The role of Toll-like receptors in neurobiology of alcoholism

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SUMMARY   Alcoholism is a global socially significant problem and still remains one of the leading causes of disability and premature death. One of the main signs of the disease is the loss of cognitive control over the amount of alcohol consumed. Among the mechanisms of the development of this pathology, changes in neuroimmune mechanisms occurring in the brain during prolonged alcohol consumption and its withdrawal have recently become the focus of numerous studies. Ethanol consumption leads to the activation of neuroimmune signaling in the central nervous system through many subtypes of Toll-like receptors (TLRs), as well as release of their endogenous agonists (high-mobility group protein B1 (HMGB1), S100 protein, heat shock proteins (HSPs), and extracellular matrix degradation proteins). TLR activation triggers intracellular molecular cascades of reactions leading to increased expression of genes of the innate immune system, particularly, proinflammatory cytokines, causing further development of a persistent neuroinflammatory process in the central nervous system. This leads to death of neurons and neuroglial cells in various brain structures, primarily in those associated with the development of a pathological craving for alcohol. In addition, there is evidence that some subtypes of TLRs (TLR3, TLR4) are able to form heterodimers with neuropeptide receptors, thereby possibly playing other roles in the central nervous system, in addition to participating in the activation of the innate immune system.

Keywords   alcoholism, brain, neuroinflammation, Toll-like receptors, neuroimmune signaling

1. Introduction

The pathological effect on neuroimmune mechanisms in the brain caused by long-term ethanol consumption has attracted increased interest among researchers over the past two decades. One of the earliest studies in this area was performed by Lewohl *et al.* in 2000 (*1*). Using DNA microarray technology, the authors discovered an unexpected abundance of changes in the genes of the innate immune system in postmortem samples of the cerebral cortex of people with alcoholism (*1*). The very first experimental data showing that long-term ethanol consumption can activate the innate immune system in the central nervous system, promoting the production of pro-inflammatory cytokines and the death of nerve cells, were published by Valles *et al.* in 2004 (*2*). These results have been repeatedly confirmed and expanded in subsequent studies (*3-8*).

Impairments in the coordinated work of mechanisms at the cellular and molecular levels in various structures of the brain induced by prolonged use of ethanol lead to serious consequences such as emotional disorders (increased levels of anxiety and anxiety, deterioration in attention, aggression) and, worst of all, loss of cognitive control over the amount of alcohol consumed. These signs serve as criteria for the transition from the abuse of alcoholic beverages (drunkenness) to the formation of a complex and incurable mental illness, alcohol dependence (*9-14*).

For our review, we use the term "alcoholism" to mean a human disease, whereas "long-term ethanol consumption" is modeling of alcoholism in animals.

Long-term ethanol consumption leads to activation of microglial cells in the brain (*15*). Microglial activation is characterized by small morphological changes and increased expression of signaling molecules involved in the immune response (components of the major histocompatibility complex, Toll-like receptors (TLRs), and pro/anti-inflammatory cytokines) (*16*). In the brain, microglial cells, being resident macrophages in the central nervous system, express diverse receptors of the innate immune system, particularly members of

the TLR superfamily. The latter plays an important role in triggering the inflammatory response to various pathogens (*17,18*).

TLRs are activated by exogenous or PAMP (pathogen-associated molecular pattern; molecular fragments associated with pathogens) ligands, as well as by endogenous or DAMP (damage-associated molecular-pattern) ligands; their level increased in the brain during prolonged use of ethanol (*4*). Animal experiments have shown that the use of ethanol leads to increased expression and extracellular release of an endogenous ligand, HMGB1 protein (High-mobility group protein B1) (*4*). The interaction of the ligand with TLRs is a signal that triggers complex intracellular cascades of reactions, activating transcription factors NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells), AP-1 (activator protein 1), and IRFs (interferon regulatory factors). This leads to a subsequent increase in expression of genes encoding various pro-inflammatory signaling molecules (pro-inflammatory cytokines, oxidases, nitric oxide synthase, proteases, and components of the major histocompatibility complex) (*3-7,18-21*). It should be noted that an increase in the level of pro-inflammatory cytokines correlates with an increase in the level of expression of certain subtypes of TLRs in the central nervous system (TLR2, TLR3, TLR4, TLR7) (*3-7*). These changes cause the development of a long-term neurotoxic effect, which further leads to proteasomal degradation of proteins, demyelination of axons, destruction of synaptic terminals due to damage of synaptic proteins and, ultimately, to the death of many cells in the central nervous system. Neurodegenerative changes in all parts of the brain of patients with alcoholism are a consequence of the neurotoxic effect of ethanol on the brain (*3,22-25*).

