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REVIEW

Monocyte chemoattractant protein-1 and the blood–brain barrier

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Abstract The blood–brain barrier (BBB) is a dynamic structure that maintains the homeostasis of the brain and thus proper neurological functions. BBB compromise has been found in many pathological conditions, including neuroinflammation. Monocyte chemoattractant protein-1 (MCP1), a chemokine that is transiently and significantly up-regulated during inflammation, is able to disrupt the integrity of BBB and modulate the progression of various diseases, including excitotoxic injury and hemorrhage. In this review, we first introduce the biochemistry and biology of MCP1, and then summarize the effects of MCP1 on BBB integrity as well as individual BBB components.

Keywords MCP1 · BBB · BMECs · Pericytes · Astrocytes · BM · Microglia · Neurons

Introduction

Monocyte chemoattractant protein-1 (MCP1, also known as CCL2) is a pro-inflammatory mediator, whose up-regulation is found in many central nervous system (CNS) disorders with blood–brain barrier (BBB) breakdown. Accumulating evidence suggests that MCP1 is able to compromise the integrity of BBB and modulate the progression of various diseases. Although several studies have explored the effects of MCP1 on BBB permeability regulation, the exact mechanism, especially the effects of MCP1 on pericytes, astrocytes, neurons, and basement membrane (BM), remain elusive. Here, we review the biochemical and biological functions of MCP1 with a focus on its role in BBB regulation. Understanding the molecular mechanisms underlying MCP1-induced BBB disruption not only broadens our knowledge on chemokines and BBB but also promotes the development of novel therapeutic reagents for many CNS diseases.

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Chemokines

Chemokines are a superfamily of structurally related small basic proteins with strong chemotactic activity. They function to induce cell-specific migration and activation of cells, especially immune cells, in response to insults [1–4]. Since the first chemokine was identified in 1977 [5], many chemokines and chemokine receptors have been identified [6]. Based on the number and position of conserved cysteine on their primary sequences, chemokines are divided into 4 sub-types: C, CC, CXC, and CXXXC [7–9]. The major functions of these chemokines include recruiting leukocytes during inflammatory conditions, maintaining ligand homeostasis between blood and tissue, and regulating developmental processes/disease progression, such as BBB permeability and myelination [10–12]. These biological functions are mediated through G-protein-coupled receptors [13]. Studies on chemokines and their receptors reveal promiscuity: one receptor may have more than one ligand and one ligand may have more than one receptor, which adds complexity to the studies of chemokines and chemokine receptors.

MCP1

MCP1, one of the most highly and transiently expressed chemokines during inflammation, is a member of the CC sub-type chemokines. In the brain, MCP1 is expressed by most cell types, including neurons, astrocytes, microglia, and brain microvascular endothelial cells (BMECs) [14–27]. MCP1 is synthesized with a signal peptide in its N-terminus, which is removed during secretion. The secreted MCP1 binds to soluble glycosaminoglycans (GAG) and GAG immobilized on the cell surface and the extracellular matrix [28–34]. This interaction is predicted to induce dimerization/oligomerization of MCP1, increase its local concentration, and promote formation of chemokine gradients [28, 35, 36].

Although the N-terminus of MCP1 is highly homologous among different species, the C-terminus is not. Human MCP1 has 76 amino acids, whereas mouse MCP1 has a C-terminal extension with about 50 amino acids. It has been shown that the C-terminal extension of mouse MCP1 is heavily O-glycosylated [37]. What is the function of the C-terminal extension of mouse MCP1? We have previously been able to show that plasmin cleaves mouse MCP1 and removes its C-terminal extension (see "Microglia" for details). Using recombinant wild-type and modified mouse MCP1, we further show that wild-type mouse MCP1 is able to dimerize, and that the mutant without C-terminus fails to dimerize [38]. Additionally, the C-terminus alone interacts with neither wild-type nor mutant mouse MCP1, suggesting that the highly glycosylated C-terminal extension of mouse MCP1 is necessary but not sufficient for dimerization. Surprisingly, human MCP1, which is highly homologous to the N-terminus of mouse MCP1, forms dimer in physiological concentration, and the residues crucial for the dimerization have been identified as amino acids 6–16 [39]. In agreement with this report, two mutant forms of human MCP1, P8A and Y13A, have been reported to be unable to dimerize [40].

