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Neuro-immune dysfunction during brain aging: new insights in microglial cell regulation

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Microglia, the resident immune cells of the brain, are at the center of communication between the central nervous system and immune system. While these brain-immune interactions are balanced in healthy adulthood, the ability to maintain homeostasis during aging is impaired. Microglia develop a loss of integrated regulatory networks including aberrant signaling from other brain cells, immune sensors, and epigenetic modifiers. The low-grade chronic neuroinflammation associated with this dysfunctional activity likely contributes to cognitive deficits and susceptibility to age-related pathologies. A better understanding of the underlying mechanisms responsible for neuro-immune dysregulation with age is crucial for providing targeted therapeutic strategies to support brain repair and healthy aging.

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Introduction

The central nervous system (CNS) and immune system are intimately coupled, and maintain homeostasis through sophisticated bidirectional crosstalk [1]. However, the aged brain's ability to preserve the balance between the protective and possibly detrimental effects of the innate immune system is profoundly impaired. The low-grade chronic neuroinflammation that accompanies normal aging likely contributes to cognitive deficits and susceptibility to a plethora of age-related pathologies such as autoimmune diseases, cancer, and neurodegenerative diseases. As microglia are the brain's innate immune effector cells, dysregulation of microglial behavior appears to be a critical component of this progression [2]. This review discusses several recent developments in the understanding of neuro-immune dysregulation with age by focusing on regulatory mechanisms that normally constrain microglia but become dysfunctional with old age. Understanding the underlying mechanisms responsible for the age-related increase in brain inflammation is critical for improving the likelihood for successful aging.

