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Review Article

Toll-like Receptors in Autism Spectrum Disorder

Kimia Kazemzadeh*

Network of Neurosurgery and Artificial Intelligence (NONAI), Universal Scientific Education and Research Network (USERN), Tehran, Iran

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Abstract

Autism Spectrum Disorder (ASD) is a multifaceted neurodevelopmental condition characterized by diverse behavioral and cognitive challenges. Despite its rising prevalence, the underlying mechanisms remain inadequately understood. Toll-like receptors (TLRs), as critical components of the innate immune system, are implicated in neuroinflammatory processes that may contribute to the pathogenesis of ASD. This narrative review delves into the relationship between TLRs and ASD. Notably, studies reveal an upregulation of TLR4 and TLR2 expression in B cells and placental tissues of individuals with ASD, correlating with increased levels of pro-inflammatory cytokines such as IL-6 and TNF-alpha. Maternal immune activation (MIA), particularly due to infections during pregnancy, has been shown to trigger TLR-mediated inflammatory responses that adversely affect fetal brain development. For instance, maternal cytomegalovirus (CMV) infection leads to heightened expression of TLR4/2 in the placenta, resulting in significant placental inflammation and altered neurodevelopmental trajectories in offspring. Furthermore, evidence indicates that individuals with ASD exhibit impaired immune responses characterized by dysfunctional natural killer (NK) cells and monocytes, which produce excessive pro-inflammatory cytokines upon TLR4 stimulation but show diminished responses to TLR9 ligands. This immune dysregulation is associated with a shift towards a TH2 cytokine profile, complicating the understanding of immune phenotype correlations with ASD symptom severity. Additionally, TLR3 activation by viral RNA has been linked to behavioral changes in murine models, underscoring the potential for maternal infections to influence neurodevelopment through TLR signaling pathways. These findings illuminate the role of TLRs in ASD pathophysiology and suggest that targeting TLR pathways may offer novel therapeutic avenues for intervention in this complex disorder.

Keywords: Autism Spectrum Disorder, Toll-Like Receptors, Innate Immune System, Immunology, Neurodevelopmental Disorder

***Corresponding Author:** Kimia Kazemzadeh
Network of Neurosurgery and Artificial Intelligence (NONAI), Universal Scientific Education and Research Network (USERN), Tehran, Iran.
E-mail: kimia_kazemzadeh@yahoo.com

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Introduction

Autism spectrum disorder (ASD) represents a complex and lifelong neurodevelopmental disorder (NDD). While the main etiology remains unclear, both genetic and environmental predispositions have been linked to the manifestation of this disorder (1). During the previous few decades, there has been a notable growth in the prevalence of ASD (2). According to a comprehensive systematic review and meta-analysis, which involves 74 articles and over 30 million participants, the global prevalence rate of ASD stands at 0.6%. It is worth noting that prevalence rates vary across different continents, with Asia exhibiting a rate of 0.4%, Europe 0.5%, America 1%, Australia 1.7%, and Africa 1% (3).

Toll-like receptors (TLRs) play a crucial role in the pathways of inflammation, as they serve to establish a connection between adaptive and innate immunity, consequently resulting in the eradication of pathogens. These receptors are capable of directly regulating inflammatory reactions and triggering both the adaptive and innate immune responses (4, 5). Identification of toll genes and their protein in Drosophila leads to the discovery of TLR, and its naming returns to its similarity with toll proteins known as the receptors on the cell surface (6, 7).

Different studies tried to evaluate the possible role of TLRs in ASD. For instance, the upregulation of TLR4 manifestation has been observed to be heightened amongst B cells of patients with ASD. This particular phenomenon has been associated with the upregulation of both NF-kB and NADPH oxidase (8). Also, the TLR4 and TLR2 may potentially participate in the inflammatory response at the maternal-fetal interface and may also be associated with NDDs such as ASD in progeny (9). In this narrative review, we aimed to integrate the results of the aforementioned studies to illustrate a better perspective of the function of TLRs in ASD. Also, we discussed the strengths and weaknesses of the existing literature, gaps in this field as future research directions, and the potential use of TLRs as therapeutic targets for ASD.

What are TLRs?

