Role of MCP-1 and CCR2 in Alcohol Neurotoxicity

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Abstract

Alcohol abuse causes profound damage to both the developing brain and the adult brain. Prenatal exposure to alcohol results in a wide range of deficits known as fetal alcohol spectrum disorders (FASD). Alcohol abuse in adults is associated with brain shrinkage, memory and attention deficits, communication disorders and physical disabilities. Monocyte chemoattractant protein-1 (MCP-1/ CCL2) is one of the key chemokines that regulate the recruitment and activation of monocytes and microglia. Both MCP-1 and its receptor C-C chemokine receptor type 2 (CCR2) expressed in the brain are involved in various neuroinflammatory disorders, such as multiple sclerosis (MS), Alzheimer's disease (AD) and Parkinson's disease (PD). However, the role of MCP1/CCR2 in alcohol-induced brain damage is unclear. Recent evidence indicates that alcohol exposure increased the activity of MCP-1/CCR2 in both mature and developing central nervous systems (CNS). MCP-1/CCR2 signaling in the brain was involved in alcohol drinking behavior. MCP-1/ CCR2 inhibition alleviated alcohol neurotoxicity by reducing microglia activation/ neuroinflammation in the developing brain and spinal cord. In this review, we discussed the role of MCP-1/CCR2 signaling in alcohol-induced neuroinflammation and brain damage. We also discussed the signaling cascades that are involved in the activation of MCP-1/CCR2 in response to alcohol exposure.

Keywords

Alcohol abuse; apoptosis; chemokines; development; glia; neurodegeneration

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Conflict of Interest

The authors declare no conflict of interest.

1. Introduction

Alcohol abuse is the third leading preventable cause of death in the United States. An estimated 88,000 people die from alcohol-related causes annually [1]. 16.6 million adults (around 7% of the population in the USA) aged 18 and older have an alcohol use disorder (AUD) [1]. The cost of excessive alcohol use in the United States reached $249 billion in 2010 [2], and is still a significant economic and medical burden to society. Alcohol contributes to more than 200 diseases and injury-related health conditions and the central nervous system (CNS) is particularly vulnerable to alcohol toxicity. Alcohol abuse causes severe damage to both developing and mature brains [4–6]. Maternal alcohol consumption during pregnancy may cause fetal alcohol spectrum disorders (FASD) which are characterized by a spectrum of physical, mental and behavioral disabilities with possible lifelong implications [2]. Fetal alcohol syndrome is the most severe form of FASD, and includes a group of pathological conditions, such as facial abnormalities, growth restrictions, learning/memory deficits, and defects of heart, kidney and bone [7]. Chronic alcohol exposure in adults results in neuronal degeneration and cognitive deficits [3]. However, the mechanisms underlying alcohol CNS neurotoxicity are unclear.

Multiple mechanisms have been proposed, including endoplasmic reticulum (ER) stress [4], oxidative stress [5, 6], interference of signaling by neurotrophic factors and disruption of microRNAs [7]. Recently, neuroinflammation has been proposed to play an important role in the pathogenesis of FASD and AUD [8–10]. Alcohol-induced neuronal death is accompanied by microglial activation and neuroinflammation in both developing and adult brains [8, 9, 11, 12]. We have recently shown that inhibition of microglial activation and neuroinflammation by minocycline or targeting monocyte chemoattractant protein 1 (MCP-1) signaling offered protection against alcohol-induced neuronal death in the developing brain and spinal cord [13–15], suggesting that of microglial activation and neuroinflammation is involved in alcohol-induced damage to in the developing CNS.

MCP-1, also known as chemokine (CC motif) ligand 2 (CCL2), is an important chemokine that regulates neuroinflammation [16–18]. MCP-1 and its receptor C-C chemokine receptor type 2 (CCR2) are mainly detected in microglia in mouse and human brains. [16]. Astrocytes are another source for MCP-1 [19], and there are reports showing that MCP-1 and CCR2 are detected in neurons [20, 21]. MCP-1/CCR2 signaling has been implicated in several neuroinflammatory disorders, such as Alzheimer's disease [22], multiple sclerosis [23] and ischemic brain damage [24]. MCP-1/CCR2 signaling is also involved in some models of alcoholism [25–27]. The administration of alcohol increases the expression of MCP-1 in the rodent brain [28, 29] and genetic studies in animals indicate that elevated MCP-1 signaling is accompanied by increased alcohol consumption [30]. In human alcoholics, MCP-1 expression is augmented in the ventral tegmental area, substantia nigra, hippocampus and amygdala of the brain autopsy [27]. This review will discuss the involvement of MCP-1/CCR2 signaling activity in alcohol's action in the CNS and potential underlying mechanisms.

