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REVIEW

Alcohol-Induced Activation of Chemokine System and Neuroinflammation Development

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Abstract—Chemokines are immunoregulatory proteins with pleiotropic functions involved in neuromodulation, neurogenesis, and neurotransmission. The way chemokines affect the CNS plays an important role in modulating various conditions that could have negative impact on CNS functions, including development of alcohol use disorders. In this review, we analyzed the literature data available on the problem of chemokine participation in pathogenesis, clinical presentation, and remission of alcohol use disorders both in animal models and in the study of patients with alcoholism. The presented information confirms the hypothesis that the alcohol-induced chemokine production could modulate chronic neuroinflammation. Thus, the data summarized and shown in this review are focused on the relevant direction of research in the field of psychiatry, which is in demand by both scientists and clinical specialists.

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INTRODUCTION

Chemokines comprise a family of small-molecule proteins that are chemoattractant cytokines with main function being recruitment of leukocytes to the sites of inflammation [1]. They play an important role in both immune system and central nervous system (CNS) [2-4]. In the CNS, chemokines, along with cytokines, are involved in a number of physiological processes, such as neuroinflammation, changes in neuronal activity, communication between neurons and glia, neuroendocrine interaction, neurogenesis, and CNS development [5, 6].

More and more publications regarding the substance use disorders indicate that alcohol intake results in activation of immune response accompanied with the development of chronic neuroinflammation and change in chemokine regulation [7].

Selection, analysis, and generalization of the experimental material, as well as the accumulated data of clinical observations on the changed content of chemokines belonging to various families in the animals exposed to alcohol, and in the patients with various patterns of alcohol consumption and during alcohol withdrawal combined in the single review is important from the point of view of determination of further vector of biomedical research involving, in particular, search for biomarkers of the initial stages of neuroinflammation and potential tools of pharmacological correction in alcohol use disorders (AUD).

Nevertheless, there have not been enough studies focused on investigation of the changes in the chemokine system during exposure to alcohol in recent years; moreover, these studies largely include works with animal models, and only one of them have been devoted to studying humans. Therefore, this review includes literature published from January 2004 to April 2024.

Our literature search was performed within PubMed and eLIBRARY databases with the keywords “alcoholism”, “alcohol use disorders” in combination

Abbreviations: AUD, alcohol use disorders; GSK-3β, glycogen synthase kinase-3 β; PND, postnatal days; TLR4, Toll-like receptor 4.

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with one of the following search terms: “chemokine”, “chemokine receptor”, “neuroinflammation”.

The aim of the review is to summarize and analyze the data available on the changes in chemokine system in alcohol intoxication in animal models and in the patients with alcohol dependence. These data could be considered in further clinical and translational studies as pathologically relevant biomarkers or therapeutic targets in the treatment of AUD.

CHEMOKINES AND THEIR FUNCTIONS IN CNS

Chemokines are important components of neuroimmune system and are involved in a number of physiological processes, such as neuroinflammation, change in neuronal activity, interaction between neurons and glia, neuroendocrine interaction, neurogenesis, and CNS development [5, 6]. Proinflammatory chemokines, such as CCL2, CCL7, CCL8, CCL12, and CCL13 have been shown to induce chemotaxis of proinflammatory cells to sites of CNS inflammation or damage. Similarly, CX3CL chemokines are involved in stimulating glial cell activation, secretion of proinflammatory cytokines, expression of ICAM-1 intracellular adhesion molecules, and recruitment of $CD4^+$ T cells to CNS during neuroinflammatory process [8]. Consequently, it has been suggested that dysregulation of chemokine signaling and neuroinflammation contribute to neurodegenerative and psychiatric diseases [9]. For example, changes in chemokine circulation [such as CCL2, also known as monocyte chemoattractant protein-1 (MCP-1), CCL11 (eotaxin-1), CXCL8, CXCL12] have been associated with neurodegenerative disorders [10-13] and psychiatric disorders [8], such as cocaine use disorders [14] affective disorders [15-19], generalized anxiety disorder [20], personality disorders [20], schizophrenia [21-25], and also correlated with the severity of psychopathological and cognitive parameters [1]. In particular, CCL11 impairs hippocampal function in aging, and prenatal exposure to CXCL8 could disrupt early neurodevelopmental periods [8].

Chemokines comprise a group of small 8-12 kDa-proteins with similar tertiary structure including a sequence of 6-10 amino acids, followed by a long loop (N-loop), a 3_{10} helix, a three-stranded β-sheet, and a C-terminal α-helix [26].

Depending on position of cysteine residues at the N-terminus, chemokine molecules are classified into four subfamilies: C, CC, CXC, and CX3C chemokines [27], while there are 27 CC chemokines, 17 CXC chemokines, 2 XC chemokines, and 1 CX3C chemokine [26]. Chemokines mediate their effects through G-protein-coupled transmembrane receptors (GPCRs), which are designated as CR1, CCR1-11, CXCR1-5, and CX3CR1 [4, 28, 29]. GPCRs transmit signals via Gαi/o proteins, through which they inhibit adenylate cyclase and reduce protein kinase A activity [30], as well as through Gq proteins, due to which they can increase intracellular levels of Ca^{2+} and protein kinase C via the phospholipase C pathway [31-33].

