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Contributions of monocytes to nervous system disorders

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Abstract

Monocytes are a class of leukocytes derived from progenitors in the bone marrow and are prevalent in the blood stream. Although the main function of monocytes is to provide innate immune defenses against infection and injury, their contributions to the central nervous system (CNS) disorders are increasingly recognized. In this review article, we summarize the molecular and physiological properties of monocytes in relation to other myeloid cells. Primarily, we discuss how monocytes (or leukocytes) may affect neuronal function in diseases that are characterized by dysregulated innate immunity and cognitive dysfunction. Under these pathological conditions, monocytes and monocyte-derived cells (1) fail to remove neurotoxic products from CNS; (2) interact with astrocytes at the periphery-brain interfaces to alter synapse development and plasticity, or (3) infiltrate into the CNS to exacerbate neuroinflammation. Through these cellular mechanisms, we speculate that monocytes and other peripheral immune cells may affect brain functioning and contribute to behavioral and cognitive deficits. Better understanding of neuroimmune interactions will help the development of strategies to ameliorate neuronal and cognitive dysfunction associated with dysregulated innate immunity.

Keywords

Monocyte; Leukocyte; Bone marrow; Synapse plasticity; Cognition; Astrocyte

Leukocytes are divided into two distinct lineages: the cells derived from myeloid progenitors, including monocytes, monocyte-derived cells (*i.e.*, macrophages and dendritic cells), neutrophils, eosinophils, tissue-resident macrophages; and cells of lymphoid lineage, including T and B cells, as well as innate lymphoid cells which are derived from lymphoid progenitors [1, 2]. Bone marrow (BM)-derived leukocytes protect the body against infectious diseases and modulate immune responses to injury. Recent studies, however, suggest that the function of BM- leukocytes may extend beyond the borders of immunology. This includes the modulation of cognitive processes such as learning and memory [3–6].

Monocytes are short-lived circulating leukocytes. Based on the expression of cell surface markers, at least two subsets of blood monocytes have been identified in both humans and rodents, namely, classical or inflammatory monocytes and non-classical or resident monocytes [7] (see reviews in [2, 8]). *Bona fide* inflammatory monocytes are early responders to infections and contribute to the central nervous system (CNS) inflammatory

diseases (see [9]), whereas resident monocytes patrol blood vessels and participate in tissue repair [10]. A recent study indicates that resident monocytes modulate learning and learning-dependent cortical synapse remodeling during viral infection [6]. Moreover, multiple classes of infiltrating monocytes and monocyte-derived cells have been observed in the CNS during the inflammatory phases of experimental autoimmune encephalomyelitis [11]. These findings open new avenues of research to explore how peripheral immune system orchestrates neural and behavioral changes in health and disease.

Origin, ontogeny and molecular characterization of myeloid cells

Monocytes and macrophages are mononuclear phagocytes. These myeloid cells were initially considered as part of a developmental continuum of cells originated from the same BM progenitors [1]. Subsequent studies showed that mononuclear myeloid cells comprise a complex multicellular network. Monocytes, monocyte-derived macrophages, dendritic cells (DC) and tissue-resident macrophages may originate from distinct progenitors [12, 13]. Monocytes originate from definitive hematopoietic stem cells (HSC) which are cells capable of self-renew and give rise to all types of blood cell precursors. Hematopoietic stem cells are located in the liver and spleen in the embryo and move to BM during postnatal life [8]. In contrast, microglial cells, a class of CNS-resident macrophages, originate from yolk sac primitive macrophages (**Fig. 1**) [12, 14, 15]. Under physiological conditions, circulating monocytes replenish a small fraction of brain macrophages (e.g. choroid plexus macrophages) [16]. Thus, the cells of myeloid lineage form a “layered system” in peripheral and brain tissues (excluding the blood) (see [17]). CNS-resident macrophages comprise microglia within the parenchyma and macrophages at the brain’s borders (e.g. leptomeninges and perivascular spaces) [16]. Monocyte-derived macrophages are continuously replenished by their BM progenitors, while tissue-resident macrophages show self-renewing dynamics [17]. The ability of microglia to self-renew during postnatal life was uncovered using a modified confetti multicolor fate mapping system [18] and *in vivo* two-photon imaging of microglia in the cortex [19].

