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Long-term altered immune responses following fetal priming in a non-human primate model of maternal immune activation

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ABSTRACT

Infection during pregnancy can lead to activation of the maternal immune system and has been associated with an increased risk of having an offspring later diagnosed with a neurodevelopmental disorders (NDD) such as autism spectrum disorder (ASD) or schizophrenia (SZ). Most maternal immune activation (MIA) studies to date have been in rodents and usually involve the use of lipopolysaccharide (LPS) or polyinosinic:polycytidylic acid (poly I:C). However, since NDD are based on behavioral changes, a model of MIA in non-human primates could potentially provide data that helps illuminate complex behavioral and immune outputs in human NDD. In this study twenty-one pregnant rhesus macaques were either given three injections over 72 hours of poly I:C-LC, a double stranded RNA analog (viral mimic), or saline as a control. Injections were given near the end of the first trimester or near the end of the second trimester to determine if there were differences in immune output due to the timing of MIA. An additional three non-treated animals were used as controls. The offspring were followed until 4 years of age, with blood collected at the end of their first (year 1) and fourth (year 4) years to assess dynamic cellular immune function. Induced responses from peripheral immune cells were measured using multiplex assays. At one year of age, MIA exposed offspring displayed elevated production of innate inflammatory cytokines including: interleukin (IL)-1β, IL-6, IL-12p40, and tumor necrosis factor (TNF)α at baseline and following stimulation. At four years of age, the MIA exposed offspring continued to display elevated IL-1β, and there was also a pattern of an increased production of T-cell helper type (T_H)-2 cytokines, IL-4 and IL-13. Throughout this time period, the offspring of MIA treated dams exhibited altered behavioral phenotypes including increased stereotyped behaviors. During the first two years, stereotyped behaviors were associated with innate cytokine production. Self-directed behaviors were associated with T_H2 cytokine production at year 4. Data from this study suggests long-term behavioral and immune activation was present in offspring following MIA. This novel non-human primate model of MIA may provide a relevant clinically translational model to help further elucidate the role between immune dysfunction and complex behavioral outputs following MIA.

1. Introduction

The recent Zika virus outbreaks have fostered public concerns about the impact that infections during pregnancy can have on fetal development (Rasmussen et al. 2016). Research over the last several decades has found associations between prenatal infections with an increased risk for altered neurodevelopmental trajectories. For example, epidemiological studies focusing on maternal infections during gestation showed associations of increased risk for developing a neurodevelopmental disorders (NDD), such as autism spectrum disorders (ASD) in the child (Atladottir et al. 2010; Brown 2012) or schizophrenia (Brown 2012) later in life. Furthermore, these studies suggest that this phenomenon is not specific to any particular infectious agent, but instead, is driven by the maternal

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immune response (Atladottir et al. 2010). Translational animal models investigating maternal immune activation (MIA) have found that in the absence of an actual infectious agent, immune stimulation alone, either by bacterial or viral products, or specific cytokines trigger an active immune response in the pregnant dam that elicits abnormal behavior, including anxiety, impaired social and repetitive behaviors in the offspring (Shi et al. 2005; Smith et al. 2007).

Most MIA studies to date have been in rodents and involve the use of lipopolysaccharide (LPS) or polyinosinic:polycytidylic acid (poly I:C); although several models have also generated MIA with live influenza virus or by injection of inflammatory cytokine, interleukin (IL)-6 to produce similar outcomes (Shi et al. 2005; Smith et al. 2007; Meyer et al. 2009; Patterson 2009; Meyer and Feldon 2010). The major findings of these studies were changes in offspring behavior, altered brain development and immune dysfunction in the offspring (Meyer et al. 2006b; Patterson 2009; Shi et al. 2009; Ito et al. 2010; Garbett et al. 2012; Hsiao et al. 2012; Malkova et al. 2012; Garay et al. 2013; Meyer 2014). Behavioral changes have varied between studies but have included such phenomena as reduced or altered ultrasonic vocalizations, reduced sociability, increased repetitive behaviors loss of latent inhibition, reduced open field exploration, deficits in reversal learning and impairments in pre-pulse inhibition (Meyer et al. 2006a; Meyer et al. 2006b; Meyer et al. 2008; Han et al. 2011; Hsiao and Patterson 2011; Malkova et al. 2012; Schwartzer et al. 2013). In human studies, immune abnormities are often observed in individuals with NDD (Ashwood et al. 2008; Potvin et al. 2008; Muller and Schwarz 2010; Ashwood et al. 2011a; Ashwood et al. 2011c; Ashwood et al. 2011b; Onore et al. 2012; Di Nicola et al. 2013; McAllister 2014; Rose and Ashwood 2014; Careaga et al. 2015). Alterations in immune function have also been observed in rodent offspring of MIA treated dams, including increased production of IL-6 and IL-17 from mononuclear cells, increased granulocyte and monocyte populations, increased production of IL-12p40 and chemokine (C-C motif) ligand (CCL)-3 from macrophages, an increased T-helper (T_H)-17 cell skewing, and altered profiles of fetal, juvenile, and adult brain cytokine and chemokine levels (Meyer et al. 2006b; Meyer et al. 2008; Mandal et al. 2011; Hsiao et al. 2012; Garay et al. 2013; Mandal et al. 2013; Onore et al. 2014; Choi et al. 2016). While rodent models of MIA provide researchers with a useful initial model to begin to investigate the intricate pathways and interactions between the developing nervous and immune systems, the disparity between human and rodent social structures limits some of the translational aspects of this model in regard to certain complex human behaviors. Moreover, since NDD are, so far at least, based solely on behavioral criteria, non-human primates may be better suited to explore the complexities of behavioral driven disorders due to their closer relationship to humans (Watson and Platt 2012; Chang et al. 2013; Meyer 2014).