TLRs represent a group of immunological receptors that have the ability to detect the molecular signature of microbial pathogens (10). Prior to the identification of TLRs, innate immunity was considered to be a primitive component of the immune system. To put it differently, immunologists referred to it as the first phase of a more complex immunity response (adaptive immunity) or as being implicated in the systemic response of the body, such as fever. Although the mechanisms for the production of innate immune components such as cytokines were well understood, the pathways responsible for enhancing antiviral interferon expression remained elusive. The discovery of TLRs provided a molecular understanding of these mechanisms and paved the way for the identification of other receptor families in innate immunity. For instance, Toll-1 of Drosophila melanogaster was first identified based on its involvement in embryonic dorsal-ventral polarity specification (11). The discovery of TLRs also presented encouraging prospects for future immunological research directions and subsequently led to the conferral of the Nobel Prize in 2011 to Jules Hoffmann and Bruce Beutler (12).

TLRs are present in innate immune cells, including macrophages and dendritic cells, in addition to non-immune cells like epithelial and fibroblast cells. The location of TLRs divides them into two different subfamilies: intracellular TLRs and cell surface TLRs. Intracellular TLRs, such as TLR13, TLR12, TLR11, TLR9, TLR8, TLR7, and TLR3, are situated in the endosome. Nucleic acids derived from viruses and bacteria are recognized by intracellular TLRs. The recognition of self-nucleic acids in some disease conditions like autoimmunities is also achievable by intracellular TLRs. On the other hand, TLRs of the cell surface, such as TLR10, TLR6, TLR5, TLR4, TLR2, and TLR1, identify microbial membrane components like lipoproteins, proteins, and lipids (13-15).

TLRs are all produced in the endoplasmic reticulum, conveyed to the Golgi complex, and then subsequently transported to intracellular compartments, including the endosome or cell surface. The localization of intracellular TLRs seems to be crucial in detecting ligands and avoiding TLR exposure to self-nucleic acids, which could potentially result in autoimmunity (16, 17). UNC93B1, a transmembrane protein with multiple passes, and PRAT4A oversee the trafficking of intracellular TLRs from the endoplasmic reticulum to endosomes (an ER-resident protein) (18, 19).

Asparaginyl endopeptidase, as well as cathepsins K, L, S, H, and B, guide nucleic acid-sensing TLRs to proteolytic cleavage in the endosome, where they become operational and initiate signaling (20, 21).

The innate immune response relies on the identification of a set of microbial constituents termed pathogen-associated molecular patterns (PAMPs) by PRRs (pattern recognition receptors). PRRs enable the human immune system to differentiate between non-self-antigen and self-antigen. TLRs are a type of PRRs implicated in recognizing peril and activating innate immune system responses. TLRs perceive pathogens in B lymphocytes, macrophages, mast cells, eosinophils, dendritic cells, neutrophils, endothelium, adipocytes, cardiomyocytes, and epithelial cells. TLR stimulation boosts the synthesis of pro-inflammatory cytokines and the synthesis of anti-bacterial substances. It also triggers dendritic cell maturation, which enhances the expression of co-stimulatory molecules and MHC antigens, rendering antigen presentation more efficacious. When innate immunity is insufficient to eradicate pathogens, TLR-activated antigen-presenting cells (APCs) release high concentrations of pro-inflammatory cytokines, including IL-12, IL-6, TNF-alpha, and chemokines. Consequently, the elevation of co-stimulatory molecules' manifestation stimulates the adaptive immune reaction. Additionally, TLRs possess a crucial function in regulating immune response by influencing CD4+CD25+ T regulatory cells, leading to immune response suppression (10, 22). Check out **Figure 1**. for information on the functions of TLRs.