Chemokines and their receptors are widely expressed on vascular bed cells, smooth muscle cells, as well as on various types of leukocytes [34]. Chemokines can reach the brain by crossing the blood-brain barrier [35]. At the same time, they can be released by neurons and glial cells directly in the brain in response to physiological or pathological conditions [36].

Regulation of chemokines and their receptors expression involves a complex and poorly understood interaction both with each other and with other systems. For example, in the case of damage or inflammation of CNS, mechanisms for increasing expression of chemokines by cerebrospinal fluid lymphocytes and T cells, migrating across the blood-brain barrier, as well as by glial cells of the brain, especially astrocytes, are triggered [37]. Moreover, in the CNS, individual chemokines of all four families perform different, but also overlapping functions.

C chemokines. The XC (or C) family includes only 2 very similar chemokines, XCL1 and XCL2, also known as lymphotactin α and β, respectively. It is known that, acting through the unique XCR1 receptor, XCL1 can cause chemotaxis of lymphocytes, but not of monocytes or neutrophils [38].

CC chemokines. Chemokines of this family have a pro-inflammatory effect through macrophage chemotaxis to the site of inflammation or damaged CNS cells, and are also involved in regulation of neural stem/progenitor cell migration [39]. Monocytic chemoattractant protein-1 (CCL2/MCP-1) is the first discovered and most studied human CC chemokine. It is one of the key chemokines that regulate recruitment and activation of monocytes and microglia. Biological function of CCL2 is mediated through the G protein-coupled CCR2 receptor. In addition to CCL2, CCR2 binds 4 more pro-inflammatory chemokines: CCL7, CCL8, CCL12, and CCL13 [40].

CCL2s, like its receptor type 2 (CCR2), are expressed in CNS neurons and cultured neuronal cell lines [41-43]. CCL2 is constitutively expressed in neurons of individual brain areas of rats, such as the cerebral cortex, hippocampus, hypothalamus, substantia nigra, cerebellum, and spinal cord [44, 45]. Thus, CCL2/CCR2 signaling could regulate functions of neurons.

In addition to its role in immune system, CCL2/CCR2 signaling is involved in the development of various neuroinflammatory diseases, such as Alzheimer’s disease [46], multiple sclerosis [47], Parkinson’s disease [40], and ischemic brain damage [48].

Although some studies have shown involvement of CCL2 in the brain damage caused by alcohol [40], its role in pathogenesis of AUD remains unclear to date.

Eotaxin-1 (CCL11), also known as the eosinophil chemotactic protein, is another chemokine of the CC family. After binding to CCR3 receptors expressed on the cell surface of eosinophils, CCL11 activates a number of intracellular signaling cascades, which leads to recruitment of eosinophils to the sites of inflammation during allergic reactions that are thoroughly investigated in asthma, allergic rhinitis, and other eosinophil-associated conditions. In particular, a systematic literature review, including 30 studies, showed that CCL11 concentrations in the blood and sputum were consistently increased in the patients with asthma, correlating negatively with lung function. This indicates the possibility of potential use of CCL11 as a biomarker for diagnosis and assessment of asthma severity and control [49]. In addition to eosinophils, CCR3 chemokine receptor is expressed on basophils, mast cells, and Th2 lymphocytes. Moreover, the last ones are involved in the production of the so-called Th2 cytokines (interleukins: IL-4, IL-5, IL-13), which implies that CCL11 is also involved in directing immune response towards the Th2 profile [1].

In the CNS, CCL11 is produced by epithelial cells of the choroid plexus, pericytes, astrocytes, and microglia under the influence of inflammatory stimuli [50]. In addition, CCL11 can enter the CNS, crossing the blood-brain barrier [51]. One study showed *in vitro* that CCL11 inhibits reversibly proliferation of neurons-progenitors in the isolated cells, neurospheres, and hippocampal slice cultures without affecting their ability to form both neurons and astrocytes [52]. It has also been demonstrated that, although no direct effect of CCL11 on neurons was found, this chemokine was able to stimulate migration and activation of microglia with subsequent production of reactive oxygen species, which enhanced death of neurons caused by glutamate [53]. A number of works show relationship of CCL11 with Alzheimer's disease [10, 13], child and adolescent psychopathology, including autism spectrum disorders [54, 55], major depression [17], bipolar disorder [56], dysthymia [57], obsessive-compulsive disorder [58], schizophrenia [22, 25], and substance use disorders [59, 60], as well as relationship of the elevated circulating CCL11 chemokine with progressive clinical deterioration observed in these disorders [61]. Thus, CCL11 is associated with a number of mental and neurodegenerative disorders, as well as with the degree of their clinical severity.

CXC chemokines. The main role of chemokines in neuroinflammation is regulation of neutrophil chemotaxis. This is the key function of CXCL1-CXCL8 chemokines, ligands of CXCR2, that are highly expressed on neutrophils. The overall effect of these chemokines on improving or worsening neuronal survival and recovery under inflammatory conditions remains unclear. In particular, there is evidence that most mice with knocked out CXC family chemokines or chemokine receptors are viable and are characterized by the absence of disturbances in the functioning of nervous tissue [39].