Being able to form a dimer does not necessarily mean it functions as a dimer. The next question is whether MCP1 functions as a monomer or dimer. Zhang and Rollins showed that chemical crosslinked human MCP1 dimer was functional in attracting monocytes in vitro [39]. Furthermore, a mutant form of MCP1, 7ND, which lacks residues 2–8, has been shown to inhibit the function of wild-type MCP1 but not crosslinked MCP1 [39], suggesting that 7ND is a dominant-negative mutant and that MCP1 functions as a dimer. P8A mutant MCP1, on the other hand, has a binding affinity for CCR2 similar to wild-type MCP1 and induces calcium influx and chemotaxis at the same level as wild-type MCP1 [40], suggesting that MCP1 works as a monomer. Additionally, 7ND MCP1 has also been shown to function as a competitive inhibitor of monomeric MCP1 [40]. Consistent with the monomer theory, we have previously been able to demonstrate that, compared to full-length mouse MCP1, the mutant variant without C-terminus, which cannot form a dimer, more efficiently activates Rac1 and promotes the formation of lamellipodia [38].

MCP1 exerts its biological functions by binding to its high affinity receptor, CCR2, which is mainly expressed by microglia, astrocytes, and BMECs in the brain [41, 42]. Although MCP1 has only one high affinity receptor, CCR2 has four more ligands (CCL7, CCL8, CCL12, and CCL13) besides MCP1 [43–45]. In rodents, only one CCR2 isoform is found, whereas two alternatively spliced CCR2 isoforms with different C-terminus [46] are found in human, denoted CCR2A and CCR2B. CCR2B is mainly expressed on monocytes and activated NK cells, whereas mononuclear cells and vascular smooth muscle cells predominately express CCR2A [47].

MCP1 AND BBB

The BBB is the largest CNS barrier, and sustains brain homeostasis and thus proper neurological functions. At the BBB, specialized endothelial cells (BMECs), astrocytes, pericytes, basement membrane (BM), neurons, and microglia can be found [48]. The BMECs connect to each other via a complex network of tight junctions, which create the primary barrier and prevent paracellular transport across endothelial cells. These cells deposit a layer of BM (endothelial BM), in which pericytes are embedded. Astrocytes, which wrap BMECs and pericytes with their endfeet, deposit another layer of BM (parenchymal BM). These two layers of BM are not distinguishable except at the post-capillary venules, where a cerebrospinal fluid-drained perivascular space separates them. Neurons and microglia, which exist in the parenchyma, have direct contact with astrocytes and BMECs. The above-mentioned cells together with both endothelial and parenchymal BM are necessary for an intact barrier of the BBB [49]. Perturbation of BBB has been found in many neurological conditions, including trauma [50, 51], brain tumors [52, 53], stroke [54–56], and neurodegenerative diseases [57–61], and BBB breakdown is one of the hallmarks that accompany the progression of these diseases. Recently, BBB compromise has been found to play a causative role in the onset of Alzheimer's disease [57] and amyotrophic lateral sclerosis [62]. A large number of molecules have been reported to affect the permeability of BBB, such as MCP1, TNF-α, IL-1β, IL-10, and IFN-γ [63–71]. Here, we focus on the effect of MCP1 on BBB integrity.

It has been shown that injection of recombinant mouse MCP1 into the brain disrupts BBB integrity [65–67, 71, 72]. We have previously been able to further demonstrate that plasmin-mediated truncation of MCP1 is indispensable

for MCP1-induced BBB compromise [71]. Consistent with our report, tPA, which converts inactive plasminogen to active plasmin, has been found to promote BBB disruption and subsequent peripheral blood mononuclear cell (PBMC) infiltration [73]. Additionally, BBB compromise and PBMC infiltration have also been found in mice deficient for plasminogen activator inhibitor-1 [74], suggesting that the effect of MCP1 on BBB is dependent on plasmin activity. MCP1, however, failed to compromise BBB integrity in CCR2$^{-/-}$ mice [67], suggesting that the effect of MCP1 on BBB also depends on CCR2. The next question then becomes how exactly MCP1-CCR2 axis affects the integrity of BBB. The roles of MCP1-CCR2 on individual BBB components are summarized below.

