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Mechanism of action and therapeutic targeting of microglia in autism spectrum disorder

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Abstract

Autism spectrum disorder (ASD) is a complex mental illness with a high incidence and considerable impact. More than half of the affected individuals have self-harm behaviors, resulting in high mortality and morbidity. The impact of ASD on education and employment opportunities, the need for family care and support, as well as the burden on families and society is enormous. The underlying pathogenesis of ASD is still unclear, and effective interventions are lacking. Microglia are key immune cells in the central nervous system (CNS), and they function far beyond classical innate immunity, as they can affect normal neuronal activity by secreting cytokines and pruning synapses through phagocytosis. On the one hand, the abnormal activity of microglia may contribute to the development of ASD; on the other hand, it provides a potential target for intervention and treatment. In this review, we comprehensively analyze the mechanism of action of microglia in ASD development and summarize the current methods for targeting microglia in treating ASD.

Keywords: Autism spectrum disorder; Microglia; Neuroinflammation; Cellular immunotherapy

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1. Introduction

Autism spectrum disorder (ASD), also known as an autistic disorder, is a developmental brain disorder. The main symptoms are social handicaps, communication disorders, narrow interests, and stereotypical repetitive behaviors[1]. ASD begins before the age of three and manifests significantly thereafter. The vast majority of children require long-term rehabilitation training and special education support. One-third of individuals with ASD may remain mute all their lives, one-half have no sense of safety or need to be restrained in their actions, nearly two-thirds of children with ASD experience bullying, and approximately one-third engage in self-harm behaviors, such as head banging, arm biting, and skin scratching[2]. The average cost for individuals with ASD is approximately $60,000/year throughout their childhood[3] and increases with the onset of intellectual disability. According to the latest screening data on ASD from the Centers for Disease Control and Prevention, one in 54 children is diagnosed with ASD, with a 24% increase in prevalence from 2016 to 2018[4].

A century ago, microglia were first recognized as a distinct cell population in the central nervous system (CNS)[5] and were primarily thought to be phagocytes responsible

for removing debris during CNS development and in pathological conditions. Microglia-mediated immune inflammation was believed to affect the stability of the CNS[6]. With the advances in genetic technologies and single-cell analysis, the connection between microglia and neurons has become more apparent. Besides being central to the inherent immune response, microglia are involved in a variety of neuropsychiatric diseases; functional abnormalities of microglia have been confirmed in anxiety, depression, and other mental disorders. Therefore, the mechanism of action of microglia in mental illness is one of the trending topics in current research. The modulation of neuronal activity by microglia is evident in many brain diseases[7].

The dysfunction of microglia may be one of the underlying mechanisms in ASD[8]. Aberrant activity of microglia, abnormal levels of associated inflammatory factors, and certain cellular pathways have been observed in autistic patients and animal models. Both genetic and environmental factors that affect microglia may be involved in the etiology of ASD[9]. The deletion or amplification of certain genes may cause ASD, and changes in the peripheral environment may affect microglia, further aggravating autism-like behaviors.

In this review, we propose that microglia are related to the development and progression of ASD. Several widely accepted theories on the etiology of ASD and ASD models are discussed. We focus on the mechanistic role of microglia in ASD from the perspective of phagocytosis and synaptic pruning, which affect neuronal activity, through the release of inflammatory factors and their influence on inflammatory pathways. We also summarize several intervention approaches for ASD by modulating microglia and analyze the current challenges in treating ASD by regulating microglia in hope that this review will make modest contributions to the early diagnosis and treatment of ASD.

2. Etiology of ASD and its pathogenesis

The etiology of ASD is still unknown. Epidemiological studies, screening for many possible risk factors, could not identify a direct contributor to the development of ASD. Although the etiology of the disease is not fully understood, genetic factors, manifested as deletion or abnormal insertion of specific genes, and gene-environment interactions might lead to autistic-like behaviors. For example, genes such as *CNTNAP2*, *NLGN3*, and *SHANK3* have been shown to be associated with ASD. *Cntnap2* knockout mice, *Nlgn3* knock-in mice, and partial knockout of *Shank3* gene are the common genotypes used to construct ASD animal models. In addition, maternal immune activation (MIA) is believed to be a cause of ASD[10]. Studies have demonstrated that infection during pregnancy, especially in the first trimester, is a risk factor for the development of ASD in offspring[11]. MIA models were induced using polyriboinosinic polyribocytidylic acid (poly[I: C]), lipopolysaccharides (LPS), valproic acid treatment, simulated viral and bacterial infections, as well as other environmental factors to activate the maternal immune system[12] and test possible autistic-like behavioral manifestations in offspring (Figure 1).

Stimulation of certain physicochemical factors during pregnancy also leads to a significantly increased risk of ASD in offspring. It has been reported that exposure to valproic acid during pregnancy increases the probability of ASD in offspring by 50%[13]. Studies of the early pregnancy diets have shown that propionic acid salt might accumulate in the gastrointestinal tract of pregnant women who consume processed foods that are rich in propionate. Propionate can pass through the placental barrier, bind with G-protein receptors 41 (GPR41) on glial progenitor cells, and cause impaired neural differentiation. Furthermore, the expression of downstream phosphatase and tensin homolog deleted on chromosome 10 (PTEN) is inhibited, and protein kinase B (PKB) pathway is activated to promote the proliferation and differentiation of glia. Subsequently, glial cells continue to produce inflammatory cytokines and release glial fibrillary acidic protein (GFAP) after maturation, thus affecting the development of the fetal nervous system.

3. Role of microglia in ASD

Microglia, the innate immune cells of the CNS, have high plasticity and can react rapidly during pathological processes. Microglia have been implicated to play a crucial role in the development of ASD associated with CNS inflammation[14]. Recent advances have shown that microglia function far beyond the classical innate immune response and are involved in various processes, such as neuronal excitation, synaptic pruning, and remodeling[15,16]. A growing number of studies have demonstrated that the biological role of microglia has a close association with ASD[17]. As early as 2005, the significant activation of microglia and astrocytes in the brains of autistic patients has been reported by Diana *et al.*[18]. Since microglia activation is the main cellular response to CNS dysfunction, it is conceivable that abnormal glial cell is inextricably linked to ASD.