The initial molecular characterization of monocytes came after searching for a common macrophage and DC precursor. These studies led to the identification of a clonogenic BM-monocyte progenitor derived from HSC, which is referred to as common macrophage and DC precursor (MDP). This precursor initially expresses the proto-oncogene c-Kit (CD117) and then CX3CR1, which commit them to differentiating into monocytes, monocyte-derived macrophages and DCs [20, 21]. A subsequent study showed that monocytes may also originate from common monocyte progenitors (cMoP) which give origin to monocytes and monocyte-derived macrophages, independently of DC development [22]. *In vivo* studies of adoptively transferred *Cx3cr1^{EGFP}* cells identified two main classes of monocytes: CX3CR1^{low}LY6C^{high} inflammatory monocytes and CX3CR1^{high}LY6C^{low} resident monocytes [7] (**Fig.2**). CX3CR1^{high}LY6C^{low} cells are less prevalent in blood than CX3CR1^{low}LY6C^{high} monocytes and reside mostly in the vasculature, which reasonably justifies their name as blood “resident monocytes”. The expression pattern of CX3CR1 and LY6C is conserved between murine and human monocytes [7]. Human monocytes are further classified into subsets based on the expression of CD14 and CD16 [23, 24].

During postnatal life, monocytes exist in various reservoirs, including BM, blood, spleen and liver. At lower prevalence, monocytes are observed in the cerebrospinal fluid (CSF) and perivascular circulation, but rarely in CNS parenchyma [25]. Recently, innovative molecular approaches have been employed to characterize peripheral monocytes [26, 27] and leukocytes in complex tissues such as CNS [11, 28]. Single-mass cytometry (CyTOF) fuses flow cytometry with basic mass spectrometry. By discriminating isotopes of different atomic weights without significant overlap, CyTOF is able to record over 40 cellular phenotypic and functional parameters. Using CyTOF, up to five subsets of LY6C-expressing monocytes have been identified in the mouse CNS (for details, see microglia subsets A, B, C and monocyte subsets D to H in [11]). Among them, only one subset (population F) was found at low incidence (<0.5%) in healthy CNS. All monocyte subsets show distinct migration dynamics and increased frequency in the CNS during experimental autoimmune encephalomyelitis. Notably, these properties of monocytes remain the same in mouse models of Huntington's disease and amyotrophic lateral sclerosis [11].

A physiological perspective for monocyte biology

Under physiological conditions, the number and composition of blood leukocytes remain relatively constant. Given the broad expression of CX3CR1 on myeloid cells [29], the lifespan of monocytes can be determined using *Cx3cr1^{creER};Rosa26^{YFP}* conditional mice in which the expression of Yellow Fluorescence Protein (YFP) is restricted to CX3CR1-expressing cells by a tamoxifen-dependent Cre recombinase, CreER [30]. Previous fate-map experiments have established that CX3CR1^{low}LY6C^{high} cells are the shorted-lived monocyte subset in circulation, exhibiting a lifespan of ~1 day; whereas CX3CR1^{high}LY6C^{low} monocytes present an extended half-life of 5–7 days [31]. Other leukocytes such as granulocytes, natural killer cells, and lymphocytes show distinct lifespans ranging from hours to weeks in the circulation. Splenic DCs and tissue-resident macrophages show longer lifespans of weeks to months [30].