2. MCP-1/CCR2 signaling

MCP-1, a member of the C-C chemokine family, is a potent chemotactic factor for monocytes. MCP-1 is the first discovered human CC chemokine. Located on chromosome 17 (chr.17, *q11.2*), human MCP-1 is composed of 76 amino acids and is 13 kDa in size [31]. The biological function of MCP-1 is mediated via its G protein-coupled receptor CCR2 [32]. CCR2 binds the five pro-inflammatory chemokines: MCP-1, CCL7, CCL8, CCL12 and CCL13 [33–35]. However, MCP-1 is the most potent among those chemokines for triggering signal transduction pathways mediated by CCR2 [36]. CCR2A and CCR2B are two alternatively spliced forms of CCR2 that differ in their carboxy-terminal tails [37]. CCR2B accounts for 90% of the CCR2 expressed on the cell-surface even though CCR2A and CCR2B can activate different signaling pathways and exert different actions [31]. The direct downstream target of CCR2 signaling is monocyte chemotactic protein-1-induced protein-1 (MCPIP1), a transcriptional activator that regulates the expression of IL-1β, MCP-1 and TNFα. Other targets of CCR2 include phosphatidylinositol-3-OH kinase (PI3K), mitogen activated protein kinases (MAPK) and protein kinase C [34, 38], indicating that a wide range of intracellular pathways may be involved in cellular responses elicited by MCP-1. MCP-1 has been demonstrated to recruit monocytes into foci of active inflammation [39] and is confirmed to be the main chemokine responsible for recruiting monocytes [40]. Besides recruiting and directing leukocyte movement, MCP-1 may also influence T-cell immunity. It has been reported that MCP-1 expression is associated with the development of polarized Th2 responses [41, 42] and that MCP-1 increases the secretion of IL-4 by T cells [43]. Gonzalo et al [44] also observed elevated expression of MCP-1 in Th2 immune-mediated diseases, such as asthma. In summary, in the peripheral immune system, MCP-1/CCR2 signaling plays a crucial role in guiding and directing immune cells in response to inflammatory challenges.

3. MCP-1/CCR2 in the CNS

In addition to its well-established role in the peripheral immune system, increasing evidence indicates that MCP-1/CCR2 plays an important role in the CNS [45–47]. MCP-1 and CCR2 are mainly expressed in microglia and astrocytes in the CNS [14, 48–52]. MCP-1/CCR2 signaling is involved in the activation of microglia. For example, Feng et al reported that expression of MCP-1 and CCR2 induced by photoreceptor apoptosis promotes the activation and migration of microglia and monocytes [53]. Another study demonstrated astrocyte-derived MCP-1 leads to increased migration of microglial cells, and inhibition of CCR2 attenuates MCP-1-mediated microglial activation [54]. Dubovy et al observed that microglial activation in periaqueductal gray (PAG) and rostral ventromedial medulla (RVM) in rats following injury was mediated by CCR2, which may be activated by neuron or astrocyte-derived MCP-1 [55].

MCP-1 and CCR2 are also expressed in CNS neurons and cultured neuronal cell lines [20, 21, 46, 47, 56]. MCP-1 is constitutively expressed in the neurons of discrete brain regions in rats, such as the cerebral cortex, hippocampus, hypothalamus, substantia nigra, cerebellum and spinal cord [20, 56]. MCP-1/CCR2 signaling can regulate neuronal functions. For instance, Zhou et al observed that MCP-1 enhances neuronal excitability and synaptic

transmission via presynaptic mechanisms in rat hippocampal slices [57]. Widera et al [58] reported that MCP-1 acts as a chemokine on neural stem cells to activate the migration capacity of rat-derived neural stem cells. Collectively, MCP-1/CCR2 signaling is involved in a variety of neurological activities in addition to its role in the immune system.