Besides microglia, neurons and neuroglial cells are able to respond to the pro-inflammatory factors of the immune system by expressing many different cytokine receptors such as TNFαR, IL-1βR, IL-6R (CD126), IFNγR, and IFNαR. Fractalin (also known as CX3CL), a protein secreted by nerve cells in the brain, is involved in the regulation of the migration of microglial cells in the central nervous system (*18,26*).

Microglial cells are considered a major component of the immune system in the central nervous system (CNS). However, accumulating evidence suggests that astrocytes serve as important effector cells and regulators of the local immunity in pathophysiological conditions due to expression of a wide spectrum of molecules involved in the innate or adaptive immune response, secretion of cytokines and complement components, and differential response to various stimuli inducing either the innate or adaptive immune response. Moreover, ethanol can alter the function of CNS glial cells including microglia and astrocytes which normally maintain homeostasis in the CNS (*27,28*).

Astrocytes are the major glial cell type in the CNS

and can also express TLR2-3 and TLR9 (*17*). Based on this, it is assumed that the effect of ethanol/ethanol metabolites on astrocytes can also be mediated through the TLR; but this assumption requires further research.

Thus, the release of pro-inflammatory cytokines can lead to the activation of an increasing number of microglial cells, as well as astrocytes, oligodendrocytes and neurons, enhancing the mechanisms of neuroimmune signaling (*2,29-31*).

This review work summarizes the results of studies that bear compelling evidence about TLR-signaling that changes in the brain when using ethanol, and that many types of brain cells are involved in this process due to the fact that different subtypes of TLR-receptors are localized on different types of nervous system cells. There is still no complete understanding of the ultimate cause that starts these changes when ethanol is consumed. It is anticipated that endogenous TLR agonists are essential to this process, because they are secreted during ethanol consumption. Such agonists, for example, include HMGB1, heat shock proteins, uric acid, microRNA (*6,32*).

The research results of recent studies bring out clearly that TLR overexpression mediates the development of a neurotoxic effect in the CNS when ethanol is used. Possibly, TLR signaling contributes to the regulation of functional activity of neurotransmitter systems, which can contribute to the formation of pathological alcohol craving. Disturbances in the coordinated operation of neuroimmune signaling mechanisms in various brain structures as a result of ethanol use may result in consequences such as emotional disrepairs (increased anxiety level, impaired concentration, aggression) and reduced cognitive control over the amount of alcohol consumed. These signs serve as criteria for change from abusive drinking behavior to the formation of a complex and incurable mental illness – alcoholism (*5,6*).

2. Toll-like receptors (TLRs)

During the last decade a large number of pattern recognition receptors (PRRs) have been discovered and intensively studied. They have been found in all multicellular organisms, ranging from invertebrates (*e.g.*, sponges) to mammals, including humans (*18*). To date, 5 families of signaling PRRs are known: Toll-like receptors (TLRs), C-type lectin receptors, scavenger receptors, Nucleotide-binding and oligomerization domain-like receptors (NLRs), and CARD (Caspase recruitment domain) helicases (*18*). All PRRs bind specifically to various molecular structures of microorganisms, including bacteria, fungi, viruses, and unicellular protozoa. PRRs specifically react to a number of plant substances and complex synthetic molecules. All these compounds serve as exogenous ligands of PRRs. PRRs can also respond to a number of substances from their own body, endogenous ligands (*18*).

TLRs represent the best studied family. In humans, 10 representatives have been found, while mice have of 13 representatives of this family (TLR1-TLR13) (*18,33*). All TLRs share a similar structure: they are integral transmembrane proteins, consisting of 3 parts that differ in their functions. The extracellular N-terminal region, responsible for ligand binding, has 19-25 leucine-rich repeats. This is followed by a cysteine-rich transition region, which is responsible for the attachment of the receptor to membrane proteins. Finally, the cytoplasmic region, represents the TIR domain (Toll / IL-1 receptor), which interacts with TLRs and adapter proteins, triggering intracellular signaling cascades (*18*) (Table 1).