BMECs

The capillary endothelium in the brain is 50–100 times tighter than that in the periphery [75]. Compared with peripheral endothelial cells, BMECs are characterized by the presence of more mitochondria, less pinocytotic activity, lack of fenestrations, and the presence of tight junctions [76–79]. BMECs connect to one another forming an impermeable monolayer. In the interendothelial space, specific structures, including adherens junctions and tight junctions, are present [68, 77, 80, 81]. Although both adherens and tight junctions act to limit paracellular permeability of endothelial cells [82], tight junctions are the primary structure that maintains the impermeability of BBB [83]. In the tight junctions, many tight junction proteins are expressed. There are two types of tight junction proteins: transmembrane ones, such as occludin and claudin-1, -5, and -11, and cytoplasmic accessory proteins, such as zonula occluden-1, -2, and -3 (ZO-1, -2, and -3) and cingulin [84, 85]. The transmembrane proteins, especially occludin, function to seal gaps between adjacent cells [86–89]. Occludin, a 60- to 65-kD transmembrane protein with its N- and C-terminus in the cytoplasm, has been shown to maintain the integrity of BBB [90–93]. There is also evidence showing that the phosphorylation state of occludin regulates BBB integrity by affecting its membrane association [94–100]. Cytoplasmic accessory proteins, on the other hand, link transmembrane proteins to cortical actin-based cytoskeleton [86–89]. ZO-1, the first identified accessory protein [101] that links occludin to actin cytoskeleton [102], plays a critical role in regulating BBB permeability. Dissociation of ZO-1 from the tight junctions has been shown to accompany the disruption of BBB [70, 71, 103–105]. In addition, ZO-1 has been detected in the nucleus and shown to co-localize with transcription factors in some conditions [106–109], suggesting its potential role as a signaling molecule.

BMECs as the major barrier of BBB have been under extensive investigations. Accumulating evidence shows that MCP1 compromises BBB integrity via redistribution of tight junction proteins from cell–cell border (probable via endocytosis) and reorganization of actin cytoskeleton in BMECs [65–67, 72]. We have previously been able to verify these data and further show that these changes are dependent on plasmin activity [71]. Mechanistic studies reveal that phosphorylation of TJP regulates their functions and locations [95, 96, 110–112]. Stamatovic and colleagues further demonstrated that the binding of MCP1 to CCR2 activated PKC (specifically PKCα and PKCζ) and Rho kinase, resulting in shift of tight junction proteins from cell border to intracellular compartments [65, 66]. Additionally, this phosphorylation event also promoted the interaction between tight junction proteins and actin cytoskeleton, resulting in a shift of tight junction proteins from Triton X-100 soluble fraction to Triton X-100 insoluble fraction [65–67, 113, 114]. These changes are not limited to MCP1, because growth factors (PDGF and VEGF) also induce phosphorylation and redistribution of tight junction proteins [115, 116], suggesting that phosphorylation of tight junction proteins may be a common mechanism to transport them to different cellular compartments. In addition to tight junction protein phosphorylation, the activated kinases, especially Rho Kinase, also phosphorylate myosin light chain phosphatase (MLCP) and inhibit its activity. The inhibition of MLCP results in enhanced phosphorylation of myosin light chain (MLC), leading to increased actin–myosin interaction and thus increased cortical force in endothelial cells [66, 117–119]. In addition, we have previously been able to demonstrate that binding of MCP1 to CCR2 on BMECs also promotes phosphorylation of Ezrin/Radixin/Moesin (ERM) proteins on conserved Threonine residues (Thr567 for ezrin, Thr564 for radixin, and Thr558 for moesin) [71]. ERM proteins are a family of highly conserved proteins that act as a linker between plasma membrane and actin cytoskeleton. Unphosphorylated ERM proteins form intramolecular interactions between the N- and C-terminus. When phosphorylated on the conserved Threonine residues, they form intermolecular interactions (N-terminus binding to membrane proteins and C-terminus binding to actin cytoskeleton) [120–122]. Our data showed that MCP1 treatment led to phosphorylation of ERM proteins and promoted interaction between ZO-1 and phosphorylated ERM proteins [71], indicating an important role of ERM proteins in translocation of ZO-1 upon MCP1 treatment. Occludin, however, did not interact with ERM proteins even upon MCP1 treatment [71], indicating different ways of regulation. Taken together, we proposed a mechanism responsible for MCP1-induced BMEC and thus BBB changes as shown in Fig. 1. The binding of MCP1 to CCR2 activates unknown kinase(s), which phosphorylates ERM proteins. The phosphorylated ERM proteins then bind to ZO-1 and actin cytoskeleton. In addition,