3.1. Microglia mediate abnormal neuronal synapse pruning/clearing

Microglia are involved in the pruning and clearance of neuronal synapses. Rosa *et al.*[19] have reported the

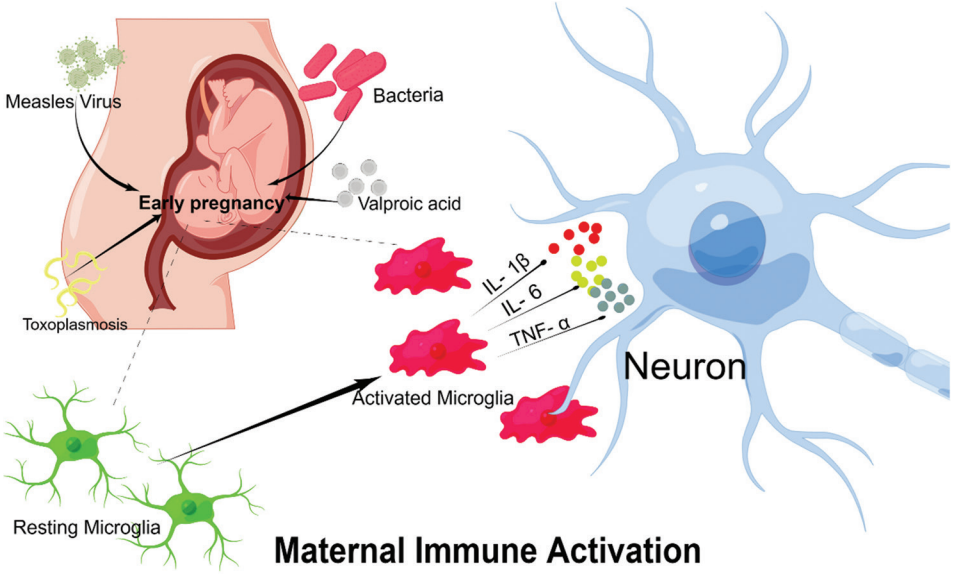

Figure 1. Maternal immune activation promotes the secretion of inflammatory cytokines through the abnormal activation of microglia. External stimulation, such as rubella virus, bacteria, and toxoplasma invasion, in the early pregnancy leads to the activation of the maternal immune system and the secretion of inflammatory cytokines through the placenta, which can activate microglia in the fetal central nervous system. Subsequently, the microglia's original branched morphology changes to amoeba-like. High levels of interleukin (IL)-1β, IL-6, and tumor necrosis factor-α interact with neurons, causing aberrant neuronal functioning and ultimately resulting in autism spectrum disorder symptoms.

importance of the active engulfment of synaptic structures by microglia during neuronal development to maintain normal neuronal functions[20]. In their physiological state, microglia have a role in removing pathogens and maintaining CNS stability, directing the remodeling of brain cells after infection, removing debris in pathogenic infection, as well as restoring affected intracellular and extracellular connections. In addition, 50% of neurons undergo programmed cell death during normal brain development, and microglia dispose of these neuronal corpses through phagocytosis. However, under specific circumstances, the impairment of microglial functions, which include phagocytosis and pruning, may lead to abnormalities in neuronal function[21], thus resulting in certain autistic manifestations.

At the postsynaptic level, previous studies have identified significant neurological changes in the brain of ASD patients, such as neuronal overgrowth and microglia activation in the frontal cortex[18]. Animal experiments have revealed that autistic mice have significantly higher dendritic spine densities[22], which could be the result of pruning abnormalities and decreased microglial ability to phagocytose synapses. In addition, the impaired synaptic connections between neurons following infection, which are caused by microglia, may also contribute to ASD[23].

At the presynaptic level, several studies have shown that the balance between excitatory (E) and inhibitory (I) synapses is disrupted in ASD patients. Appropriate pruning of synapses by microglia is vital to ensure the function of excitatory and inhibitory neurons. Therefore, the aberrant pruning effect of microglia on the E/I balance may be one of the pathological reasons for ASD. It has been confirmed in animal experiments that abnormal microglial phagocytosis will break the balance of E-I synapses and eventually lead to the development of neurodevelopmental disorders and advanced brain dysfunction, such as Alzheimer's disease and ASD[24]. However, the role of E/I signaling in ASD is still controversial. Some studies have found that the function of excitatory synapses is inhibited in *Shank2*- and *Shank3*-deficient mice[25]. In contrast, other studies have shown that the synaptic E/I balance in the hippocampus inclines toward excitability[26]. Recent studies have observed the preferential elimination of excitatory synapses by microglia in pathological and physiological brains of mature rats. The impairments of microglia in pruning excitatory synapses would result in a predominance of excitatory signaling compared to inhibitory signaling in the ASD brain. The intervention of the abnormal pruning process will be a practical approach for ASD treatment in the future[16].

3.2. Microglia contribute to the progression of ASD by mediating inflammatory pathways and cytokines

Microglia are key immune cells of the CNS, and the neuroinflammation that is mediated by microglia is

centrally involved in CNS injury and disease processing. Microglia can be categorized into two phenotypes, the pro-inflammatory M1 type and the anti-inflammatory M2 type[27]. M1 microglia, which induce neurotoxicity, can secrete pro-inflammatory cytokines and chemokines such as interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α. The neuroprotective M2 phenotype contributes to regulating inflammation with specific cytokines, such as IL-10 and transforming growth factor (TGF)-β, as well as neurotrophic growth factors, such as brain derived neurotrophic factor (BDNF) and glial cell-derived neurotrophic factor (GDNF)[28]. The balance of M1/M2 paradigm underlies different neuropathological conditions in ASD and is essential for CNS hemostasis. It is widely believed that an abnormal inflammatory response induced by M1 microglia may be an etiology of ASD. Microglia regulate neuroinflammation through specific inflammatory pathways, such as nuclear factor kappa B (NF-κB), mitogen-activated protein kinase (MAPK), IL-1β, IL-6, IL-8, IL-17, and TNF. The dysregulation of these pathways will affect neuronal activity and result in ASD-like symptoms.

3.2.1. Inflammatory pathways

The inflammatory pathway of NF-κB that is regulated by microglia plays an important role in mediating immune response[29]. The initiation of the NF-κB signaling pathway and the transcription of cytokines involved in directing the immune response are required following an infection to clear the invading pathogenic bacteria. Therefore, the NF-κB signaling pathway is closely associated with inflammatory diseases[30] and regulates the expression of genes encoding immune response proteins[31]. Inflammatory cytokines, infection markers, or stress-activated protein kinases can induce the activation of the NF-κB pathway[32]. Within the CNS, the abnormal activation of the NF-κB signaling cascade leads to an abnormal expression of pro-inflammatory cytokines, which may disrupt normal neuronal activity[33]. A large number of literature works have reported that the activation of microglial NF-κB pathway may be involved in the regulation of ASD[34].

The MAPK signaling pathway also plays a key role in inflammation and can be activated by cytokines, stress mediators, and inflammatory markers. Microglia activation can also induce the MAPK signaling cascade, which is primarily influenced by the activation of toll-like receptors during inflammation, resulting in the phosphorylation of transcription factors[35]. Once phosphorylated, the transcription factors translocate to the nucleus and transcribe genes encoding pro-inflammatory cytokines, such as IL-6 and TNF-α, thus promoting a neuroinflammatory environment[17] and leading to an increased risk of ASD. Besides, IL-6 cytokines form a positive feedback loop by activating MAPK signaling, thus exacerbating inflammation.

3.2.2. Inflammatory cytokines

The increased release of inflammatory factors is closely associated with the development of ASD. The expression of inflammatory molecules, such as IL-1β, IL-6, IL-8, IL-17, and TNF, has been found to be elevated in ASD, and it has been suggested that the severity of the ASD phenotype might be positively correlated with the pro-inflammatory cytokine concentration[36]. Among them, pro-inflammatory cytokines IL-1β and IL-6, released by activated microglia, might have a role in neuronal damage and significantly affect ASD[37]. In addition, maternal infection or inflammatory immune activation during pregnancy may drive microglia activation[18] and promote pro-inflammatory cytokine release, which would, in turn, exacerbate the inflammatory response.