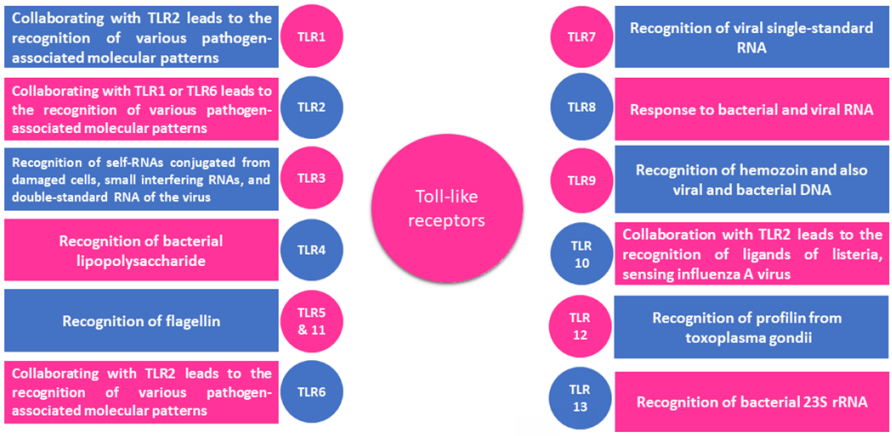

Figure 1. Functions of Different Types of TLRs

TLRs and Neurodevelopmental Disorders

There has been an increasing recognition of the crucial role played by inflammation in gene-environment interactions among patients with NDDs. The impact of unbalanced immune responses against environmental stimuli among both the child and the mother can have a significant effect on neuroimmune signaling, which is integral to brain development. TLRs serve as the most well-known sensors for danger recognition and innate immune patterns to a wide range of environmental threats. Within animal models, MIA (maternal immune activation), which is secondary to inflammatory factors such as obesity, stress, diabetes, and maternal gestational infection, leads to the activation of the TLR pathway in the fetal brain, placenta, and maternal blood. This is associated with neurobehavioral abnormalities in the offspring (23). A systematic review revealed that elevated levels of TLR4 and TLR2 mRNA and/or protein were frequently noticed in the peripheral blood of patients with diabetes mellitus, depression, obesity, autoimmune thyroid disease, and rheumatoid arthritis. In individuals with depression and autoimmune diseases, TLR 9, 8, 7, and 3 activations in peripheral blood were found to a lesser extent. During pregnancy, heightened levels of TLR4 mRNA were detected in the pe-

ripheral blood of females with systemic lupus erythematosus and diabetes mellitus. Moreover, activation of placental TLR was detected among mothers with diabetes or obesity. Following birth, a disturbed response of TLR to stimulation was observed in the peripheral blood of patients with NDDs. While the aforementioned study has revealed emerging evidence that activation of TLR could potentially provide a mechanistic connection between offspring NDD and maternal inflammation, the literature is not exhaustive, and there is a dearth of longitudinal outcome studies. By identifying pathogenic mechanisms in MIA, therapeutic and preventive opportunities can be created to reduce the severity and prevalence of NDD (24).

TLRs in ASD

There is proof that there is a hindrance in the functioning of immune cells and their response to immune challenges in patients with ASD. Patients suffering from ASD have defective natural killer (NK) cells, which are unable to perform their usual function of lysing infected cells upon challenge. Moreover, monocytes that are extracted from patients with ASD also depict inadequate responses to challenges. They tend to secrete an excessive amount of pro-inflammatory cytokines after being challenged with ligands for TLR-4, but their production of similar cytokines diminishes when challenged with ligands for TLR-9 (25-27). Similarly, the levels of CD4+ T cells circulating within the body are diminished, and a preference towards an anti-inflammatory (TH2) profile is displayed while also exhibiting a dysfunctional response to the stimulation among patients with ASD. Efforts aimed at linking specific cellular immuno-phenotypes to symptom severity have resulted in seemingly contradictory outcomes. Specifically, the inclination of T-cells towards a TH2 phenotype (which is widely regarded as 'anti-inflammatory' and is observed in a subgroup of the population with ASD) has been linked to better adaptive as well as cognitive behavioral outcomes (28-31). In another investigation, heightened concentrations of the classic TH2 cytokine IL-4 have been linked to more pronounced deficits in non-verbal interaction. However, adequate proof suggests that overall aberrations in peripheral immunity are known to be a common characteristic among individuals with ASD (32).