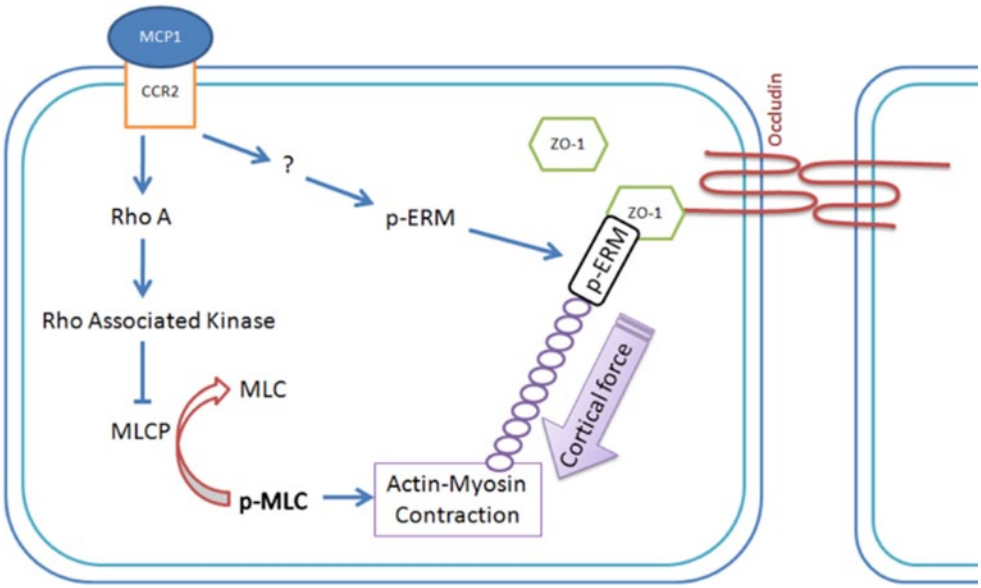

Fig. 1 Proposed model for MCP1-induced BBB compromise. By binding to CCR2, MCP1 induces phosphorylation of ERM proteins, which then bind to ZO-1 and actin cytoskeleton. Additionally, MCP1 also activates Rho-associated kinases, which phosphorylate and inactivate MLCP, resulting in increased phosphorylation of MLC. The over-phosphorylation of MLC induces enhanced and prolonged actin–myosin contraction, which generates forces that pull ZO-1 away from the cell–cell border, leading to BBB compromise. Adapted from [71]

Rho-associated kinase, which is activated by MCP1, phosphorylates MLCP, resulting in decreased phosphatase activity. The imbalance of MLCP and MLC kinase activity results in over-phosphorylation of MLC, and thus increased and prolonged actin–myosin contraction. This contraction in turn pulls ZO-1 away from the tight junction complex, leading to disruption of BBB integrity. In the BMEC–astrocyte co-culture system, Stamatovic and colleagues further demonstrated that lack of expression of CCR2 in BMECs was sufficient to prevent the leakage of BBB upon MCP1 treatment [67], suggesting that MCP1-induced BBB compromise is dependent on endothelial CCR2.

