-resident macrophages under homeostatic conditions are largely self-renewing. Under very defined, specific conditions where tissue-resident macrophages are ablated or the tissue is irradiated prior to a bone marrow transplant, monocytes are capable of replenishing the tissue-resident macrophage pool. d) When a tissue becomes inflamed, monocytes traffic inside and differentiate into inflammatory macrophages. Tissue-resident macrophages also become inflamed. e) At the conclusion of inflammation, the majority of inflammatory macrophages undergo apoptosis, and tissue-resident macrophages resume their role in maintenance of tissue homeostasis, which includes clearing away inflammatory debris.

tissue-resident macrophages do not derive, at least initially, from circulating monocytes or hematopoietic stem cells [15], although under certain circumstances they can be derived from these precursors [32-34]. While monocyte-derived inflammatory macrophages and tissue-resident macrophages share many phenotypic and functional similarities, it is becoming increasingly clear that their physiological and functional differences are greater than originally appreciated [18].

Recent evidence suggests that there exists an entirely separate lineage of myeloid cells derived from yolk sac macrophages, as opposed to those derived from hematopoietic stem cells [15]. Although it had been previously shown that some tissue-resident macrophage-type cells, such as microglia, derive from primitive, embryonic macrophages [35], the exact relationship between yolk sac macrophages and traditional hematopoietic cells was not clear until recently [15]. Specifically, Schulz *et al.* [15] showed that embryonic mice null for the transcription factor Myb (which is critical for the development of hematopoietic stem cells) lacked $CD11b^{hi}$ monocytes and macrophages but still developed $F4/80^{bright}$ macrophages in multiple organs. These $F4/80^{bright}$ cells were found to be tissue-resident macrophages, such as Kupffer cells and microglia [15]. The separate nature of the tissue-resident macrophage lineage is not entirely surprising, as it had been previously shown that microglia, for instance, are capable of self-renewal independent of hematopoietic progenitors [36]. This evidence suggests that while inflammatory and tissue-resident macrophages express similar markers, and can perform certain functions in a similar manner, their origin, development, and life cycles are distinct under homeostatic conditions.

