

Microglia and Autism Spectrum Disorder: Overview of Current Evidence and Novel Immunomodulatory Treatment Options

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Autism spectrum disorder is a rapidly increasing heterogeneous neurodevelopmental syndrome, remarked by persistent deficit in social communication, and restricted, repetitive patterns of behavior and interest. Lately, maternal immune activation and microglial dysfunction in the developing brain have been gaining mounting evidence and leading to studies of various novel agents as potential treatment options. A few immunomodulatory treatment options—luteolin, minocycline, suramin, vitamin D, gut microbiota—are discussed in the current article, regarding the current understanding of their mechanisms and evidence for potential clinical use. More studies are warranted to understand their exact mechanisms of action and to verify efficacy and safety in human subjects.

KEY WORDS: Autism spectrum disorder; Maternal immune activation; Microglia; Immunomodulatory therapy.

INTRODUCTION

Characterized by persistent deficit in social communication, and restricted, repetitive patterns of behavior and interest, autism spectrum disorder (ASD) is now one of the most commonly diagnosed neurodevelopmental disorders, with its latest prevalence measured at 1 out of 68 children aged 8 years.¹⁾ The prevalence of the disorder has rapidly and continuously been increasing.¹⁾ Some studies suggest an even higher prevalence of 2.64%.²⁾ In order to account for its rapidly increasing prevalence over the last couple of decades, many etiologic theories have been proposed with varying degrees of supporting evidence. Some of the popular theories include increased public awareness, higher diagnostic rates, environmental changes, etc.³⁻⁵⁾ However, in large, exact pathogenesis

still remains unknown,⁶⁾ and so far, no biomarker has been identified with reliable diagnostic or prognostic values.⁷⁾ A growing body of evidence indicates that infectious or inflammatory immune activation in pregnant mothers lead to higher risk of neurodevelopmental disorders including ASD in the offspring.⁸⁾ To account for such findings, maternal immune activation (MIA) and resulting microglial dysfunction in the developing brain have been studied.⁹⁾ Meanwhile, a few novel agents targeting microglial activation in particular have shown positive results in animal studies.¹⁰⁻¹²⁾ In this article, we aim to overview current understanding of MIA and microglial dysfunction as a potential cause for a subtype of ASD and novel treatment ideas.

MATERNAL IMMUNE ACTIVATION

The potential role of MIA in increased risk for ASD in the offspring has been repeatedly reported in studies of rodent and rhesus monkey.^{13,14)} Increased maternal interleukin (IL)-6 induced by inflammation has been shown to directly cause changes in transcription in the frontal cortex of the offspring.¹⁵⁾ In animal studies, maternal immune-activated pro-inflammatory cytokines, such as

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IL-1 β , IL-6, tumor necrosis factor (TNF)- α have been shown to readily cross over to the fetal brain^{16,17} and cause direct impact on the fetal neurodevelopment.¹⁸ Several animal studies have demonstrated that autism-like behaviors were observed in the offspring, following the exposure of pregnant mothers to the polyinosinic:polycytidylic acid (poly I:C)—a viral mimetic, an influenza virus, or the lipopolysaccharides (LPS)—a bacterial component.¹⁹ Growing evidence for the presence of inflammation in the fetal brain includes microglial activation and proliferation in addition to the presence of increased pro-inflammatory cytokines.^{20,21}

MICROGLIA

Microglia are well-known as macrophages of the central nervous system (CNS), whose major function is ambient surveillance and activation during insults to CNS, such as damage, infection, or disease.²² Microglial progenitors are believed to arrive and occupy the developing fetal brain, preceding the completion of the blood brain barrier (BBB).²³ Unlike traditionally believed, the non-inflammatory role of microglia—synaptic pruning—has been implicated more as essential part of CNS development.^{24,25} Therefore, in addition to inflammatory role of microglia implicated in dysfunctional microglial activation and pathogenesis of ASD,²⁶ dysfunction in

such microglia-mediated synaptic pruning process during the neural development in the offspring has been associated with neurodevelopmental disorders, such as ASD.²⁷ Studies also demonstrate that disruption in such essential function of microglia during neural development at any time from pregnancy to early postnatal period can lead to neurodevelopmental disorders, such as ASD.²⁸