Astrocytes

The unique properties of BMECs (increased mitochondria number, few pinocytotic vesicles, and the presence of tight junctions [76–79]) lead to the question: is it due to intrinsic characteristics of BMECs or the microenvironment in the brain? Stewart and Wiley elegantly demonstrated that nonvascularized brain tissue grafted into the coelomic cavity developed capillaries with BMEC properties, whereas somite tissue grafted into cerebral ventricles failed to do so [123], strongly suggesting that interaction between vascular tissue and CNS tissue contributes to the properties of BMECs. In the brain, astrocytic endfeet together with the parenchymal BM establish the glia lamitans, which defines the parenchymal border in the CNS [49]. Astrocytic endfeet cover more than 99 % of the vascular surface [124, 125], suggesting that astrocytes may confer on BMECs those unique properties and thus contribute to the impermeability of BBB. Janzer and Raff found that astrocytes induced tight junction in endothelial cells within the eyes [52], indicating that astrocytes can enhance the impermeability of endothelial cells lining the capillaries in the eyes. In consistent with these data, the BMEC–astrocyte co-culture system showed a higher transendothelial electrical resistance (TEER) and less infiltration of tracers across the in vitro BBB than BMECs alone [71, 126– 129]. Additionally, the temporary focal loss of astrocytes has been found to parallel the compromise of BBB integrity in vivo [130]. Further experiments reveal that the role of astrocytes in BBB integrity is due to the release of soluble factors, including Ang1, TGF-β, GDNF, and FGF2 [131–133], as well as the direct contact with BMECs [134]. Recently, polarized distribution of intramembranous orthogonic arrays of particles (OAPs), which contain water channel aquaporin 4 (AQP4), the potassium channel Kir4.1, and a dystroglycan–dystrophin complex, has been reported in astrocytic endfeet [135]. Given the functions of AQP4 and Kir4.1 (regulation of water and ion homeostasis at the glial–endothelial interface, respectively), OAPs have been speculated to influence BBB permeability [133, 135]. Together, these data support that astrocytes contribute to the impermeability of BBB. It should be noted that the effect of astrocytes in BBB integrity only takes place in adulthood, because astrocyte development starts after birth [136]. There is also evidence, however, suggesting astrocytes may not contribute to the BBB integrity [137]. This inconsistency may be due to different experimental conditions or methodology.

Since astrocytes are a main source of MCP1, transgenic mice over-expressing MCP1 in astrocytes have been developed and used to study the function of MCP1. In addition to enhanced nociceptive responses in these transgenic mice [138], chronic expression of MCP1 in astrocytes induces BBB compromise in vivo, indicating a

crucial role of astrocytic MCP1 in BBB regulation [139]. In an in vitro system, it has been shown that MCP1 binding in human astrocytes is mediated by both CCR2 and D6 decoy chemokine receptors [140] and activation of CCR2 promotes the survival of astrocytes [141]. Additionally, caveolin-1 has been found to mediate the effects of MCP1 in astrocytes [142]. Knockout of CCR2 in astrocytes, however, failed to affect BBB integrity in the BMEC–astrocyte co-culture model [67], suggesting that CCR2 in astrocytes is dispensable for MCP1-induced BBB breakdown. This discrepancy may be due to the over-simplified in vitro BBB models used. Future studies should focus on in vivo studies or use in vitro BBB models that replicate both the anatomical and physiological characteristics of the BBB.

BM

Two layers of BM are found at the BBB: endothelial BM and parenchymal BM [49, 143]. The two layers are morphologically indistinguishable except at the post-capillary venules, where a cerebrospinal fluid-drained perivascular space separates them [49]. BM consists of a mixture of extracellular matrix (ECM) proteins, including collagens, laminins, heparin sulfate proteoglycans, fibronectin, vitronectin, nidogens, perlecan, and agrin [131, 144–147]. BM assembly mainly involves polymerization of laminins and collagens, which is cross-linked by nidogens [147, 148]. Although most ECM proteins are ubiquitously expressed at both endothelial and parenchymal BM, the former mainly expresses laminin α4 and α5 [51] and the latter predominantly expresses laminin α1 and α2 [51, 143, 149].

BM has been proposed to regulate BBB integrity directly as a physical barrier [150], given its anatomical location: i.e. between BMECs and astrocytes. Loss of BM leads to breakdown of BBB [151–156]. In addition, the BM also contributes to the integrity of BBB through individual ECM proteins, which not only anchor different cells in place at BBB but also regulate cellular processes [51, 131, 143, 144, 157]. It has been shown that laminin, collagen type IV, and fibronectin increase TEER of brain capillary endothelial cells [158]. Dystroglycan, a major receptor for ECM proteins, is selectively cleaved at the parenchymal BM at sites of leukocyte infiltration during experimental autoimmune encephalomyelitis [144]. Additionally, laminin α5, an endothelial BM-specific component, has been shown to serve as a barrier for leukocyte translocation [51]. These results suggest that ECM proteins and their receptors also contribute to the integrity of BBB.