IL-1β is a key pro-inflammatory cytokine that is involved in various autoimmune inflammatory responses and cellular activities, such as cell multiplication, differentiation, and apoptosis. The synthesis and release of IL-1β are tightly regulated, and its expression is upregulated[38] in acute inflammatory, chronic inflammatory, and auto-inflammatory diseases[39]. Microglia can produce large amounts of IL-1β within the CNS[40]. Abnormal IL-1β levels may be responsible for neurological deficits of the brain in ASD. In a study of serum extracellular vesicles in children with ASD, Tsilioni *et al.*[41] found that the IL-1β levels in these children were remarkably higher than the controls following microglia activation. Another study[42] found elevated IL-1β levels in children with degenerative ASD and that IL-1β regulates the proliferation of neural precursor cells, suggesting that elevated IL-1β levels in the brain of ASD patients may alter neuronal development[43] and affect postnatal neural development[36].

IL-8 is a pro-inflammatory cytokine, secreted by various cells, including macrophages and microglia. IL-8 functions primarily as a neutrophil chemotactic factor in the blood. This pro-inflammatory cytokine that is released from microglia increases in response to pro-inflammatory stimuli. Elevated peripheral IL-8 levels have been observed in psychiatric disorders, such as major depression, bipolar affective disorder, schizophrenia, sleep disorders, ASD, anxiety disorders, and dementia[44]. In a clinical study conducted by Bryn *et al.*[45], serum IL-8 concentrations were significantly elevated in children with ASD compared to healthy controls; this finding validates the role of peripheral IL-8 associated with microglia in ASD. Another study found that ASD patients have higher IL-8 levels in the

frontal cerebral cortex[46] and that the local inflammation caused by IL-8 can affect frontal cortical processing in these patients. The frontal cortex is critical for cognitive, emotional, and social behavior[47]. Its abnormal function is closely tied to the pathological mechanisms of ASD[48] and may lead to abnormal cognitive-emotional manifestations.

IL-6 is one of the most important neuroimmune factors that are produced over a short period of time by activated microglia in response to infection and tissue injury. It promotes host defense by stimulating acute phase, hematopoietic, and immune responses[49]. IL-6 has long been shown to be associated with physiological brain development and neurological disorders, such as schizophrenia, major depression, and Alzheimer's disease[50]. Studies have shown that ASD patients have elevated IL-6 levels in their blood, which is strongly associated with neuronal cell adhesion damage, migration, and synapse formation[51]. In a study conducted by Smith *et al.*[52], ASD-like behavioral traits were found in the offspring of pregnant female rats following IL-6 injection. ASD behavior was prevented by simultaneous injection of anti-IL-6 antibodies in another experimental group, suggesting that IL-6 and abnormal microglia could influence ASD.

Using the adenoviral gene delivery approach, Wei *et al.*[53] developed a mouse model that overexpressed IL-6 centrally and confirmed that the overexpression of IL-6 is an important mediator of autistic-like behaviors. When IL-6 levels were elevated in the brains of mice, autistic-like behaviors were observed. Elevated IL-6 levels have also been reported to cause abnormal changes in the shape, length, and distribution pattern of dendritic spines, suggesting that elevated IL-6 levels may mediate ASD-like behaviors through an imbalance of neural circuits and impairment of synaptic plasticity. In the CNS, astrocytes, microglia, neurons, and endothelial cells of the cerebral microvascular system are the cellular sources of IL-6, and its levels are significantly affected by abnormal microglia activation.

3.3. Microbial-gut-brain axis and microglia

In the past few decades, the "microbial-gut-brain axis" has garnered widespread attention from researchers[54], leading to the discovery of the two-way communication between the gut and brain through pathways involving neural, endocrine, and immune systems. Intestinal microbiota is extensively involved in each pathway of the brain-gut axis, influencing neural development, cognition, and behavior by regulating the two-way communication between the gut and the CNS. Studies have shown that gut microbes play an important role in brain function[55]. Many studies have explored the effect of intestinal microbes on microglia-associated neuroinflammation and found immune abnormalities in the gastrointestinal tract of autistic patients, such as increased intestinal immunoglobulin (Ig) G, intestinal CD8 lymphocyte, and T-cell infiltration in the mucosal lamina propria. The deposition of IgG1 and IgG4 has been observed in the basement membrane of intestinal epithelium, along with an increased production of TNF-α in intestinal lymphocytes. Peripheral circulating immune cells may penetrate the blood-brain barrier (BBB) and subsequently affect neurons and glial cells, thereby perpetuating the immune response. Microglia respond to local signals within the brain and receive inputs from the periphery, including the gastrointestinal tract. There is evidence showing altered microbial community composition in nervous system diseases. Recent clinical research has revealed that gut microbes play a critical role in regulating microglial cell maturation and function[56]. However, compared with the physiological state, when mucosal rupture occurs as a result of gastrointestinal inflammation or other factors, many intestinal bacterial-related antigens, cytokines, and chemokines that damage the BBB are released continuously. The hyperactivity of the peripheral immune system promotes CNS inflammation, on which microglia are affected, thus altering the synaptic pruning of neurons and triggering ASD[57] (Figure 2).

4. Therapeutic strategies targeting microglia

In the previous section, we discuss the key roles microglia play in the neuroinflammatory process of ASD. As a dynamic cellular mediator in the CNS, microglia can disrupt the balance of the nervous system by affecting the microenvironment and deranging the expression of important neuroinflammatory molecules[27,58]. Preclinical assessment is anticipated to benefit from treatments targeting microglia dysfunction.

4.1. Microglia and inflammatory interventions

Microglia are the primary immune cells involved in neurodegenerative diseases of the CNS, such as ASD[59]. Microglia express various immune receptors that secrete numerous cytokines and chemokines. The malfunctioning of these molecules in the pathologic signaling pathways of ASD provides neuroscientists and clinicians with potential treatment targets.