CXCL9-CXCL11 chemokines, in contrast, exert a more pronounced pro-inflammatory effect through the CXCR3-mediated chemotaxis of natural Th1 killer cells and associated macrophages. For example, blockade of CXCR3 has been shown to reduce Th1 and macrophage infiltration, as well as tissue damage. Moreover, increase in the hippocampal neurogenesis has been demonstrated in the adult mice with knockout of the *CXCR5*$^{-/-}$ chemokine receptor (CXCL13 ligand), while change in the structure of the cerebellum is observed in the mice with knockout of the *CXCL12* and its CXCR4 receptor [8].

CX3C chemokines. CX3CL1 chemokine, also known as fractalkine, is highly expressed in mature neurons and astrocytes, while its CX3CR1 receptor is expressed mainly on microglial cells, but, also, on mature neurons [62, 63]. CX3CL1 has been shown to have multiple effects on the CNS: on the one hand, it prevents excessive activation of microglia in the absence of injury, while promoting activation of microglia and astrocytes during neuroinflammatory processes, including secretion of proinflammatory cytokines, expression of ICAM-1, and recruitment of $CD4^+$ T cells in the CNS [64-66].

Both soluble and membrane-bound forms of CX3CL1 attenuate microglial activation and the lipopolysaccharide-induced increase in proinflammatory cytokines in primary microglia and neuron cell cultures.

Thus, while a number of chemokines have been shown to have significant effects on regulation of neuroinflammation, it is unclear for most of the chemokines whether their functions have the same relevance to regulation of chronic inflammation, which is assumed to be one of the mechanisms of pathogenesis in many psychiatric disorders.

STUDIES OF CHEMOKINE SYSTEM IN ANIMAL MODELS OF ALCOHOL USE DISORDERS

C chemokines. Studies of this chemokine family and their receptors are very few, including in animal models. It was revealed that the C3AR1 receptor expression is induced by ethanol, which leads to the change in phagocytosis of microglia [67]. Earlier studies have shown that the C5AR1 receptor is involved in the alcohol-induced inflammation [68, 69].

In the study by Holloway et al. some potential mechanisms by which the ethanol-induced neuroin-

flammation could contribute to the onset of neuropathology were shown [70]. Using the postnatal mouse model of fetal alcohol syndrome equivalent to the third trimester of human pregnancy, transcriptomic changes caused by ethanol exposure at a dose of 4 g/kg/day were evaluated in the cerebellum on the postnatal days 5 (PND5) and 6 (PND6), after 1 or 2 days of ethanol exposure, in order to detect changes in the early stages of emergence and development of this syndrome. In elucidating possible mechanisms by which ethanol induces early immune activation, Holloway et al. [70] identified immune-associated transcripts, expression of which was strongly altered by ethanol. On the PND5 and PND6, upregulated transcripts included C5AR1 and C3AR1 receptors, MSR1 (macrophage scavenger receptor 1), CCL3 chemokine (macrophage inflammatory protein-1 α, MIP-1α), and CD14.

CC chemokines. More studies are focused on investigation of the CCL2 chemokine belonging to this family. Ethanol has been found to induce CCL2 expression in the animal models of AUD [70], and genetic studies in animals show that the enhanced CCL2 signaling is accompanied by the increased alcohol consumption [71].

CCL2/CCR2 signaling plays an important role in the alcoholic neuropathology both in adult and developing CNS. Studies of the ethanol impact on the developing spinal cord in a mouse model equivalent to the third trimester of pregnancy [72] found that exposure to ethanol at a dose of 2.5 g/kg/day during development caused irreversible loss of spinal cord neurons, and CCR2 signaling played an important role in ethanol neurotoxicity. The ethanol-induced apoptosis and neurodegeneration in dorsal horn neurons in the mice in early postnatal period, which was accompanied by the glial activation, macrophage infiltration, and increased CCR2 expression. The ethanol-induced neuronal death during development resulted in irreversible loss of the spinal cord neurons in the adult mice. The study revealed that ethanol-induced endoplasmic reticulum stress and oxidative stress, as well as activated the glycogen synthase kinase-3 β (GSK-3β) and c-Jun N-terminal kinase (JNK) pathways. Knockout of the *CCL2* or *CCR2* genes made mice resistant to the ethanol-induced apoptosis, endoplasmic reticulum stress, glial activation, GSK-3β and JNK activation. On the other hand, knockout of the *CCR2* gene provided much better protection against the ethanol-induced spinal cord injury. Hence, the effect of ethanol on the mouse embryonic development led to irreversible losses of spinal cord neurons, and CCR2 signaling played an important role in the ethanol neurotoxicity [72].

In the study by Chang et al. [73], pregnant rats from the embryonic day 10 to 15 (during peak neurogenesis) were orally administered ethanol at a moderate dose (2 g/kg/day) or peripherally injected with CCL2 or CCR2 antagonist to examine the role of the CCL2/CCR2 system in the mechanisms of ethanol effects. Maternal administration of ethanol has been demonstrated to increase the radial glia cell density in embryos and simultaneous stimulation of the CCL2/CCR2 system, and these effects are mimicked by the maternal CCL2 administration and blocked by the CCR2 antagonist. By stimulating colocalization of CCL2 with radial glia and neurons, but not microglia, ethanol increases amount of neuronal melanin-concentrating hormone (MCH) near radial glia cells and establishes contact along their processes protruding into the lateral hypothalamus. Further tests identified that the CCL2/CCR2 system in the neuroepithelium is the primary source of ethanol sexual dimorphism. These results provide new knowledge on how the inflammatory chemokine pathway functions in neuroprogenitor cells mediating long-lasting stimulatory effects of ethanol on neuropeptides linked to adolescent behavior associated with alcohol consumption [73].