TLR 2 and ASD

The literature review demonstrates that there is an increasing amount of data indicating that maternal cytomegalovirus (CMV) infection can be correlated with NDDs in the offspring (33-35). Clinical observation has indicated a potential association between CMV-related autism and placental inflammation. Multiple studies have noted the presence of abnormal expression of TLR4 and TLR2 in the placenta of patients with chorioamnionitis. Additionally, two important maternal inflammatory mediators, IL-6 as well as IL-10, have been implicated in neurodevelopmental disorders (23, 36). In a study by Liao et al., a murine model was established to simulate acute murine cytomegalovirus infection during pregnancy with pre-pregnant mice that were either infected with murine CMV (MCMV), treated with lipopolysaccharide (LPS), or uninfected serving as controls. At E18.5, E14.5, and E13.5, fetal brains and placentas were collected, and the levels of expression of placental TLR4/2 and IL-10/6 mRNA were scrutinized. The findings revealed that following acute MCMV infection, there was a noticeable increase in the levels of expression of placental TLR4/2 as well as IL-6 at E13.5, which was accompanied by marked placental inflammation and a decline in placental and fetal brain weights. Nevertheless, the LPS administration at a dose of 50 μg/kg was observed to decrease the expression of EL-6 at both E13.5 and E14.5. This implies that the onset of acute infection with MCMV during pregnancy may result in the TLR4/2 upregulation gene expression in the trophoblasts of the placenta and their subsequent activation, thereby increasing the production of IL-6 as a proinflammatory cytokine. In the advanced stages of pregnancy, exposure to an elevated dose of LPS stimulation (50 μg/kg) may result in a decline in IL-6 levels. An imbalance in IL-6 expression in the placenta may be linked to NDDs like autism in offspring (9).

TLR 3 and ASD

Maternal infection during the gestational period with diverse DNA and RNA viruses is linked to an augmented predisposition for autism in their progeny. A recurrent characteristic of these exposures is that replication of the virus triggers

an innate immune response via interaction with TLRs. The TLR family has a significant role in regulating the type I IFN response, along with other chemokine and cytokine responses against viral pathogens. In vivo, immune cells employ distinct mechanisms to sense the RNA of viruses and to produce type I IFNs. In certain viral infections, mechanisms of TLR3-independent signaling, including TLR9/TLR7-mediated pathways or mitochondrial antiviral signaling (MAVS), induce innate immunity despite viral pathogens. (37). Administration of Poly(I·C) results in significant expression of proinflammatory cytokines TLR3 activation (38). Maternal immune activation caused by influenza infection and exposure to lipopolysaccharide, or Poly(I·C), leads to modified levels of cytokine in maternal serum, placenta, fetal brain, and amniotic fluid (39-41). Interleukin 6 (IL-6) has been identified by Smith et al. as a mediator of the consequences of Poly(I·C) on the development of fetal murine, with behavior serving as an outcome (42). In vivo, Poly(I·C) administration has been shown to generate transient, viral-infection-like sickness behavior, like changes in weight loss, anorexia, and body temperature (43). The abrogation of side effects of poly(I·C) on the proliferation of embryonic NPC is largely achieved by eliminating TLR3 in knockout mice. However, it is yet to be determined whether the effects of poly(I·C) are solely mediated by immune activation of the embryo or/and the mother. In vitro studies have demonstrated direct impacts of poly(I·C) on the neural element, where decreased cell proliferation in cortical neurospheres of the embryo of WT mice was observed, but not in NPC derived from TLR3−/− mice (44).

The activation of Poly(I·C) leads to signaling through TLR3, which subsequently induces proinflammatory cytokines like IL-8, IL-6, IL-1, RANTES, and TNF-α as well as type II and I interferons via an NF-κB-dependent mechanism (see **Figure 2**) (42, 45). Carprofen, classified as a Cyclooxygenase (COX) inhibitor, is utilized in clinical settings to alleviate inflammation, fever, and pain. Furthermore, some nonsteroidal anti-inflammatory drugs (NSAIDs) not only inhibit COX but also suppress producing IFN and proinflammatory cytokines via NF-κB transactivation (46, 47). In a study by Miranda et al., the employment of NSAID intervention might negate the TLR3-dependent poly(I·C) impact through a comparable cytokine-mediated pathway. Extrapolating from murine prototypes to the human populace may pose a potential risk. Nevertheless, the hampered cerebral inflammatory and corticogenesis retort while congenital viral infections indicate that an inborn immune-centered approach may underscore the postnatal evolution of behavioral anomalies that are indicative of numerous neuropsychiatric ailments. Furthermore, the monitoring that a COX inhibitor abolishes poly(I·C) impacts on embryonic neuronal stem cell/progenitor proliferation may furnish a reasonable justification for the utilization of anti-inflammatory agents in prenatal infections, within periods of neural development while the fetus is exceptionally susceptible to CNS impairment (48).