4.1.1. Targeting microglia in synapse development

Insufficient pruning of weak or nonfunctional synapses by microglia can perturb neurodevelopment due to decreased levels of complement receptor 3 (CR3) or complement component 3 (C3)[60]. Microglia regulate synaptic plasticity

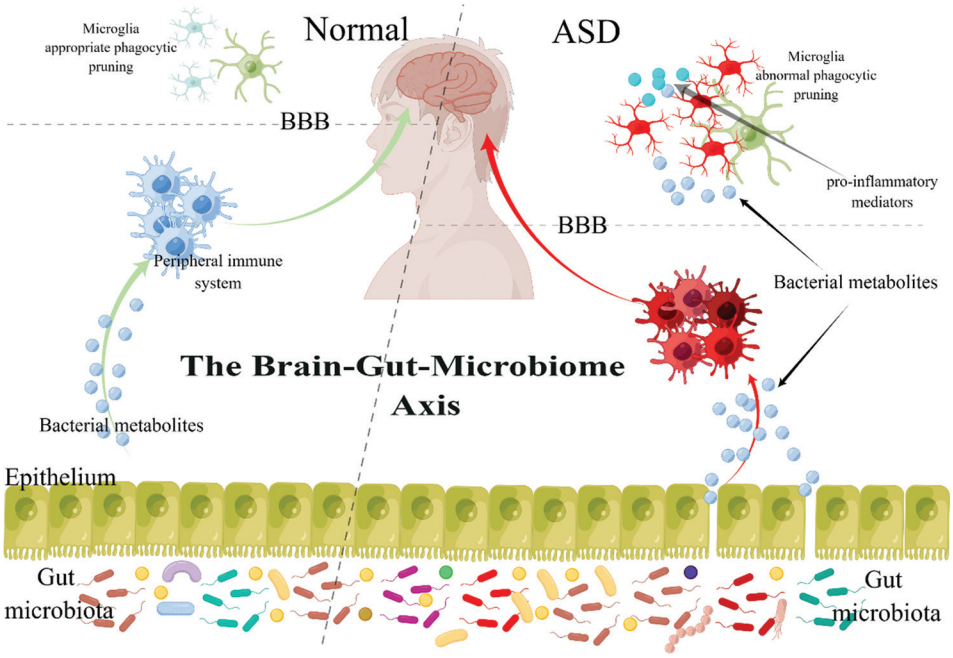

Figure 2. Involvement of microbial-gut-brain axis in autism spectrum disorder (ASD) development. The microbial-gut-brain axis plays a role in ASD development by regulating body immunity. On the left is the physiological state: the secreted metabolites from intestinal flora activate the peripheral immune system, causing the production of inflammatory factors, which reach the central nervous system through the BBB and act on microglia for normal phagocytosis and pruning. On the right is the pathological state: the integrity of intestinal epithelial cells is destroyed, and the metabolites entering the periphery are significantly increased, enhancing the peripheral immune response, and affecting the activity and physiological function of microglia.

through the secretion of reactive oxygen species (ROS) and BDNF, which engages the Trk receptors that modulate the activities of various synapses. Synaptic pruning by microglia is impaired if CX3CR1-CX3CL1 interactions are imbalanced[61]. By blocking inflammatory microglia functions, humanized antibodies or other reagents that target the aforementioned molecular markers may have beneficial effects. The inverse functions of M1/M2 microglia phenotypes provide us with a promising therapeutic idea that emphasizes on the balance of polarizations. Shifting the polarization from M1 to M2 helps in neural regeneration and regulates neuroinflammation in neurodevelopmental diseases, like ASD. The simultaneous inhibition of M1 microglia and acceleration of M2 microglia transformation might be useful for treating ASD[62]. However, due to the lack of animal experiments using M1 depressants like cyclooxygenase inhibitors, and anti-inflammatory drugs like aspirin, for treating ASD, the eligibility and availability of these drugs still require further testing. Recently, the research focus has been on microglia autophagy. It has been reported that the loss of mammalian target of rapamycin (mTOR)-dependent macro-autophagy can lead to ASD-like spine densities and a decrease in presynaptic markers, like light chain protein 3-II (LC3-II)[63]; additionally, malfunctioning autophagy can also be induced by infected microglia[64]. Hence, the potential relationship between mTOR-dependent autophagy and infected microglia may provide researchers with interesting targeting methods (Figure 3).

4.1.2. Targeting microglia in maternal immune activation

Infection during prenatal period induces pro-inflammatory cytokines, such as TNF-α, IL-6, and IL-β, causing neuroinflammation effectors that activate microglia[65]. Coadministration of anti-IL-6 and -IL-1β antibodies has also been demonstrated in certain animal models. Suramin has shown to be effective in improving core ASD behaviors by recovering dysregulated purinergic metabolism in the poly I: C-exposed MIA model of adult ASD mice[66] (Figure 4).

4.2. Metabolic deficiency and microglia abnormalities

Omega-3 polyunsaturated fatty acids (n-3 PUFAs) are essential in maternal diet. A deficiency of n-3 PUFAs alters the offspring's microglia lipid composition and oxylipin signature, which are implicated in inflammation, resulting in microglia polarization toward a phagocytic phenotype, possibly by the overexpression of 12-hydroxyeicosatetraenoic (12-HETE)[67,68]. Phagocytic

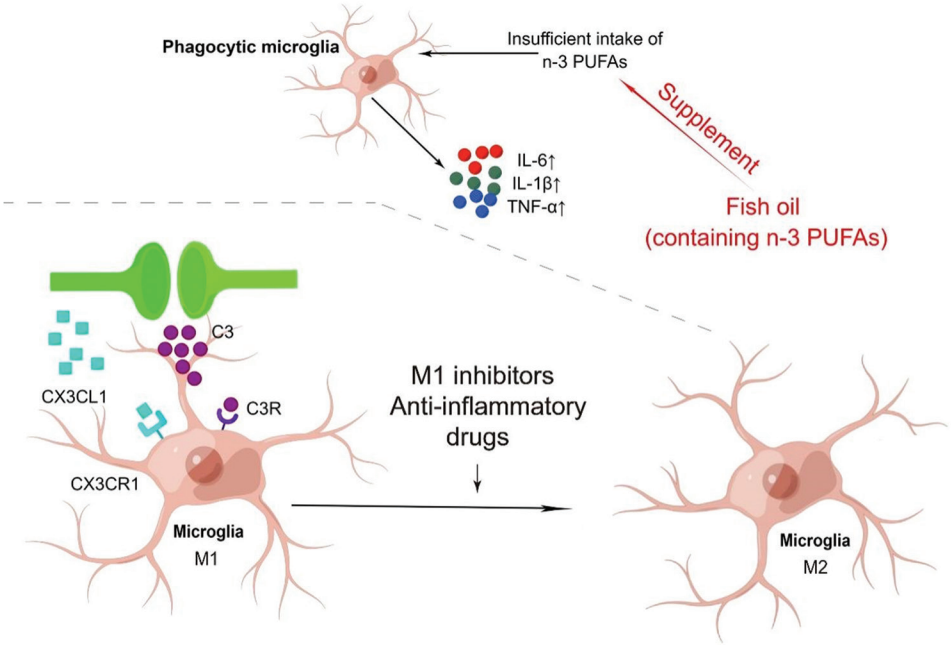

Figure 3. Treatment of autism spectrum disorder (ASD) through inflammatory and metabolic methods. Loss of omega-3 polyunsaturated fatty acids (n-3 PUFAs) leads to changes in the lipid composition and oxygen-lipid characteristics of progeny microglia, ultimately polarizing microglia toward the phagocytic phenotype and resulting in the secretion of neuroinflammatory cytokines that impair spine density and/or synaptic pruning, such as tumor necrosis factor-α, interleukin (IL)-1β, and IL-6. Supplementation of n-3 PUFAs from weaning to adulthood improves ASD-like social deficits in a mouse ASD model. Therefore, fish-oil capsules, containing n-3 fatty acids, with vitamin E supplements may be one of the key treatments for ASD patients. Shifting the polarization from M1 to M2 has potential application using different kinds of drugs.

microglia produce a cascade of neuroinflammation cytokines that impair spine refinement or synaptic pruning. Supplementing with n-3 PUFAs from weaning to adulthood has led to significant improvements in ASD-like social deficits in ASD mouse models[69]. Therefore, n-3 PUFAs supplementation through fish-oil capsules containing eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) with vitamin E may be a pivotal treatment for ASD patients (Figure 3).