4. MCP-1/CCR2 in neurological disorders

An increased MCP-1 expression has been observed in the CNS astrocytes and microglia under pathological conditions. Elevated MCP-1 is an important mediator of the neuroinflammatory responses in brain trauma [59], ischemic brain injury [24, 60] and various neurodegenerative disorders, such as multiple sclerosis [23, 61] and Alzheimer's disease [62, 63]. MCP-1 interacts with CCR2 to regulate neuroinflammatory processes in the CNS [63, 64]. We discuss several examples in which MCP-1/CCR2 is involved in CNS damage and neurological disorders.

4. 1. MCP-1/CCR2 in Alzheimer's disease

Alzheimer's disease (AD) is the most common neurodegenerative disorder characterized by the accumulation of β-amyloid peptide (Aβ). Elevated levels of MCP-1 have been observed in the brains of AD patients and in transgenic mouse models of the disease. Galimberti et al [22] reported that the transition from mild cognitive impairment to AD is associated with an elevated MCP-1 level in the cerebrospinal fluid (CSF). Several studies have also demonstrated that increased levels of β-amyloid induces MCP-1, which results in disrupting the blood–brain barrier (BBB) and facilitating recruitment of immune cells into the CNS [65]. However, the role of CCR2 in AD is controversial. Some studies using mouse models show that monocytes migrate from the bone marrow, infiltrate into the brain in a CCR2-dependent manner, and then differentiate into microglia or macrophages; others argue that CCR2 deficiency accelerates the onset of spatial and contextual memory deficits and aggravates amyloid pathology in a mouse model of AD [66]. Nevertheless, a majority of data support a role for the MCP-1/CCR2 system in the pathogenesis of Alzheimer's disease. MCP-1 in peripheral blood may attract blood-derived monocytes to migrate into the brain [67], and a higher plasma MCP-1 level is associated with greater severity and faster cognitive decline.

4. 2. MCP-1/CCR2 in Parkinson's disease

Parkinson's disease (PD) is the second most common neurological disorder in the elderly after AD [68]. Although the origin of this neuronal degeneration is unknown, substantial evidence has indicated the involvement of inflammatory processes in the pathology of PD [69, 70]. Several studies propose that PD is caused by programed cell death (apoptosis) due to increased levels of cytokines, such as TNFα and MCP-1 [71–76]. Elevated MCP-1 expression was observed in peripheral blood mononuclear cells (PBMCs) in PD patients compared with healthy control subjects [77]. Moderate MCP-1 over-induction led to increased neurotoxicity in MPTP mice (a mouse model of PD), likely due to the increased CCR2^{+} monocyte infiltration [78]. Liu et al reported that Nurr1 overexpression played neuroprotective and anti-inflammatory roles via down-regulating MCP-1 in both *in vivo* and *in vitro* PD models [79]. In another study of PD, Kempuraj et al observed that MPP[+]

activates mouse and human mast cells to release MCP-1 [80]. In addition, Lindqvist et al reported that MCP-1 levels in CSF were correlated with increased non-motor symptoms of PD, such as depression [81]. Furthermore, Nishimura et al found that MCP-1–2518A/G genotype affected the age-at-onset of PD patients [82], which suggested an association between the MCP-1 and CCR2 gene polymorphisms and PD risk.

4. 3.   MCP-1/CCR2 in ischemic stroke

Accumulating evidence indicates that MCP-1 and CCR2 are involved in postischemic inflammation. An augmented MCP-1 expression has been observed in both the serum and CSF of patients after cerebral stroke [83, 84]. MCP-1$^{-/-}$ mice exhibit decreased activated microglia and phagocytic macrophage accumulation in the brain and smaller infarcts following permanent middle cerebral artery occlusion [85]. The expression of a nonfunctional MCP-1 gene (an N-terminal deletion mutant of human MCP-1) in rats significantly attenuated the infarct volume and macrophage infiltration [86]. CCR2 $^{-/-}$ mice have reduced blood–brain barrier permeability, decreased level of inflammatory cytokines and smaller infarct size in the affected ischemic hemisphere [87]. In summary, these data suggest that inhibition of MCP-1/CCR2 could improve the treatment of ischemic stroke.