Microglia at the nexus of immune-brain communication

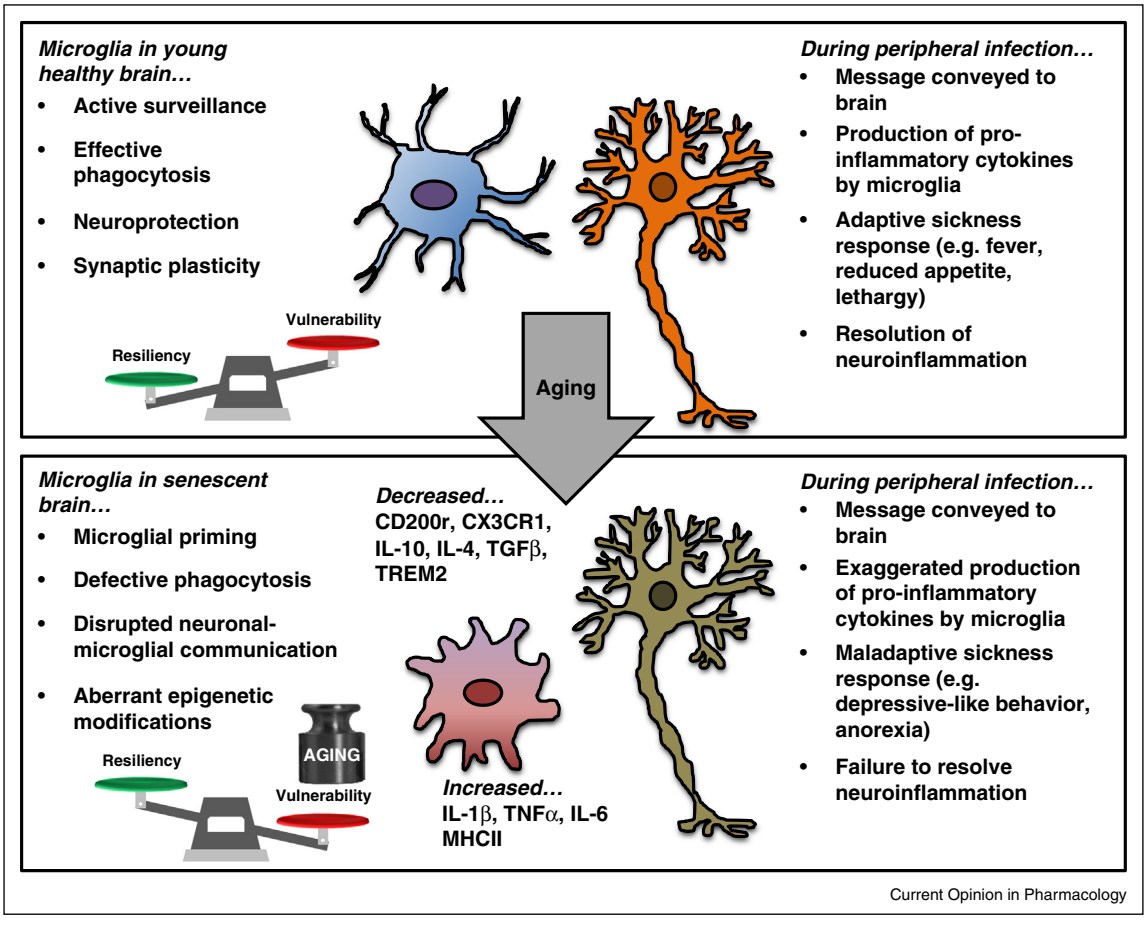
The brain was once thought to be an immune-privileged organ, devoid of lymphatic vasculature and considered pathological with immune cell entry. Now it is clear that interactions between the immune system and the brain are integral in eliciting behavioral, endocrine, and physiological modifications in the presence of peripheral inflammation. A key player in this interaction is the microglial cell, as this resident immune effector cell in the brain is responsive to signals emerging from the periphery [3,4]. The peripheral immune system is able to stimulate microglial cell activity through at least four major communication pathways: (1) diffusion of cytokines through leaky regions of the blood–brain barrier (e.g. choroid plexus and circumventricular organs), (2) passage of cytokines through energy-dependent, saturable transport systems on brain endothelium, (3) activation of cytokine receptors on endothelial cells that subsequently release cytokines or second messengers, and (4) transmission of cytokine signals via afferent nerve fibers, such as the vagus nerve. Further, with the discovery of functional lymphatic vessels lining the dural sinuses [5••,6], there is potential for yet another way in which brain-resident immune cells could communicate with peripheral immune cells. The lymphatic vessels have molecular characteristics of lymphatic endothelial cells, and act as direct clearance routes for the brain and cerebrospinal fluid (CSF) into the deep cervical lymph nodes. These lymphatic vessels also absorb CSF from the adjacent subarachnoid space and brain interstitial fluid via the recently discovered glymphatic system [7]. Irrespective of the communication pathway employed, a common outcome appears to be stimulation of microglial cells and induction of a neuroinflammatory response that elicits behavioral, endocrine, and physiological responses [8]. The large body of work in this area established beyond doubt the relevance of neuroimmune interactions and highlighted the fundamental role of microglia in neuroinflammation. The new findings concerning the glymphatic and lymphatic systems may form the basis for innovative therapeutic strategies to regulate microglia, for example

boosting growth of meningeal lymphatic vessels to help clear debris that becomes harder for microglia to phagocytize with age.

Microglia were first thought to be similar to macrophages, but it is now apparent that these cell types differ in ontogeny and functional capacity [9]. Unlike bone marrow-derived macrophages, microglia are derived from yolk sac progenitors and migrate to the CNS at approximately embryonic day 8.5 in mice [10]. Fate-mapping studies have also established that the transcription factor Myb, required for hematopoiesis, is not necessary for the generation of microglia [11], and that microglia specifically originate from CD45-c-kit+ erythromyeloid progenitor cells [12]. Therefore, microglia are not hematopoietically derived in the same way as macrophages. An additional unique feature is that in the adult brain, the microglial population is maintained by local self-renewal without significant contribution from bone marrow derived progenitors. Specifically, colony-stimulating factor 1 receptor (CSF-1R), but not colony-stimulating factor 1, is necessary for regulating the microglial lineage, because inhibition of CSF-1R in mice leads to depletion of 99% of the microglial population [13•].