4.3. Gut-brain axis and microglia

The gut-brain axis plays an intrinsic role in brain homeostasis. Microbiome-microglia crosstalk research has generated novel therapeutic approaches for ASD patients. There have been reports of several microbiota metabolisms that impact the physiological characteristics of microglia. As bacterial metabolites derived from microbial fermentation, the three main short-chain fatty acids (SCFAs) – propionate, butyrate, and acetate – can be beneficial or detrimental for ASD treatments[63]. Using antibiotics against *Clostridia* and *Bacteroidetes*, the main producers of propionate can help prevent the increased activation of microglia. The treatment with butyrate or its byproducts generated by *Clostridium butyricum* promotes anti-inflammatory effects in microglia, contributing to the improvement of neuropsychiatric symptoms associated with ASD[70]. It has been suggested that *p*-Cresol, which is derived from *Blautia hydrogenotrophica* and *Clostridium* spp., induces microglia activation and neuroinflammation[71]. Recolonizing patients with various microbiota or feeding SCFAs can sufficiently modulate microglia activation and alleviate ASD syndrome[72].

5. Challenges

ASD is a psychosocial condition that persists throughout life. ASD patients experience social, speech, and behavioral changes that can severely disrupt their daily life and work. At present, there are many challenges in the treatment and prevention of ASD. Since the disorder cannot be cured, it becomes a lifelong problem for patients. Furthermore, the detection of ASD is not feasible at an early stage or through pregnancy screening. The diagnosis of ASD mainly relies on behavioral observations, and its symptoms cannot be detected under the age of three, thus missing the critical period of intervention.

MIA during pregnancy is one of the well-recognized mechanisms that have been successfully used to construct animal models. Altered cytokine expressions could facilitate the identification of ASD phenotypes and provide

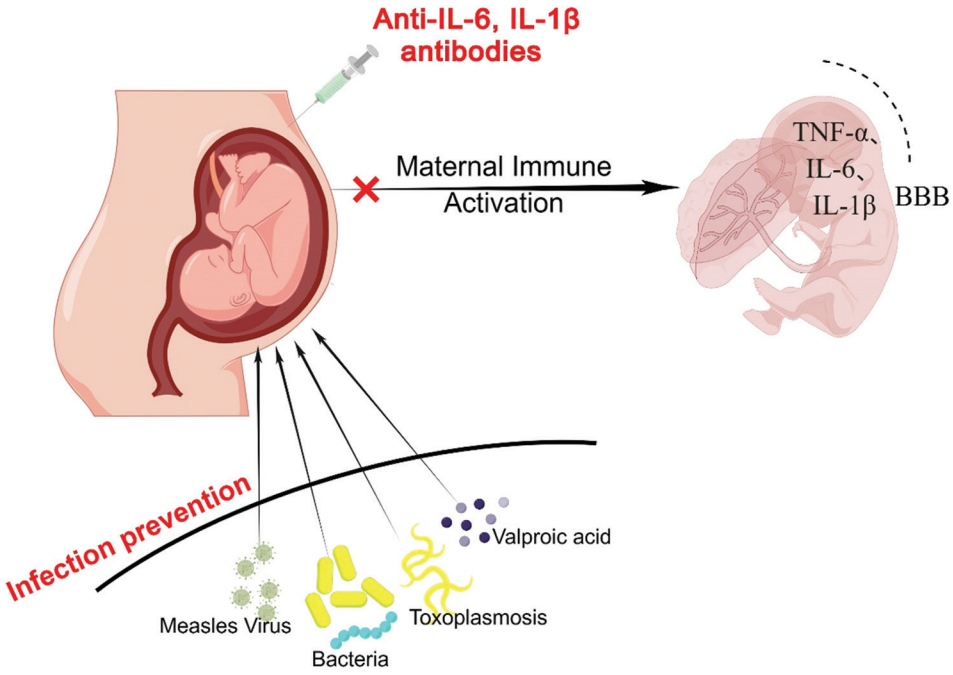

Figure 4. Targeted treatment of immune activation in pregnancy. In the early stage of pregnancy, the exposure of the mother to external stimuli, such as rubella virus, bacteria, and *Toxoplasma*, leads to the activation of microglia and the maternal immune system, as well as an increased secretion of cytokines. Treatments that are commonly used include the prevention and removal of pathogens to reduce maternal infection by stimulating the immune system and inhibiting maternal immune activation signaling; a concomitant administration of anti-interleukin (IL)-6 and -IL-1β antibodies in poly(I: C) models could take effect in animal models.

biomarkers of response to treatment[73,74]. A timely analysis of gestational samples is an excellent opportunity for pathological diagnosis and future behavioral interventions in the early ASD childhood. However, the average age of ASD children at which atypical behaviors are first noticed by the parents is 4.6 years[75]. This means that sample analysis during pregnancy may not be a suitable solution due to parental acceptance. Some animal experiments have shown promising results concerning therapeutic targeting T helper 17 (Th17) cells in preventing inflammation-induced ASD-like behaviors in offspring of susceptible mothers[76]. However, the specific inflammatory factors that cause ASD are poorly understood, and most existing immunomodulatory tools are ineffective. There are still many challenges in reversing the pathological changes in gestation to ameliorate ASD-like behaviors in offspring; however, fostering routine sample analysis and clinical trials would help address these challenges.

Microglia are innate immune cells of the CNS. An important pathogenic mechanism of ASD is the decreased ability of microglia to engulf and prune unwanted synapses, resulting in CNS inflammation. A number of studies have demonstrated that microglia release IL-1β, IL-6, IL-8, and other inflammatory factors, and these factors are significantly increased in the peripheral blood and CNS of autistic patients. Insights into possible targets associated with microglia would aid the development of potential medical therapies for various CNS disorders. A recent study identified a novel pharmacological anti-neuroinflammatory agent and GIBH-130, for Alzheimer's disease through microglia-based phenotypic screening, the method by which novel favorable targets in ASD models may be introduced[77]. Further studies are needed to confirm the specificity of inflammatory factor release and the intricate interactions between microglia and inflammation. Besides, glial cell modulation cannot be precisely controlled, and microglia modulation may have other adverse effects on the CNS, thus posing a challenge for ASD treatment.

6. Conclusion

Our work offers a summary of the main pathological mechanisms of microglia in ASD and several targeted therapeutic methods. There are limitations in the existing mechanisms, such as the ambiguous pruning preference for which type of synapses and the lack of explicit cause-effect relationship between studies, like MIA and microglia polarization. Focusing on new insights such as glial cell interactions and autophagy may bring promising results. As for effective interventions, most therapeutic methods are still

in the theoretical stage and have not been translated from ongoing animal experiments to clinical trials. The precise regulation of microglia should be the focus of future research, but elucidating the explicit circuits still remains a priority.

