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Review Article

Modulation of microglia activation and Alzheimer's disease: CX3 chemokine ligand 1/CX3CR and P2X$_7$R signaling

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Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by cognitive deficits. Two hallmarks of AD that cause chronic inflammation and lead to neuronal dysfunction and damage are tau tangles and amyloid plaques. Microglial cells, the primary immune cells of the central nervous system, maintain a homeostatic active/inactive state via a bidirectional, dynamic communication with neurons. Several studies have revealed that dysregulated microglial activation leads to AD pathology. Therefore, we reviewed the relationship between AD and two important signaling complexes, CX3 chemokine ligand 1 (CX3CL1)/CX3CR1 and ATP/P2X$_7$R, that play critical roles in the regulation of microglial activation. CX3CL1/CX3CR1 is one important signaling which controls the microglia function. Altering this pathway can have opposite effects on amyloid and tau pathology in AD. Another important molecule is P2X$_7$R which involves in the activation of microglia. Over activation of P2X$_7$R is evident in AD pathogenesis. In this review, we discuss influence of the two signaling pathways at different stages of AD pathology as well as the drug candidates that can modulate CX3CL1/CX3CR1 and ATP/P2X$_7$R.

Keywords: *Alzheimer's disease, CX3 chemokine ligand 1/CX3CR1, Microglia, P2X$_7$R*

Introduction

Microglia are resident immune cells in the central nervous system (CNS) and are characterized based on its active or inactive state. They are the part of the CNS defense system, also the main source of inflammatory cytokine release in the brain and are involved in phagocytosis activity. Several studies have indicated the role of microglia activation in the pathological conditions such as Alzheimer's disease (AD) [1], stroke [2], amyotrophic lateral sclerosis (ALS) [3], and Parkinson's disease (PD) [4]. The major cause of these diseases is the dysregulation of microglial activity. We, therefore, discussed the modulation of microglia activation in AD pathogenesis in this review.

AD, one of the most devastating neurodegenerative disorders, along with jeopardizing patients' lives also places an enormous, physical, mental, and medical care burden on their families. First identified by German psychiatrist Alois Alzheimer in 1901, AD has now become the most common form of dementia. Amyloid plaque deposition and accumulation of hyperphosphorylated Tau protein were believed to be the two hallmarks of AD. However, research spanning more than a century to generate the drugs targeting Aβ and Tau achieved limited success. Therefore, recent AD drug development shifted to reducing neuroinflammation caused by microglial overactivation in the initiation and progression of the disease. Thus, the novel drug candidates for AD were the several compounds targeting CX3 chemokine ligand 1 (CX3CL1)/CX3CR and P2X$_7$R signaling, responsible for the inhibition and promotion of microglial activation, respectively. We thoroughly reviewed the CX3CL1/CX3CR and P2X$_7$R signaling pathways and their involvement in the regulation of microglial activation for physiological homeostasis as well as initiation and progression of AD.

Role of microglia in physiological and pathological conditions

Microglia are part of the innate immune system, which acts as the first-line defense in the CNS. They account for 5%–20% of the total population of glial cells. During the development, microglia perform the same function as the phagocytes of the brain and help eliminate neurons, glia, and synapses (synaptic stripping) to shape and wire new neural