4. 4.   MCP-1/CCR2 in multiple sclerosis

Multiple sclerosis (MS) is a demyelinating autoimmune disease leading to severe and progressive neurological impairment. Activated microglia, infiltration of macrophages and lymphocytes, and reactive astrocytes are the major characteristics of MS [88, 89]. Increased expression of MCP-1 has been detected in patients with both acute and chronic MS [16]. It has been demonstrated that MCP-1 is expressed by astrocytes and macrophages within actively demyelinating MS plaques [61]. In experimental autoimmune encephalomyelitis (EAE), an animal model for MS, increased MCP-1 expression correlates with the severity of the disease [16]. Also in EAE, knocking out CCR2 inhibited mononuclear cell inflammatory infiltration and proinflammatory cytokine expression in the CNS of mice [90].

5.   MCP-1/CCR2 in alcohol-induced neuropathology

In addition to the involvement in neurological disorders, recent studies indicates that MCP-1/CCR2 signaling also plays and important role in alcoholic neuropathology of both the adult CNS and the developmental CNS. These findings are discussed below.

5.1.   MCP-1/CCR2 in alcohol's action in the adult CNS

Heavy alcohol exposure causes neuroinflammation. For example, Increased MCP-1 expression and microglial activation have been observed in the brain of human alcoholics [27]. Greater amounts of TNFα were observed in the monocytes isolated from the blood of alcoholics [91]. Leclercq et al observed that lipopolysaccharides and peptidoglycans from the gut microbiota stimulate IL-8 and IL-1β in peripheral blood mononuclear cells that are correlated with alcohol craving [92]. Studies using animal models confirmed that alcohol increased the expression of multiple neuroimmune genes, such as cyclooxygenase 2 (COX2), NF-κB and cyclic AMP-responsive element binding protein (CREB) in the brain

and that these alterations may persist over long periods even after alcohol withdrawal [93–95].

MCP-1 has been shown to regulate neuroinflammation and microglia activity [96]. As the first responder to environmental insults in the CNS, microglia are vital in neuroinflammation. Under resting conditions, microglia is in the ramified form, having long branching processes and a small cellular body [97]. In response to injury or pathogen invasion, quiescent ramified microglia proliferate and transform into reactive ameboid microglia, which have fewer and thicker processes with a larger cell body. The marker Iba-1 is upregulated in reactive microglia and is often used to visualize these cells [98]. It appears that alcohol could stimulate inflammatory pathways by activating microglia in the CNS and that MCP-1 plays an important role in these processes. For example, He et al [27] observed elevated MCP-1 levels and microglial markers, such as Iba-1 and Glucose transporter-5 (GluT5), in the ventral tegmental area (VTA), substantia nigra (SN), hippocampus and amygdala in the brains of human alcoholics. Alcohol exposure activated microglia, increased the expression of MCP-1 and other proinflammatory cytokines, such as TNF-α, IL-6 and IL-1β, and induced neuronal death in rats [8, 99]. Qin et al [28] revealed that pretreatment with alcohol potentiated LPS-induced increase in MCP-1 and microglial activation in the brain of adult mice. It has been proposed that MCP-1 lowers the "threshold sensitivity" for microglia as a "priming" stimulus and enhances the synthesis of proinflammatory cytokines in response to subsequent insult [100]. With a neuron/microglia co-culture system, Yang et al showed that MCP-1-induced neurotoxicity requires the presence of microglia and that exogenous MCP-1 was able to activate and stimulate microglia to produce cytokines [101]. An MCP-1 neutralizing antibody inhibited MCP-1-induced microglia activation and neuronal death in culture and in the thalamus [101].

Several studies have demonstrated that MCP-1/CCR2 signaling is involved in alcohol drinking behavior as well. Deletion of CCR2 and MCP-1 (in female mice) reduced alcohol preference and consumption in a two-bottle choice test, and alcohol administration produced a stronger conditioned taste aversion in CCR2$^{-/-}$ and MCP-1$^{-/-}$ mice [25]. The delivery of MCP-1 or TLR4 siRNA into the central nucleus of the amygdala (CeA) and ventral tegmental area (VTA) in alcohol preferring rats inhibited target gene expression and blunted binge drinking [96]. Although exactly how MCP-1 regulates drinking behavior is not clear, a potential explanation is that MCP-1 activates the dopamine system [102]. Taken together, MCP-1/CCR2 may participate in alcohol-induced brain damage and drinking behavior through the regulation of microglial activation and neurotransmission.