Consistent with the detrimental role of MCP1 in BBB integrity, MCP1 has been found to promote hydrogen peroxide induced ECM protein degradation [159]. Contrary to this finding, there are reports showing that MCP1 directly contributes to the production and accumulation of ECM, especially collagen, in many disease models, including diabetic nephropathy [160], systemic sclerosis [161], and peritoneal dialysis-related epithelial–mesenchymal transition [162]. This discrepancy may be explained by different disease models and/or indirect effects of MCP1. For example, leukocytes recruited by MCP1 may secrete proteases that degrade ECM proteins [163, 164]. The exact role of MCP1 in BM needs further investigations.

Pericytes

Discovered more than 100 years ago [165], pericytes are sandwiched inbetween the abluminal side of BMECs and luminal side of astrocytic endfeet [166]. Specifically, pericytes are embedded in the endothelial BM in CNS capillaries [49, 167], and the degree of its coverage on endothelial cells varies depending on tissue type and species [166]. It has been shown that the pericyte-to-endothelial ratio is 1:1 in retina, 1:3 in brain, and 1:100 in skeletal muscles, respectively [166, 168]. The average pericyte-to-endothelial ratio is lower in rat capillaries (1:5) and relatively higher in mouse and human capillaries (1:4 and 1:3–4, respectively) [169, 170]. Although brain capillaries have high pericyte coverage, the precise percentage of capillary surface covered by pericytes varies significantly depending on different research groups, ranging from 22 to 99 % [168, 171, 172]. This difference may be due to the different pericyte markers used. There are no specific markers for pericytes so far, although several markers, including α-smooth muscle actin (SMA) [169, 173–175], PDGFRβ [25, 176], Desmin [177], CD13 [178, 179], NG2 [6, 180, 181], and RGS-5 [182, 183], have been used to identify pericytes. It should be noted that (1) these markers are also expressed by other types of cells besides pericytes, such as smooth muscle cells, myofibroblasts, and neuronal progenitors [167, 184, 185], and (2) the expression of these markers also depends on the differentiation stage of pericytes. It has been shown that TGF-β-treated (further differentiated) pericytes express significantly more SMA, VEGF, MMP-2, and MMP-9 than bFGF-treated (less differentiated) pericytes [186]. Fate-mapping experiments have shown that pericytes have several different developmental origins [167]. For example, ectoderm-derived neural crest gives rise to pericytes in the brain and thymus [187–189], whereas pericytes in the lungs [190], liver [191], and gut [192] are from mesothelium. In addition, there is also evidence suggesting that some pericytes are derived from hematopoietic stem cells [174, 193].

Pericytes have been reported to exert different functions, including BBB regulation, vascular development, homeostasis maintenance, and serving as contractile and multipotent cells [169, 173, 194]. Here, we focus only on its role in BBB regulation. It has been found that addition of pericytes to the BMEC–astrocyte co-culture system significantly enhances TEER [195, 196], suggesting that

pericytes contribute to the integrity of BBB. In addition, pericytes have been shown to up-regulate P-glycoprotein functional activity in endothelial cells and control tight junction permeability [197–199], suggesting that the interaction between endothelial cells and pericytes may play an important role in BBB integrity regulation. Using mice with defects in pericyte generation, researchers have demonstrated that pericyte coverage positively correlates with tight junction tightness [134] and negatively correlates with the BBB permeability [136]. Consistently, a correlation has been reported between pericyte loss and BBB breakdown [57, 200]. Further mechanistic studies suggest that the diminished expression of BBB-specific genes in endothelial cells and lack of polarity in astrocytic endfeet are responsible for pericyte loss-induced BBB breakdown [200]. In pathological conditions, such as hypoxia or traumatic brain injury, which lead to the disruption of BBB, pericytes have been found to migrate away from the micro-vasculatures [201, 202], but the relationship between migration of pericytes and compromise of BBB has not yet been studied.

Increased MCP1 levels have been reported in many disorders, including neurodegeneration, neuroinflammation, and kidney fibrosis [203, 204]. Additionally, MCP1 concentration has also been revealed to correlate with heavily oxidized LDL-induced pericyte injury [205]. However, due to the recent discovery of the critical role of pericytes in BBB regulation, only limited data are available to answer the question that how MCP1 affects pericytes.