The emphasis of future research on ASD should include a range of relevant mechanisms: (1) the involvement of microglia in the development of ASD, and the molecular mechanisms of microglia activation, which are crucial for precise intervention; (2) the modulation of cytokines secreted by microglia, including IL-1β, IL-6, and IL-8 in both peripheral blood and CNS of ASD patients, and the inflammatory pathways they participate in, since there might be association between the role of inflammatory factors and the abnormal phagocytic pruning by microglia; (3) the characterization of microglial activity and marker molecules in the early stages of ASD, which is crucial for the early identification and intervention of ASD.

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Conflict of interest

The authors declare that they have no competing interests.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

The supporting data can be obtained from the corresponding author upon reasonable request.

References

1. Maenner MJ, Shaw KA, Bakian AV, *et al*., 2021, Prevalence and characteristics of autism spectrum disorder among children Aged 8 years - autism and developmental disabilities monitoring network, 11 sites, United States 2018. *MMWR Surveill Summ*, 70(11): 1–16.

   https://doi.org/10.15585/mmwr.ss7011a1

2. Zeidan J, Fombonne E, Scorah J, *et al*., 2022, Global prevalence of autism: A systematic review update. *Autism Res*, 15(5): 778–790.

   https://doi.org/10.1002/aur.2696

3. Elsabbagh M, Divan G, Koh YJ, *et al*., 2012, Global prevalence of autism and other pervasive developmental disorders. *Autism Res*, 5(3): 160–179.

   https://doi.org/10.1002/aur.239

4. Shaw KA, Maenner MJ, Bakian AV, *et al*., 2021, Early identification of autism spectrum disorder among children Aged 4 Years - autism and developmental disabilities monitoring network, 11 sites, United States, 2018. *MMWR Surveill Summ*, 70(10): 1–14.

   https://doi.org/10.15585/mmwr.ss7010a1

5. Borst K, Dumas AA, Prinz M, 2021, Microglia: Immune and non-immune functions. *Immunity*, 54(10): 2194–2208.

   https://doi.org/10.1016/j.immuni.2021.09.014

6. Cserep C, Posfai B, Denes A, 2021, Shaping neuronal fate: Functional heterogeneity of direct microglia-neuron interactions. *Neuron*, 109(2): 222–240.

   https://doi.org/10.1016/j.neuron.2020.11.007

7. Badimon A, Strasburger HJ, Ayata P, *et al*., 2020, Negative feedback control of neuronal activity by microglia. *Nature*, 586(7829): 417–423.

   https://doi.org/10.1038/s41586-020-2777-8

8. Xu ZX, Kim GH, Tan JW, *et al*., 2020, Elevated protein synthesis in microglia causes autism-like synaptic and behavioral aberrations. *Nat Commun,* 11(1): 1797.

   https://doi.org/10.1038/s41467-020-15530-3

9. Velmeshev D, Schirmer L, Jung D, *et al*., 2019, Single-cell genomics identifies cell type-specific molecular changes in autism. *Science*, 364(6441): 685–689.

   https://doi.org/10.1126/science.aav8130

10. Elsabbagh M, 2020, Linking risk factors and outcomes in autism spectrum disorder: Is there evidence for resilience?. *BMJ*, 368: l6880.

    https://doi.org/10.1136/bmj.l6880

11. Kalish BT, Kim E, Finander B, *et al*., 2021, Maternal immune activation in mice disrupts proteostasis in the fetal brain. *Nat Neurosci*, 24(2): 204–213.

https://doi.org/10.1038/s41593-020-00762-9

12. Jensen AR, Lane AL, Werner BA, *et al*., 2022, Modern biomarkers for autism spectrum disorder: Future directions. *Mol Diagn Ther*, 26(5): 483–495.

https://doi.org/10.1007/s40291-022-00600-7

13. Seiffe A, Ramirez MF, Sempe L, *et al*., 2022, Juvenile handling rescues autism-related effects of prenatal exposure to valproic acid. *Sci Rep*, 12(1): 7174.

https://doi.org/10.1038/s41598-022-11269-7

14. De Luca SN, Soch A, Sominsky L, *et al*., 2020, Glial remodeling enhances short-term memory performance in Wistar rats. *J Neuroinflammation*, 17(1): 52.

https://doi.org/10.1186/s12974-020-1729-4

15. Barahona RA, Morabito S, Swarup V, *et al*., 2022, Cortical diurnal rhythms remain intact with microglial depletion. *Sci Rep*, 12(1): 114.

https://doi.org/10.1038/s41598-021-04079-w

16. Choudhury ME, Miyanishi K, Takeda H, *et al*., 2020, Phagocytic elimination of synapses by microglia during sleep. *Glia*, 68(1): 44–59.

https://doi.org/10.1002/glia.23698

17. Jin X, Simmons SK, Guo A, *et al*., 2020, *In vivo* perturb-seq reveals neuronal and glial abnormalities associated with autism risk genes. *Science*, 370(6520): eaaz6063.

https://doi.org/10.1126/science.aaz6063

18. Vargas DL, Nascimbene C, Krishnan C, *et al*., 2005, Neuroglial activation and neuroinflammation in the brain of patients with autism. *Ann Neurol*, 57(1): 67–81.

https://doi.org/10.1002/ana.20315

19. Paolicelli RC, Bolasco G, Pagani F, *et al*., 2011, Synaptic pruning by microglia is necessary for normal brain development. *Science*, 333(6048): 1456–1458.

https://doi.org/10.1126/science.1202529

20. Donzis EJ, Tronson NC, 2014, Modulation of learning and memory by cytokines: Signaling mechanisms and long term consequences. *Neurobiol Learn Mem*, 115: 68–77.

https://doi.org/10.1016/j.nlm.2014.08.008

21. Donovan AP, Basson MA, 2017, The neuroanatomy of autism- a developmental perspective. *J Anat*, 230(1): 4–15.

https://doi.org/10.1111/joa.12542

22. Varghese M, Keshav N, Jacot-Descombes S, *et al*., 2017, Autism spectrum disorder: Neuropathology and animal models. *Acta Neuropathol*, 134(4): 537–566.

https://doi.org/10.1007/s00401-017-1736-4

23. Li Q, Zhang L, Shan H, *et al*., 2022, The immuno-behavioural covariation associated with the treatment response to bumetanide in young children with autism spectrum disorder. *Transl Psychiatry*, 12(1): 228.

https://doi.org/10.1038/s41398-022-01987-x

24. Koyama R, Ikegaya Y, 2015, Microglia in the pathogenesis of autism spectrum disorders. *Neurosci Res*, 100: 1–5.

https://doi.org/10.1016/j.neures.2015.06.005

25. Won H, Lee HR, Gee HY, *et al*., 2012, Autistic-like social behaviour in Shank2-mutant mice improved by restoring NMDA receptor function. *Nature*, 486(7402): 261–265.

