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Maternal immune activation leads to activated inflammatory macrophages in offspring

Charity E. Onore [a,b], Jared J. Schwartzer [b,c,e], Milo Careaga [a,b], Robert F. Berman [b,c,d], Paul Ashwood [a,b,*]

[a] Department of Medical Microbiology and Immunology, University of California, Davis, One Shields Avenue, Davis, CA 95616, United States
[b] The M.I.N.D. Institute, University of California, Davis, One Shields Avenue, Davis, CA 95616, United States
[c] Department of Psychiatry and Behavioral Sciences, University of California, Davis, One Shields Avenue, Davis, CA 95616, United States
[d] Department of Neurological Surgery, University of California, Davis, One Shields Avenue, Davis, CA 95616, United States
[e] Department of Psychology and Education, Mount Holyoke College, 50 College Street, South Hadley, MA 01075, United States

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ABSTRACT

Several epidemiological studies have shown an association between infection or inflammation during pregnancy and increased risk of autism in the child. In addition, animal models have illustrated that maternal inflammation during gestation can cause autism-relevant behaviors in the offspring; so called maternal immune activation (MIA) models. More recently, permanent changes in T cell cytokine responses were reported in children with autism and in offspring of MIA mice; however, the cytokine responses of other immune cell populations have not been thoroughly investigated in these MIA models. Similar to changes in T cell function, we hypothesized that following MIA, offspring will have long-term changes in macrophage function. To test this theory, we utilized the poly (I:C) MIA mouse model in C57BL/6J mice and examined macrophage cytokine production in adult offspring. Pregnant dams were given either a single injection of 20 mg/kg polyinosinic–polycytidylic acid, poly (I:C), or saline delivered intraperitoneally on gestational day 12.5. When offspring of poly (I:C) treated dams reached 10 weeks of age, femurs were collected and bone marrow-derived macrophages were generated. Cytokine production was measured in bone marrow-derived macrophages incubated for 24 h in either growth media alone, LPS, IL-4/LPS, or IFN-γ/LPS. Following stimulation with LPS alone, or the combination of IFN-γ/LPS, macrophages from offspring of poly (I:C) treated dams produced higher levels of IL-12(p40) ($p < 0.04$) suggesting an increased M1 polarization. In addition, even without the presence of a polarizing cytokine or LPS stimulus, macrophages from offspring of poly (I:C) treated dams exhibited a higher production of CCL3 ($p = 0.05$). Moreover, CCL3 levels were further increased when stimulated with LPS, or polarized with either IL-4/LPS or IFN-γ/LPS ($p < 0.05$) suggesting a general increase in production of this chemokine. Collectively, these data suggest that MIA can produce lasting changes in macrophage function that are sustained into adulthood.

1. Introduction

There is increasing evidence suggesting that an immune insult during gestation can have a profound effect on the developing fetus (Brown, 2012). For over 30 years, epidemiological research has continued to find associations between maternal infection and increased risk of autism (Atladottir et al., 2010; Chess, 1971, 1977; Mednick et al., 1988). A recent large case-control population based study revealed an increased risk of developing autism spectrum disorder (ASD) with maternal fever, which was attenuated if pregnant mothers used a fever reducing agent (Zerbo et al., 2013). In addition, reports highlight associations between risk of having a child with autism and increased levels of inflammatory mediators in both the maternal sera and amniotic fluid. These increased inflammatory markers, including macrophage chemotactic protein (MCP)-1/CCL2, matrix metalloproteinase (MMP)-9, C-reactive protein (CRP), interleukins (IL)-4, IL-5, and interferon (IFN)-γ (Abdallah et al., 2012a,b; Brown et al., 2013; Goines et al., 2011), supporting a relationship between maternal immune activation (MIA), aberrant fetal neurodevelopment, and risk for neurodevelopmental disorders such as autism.

Murine models add further support for a role of MIA in altering fetal neurodevelopment (Patterson, 2009; Patterson et al., 2009). Many of these studies utilize either a maternal influenza infection

* Corresponding author at: The M.I.N.D. Institute, 2825 50th Street, Sacramento, CA 95817, United States. Tel.: +1 916 703 0405; fax: +1 916 703 0367.
E-mail address: pashwood@ucdavis.edu (P. Ashwood).

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or polyinosinic–polycytidylic acid [poly(I:C)] a synthetic toll-like receptor (TLR)-3 agonist that mimics viral infection. Maternal influenza infection or poly (I:C) induced MIA in pregnant dams produces a number of physiological changes in resultant offspring, including changes in brain morphology, decreased reelin, astrogliosis, fewer purkinje cells (PC), the presence of heterotopic PCs, and delayed migration of cerebellar granule cells (Fatemi et al., 2002a,b, 1999; Shi et al., 2009). In addition to these neurobiological effects of MIA, there are a number of behavioral alterations observed in offspring that parallel many features of autism and schizophrenia, including reduced exploratory behavior, and impaired social interaction (Malkova et al., 2012; Schwartzer et al., 2013; Shi et al., 2003).