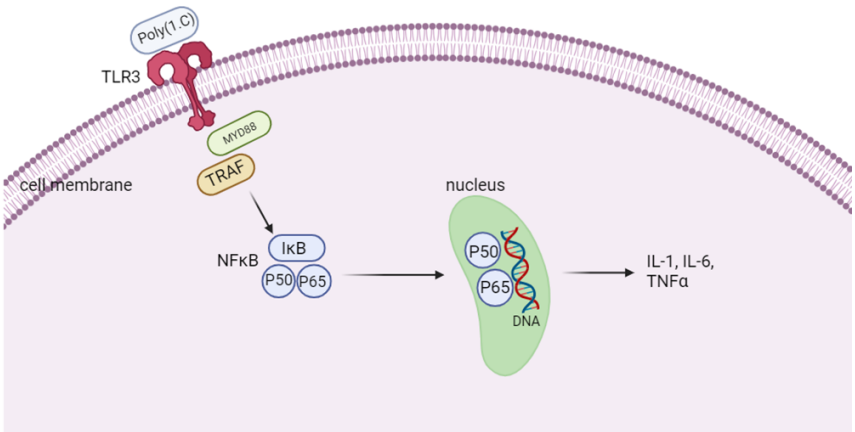

Figure 2. The activation of Poly(I·C) results in signaling through TLR3. Via an NF-κB-dependent mechanism, proinflammatory cytokines are induced.

TLR 4 and ASD

Reportedly, children with ASD exhibit dysregulated biochemical and cellular processes such as intestinal dysbiosis, impairment of mitochondrial function, immune system dysfunction, elevated exposure to toxic metals, and stimulation of neuroglial cells (49-52). Recent findings suggest that the determination of ASD pathogenesis is influenced by the balance of oxidant-antioxidant and TLR4-related signaling. Also, they indicate that B cells display increased NF-kB/ TLR4 expression levels, which is concurrent with oxidant stress as evidenced by elevated NOX2/nitrotyrosine expression. B lymphocytes are intricate, adaptable immune cells. They execute multifaceted functions in the circulation and can exert a modulating influence on oxidative inflammation. Various investigations have corroborated a significant function of B lymphocyte mediators in the inflammatory response (53, 54). To elucidate, B lymphocytes elicit an excessive production of oxidants and inflammatory cytokines in response to microbes, as well as environmental agents. This observation is further substantiated by human and animal studies wherein B lymphocyte depletion has been found to lead to a mitigation of autoinflammatory disorders like arthritis and lupus (55, 56).

There exists a compelling collaboration concerning the function of TLR4 signaling in a variety of immune-mediated and neuropsychiatric ailments such as multiple sclerosis, psoriasis, ASD, and anxiety (57, 58). When it comes to B cells, activation of TLR4 is linked with NF-kB and other signaling pathways like SYK, which may lead to the production of different inflammatory and oxidative mediators within these cells (59-61). There is an increase in TLR4 expression in B cells, which is associated with elevated expression of NF-kB. These discoveries may have significance with regard to systemic and neuroinflammation. It is common knowledge that ASD patients have an imbalanced gut environment, resulting in disturbance in the function of the intestinal barrier. An increase in the gut has been claimed permeability as a consequence of bacterial product transmigration that could activate TLR4 in peripheral B cells (62-64). The regulation of NOX2 within immune cells like T cells may be influenced by TLR4 signaling. It is plausible that oxidative stress mediated by NOX2 in B cells contributes to systemic oxidative inflammation. A well-documented phenomenon is producing ROS by neutrophils, monocytes, as well as T cells, which has a significant impact on neuroinflammation by affecting the vascular barrier among adhesion of immune cells to the vascular endothelium as well as immune cell infiltration into neuronal tissue (65-67).

An animal study provided evidence that TLR4 activation during pregnancy resulted in developmental defects in the fetal brain, likely due to oxidative stress derived from NOX2. These findings claim that increased NOX2/TLR4 signaling in B cells of individuals with ASD may give rise to systemic oxidative inflammation, which could impair neuronal function (68, 69).