https://doi.org/10.1038/nature11208

26. Chao HT, Chen H, Samaco RC, *et al*., 2010, Dysfunction in GABA signalling mediates autism-like stereotypies and Rett syndrome phenotypes. *Nature*, 468(7321): 263–269.

https://doi.org/10.1038/nature09582

27. Colonna M, Butovsky O, 2017, Microglia function in the central nervous system during health and neurodegeneration. *Annu Rev Immunol*, 35: 441–468.

https://doi.org/10.1146/annurev-immunol-051116-052358

28. Sanjay, Park M, Lee HJ, 2022, Roles of fatty acids in microglial polarization: Evidence from *in vitro* and *in vivo* studies on neurodegenerative diseases. *Int J Mol Sci*, 23(13): 7300.

https://doi.org/10.3390/ijms23137300

29. Gilchrist SE, Pennelli GM, Hafizi S, 2021, Gas6/TAM signalling negatively regulates inflammatory induction of GM-CSF in mouse brain microglia. *Cells*, 10(12): 3281.

https://doi.org/10.3390/cells10123281

30. Hyder U, Mccann JL, Wang J, *et al*., 2020, The ARF tumor suppressor targets PPM1G/PP2Cgamma to counteract NF-kappaB transcription tuning cell survival and the inflammatory response. *Proc Natl Acad Sci U S A*, 117(51): 32594–32605.

https://doi.org/10.1073/pnas.2004470117

31. Diamant G, Dikstein R, 2013, Transcriptional control by NF-kappaB: elongation in focus. *Biochim Biophys Acta*, 1829(9): 937–945.

https://doi.org/10.1016/j.bbagrm.2013.04.007

32. Karin M, Delhase M, 2000, The I kappa B kinase (IKK) and NF-kappa B: Key elements of proinflammatory signalling. *Semin Immunol*, 12(1): 85–98.

https://doi.org/10.1006/smim.2000.0210

33. Roberti A, Chaffey LE, Greaves DR, 2022, NF-kappaB signaling and inflammation-drug repurposing to treat inflammatory disorders? *Biology* (*Basel*), 11(3): 372.

https://doi.org/10.3390/biology11030372

34. Okun E, Griffioen KJ, Lathia JD, *et al*., 2009, Toll-like receptors in neurodegeneration. *Brain Res Rev*, 59(2): 278–292.

https://doi.org/10.1016/j.brainresrev.2008.09.001

35. Kaminska B, Gozdz A, Zawadzka M, *et al*., 2009, MAPK signal transduction underlying brain inflammation and gliosis as therapeutic target. *Anat Rec* (*Hoboken*), 292(12): 1902–1913.

https://doi.org/10.1002/ar.21047

36. Ashwood P, Krakowiak P, Hertz-Picciotto I, *et al*., 2011, Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome. *Brain Behav Immun*, 25(1): 40–45.

https://doi.org/10.1016/j.bbi.2010.08.003

37. Theoharides TC, Tsilioni I, Patel AB, *et al*., 2016, Atopic diseases and inflammation of the brain in the pathogenesis of autism spectrum disorders. *Transl Psychiatry*, 6(6): e844.

https://doi.org/10.1038/tp.2016.77

38. Kaneko N, Kurata M, Yamamoto T, *et al*., 2019, The role of interleukin-1 in general pathology. *Inflamm Regen*, 39: 12.

https://doi.org/10.1186/s41232-019-0101-5

39. Li DJ, Tsai CS, Hsiao RC, *et al*., 2022, Associations between Allergic and autoimmune diseases with autism spectrum disorder and attention-deficit/hyperactivity disorder within families: A population-based Cohort study. *Int J Environ Res Public Health*, 19(8): 4503.

https://doi.org/10.3390/ijerph19084503

40. Alcala A, Osborne B, Allen B, *et al*., 2022, Toll-like receptors in the mechanism of tributyltin-induced production of pro-inflammatory cytokines, IL-1beta and IL-6. *Toxicology*, 472: 153177.

https://doi.org/10.1016/j.tox.2022.153177

41. Tsilioni I, Theoharides TC, 2018, Extracellular vesicles are increased in the serum of children with autism spectrum disorder, contain mitochondrial DNA, and stimulate human microglia to secrete IL-1beta. *J Neuroinflammation*, 15(1): 239.

https://doi.org/10.1186/s12974-018-1275-5

42. Qin Y, Cao L, Zhang J, *et al*., 2022, Whole-transcriptome analysis of serum L1CAM-captured extracellular vesicles reveals neural and glycosylation changes in autism spectrum disorder. *J Mol Neurosci*, 72(6): 1274–1292.

https://doi.org/10.1007/s12031-022-01994-z

43. Che L, Paliogiannis P, Cigliano A, *et al*., 2022, Corrigendum: Pathogenetic, prognostic, and therapeutic role of fatty acid synthase in human hepatocellular carcinoma. *Front Oncol*, 12: 874053.

https://doi.org/10.3389/fonc.2022.874053

44. Tsai SJ, 2021, Role of interleukin 8 in depression and other psychiatric disorders. *Prog Neuropsychopharmacol Biol Psychiatry*, 106: 110173.

https://doi.org/10.1016/j.pnpbp.2020.110173

45. Bryn V, Aass HC, Skjeldal OH, *et al*., 2017, Cytokine profile in autism spectrum disorders in children. *J Mol Neurosci*, 61(1): 1–7.

https://doi.org/10.1007/s12031-016-0847-z

46. Lin CW, Septyaningtrias DE, Chao HW, *et al*., 2022, A common epigenetic mechanism across different cellular origins underlies systemic immune dysregulation in an idiopathic autism mouse model. *Mol Psychiatry*.

https://doi.org/10.1038/s41380-022-01566-y

47. Li X, Chauhan A, Sheikh AM, *et al*., 2009, Elevated immune response in the brain of autistic patients. *J Neuroimmunol*, 207(1-2): 111–116.

https://doi.org/10.1016/j.jneuroim.2008.12.002

48. Weerasekera A, Ion-Margineanu A, Nolan G, *et al*., 2022, Subcortical brain morphometry differences between adults with autism spectrum disorder and schizophrenia. *Brain Sci*, 12(4): 439.

https://doi.org/10.3390/brainsci12040439

49. Tanaka T, Narazaki M, Kishimoto T, 2014, IL-6 in inflammation, immunity, and disease. *Cold Spring Harb Perspect Biol*, 6(10): a016295.

https://doi.org/10.1101/cshperspect.a016295

50. Garay PA, Mcallister AK, 2010, Novel roles for immune molecules in neural development: Implications for neurodevelopmental disorders. *Front Synaptic Neurosci*, 2: 136.

https://doi.org/10.3389/fnsyn.2010.00136

51. Guma E, Bordeleau M, Gonzalez Ibanez F, *et al*., 2022, Differential effects of early or late exposure to prenatal maternal immune activation on mouse embryonic neurodevelopment. *Proc Natl Acad Sci U S A*, 119(12): e2114545119.

