

# Immune Dysregulation and the Pathogenesis of Autism

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**Autism spectrum disorder (ASD) is a devastating disease affecting 1 in 68 children in the United States. Although much has been learned, the etiology and pathogenesis of autism remain largely elusive. More recently, mutations or variants of genes encoding proteins involved in immune regulation have been frequently found in ASD patients, implicating a key role of immune dysregulation in the pathogenesis of ASD. In support of this notion, parental, especially maternal, immune disorders are also identified to be associated with ASD. Further, altered immune responses are constantly observed in ASD patients. On the other hand, restoration of a normal immune system in ASD patients and in animal models has shown promising therapeutic effects, further linking ASD to an abnormal immune state. Here we review current literatures and discuss the potential role of immune dysregulation in driving the pathogenesis of autism.**

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## INTRODUCTION

Autism spectrum disorders (ASD) are a hierarchical group of pervasive developmental disorders of the neural system. The diseases in the spectrum include the autistic disorder, Rett's disorder, childhood disintegrative disorder, Asperger's disorder, and pervasive developmental disorder not otherwise specified.<sup>1</sup> The average onset of ASD is 30 months. Its manifestation is characterized by a lack of social interest, impaired language development, obsessive insistence on sameness, and repetitive motor behavior.<sup>1</sup> Prognosis of ASD is usually poor, making it a devastating disease to patients and to their families. Moreover, the prevalence of ASD has increased rapidly in recent years, rising from approximately 1 in 500 children in 1992 to the now highly concerning 1 in 68 children in the United States.<sup>2</sup> Although some researchers in past contributed this rise to high public awareness of ASD and wider diagnostic criteria use to diagnose ASD, studies now indicate that none of these factors could account for most of the rise in the number of ASD.<sup>3</sup> Thus, other factors must have been involved in driving the development of ASD.

The immune system is designed to ward off invasion of pathogens such as bacteria and viruses. Interestingly, the immune system also plays a critical role in the nervous system: that is to regulate cognition and behavior. Immune cells, especially T cells are indispensable for normal brain development and function.<sup>4,5</sup> Deficiency of lymphocytes in genetically engineered mice leads to profound impairment in learning and memory capability. These animals also exhibit increased repetitive behaviors and anxiety, reminiscent of

typical ASD symptoms.<sup>6,7</sup> Most strikingly, adoptive transfer of wild-type splenocytes or bone marrow cells to reconstitute the lymphocyte population in the experimental mice could reverse the defects and significantly improve their learning capability as well as reduce their repetitive behavior.<sup>8,9</sup> On a genetic level, whole-genome sequencing, gene association studies, copy number variation and SNP analyses have revealed a large number of genes that are associated with ASD and many of those are also involved in immune regulation.<sup>10,11</sup> Together, these findings suggest that an altered immune system is associated with the development of ASD.<sup>12-15</sup>

In this review, we present evidence in the current literatures that suggest a role of immune dysregulation in the pathogenesis of autism. We also discuss potential immune modulation as a new way for treating ASD.

## THE GENETICS UNDERLYING ASD

The development of ASD has a genetic basis. In twins, the concordance for autism development in monozygotic twins is much higher than that in dizygotic twins.<sup>16,17</sup> In non-twin siblings, the odds of developing ASD is 25-fold higher as compared to the general population.<sup>18</sup> Moreover, first-degree family members of ASD patients tend to have a higher incidences of anxiety disorder, major depressive disorder, and motor tics that are also seen in ASD patients.<sup>19</sup> It has been estimated that approximately 38-54% of ASD are inherited, suggesting a link between genetic composition and the development of ASD.<sup>20,21</sup>

The understanding of individual genes associated with ASD came from studies of syndromic disorders with single gene

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mutations. In these disorders, the occurrence of ASD is higher than the general population. Within those, the classical ASD genes identified include *MeCP2* in the Rett's disorder,<sup>22</sup> *FMRI* in Fragile X syndrome,<sup>23</sup> *TSC1* and *TSC2* in Tuberous Sclerosis,<sup>24</sup> *PTEN* in Cowden's Syndrome,<sup>25</sup> and *UBE3A* in Angelman's Syndrome.<sup>26</sup> Interestingly, some of these genes are integral to the immune system. For example, proteins encoded by the *PTEN*, *TSC1* and *TSC2*, and the later identified *MET* gene are all within the phosphoinositide-3-kinase (PI3K)-mTOR pathway.<sup>27</sup> A key function of this pathway is to place a check on the production of pro-inflammatory cytokines such as interleukin (IL)-12 by the antigen presenting cells. This check in turn prevents excessive T(H)1 polarization that may cause undesirable immune responses.<sup>27-29</sup> Through the years more genes involved in immune regulation have been found to associate with ASD. These include the *major histocompatibility complex type 1 and 2 (MHC-I and MHC-II)*,<sup>15</sup> *C4B* of the complement pathway,<sup>30,31</sup> *IL-1*,<sup>32,33</sup> and the *macrophage inhibitory factor (MIF)*.<sup>34</sup>

