Crosstalk Between the Microbiome and Gestational Immunity in Autism-Related Disorders

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The etiologies of most neurodevelopmental disorders, including autism spectrum disorder (ASD), remain incompletely understood. However, recent epidemiological and experimental data suggest that dysregulated maternal immune activation (MIA) can impede normal brain maturation and promote the development of autism-related phenotypes. Indeed, our studies and work by others demonstrate that offspring born to pregnant animals that were exposed to immune activators develop many of the defining behavioral features of ASD, including abnormalities in social preference, communicative impairments, and repetitive/stereotyped behaviors. Although mounting evidence implicates key roles for hyperactive gestational inflammatory responses in neurodevelopmental disorders, the specific immune pathways that provoke autism-related phenotypes remain poorly described. The microbiome is recognized as a key modulator of immune responses, and emerging studies suggest that microbiota composition is a pivotal regulator of central nervous system function and disease. There has been growing speculation that changes in human microflora diversity contribute at some level to the recent rise in autism incidence. This has largely stemmed from reports of dysbiosis and gastrointestinal inflammation in autistic individuals. Given these clinical findings and the well-described role of the microbiome in calibrating the immune system, our group and others have recently become interested in investigating how changes in microbiota landscape influence neurodevelopmental disorder pathogenesis. In this review, we highlight emerging data describing roles for microbiota in the development of autism-related behavioral abnormalities. These recent findings identify the immune system as a link between gut microbiota and the brain in neurodevelopmental disorders, and suggest that targeting the microbiome and maternal immune responses during gestation may offer strategies to limit autism development in at-risk pregnancies.

Keywords: maternal immune activation, autism, neurodevelopmental disorders, microbiota, dysbiosis, IL-17a

Introduction

THE INCIDENCE OF AUTISM spectrum disorder (ASD) has increased at unprecedented rates over the past two decades and it is now estimated that 1 in every 59 children in the United States will develop autism (Baio *et al.*, 2018). ASD is a highly complex disorder with cases varying in symptoms and phenotypic severity, although most cases generally are marked by impaired social ability, verbal and nonverbal communicative deficits, and repetitive/stereotyped behaviors. The etiology of ASD currently remains unknown; however, emerging clinical and experimental data have increasingly linked dysregulated gestational and perinatal immune responses with ASD (Atladdottir *et al.*, 2010; Ashwood *et al.*, 2011; Garay *et al.*, 2013; Knuesel *et al.*, 2014; Estes and McAllister, 2015, 2016). In particular, pregnant mothers who suffer from infectious disease, inflammatory disorders, or autoimmune conditions are at a

significantly higher risk of having children with ASD (Atladdottir *et al.*, 2009; Braunschweig and Van de Water, 2012; Krakowiak *et al.*, 2012; Brimberg *et al.*, 2013; Bilbo *et al.*, 2018) (Fig. 1). Moreover, patients with autism frequently present with elevated plasma levels of proinflammatory cytokines, which often correlates with ASD symptom severity (Ashwood *et al.*, 2011; Jones *et al.*, 2017).

Animal models of maternal immune activation (MIA) have provided some of the strongest support for maternal immune dysregulation as a contributor to neurodevelopmental disorders (Smith *et al.*, 2007; Malkova *et al.*, 2012; Weber-Stadlbauer *et al.*, 2017; Brown and Meyer, 2018). Indeed, our laboratory and others have shown that autism-related phenotypes can be induced by treating pregnant mice with the viral mimetic polyinosinic-polycytidylic acid (PolyI:C) between embryonic days 11.5 (E11.5) and E12.5 of development (Shi *et al.*, 2003; Smith *et al.*, 2007; Malkova *et al.*, 2012; Hsiao *et al.*, 2013; Lammert *et al.*, 2018). Importantly, mice that mature in this

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FIG. 1. Dysregulated maternal immune responses can lead to impaired neurodevelopment and autism-related disorders. Immune-mediated inflammation in pregnant mothers can promote the acquisition of autistic-like behaviors in offspring. Autism-provoking inflammation in the pregnant mother can result from infection, autoimmune disease, obesity-induced inflammation, and disruptions in microbiome homeostasis (dysbiosis). The release of inflammatory mediators by the maternal immune system perturbs proper neurodevelopment and promotes the development of autistic-like behaviors.

inflammatory maternal environment develop many of the core symptoms used to diagnose ASD. Namely, PolyI:C treatment during pregnancy causes communicative deficits, abnormalities in social preference, and repetitive/stereotyped behaviors in MIA offspring (Shi *et al.*, 2003; Smith *et al.*, 2007; Malkova *et al.*, 2012; Hsiao *et al.*, 2013).

