Brain, Behavior, and Immunity 63 (2017) 99–107

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Special Issue on Perinatal Inflammation

Behavioral impact of maternal allergic-asthma in two genetically distinct mouse strains

Jared J. Schwartzer [a,*], Milo Careaga [b,c], Morgan A. Coburn [a], Destanie R. Rose [b,c], Heather K. Hughes [b,c], Paul Ashwood [b,c,*]

[a] Program in Neuroscience and Behavior, Department of Psychology and Education, Mount Holyoke College, 50 College Street, South Hadley, MA 01075, United States
[b] The M.I.N.D. Institute, University of California, Davis, 2825 50th Street, Sacramento, CA 95817, United States
[c] Department of Medical Microbiology and Immunology, University of California, Davis, One Shields Avenue, Davis, CA 95616, United States

ARTICLE INFO

Article history:
Received 28 July 2016
Received in revised form 8 September 2016
Accepted 9 September 2016
Available online 10 September 2016

Keywords:
Allergies
Asthma
Animal model
Autism
ASD
Translational
Maternal immune activation

ABSTRACT

Recent population-based studies of expecting mothers identified a unique profile of immune markers that are associated with an increased risk of having a child diagnosed with autism spectrum disorder (ASD). This immune profile, including increased levels of maternal and placental interleukin (IL)-4 and IL-5, is consistent with an immune response found in an allergic-asthma phenotype. Allergies and asthma reflect an imbalance in immune responses including polarization towards T-helper type 2 (T_H2) responses, with both genetic susceptibility and environmental factors affecting this T-cell polarization. Mouse strains provide a known and controlled source of genetic diversity to explore the role of genetic predisposition on environmental factors. In particular, the FVB background exhibits a skew towards T_H2-mediated allergic-asthma response in traditional models of asthma whereas the C57 strain exhibits a more blunted T_H2 polarized phenotype resulting in an attenuated allergic-asthma response. C57BL/6J (C57) and the sighted FVB.129P2-Pde6b(+) Tyr(c-ch)/Ant (FVB/Ant) lines were selected based on their characteristic high sociability and differing sensitivity to T_H2-mediated stimuli. Based on the distinct allergy-sensitive immune responses of these two strains, we hypothesized that unique developmental consequences would occur in offspring following maternal allergy-asthma exposure. Female C57 and FVB/Ant dams were primed/sensitized with an exposure to ovalbumin (OVA) before pregnancy, then exposed to either aerosolized OVA or PBS-vehicle throughout gestation. Sera from pregnant dams were analyzed for changes in cytokine profiles using multiplex-arrays and offspring were assessed for changes in autism-like behavioral responses. Analysis of maternal sera revealed elevated IL-4 and IL-5 in OVA-treated dams of both strains but only C57 mice expressed increased levels of IL-1β, IL-6, TNFα, and IL-17. Behavioral assessments revealed strain-dependent changes in juvenile reciprocal social interaction in offspring of maternal allergic asthma dams. Moreover, mice of both strains showed decreased repetitive grooming and increased marble burying behavior when born to OVA-exposed dams. Together, these findings support the important role genetic predisposition plays in the effects of maternal immune activation and underscore differences in ASD-like behavioral outcomes across mouse strains.

1. Introduction

Autism spectrum disorders (ASD) are a phenotypically diverse class of neurodevelopmental disorders characterized by social-communication deficits and restrictive/repetitive behaviors (Association, 2013). Twin studies indicate that ASD is highly heritable (Hallmayer et al., 2011) and research suggests that familial history of autoimmune diseases like rheumatoid arthritis, celiac disease, and type-1 diabetes increase the risk for an ASD diagnosis (Atladottir et al., 2009). However, the hundreds of genes linked to predisposition and the wide variation in the genomic pattern from individual to individual has made it difficult to identify a distinct genetic marker (Vijayakumar and Judy, 2016). Consequently, research is looking beyond exclusively genetic origins of ASD and considering environmental influences, particularly the connection to immune system perturbations. Epidemiological studies have

* Corresponding authors at: Program in Neuroscience and Behavior, Department of Psychology and Education, Mount Holyoke College, United States (J.J. Schwartzer), Department of Medical Microbiology and Immunology, University of California, Davis, United States (P. Ashwood).

E-mail addresses: jjschwar@mtholyoke.edu (J.J. Schwartzer), pashwood@ucdavis.edu (P. Ashwood).

http://dx.doi.org/10.1016/j.bbi.2016.09.007
0889-1591/

found that gestational exposure to air pollutants associated with freeways can increase the risk for an ASD diagnoses in offspring (Raz et al., 2015; Volk et al., 2011), and maternal inflammation, most notably by viral or bacterial pathogen, during gestation can also affect neurodevelopment (Jiang et al., 2016).

Most models of immune system dysfunction and ASD evaluate the effects of an immune responses using polyinosinic:polycytidylic acid (Poly[I:C]) to mimic the double stranded RNA in viruses or lipopolysaccharide (LPS), a bacterial membrane protein, to activate pathways of innate immunity. A less studied pathway of maternal immune activation postulates that allergic asthma and T-Helper 2 (T_H2) immune responses may contribute to an ASD diagnosis in offspring. Epidemiological studies have shown that mothers with allergies or asthma during pregnancy were at increased risk for having a child with ASD (Croen et al., 2005; Lyall et al., 2014). Furthermore, a case-control study conducted by Goines and colleagues revealed elevated gestational levels of interleukin (IL)-4 and IL-5 in mothers who had children with autism, describing a 50% increased risk of ASD diagnosis in offspring (Goines et al., 2011). These cytokines are consistent with the profiles associated with allergy/asthma immune responses, highlighting the correlations between maternal T_H2 immune responses and increased incidence of ASD in the child. Interestingly, elevated levels of IL-4 have been detected in the amniotic fluid of children who were later diagnosed with ASD (Abdallah et al., 2013). More recently, an analysis of cytokine levels in newborn blood spots linked elevations in IL-4 at birth with more severe ASD symptoms and cognitive deficits (Krakowiak et al., 2015). Together, these findings implicate T_H2-associated maternal-fetal signaling as a mediating factor in the etiology of ASD and underscore the need to investigate causal links between maternal allergic-asthma and ASD-like behavioral outcomes.