Monocytes circulate between BM and blood. The egression of inflammatory monocytes from the BM is dependent on CC-chemokine receptor 2 (CCR2) [2]. Under physiological conditions, CX3CR1^{low}LY6C^{high} cells are released into the circulation and a fraction of them mature into CX3CR1^{high}LY6C^{low} monocytes. CX3CR1^{low}LY6C^{high} monocytes may also traffic from the blood stream to BM where they can differentiate into CX3CR1^{high}LY6C^{low} monocytes [31]. Under inflammatory conditions, however, circulating monocytes are rapidly recruited into the infected/damaged tissues in a CCR2-dependent manner and differentiate into inflammatory macrophages or DCs [2]. Both human and murine blood resident LY6C^{low} and LY6C^{int} monocytes show a relatively low turnover as compared to their immediate precursors [31, 32]. CX3CR1^{high}LY6C^{low} monocytes can “patrol” blood vessels, preserving vascular integrity and mediating the clearance of toxic products. Resident monocytes are recruited into the infected tissues in a CX3CR1-dependent manner [10]. They may also enter healthy tissues and differentiate into CD11b⁺ DCs [33] (**Figs. 2 and 3**). Understanding the dynamics properties of monocytes (*i.e.*, its half-life, turnover, and trafficking) is important for distinguishing them from longer-lived resident immune cells and investigating their distinct contributions to nervous system diseases.

The number of leukocytes in blood is also regulated by the autonomic nervous system. The autonomic sympathetic division regulates the release of leukocytes from BM and controls the production and activity of BM HSC [34, 35]. Physiological stress enhances the levels of norepinephrine as well as circulating hormones associated with hypothalamus pituitary axis. These hormones are released from sympathetic post-ganglionic nerves. Both glucocorticoids and catecholamines control the trafficking and activation of monocytes [36]. Pro-inflammatory cytokines stimulate the parasympathetic division of the autonomic nervous system, triggering a vagal-mediated inflammatory reflex which prevents the prolonged inflammatory responses [37].

Circadian oscillations of norepinephrine and CXCL12 regulate the number of blood leukocytes [38, 39]. Diurnal waves of CX3CR1^{low}LY6C^{high} cell egression from BM transiently elevate the number of inflammatory monocytes in the blood; a process hypothesized to prevent potential infections and sometimes referred to as “anticipatory inflammation”. These waves are not observed in the CX3CR1^{high}LY6C^{low} subset. Interestingly, lack of a circadian pattern alters monocyte homeostasis and renders mice susceptible to pathogenic inflammation, e.g, premature death after acute infections and increased immune responses to obesity [8, 40].

Roles of monocytes in neuropathology

We review several pathological conditions in which leukocytes, in particular monocytes and monocyte-derived cells, contribute to neuronal and cognitive dysfunction.

Alzheimer's disease.

Alzheimer's disease (AD) is a chronic and progressive neurodegenerative disorder with high prevalence in aged population. The neuropathological features of AD include the accumulation of fibrillogenic A β (42) forms, aggregation of tau tangles and alteration of innate immunity in the brain. As phagocytic cells, monocytes and resident macrophages may regulate A β deposition and clearance. Initial studies, however, showed that the absence of parenchymal macrophages has no effect on A β accumulation in various transgenic AD (Tg-AD) mouse models [41]. In Tg-AD mice depleted of resident microglia, the repopulated microglia in the brain parenchyma do not affect A β clearance [42]. Although the origin (peripheral vs. central) of these repopulated microglial cells has been debated, a recent study showed that repopulated microglia originate from CNS-resident cells rather than from peripheral precursors [43]. A distinct subset of microglia has recently been identified near senile plaques of 5XFAD mice. These cells express the macrophage activation marker CD11c and phagocyte A β *in vivo* at early stages of disease. These microglial cells are not present in healthy brain and are thus referred to as disease-associated microglia (DAM) [44]. DAM has also been identified in APP/PS1 mice by CyTOF [28]. The turnover of DAM has not been described and it is unknown whether DAM are derived from infiltrating monocytes or share origin and phenotype with the activated microglia subsets described in [11] (populations B and C). Ajami and colleagues confirmed that activated B and C microglia subsets (phenotypically characterized from sorted CD45^{low} brain myeloid cells) are resident macrophages by using *Cx3cr1^{creER} Rosa26^{YFP}* conditional mice. In these mice, resident

macrophages express YFP >7 days after tamoxifen administration, whereas peripheral blood monocytes express YFP for a period shorter than 7 days.