All subtypes of TLRs are expressed in the central nervous system: TLR1-TLR9 are expressed by microglial cells, TLR3 and TLR7-9 are expressed by neurons, TLR2-3 and TLR9 are expressed by astrocytes, and TLR2-3 are expressed by oligodendrocytes (*17,34*) (Figure 1).

It is important to note that TLR1-2 and TLR4-6 are expressed on the surface of the cytoplasmic membrane, while TLR3 and TLR7-13 are expressed on endosomes inside the cell (*35,36*).
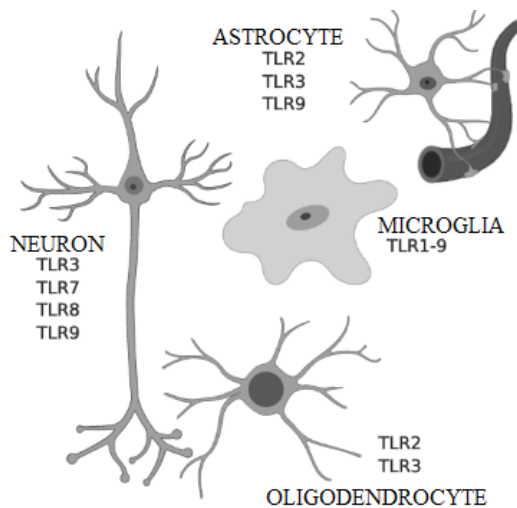
Table 1. TLR adaptor proteins (modified from (*53*))

Receptor	Adaptor protein
TLR2/TLR1 (heterodimer)	Myd88/TIRAP
TLR3	TRIF
TLR4	Myd88/TIRAP; TRIF/TRAM
TLR2/TLR6 (heterodimer)	Myd88/TIRAP
TLR7	Myd88
TLR8	Myd88
TLR9	Myd88
TLR11/TLR12 (heterodimer)	Myd88
TLR13	Myd88

3. TLR signaling cascades

All TLRs function as homo- or heterodimers: TLR2 forms heterodimers with TLR1 or TLR6, TLR11 forms heterodimers with TLR12, while TLR3-5, TLR7-9, and TLR13 form homodimers (*36*). After ligand recognition (Table 2) TLR undergoes dimerization with formation of a heterodimer or homodimer, followed by conformational changes in the receptor necessary for the interaction of the cytoplasmic TIR domain with intracellular adapter proteins and subsequent activation of the intracellular signaling cascade (*32*).

The most common case includes binding of the TIR receptor domain of TLR to the adapter protein MyD88 (Myeloid differentiation primary response 88) (Figure 2). After that, MyD88 interacts with kinases

Figure 1. Expression of TLRs in cells of the central nervous system.

Table 2. TLR ligands (summarized using data from (*47-53*))

Receptor	Exogenous ligands	Endogenous ligands
TLR1	Triacetylated peptides	Unknown
TLR2	Zymosan, diacetylated peptides, triacetylated peptides, lipoteichoic acid	rHSP70, gp96, HMGB1, uric acid, hyaluronic acid, -synuclein
TLR3	Double- stranded RNA (dsRNA), poly (I:C)	mRNA, statmin,
TLR4	Lipopolysaccharide (LPS)	HMGB1, HSP60, HSP70, HSP72, hyaluronic acid, fibrinogen, protein S100, uric acid, heparan sulfate fragments, tenascin-C
TLR5	Flagellin	Unknown
TLR6	Zymosan, diacetylated peptides, triacetylated peptides, lipoteichoic acid, lipoarabinoman	Unknown
TLR7	Imiquimod, gardiquimod, single-stranded RNA (ssRNA), miRNAs let-7, microRNA-21, imidazoquinoline, loxoribine, bropyrimin	Unknown
TLR8	Single-stranded RNA (ssRNA), ssRNA40/Lyovec, gardiquimod	Unknown
TLR9	DNA with unmethylated CpG oligodeoxynucleotides	Chromatin-IgG complexes
TLR10[*]	Double- stranded RNA (dsRNA)	Unknown
TLR11[**]	Profilin and profilin-like proteins	Unknown
TLR12[**]	Profilin	Unknown
TLR13[**]	Single-stranded RNA (ssRNA)	Unknown