Several studies have shown that the CCL2/CCR2 signaling is also involved in the behaviors associated with alcohol use. Thus, deletion of the *CCR2* and *CCL2* genes (in female mice) reduced preference for ethanol in the case of free choice between ethanol solution and water, while alcohol administration resulted in the stronger conditional aversion to its taste in the $CCR2^{-/-}$ and $CCL2^{-/-}$ mice [68]. The study by Holloway et al. [74] in mice showed that alcohol activates the Toll-like receptor 4 (TLR4) signaling, leading to induction of proinflammatory cytokines and chemokines in the CNS. Evaluation of mRNA expression with qRT-PCR showed that ethanol induced increase of the levels of IL-1β cytokines and TNF-α tumor necrosis factor, CCL2 chemokine, COX2 cyclooxygenase, as well as FosB and JunB proteins in the cerebellum of the wild-type and TLR4-deficient mice (TRIF). Although it is not exactly clear how CCL2 regulates alcohol behavior, a possible explanation is that CCL2 activates dopamine system [75]. Based on the presented information it could be concluded that CCL2/CCR2 signaling likely participates in the alcohol-induced brain damage by regulating microglial activation and neurotransmission [40]. In the study by June et al. [76] it has been reported that the P-line rats preferring alcohol have innately elevated levels of TLR4 and CCL2 chemokine, which are localized in the neurons of the central nucleus of amygdala (CeA) and ventral tegmental region (VTA). To investigate potential role of the TLR4/CCL2 signal, herpes simplex virus (HSV) vectors (amplicons) that preserve neurotropism *in vivo* have been used. Introduction of the TLR4 or CCL2 miRNA amplicons into the CeA or VTA of the P rats inhibited expression of the target genes and reduced alcohol dependence. The similarly delivered amplicon of scrambled miRNAs

did not inhibit TLR4 or CCL2 expression or reduce alcohol overconsumption, thus identifying the neuronal TLR4/CCL2 signal as the signal that regulates onset of the voluntary self-administered alcohol consumption. This signal was sustained during alcohol consumption through increased expression of the corticotropin-releasing factor (CRF) and its feedback regulation of TLR4 expression, which, likely, contributed to the transition to alcohol dependence [76].

In the study by Zhang et al. [77] exposure to ethanol was shown to increase expression of CCL2, but not of CCR2, in the mouse brain on the PND4 and in the microglial cells (SIM-A9). The CCL2 synthesis inhibitor, bindarite, and the CCR2 antagonist, RS504393, inhibited ethanol-induced neuroapoptosis, microglial activation, and expression of proinflammatory factors. Further studies using the gene knockout mice confirmed that CCL2 or CCR2 deficiency made the mice more resistant to the ethanol-induced neurodegeneration. Moreover, ethanol and CCL2 caused greater neuronal death in the neuron/microglia co-culture system than in the neuronal culture alone. Blocking the CCL2/CCR2 signaling protected primary cortical and cerebellar neurons from the ethanol-induced death in co-cultures of neurons and microglia. The TLR4 receptor involved in innate immunity along with the GSK-3β kinase were found to mediate the ethanol-induced activation of microglia and proinflammatory cytokines in the cultured microglia cells, and there was a significant interaction between the TLR4, GSK-3β, and CCL2/CCR2 signaling in response to ethanol exposure [77]. Also, Yang et al. [78] have shown using co-culture of neurons and microglia that the CCL2-induced neurotoxicity requires microglia and that exogenous CCL2 is able to activate and stimulate microglia to produce cytokines. This study revealed that the neutralizing CCL2 antibodies inhibit the CCL2-induced microglial activation and neuronal death in the culture and thalamus.

The levels of CCL2 transcript are increased by exposure to ethanol. *CCL2* and *CCL3*, which are target genes of the NF-κB transcription factor, are induced by ethanol and are key mediators of CNS inflammation and alcohol-associated behavior [68, 69]. Ethanol has previously been shown to induce CCL2 expression in the animal models of fetal alcohol syndrome [79] and in the adult animal models of alcohol use disorders [80].

Alcohol exposure activates microglia, increases expression of CCL2 and other proinflammatory cytokines, such as TNF-α, IL-6, and IL-1β, and leads to neuronal death in the rats [81-83]. Qin et al. [84] reported that ethanol, administered at a dose of 5 g/kg/day *per os*, potentiated the lipopolysaccharide-induced CCL2 increase and microglial activation in the brain of adult mice. It was suggested that CCL2 reduces the "threshold sensitivity" of microglia as a "priming" stimulus and enhances synthesis of proinflammatory cytokines in response to subsequent exposure [85].