https://doi.org/10.1073/pnas.2114545119

52. Smith SE, Li J, Garbett K, *et al*., 2007, Maternal immune activation alters fetal brain development through interleukin-6. *J Neurosci*, 27(40): 10695–10702.

https://doi.org/10.1523/JNEUROSCI.2178-07.2007

53. Wei H, Chadman KK, Mccloskey DP, *et al*., 2012, Brain IL-6 elevation causes neuronal circuitry imbalances and mediates autism-like behaviors. *Biochim Biophys Acta*, 1822(6): 831–842.

https://doi.org/10.1016/j.bbadis.2012.01.011

54. Cryan JF, O'Riordan KJ, Cowan CSM, *et al*., 2019, The microbiota-gut-brain axis. *Physiol Rev*, 99(4): 1877–2013.

https://doi.org/10.1152/physrev.00018.2018

55. Sampson TR, Debelius JW, Thron T, *et al*., 2016, Gut microbiota regulate motor deficits and neuroinflammation in a model of parkinson's disease. *Cell*, 167(6): 1469–1480 e12.

https://doi.org/10.1016/j.cell.2016.11.018

56. Needham BD, Tang W, Wu WL, 2018, Searching for the gut microbial contributing factors to social behavior in rodent

models of autism spectrum disorder. *Dev Neurobiol*, 78(5): 474–499.

https://doi.org/10.1002/dneu.22581

57. Abdel-Haq R, Schlachetzki JCM, Glass CK, *et al*., 2019, Microbiome-microglia connections via the gut-brain axis. *J Exp Med*, 216(1): 41–59.

https://doi.org/10.1084/jem.20180794

58. Wright-Jin EC, Gutmann DH, 2019, Microglia as dynamic cellular mediators of brain function. *Trends Mol Med*, 25(11): 967–979.

https://doi.org/10.1016/j.molmed.2019.08.013

59. Kim JW, Hong JY, Bae SM, 2018, Microglia and autism spectrum disorder: Overview of current evidence and novel immunomodulatory treatment options. *Clin Psychopharmacol Neurosci*, 16(3): 246–252.

https://doi.org/10.9758/cpn.2018.16.3.246

60. Yuan T, Orock A, Greenwood-Vanmeerveld B, 2022, An enriched environment reduces chronic stress-induced visceral pain through modulating microglial activity in the central nucleus of the amygdala. *Am J Physiol Gastrointest Liver Physiol*, 322(2): G223–G233.

https://doi.org/10.1152/ajpgi.00307.2021

61. Subbarayan MS, Joly-Amado A, Bickford PC, *et al*., 2022, CX3CL1/CX3CR1 signaling targets for the treatment of neurodegenerative diseases. *Pharmacol Ther*, 231: 107989.

https://doi.org/10.1016/j.pharmthera.2021.107989

62. Yang Z, Liu B, Yang LE, *et al*., 2019, Platycodigenin as potential drug candidate for Alzheimer's disease via modulating microglial polarization and neurite regeneration. *Molecules*, 24(18): 3207.

https://doi.org/10.3390/molecules24183207

63. Davoli-Ferreira M, Thomson CA, Mccoy KD, 2021, Microbiota and microglia interactions in ASD. *Front Immunol*, 12: 676255.

https://doi.org/10.3389/fimmu.2021.676255

64. Alirezaei M, Kiosses WB, Flynn CT, *et al*., 2008, Disruption of neuronal autophagy by infected microglia results in neurodegeneration. *PLoS One*, 3(8): e2906.

https://doi.org/10.1371/journal.pone.0002906

65. Estes ML, Mcallister AK, 2016, Maternal immune activation: Implications for neuropsychiatric disorders. *Science*, 353(6301): 772–777.

https://doi.org/10.1126/science.aag3194

66. Naviaux JC, Schuchbauer MA, Li K, *et al*., 2014, Reversal of autism-like behaviors and metabolism in adult mice with single-dose antipurinergic therapy. *Transl Psychiatry*, 4: e400.

https://doi.org/10.1038/tp.2014.33

67. Martinat M, Rossitto M, Di Miceli M, *et al*., 2021, Perinatal dietary polyunsaturated fatty acids in brain development, role in neurodevelopmental disorders. *Nutrients*, 13(4): 1185.

https://doi.org/10.3390/nu13041185

68. Rey C, Nadjar A, Joffre F, *et al*., 2018, Maternal n-3 polyunsaturated fatty acid dietary supply modulates microglia lipid content in the offspring. *Prostaglandins Leukot Essent Fatty Acids*, 133: 1–7.

https://doi.org/10.1016/j.plefa.2018.04.003

69. Van Elst K, Brouwers JF, Merkens JE, *et al*., 2019, Chronic dietary changes in n-6/n-3 polyunsaturated fatty acid ratios cause developmental delay and reduce social interest in mice. *Eur Neuropsychopharmacol*, 29(1): 16–31.

https://doi.org/10.1016/j.euroneuro.2018.11.1106

70. Wenzel TJ, Gates EJ, Ranger AL, *et al*., 2020, Short-chain fatty acids (SCFAs) alone or in combination regulate select immune functions of microglia-like cells. *Mol Cell Neurosci*, 105: 103493.

https://doi.org/10.1016/j.mcn.2020.103493

71. Gabriele S, Sacco R, Altieri L, *et al*., 2016, Slow intestinal transit contributes to elevate urinary p-cresol level in Italian autistic children. *Autism Res*, 9(7): 752–759.

https://doi.org/10.1002/aur.1571

72. Vuong HE, Hsiao EY, 2017, Emerging roles for the Gut microbiome in autism spectrum disorder. *Biol Psychiatry*, 81(5): 411–423.

https://doi.org/10.1016/j.biopsych.2016.08.024

73. Comi AM, Zimmerman AW, Frye VH, *et al*., 1999, Familial clustering of autoimmune disorders and evaluation of medical risk factors in autism. *Child Neurol*, 14(6): 388–394.

https://doi.org/10.1177/088307389901400608

74. Jones KL, Croen LA, Yoshida CK, *et al*., 2017, Autism with intellectual disability is associated with increased levels of maternal cytokines and chemokines during gestation. *Mol Psychiatry*, 22(2): 273–279.

https://doi.org/10.1038/mp.2016.77

75. Crane L, Chester JW, Goddard L, *et al*., 2016, Experiences of autism diagnosis: A survey of over 1000 parents in the United Kingdom. *Autism*, 20(2): 153–162.

https://doi.org/10.1177/1362361315573636

76. Choi GB, Yim YS, Wong H, *et al*., 2016, The maternal interleukin-17a pathway in mice promotes autism-like phenotypes in offspring. *Science*, 351(6276): 933–939.

https://doi.org/10.1126/science.aad0314

77. Zhou W, Zhong G, Fu S, *et al*., 2016, Microglia-based phenotypic screening identifies a novel inhibitor of neuroinflammation effective in Alzheimer's disease models. *ACS Chem Neurosci*, 7(11): 1499–1507.

https://doi.org/10.1021/acschemneuro.6b00125