CCR2 is a chemokine receptor expressed on peripheral inflammatory monocytes but not CNS resident macrophages (**Fig. 2**). In Tg-AD mice lacking CCR2, myeloid cells are not observed in senile plaques and do not eliminate brain A β [45]. Repetitive adoptive transfer of activated and genetically engineered monocytes into Tg-AD mice prevents A β accumulation and A β -associated pathology, reduces synapse loss and improves behavioral outcomes [46–48]. Transplantation of BM cells obtained from CCR2^{+/+} mice into irradiated symptomatic Tg-AD mice increases vascular A β clearance, but has no major effect on A β load in the brain. Inflammatory monocytes are recruited into senile plaques in Tg-AD mice that have been irradiated without head protection, but not when cranial radiation is excluded. In this study, the size of the plaques, A β load, and A β deposition are not associated with the accumulation of myeloid cells in the plaques [49]. Other studies, in contrast, reported that adoptively transferred monocytes are recruited into senile plaques of Tg-AD mice that have not undergone lethal irradiation. Moreover, selective depletion of CX3CR1^{high}LYC6^{low} monocytes *in vivo* increased A β load in both cortex and hippocampus [50], indicating a role of monocytes in eliminating vascular A β in Tg-AD mice. Consistent with these findings, monocytes collected from AD patients show reduced phagocytosis in response to A β [41]. In human patients, the CD33 risk allele is associated with the elevation of surface levels of CD33 in circulating monocytes, diminished internalization of A β 42, and amyloid pathology. CD33 risk allele is also associated with morphological signs of microglia activation and cognitive decline [51]. Nevertheless, all these studies suggest that distinct monocyte subsets and monocyte-derived cells contribute, although at different extents and with different efficiency, to removing toxic products from mouse brain. Whether the clearance of A β from brain senile plaques involves the recruitment of monocyte-derived cells, however, requires further investigation.

Leukocytes can circulate through a perivascular pathway situated alongside basement membranes of capillaries and outer wall of penetrating arteries [52], facilitating immune surveillance in conjunction with perivascular macrophages [16, 25]. Both leukocytes and CSF- borne macromolecules are thought to be drained into the dural lymphatic vessels [53, 54]. The perivascular circulation and meningeal lymphatics likely operate in conjunction and mediate the clearance of waste products and potentially toxic metabolites under pathological conditions [55]. We speculate that monocytes may use this extravascular pathway to clear neurotoxic products from brain parenchyma. It would be interesting to study whether these systems modulate the brain's innate immune responses during AD progression and under other pathological conditions.

A recent study on human frontal cortex showed that cell-specific co-expressed genes (namely modules) are related to immunity and mediate A β -associated pathology. Moreover, the modules implicated in cortical AD pathology are often different from those associated with cognitive decline [56]. With increasing evidence revealing the complexity of immune mechanisms in AD, it seems challenging to achieve translational results solely by enhancing the phagocytic function of myeloid cells. Besides promoting intravascular and extravascular

clearance of toxic products from brain parenchyma, using a comprehensive approach to correct the abnormal immune responses in AD brain seems necessary.

Traumatic CNS injury.