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circuits in the various regions of the brain [5]. Thus, these cells play important roles in establishing brain circuits by enabling the formation, elimination, and migration of synapses. Microglia also aid the proliferation and survival of neuronal progenitor cells. Microglial cells demonstrate a high diversity in their morphology and functions. A previous study mentioned several existing microglia-sensing apparatus called "sensomes" [6] that are responsible for the direct communication between neurons and microglial cells. These sensomes comprise the receptor of the neuronal chemokine fractalkine (FKN) (CX3CR1) [7], and purinergic receptors such as P2rx7, P2ry12, P2ry13, and P2ry6 [8]. Under normal physiological conditions, microglial cells exist in ramified forms throughout the CNS. Although the ramified microglial cell body is present in a particular brain region, their processes disseminate for surveillance of the area. Critical conditions, such as injury, pathogen infection, or disease development, cause activation of the microglia in response to stimuli called "find-me" signals from the environment. They then take an amoeboid shape by retracting their processes and engage in phagocytosis of pathogens and clearance of cellular debris by extending dynamic protrusions. They also respond swiftly to pathological changes in the CNS by expressing a variety of neurotransmitters, neuropeptides, and immune receptors [9]. Their versatile and immediate response to insults from the surroundings makes them an invaluable part of the CNS defense system. Therefore, dysregulation in their activation results in multiple neurodegenerative diseases including AD [10], PD [11], and ALS [12]. Microglia typically show classically activated (M1) and alternatively activated (M2) states based on types of stimuli such as lipopolysaccharides (LPS), interferon gamma (INF-γ), interleukin 4 (IL-4), and IL-13 [13]. The classically activated M1 state can be characterized by the production of pro-inflammatory cytokines including IL-1β, tumor necrosis factor-α (TNF-α), and IL-6, whereas the M2 state generates anti-inflammatory cytokines, such as IL-10, and neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), insulin-like growth factor 1 (IGF-1), transforming growth factor-beta (TFG-β), and glial cell line-derived neurotrophic factor (GDNF) [14]. Thus, the M1 microglia causes inflammation in the CNS, which can be attenuated by the M2 microglia because of their contradictory functions. In this study, we reviewed the two most important signaling molecules involved in the activation and modulation of microglia in both normal and pathogenic circumstances of AD CX3CL1/CX3CR1 and $P2X_7R$. The modulation of microglia activation by the two signaling molecules is presented in [Figure 1].

Therapeutic agents of CX3 chemokine ligand 1/CX3CR1 and $P2X_7R$

Since the CX3CL1/CX3CR1 and $P2X_7R$ signaling pathways modulate microglial activation involved in the pathogenesis of various diseases, they can be therapeutic drug candidates. They are differentially expressed at the different stages of AD, therefore, the expression level of these molecules should be considered before deciding the drug targets. The different antagonists of CX3CL1/CX3CR1 and $P2X_7R$ selected to treat different diseases including AD, neuropathic pain, and psychiatric disorders are listed in Table 1. JNJ-54175446, a $P2X_7R$ antagonist, is one of the promising compounds under clinical trial to treat symptoms of depression. The table presents the compounds that have been used in treating different diseases. It is important to identify the dosage and duration of treatment based on the stage and severity of AD pathology. Because these two signaling molecules can either be protective or detrimental depends on the progression of the AD pathology. Identifying an effective compound to manipulate these two signaling pathways might lead to the discovery of a new promising therapeutic targets for treating AD.

Role of CX3 chemokine ligand 1/CX3CR1 signaling in Alzheimer's disease

Expression of CX3 chemokine ligand 1 and CX3CR1 at various stages of Alzheimer's disease progression

Studies have reported that when CX3CL1 binds to its receptor, CX3CR1, it forms a CX3CL1/CX3CR1 complex, facilitating a bidirectional interaction between neurons and microglia [28-30]. CX3CL1 released from the neurons can regulate the activity status of microglia, in return, cytokines and neurotrophic factors released from microglia can modulate the neuronal function. This reflects the bidirectional communication between neurons and microglia cells. The disruption of this signaling has been reported in the progression of AD [31,32]. Previous research has reported that patients with mild–moderate AD demonstrated higher CX3CL1 levels in plasma than those with severe AD [33].

Another study using real-time PCR shows the expression of *CX3CL1* mRNA level was dramatically decreased in the hippocampus, but not in the brainstem and cerebellum of AD patient brain [34]. Moreover, the samples collected from the CSF may be sufficient to detect the level of soluble CX3CL1 through ELISA [35] and used as a biomarker to indicate the AD progression.

Neuroprotective and neurotoxic effects of CX3CL1/CX3CR1 signaling in Alzheimer's disease

The CX3CL1, also known as FKN (in humans) or neurotactin (in mouse), is a member of the CX3C family [36-38]. This chemokine exists as either membrane-bound or soluble form [39] and expressed in neurons throughout the brain, but the expression is especially high in the hippocampus [40]. The CX3C chemokine receptor 1 (CX3CR1) expresses exclusively in microglia and is the specific receptor to the CX3CL1 [7,41]. Neurons are known to modulate microglial function through CX3CL1/CX3CR1 signaling [39]. The disruption of this signaling results in the dysregulation of microglial activation, leading to AD pathogenesis [42,43].