Within all the ASD candidate genes, the *MET* gene seems to be most associated with ASD. *MET* encodes hepatocyte growth factor receptor. In addition to its role in the immune system, it is critical for brain development.<sup>32</sup> Variation of the *MET* gene, such as a G-C SNP in its promoter is associated with ASD.<sup>13,35,36</sup> On the molecular level, the SNP leads to reduced *MET* expression and the disruption of its downstream signaling.<sup>35</sup> More strikingly, postmortem examination of the brain of ASD patients revealed reduced *MET* expression in the temporal lobe.<sup>13,37,38</sup> Moreover, patients with the G-C SNP exhibited reduced structural and functional connectivity in the temporoparietal lobes, which is consistent with *MET*'s role in brain development.<sup>38</sup>

While our understanding of the genetic basis of autism has advanced greatly, no single gene has been shown to convey substantially increased risk for developing ASD.<sup>39</sup> On the contrary, ASD is shown to be associated with a large number of gene variations and mutations.<sup>40,41</sup> More importantly, some of these variations are also present in individuals that do not have ASD, suggesting that the genetic background may only contribute to a vulnerable state, and when other stressors from the environment strike, individuals with such vulnerability of the developing brain will have developmental defects and express ASD.

#### **THE MATERNAL IMMUNE ACTIVATION THEORY**

The immune system protects the host from pathogen invasion from the environment. During early development in the uterus and shortly after birth the immune system is programmed. In the same period, there are also extensive cross-talk between the immune system and the nervous system, which facilitates the latter to develop normally.<sup>42</sup> Should this process be interrupted, profound impairment in both brain development and cognition occurs.<sup>43</sup> Consistent with this idea, maternal immune activation (MIA) has been found to increase ASD risk in the offsprings.<sup>44</sup>

#### **Maternal Infection**

The most classical way of inducing MIA is infection. The initial study of the relationship between maternal infection and ASD came after a rubella epidemic that swept the US in 1964. Children born to mothers who were pregnant during the epidemic were found to have a significantly increased rate of acquiring ASD.<sup>45-47</sup> Similarly, in a retrospective study using the Danish database, it was found that children born to mothers who were hospitalized for a viral or bacterial infection during the first or second trimester of pregnancy had a 3 fold increase in the risk for ASD.<sup>48,49</sup> In addition to that, various case reports have suggested that ASD is associated with many other infectious diseases such as varicella, toxoplasmosis, syphilis, and CMV infections.<sup>44</sup> The vast diversity of the pathogens involved in these reports suggests that ASD is associated with immune activation in general rather than with the activation of the immune system by a specific bacteria, virus, or parasite.

Our understanding of the role of MIA in ASD has also been greatly advanced by studies in animal models. Injection of poly (I:C), which mimics viral infection, into mid-gestation in mice yielded offspring that exhibited behavior defects characteristic in ASD. These include decreased prepulse inhibition and impaired social interactions.<sup>43</sup> Very similar phenotypes were later observed in rhesus monkeys with poly IC induced MIA<sup>50</sup> and in rodents where MIA was induced through exposure to LPS, periodontal bacteria, or influenza virus.<sup>43</sup> From a brain pathological point of view, spatially restricted deficit in Purkinje cells was observed in the influenza MIA model, which is a common histological feature in humans with ASD.<sup>51-53</sup> In addition, expression of the presynaptic markers SNAP-25, nNOS, and reelin was altered in these experimental animals, suggesting a defective neural development.<sup>54,55</sup>

Interestingly, in all MIA-ASD mouse models, the maternal cytokines seems to play an essential role. First, interleukin 6 (IL-6) levels in the animal's brains increased after maternal poly IC injection and this high level of IL-6 stayed for up to 24 weeks.<sup>56</sup> Similarly, cytokines IL-2 and IL-10 were also found altered.<sup>57</sup> In demonstrating the pathogenic role of these cytokines, IL-6 was injected into mice at mid-gestation.<sup>58</sup> The results were striking: the offspring displayed aberrant social interactions, repetitive behavior, and increased anxiety, all features of ASD.<sup>58</sup> Similar phenotypes were observed in experiments where low dose IL-2 was injected daily between gestational days 12 and 16 further supporting a causal role of cytokines in ASD.<sup>59</sup> Even more interestingly, the ASD symptoms induced by MIA were ablated if poly(I:C) was injected into a Il6-knockout mouse, or when poly IC was administered together with an IL-6 neutralizing antibody.<sup>58</sup> In addition, overexpression of the anti-inflammatory cytokine IL-10 could also prevent MIA-caused ASD symptoms.<sup>60</sup> These studies thus support a model in which MIA leads to overproduction of proinflammatory cytokines which in turn leads to the development of ASD.