After traumatic CNS injuries, peripheral immune cells and molecules may permeate the CNS boundaries and contribute to secondary inflammation and neurodegeneration. The prevailing hypothesis was that monocytes enter the CNS through the disrupted BBB. However, a recent study showed that the monocytic precursors of pro-inflammatory macrophages enter the injured spinal cord through leptomeninges adjacent to the lesion site, whereas anti-inflammatory monocyte-derived macrophages access the injury sites through a remote process that is orchestrated by the brain's choroid plexus and requires intact CSF circulation [57] (**Fig. 4**). Once in the injured CNS, infiltrating monocytes differentiate into two distinct classes of macrophages: classically activated pro-inflammatory (M1) macrophages and alternatively activated anti-inflammatory (M2) macrophages. M1 macrophages are neurotoxic and more prevalent at the sites of lesion within the first week after injury, whereas M2 macrophages are abundant one week after injury and thought to be involved in CNS repair and regeneration [58]. Interestingly, CX3CR1 deficiency in BM-derived leukocytes ameliorates spinal cord injury-induced neuropathology and functional deficits [59], possibly by altering the development of these macrophage subsets at the lesion sites or by affecting monocyte trafficking into the spinal cord. Consistently, in a mouse model of traumatic brain injury, better neurocognitive outcomes are observed either in CX3CR1 null mice that have reduced number of patrolling monocytes in circulation, or in wild type mice that have been depleted of this monocyte subset [60]. Furthermore, systemic depletion of mononuclear phagocytes (monocytes, macrophages and DCs) by Clodronate promotes recovery after spinal cord injury [61], while a microenvironment rich in tumor necrosis factor (TNF)- α favors the development and prevalence of M1 macrophages at the site of injury and the progression of secondary neurodegeneration after spinal cord injury [62]. Shechter et al have been able to promote recovery from spinal cord injury by enhancing monocyte infiltration and differentiation into an IL-10-producing antiinflammatory macrophage subset [63], which resembles the M2 phenotype. Together, these studies suggest that infiltrating monocytes contribute to secondary inflammation and tissue damage, and that the net balance of pro- and anti-inflammatory signals may determine the neurological outcomes after traumatic CNS injuries.

Stress.

Social stress is another pathological condition in which monocytes may infiltrate into CNS and affect behavior. In particular, social stress causes the recruitment of inflammatory monocytes into the brain regions that have been implicated in anxiety-like behaviors in mice [64]. In a rodent model of chronic stress, disruption of neuroimmune communication in the spleen abolished monocyte trafficking into the brain and prevented the development of anxiety after repeated social defeat stress (RSDS) [65], suggesting that the spleen is a major reservoir for the primed monocytes. Subthreshold stress with a single cycle of social defeat was enough to promote monocyte release from the spleen, recruitment to the brain and re-establishment of anxiety 1 month after the first cycle of RSDS. Understanding whether these infiltrating monocytes differentiate into M1 or M2 macrophages, as what occurs after spinal

cord injury, will help identify the immune signaling that mediates anxiety. In another study, BMCs obtained from stress-susceptible mice, in which leukocytes produce high levels of IL-6 after *ex vivo* LPS challenge, were transplanted into WT recipient mice that had been ablated of peripheral leukocytes before RSDS exposure. The results from this study show that stress responsive IL-6- producing leukocytes drive the development of social avoidance [66]. These results from laboratory animals resonate with clinical findings that patients with major depressive disorder have elevated levels of circulating CX3CR1^{low}LY6C^{high} monocytes and neutrophils and various serum proinflammatory cytokines including IL-6 and IL1- β [67].

Neurodevelopmental disorders.

Pre-natal and neonatal inflammation are risk factors for developing cognitive disorders later in life, such as autism and schizophrenia [68]. In rodents, following maternal immune activation (MIA), adolescent offsprings have elevated brain innate immune responses to physical and environmental stressors and are more susceptible to developing cognitive impairments later in life [69]. Maternal immune activation causes the elevation of IL-17 in maternal circulation, which is associated with autistic-like behaviors in offsprings [70]. Interestingly, monocyte-derived DCs, which are located in pregnant mother's small intestine, amplify Th17-mediated immune responses, and further contribute to cognitive impairment later in life [71]. As for MIA model, brain injury-induced neonatal neuroinflammation is associated with abnormal behaviors that resemble some of those observed in autistic children. Blocking Th17 lymphocyte trafficking into brain ameliorates tissue damage and neuronal dysfunction in a mouse model of neonatal inflammation [72]. These data suggest that peripheral leukocytes are key players in driving neuronal and cognitive dysfunction in neurodevelopmental disorders associated with perinatal inflammation.