Research states that under pathological insults or during injury, CX3CL1 levels are elevated in neuronal cells, promoting the migration of microglia to the site of injury or inflammation [44-46], where they become activated and release pro-inflammatory cytokines [47-49]. This is the critical time point where several enzymes, such as a disintegrin and metalloprotease (ADAM) 10 [50], ADAM 17 [51], cathepsin S [52] and α-, β-, and γ-secretase [53], cleave the membrane-bound form of CX3CL1 to release various forms

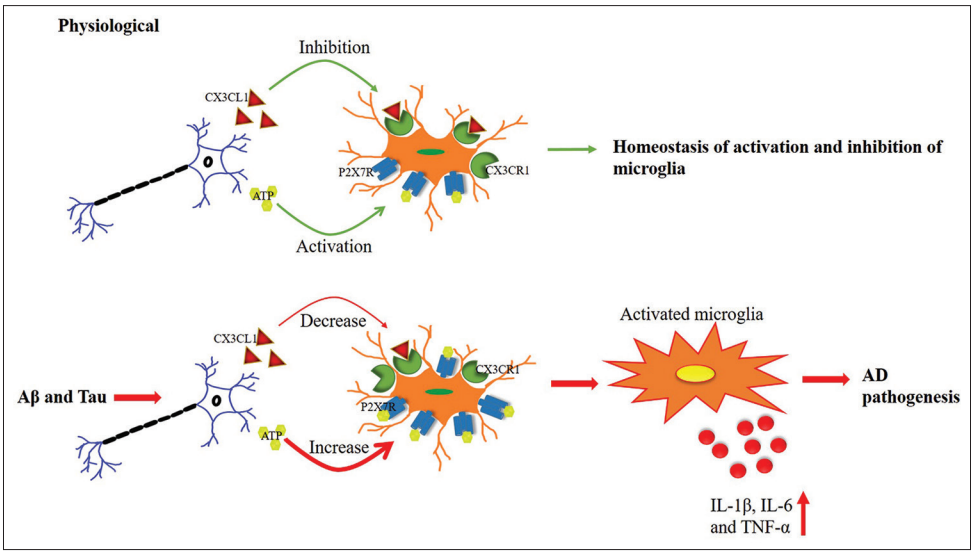

Figure 1: Modulation of microglia by CX3CL1/CX3CR1 and $P2X_7R$ signaling. A schematic of CX3CL1/CX3CR1 and $P2X_7R$ signaling under normal and pathological conditions. The CX3CL1/CX3CR1 pathway can stop the activation of mic, whereas ATP binding with $P2X_7R$ can induce microglial activation. Alzheimer's disease affects the regulation of these two signaling molecules, leading to microglial overactivation, thus resulting in pathological changes

Table 1: Drug targets CX3 chemokine ligand 1/CX3C chemokine receptor 1 and $P2X_7R$

Drug	Disease	Subject	Purpose	Effects	References
BBG	AD	Mice	Noncompetitive antagonist of $P2X_7R$	Improvement in cognitive and spatial learning function, inhibited the release of pro-inflammatory cytokines	[15]
JNJ-47965567	Neuropathic pain, schizophrenia	Rat, mice	$P2X_7R$ antagonist	Attenuated amphetamine-induced hyperactivity	[16]
				Alleviate schizophrenia-like behavioral alterations	[17]
					[18]
JNJ-54175446	Depression (clinical trial ongoing)	Human	$P2X_7R$ antagonist	Attenuated *ex vivo* lipopolysaccharide-induced interleukin-1β release	[19]
					[20]
F1	Peritonitis	Cell line	Antagonist of hCX3CR1	Inhibited the CX3CL1-induced calcium flux	[21]
AZ12201182	Primary human SMCs	Cell line	antagonist of CX3CR1	Abrogates the mitogenic and anti-apoptotic effects of CX3CL1	[22]
AZD8797	SCI, MS	Rat, mice	CX3CR1 inhibitor	Suppressing apoptosis, necrosis, and inflammatory responses	[23-25]
BI 655088	Atherosclerosis	Mice	CX3CR1 antagonist	Reduced the descending aorta plaque	[26]
WIN55,212-2	Human astrocytes	Cell line	suppression of CX3CL1	Inhibition of CX3CL1 through p38 MAPK signaling	[27]