In addition to their unique ontological properties, microglia perform a number of vital functions in maintaining brain homeostasis (Figure 1). During development, microglia regulate neurons in at least two important ways. First, microglia act as 'gate keepers' to monitor the number of neural precursor cells in the developing cerebral cortex. Cunningham *et al.* demonstrated that microglia selectively colonize the cortical proliferative zones in the developing neocortex, and phagocytize neural precursor cells in late

Figure 1



Age-related changes in microglia. Microglia participate in brain development by regulating the number of neuronal precursors and engaging in synaptic pruning. In adulthood they maintain tissue homeostasis through active surveillance of the brain parenchyma, effective phagocytosis to clear harmful debris such as Aβ aggregates, and facilitate synaptic plasticity by either supporting or removing synapses. Upon stimulation by signals emerging from the peripheral immune system, microglia produce pro-inflammatory cytokines to initiate adaptive behavioral and physiological responses. During aging however, microglia are primed to be more reactive to inflammatory stimuli, overproducing pro-inflammatory cytokines for a longer period of time resulting in maladaptive sickness responses. Age-related changes in neural-microglial communication and epigenetic modifications may underlie microglial cell hyperactivity.

stages of cortical neurogenesis [14•]. By activating microglia with lipopolysaccharide (LPS) or eliminating them with liposome clodronate, the neural precursor numbers were either reduced or increased, respectively, thus identifying a crucial role for microglia in controlling the size of the precursor population pool. Second, precursor cells that differentiate into neurons often make far more synapses than needed, and many become eliminated by microglia in a process called synaptic pruning. The complement system has been suggested to play a major role in mediating microglia-synapse interactions, and one study revealed that CR3/C3 signaling is specifically involved, as CR3 and C3 deficient mice have dramatic pruning defects [15].

In adulthood microglia are said to be 'resting' or 'quiescent', but they are in fact active surveyors of the CNS parenchyma, continually scanning the surrounding extracellular space to communicate with other brain cells to maintain tissue homeostasis [16]. Using *in vivo* two-photon microscopy of fluorescent-labeled microglia, it was established that processes of microglia make contacts with neuronal synapses for a brief duration of about 5 minutes, occurring about once every hour [17]. Moreover, after cerebral ischemia, the duration of these microglia–synapse contacts are prolonged for over an hour, and frequently followed by synapse elimination, suggesting that microglia can restore synapse function or initiate future removal.

Lastly, microglia play a fundamental role in clearing amyloid-β (Aβ) aggregates, which is thought to regulate the development and progression of Alzheimer's disease [18]. It is known that Aβ deposits have potent chemoattractant activity on microglia, but direct phagocytosis of Aβ by microglia has not been demonstrated *in vivo*. Although outside the scope of this review, it is important to highlight microglia as a key player in eliminating harmful debris within the brain parenchyma. As evidenced later, the aging of microglia progressively diminishes their normal regulatory capacity, potentially leading to insufficient phagocytic activity along with a host of other complications.