IMMUNOMODULATORY TREATMENT

Along with accumulating evidence for MIA and microglial impact as a potential etiologic cause for a subtype of ASD, a few novel treatment options have been proposed and studied in animal and/or human trials. Some of the most studied agents include *luteolin*, *minocycline*, *suramin*, *vitamin D*, and *gut microbiota*, which will be reviewed here (Table 1 and Fig. 1).

Luteolin

Luteolin is an antioxidant known to block microglial activation²⁹ and reduce the neurotoxic effect.³⁰ Luteolin appears to exert its effects by dampening histamine, IL-6, IL-8, TNF, tryptase from mast cells.³¹ It is considered safe with minimal to no side effects.³²⁻³⁵ In an animal model study (mice ASD), luteolin has been shown to attenuate autism like behaviors in mice.³³ In the most recent animal studies, luteolin has been observed to decrease the levels

Table 1. Overview of immunomodulatory agents for autism spectrum disorder (ASD)

Name of treatment	Mechanism of action	Effects	Side effects	Trials
Luteolin	Antioxidant Block microglial activation ²⁹ Reduce neurotoxic effect ³⁰	Decrease inflammatory cytokines, TNF- α , IL-1 β ^{10,31}	Considered safe with minimal to no side effects ³²⁻³⁵	Attenuated autism-like behaviors in mice ³³ Improved sociability in humans ^{10,34,35}
Minocycline	Tetracycline antibiotic Pleotropic anti-inflammatory and neuroprotective activities ^{36,37}	Modulate microglial activation ^{39,40}	Deemed safe (as add-on to risperidone for children with ASD) ⁴⁴	Improved autistic, anxiety behavior in mice ^{11,41} Inconsistent results in humans ^{43,44}
Suramin	Competitive inhibition of purinergic signaling ⁴⁵	Recover dysregulated purinergic metabolism ¹²	Tolerated without major adverse effects (in pilot trial for children with ASD) ⁴⁸	Improved core symptoms of autism in mice ^{12,46,47} and humans ⁴⁸ Mixed results in RCTs ^{53,54}
Vitamin D	Active neuro-steroid.	Modulate pro-inflammatory cytokine release from microglia ^{51,52}		
Gut microbiota	Microbiome	Modulate environmental and genetic risk factors for ASD ^{62,63}		<i>Bacteroides fragilis</i> improved behavioral disturbance in mice ⁶¹

TNF, tumor necrosis factor; IL, interleukin; RCT, randomized controlled trial.