Mouse models afford researchers a powerful tool to directly evaluate the causative effects of maternal immune activation. Though ASD is a strictly human disorder, several indicative behaviors like repetitive stereotypies or social avoidance can be modeled in mice (Silverman et al., 2010). One novel model precipitates ASD-like behavioral deficits in offspring exposed to maternal allergic asthma (MAA) (Schwartzer et al., 2015); this model sensitizes female mice to ovalbumin (OVA) protein followed by induction of airway hypersensitivity through aerosolized OVA exposure at gestational days (G)9.5, 12.5, and 17.5 to mimic early, middle, and late gestation in humans. Importantly, variations in genetic background between mouse strains result in varying severity of immune responses to allergic asthma induction. For example, the FVB/NJ strain displays heightened T_H2-responses compared to C57Bl/6J (C57) mice in OVA-induced allergy models (Zhu and Gilmour, 2009). Additionally, all FVB lines carry the Fv-1^b allele (van Wyk et al., 2015), leading to increased histamine sensitivity and a significantly greater airway hyperreactivity in OVA-induced asthma (Whitehead et al., 2003). These phenotypic differences in immune responses predispose the FVB line to heightened immune activation. However, it remains unknown whether this heightened immune response in FVB strains will result in greater behavioral deficits in offspring following MAA.

To this end, we explored whether genetic predisposition in FVB strains results in greater offspring behavioral deficits following MAA exposure. Two highly social strains of mice, C57 and a sighted hybrid FVB.129P2-Pde6b(+) Tyr(c-ch)/Ant (FVB/Ant), were selected for their distinct genetic backgrounds and immune profiles. C57 and FVB lines are widely studied inbred strains and known to display typical social and repetitive behaviors (Moy et al., 2007). FVB/Ant mice are a sighted hybrid of the FVB/NJ, characterized by high sociability and low self-grooming, comparable to the C57 strain, and this strain has previously been used as a highly social control strain (Yang et al., 2013). Offspring of OVA- and PBS-treated dams were assessed for changes in sociability at a juvenile age using the reciprocal social interaction task. This behavioral paradigm has the benefit of measuring complex and intricate social behaviors, providing new insights into underlying features of social interaction. While this task often requires a trained observer, demanding significant human resources and increased sensitivity to human error (Silverman et al., 2010), we employed automated tracking technology to produce unbiased measures of juvenile play behavior in offspring. Then, mice were measured for changes in restricted/repetitive behaviors using the self-grooming and marble burying tasks. In addition, differences in cytokine responses were assessed in pregnant dams using multiplex bead-based arrays.

2. Materials and methods

2.1. Animals

Male and Female C57Bl/67 (Jackson Laboratory, Bar Harbor, Maine USA & Sacramento, CA USA) and FVB/Ant (Jackson Laboratory, Bar Harbor, Maine USA) mice were bred and maintained at Mount Holyoke College and the Center for Laboratory Animal Research, at the University of California, Davis. Mice were maintained at ambient room temperature on a 12 h light/dark cycle (lights on at 0800 h). Each strain was housed separately in standard plastic cages with same-sex littermates, and food and water were provided *ad libitum*. Mice were group-housed 2–4 per cage and pregnant female mice were single housed following the start of pregnancy. All mice were provided nestlets for enrichment. Behavioral procedures were performed during the first 4 h of the light cycle and all procedures were approved by Mount Holyoke College, Institutional Animal Care and Use Committee and the University of California, Davis Institutional Animal Care and Use Committee in accordance with the guidelines provided by the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Maternal allergy/asthma induction

Sexually naïve female C57 and FVB/Ant mice were randomly assigned to either the allergic asthma (OVA) or the control group (PBS) and sensitized with 10 μg ovalbumin (OVA, Sigma, St Louis, MO, USA) in 1 mg (Al)OH3 (InvivoGen, San Diego, CA, USA) dissolved in 200 μl phosphate-buffered saline (PBS) or vehicle alone on postnatal day (P)42 and again 1 week later at P49. One week following the second sensitization period, mice were mated overnight and females were checked daily for the presence of seminal plugs, noted as gestational day 0.5 (G0.5). Pregnant mice were exposed to either an aerosolized solution of 1% (wt/vl) OVA in PBS (OVA group) or PBS control for three 45-min induction sessions throughout gestation. Specifically, these induction sessions occurred at gestational days 9.5, 12.5, and 17.5, to correspond with early, middle, and late gestation as previously described (Schwartzer et al., 2015). Four hours after the final induction, 100 μl of blood was drawn and collected from the saphenous vein for cytokine analysis and mice were returned to their home cages, single housed, and left undisturbed until the birth of their litters. Pups remained with their mother until weaning on P21, at which time the offspring were group housed with same-sex littermates. A total of 34 C57 dams (17 PBS and 17 OVA) were used to generate 152 offspring (67 PBS, 85 OVA) and a total of 22 FVB/Ant dams (10 PBS and 12 OVA) were used to generate 63 offspring (25 PBS, 38 OVA) (Table 1).