5. 2. MCP-1/CCR2 in alcohol neurotoxicity in the developing CNS

FASD is the leading cause of mental retardation in North America, ahead of Down syndrome and cerebral palsy [103–106]. FASD is estimated to affect as high as 5% of people in the United States and some Western European countries, and the economic burden of FASD in the US is significant [107]. The developing CNS is particularly susceptible to alcohol exposure, so even moderate maternal drinking could lead to cognitive and behavioral impairments [103, 108–111]. Despite attempts to raise awareness about the dangers of

drinking during pregnancy, numbers of women drinking during pregnancy in the USA have not declined [30].

Microglial activation and neuroinflammation have been implicated in alcohol neurotoxicity in the developing CNS [8, 13–15, 112]. It has been proposed that microglia primed by ethanol exposure may lead to excess neuroinflammation and the subsequent neurotoxicity observed in AUD and FASD [8, 112]. Since MCP-1/CCR2 plays an important role in microglial activation and neuroinflammation, we investigated the involvement of MCP-1/CCR2 in alcohol neurotoxicity in the developing CNS. Using a third trimester equivalent mouse model of alcohol exposure, we compared the effects of alcohol on the developing spinal cord among wild type, MCP-1 deficient (MCP-1$^{-/-}$) and CCR2 deficient (CCR2$^{-/-}$) mice [14]. Alcohol caused apoptotic cell death in the dorsal horn of the spinal cord, which was accompanied by glial activation and macrophage infiltration. MCP-1 or CCR2 deficient mice were resistant to alcohol-induced apoptosis, inflammation, and glial activation. It appeared that deletion of CCR2 was more effective than MCP-1 in the protection against alcohol-induced damage to the spinal cord [14]. Therefore, MCP1/CCR2 signaling may mediate alcohol-induced microglial activation and neuronal death in the developing spinal cord.

The importance of MCP-1/CCR2 signaling in mediating alcohol-induced microglial activation and neuroinflammation is also demonstrated in the developing brain [15]. In the third trimester equivalent mouse model of alcohol exposure, alcohol caused widespread neuroapoptosis, microglial activation and neuroinflammation in the brain. MCP-1 synthesis inhibitor Bindarit and CCR2 antagonist RS504393 each significantly inhibited alcohol-induced microglial activation/neuroinflammation and neuroapoptosis in the brain of early postnatal mouse pups [15]. Further studies using MCP-1$^{-/-}$ or CCR2$^{-/-}$ mice also confirmed that the deficiency in MCP-1 or CCR2 made mice more resistant to alcohol-induced neurodegeneration. It appeared MCP-1 deficiency offered better protection against alcohol-induced damage to the developing brain. Moreover, alcohol and MCP-1 caused more neuronal death in the neuron/microglia co-culture system than the neuronal culture alone, suggesting that microglia are required for alcohol/MCP-1-induced neurotoxicity. Bindarit and RS504393 effectively protected neurons against alcohol/MCP1-induced neuronal death in the co-cultures, indicating that MCP-1/CCR2 signaling is involved in microglial contribution to ethanol neurotoxicity [15].

Minocycline is an antibiotic that inhibits microglial activation and alleviates neuroinflammation. Using the same third trimester equivalent mouse model of alcohol exposure, we showed that minocycline significantly inhibited alcohol-induced caspase-3 activation, and blocked alcohol-increased MCP-1/CCR2 expression as well as microglial activation in the developing brain [13]. Minocycline was also effective in protecting neurons against alcohol-induced neuronal death in neurons/microglia co-cultures. It is possible that minocycline-mediated neuroprotection operates through its inhibition of MCP-1/CCR2 signaling. Taken together, MCP-1/CCR2 is an important mediator for alcoholic neuropathogenesis.

6. Proposed mechanisms underlying the interaction between MCP-1/CCR2 and alcohol in microglial activation and neuroinflammation

The above evidence indicates that MCP-1/CCR2 signaling plays an important role in alcohol-induced neuroinflammation and microglial activation in both the adult and developing CNS. It is important to understand the mechanisms underlying MCP-1/CCR2induced microglia activation and neuroinflammation in the context of alcohol neurotoxicity.