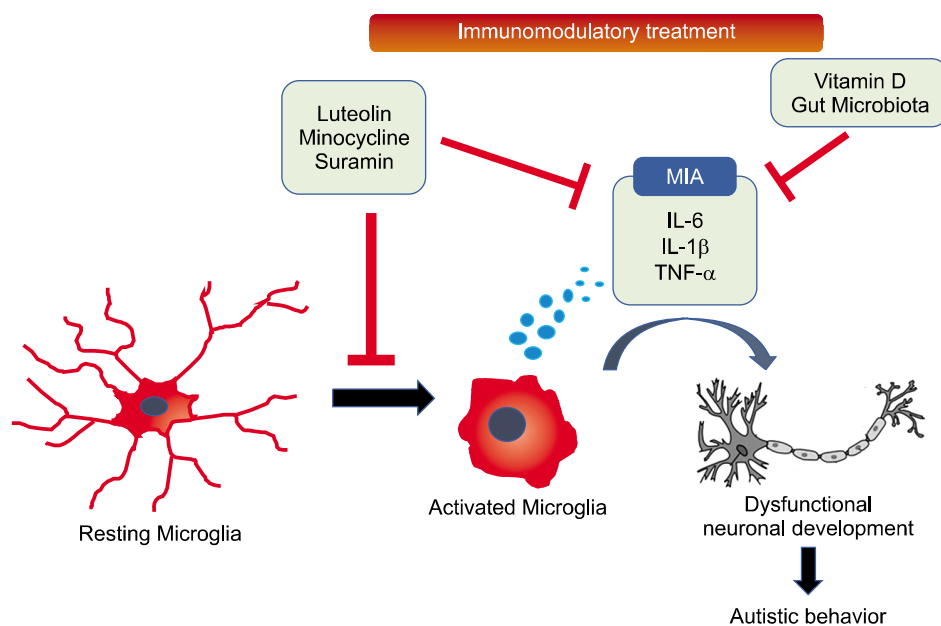


Fig. 1. Schematic diagram of microglia and immunomodulatory treatments. Maternal immune activation (MIA) during pregnancy may be bridged to an increased risk of autistic behaviors in the offspring by defective microglial activation. Microglial activation results in the production of pro-inflammatory cytokines such as interleukin (IL)-1 β , IL-6 and tumor necrosis factor (TNF)- α . Luteolin, minocycline, and suramin have been shown to block microglial activation and attenuate the levels of inflammatory cytokines. Vitamin D and gut microbiota are believed to reduce pro-inflammatory cytokines released from microglia.

of inflammatory cytokines, TNF- α and IL-1 β in valproic acid (VPA)-induced ASD animal models.¹⁰ In particular, the study by Bertolino *et al.*¹⁰ included the results of animal experiments as well as that of clinical trial of a 10-year-old boy with ASD. The boy showed improved symptoms overall, but particularly in the social area. He also showed significant improvement in enuresis, a most difficult symptom reported by parents. In clinical studies, luteolin also has proven effective in ameliorating comorbid symptoms of ASD patients, such as attention and sociability.^{34,35}

Minocycline

Minocycline is a tetracycline antibiotic; whose mechanism is via its known pleiotropic anti-inflammatory and neuroprotective activities.^{36,37} Another proposed key mechanism of action for minocycline is via hindering matrix metalloproteinase (MMP).³⁸ A growing evidence has suggested the modulating effect of minocycline in microglia activation.^{39,40} A series of animal studies have proven minocycline as an effective treatment for autistic behaviors. In their study with *patDp/+* mice ASD model (human 15q11-q13), Shigemori *et al.*⁴¹ showed that prenatal treatment with minocycline led to recovery of microglial activation markers as well as improved anxiety behaviors. In VPA-induced rat ASD model, minocycline was also shown to improve autistic behaviors.¹¹ Minocycline has also been shown promising in few yet

accumulating human trials. In an open-label add-on trial for fragile X syndrome, the most common known genetic cause of ASD, minocycline significantly improved irritability in patients, as evidenced by Aberrant Behavior Checklist-Community (ABC-C), irritability subscale.⁴² Human trials, however, have not shown consistent results. A small pilot trial (n=10) with minocycline up to six months showed only minimal improvement, and neither yielded any significant behavioral improvement in ASD nor showed any observable changes in key inflammatory cytokines, such as IL-1 β , IL-6, TNF- α , or MMP.⁴³ More lately, however, minocycline was deemed a safe and effective choice as an add-on to risperidone for children with ASD, based on ABC-C irritability subscale.⁴⁴ Further studies looking at clinical utility of minocycline as a monotherapy or an add-on to risperidone for symptoms of ASD in not only child/adolescent but also adult patients are needed.