Rett's syndrome (RTT) and Mecp2 duplication syndrome are neurodevelopmental disorders caused by mutations in a gene encoding methyl CpG binding protein 2 (*Mecp2*) [73]. Children with Mecp2 duplication syndrome show reduced lymphocytes and NK cell responses [74]. Furthermore, a recent study showed that Mecp2 deficiency exacerbates innate immune responses in peripheral monocytes and CNS macrophages in mice [75]. Although brain-resident macrophages appear not to be essential for the pathogenesis of RTT, microglial cells have been shown to contribute to neuronal circuit deficits in *Mecp2*^{-y} mice [76] as well as in Tg-AD mice [77]. Whether dysregulated peripheral immunity contributes to neuronal and cognitive deficits in Rett's and Mecp2 duplication syndromes remains unclear. Meninges are immediately adjacent to the CNS and can generate T cell- and macrophage-mediated long-lasting immune signals. Given the importance of immune signaling in social behaviors [5, 78], it would be interesting to study the involvement of meninges in the pathogenesis of neurodevelopmental disorders.

Infectious diseases.

Infections are often associated with changes in mood, sense of well-being, learning and memory. Some pathogens such as Lymphocytic Choroidmeningitis virus (LCMV) may enter the meninges from the circulation through the fenestrated vessels in the arachnoid membranes, choroid plexus and ventricles [79]. Learning deficits are observed in mice

persistently infected with LCMV and also those in which LCMV have been cleared by CD8⁺ T cells, suggesting that infection-induced brain dysfunction may persist after the clearance of the pathogen [80]. Furthermore, experimental challenge to meningeal immunity by intracranial LCMV infection initiates a powerful CD8-mediated immune response that causes the recruitment of monocytes and neutrophils into the meninges, leading to vascular pathology, epileptic seizure and death [81].

Through paracellular or transcellular paths across the blood-brain barrier (BBB), the brain is accessible to circulating pathogens [79]. The cellular components of the BBB (e.g. endothelial cells, pericytes, and astrocytic end feet) provide a rich niche for initiating peripheral and central innate immunity. Pathogen associated molecular patterns activate pattern recognition receptors expressed by the BBB, leading to the production of various cytokines including type I and type III interferons, IL1- β , and TNF- α [82]. While interferons may promote the restoration of BBB during inflammation, TNF- α and IL1- β can induce BBB breakdown. We have shown that peripheral immune responses to viral infection are sufficient to alter the dynamics of postsynaptic dendritic spines in the mouse cortex and cause learning deficits [6]. These synaptic and behavioral changes are mediated by CX3CR1^{high}LY6C^{low} monocytes through TNF- α signaling initiated in the periphery and do not require the function of CNS-resident macrophages (including microglia) (**Fig. 5**). Moreover, changes in dendritic spine remodeling occurred in the presence of a MMP-9 blocker which ameliorates BBB disruption induced by plasma elevation of IL1- β and TNF- α [6]. In blood, TNF- α may be necessary for the normal development of CX3CR1^{high}LY6C^{low} monocytes [83] and cell-cell interactions that allow the rolling and adhesion of monocytes on brain endothelial cells during peripheral inflammation [84]. Moreover, activation of TNF receptor in endothelial cells promotes IFN- β -mediated monocyte recruitment [85]. During systemic infections, IFN- β and CXCL10 signaling in brain endothelial cells plays a key role in altering synaptic plasticity and learning [86].

Similar to peripheral and meningeal infections, infections within CNS parenchyma also cause brain dysfunction. During CNS infection, parenchyma-resident cells, such as astrocytes and microglia, are the driving force for inducing neuronal dysfunction [79]. For example, brain infection by a neuroinvasive West Nile Virus (WNV) strain causes synapse loss through a complement-microglia-axis-dependent mechanism, leading to hippocampal memory impairment. Weeks after initial WNV infection, microglia retain abnormal phagocytic activity and disrupt synaptic connections in the absence of viral titers in the brain [87]. These findings provide another example in which the innate immune responses to infection, rather than the viruses per se, contribute to chronic cognitive decline.