The oxidative signaling pathway, specifically NOX2, has reportedly exhibited an increase in immune cells among patients with ASD and BTBR mice (65, 70, 71). Additionally, various inflammatory receptors in the immune cells of ASD patients have been activated, leading to an oxidant stress upregulation. This suggests a significant role of oxidant-antioxidant imbalance in inflammation modulation. Furthermore, research has documented oxidative stress in the form of ROS and lipid peroxides in the plasma/ blood of ASD children (65, 70, 72-74). However, the oxidizing agent-producing enzyme NOX2 and its correlation with enzymatic antioxidants were not explored in the B lymphocytes of individuals with ASD. Investigation demonstrates an escalation in oxidizing stress indicators, such as nitrotyrosine and NOX2, among B lymphocytes of ASD patients. Although NOX2 is predominantly acknowledged for its participation in the antimicrobial response during pathogen phagocytosis, the oxidase plays a substantial role in several other functions of B lymphocytes. The functional existence of NOX2 in B lymphocytes leads to the generation of ROS. Multiple studies have indicated that NOX2 is implicated in the proliferation, activation, as well as overall operation of B lymphocytes. Moreover, heightened activation of NOX2 and ensuing ROS generation may affect the antibody responses and activation/proliferation of B lymphocytes in ASD patients, which is possible to contribute to systemic oxidative inflammation. (75, 76).

Alteration of enzymatic antioxidants is observed in the blood and cerebral specimens of individuals with ASD. Enzymatic antioxidants, including peroxiredoxins, GPx, SOD, as well as GR in brain/red blood cells/serum, have been demonstrated to be in a state of dysregulation in children affected by ASD (8). Additionally, recent research has manifested that enzymatic antioxidants are also dysregulated in specific immune cells, such as neutrophils and monocytes (72). The reduction in antioxidants could be attributed to the generation of peroxynitrite, which has been documented to trigger proteolysis and deterioration of antioxidant enzymes due to oxidative modification. Antioxidants play a pivotal role in the prevention of inflammatory processes, which implies that their dysregulation may result in the amplification of inflammatory signaling in individuals with ASD (77, 78).

Prior investigations have demonstrated that the occurrence of oxidative stress serves as a contributory element in the manifestation of hypersensitivity to environmental toxicants, such as thimerosal. The utilization of immortalized lymphoblastoid cells, for instance, B cells, revealed a higher level of reactive oxygen species (ROS) production at baseline in ASD patients compared to normal controls or their unaffected siblings. The aforementioned cells, when faced with thimerosal, a glutathione-depleting agent, exhibited an elevated production of ROS, thereby indicating a dysregulated antioxidant defensive mechanism (70, 79). However, neither the oxidant-generating enzyme, NOX2, nor the antioxidant enzymes, GPx and SOD, were assessed in the aforementioned studies. Our research contributes further insight into this domain by demonstrating that an insufficiency in antioxidants and an augmentation in the generating enzyme, NOX2, may be held accountable for the heightened vulnerability of B cells to environmental oxidants/toxicants. To conclude, the heightened TLR4/ NF-kB signaling is concurrent with NOX2-mediated oxidative stress in the B cells of patients with ASD. This may ultimately result in immune dysfunction within the B cells, which could potentially contribute to systemic inflammation among patients with ASD (8).

The potential role of TLRs in ASD were summarized in **Table 1**. However, more investigations are required to find the exact role of these TLRs.

Gaps and Challenges

TLRs are an essential component of the innate immune system, responsible for detecting and responding to pathogens and initiating an immune response. In recent years, there has been growing interest in exploring the role of TLRs in