There is a high incidence of aberrant immune responses commonly reported in individuals with autism (Onore et al., 2012), including increased monocyte numbers (Sweeten et al., 2003). Plasma cytokine profiles also suggest an increase in monocyte derived cytokine and chemokine activation (Ashwood et al., 2011a,b). Moreover, there is evidence of differential TLR signaling in autism, including increased inflammatory cytokine production following TLR4 stimulation with lipopolysaccharide (LPS) (Enstrom et al., 2010; Jyonouchi et al., 2008), further suggesting aberrant myeloid function in this disorder. Similar to immune dysfunction in human subjects, recent data in mice suggest there are long-term alterations in immune function as a result of MIA with increased numbers of CD11b+ cells in MIA offspring (Hsiao et al., 2012). Increased CD11b+ cells may implicate atypical myeloid cell activity; however, little has previously been reported regarding specific macrophage responses in MIA offspring.

Macrophage phenotypes are generally divided into major subsets characterized as M1 and M2 (Mantovani et al., 2005; Martinez et al., 2008). M1 macrophages, also known as classically activated macrophages, are associated with bacterial or viral infection (Benoit et al., 2008). Polarization to an M1 phenotype is induced by exposure to IFN-γ and results in high expression of the natural killer cell and T_H1 cell activating cytokine IL-12 as well as very low levels of the anti-inflammatory cytokine IL-10. In contrast, M2 macrophages, also referred to as alternatively activated macrophages, are associated with defense against helminth infections and wound healing (Kreider et al., 2007; Rodero and Khosrotehrani, 2010). Macrophages are polarized to an M2 phenotype in the presence of IL-4 or IL-13 and generally produce high levels of IL-10, very low levels of IL-12, and generally lower levels of inflammatory cytokines compared with M1 macrophages (Zhang et al., 2008).

Growing evidence suggests that manipulating macrophage phenotype can profoundly affect normal neurodevelopment and cognitive function in animal models (Derecki et al., 2013). For example, increased M1 polarization has been implicated in several neurological diseases including multiple sclerosis and Alzheimer's disease (Gate et al., 2010; Mikita et al., 2011), and promoting an M2 phenotype may be beneficial to cognitive function (Derecki et al., 2010a,b, 2011). Together, these data suggest that M1 macrophage polarization may have detrimental effects on normal brain development and function. Given the association between M1 polarization and neurological dysfunction and the mounting research linking MIA with altered brain and behavior function, we hypothesized that macrophages from mice born to MIA dams would be preferentially skewed towards the M1 phenotype and exhibit a pro-inflammatory cytokine profile.

To investigate the potential long term effects of MIA in offspring macrophage polarization, we utilized the poly(I:C) model of MIA in C57BL/6J mice and tested macrophage responses *in vitro*. Bone marrow-derived macrophages were obtained from offspring exposed to poly (I:C) [poly (I:C) group here-in] or to saline control (referred to as saline group here-in) *in utero*. To test for specific effects on macrophage function, we measured macrophage cytokine profiles in response to TLR4 activation with and without the presence of polarizing cytokines IFN-γ (M1) and IL-4 (M2). In this study we describe a long-term effect in macrophage polarization in murine MIA offspring.

2. Methods

2.1. Mice

C57Bl/6J (C57) (Jackson Laboratory, Sacramento, CA) mice were maintained by the Campus Laboratory Animal Services, at University of California, Davis at ambient room temperature on a 12 h light/dark cycle with food and water available *ad libitum*. All procedures were performed with approval by the University of California, Davis Institutional Animal Care and Use Committee and in accordance with the guidelines provided by the National Institutes of Health for the ethical treatment of animals.

2.2. Maternal immune activation

Mice were mated overnight and females were checked daily for the presence of seminal plugs, noted as gestational day 0.5 (G0.5). MIA was induced as previously described (Schwartzer et al., 2013). Briefly, on gestational day 12.5, pregnant female mice were weighed and injected with a single dose (20 mg/kg body weight, intraperitoneally) of poly (I:C) (Sigma Aldrich; St. Louis, MO) or saline solution. Standard procedure for intraperitoneal injections included aspirating after needle insertion, and prior to injection, to verify needle placement in the intraperitoneal space and no entry into any of the surrounding organs. Litters receiving either saline or poly(I:C) injections appeared to be healthy, and did not display any outward signs of damage due to injection. Dams were returned to their cage and remained undisturbed until parturition. Pups remained with the mother until weaning on postnatal day (PND) 21, at which time mice were group housed 2–4 mice per cage with same-sex littermates. For all behavioral and biological assays, one male and one female from each litter were used to reduce the likelihood of litter effects. A total of 40 mice were tested [poly(I:C) group, $n = 22$; saline group, $n = 18$].

2.3. Three chamber social approach

On PND70, mice were assessed for social approach behavior as previously described (Schwartzer et al., 2013). One male and one female from each litter were habituated for 10-min in the center chamber with doors closed followed by an additional 10-min habituation to the entire apparatus. Habituation sessions were video recorded and analyzed to confirm a lack of innate side preference. Following habituation, experimental mice were returned to the center chamber and a novel 129/SvImJ mouse was placed under an inverted wire cup in one side chamber and an identical empty wire cup was placed on the other side. Offspring of poly(I:C) and vehicle-treated dams were then given a 10 min test period and measured for the time spent in the chamber with the novel mouse and novel object. A sociability score was calculated as the time in social chamber minus time in novel object chamber. All testing chambers were thoroughly cleaned with 70% ethanol in between each testing session.

2.4. Marble burying

Perseverative marble-burying behavior in mice is an analogous index to the restricted repetitive patterns of behavior observed in ASD (American Psychological Association, 2013; Silverman et al.,