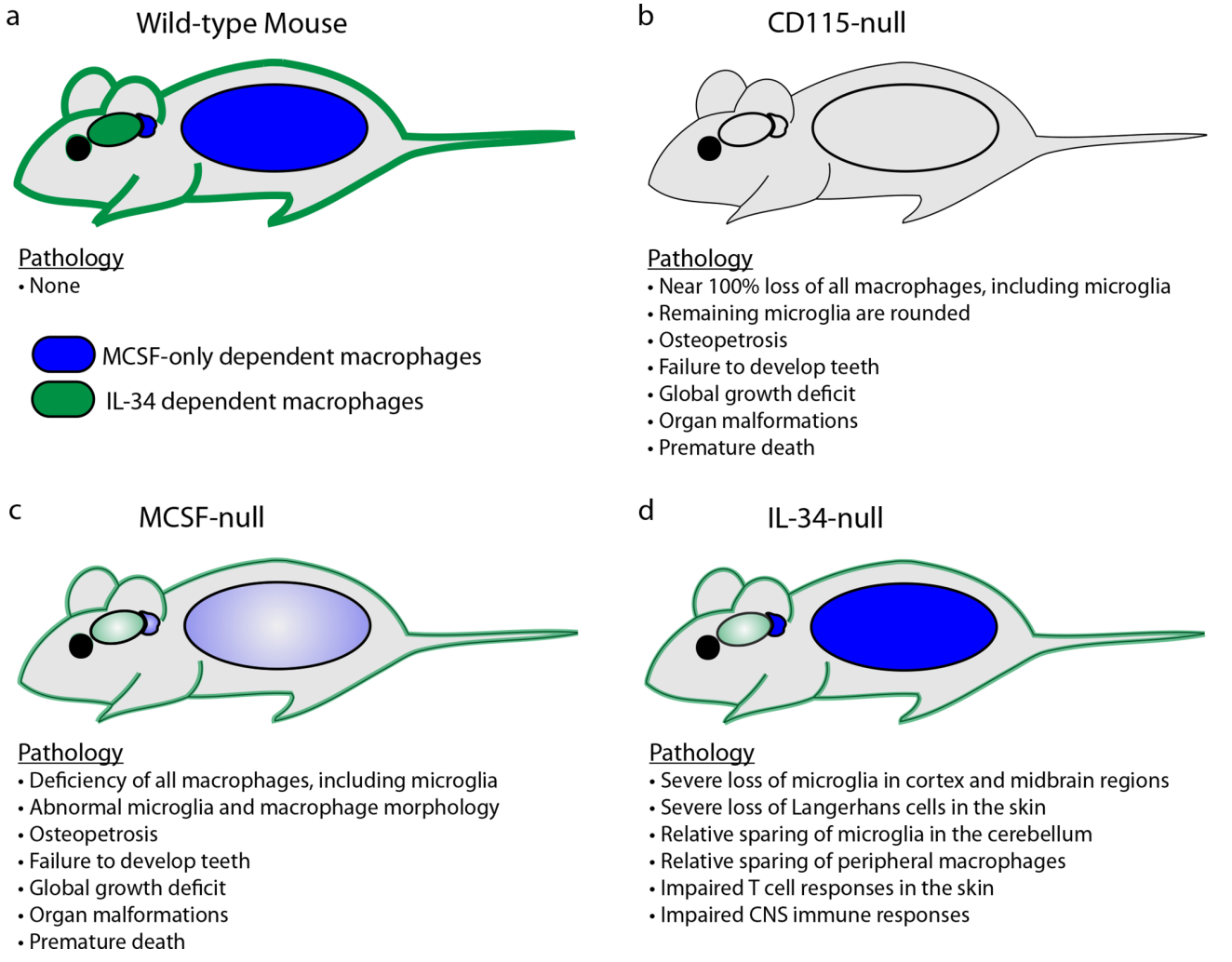
Although two distinct lineages of macrophages were identified, it was also found that PU.1-null mice contain neither yolk sac nor hematopoietic macrophages [15]. It was also recently shown that PU.1 is essential for microglia development [37]. PU.1 is also a critical transcription factor for macrophage development [38], therefore providing evidence of genetic similarity between hematopoietic-derived macrophages and yolk sac-derived macrophages. Similarly, bone marrow transplantation with immune ablation results in tissue-resident macrophage engraftment by the donor bone marrow [34]. In steady-state conditions, however, it has been shown that circulating monocytes contribute very little to the maintenance of tissue-resident macrophage populations, which are all largely self-renewing [39]. In regards to the brain specifically, it has been shown that very little or no replacement of microglia occurs without irradiation followed by bone marrow transplantation [32,36], chronic inflammation [40], or specific removal of microglia from the mature brain [41], strongly supporting the concept that under normal circumstances, microglia are entirely self-renewing and therefore independent of hematopoietic stem cells or monocytic progenitors.

CD115, MCSF, and IL-34: essential growth factor signaling

CD115 is the only known receptor for MCSF and IL-34, which are functionally overlapping, tissue-specific growth factors for macrophage differentiation, survival, and proliferation [42-44]. Severe growth and developmental defects occur in mice in which the critical receptor CD115 (also known as CSF1R and MCSF-R) has been knocked out [45,46] (Figure 2). Importantly, the near-complete absence of microglia in CD115 knockout mice results in perturbations of postnatal brain architecture and function, including enlarged ventricles and parenchymal compression [3]. While it cannot be completely ruled out that signaling through CD115 is important for processes other than macrophage development, the phenotypes of CD115 knockout mice are striking. They include, among others, osteopetrosis, lack of teeth, general growth deficit, organ malformation, and premature death [45,46]. These phenotypes were all reported to be similar to the $Csf1^{op/op}$ mouse (an inactivating mutation of CSF-1/MCSF [47-49]), in which mice that survive weaning have a 40% chance of dying before 12 months of age [47]. It has been reported that CD115 knockout mice are about 99% depleted of microglia, and those microglia that do exist have a rounded phenotype (unlike microglia in adult wildtype mice, which are heavily ramified [3]). Rounded macrophages/microglia can be a symptom of MCSF withdrawal, which leads to apoptotic cell death [50,51]. Rounding and apoptosis would logically result from IL-34 withdrawal as well, but this has not been directly shown. Since CD115 knockout mice are incapable of receiving MCSF or IL-34 signals, the rounded cells that do exist are likely in a state of growth factor withdrawal, and are therefore supposedly dying.