Contribution of astrocytes to neuronal dysfunction

Peripheral inflammation may activate astrocytes and modulate neural plasticity and function. As the essential elements of tripartite synapse, astrocytes are integral part of neuronal circuits and respond to numerous neurotransmitters. Astrocytes also release glutamate, ATP and D-Serine, the so-called gliotransmitters (for review see [88]), as well as synaptogenic factors. Astrocyte-derived thrombospondins promote the formation of silent synapses during early postnatal development, while glypicans 4 and 6 induce functional glutamatergic

synapses. Astrocytes also produce molecules (e.g. SPARCS) that promote synapse elimination [89–91]. Astrocytic secretion of synaptogenic signals is increased after mechanical injury-induced inflammation *in vitro* [92]. The abnormal expression of these synaptogenic factors has been associated with synaptic malfunction in various neurodevelopmental diseases including RTT, Down and fragile X syndromes [91]. Therefore, it is likely that astrocyte-derived factors participate in inflammation-related synaptic alteration. Indeed, previous studies have shown that local increase of TNF- α in the hippocampal dentate gyrus activates astrocyte TNFR1, triggering calcium- dependent glutamate release, which leads to functional modification of hippocampal excitatory synapses [93, 94] (**Fig. 5**). Moreover, a recent study reported that in response to WNV infection, activated astrocytes release IL1 which subsequently disrupts neuronal circuits related to hippocampal memory through IL1R-dependent mechanisms [95].

Connexins may serve as a molecular link between peripheral inflammation and synapse plasticity. Connexins oligomerize in hexamers and form hemichannels that dock in the apposed membranes of two adjacent cells to form aggregates of intercellular gap junction channels (referred to as gap junctions) [96]. Connexin43 (Cx43) and Cx30 are highly expressed at astrocytic end feet, enabling the formation of astroglial gap junction networks that regulate synaptic plasticity by providing an activity-dependent intercellular pathway that sustains the supply of energetic metabolites from blood vessels to distal neuronal synapses [97]. Previous studies have shown that inflammatory mediators reduce gap junction-mediated intercellular communication [98, 99], which may disrupt astrocyte networks at the brain's boundaries (i.e., BBB and cortical layers near to meninges). We speculate that activated monocytes (or other leukocytes) generate an inflammatory microenvironment at the BBB and/or meningeal and perivascular spaces. This local inflammation alters intercellular gap junction communication in astrocytes, which in turn alters the metabolic supply to synapses. Lastly, in a scenario in which monocytes interact with the vasculature, endothelial connexins and pannexins could contribute to the permeation of cytokines through the blood-brain barrier and regulate astrocyte and neuronal signaling. It has been proposed that connexins and pannexins may either provide a direct diffusion pathway across the endothelial monolayer, or more likely, control $[Ca^{2+}]_i$ in endothelial cells and regulate the transportation and release of vesicles at the abluminal side of vasculature [100].

Conclusions

Although the major function of monocytes is to provide defenses against infection and injury, their impacts on brain function have been increasingly recognized. Under pathological conditions (e.g. traumatic injury, chronic stress, infections), monocytes may permeate the blood-CNS barriers, differentiate into macrophages and modulate neuronal function via secreting inflammatory mediators. In the diseased brain (e.g. AD), monocytes may fail to remove neurotoxic molecules (e.g. A β), which could adversely affect the environment in CNS parenchyma. Pro-inflammatory cytokines and other neurotoxic molecules alter synaptic connections and neural circuitry that are important for learning and memory, anxiety and social behaviors. Finally, monocytes can cause neuronal and behavioral deficits even in the absence of the brain infiltration, for example, by causing a cytokine-

mediated inflammatory cascade initiated in peripheral tissues and subsequently continued by CNS-resident glial cells.

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Figure 1. The origin and ontogeny of CNS-resident macrophages.