GSK3β, TLR4, JNKs, and p38 MAPK are key regulators of microglia activation and neuroinflammation [113–115]. GSK3β is a multifunctional serine/threonine kinase and plays an important role in neurogenesis, neuronal differentiation, neuronal migration and survival in the developing CNS [116–119]. The activity of GSK3β is inhibited by the phosphorylation at Ser9 but stimulated by the phosphorylation at Tyr216 [120, 121]. GSK3β activation promotes microglia migration and the production of inflammatory molecules [115]. Over-expression of GSK3β makes neurons more sensitive to alcohol-induced neuronal death while inhibition of GSK3β ameliorates alcohol neurotoxicity in vitro and in vivo [122]. Lithium, a GSK3β inhibitor, protects neurons against alcohol-induced neurodegeneration and inhibition of neurite outgrowth [122, 123].

GSK3β was activated and involved in alcohol-induced microglial activation and up-regulation of proinflammatory cytokines *in vitro* and *in vivo* [15]. Blocking MCP-1/CCR2 signaling partially mitigated alcohol-mediated activation of GSK3β. TLR4 was also activated by alcohol in the developing brain and cultured microglial cells [15]. Inhibition of MCP-1/CCR2 signaling significantly blocked alcohol-induced increase of TLR4. These results suggest that MCP-1/CCR2 signaling is involved in alcohol-induced activation of GSK3β and TLR4. In addition, there is a cross-talk among MCP-1/CCR2 signaling, GSK3β, and TLR4 in response to alcohol exposure. Blocking TLR4 inhibited ethanol-induced activation of GSK3β and up-regulation of MCP-1 in cultured microglial cells. Furthermore, blocking GSK3β also attenuated ethanol-induced up-regulation of TLR4 and MCP-1 [15]. Similarly, alcohol-induced activation of GSK3β and JNKs were reduced in the developing spinal cord of MCP-1$^{-/-}$ mice and CCR2$^{-/-}$ mice compared to wild type mice [14]. Furthermore, minocycline inhibited alcohol-induced microglial activation and neuroinflammation in the developing brain; it also attenuated alcohol-mediated activation of MCP-1, GSK3β, and JNKs, supporting the notion that the interaction among MCP-1, GSK3β, and JNK contributes to alcohol-induced neuroinflammation [13]. Alcohol did not significantly affect p38 MAPK signaling in this mouse model of third trimester equivalent exposure [13].

According to these findings, we propose a model of alcohol-induced neuroinflammation and microglial activation (Fig. 1). In this model, alcohol up-regulates the expression of MCP-1 and activates CCR2 signaling. It is also possible that alcohol could directly activate CCR2; this possibility remains further investigation. Activated MCP1/CCR2 signaling regulates pro-inflammatory transcription factors, such as AP-1 and NF-κB through GSK3β or JNK. These transcription factors stimulate the expression of inflammatory cytokines and chemokines including MCP-1 itself, causing neuroinflammation and neuronal death. On the

other hand, alcohol could activate TLR4 either directly or indirectly and turn on its down-stream effectors such as TRIF, MyD88 and JNK; at the same time, the active TLR4 may stimulate GSK3β, which further activates TLR4 and JNK. Consequently, the activation of TRIF, MyD88, JNK and GSK3β up-regulates transcription factors, resulting in an increase of proinflammatory cytokines (e.g. MCP-1, IL6, and TNF-α). The released MCP-1 binds its receptor CCR2 which further activates TLR4, GSK3β, and JNK; this positive feedback loop amplifies the neuroinflammation and neurotoxicity. In addition to its role in neuroinflammation and neurotoxicity, MCP-1 may regulate alcohol self-administration through unknown mechanisms.

7. Conclusions and future studies

Available evidence suggests that MCP-1/CCR2 interacting with GSK3β, TLR4, and JNKs may mediate alcohol's action on neuroinflammation and neurotoxicity in the CNS, particularly the developing CNS. Therefore, suppression of MCP-1/CCR2 activity by selective inhibitors or genetic manipulation may ameliorate alcohol-induced damages to the CNS. These findings establish MCP-1/CCR2 signaling as a potential target for therapeutic efforts. Although there has been no definitive clinical proof-of-concept for antiMCP-1/CCR2 therapeutics, there has been great interest in targeting MCP-1/CCR2 signaling in treating some neurological disorders [124]. For this purpose, innovative technologies that facilitate effective and specific targeting of MCP-1/CCR2 signaling in neuroinflammation have been developed, such as dominant negative mutants of MCP-1, RNAi and potent small-molecule antagonists of CCR2 to block receptor activation [124–126]. Although the excessive MCP-1/CCR2 activation has detrimental effects on neurons, basal MCP-1/CCR2 signaling is required for normal immune response against disease [19]. It is therefore important to carefully design and evaluate anti-MCP-1/CCR2 therapy.