Microglia

Microglia, which account for 10–20 % of glial cells, are brain-resident immune cells. Their number in the brain (100–200 billion depending on the condition) is comparable to that of neurons. It had long been believed that microglia originated from myeloid progenitors in the bone marrow [206–210] and that these macrophage-like cells migrated into the brain during early development (before the formation of BBB). A recent lineage tracing study has shown that adult microglia derive from colony-stimulating factor-1 receptor (CSF1R) positive primitive myeloid progenitors that arise before embryonic day 8 [211]. Consistently, fate-mapping experiments support that microglia mostly originate from *Myb*-independent, *FLT3*-independent, but *PU.1*-dependent myeloid progenitors that express CSF1R at embryonic day 8.5 [212–216]. Microglia have two states: a resting state with ramified morphology and an activated state with amoeboid morphology. Compared to the amoeboid morphology, the ramified structure has a smaller cell body surrounded by many long, thin, and highly dynamic processes. In the brain parenchyma, ramified microglia extend and retract their processes continually to sense changes in the surrounding microenvironment [217]. It is estimated that microglia can survey the entire brain in a few hours [217]. When there is an injury or disturbance of homeostasis in the CNS, microglia become activated. The activation involves changes of morphology and gene expression. The activated microglia then migrate to the injury site and proliferate locally. Additionally, these cells also secrete both pro- and anti-inflammatory cytokines, phagocytose cellular debris, process antigens, and present them to T cells via MHC class I molecules [218–225]. Whether microglia play a beneficial or detrimental role in CNS injury is highly controversial. There is evidence showing that microglia play neuroprotective roles by clearing cell debris and secreting factors promoting neurite growth and neuronal survival, such as neurotrophin-3 and brain-derived neutrophic factor (BDNF) [226–228]. In contrast, microglia have also been shown to produce pro-inflammatory cytokines, including TNF-α and IL-1β, which induce direct cytotoxicity by binding to their receptors (TNFRs and IL-1RI, respectively) and activating downstream cell death pathways [229]. Given that microglia are found in the perivascular space, it is speculated that microglia may play an important role in regulating BBB integrity by interacting with other BBB components, including BMECs, astrocyte endfeet, and pericytes [230]. There is evidence showing that microglial activation restores BBB integrity after the disruption of BBB [231]. In contrast, TNF-α released from activated microglia has been shown to impair BBB integrity [232], possibly through TNF-α-induced direct cytotoxicity on BMECs. This discrepancy may be due to different injury models and different timing after injury. Therefore, further investigations are needed to clarify the role of microglia in BBB permeability.

As indicated by its name, MCP1 is a potent chemoattractant for monocytes and microglia. MCP1-induced chemotaxis of monocytes and microglia has been found in many CNS injuries, including ischemia, excitotoxicity, and hemorrhage [72, 204, 221, 233–237]. The trafficking of microglia and leukocytes, however, is impaired in mice lacking CCR2, suggesting that MCP1-induced chemotaxis is dependent on CCR2 [238, 239]. Our laboratory has previously shown that microglial activation/migration induced by excitotoxic injury is attenuated in MCP1$^{-/-}$ mice [204]. Similar results were found in rats or mice injected with MCP1 blocking antibody [204, 240]. Interestingly, like MCP1$^{-/-}$ mice, excitotoxicity-induced microglial activation/migration is decreased in mice lacking plasminogen (plg) or tissue plasminogen activator (tPA), which converts plg to active plasmin [241, 242]. These results indicate that mouse MCP1 and the plg activation system may use converging pathways. Further studies in our laboratory have revealed that plasmin, generated by the action of tPA on plg in the mouse CNS (or urokinase plasminogen activator in other systems), cleaves MCP1 at lysine (K) 104 [204]. This cleavage removes the highly glycosylated