Table 1. Potential Role of TLRs in ASD

Type of TLR	Potential role of TLR in ASD	Reference
TLR2	Activation of TLR-2 increases the production of inflammatory cytokines like IL-1β and TNFα, which are linked to neuroinflammation and behavioral symptoms in ASD. Monocytes from individuals with ASD exhibit an exaggerated response to TLR-2 stimulation, suggesting immune dysregulation that may contribute to ASD development.	(80, 81)
TLR3	It is known to respond to viral RNA and trigger inflammation. Dysregulated TLR-3 signaling during critical developmental periods may disrupt neurodevelopment through altered cytokine production.	(82)
TLR4	TLR-4 expression is elevated in T cells of ASD patients, leading to higher levels of ROS through NOX-2 pathways. This activation is associated with increased inflammatory cytokines and has been implicated in neuroinflammation and ASD-related symptoms.	(83, 84)
TLR5	TLR-5, which detects bacterial flagellin, may influence gut-brain interactions. While its role in ASD is not well-studied, its activation could affect the gut microbiota and immune responses that impact brain development.	(85)
TLR9	In monocytes from individuals with ASD, TLR-9 activation appears to reduce pro-inflammatory cytokine responses, indicating an atypical immune regulation that might negatively affect neurodevelopment.	(80)

ASD. While significant progress has been made in understanding the genetic and environmental factors contributing to ASD, the exact mechanisms underlying its development remain elusive. Exploring the involvement of TLRs in ASD could provide valuable insights into the pathogenesis of this complex neurodevelopmental disorder. Several studies have suggested that dysregulation of TLR signaling could be implicated in ASD. These studies have reported alterations in TLR expression, activation, and downstream signaling pathways in individuals with ASD compared to typically developing individuals. However, it is important to note that the findings have not been consistent across all studies, leading to significant gaps in our understanding of the role of TLRs in ASD. One of the challenges in studying TLRs in ASD is the heterogeneity of the disorder itself. ASD is a spectrum disorder with significant variability in symptoms and underlying biology. This heterogeneity makes it difficult to draw definitive conclusions from studies, as variations in TLR expression or activity could be specific to certain subgroups of individuals with ASD rather than a general characteristic of the entire spectrum. Another gap in our knowledge is the lack of longitudinal studies that investigate the temporal relationship between TLR dysregulation and the development of ASD. Most existing studies have focused on examining TLR expression or activation at a single time point, providing only a snapshot of the immune response. Longitudinal studies could help elucidate whether TLR dysregulation is a cause or consequence of ASD or if it occurs concurrently. Furthermore, the specific mechanisms through which TLR dysregulation could contribute to ASD remain unclear. TLRs play a crucial role in neurodevelopment, synaptic plasticity, and immune-brain crosstalk. Dysregulation of TLR signaling could disrupt these processes, leading to altered neural development and aberrant behavior. More research is needed to unravel the intricate molecular pathways and cellular interactions involved in TLR-mediated effects on neurodevelopment and their potential contribution to ASD. To address these gaps and advance our understanding of the role of TLRs in ASD, several future directions should be considered. Firstly, large-scale, well-designed studies need to be conducted to overcome the limitations of previous studies and establish the extent and consistency of TLR dysregulation in individuals with ASD. These studies should include diverse ASD subgroups, account for potential confounding factors, and utilize advanced techniques such as single-cell RNA sequencing to capture the complexity of immune responses in ASD. Secondly, longitudinal studies that track TLR dysregulation across different developmental stages are essential to provide insights into the timing and progression of immune abnormalities in individuals with ASD. By following individuals from infancy through childhood and adolescence, we can determine if TLR dysregulation is a prodromal marker, a persistent feature, or a result of ongoing processes in ASD. Additionally, more mechanistic studies are needed to unravel the downstream effects of TLR dysregulation in the context of neurodevelopment. Animal models and in vitro experiments can provide valuable insights into the cellular and molecular processes linking TLR dysregulation to altered brain development and an increased risk of ASD (50, 86-88).

Expert Opinion

While TLRs primarily function as part of the innate immune system, their potential therapeutic implications in ASD stem from their role in immune regulation, neuroinflammation, and the modulation of neurodevelopmental processes. However, it's important to note that the field is still in its early stages, and more research is needed to fully understand the therapeutic potential and prognostic relevance of TLRs in ASD. It is noteworthy that targeting TLR signaling pathways can have beneficial effects on ASD-like behaviors in animal models. For instance, modulation of TLR signaling in rodents through pharmacological interventions or genetic manipulations has been shown to improve social interaction deficits, repetitive behaviors, and cognitive impairments associated with ASD-like phenotypes. These findings suggest that targeting TLR modulation could potentially be a promising therapeutic approach for individuals with ASD. One potential therapeutic avenue involving TLRs is the use of antagonists that specifically target TLRs to modulate immune responses. TLR agonists can stimulate the immune system and promote an inflammatory environment, while TLR antagonists can