There are several interesting points for future study. First, although MCP-1 and CCR2 are mainly expressed by microglia, they are also expressed by astrocytes [19]. Mouse studies have demonstrated that MCP-1/CCR2 signaling could mediate astrocytosis in familial ALS [127] and that MCP-1 produced by spinal cord astrocytes contributes to central sensitization and neuropathic pain [52]. Astrocytes play critical roles in AUD by modulating neurotransmission [128]. It has also been demonstrated that astrocytes are involved in animal models of FASD [12, 129]. Therefore, it would be interesting to investigate the contribution of MCP-1/CCR2 to the activation of astrocytes in the context of alcohol neurotoxicity.

Second, several animal models of FASD have demonstrated that alcohol exposure could produce long lasting neurobehavioral deficits in adolescent and adult mice [130–139]. Since MCP-1$^{-/-}$ mice and CCR2$^{-/-}$ mice are more resistant to alcohol-induced neuronal death in the developing CNS, it would be important to determine whether deficiency of MCP-1/CCR2 protects mice against alcohol-induced behavioral deficits.

Third, it is well-established that the vulnerability of the developing brain to alcohol is temporal- and regional-dependent. Accordingly, is MCP-1/CCR2 developmentally

regulated? Does the expression pattern of MCP-1/CCR2 contribute to the differential sensitivity to alcohol during the development? These questions warrant further investigation.

Lastly, in addition to microglial activation and neuroinflammation, MCP-1/CCR2 signaling may participate in other cellular stress processes, which contribute to alcohol neurotoxicity. It has been well-established that oxidative stress and endoplasmic reticulum (ER) stress play an important role in alcohol neurotoxicity [4, 140–142]. MCP1/CCR2 signaling may regulate ER stress. For example, the activation of MCP-1/CCR2 signaling may cause ER stress through the upregulation of MCPIP in cardiomyocytes and osteoclasts [143]. CCR2 inhibitor attenuates ER stress and reduces the expression of inflammatory cytokines in the liver of type 2 diabetic mice [144]. We recently showed that MCP-1$^{-/-}$ and CCR2$^{-/-}$ mice are more resistant to alcohol-induced ER stress in the developing spinal cord [14]. It appears that MCP-1/CCR2 signaling may also be involved in oxidative stress. Kim et al reported that MCP-1 deficiency attenuates oxidative stress and protects against ovariectomy-induced chronic inflammation in mice [145]. Therefore, it would be interesting to determine the interplay of MCP-1/CCR2 signaling, ER stress, and oxidative stress, as well as how the interplay contributes to alcohol neurotoxicity.

Acknowledgments

Abbreviation

AD	Alzheimer's disease
AUD	Alcohol use disorders
BBB	Blood–brain barrier
CNS	Central nervous system
CSF	Cerebrospinal fluid
CCR2	Chemokine (C–C motif) receptor 2
COX2	Cyclooxygenase 2
ER	Endoplasmic reticulum
EAE	Experimental autoimmune encephalomyelitis
FASD	Fetal alcohol spectrum disorder
GSK3β	Glycogen synthase kinase 3 beta
Iba-1	Ionized calcium binding adaptor molecule 1
IL	Interleukin

MCP-1	Monocyte chemoattractant protein-1
MS	Multiple sclerosis
PD	Parkinson's disease
TLR4	Toll-like receptor 4
TNF-α	Tumor necrosis factor-α