Microglial dysfunction with age

Despite the dynamic role of microglia in maintaining homeostasis, their long-lived nature and general inability to be replaced by circulating peripheral cells makes them particularly sensitive to oxidative stress, DNA damage, and a lifetime of inflammatory insults. Over time, these cells may become hypersensitive or primed in the healthy aged brain (Figure 1) [19]. The traditional view holds that microglia in the aged (but otherwise healthy) brain are primed towards a pro-inflammatory M1 phenotype with diminished anti-inflammatory (M2) properties. Approximately 25% of microglia isolated from aged mice stain positive for major histocompatibility complex class II compared to less than 3% of microglia isolated from young adults [20]. Furthermore, aging reduces expression of IL-4 receptor by microglia, rendering them less responsive to the M2-promoting effects of IL-4 [21]. This results in exaggerated immune responsiveness typified by increased pro-inflammatory cytokine production and signaling including upregulation of interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α both in whole brain and specifically in microglia [22,23]. Accordingly, when the immune system is challenged by a pro-inflammatory agent such as LPS, microglial activation is amplified and prolonged in the aged brain compared with adults, leading to exaggerated neuroinflammation, sickness behavior and cognitive deficits (Figure 1) [20,24]. This age-related microglial activity can also be brain-region specific [25,26]. For example, release of pro-inflammatory cytokines from microglia impair hippocampal-dependent memory by way of blocking long-term potentiation [25], and can activate NF-kB signaling in hypothalamic neurons to hinder neurogenesis and accelerate cognitive decline [26].

Given the complexity of microglial behavior, classifying them as only 'damaging' M1-phenotype and 'healing' M2-phenotype fails to recognize the subtle nuances within the spectrum of phenotypes and activation patterns necessary to understand neuroinflammation in various environmental conditions [27]. An increasing number of transcriptomic studies using primary microglia have revealed gene signatures extending far beyond upregulation of pro-inflammatory genes with age [28••,29,30]. Notably, Hickman *et al.* identified 100 genes specifically enriched in microglia that were collectively named the microglial sensome, thus creating a new way to discriminate between other CNS cells and immune cells [28••]. Surprisingly, the most upregulated genes in aged microglia belonged to neuroprotective pathways, whereas genes in neurotoxic pathways were downregulated. Therefore, studies of highly purified microglia taking into account the transcriptional profile instead of a limited number of markers are rapidly changing the way microglia are viewed [31].

Mechanisms that mediate microglial inflammation in aging

Within the aged brain, control of immune-sensing and regulatory pathways begins to break down, producing microglial priming and heightened responsiveness to inflammatory stimuli. Considerable findings have suggested an instrumental role of immune sensors such as inflammasomes [32], as they are necessary for activating caspases, which then cleave the precursor forms of pro-inflammatory cytokines into their active forms. Of note, it was found that aged nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3 $(NLRP3)^{-}/^{-}$ mice were protected from age-related increases in astrogliosis, microglia activation, elevation of IL-1β and TNF-α, and displayed cognitive improvement [33]. In addition, aging-associated increases in interferon and complement pathways were reduced in mice

deficient in NLRP3 or its associated inflammasome adapter proteins.

To maintain control of microglial activation, neurons produce several immunomodulatory factors that interact with microglial receptors, including the most well known ligand-receptor pairs CX3CL1-CX3CR1 and CD200-CD200r. Aging is associated with reduced signaling of both of these pairs [34,35], and mice deficient in either CD200 or CX3CR1 exhibit an activated microglial phenotype [36,37]. In addition, triggering receptor expressed on myeloid cells 2 (TREM2) is a microglial surface receptor that belongs to the immunoglobulin superfamily. TREM2 is reduced in aged microglia [28••], and is suspected to be required for removal of damaged myelin and support myelin regeneration by oligodendrocytes. TREM2 deficiency produced fewer microglia with an abnormal morphology in aged mice, particularly in the corpus callosum, which is rich in myelin and may provide a major stimulus for TREM2 signaling [38]. Transforming growth factor β1 (TGF-β1), a protein that has protective properties in the brain, was demonstrated to be critical for microglia to populate the brain [39••]. By transcriptional profiling of purified microglia, Butovsky *et al.* uncovered a unique gene signature present in both mouse and human primary microglia, but absent in cultured microglia, recruited monocytes, and other brain cells. This signature reemerged in cultured microglia after addition of macrophage colony-stimulating factor and TGF-β1. To demonstrate an *in vivo* role for TGF-β1, mice deficient for TGF-β1 in the CNS had microglial loss, late onset motor dysfunction, and defects related to glutamate recycling and synaptic plasticity [39••,40]. Additionally, inhibition of TGFβ signaling in the brain produced exaggerated microglial activation and sickness behavior after LPS [41].