Interestingly, evidence suggests that although MCSF and IL-34 share the CSF-1 receptor (CD115), they are evolutionarily distinct and exhibit somewhat different effects [52,53]. However, expression of IL-34 at the MCSF locus can rescue defects seen in $Csf1^{op/op}$ mice [44], indicating at least some functional overlap between these distinct ligands. In wildtype mice, there are tissue expression differences between MCSF and IL-34 [43,44]. IL-34 LacZ reporter mice, in which the IL-34 locus has been replaced with a *LacZ* reporter gene and are therefore functional IL-34 knockouts, revealed that IL-34 is expressed in a tissue-specific manner, and is particularly important in certain regions of the CNS and in the

Figure 2. Role of CD115, Macrophage colony-stimulating factor, and interleukin-34 in microglia and macrophage development



a) Distribution of microglia and macrophages in a normal mouse. b) CD115-null mice lack nearly all microglia and macrophages, and have significant pathology. c) Macrophage colony-stimulating factor (MCSF)-null mice have severe deficiency of all microglia and macrophages, and similar pathologies to CD115-null mice. d) interleukin-34 (IL-34)-null mice have microglia and Langerhans cell deficiencies, and specific immune response defects.

skin. In these organs, IL-34 was found to be expressed by neurons and keratinocytes, respectively. Lack of IL-34 resulted in a loss of microglia in the CNS and Langerhans cells (skin-resident dendritic cells). However, Wang *et al.* noted that in the cerebellum, microglia numbers were near normal [43]. A plausible explanation for this is that MCSF is more important for microglia in the cerebellum, whereas IL-34 is the critical growth factor for microglia in other regions, including the cerebral cortex, hippocampus, and striatum. Together, the evidence shows that IL-34 and MCSF are critical for macrophage (and specifically microglial) development and that they have tissue-specific expression patterns, but it is unclear whether or not functional differences exist.

Functional and genetic relationships of microglia to other tissue-resident macrophages

The complexity and diversity of the macrophage family is further complicated by genetic analysis. Gautier *et al.* performed a study comparing the gene expression profiles of multiple macrophage and dendritic cell populations [54]. They found that dendritic cells from different organs clustered closely together, while different populations of tissue-resident macrophages had significant genetic diversity. These data suggest that tissue-resident macrophages are not only derived from a different source than monocyte-derived macrophages, but the diversity within tissue-resident macrophages is larger than their closest cellular cousins. In other words,

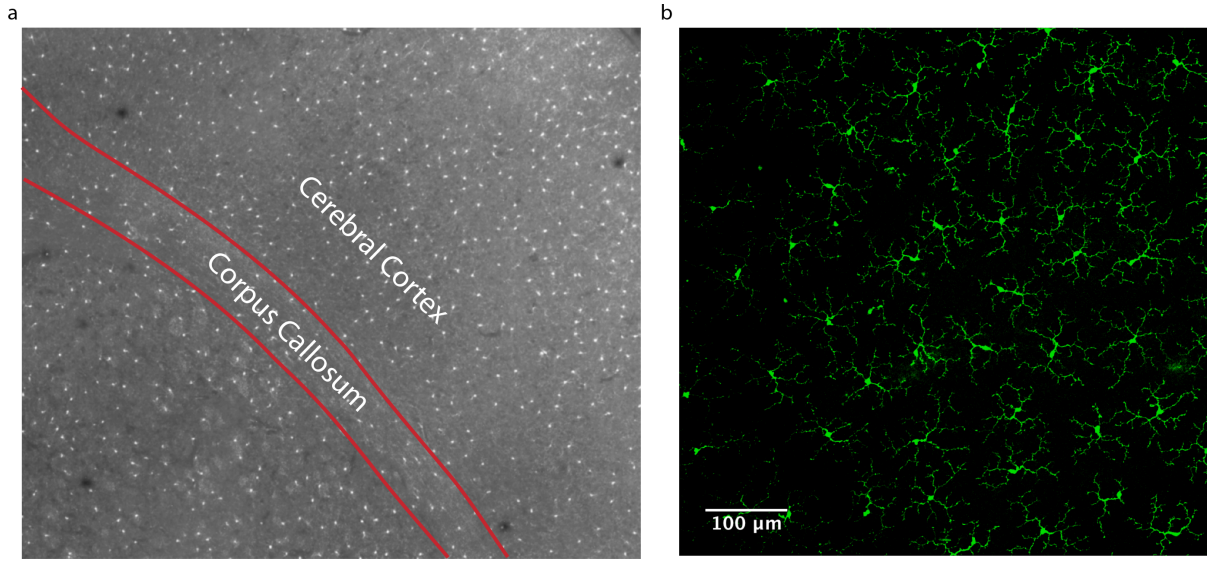
it is probably not unreasonable to assume that microglia, red pulp macrophages, and Kupffer cells may behave and function differently. Not surprisingly, the diversity of tissue-resident macrophages is supported by flow cytometric analysis, as identification of macrophages from each organ by flow cytometry typically requires different sets of markers specific to each organ's population [54]. Dendritic cells, on the other hand, tend to have common markers that can be utilized for any tissue [55]. Although a somewhat crude measure of cellular diversity, differences in surface markers between tissue-resident macrophage populations reflect the gene expression differences between each organ-specific population.