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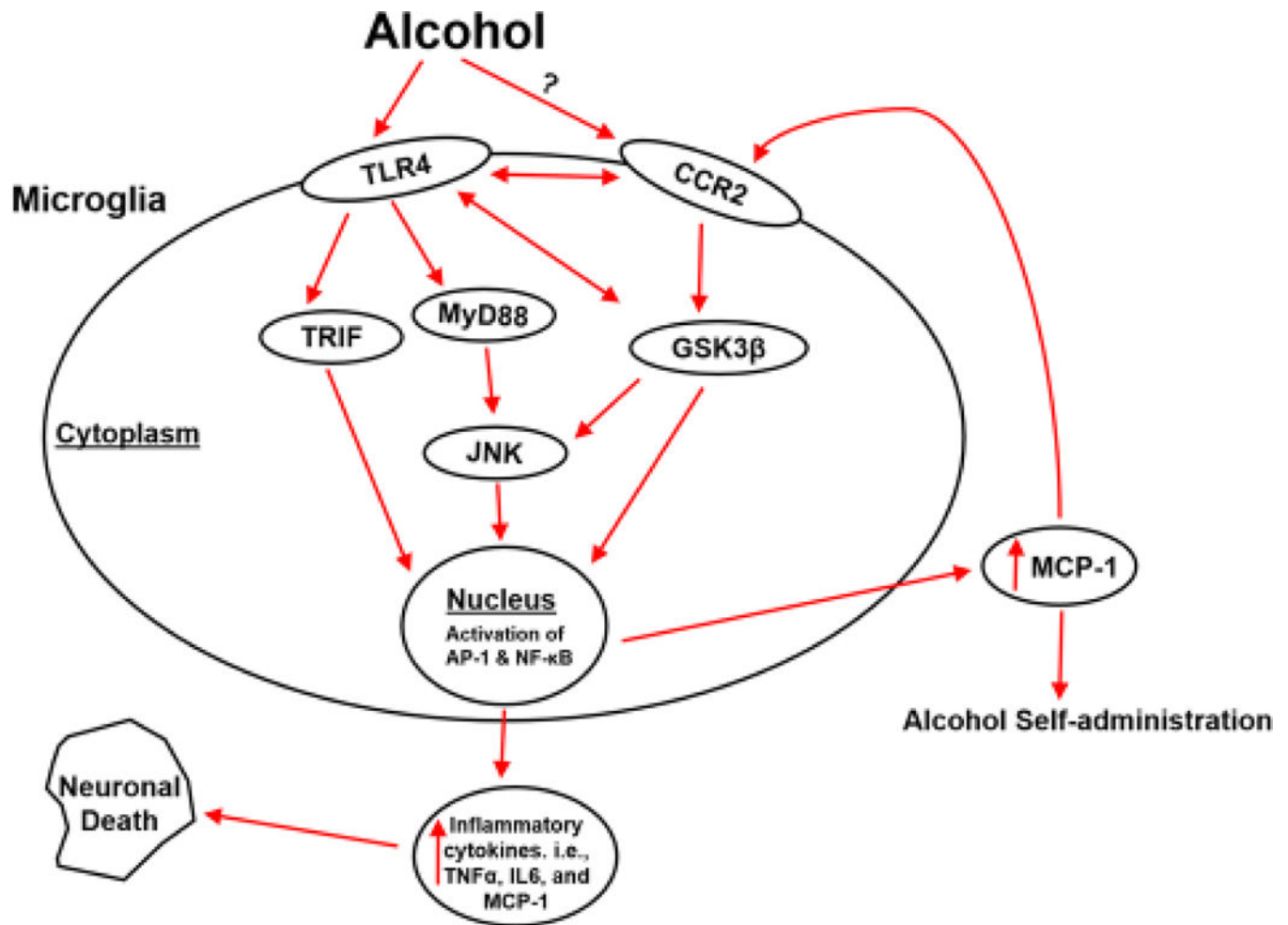
Figure 1.

The cascade of MCP-1/CCR2 signaling in alcohol-induced neuroinflammation and neurotoxicity. Alcohol increases the expression of MCP-1 and activates CCR2 signaling. Activated MCP-1/CCR2 signaling regulates pro-inflammatory transcription factors, such as AP-1 and NF-κB through GSK3β or JNK. These transcription factors stimulate the expression of inflammatory cytokines and chemokines including MCP-1 itself, causing neuroinflammation and neuronal death. On the other hand, alcohol could directly or indirectly activate TLR4 and turn on its down-stream effectors such as TRIF, MyD88 and JNK; the active TLR4 meanwhile may stimulate GSK3β, which may further activate TLR4 and JNK. The activation of this cascade results in an increase of proinflammatory cytokines (e.g. MCP-1, IL6, and TNF-α). The released MCP-1 binds its receptor CCR2 which further activates TLR4, GSK3β, and JNK; this positive feedback loop amplifies the neuroinflammation and neurotoxicity. In addition to its role in neuroinflammation and neurotoxicity, MCP-1 is also reported to regulate alcohol self- administration.