Tissue-resident macrophages originate from yolk sac progenitors known as eritromyeloid progenitors (EMP) that subsequently develop into c-Kit⁺CX3CR1⁻ (A1) and c-Kit⁻CX3CR1⁺ (A2) precursors. A1 and A2 progenitors populate peripheral tissues and mouse brain between embryonic days 9 and 10 (E9.5). They differentiate and give rise to early embryonic macrophages. In the CNS, these embryonic cells develop into mature macrophages that include parenchymal microglia and meningeal and perivascular macrophages at the periphery-brain interfaces.

Figure 2. The origin and fates of monocytes under physiological conditions.

The origin and fates of monocytes under physiological conditions. Monocytes originate from hematopoietic stem cells in the bone marrow (BM) through macrophage and DC precursors (MDP, which are c-Kit⁺CD115⁺CD135⁺LY6C⁻CD11b⁻) and common monocyte precursor (cMoP, which are c-Kit⁺CD115⁺CD135⁻LY6C⁺CD11b⁻). The inflammatory monocytes express CCR2, high levels of LY6C and low levels of CX3CR1 (CD11b⁺CD115⁺MHCII⁻CCR2⁺CX3CR1^{low}LY6C^{high} subset, abbreviated hereinafter and in the text as CX3CR1^{low}LY6C^{high} monocytes). Inflammatory monocytes egress out of the BM and into the circulation where they develop into a monocyte subset that lacks CCR2 and shows high and low expression levels of CX3CR1 and LY6C, respectively (CD11b⁺CD115⁺MHCII⁻CCR2⁻CX3CR1^{high}LY6C^{low} subset, abbreviated as CX3CR1^{high}LY6C^{low}). A heterogeneous monocyte subset expressing intermediate levels of LY6C (LY6C^{int}) has also been described in mice. Under physiological conditions, a small fraction of blood monocytes may infiltrate tissues and differentiate into monocyte-derived macrophages or DCs.

Figure 3. In vivo two-photon imaging of CX3CR1⁺ cells at the brain/leptomeninges interface. Time series of the same brain region of a living mouse showing the extension and retraction of CNS-macrophage processes (red arrow head) over 50 s. White arrow heads indicate a CX3CR1- expressing monocyte patrolling a vessel as it appears to crawl on one side of the vasculature at T₀₋₂₅, and move to the opposite side at T₅₀. CX3CR1-expressing cells are shown in green (GFP) and meningeal vessels were labeled with rhodamine-conjugated dextran (red). Images were collected at 0.5 frames per second with a two-photon microscope (4 representative images are shown at time 0, 12.5, 25, and 50 sec). These images are shown for the purpose of this review only. *In vivo* transcranial two-photon imaging was performed as previously described (see [6]).

Figure 4. Recruitment of monocytes into CNS in inflammatory diseases.

Under pathological conditions in which BBB is compromised (e.g. spinal cord injury), the defined conditions driven by local growth factors, cytokines, and pathogen products produced by CNS-resident cells favor the recruitment of circulating blood monocytes into CNS. Infiltrating monocytes differentiate into two classes of macrophages, classically activated pro-inflammatory M1 macrophages and alternatively activated anti-inflammatory M2 macrophages.

Figure 5. Astrocytes may transmit the inflammatory signaling initiated in the microvasculature, perivascular spaces and leptomeninges toward neurons in the brain.

Astrocytes show different subdomain specialization. Astrocytic end-feet represent an integral part of glio-vascular unit, parenchymal basal lamina surrounding the perivascular spaces, and leptomeninges that constitute the glia limitant. The terminal astrocytic processes (or leaflets) are closely related to the synapses. In the superficial layer of the cortex, the brain surfaces are represented by the leptomeninges and glia limitant (A). At the capillary level (B), there is little space between the vasculature and parenchyma, and the glial extensions and vascular basement membranes fuse together to form an integral part of the BBB.

Gap junction-mediated intercellular communication provides ultrastructural cytoplasmic continuity between adjacent astrocytes and allows the formation of astrocyte networks. These networks are prominent at the gliovascular interfaces and superficial layers of the cortex. Inflammation may reduce gap junction coupling between astrocytes, affecting BBB permeability and metabolic supply to the synapses.

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