To date few studies have explored MIA in non-human primates (Short et al. 2010; Willette et al. 2011; Bauman et al. 2014; Machado et al. 2015; Weir et al. 2015). In one study, maternal influenza infection during early third trimester, led to offspring with smaller brain volume and reduced gray matter, particularly in the cingulate and parietal areas (Short et al. 2010). Prenatal LPS exposure during early third trimester led to increases in global white matter in the brains of the offspring and a trend for larger brain volume, which was accompanied by altered behaviors including reduced response to prepulse inhibition acoustic startle (Willette et al. 2011). However, we do not know of any study that has looked at immune responses in the MIA model in non-human primates.

In this study we sought to examine the effects of MIA on offspring immune activation in a non-human primate model. Previously we reported finding increased repetitive behaviors, motor stereotypies, decreased affiliative vocalizations, and abnormal social behaviors in offspring of non-human primate dams injected with poly I:C to induce MIA (Bauman et al. 2014); offspring also had abnormal gaze patterns when presented with various rhesus monkey facial expressions (Machado et al. 2015). In addition, we also reported findings of altered dendritic morphology of increased number of oblique dendrites and narrower apical dendritic diameter, in MIA offspring compared to saline controls. (Weir et al. 2015). In the present study, we examined plasma cytokine concentrations and dynamic induced cellular responses of peripheral immune cells from offspring of MIA treated dams during the first (year 1) and fourth (year 4) year of life and found elevated production of innate cytokines and chemokines at year 1, and a pattern of elevated T_H2 cytokines during year 4. Moreover, many of the measured cytokines correlated with the emergence of repetitive behaviors in MIA exposed offspring and may provide insight into observations of increased immune activation and increased impairment in symptoms of NDD (Ashwood et al. 2011a; Ashwood et al. 2011c).

2. Methods and materials

The experimental methods used were developed in consultation with the California National Primate Research Center veterinary staff. The University of California, Davis Institutional Animal Care and Use Committee approved all protocols used. All attempts were made (in terms of social housing, enriched diet, use of positive reinforcement strategies, and minimizing the duration of daily training/testing sessions) to promote the psychological well-being of the animals that participated in this research. Detailed methods on the administration of polyinosinic:polycytidylic acid stabilized with poly-L-lysine (poly I:C-LC), rearing conditions and behavioral observations have been previously published (Bauman et al. 2014) and are described below:

2.1. Maternal administration of poly ICLC

A total of 24 rhesus monkeys with timed pregnancies were placed into two main treatment groups, namely; controls or MIA and each dam gave birth to a single infant (Supplemental Table 1). Maternal injection of poly I:C-LC or saline took place at 8 am on gestational days 43, 44, and 46; a total of 10 dams received injections of either poly I:C (n = 6) or saline (n = 4), in the first trimester group. Second trimester dams were injected at 8 am on gestational days 100, 101 and 103 where 11 dams received injections of poly I:C (n = 7) and saline (n = 4). For animals that were in the MIA cohort, three injections of 0.25 mg/kg of poly IC-LC (Oncovir, Inc., Washington, DC) were administered. Three untreated (no administration of saline or poly I:C) animals were also included to determine whether there was an effect of saline on behavioral and immune outcomes.

2.2. Rearing conditions

Mother and infant were housed in individual cages with continual visual access to other animals. Enrichment and species-typical social development was facilitated by placing four mother-infant pairs and an adult male in large chain link enclosures for three hours a day. Each of these socialization groups consisted of both control and treatment males and females. Infants were weaned at 6 months of age, but continued daily peer group interactions through approximately 2 years of age. At the time of the current study, all animals were housed indoors in social pairs 24 h per day, 7 days per week.