The diversity of tissue-resident macrophages is most likely derived from functional necessity, as each population must perform both general homeostatic and organ-specific tasks. When cells undergo apoptosis, or die via necrosis, it is the job of tissue-resident macrophages to clean up the debris [24]. In the brain, microglia are critical for the pruning of unneeded neuronal synapses in a complement-dependent manner [4,5] and removal of apoptotic cells during brain development and remodeling [7,19,24], among other functions. Red pulp macrophages in the spleen remove old, defunct erythrocytes from the blood [20]. Kupffer cells in the liver are critical for bilirubin metabolism [21]. Macrophages in general have been shown to be a major source of IGF-1, and it has been suggested that they are the largest extrahepatic source of this critical growth factor [22]. In addition, as previously discussed, a near-complete removal of macrophages via knocking out CD115 results in severe developmental defects [45,46]. It could be hypothesized that at least some of these growth and developmental defects are directly linked to macrophage-derived trophic factor deficiency. In summary, it is logical that the transcriptional diversity of tissue-resident macrophages is linked to the unique functions required for the maintenance of individual organs, but all tissue-resident macrophages share in common their primary role in the maintenance of tissue health, normal growth, homeostasis, and immunologic defense.

The homeostatic role of microglia

For many years, prevailing scientific thought dictated that microglia were static cells in the brain until an injury or infection occurred, at which point they became activated, inflamed, and performed their role as immune defenders of the CNS. Since then, advanced imaging techniques have revealed—to considerable surprise—that microglia are in constant motion [6], extending and retracting their processes, presumably sampling their environment in search of salient stimuli. Microglia maintain a regular pattern in the CNS, with individual cells covering a relatively non-overlapping and constant sized area within the tissue (Figure 3). Upon insult to the brain parenchyma, it has been observed that nearby microglia break their regular pattern and some