CX3CR1: CX3C chemokine receptor 1, CX3CL1: CX3 chemokine ligand 1, SCI: Spinal cord injury, SMC: Smooth muscle cells, AD: Alzheimer's disease, BBG: Brilliant Blue G, MS: Multiple Sclerosis

of soluble CX3CL1. While CX3CL1 extracellular domains usually act as chemoattractant to recruit microglia to the site of injury in the brain [54], its soluble form has shown more neuroprotective effect, at least in PD than its membrane-bound form [55]. In AD, excessive γ-secretase complex activation enhances cleavage of amyloid precursor protein (APP) into the various lengths of Aβ peptide including Aβ42 which in turn lead to increased amyloid beta deposition [56]. The finding of the novel cleavage process of CX3CL1 by γ-secretase may suggest the possible neurotoxic role of this cleavage enzyme by enhancing soluble CX3CL1 clearance during AD pathogenesis.

Furthermore, studies on mice demonstrated dysregulated microglia activation and neurotoxicity upon LPS injection into *Cx3cr1*$^{-/-}$mice [30], indicating the neuroprotective effect of CX3CR1 on LPS-induced neuronal damage [30]. In a study that used mating *Cx3cr1* knockout mice with two different models of amyloid deposition (either rapid deposition of $A\beta_{42}$ [APPPS1] mice or gradual deposition of $A\beta_{40}$ (R1.40) mice), the neuroprotective effect of CX3CR1 signals were demonstrated by activated microglia, which reduced amyloid plaque deposition [31]. Another research used the *Cxcr1*$^{-/-}$mouse model and demonstrated that after LPS administration, MAPT phosphorylation was elevated in the neurons of knockout mice, which is related to microglia activation, in turn activating toll-like receptor 4 (TLR4) and IL-1 receptors [57]. This hyperphosphorylation of MAPT within neurons is correlated with microglia activation, which activates TLR4 and IL-1 receptors [58].

These findings indicate that CX3CR1 deficiency enhances the MAPT phosphorylation through microglial-mediated neuroinflammation. Moreover, CX3CL1 and CX3CR1 expression levels reportedly decreased only in the late, but not the intermediate stage of AD [34,35]. Further, animal-based studies have demonstrated the upregulation of CX3CR1 caused by the exposure of endothelial progenitor cells to 25 μmol/L $A\beta_{1-40}$ ($A\beta_{1-40}$),

which can be ameliorated by blocking FKN [58]. These data indicate that $A\beta_{1-40}$ induces elevation of CX3CR1 through the activation of FKN. Similarly, microinjection of $A\beta_{1-40}$ into the hippocampal CA1 area showed a significant increase in CX3CR1 expression [59]. Another research that used the 5xFAD mouse model demonstrated that CX3CR1 deficiency can rescue neuronal loss [60]. These results highlight the fact that microglial activation and the role of CX3CR1/CX3CL1 are complex and can be either protective or detrimental depending on the progression stages of the disease. The correlation of the CX3CR1/CX3CL1 signaling level with microglia activation and the effect at various stages of AD such as early, mild cognitive impairment (MCI), and late stage are presented in Figure 2.

Relationship between the $P2X_7R$ receptor and Alzheimer's disease

Expression of $P2X_7R$ in Alzheimer's disease pathology

Purinergic type-2 (P2) receptor family is classified into P2Y-G protein-coupled receptors and P2X-ligand-gated ion channels. Of the seven family members (P2X1–7) of P2X receptors, the immunological functions of $P2X_7R$ have been studied extensively. ATP reportedly activates this receptor under different types of stimulations, including injury, infection, or pathological conditions, triggering pore formation, which allows the molecules up to 900 Da to enter the cells [61]. The $P2X_7R$ has been associated with several neurodegenerative diseases including AD and PD [62]. Another research performed a postmortem investigation of the brain of patients with AD and demonstrated that $P2X_7R$ expression was upregulated in the microglia of the frontal cortex in those brains near the senile plaque [63]. Microglial cells monitor the brain, responding to disturbances with the help of cell surface receptors. Upon injury or insult of the brain, ATP is generated abundantly by neurons that signal the $P2X_7R$ to increase the expression in the microglia. A study cultured microglia from the brains of patients with AD and showed higher $P2X_7R$ upregulation compared with the control group [64]. These human studies confirm the strong link between $P2X_7R$ and microglia activation in AD.