[*]- Found only in humans, [**]- found only in mice.
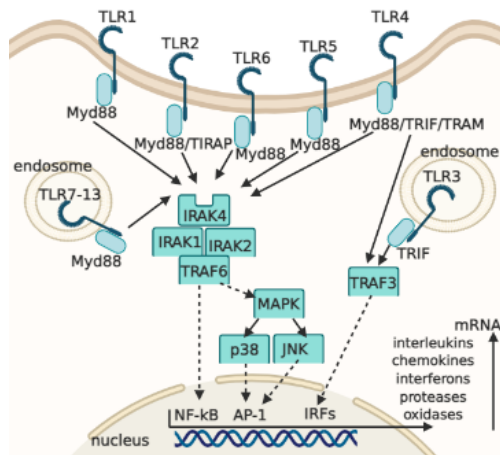
Figure 2. Signaling cascades realizing TLRs effects.

of the IRAK family (IL-1 receptor associated kinase); the family consists of several enzymes: IRAK1-4 and IRAK-M. Initially, IRAK4 is activated, and then IRAK1. Activated IRAK1 interacts with TRAF6 (TNF receptor-associated factor 6). This factor can trigger two signaling pathways including: (*i*) activation of the transcription factor AP-1 by MAP-kinases (mitogen-activated protein kinases), JNK-kinases (c-Jun N-terminal kinases), and p38; (*ii*) activation of the TAK1/TAB (Transforming growth factor-β (TGF-β)-activated kinase 1/ TAK1-binding protein) and IKK (IκB kinase) complex. After IKK activation, the inhibitory protein IκB is phosphorylated and degraded; this leads to release of the NF-κB dimer and its subsequent translocation into the nucleus, where NF-κB binds to the promoter regions of genes that activate and regulate the development of the inflammatory response. This intracellular signaling mechanism functions when almost all known TLRs (with the exception of TLR3) are activated. This indicates that different pathogens that activate different TLRs initiate a common universal pathway for the activation of the inflammatory response (*18*). The Toll/ interleukin-1 (IL-1) receptor (TIR) domain of TLR3 and TLR4 can interact with the adapter protein TIR domain containing adapter inducing IFNβ (TRIF) (*37*). The TRIF protein activates TRAF6 (TNF receptor-associated factor 6) and TRAF3 (TNF receptor-associated factor 3). This results in activation of the intracellular factor TBK1 (TANK-binding kinase 1) followed by activation of IRF3 (Interferon regulatory factor 3). Activated IRF3 triggers expression of the IFNα and IFNβ genes required for the development of an antiviral response (*18*).

Other adapter proteins, besides those considered above, required for signal transduction from certain TLRs have been identified. These include TIRAP protein (TIR-domain-containing adapter protein), which together with MyD88 participates in signal transduction from TLR2 and TLR4, but not from other TLRs (*38*). The adapter protein TRAM (TRIF-related adapter molecule), which interacts with TRIF, is used for signal transduction

from TLR4 (*39*).

The activation of TLRs triggers several intracellular signaling pathways. This results in activation of complex intracellular cascades, which can cause both the enhancement and inhibition of the final effect of cytokine expression. For example, activation of TLR3 and TLR4 can enhance expression of TLR2 on the surface of macrophages in a Myd88-independent manner, while activation of TLR7 and TLR9 induces its expression in a Myd88-dependent manner (*40*). TLR4 activation can positively regulate TLR2, TLR4, and TLR9 (*39*). Such regulation (TLR-TLR) often leads to an enhancement of the immune response, attracting more TLRs; however, the initial stimulating dose and the activation time of the second TLR involved in the process can have a significant impact on the immune response (*18,40-43*). In addition, different subtypes of TLRs can jointly form a synergistic effect or create feedback with respect to another TLR. Stimulation of a dendritic cell with a TLR2 agonist counteracts the expression of IL10 and IL12, which is initiated by TLR3 and TLR4 agonists. TLR8 inhibits TLR7 and TLR9, while TLR9 inhibits TLR7 as a result of direct or indirect interactions between them (*44*).

4. The role of TLRs in neuroimmune mechanisms of alcoholism development

Ethanol consumption promotes TLR-mediated activation of the innate immune system manifested by increased levels of pro-inflammatory signaling molecules (*7*). Among TLRs involved in the pathogenesis of alcoholism, researchers pay particular attention to TLR3, TLR4, TL7 (*3-7,17, 22,45-48*).