In the experiment of Valenta et al. [86] involving the CCL2 signaling enhancement through direct injection of it into the brain, there was correlation observed between the dose of CCL2 and consumption of the sweetened ethanol solution by the Long-Evans rats during the first 4 weeks (while pumps were flowing) and during the entire 8-week experiment. Animals receiving the highest dose of CCL2 (2 μg/day) consumed the most ethanol during weeks 3 through 8. This study proves that neuroimmune signaling could increase directly chronic voluntary ethanol consumption, and that this increase persists beyond the cytokine administration. The ethanol-induced increase in CCL2 or increase in CCL2 due to various other neuroimmune mechanisms could further facilitate ethanol consumption. Further investigation of this mechanism, particularly using alcohol dependence models, could help to determine whether the effects on CCL2 signaling possess therapeutic potential in the treatment of AUD. Bray et al. [87] in their work attributed increased CCL2 expression levels in the transgenic mouse astrocytes to the increased alcohol consumption in the alcohol consumption tests, and to spatial and associative learning, thereby supporting the following hypothesis that the increased CCL2 levels induce neuroadaptive changes, which, in turn, alter the alcohol impact on CNS.

Lowe et al. [88] were searching for a potential therapeutic approach in the treatment of alcohol-associated neuroinflammation, and have suggested that chronic alcohol consumption leads to infiltration of peripheral immune cells into the CNS. Since chemotaxis through the CCL2/CCR2 signaling axis is critical for recruitment of macrophages in the periphery and center areas, it has also been hypothesized that blockade of the CCL2 signaling by the dual CCR2/5 inhibitor cenicriviroc would prevent the alcohol-induced infiltration of peripheral macrophages into the CNS and alter neuroinflammatory state of the brain caused by chronic alcohol consumption. Investigation of the female mice demonstrated that chronic alcohol consumption caused microglial activation and infiltration of peripheral macrophages in the CNS, especially in the hippocampus. Cenicriviroc eliminated the ethanol-induced recruitment of peripheral macrophages and reversed partially microglial activation. In addition, chronic alcohol consumption enhanced expression of the pro-inflammatory markers in various brain areas, including cortex, hippocampus, and cerebellum. The CCR2/5 inhibition reduced alcohol-mediated expression of inflammatory markers. This fact is considered by the authors as a potential therapeutic approach in the treatment of alcohol-associated neuroinflammation. In another work, Lowe et al. [89]

revealed that alcohol consumption increased significantly expression of the proinflammatory cytokines, such as TNF-α, IL-17, and IL-23, CCL2 chemokine, and Hmgb1 (high-mobility group box 1) cytokine mediator in the brain and intestine. Thus, decrease in the bacterial load of intestine, resulted from the use of antibiotics, weakened expression of all pro-inflammatory cytokines both in the brain and in the small intestine. Alcohol consumption led to microglial activation and morphological changes in the cortex and hippocampus, characterized by the reactive phenotype. These alcohol-induced changes were corrected after the prescribed antibiotics weakened the intestinal microbiome. Unexpectedly, antibiotic use led to the increased expression of mRNAs of some inflammation components in both brain and intestine [89].

The data obtained by Huang et al. [90] in humans and mice, show that chronic alcohol consumption is associated with the increased levels of the CCL11 chemokine. CCL11 levels correlate with severity of alcohol dependence and could serve as its potential indicator. The reduced CCL11 level after alcohol withdrawal is associated with the relief in clinical symptoms. The authors conclude that CCL11 participates in the neurobiological mechanisms underlying alcohol dependence. Chronic and excessive ethanol administration has also been shown to induce expression of inflammatory molecules (CCL2) in the adult mice [83].

Specific sex-dependent effects of alcohol on microglia in the developing rat hippocampus were investigated to simulate acute effects following a single-day alcohol exposure (2 feedings, 2 h apart, at a total ethanol dose of 5.25 g/kg) on PND4 [91]. Neuroimmune response was evaluated by measuring microglia number and chemokine gene expression on the PND5 and PND8. In many hippocampal subregions on the PND5, the male pups had higher microglial number compared to the females, but this difference disappeared by the PND8, unless exposed to alcohol. After alcohol exposure, the level of C-C motif chemokine ligand 4 (CCL4) was significantly increased in the female pups on the PND5 and PND8. The results demonstrate clear difference between the neuroimmune response to ethanol exposure in females and males [91].

Effects of alcohol on activation of immunity in the aged animals has not been investigated extensively, despite the fact that alcohol abuse has a significant impact on the health of the elderly population. Kane et al. [92] compared influence of ethanol on the chemokine and cytokine expression in the hippocampus, cerebellum, and cerebral cortex of the aged C57BL/6 mice. The mice were gavaged with 6 g/kg of ethanol for 10 days, tissue were harvested 1 day after treatment. Ethanol exposure caused selective increase of the CCL2 mRNA levels in the hippocampus and cerebellum, but not in the cortex of the aged mice compared to the control animals. According to this paradigm, ethanol did not affect the mRNA levels of the cytokines IL-6 or TNF-α in any of the studied brain areas of aged animals. Collectively, these data indicate area-specific susceptibility to ethanol regulation of neuroinflammatory and addiction-associated molecules in the aged mice. The authors conclude that these studies could be of great importance regarding to alcohol-induced neuropathology and alcohol dependence in older adults [92].