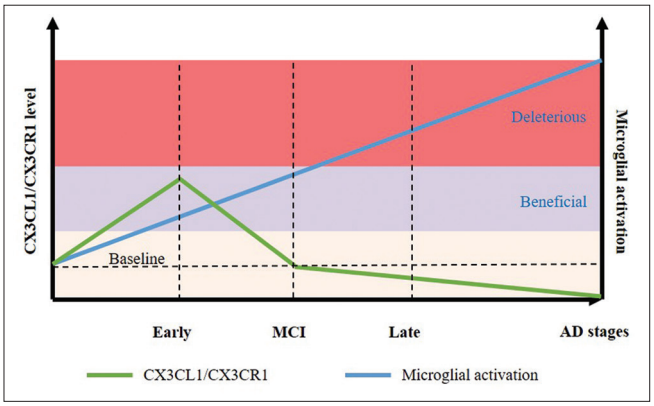

Figure 2: Expression of CX3CR1/CX3CL1 varies at different stages of Alzheimer's disease. During the early stage, CX3CR1/CX3CL1 signaling is low, and moderate microglial activation protects the neurons. At a later stage, when the disease progresses because of neuronal loss, CX3CR1/CX3CL1 signaling diminishes, resulting in microglial over activat

Neuroprotective and neurotoxic effects of $P2X_7R$ signaling in Alzheimer's disease

$P2X_7R$ is also known to regulate neuroinflammation and is widely expressed in microglial cells. A study that explored the connection between this receptor and microglia observed that $P2X_7R$ was overexpressed in the primary hippocampal culture of the rat. Time-lapse microscopy indicated that the overexpression of $P2X_7R$ itself is sufficient to cause microglia activation and proliferation [65]. Another study using a coculture of neurons and microglia demonstrated that $P2X_7R$ can regulate microglial activation and protect neurons from cell death. Administration of the $P2X_7R$ agonist reportedly triggered the release of neuroprotective TNF from the microglia and protected the neurons from glutamate toxicity [66]. Several other studies have also demonstrated that the release of pro-inflammatory cytokines from the microglia is controlled by $P2X_7R$. A study wherein the authors blocked $P2X_7R$ in experimental rats with autoimmune encephalomyelitis found that microglial activation was reduced along with the expressional decrease of pro-inflammatory cytokines including IL-1β, IL-6, and TNF-α [67]. The aforementioned results evidence the importance of this receptor in the regulation of different activities of the microglia.

Under pathological conditions, ATP can be released in large amounts and activate the $P2X_7R$, in turn, causing neuroinflammation and cytotoxicity [68]. Since AD is now believed to result from the conditions of chronic inflammation [69], loss or gain of function of $P2X_7R$ might influence the pathogenesis of AD. Another study used intrahippocampal injections of Aβ oligomers in wild-type mice and demonstrated a significant increase in IL-1β secretion from WT microglia compared with $P2X_7R^{-/-}$ microglia [70]. This indicates that by blocking $P2X_7R$ signaling, IL-1β secretion induced by the Aβ oligomers can be reversed. Another study that used APPPS1 mice, an AD mouse model, showed that the absence of $P2X_7R$ reduced the amyloid-beta load in isocortical regions and rescued spatial memory deficit. Notably, $P2X_7R$ deficiency in this AD mouse model did not alter IL-1β secretions [71]; however, it lowered the levels of chemokines, such as CCL3, CCL4, and CCL5. These inconsistent results could be because of the use of different animal models. An *in vivo* study that used a transgenic mouse model of AD (Tg2576) demonstrated an increase in $P2X_7R$ expression around the amyloid plaques in the hippocampus. Another analysis that used the Tg2576-AD mouse model also demonstrated the important role of $P2X_7R$ receptor in the generation of superoxide [72]. Taken together, these studies suggest that $P2X_7R$ can be considered as a therapeutic target for AD.

Conclusion

This article reviewed the importance of microglial activation in AD. We discussed the physiological as well as the pathological roles of microglia in the hope to provide an understanding of how microglial activation and inactivation signals by CX3CL1-CX3CR and $P2X_7R$ help the diagnosis and treatment of this disease. The significance of microglial cells in the progression of AD must be considered together with the neurons. Depending on the activating factors, microglia can

act as either as a savior or destroyer in AD. More studies are necessary to understand how microglial activation should be regulated to prevent or treat progression of AD.

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Conflicts of interest

There are no conflicts of interest.

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