Increased levels of pro-inflammatory cytokines play an important role in the development of neurotoxicity, with the subsequent death of many cells in the central nervous system. Although microglia are considered the main source of pro-inflammatory cytokines in the central nervous system, the role of neurons in the ethanol-induced neuroimmune signaling networks is not fully understood (*26*). The activity of TLRs depends on the level of exogenous and endogenous ligands by which these receptors are activated. Ethanol consumption is accompanied by an increase in the level of endogenous ligands, such as HMGB1 (*7*), heat shock proteins (*49*), proteins involved in extracellular matrix degradation (*50*), and various variants of microRNA (*51-55*). Endogenous ligands are released in response to the activation of the inflammatory process in the brain and during apoptotic cell damage in the central nervous system (*3-7*). Exogenous ligands and various cytokines can be transported by blood to the central nervous system from the periphery (*6*).

5. The role of TLRs in the pathogenesis of alcoholism

5.1. The Role of TLR3 in the pathogenesis of alcoholism

An increased level of TLR3 mRNA was found in postmortem samples of the brain's orbifrontal cortex from patients with alcoholism (*4*). Rodent experiments have shown that TLR3-dependent signaling affects the level of voluntary ethanol consumption (*56,57*). Alcoholization of mice for 10 days increased the brain level of TLR3 mRNA and the expression level of the TLR3 protein in the orbifrontal and entorhinal cortex (*4*).

A single intraperitoneal injection of a TLR3 agonist poly (I:C) (poly-inosine-polycytidylic acid, a synthetic analogue of the viral double-stranded RNA) to mice increased the level of voluntary ethanol consumption in the two-bottle choice test (ethanol solution or water); at the same time, the increase in the level of ethanol consumption developed during several days (*57*).

The study of the poly (I:C) effects on gene expression in the rat brain nucleus accumbens has shown that activation by the TLR3 agonist leads to an increase in the mRNA levels of TLR3, COX2 (cyclooxygenase 2) and genes of the glutamatergic system (mGluR2 - metabotropic glutamate receptor 2; mGluR3; GLT1 - glutamate transporter 1), as well as the BDNF gene (brain-derived neurotrophic factor). Moreover, an increase in the mRNA of each of these genes correlated with an increase in TLR3 mRNA (*58*).

The use of poly (I:C) led to an increase in the expression of a number of pro-inflammatory genes (CCL5, CCL2, IL-1b, IL-6, *etc.*) in the prefrontal cortex of the mouse brain. Under conditions of free access to ethanol (two-bottle choice drinking) during the peak activation of the pro-inflammatory response in the brain, the level of voluntary ethanol consumption by mice decreased and remained unchanged when access to ethanol was provided to mice with descending limb of activation of the innate immune system. These results suggest that a gradual increase in the inflammatory response may indirectly contribute to an increase in alcohol craving in mice. According to the authors (*57*), specific pathways and the balance between cytokines can regulate the level of craving for alcohol.

Using a model of 10-day alcoholization of mice with ethanol, it was shown that a single administration of the TLR3 agonist poly (I:C) resulted in an increase in the TRAIL (TNF-related apoptosis-inducing ligand) mRNA level in the orbitofrontal and entorhinal cortex of the mouse brain (*59*). Treatment of cell cultures with ethanol activated TLR3, which promoted release of IFNβ and IFNγ by neurons and astrocytes. Afterward, addition of poly (I:C) into the cell culture resulted in increased activity of the TRAIL gene. TRAIL blockage *via* neutralizing antibody led to a decrease in the levels of IFNβ and IFNγ in both astrocytes and neurons. The combined effect of ethanol and a TLR3 agonist (poly (I:C)) showed an increase in the levels of TNF-α, IL-1β, and IL-6 mRNA, as well as an increase in the levels of p38 and IRF3 proteins in microglial and neuronal cell cultures (*59*).

TLR3 mRNA and components of the TRIF-dependent pathway were increased in the prefrontal cortex of mice 24 hours after ethanol withdrawal (*56*). Expression of TLR3-related components of the TRIF-dependent pathway increased in the nucleus accumbens, but decreased in the amygdala. In addition, Amlexanox, an inhibitor of the IKKε/TBK1 complex, reduced immune activation of the TRIF-dependent pathway in the brain and decreased ethanol consumption. This suggests that the TRIF-dependent pathway regulates the level of ethanol consumption (*56*).