Figure 3. Appearance of microglia in the normal mouse central nervous system



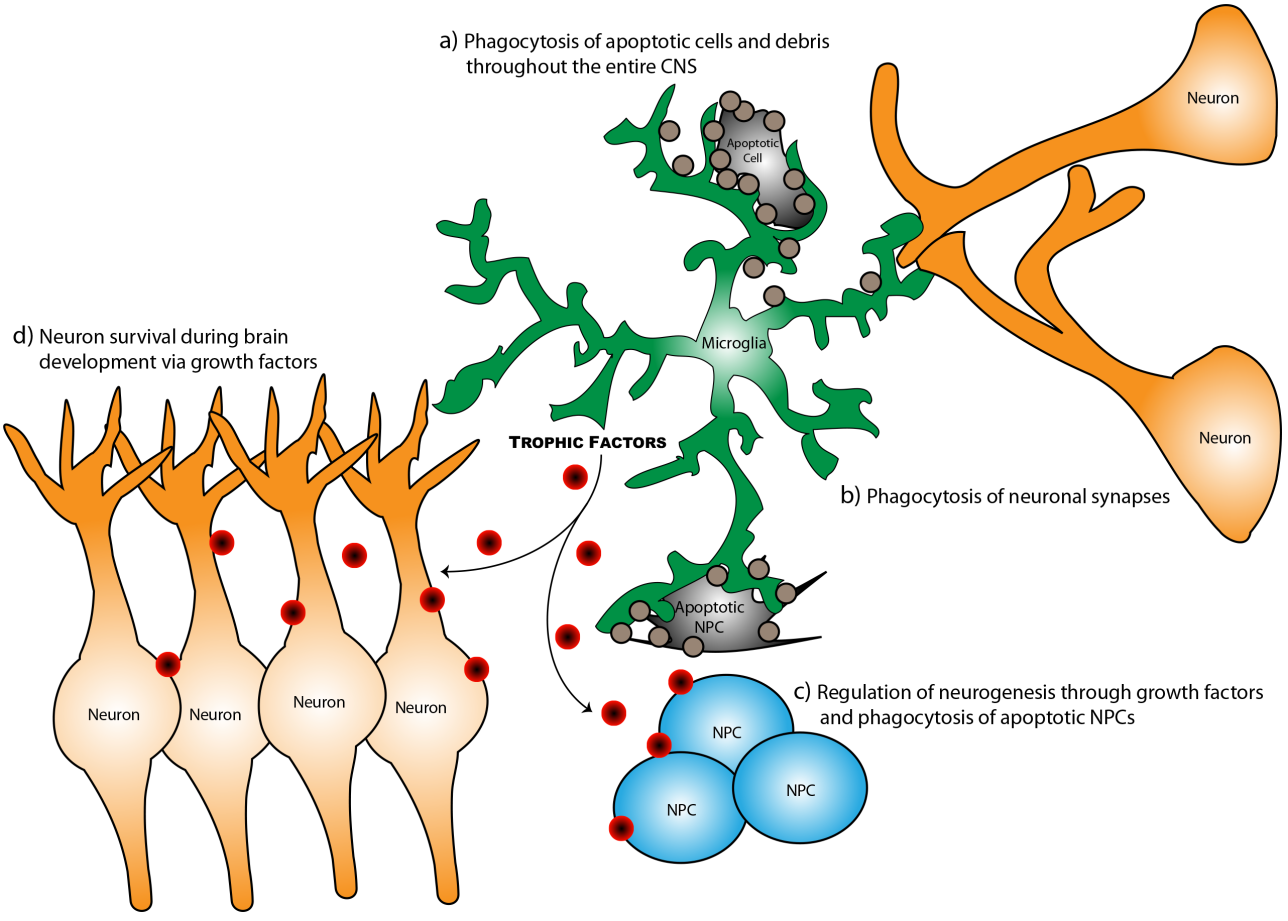
a) Representative image of mouse cerebral cortex from a CX3CR1$^{GFP/+}$ mouse, in which all microglia express green fluorescent protein (GFP). Microglia are regularly distributed throughout all brain regions. b) Representative image of microglia from the mouse retina (CX3CR1$^{GFP/+}$) demonstrating that each cell covers a defined, non-overlapping region.

will migrate quickly to the area of injury [6,56], suggesting that these cells truly are first responders to any breach of normal CNS homeostasis, with the presumed job of maintaining and returning the CNS to a healthy, homeostatic state.

Recent evidence suggests that microglia perform many homeostatic functions (Figure 4), such as regulation of neuronal synapses [4,5,11,12]. Interestingly, the CNS appears to utilize the complement system to achieve this goal. Complement is used in the periphery to tag and expedite elimination of apoptotic cells and pathogens [57]. One-way complement does this by facilitating phagocytosis by professional phagocytes via complement receptors on the surface of the cell. In the CNS, microglia phagocytose neuronal synapses that have been tagged with complement proteins via the membrane-bound complement receptor 3 [4]. This function appears to be critical for the elimination of weak or dysfunctional synapses during postnatal neurodevelopment [4,5].