## Autoimmune Disorders

Autoimmune disease is another cause of inappropriate immune activation. In fact, the incidences of allergies, asthma, and autoimmune disorders are disproportionately high in families of ASD patients.<sup>61-63</sup> Children born to mothers who have rheumatoid arthritis, SLE or type 1 diabetes also have a much increased risk of developing ASD.<sup>61</sup> Moreover, autoimmune disorders are over-represented in the population of individuals with ASD.<sup>64</sup> These findings together suggest immune dysregulation during fetus development and perhaps continued immune dysregulation in adulthood is in the pathogenesis of ASD.

One possible mediator that links maternal autoimmunity to ASD development in the offspring is the IgG autoantibodies that can diffuse through the placenta from the mother to the fetus. It has been found that in about 12% of the mothers whose children have ASD, there are autoantibodies present in their blood that can react with fetal brains. But such antibodies are not found in mothers whose children are developing normally.<sup>65-68</sup> In autoimmune diseases such as SLE, maternal antibodies reactive to fetal brain have been shown to impede brain development.<sup>69,70</sup> On the other hand, purification of fetal brain reactive antibodies from mothers of ASD patients and injection of these antibodies into pregnant rhesus macaques can lead to ASD-like symptoms in the monkeys.<sup>71</sup> Similar transfer of such antibodies into mice also led to behavioral changes and neuro-pathology reminiscent of ASD, once again pointing auto-reactive antibodies as the mediator of pathogenesis in MIA-related ASD.<sup>68</sup> Interesting enough, however, is that autoimmune diseases in fathers also seem to contribute to the increased risk of ASD, suggesting that in addition to autoantibodies and cytokines, there is a heritable trait, either genetic or epigenetic, is accountable for the association between autoimmunity and ASD.<sup>61</sup>

## Inflammation in the CNS

The presence of neuro-inflammation in ASD patients has also been studied. Although with some controversies, a myriad of studies suggest inflammation in the brains of ASD patients.<sup>57,72-75</sup> Analyses of postmortem brains from ASD patients revealed markedly elevated inflammation markers that include the proinflammatory cytokines and chemokines GM-CSF, IL-1 $\beta$ , IL-6, IL-8, IL-12p40, TNF, IFN $\gamma$ , and CCL2.<sup>75-77</sup> In addition, global gene expression analyses of the brains of ASD patients showed alterations in the expression of genes that are fundamental to the immune system.<sup>37,78</sup> Correlating with these molecular findings is the prominent activation of microglia, which are macrophages-related phagocytic cells that participate in immune responses in the CNS.<sup>79,80</sup> Taking these observations together, it is thus evident that an ongoing dysregulation of the immune system is likely in the CNS of ASD patients.

## THE ALTERED IMMUNE RESPONSE IN ASD PATIENTS

While maternal immune alterations clearly contribute to the development of ASD, it has been constantly observed that the immune functions in individuals with ASD are also altered.<sup>81</sup>

## Altered Cellular Immune Responses

In individual with ASD, T cell responses are defective.<sup>82</sup> Circulating T cells from ASD patients bear more activation markers such as MHC-II molecules and CD26<sup>83</sup> and the T cell effectors adapts predominantly a Th2 phenotype: that is the T cells produce IL-4 but not IFN- $\gamma$ .<sup>84</sup> TNF is a proinflammatory cytokine that can be produced by T cells. Increased TNF- $\alpha$  has been shown to correlate with increased stereotypical behaviors typical for ASD.<sup>85</sup> Analysis of cytokine production by peripheral and intestinal T cells collected from children with ASD revealed that there is an increased frequency of TNF- $\alpha$  T cells in these patients.<sup>86,87</sup> At the same time, IL-10 producing T cells have a reduced frequency, suggesting a proinflammatory state in ASD patients.<sup>86,87</sup>

T cells from ASD patient also express lower levels of adhesion molecules such as sPECAM-1, sP-Selectin and sL-selectin.<sup>88,89</sup> Interestingly, the severity of ASD symptoms correlated with how low the expression of these adhesion molecules.<sup>88</sup> Because interaction between t cells and the CNS is essential for its development and maintenance of function,<sup>90,91</sup> it is postulated that the reduction of adhesion molecules causes ASD-like symptoms by inhibiting t cells from egressing through the blood-brain barrier. An interesting phenomenon that may support this notion is that some ASD patients may have a temporary improvement of their symptoms when they develop fever, whereas fever is known to transiently upregulate adhesion molecules.<sup>92</sup> Although the effect is temporary, and the patients all fell back to their original disease state when fever is over, it is possible that fever may evoke a transient increase in T cell-brain interactions and hence an improvement in behavior.