The level of CCL2 in microglia obtained in animal models of AUD with forcible ethanol administration is a major marker of neuroinflammation. However, there is conflicting evidence on whether the CCL2 levels increase in the case of voluntary ethanol intake. Thus, the key role of CCL2 in stimulating motivation to consume ethanol is challenged. In the study of Berríos-Cárcamo et al. [93] the levels of CCL2 mRNA levels were studied in the brain areas of the C57BL/6-line mice associated with motivation to consume alcohol, particularly in the prefrontal cortex, hippocampus, and striatum, as well as in the cerebellum, which is the brain area highly sensitive to ethanol; all experiments were carried out with the animals that consumed ethanol voluntarily for two months. A significant increase in the CCL2 mRNA levels was found in the cerebellum of mice exposed to ethanol compared to the control animals. At the same time, no significant changes were observed in the prefrontal cortex, hippocampus, striatum, and microglia isolated from the hippocampus and striatum. These results suggest that stimulation of the voluntary ethanol consumption by the C57BL/6 mice does not require neuroinflammation in the areas associated with motivation. Moreover, cerebellar susceptibility to neuroinflammation could be the cause of cerebellar degeneration in humans after chronic ethanol consumption [93].

CXC chemokines. It was revealed in the recent study with mice that overexpression of *CXCL14*, assessed by qPCR and ELISA, enhances alcoholic liver damage, as evidenced by the measurements of ALT (alanine aminotransferase) and AST (aspartate transaminase) levels in the plasma, as well as of triglycerides in the liver [94]. In addition, co-expression of *BRG1* (Brahma-related gene 1) and *CXCL14* genes was found to be positively correlated with the neutrophil infiltration in this study. In the work by Kusumanchi et al. [95] a novel role of the *FKBP5* gene (encoding FK506-binding protein 51) in pathogenesis of the alcoholic liver disease was identified. *FKBP5* loss alleviates the alcohol-induced liver damage through the CXCL1 signaling, indicating its potential role as a target in the treatment of alcohol-induced liver damage.

The alcohol-induced changes in circulating chemokines have also been studied in preclinical models of alcohol consumption using male Wistar rats [59].

The rats subjected to repeated ethanol administration (3 g/kg, gavage) had lower CXCL12 concentrations and higher CCL11 concentrations relative to the control group. Moreover, the elevated CCL11 concentrations in the ethanol-exposed rats were further increased by the prior stress exposure. Accordingly, acute ethanol exposure caused changes in the CXCL12 and CCL11 levels, similarly to the effect of repeated exposure. Another study showed that the moderate prenatal ethanol exposure stimulates chemokine system of CXCL12/CXCR4 in the radial glial progenitor cells in hypothalamic neuroepithelium and also in neuropeptide system in the lateral hypothalamus of embryo and postnatal rat offsprings [96].

A study conducted with adult male cynomolgus macaques evaluated plasma protein levels during the 32-month experimental protocol: at baseline, induction of water and ethanol (4% *w/v* in water) self-administration, after 4 months, after 12 months of simultaneous 22-hour daily access to ethanol and water [97]. Chronic ethanol intake in primates has been shown to result in the allostatic state of physiological compromise with regards to circulating proteins associated with immunity and stress in the NF-κB and STAT/JAK-associated pathways, which correlates with the altered endocrine activity.

In another animal model, *Danio rerio* fish, participation of the CXCL2a/CXCR4b chemokine system in mediating the stimulating effect of ethanol on neuron density in the hypothalamus of embryos was studied. The results provide clear evidence that the stimulating effects of ethanol in small and moderate doses on the number of hypothalamic neurons in the early stages of development are mediated, in part, by enhanced transcription and intracellular activation of the CXCL2a/CXCR4b chemokine system, probably due to autocrine CXCL2a signaling to the CXCR4b receptor on neurons [98].

CX3C chemokines. It was shown in the work by García-Marchena et al. [59] that there was no significant difference in the CX3CL1 chemokine concentrations in the plasma of male Wistar rats between the group subjected to repeated ethanol exposure (3 g/kg, gavage) and the control group.

Investigation of gender differences in the inflammatory chemokine profiles caused by the excessive ethanol consumption during adolescence revealed that in the female wild-type adolescent mice, intermittent ethanol administration increased the CCL2, CCL3, and CXC3CL1 chemokine levels in the prefrontal cortex and serum (CCL2 and CCL3), but significant differences in the CX3CL1 levels in the prefrontal cortex were observed only in the male mice. In the ethanol-treated *TLR4* genetic knockout mice, male or female, no changes in the chemokine levels were found in the serum or prefrontal cortex. These results showed that the females are more vulnerable to the inflammatory effects of binge ethanol consumption than the males; and suggested that TLR4 is an important target of the ethanol-induced inflammation and neuroinflammation in adolescence [99].

Pascual et al. [100] in their work revealed that chronic ethanol consumption increased the CX3CL1 chemokine levels in striatum and serum of the wild-type mice. Twenty-four hours after ethanol withdrawal, high CX3CL1 level was maintained in the striatum. The authors associate this with the increase in anxiety behavior assessed with the light/dark transition and elevated plus maze tests. Notably, the mice lacking TLR4 or TLR2 receptors are largely protected from the ethanol-induced chemokine release, as well as from the behavioral effects during the alcohol withdrawal. These data confirm the role of TLR4 and TLR2 responses in neuroinflammation and anxiety-associated behavioral effects during ethanol deprivation. This evidence also proves that chemokines could serve as biomarkers of the ethanol-induced neuroimmune response [100].

Thus, the results of the above-mentioned studies in animal models suggest involvement of chemokines in the mechanisms of AUD. Majority of the studies involve CCL2 chemokine and its receptor. CCR2 signaling has been shown to play an important role in the ethanol neurotoxicity. The results of the presented works support the general neuroimmune hypothesis of dependence. The alcohol-induced chemokine production could modulate alcohol impact on regulation of chronic inflammation.