Decreased activity of the MyD88-dependent pathway correlates with decreased ethanol consumption and increased levels of the TRIF-dependent pathway. To test the mediated action of poly (I:C) *via* MyD88, female Myd88 knockout mice were used and showed that administration of poly (I:C) did not alter alcohol consumption in Myd88 knockouts, indicating that poly (I:C)-induced changes in alcohol consumption depended on the MyD88-dependent pathway (*57*).

Based on the experimental data obtained using various models, it can be concluded that TLR3 plays an important role in the pathogenesis of alcoholism; however, the exact mechanisms of TLR3-dependent signaling remain completely unclear.

5.2. The Role of TLR4 in the Pathogenesis of Alcoholism

In the context of alcoholism, most studies were focused on the involvement of TLR4 in the mechanisms of pro-inflammatory signaling activation as a result of ethanol consumption (*37,60-65*).

A large amount of data was obtained on rats and mice using genetic and pharmacological manipulations (TLR4 knockout and antagonist use); these studies showed that although TLR4 activity did not regulate the level of ethanol consumption, subsequently consumed alcohol influenced TLR4-mediated signaling (*61,62*).

Ethanol consumption by mice for 2 weeks led to the activation of TLR4-dependent pro-inflammatory processes, which were characterized by the activation of MAP kinases and NF-κB, followed by release of COX-2 (Cyclooxygenase 2), iNOS (Inducible nitric oxide synthase), and HMGB1. The development of the inflammatory process under conditions of increased activity of these pro-inflammatory signaling molecules led to demyelination of axons and structural synaptic changes due to the damage to myelin proteins and synaptic proteins. Subsequently, such mice were characterized by impaired parameters in tests of object recognition, passive avoidance, and olfactory behavior (*66*). Knockdown of the TLR4 gene was accompanied by inhibition of the production of pro-inflammatory mediators, blockade of the activation of MAP kinases and NF-κB pathways in astrocytes (*67*). *Tlr4* gene knockout mice were protected from an increase in the concentration of cytokines and chemokines in the brain

caused by prolonged consumption of ethanol (for 5 months), while the presence of a functionally competent *Tlr4* gene led to an increase in the concentration of cytokines (IL-1β, IL-17, TNF-α) and chemokines (MCP-1, MIP-1α, CX3CL1) in the blood and striatum (*39*).

It was shown that ethanol caused accumulation of polyubiquitinated forms of proteins in the cerebral cortex and promoted activation of immunoproteasomes and autophagolysosomes (*48*). Mice lacking TLR4 receptors were protected from such changes induced by ethanol (*64*).

There is evidence that TLR4/MCP-1-mediated signaling in the amygdala and ventral tegmental area (VTA) predisposes rats to increased ethanol consumption. This signaling is supported by increased expression of corticotropin-releasing factor (CRF), which is capable of downregulating TLR4 (*61*). In addition, there is evidence that the level of MCP-1, with a simultaneous increase in the level of microglia activity, was increased in the VTA, amygdala, substantia nigra, and hippocampus of postmortem brain samples from patients with alcoholism (*68*).

It is suggested that TLR4-MyD88-dependent signaling mediates acute depressive disorders, which develop after ethanol consumption, and may also be involved in the regulation of GABAergic transmission in the central nervous system (*61*). TLR4 gene knockdown rats had a decreased level of voluntary alcohol consumption (*60*). It is suggested that this was associated with a decrease in the expression of the GABAA a2 subunit in the amygdala (*60*). Intraperitoneal administration of the TLR4 ligand, LPS (lipopolysaccharide), accelerated the development of anxious behavior in animals subsequently exposed to ethanol (*69*). Mice lacking TLR4 or MyD88 became less sensitive to the sedative and intoxicating effects of ethanol, while mice lacking TLR2 did not differ from control mice in these tests (*69*).

These data suggest that TLR4 may indirectly interact with neurotransmitter receptors (or other targets), thereby indirectly regulating the level of ethanol consumption.