A significant subset of patients with ASD also suffer from gastrointestinal (GI) comorbidities, including constipation, diarrhea, gastroesophageal reflux disease, increased gut permeability (also called “leaky gut”), GI inflammatory disease, and gut dysbiosis (Parracho *et al.*, 2005; Buie *et al.*, 2010; de Magistris *et al.*, 2010; Finegold *et al.*, 2010; Adams *et al.*, 2011). Growing interest in the microbiome has revealed an axis of interplay between the gut, microbiota, and the brain, showing gut microflora to be consequential in brain development, central nervous system (CNS) disease progression, and behavior (Hsiao *et al.*, 2013; Fung *et al.*, 2017).

Reports of ASD patients with abnormal gut microflora compositions (Finegold *et al.*, 2010; Kang *et al.*, 2013; Tomova *et al.*, 2015) have led to growing speculation about a role for gut dysbiosis in the pathogenesis of ASD-related GI dysfunction, as well as the abnormal neurodevelopment seen in ASD. Importantly, an ever-increasing body of work

in rodent models is beginning to show immune modulation as a key component of microbiome-mediated influence on brain and behavior (Kim *et al.*, 2017; Lammert *et al.*, 2018). This suggests that a gut–immune–brain axis of interactions may be contributing to pathogenesis and progression of a subset of ASD cases, and could provide further insight into how gut microflora can be therapeutically targeted in ASD.

The MIA model of autism

ASD is a heterogeneous neurodevelopmental disorder and it is likely that a complex combination of genetic, environmental, and immune factors ultimately contributes to ASD etiology. It can, therefore, be difficult to model all aspects of ASD pathophysiology in animal models. However, the PolyI:C MIA rodent model has emerged as a well-accepted model to study autism given that it recapitulates many of the behavioral and cortical abnormalities frequently observed in ASD patients (Shi *et al.*, 2003; Smith *et al.*, 2007; Malkova *et al.*, 2012; Hsiao *et al.*, 2013; Lammert *et al.*, 2018).

In this model, synthetic double-stranded RNA in the form of PolyI:C is administered to pregnant mice to evoke maternal immune responses consistent with viral infection. Injection is typically administered between embryonic day (E) 11.5 and 12.5, resembling the late first trimester of human pregnancy during which maternal viral infection is a noted risk factor for offspring developing ASD (Atladdottir *et al.*, 2010). Offspring from pregnant mothers injected with PolyI:C typically present with core symptoms analogous to those of ASD, including communicative deficits, social impairment, and repetitive/stereotyped behaviors (Malkova *et al.*, 2012). This MIA model has recently been employed by our group and others to further investigate how crosstalk between the gut–immune–brain axis contributes to ASD-associated behavioral abnormalities and altered CNS maturation.

Microbiome–immune crosstalk at the interface of neurodevelopmental health and disease

In recent work from Hsiao *et al.*, it was shown that offspring of MIA mothers display concurrent increases in intestinal permeability in addition to ASD-related behavioral abnormalities (Hsiao *et al.*, 2013). Not only was the increase in intestinal permeability ameliorated by probiotic treatment with the human commensal microbe, *Bacteroides fragilis*, but Hsiao *et al.* also showed improvement in hallmark behavioral symptoms of the MIA model (Hsiao *et al.*, 2013). Moreover, they reported that *Bacteroides fragilis* probiotic treatment of pregnant MIA mothers leads to downregulation of the proinflammatory cytokine interleukin-6 (IL-6) in the colon (Hsiao *et al.*, 2013). Interestingly, IL-6 has previously been implicated as a requisite component of the maternal immune response driving behavioral and neurodevelopmental abnormalities in the MIA rodent model (Smith *et al.*, 2007; Hsiao and Patterson, 2011; Wu *et al.*, 2017), suggesting that crosstalk between gut microbiota and the immune system may potentially be influential in promoting the neurodevelopmental disorders seen in the MIA model.

More recent studies have explored interactions across the gut–immune–brain axis downstream of IL-6. IL-17a, an inflammatory cytokine generated downstream of IL-6, has also recently been identified to be a critical instigator of behavioral and cortical abnormalities in the MIA model

(Choi *et al.*, 2016; Shin Yim *et al.*, 2017). Interestingly, findings from our group and others indicate that maternal immune responses along this pathway provoke MIA-associated behavioral abnormalities, and are linked to specific bacterial species in the gut microbiome (Hsiao *et al.*, 2013; Kim *et al.*, 2017; Lammert *et al.*, 2018) (Fig. 2). In a recent article by Kim *et al.*, the MIA model was used to explore the mechanisms underpinning MIA-triggered increases in systemic IL-17a production (Kim *et al.*, 2017). The authors found that offspring of pregnant PolyI:C-injected mothers treated with vancomycin—a broad-spectrum antibiotic—did not develop cortical lesions or core behavioral abnormalities typically seen in the MIA model. Moreover, treatment of PolyI:C-injected mothers with vancomycin led to lower levels of circulating maternal IL-17a.

This suggests that vancomycin-sensitive gut microflora are required for the induction of IL-17a production by the maternal immune system, as well as, the development of MIA-associated behavioral abnormalities in the offspring.