In addition, microglia are important for neurogenesis. It has been shown that they can both influence differentiation of neural progenitors [23] and are also one of the phagocytes responsible for clearing the many progenitors that undergo apoptosis in the subgranular zone of the dentate gyrus [7]. Importantly, the ability of microglia to influence progenitor differentiation was linked to the induction of IGF-1 production, which was enhanced by the treatment of microglia with IL-4 [23], a molecule our lab has previously identified as important for learning and memory [58]. It has also been shown that

Figure 4. Homeostatic functions of microglia



a) Microglia are responsible for clearing apoptotic cells in the central nervous system (CNS) by phagocytosis both during postnatal neurodevelopment and throughout adult life. b) Microglia remove neuronal synapses that have been targeted for elimination. c) Microglia assist in neurogenesis both via aiding in the removal of apoptotic neural progenitor cells (NPC) and trophic support. d) Microglia support neuronal survival via release of trophic factors during neurodevelopment.

microglial-derived IGF-1 supports the survival of layer V cortical neurons during development [8]. As the brain develops, microglia are critical for inducing apoptosis and clearing apoptotic Purkinje neurons in the cerebellum [13], and also regulate the number of cortical neural precursor cells [9], placing microglia as key players in the maturing CNS, and therefore presumably its downstream function. As previously mentioned, CD115 knockout mice, which almost entirely lack microglia, have perturbations of postnatal brain development [3]. This suggests that microglia are primarily important for homeostasis and proper postnatal development of the brain, and in their absence, the brain is unable to properly maintain itself, eventually experiencing not only functional but also physical pathology.

Several studies have shown that disruption of normal, non-inflammatory microglial function appears to contribute to CNS disease. The gene encoding TREM2 (triggering receptor expressed on myeloid cells-2), which is only expressed in microglia in the CNS from an early developmental timepoint [59], has been implicated as an important molecule for promoting microglial phagocytosis of apoptotic neurons [14]. Mutations of TREM2 have been linked to the neuropsychiatric/degenerative Nasu-Hakola disease, suggesting that dysfunction of microglial phagocytosis may play a central role in the pathogenesis [59]. Mice that are mutant for the transcription factor *Hoxb8* exhibit pathologic grooming behavior, and are thought to be a model of trichotillomania, an obsessive-compulsive disorder (OCD)-spectrum disorder. It was identified that microglia express *Hoxb8* in the mouse brain, and that engraftment of wildtype microglia-like cells via bone marrow transplantation was able to rescue the pathologic, compulsory grooming behavior in mice [60]. Similarly, in Rett syndrome (an X-linked neurodevelopmental disease caused by mutations of *MECP2*), our group showed that bone marrow transplantation with engraftment of wildtype microglia-like cells, or selective genetic rescue of myeloid cells, including some microglia, in otherwise *Mecp2*-null mice ameliorates some disease symptoms and extends lifespan [61]. Interestingly, in both the *Hoxb8* and *Mecp2* studies, only certain pathologies were affected by engraftment of wildtype microglia-like cells, while others remained unaffected, presumably due to neuron or other CNS cell-specific defects. This suggests that as we learn more about these complex disorders, even those that are monogenic, the full picture of pathology is likely to be complex and multifactorial. However, it gives hope that we might be able to treat these disorders via the immune system, and more specifically, replacement or treatment of microglia to encourage healthy and/or restorative behaviors.

Conclusions

Microglia were originally discovered and described as unique immune cells of the brain, playing the role of immune defenders in an otherwise immunocompromised organ. As such, microglia have long been blamed for pathology in the context of inflammation and disease. We now understand that microglia, unlike inflammatory macrophages and other myeloid cells, are part of a larger family of cells: the tissue-resident macrophages. The primary job of these cells may be to maintain homeostasis and the health of tissues, rather than to fight off infection. In support of this concept, it has been shown that tissue-resident macrophages, including microglia, are derived from an entirely different developmental source than the rest of the immune system, arising early in development and engrafting as permanent residents of nearly every tissue, playing their role as caretakers of the body from an ontologically primitive stage. As such, the classical view of microglia as mediators of disease should be revised, and in the context of disease we might consider how these cells could be utilized, or encouraged, to perform their intended role in maintenance of health and CNS homeostasis.

Abbreviations

CNS, central nervous system; IGF-1, insulin-like growth factor 1; IL-4, interleukin 4; IL-34, interleukin 34; MCSF, macrophage colony-stimulating factor; MECP2, methyl-CpG binding protein 2; OCD, obsessive-compulsive disorder; TREM2, triggering receptor expressed on myeloid cells-2.

Disclosure

The authors declare that they have no disclosures.

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