CHANGES IN THE HUMAN CHEMOKINE SYSTEM UNDER THE INFLUENCE OF ALCOHOL

There is sufficient evidence in the literature to support involvement of many chemokines in pathogenesis of AUD. At the same time, most of the studies are focused on the research in animal models, while only few studies have been conducted with biomaterial from the patients with AUD.

Alcohol has been shown to stimulate inflammatory pathways by activating microglia in the CNS in both adult and developing brain [101]. Recent studies indicate that chemokines are key mediators of the ethanol-induced neuroimmune response and neuroadaptive changes in the CNS, together with cytokines, such as IFN-2α [102], TNF-α, IL-1β, IL-6, IL-10 cytokines [103].

In particular, the study of postmortem brain tissues of 5 people with alcoholism revealed that CCL2 concentration in the ventral tegmental area, substantia nigra, hippocampus, and amygdala of the brain

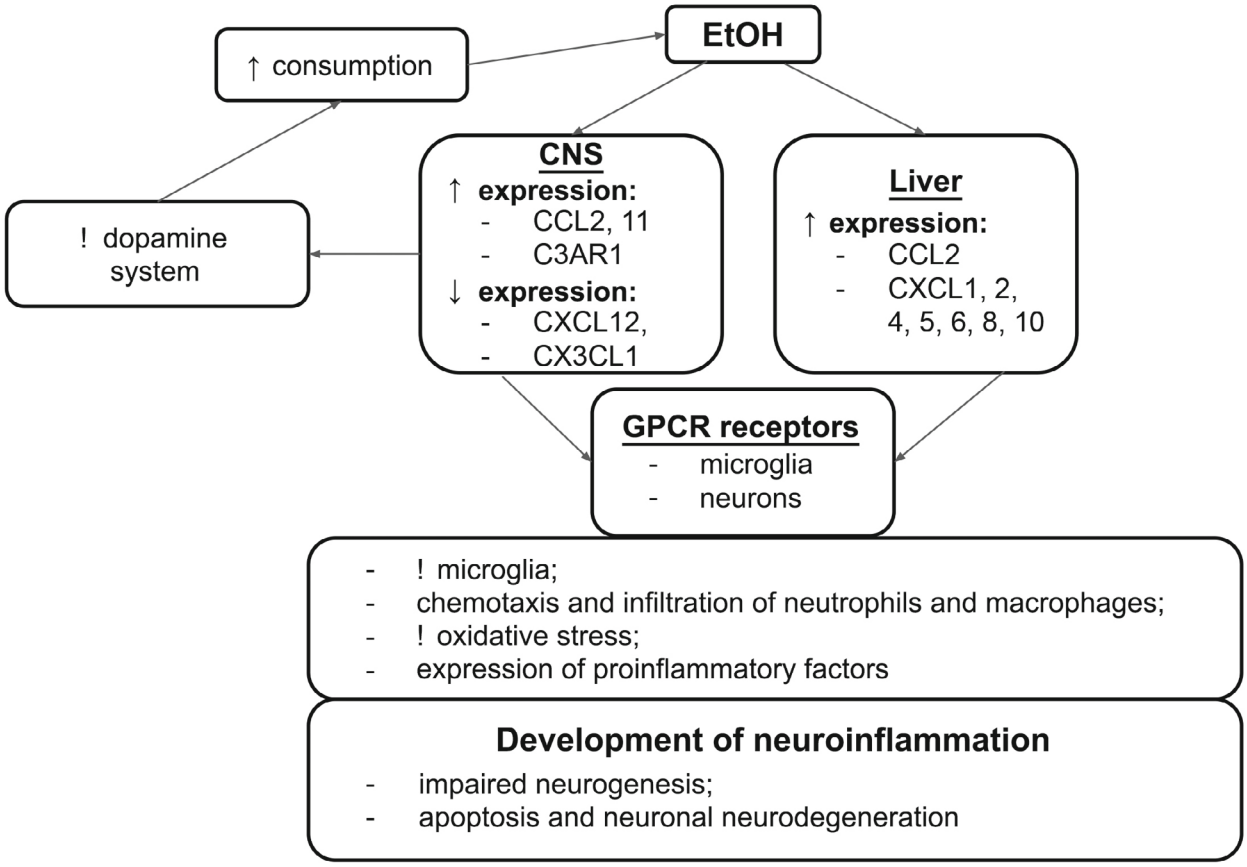

Fig. 1. Contribution of chemokines to neuroinflammation in alcohol intoxication. Ethanol exposure (EtOH), affecting liver and CNS cells, leads to the increased expression (↑) of some chemokines and their receptors (CCL2, -11, C3AR1, CXCL1, -2, -4, -5, -6, -8, -10) and decreased expression (↓) of others (CXCL12, CX3CL1). Chemokines, in turn, interacting with their GPCR receptors on the surface of microglia and neurons, contribute to activation (!) of microglia, chemotaxis, and infiltration of neutrophils and macrophages into the inflammation site, as well as to activation of oxidative stress and expression of a number of pro-inflammatory factors. Altered expression of chemokines in the CNS also leads to activation of the dopamine system and increased alcohol consumption, which, in turn, results in an even greater change in the chemokine expression. Thus, the development of neuroinflammation leads to impaired neurogenesis, neurodegeneration, and apoptosis of neurons.