inhibit excessive immune responses and reduce inflammation. By targeting TLR signaling, it may be possible to attenuate neuroinflammation, restore synaptic plasticity, and improve behavioral outcomes in individuals with ASD. Furthermore, since TLRs play a vital role in the gut-brain axis and the communication between the immune system and the central nervous system, interventions targeting the gut microbiota and its interaction with TLRs hold promise for ASD treatment. Imbalances in the gut microbiota, known as dysbiosis, have been observed in individuals with ASD. Emerging evidence suggests that gut dysbiosis can lead to alterations in TLR signaling, triggering systemic inflammation and potentially influencing neurodevelopment and behavior. Strategies like probiotic supplementation, fecal microbiota transplantation (FMT), and dietary interventions aim to restore a healthy gut microbial composition and modulate TLR activation, potentially improving the symptoms and prognosis of ASD. However, it is important to approach these potential treatments with caution, as TLRs are involved in complex immune regulatory processes, and their modulation needs to be carefully tailored to avoid unintended consequences. The development of targeted therapies that selectively modulate specific TLRs, along with advancements in our understanding of TLR signaling pathways and their interactions, will be crucial for the safe and effective implementation of TLR-based interventions in ASD. Regarding the prognosis of ASD, there is limited evidence thus far regarding the prognostic role of TLRs. However, TLRs could potentially serve as biomarkers for disease severity or treatment response. Some studies have reported associations between TLR expression levels and clinical features of ASD, such as language impairment, cognitive abilities, and behavioral traits. Longitudinal studies that investigate the relationship between TLR dysregulation and long-term outcomes could provide insights into the prognostic relevance of TLRs in ASD. While the research on the therapeutic and prognostic implications of TLRs in ASD is still developing, it holds promise for advancing our understanding and potentially improving the lives of individuals with ASD. Further research, including well-designed clinical trials and longitudinal studies, is necessary to establish the efficacy, safety, and long-term effects of TLR-targeted interventions in the treatment and prognosis of ASD (50, 89-91) Check out **Figure 3**.

Conclusion

In conclusion, we tried to review the role of TLRs in ASD in this narrative review. Generally, the elevated gene expression of placental TLR2 in the MCMV group may cause placental inflammation, leading to the production of IL-6, which has an imbalanced expression in the placenta that could be linked to NDDs such as ASD in offspring. In addition, TLR4 signaling is elevated in B cells of ASD patients, accompanied by oxidative stress mediated by NOX2. This could cause immune dysfunction and potentially contribute to systemic inflammation in ASD. However, more studies on larger scales are needed to evaluate the

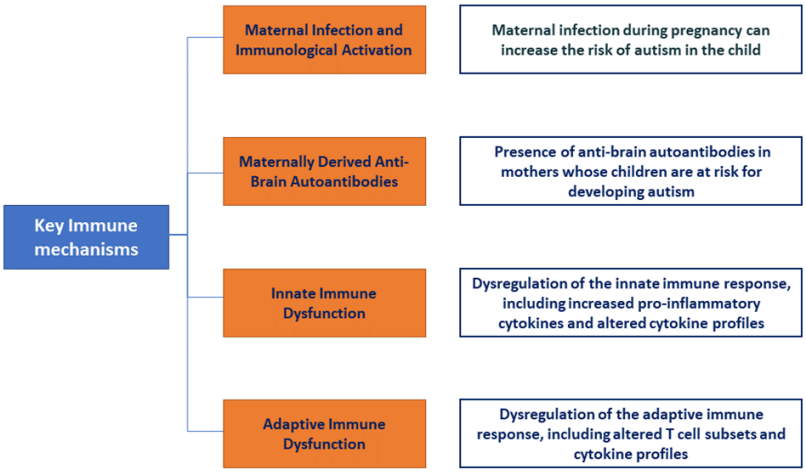

Figure 3. Summarizing Key Immune Mechanisms Suggested to be Involved in ASD Development

role of other TLRs in ASD.

Conflict of interests

There is no conflict of interests.

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