Unlike most other tissue macrophages, adult microglia express high levels of the fractalkine receptor CX_3CR1. Several recent studies have utilized unique mouse models that for the first time allow for specific genetic targeting of microglia in the adult brain, particularly the generation of $Cx3Cr1^{creER}$ mice [42–44]. For example, the specific deletion of transforming growth factor-b-activated kinase 1 in microglia reduced disease course of experimental autoimmune encephalomyelitis [43], and conditional removal of brain-derived neurotrophic factor from microglia resulted in deficits in motor-learning tasks and learning-dependent synapse formation [44]. A method for generation of microglial cells derived from embryonic stem (ESdM) cells has also been described [45]. ESdM develop in a mixed neural culture through a yolk sac-like primitive macrophage intermediate, which mirrors the *in vivo* situation of microglial development. When these cells were genetically modified to express the neuroprotective neurotrophin 3, mice recovered from EAE. This suggests that microglia could be used as delivery vehicles or even as therapeutic targets themselves.

Epigenetics and microglial regulation

The cellular and physiological phenotypic trait variation evident in aged microglial cells is likely related to epigenetic changes such as DNA methylation and histone modifications. To date, only a handful of studies have used high-throughput sequencing to address the epigenetic landscape of primary microglia [46,47], and there is yet to be a study comparing young to old. Fortunately, a number of recent studies have investigated specific epigenetic regulators in microglia. Sirtuin 1 (SIRT1) is a member of class III histone deacetylases and is known to play a role in longevity [48]. SIRT1 deficiency in aged microglia is related to increases of IL-1β transcription and decreased methylation of CpG sites within the IL-1β proximal promoter [49•]. As epigenetic regulation of other pro-inflammatory genes such as TNF-α and IL-6 has been demonstrated in other immune cell types [50,51], it will be pertinent to explore this possibility in microglia. It has also been observed that the suppressing histone H3K27me3 demethylase Jumonji domain containing 3 (Jmjd3) in the substantia nigra *in vivo* caused exaggerated microglial activation and provoked greater dopamine neuron death in a mouse model of Parkinson's disease [52]. Moreover, the Jmjd3 level was lower in the midbrain of aged mice, which was accompanied by an elevated level of H3K27me3.

Micro-RNAs (mi-RNAs) are small non-coding mRNAs that can negatively control their target gene expression post-translationally, and have been suggested to participate in epigenetic machinery [53]. Importantly, an increasing number of miRNAs are being associated with specific microglial phenotypes. Of note, mirR-155 is the most upregulated miRNA in primary microglia stimulated with LPS, and an emerging hypothesis is that miR-155 is required to skew microglia to a more pro-inflammatory M1-like phenotype [54]. Another report also indicates that insulin-like growth factor 1 and CX3CL1 are reduced in aged microglia and associated with increased levels of miR-29b [55]. Lastly, dietary factors such as resveratrol, luteolin, and curcumin regulate microglia [56], and have been shown to modify the epigenome [57].

Coda

The pathways shared between the CNS and peripheral immune system represent therapeutic targets in preventing neuro-immune dysregulation in aging. As microglia are at the center of communication between these two systems, uncovering ways to protect the balance between protective and detrimental effects of these cells is a high priority. Aging results in the loss of integrated regulatory networks needed to maintain appropriate microglial function, including aberrant signaling from other brain cells, immune sensors such as inflammasomes, and epigenetic

modifiers such as histone deacetylases and miRNAs. Pharmacological manipulation of these regulators could direct microglia towards conditions that support brain repair and tissue homeostasis, through subtle adjustment of their continuum of phenotypes.

Conflict of interest statement

Nothing declared.

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