Suramin

Suramin is best known as a treatment for African sleeping sickness, whose mechanism of action is via competitive inhibition of purinergic signaling.⁴⁵ In the first study of its kind, Naviaux *et al.*⁴⁶ showed significant effect of suramin on improving core behavioral and comorbid symptoms in poly I:C-exposed MIA model of ASD mice. In their subsequent study, Naviaux *et al.*¹² demonstrated that a single dose of suramin was able to improve core

symptoms of autism, such as sociality, novelty-seeking, as well as recover dysregulated purinergic metabolism in poly I:C-exposed MIA model of adult ASD mice. Antipurinergic therapy using suramin also proved effective for improving autistic features in fragile X mouse model, showing its efficacy across different etiologic models of ASD.⁴⁷⁾ More recently, in the first translational pilot trial, low-dose suramin treatment was well tolerated without major adverse effects and associated with significant improvement in core symptoms of ASD in a small number of child and adolescent male patients.⁴⁸⁾ A study has shown that suramin also inhibits spinal cord microglia activation followed by long-term hyperalgesia.⁴⁹⁾ The exact mechanism of action of suramin on microglia with regards to ASD is yet unclear and requires further studies.

Vitamin D

Vitamin D deficiency has been increasingly implicated as a risk factor for development of ASD.⁵⁰⁾ Vitamin D is an active neuro-steroid, believed to modulate pro-inflammatory cytokine release from microglia.^{51,52)} Despite being seemingly one of the most studied supplemental treatment for ASD, studies targeting use of vitamin D as a treatment or prophylaxis for ASD; however, have been yielding mixed results. One of the first randomized controlled trials (RCTs) involving a high dose vitamin D supplementation in child ASD patients showed significant improvement in symptoms.⁵³⁾ However, another RCT involving a lower dose vitamin D supplementation in child ASD patients failed to show consistent benefits.⁵⁴⁾ Furthermore, a recent study showed low serum levels of vitamin D in 60 patients with ASD.⁵⁵⁾ Such discrepancy and inconsistent results from trials have been speculated to originate from failures to account for various forms of vitamin D metabolites with different bioavailability as well as its receptors and binding proteins.⁵⁰⁾ Further studies with methodological designs that would allow delineating such discrepancies are called for.

Gut microbiota

It is widely known that ASD commonly and frequently presents with gastrointestinal disturbances.⁵⁶⁾ Studies also have suggested relationship with symptom severity in ASD patients and gastrointestinal disturbances.⁵⁷⁾ Gut bacteria have been frequently implicated in ASD,⁵⁸⁾ and animal and human studies have demonstrated its correla-

tion with behavioral disturbances.⁵⁹⁻⁶¹⁾ Interestingly, modeling MIA-induced ASD offspring in mice resulted not only in behavioral disturbance but also alterations in gut microbiota, which were however reversed by treatment with *Bacteroides fragilis* (*B. fragilis*).⁶²⁾ More recent findings have suggested that the microbiome plays a crucial role in modulating environmental and genetic risk factors for development of ASD.^{63,64)} The relationship between microbiota and microglial activation has lately been further studied. Short-chain fatty acids, microbiota-derived bacterial fermentation products, are identified to regulate microglia homeostasis, and the depletion of short-chain fatty acid receptors mimics microglia defects.⁶⁵⁾ Further clinical studies involving patients with ASD are called for based on this finding.

CONCLUSION

ASD is an immense heterogeneous syndrome that is believed to be a product of complex interactions among many known and unknown risk factors. MIA-induced microglial activation in the offspring brain has been implicated as an important etiologic mechanism for a subset of ASD, opening windows into a few novel treatment options. A few immunomodulatory treatment options—such as luteolin, minocycline, suramin, vitamin D, gut microbiota—have been discussed in our paper about the current understanding of their mechanisms and the mounting evidence for potential clinical use. Although many of these novel treatment options appear to be promising and hopeful, their exact mechanisms of action and clear explanation as to how microglia activation is being recruited and modulated upon each of the optional treatments are to be further studied, and more translational trials are in order to verify efficacy and safety in human subjects. Given that immunomodulatory treatment options described here modulate autistic behaviors, it is plausible to test whether these treatments can be mediated by microglial activation. Such further clinical/experimental studies could potentially allow for better understanding and development of treatment options specifically targeting a process or cascades involved in microglial activation and/or dysfunction.

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