Because segmented filamentous bacteria (SFB), a commensal bacteria found in some laboratory mice, are well-known inducers of IL-17a-mediated immune responses (Ivanov *et al.*, 2009; Sivan *et al.*, 2015) and are also susceptible to vancomycin, the authors then interrogated a role for SFB in the development of autism-related disorders in the MIA model. To this end, they exploited differences in gut microbiota of laboratory mice originating from The Jackson Laboratory and Taconic Biosciences (Ivanov *et al.*, 2009; Sivan *et al.*, 2015). Jackson mice have been shown to lack SFB colonies, whereas Taconic mice contain SFB among their gut microflora (Ivanov

FIG. 2. Microbiota-mediated calibration of maternal IL-17a responses affects neurodevelopmental disorder susceptibility in the MIA model of autism. **(A)** Pregnant animals that harbor segmented filamentous bacteria (SFB) or other commensals capable of promoting robust IL-17a responses produce higher levels of IL-17a in response to MIA induction with the viral analog PolyI:C, and offspring from these mothers develop autism-related behavioral abnormalities. Previous studies have shown that IL-6 is critical in this process and contributes to the activation of IL-17a-secreting immune cells. **(B)** In contrast, MIA offspring from mothers possessing microflora communities that are poor inducers of systemic IL-17a responses do not exhibit abnormalities in autism-related behaviors or cortical development. MIA, maternal immune activation; PolyI:C, polyinosinic-polycytidylic acid; IL, interleukin.

et al., 2009; Sivan *et al.*, 2015). Notably, Taconic mice are also known to have significantly higher numbers of intestinal IL-17a producing CD4⁺ T cells (also referred to as T_H17 cells) capable of driving the IL-17a responses required to promote the development of ASD-associated phenotypes in MIA mice (Ivanov *et al.*, 2009; Sivan *et al.*, 2015). Indeed, the authors demonstrated that offspring of PolyI:C-injected Jackson mothers lacking SFB colonization did not develop behavioral abnormalities typically seen in the MIA model, whereas Taconic mothers with SFB colonization did. Furthermore, Jackson mothers whose intestinal tracts were colonized with SFB through oral gavage showed an increase in intestinal T_H17 cells, and offspring from these MIA mothers developed ASD-related behavioral abnormalities consistent with MIA.

Our group has also obtained similar findings as Kim *et al.* on the role of the maternal microbiome and its regulation of neurodevelopmental disease progression in the MIA model of autism (Lammert *et al.*, 2018). In these recently published studies, we also leveraged differences in gut microbiota between Jackson and Taconic mice to explore how maternal microbiota landscape influences gestational IL-17a immune responses and the subsequent development of ASD-like phenotypes. In agreement with Kim *et al.*, we showed that microbiota transplantation can transfer autism susceptibility and that this is mediated by microbiota-dependent calibration of maternal IL-17a inflammatory responses.

More specifically, we found that offspring of Taconic PolyI:C-injected mothers with SFB displayed MIA-associated behavioral abnormalities. Likewise, we also observed that offspring of Jackson mothers were protected in the MIA model. Interestingly, transfer of the Taconic microbiome to Jackson mothers through a 2-week cohousing period negated this protective effect, as transfer of SFB to Jackson mothers before PolyI:C injection was sufficient to promote elevated maternal IL-17a responses, as well as ASD-associated behavioral abnormalities in MIA offspring. Furthermore, we showed that IL-17a neutralization in SFB-positive MIA mothers prevents the development of ASD-associated phenotypes in the offspring.

Collectively, these studies suggest that environmental factors that alter microbiome composition may contribute to increased risk of autism through modulation of the maternal immune response. Importantly, these results may help to provide mechanistic explanation for the growing speculation that environmental factors play key roles in autism pathogenesis.

Conclusions

Mounting evidence demonstrates that ASD is often associated with immune dysfunction, GI inflammation, and gut dysbiosis, and recent findings in rodent models suggest that these factors may contribute to the abnormal neurodevelopment in patients with ASD. Furthermore, subsets of patients with autism present with elevated levels of both IL-6 and IL-17a (Ashwood *et al.*, 2011; Al-Ayadhi and Mostafa, 2012), perhaps alluding to the clinical relevance of this pathway in human ASD. As highlighted in this study, gut microbiota can shape the gestational inflammatory responses thought to promote some forms of neurodevelopmental disease.

To date, there has been little clinical research conducted to explore the therapeutic potential of probiotics in autism,

and the few studies that do exist have primarily focused on alleviating GI dysfunction in this patient population (Patusco and Ziegler, 2018). Additional research is needed to more fully define the mechanisms through which the gut-immune-brain axis contributes to the pathogenesis of ASD. In so doing, significant progress can be made in alleviating the broad range of neurodevelopmental, GI, and immune symptoms associated with autism.

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Authors' Contributions

M.J.P. wrote the article, critically reviewed the text, and edited the article. A.C.B. participated in writing and editing, and created the artwork presented in Figure 2. J.R.L. designed the article, participated in writing and proofreading, and oversaw the entire process of article preparation.

Disclosure Statement

No competing financial interests exist.

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