5.3. The Role of TLR7 in the Pathogenesis of Alcoholism

In addition to the above described studies on TLR3 and TLR4, there are a small number of studies aimed at studying TLR7 in the pathogenesis of alcoholism. For example, TLR7 expression was higher in the hippocampus of postmortem human brain samples (*47*). There is evidence that ethanol induces secretion of the TLR7 agonist (miRNA let-7b), which leads to TLR7-mediated activation of neurodegenerative processes in the central nervous system (*47*). The effect of ethanol on TLR7 and let-7b was studied in a cultured section of the hippocampal-entorhinal cortex of rats: the tissue of the alcoholized hippocampus was characterized by

increased expression of TLR7 (*47*). In addition, it was found that ethanol induced formation of HMGB1-miR-let-7 complexes in microvesicles, which induced the development of a neurotoxic effect through TLR7 activation (*47*). Ethanol causes an increase in TLR7 expression and release of let-7b and HMGB1 from microglia. Inhibition of HMGB1 by glycyrrhizin prevented the development of neurotoxicity (*47*).

Findings obtained in one of the recent research papers attest to the fact that TLR7 may serve as one of their potential targets during development of treatment therapy options for alcoholism. The chemical mixture imidazoquinoline R848 and protagonist TLR7 used in the work, lead to a decrease in ethanol absorption with single dosing to Kunming mice, but prolonged activation of TLR7 lead to an increase in ethanol absorption. It is anticipated that these effects are associated with molecular non-responsiveness of intracellular signaling cascades and with the fact that the access time connected with signaling of constitutive immunity plays an important role in regulating ethanol absorption behavior. In addition, there is available evidence in favor of the fact that the peak of neuroimmune response is crucial for ethanol consumption-related behavior. The peak immune activation response results in reduced ethanol consumption, but ethanol consumption increases several hours or days after immune system activation. There are some suggestions that IRF7 and the genes regulated by it in this case may become potential targets for correcting ethanol consumption behavior, since Irf7 is the only gene that was activated after 24 hours following both a single dose of R848 (the TLR7 protagonist) and repeated injection of R848 (*70*).

Our laboratory also obtained information regarding the proportion of mRNA TLR7 in various cerebral structures of rats after alcoholization with a 20% ethanol solution during 1 month. In the group of prolonged alcoholization, there were no changes in mRNA levels in any of the brain structures that we studied. However, during the period of alcohol cessation, on the 1st day, there was an increased level of TLR7 mRNA in the hippocampus and amygdala and a decrease in the striatum. This data once more emphasizes the fact that the TLR7 mRNA level can have multidirectional changes not only in different cerebral structures, but also during different conditions of the organism. Alcohol ingestion and cessation of alcohol at different times serves as the reason for multidirectional changes in the proportion of TLR7 mRNA. It is important to consider this when searching for targets and choosing the date for beginning pharmaceutical correction of alcoholism (*10*).

6. Conclusions

Over the past 20 years, research on the role of TLRs in the pathogenesis of alcoholism has been focused mainly on two subtypes of TLRs; TLR3 and TLR4. Several

studies have investigated the role of TLR7. Attention was mainly paid to the analysis of the expression of these receptors, as well as the components involved in intracellular signaling (mainly at the mRNA level), mediated by the interaction of TLRs with their specific ligands. Most of the work was carried out on cell cultures and mice exposed to various models of alcohol intoxication. The results presented in these works convincingly show that TLRs mediate the development of a neurotoxic effect in the central nervous system when ethanol is consumed. Moreover, TLR signaling not only contributes to the development of the neuroinflammatory process in the brain, but is probably also involved in the mechanisms of regulation of the functional activity of neurotransmitter systems, which may contribute to the formation of a pathological craving for alcohol. However, it would be interesting to study how the TLR signaling components change during alcohol withdrawal at different withdrawal periods and how long in this case the neuroinflammatory process in the central nervous system, mediated by TLRs, persists. The level of cytokine expression at the protein level in the brain in pathological conditions caused by exposure to ethanol needs detailed investigation. It would also be interesting to investigate how the expression level of TLRs and components of intracellular signaling change in various brain structures that are primarily involved in changes in the course of alcohol intoxication. Understanding the intracellular mechanisms mediated by TLR activation may open up new targets for the development of effective drugs for the treatment of alcoholism.

Conflict of Interest: The authors have no conflicts of interest to disclose.

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