was increased in the alcoholic patients with compared to the control group [69]. Another research of the postmortem brain tissues of 10 men with AUD [104] showed increase in the expression of CCL8, CCL7, CCL13, CCL5, CXCL8, CXCL12 chemokines and their receptors (CCR1 and CCR2, CXCR3 and CXCR4) in orbitofrontal cortex of the patients compared to the control group. Although the sample size in these two studies is rather small, the demonstrated data suggest that the ethanol-induced increased expression of chemokines and their receptors in the brain could modulate the effects of alcohol exposure/withdrawal on synaptic function, as well as contribute to AUD [105]. In the study by García-Marchena et al. [59] content of a number of chemokines (CXCL8 and -12, CX3CL1, CCL2, -3, and -11) was examined and the statistically significant association of just a few chemokines with AUD was demonstrated: plasma concentrations of CXCL12 and CX3CL1 chemokines were lower in the patients compared to the control group.

In the same work, the topic of gender differences in terms of immune response to alcohol overconsumption was noted. In particular, plasma concentration of CCL11 was much lower in the women with alcoholism than in the men [59]. Experiments conducted in adolescents (humans and mice) by Pascual et al. [99] also showed that women are more vulnerable to the inflammatory effects of ethanol overconsumption than men: at equivalent blood alcohol levels, cytokine and chemokine levels (IFN-γ, IL-10, IL-17A, IL-1β, IL-2, IL-4, IL-6, IL-8, CX3CL1, CCL2, and CCL3) in the plasma were found to be higher in the adolescent women than in the male adolescents after severe alcohol intoxication. Thus, in addition to the structural and functional gender differences in the effects of ethanol on the brain of adolescents [106], new evidence suggests existence of sex differences also in the immune and neuroimmune responses induced by ethanol [107].

A study of the blood plasma of 151 patients with alcohol dependence showed that the CCL11 levels

were higher in the patients than in the control group, and the CCL11 levels decreased during detoxification. In addition, the CCL11 levels were shown to correlate positively with the alcoholism severity assessed by the SADQ scale (The Severity of Alcohol Dependence Questionnaire) [90]. In another work focused on analysis of the serum of male patients with excessive alcohol consumption, higher levels of CCL2 were observed along with the increased levels of IL-6 and IFN-γ, as well as lower levels of TGF-β1 (transforming growth factor β1) compared to the control group [108]. Moreover, relationship between the CCL2 plasma concentration and clinical remission in the patients was shown: increase in the number of days since last drink was associated with the lower CCL2 concentration [109], while the higher CCL2 concentration was associated with greater cravings for alcohol [110]. Higher CCL2 levels were also associated with poor sleep quality, higher rates of anxiety and depression, as well as with the increase of the following parameters: number of drinking days, average amount of alcohol drunk per day, number of days of excessive alcohol consumption, and total amount of alcohol consumed [111].

When examining cerebrospinal fluid of 28 alcoholics and 13 healthy volunteers, the researchers found that CCL2 concentration in the patients was significantly higher both on the 4th and 25th day after detoxification. The CCL2 concentration also correlated positively with activity of the liver enzymes: GGT (gamma-glutamyl transpeptidase) and AST [112]. These data support the hypothesis that the CCL2-mediated neuroinflammation could be associated with alcohol-dependent liver inflammation.

Hence, it seems reasonable to suggest that in the AUD, chemokines are usually evaluated particularly in the context of alcoholic hepatitis [113-115]. Moreover, along with the IL-1 inhibitors and pan-caspases, CCL2 inhibitors are currently considered as new therapeutic drugs in the treatment of alcoholic hepatitis [116].

In particular, increased expression of CXC (CXCL8, CXCL2, CXCL5, CXCL6, CXCL10, and CXCL4) and CC (CCL2, but not CCL5) chemokines was observed in the liver cells of the patients with alcoholic hepatitis in comparison with the control [115]. In addition, higher expression levels of CXCL8, CXCL5, CXCL2, and CXCL6 were associated with the worst disease prognosis [115]. Another study performed using RNA sequencing also showed that activity of the CXCL chemokines (CXCL1, CXCL6, and CXCL8) was increased in the liver of patients with alcoholic hepatitis [117]. A weighted gene coexpression network analysis (WGCNA) showed that the individual members of the CXC chemokine (CXCL8) and CC chemokine (CCL20) families were highly associated with alcoholic hepatitis, compared to the controls, but have no relationship with the liver diseases of another etiology [118].

Taken together, this evidence suggests potential mechanisms through which the ethanol-induced neuroinflammation could contribute to neuropathology in both the developing and adult organism.

CONCLUSION

To date, there are not many works in the literature focused on investigation of chemokines in the context of alcohol dependence. Most of them include studies in animal models, and only few study patients with AUD.

Based on the presented data, we propose a diagram illustrating contribution of the ethanol-induced activation of chemokine system to the development of neuroinflammation (Fig. 1).

Generally, in the context of alcohol abuse, most of the chemokines have not been explored and not represented sufficiently in the literature, especially in the studies performed with patients. Feasibility of using these proteins as pathologically relevant biomarkers or therapeutic targets should be considered in future clinical and translational studies.

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