## The Microbiota

It is worth noting microbiota in the setting of ASD. Microbes in the intestine play a central role in priming the immune system during early infancy as well as maintaining the homeostasis of the immune system through adulthood.<sup>93-95</sup> The composition of the microbiota in the intestine affects the repertoire of T cell clones in the host and affect their effector phenotypes.<sup>96,97</sup> Some of these T cell clones are likely playing a role to support brain development.<sup>98</sup>

In the poly IC induced MIA ASD mouse models, the Offspring from the poly(I:C) displayed changes in gut flora with excessive levels of *Clostridium* and *B. fragilis* spp. Interestingly, in children with ASD, there is a similar increase of *Clostridium* spp. in their gut microbiome.<sup>99-102</sup> Moreover, it is observed in the animal model that there is increased permeability and altered cytokine profiles in the intestine which was also observed in humans with ASD that are found in some humans with ASD.<sup>103-105</sup>

## RESTORING THE NORMAL FUNCTION OF THE IMMUNE SYSTEM AS A NOVEL THERAPY OF ASD

Because immune dysregulation has clearly been shown to be associated with ASD, the idea of restoring a normal state of the immune system as a way of therapy has been tested. In

the MIA-ASD mouse model, treatment of *B. fragilis* present at high levels in the gut of these mice, not only corrected the changes in gut permeability and cytokine profiles but also improved ASD-associated symptoms.<sup>105</sup> With a more drastic approach, experiments were conducted in two murine ASD models (the MIA model and *Mecp2*<sup>-y</sup> model of Rett syndrome), where the whole immune system of the ASD animals were reconstituted via bone marrow transplantation.<sup>106</sup> The outcome is striking, after transplantation of the immunologically normal bone marrow, ASD-associated symptoms such as the repetitive and anxiety like behaviors were all corrected in these mice.<sup>106</sup> The studies thus support the idea of treating ASD patients by restoring their immune system. On the basis of this idea and data from the animal models, clinical trials of reconstituting a normal immune cell population in ASD patients are now underway.<sup>107,108</sup>

On the other hand, another approach aimed to suppress the inflammatory state in ASD patients has also been tested. Minocycline is an antibiotic but with immune suppressive effects., in *Fmr1*-knockout mice, administration of minocycline corrects synaptic abnormalities, anxiety and social abnormalities.<sup>109,110</sup> Similarly in children with the fragile X syndrome, treatment with minocycline improved ASD-like symptoms.<sup>111</sup> To some extent, antibiotic treatment seems to also improve symptoms in some forms of ASD.<sup>112-114</sup> Despite these studies, the exact mechanism through which minocycline renders its function is still waiting for more investigation.

## CONCLUSIONS

A large collection of studies has shown that the immune system facilitates the development of a normal nervous system whereas its alteration would affect profoundly neurodevelopment as well as cognition and behavior. In ASD, accumulating evidence indicates the presence of an aberrant immune system in both patients and animal models that includes changes in cellular, humoral immune responses and cytokine production. These findings combined with the fact that restoring a normal immune system provides improvement of ASD symptoms demonstrate clearly an important role of immune dysregulation in the pathogenesis of ASD.

ASD is a heterogeneous group of disorders. Various types of stimuli or assaults are implicated in causing the defects observed in ASD. But there are commonalities in manifestation shared by the spectrum of disorders, especially in the altered behavior. Given the importance of immune system in neural function, it is plausible to think that immune dysregulation is the converging point, where a diversity of factors could contribute to similar symptoms.

On the other hand, cytokine profiling and phenotypical analyses of effector immune cells in ASD patients have revealed a proinflammatory state and restoring normal immune cells via bone marrow transplantation or immune suppression have provided some promising therapeutic

effects. However, the exact mechanisms underlying these effects are still unclear. Thus, a better characterization of the immune dysfunction in ASD and mechanistic studies of immune function and neurodevelopment and function warrants further research.

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## CONFLICT OF INTEREST

None.

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