C-terminal extension and generates a N-terminal fragment that is highly homologous to human MCP1. The chemotactic potency of plasmin-cleaved MCP1 is higher than that of intact MCP1 and comparable to human MCP1 [38, 204], suggesting that plasmin is an activator of mouse MCP1. In accordance with this, infusion of plasmin-cleaved MCP1 into the CNS restored excitotoxicity-induced microglial activation/migration in $plg^{-/-}$ mice, whereas infusion of FL-MCP1 failed to do so. Furthermore, we have shown that mouse MCP1 C-terminus, when fused to human MCP1, is also inhibitory [70]. These data indicate that plasmin-mediated cleavage may be a mechanism used by cells to activate MCP1 and initiate downstream signaling cascades in mice. Importantly, the effects of plasmin-cleaved MCP1 are comparable to that of human MCP1 [38], suggesting that human MCP1 may be regulated similarly by an unidentified protein or differently at transcriptional/translational, transport, or signaling levels. We favor the latter, because we were not successful in pulling down proteins that specifically interact with human MCP1. In addition, the two CCR2 isoforms in human (CCR2A and CCR2B) have been shown to be able to activate different signaling pathways. Calcium influx has been found in chemotaxis of CCR2B-positive cells, but not in CCR2A-positive cells [243, 244], suggesting that the MCP1-CCR2 axis may also be regulated at the receptor or signaling levels in human.

Although human MCP1 does not have a highly glycosylated C-terminus, it can also be truncated in the C-terminus. A fragment with 69 amino acids (1–69) has been found and this fragment has the same activity as the wild-type MCP1 [245]. Which enzyme(s) is responsible for this cleavage, however, is not clear. In the N-terminus, human MCP1 has been reported to be cleaved by matrix metalloproteinase-1, -3, -8, and -12 between aminoacid 4 and 5 [246, 247]. This cleavage generates a fragment (5-76) that functions as an antagonist for CCR2 [245–248]. Consistently, the MCP1 mutant lacking amino acids 2-8 (7ND) has been shown to inhibit MCP1-CCR2 signaling both in vitro and in vivo [39, 249, 250]. There is no report on whether these matrix metalloproteinases cleave mouse MCP1. However, it would be reasonable to assume they do, based on the similarity of human and mouse MCP1 sequence. The first eight amino acids for human and mouse MCP1 are QPDAINAP and QPDAVNAP, respectively.

Neurons

It is estimated that in human brain every neuron has its own capillary [251], which together with neurovascular coupling (local neuronal activity and metabolism regulate cerebral blood flow) [252] indicates that neurons may regulate BBB functions. Anatomical studies have shown that BMECs and astrocytic processes are directly innervated by noradrenergic, serotonergic, cholinergic, and GABAergic neurons [253–259]. The presence of neurons has been shown to increase the integrity of BBB in vitro [260]. In addition, neurons have been shown to promote the expression of specific enzymes on BMECs [187]. These data suggest that neurons contribute to the integrity of BBB.

MCP1 exerts profound functions on neurons both directly and indirectly. MCP1 has been shown to directly bind to CCR2 on rat spinal neurons and activates Akt pathway. The binding of MCP1 on these neurons potently and efficiently inhibits GABA(A)-mediated GABAergic responses [261]. The MCP1–CCR2 axis has also been shown to contribute to neuropathic pain via interaction between astrocytes and neurons [262–265]. Contrary to these reports, MCP1 has been found to protect against methylmercury neurotoxicity [266], and early expression of MCP1 in neurons is necessary for hypoxic preconditioning-induced ischemic tolerance to focal stroke [267]. MCP1-induced molecular changes in neurons may modulate the function of other BBB components, and thus affect the integrity of BBB. In accordance with this hypothesis, MCP1 level has been found to positively correlate with the permeability of BBB and progression of disease, including Gaucher's disease, brain inflammation, stroke, and excitotoxic injury [71, 72, 204, 221, 233–237, 268, 269]. Consistently, lack of MCP1 or CCR2 prevents neuronal death, decreases BBB permeability, and improves neuronal function in many disorders, including hemorrhage and ischemia–reperfusion injury [72, 270].

Future directions

Although it is clear that plasmin removes the C-terminus of mouse MCP1 and enhances its chemotactic activity [38, 204], how human MCP1, which lacks the heavily glycosylated C-terminus, is regulated is still elusive. Future work should focus on the regulation/activation of human MCP1. In addition, how MCP1 regulates individual BBB components, especially pericytes, astrocytes, neurons, and BM, needs investigations. Such studies will shed light on our understanding of MCP1–CCR2 signaling/functioning, and may provide new molecular targets for the treatment of many CNS diseases, including brain trauma, stroke, and neurodegenerative diseases.

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