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Maternal immune activation causes age- and region-specific changes in brain cytokines in offspring throughout development

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ABSTRACT

Maternal infection is a risk factor for autism spectrum disorder (ASD) and schizophrenia (SZ). Indeed, modeling this risk factor in mice through maternal immune activation (MIA) causes ASD- and SZ-like neuropathologies and behaviors in the offspring. Although MIA upregulates pro-inflammatory cytokines in the fetal brain, whether MIA leads to long-lasting changes in brain cytokines during postnatal development remains unknown. Here, we tested this possibility by measuring protein levels of 23 cytokines in the blood and three brain regions from offspring of poly(I:C)- and saline-injected mice at five postnatal ages using multiplex arrays. Most cytokines examined are present in sera and brains throughout development. MIA induces changes in the levels of many cytokines in the brains and sera of offspring in a region- and age-specific manner. These MIA-induced changes follow a few, unexpected and distinct patterns. In frontal and cingulate cortices, several, mostly pro-inflammatory, cytokines are elevated at birth, followed by decreases during periods of synaptogenesis and plasticity, and increases again in the adult. Cytokines are also altered in postnatal hippocampus, but in a pattern distinct from the other regions. The MIA-induced changes in brain cytokines do not correlate with changes in serum cytokines from the same animals. Finally, these MIA-induced cytokine changes are not accompanied by breaches in the blood–brain barrier, immune cell infiltration or increases in microglial density. Together, these data indicate that MIA leads to long-lasting, region-specific changes in brain cytokines in offspring—similar to those reported for ASD and SZ—that may alter CNS development and behavior.

1. Introduction

Autism spectrum disorder (ASD) and schizophrenia (SZ) are devastating disorders that each affect cognitive and social functions of approximately 1% of the population (Kogan et al., 2009). Although the etiology of these disorders is unclear, genetics and environmental factors appear to interact to increase risk (Nawa et al., 2000; Patterson, 2009). Indeed, many of the environmental insults linked to SZ and ASD involve the maternal-fetal environment. Large SZ and ASD twin studies highlight the fact that concordance for dizygotic twins is much greater than that for siblings (Brown and Patterson, 2011; Hallmayer et al., 2011; Patterson, 2007; Rosenberg et al., 2009; Szatmari, 2011) and concordance for SZ is also higher for monochorionic twins, who share a placenta, than for dichorionic twins, who do not (Davis et al., 1995). Together, these studies indicate a significant role for the fetal environment in these disorders. In addition, maternal infection greatly increases the risk for SZ and ASD in offspring (Brown and Derkits, 2010; Brown and Patterson, 2011; Patterson, 2011a), and maternal viral infection is associated with increased risk of ASD as well as a 3–7-fold increased risk of SZ in the offspring (Atladottir et al., 2010; Brown et al., 2004; Brown and Patterson, 2011). Since different types of viral, bacterial, and parasitic infections are associated with ASD and SZ, the critical link between prenatal maternal infection and postnatal brain and behavioral pathology appears to be the maternal immune response and factors that mediate that response, such as cytokines (Deverman and Patterson, 2009; Garay and McAllister, 2010).

These correlations from epidemiological studies are supported by work in rodent models of maternal infection. Adult offspring of pregnant mice given intranasal influenza virus exhibit behavioral abnormalities and changes in gene expression, neuroanatomy, and neurochemistry consistent with both SZ and ASD (Fatemi et al., 2002; Fatemi et al., 1998). Because these outcomes are also elicited in the absence of infection by maternal injection of synthetic dsRNA (poly(I:C)), which mimics the acute phase response to viral infection (Traynor et al., 2004), it is maternal immune activation (MIA) that drives the changes in fetal brain development (Shi et al., 2005). Offspring born to pregnant mice

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injected with poly(I:C) at embryonic day 12.5 (E12.5) display the three core behavioral symptoms of ASD: stereotyped and repetitive behaviors, deficits in social interaction, and deficits in communication (Malkova et al., 2012; Smith et al., 2007). These offspring also display behaviors that are consistent with both SZ and ASD, including elevated anxiety and deficits in prepulse inhibition (PPI), latent inhibition (LI), and working memory (Patterson, 2011b), some of which can be alleviated by treatment with anti-psychotic drugs (Meyer and Feldon, 2010; Meyer et al., 2010; Piontkewitz et al., 2009; Shi et al., 2003). Adult MIA offspring also exhibit abnormalities in gene expression and neurochemistry similar to those noted in SZ and ASD (Meyer et al., 2011). Finally, neuropathology is also seen in this model, including enlarged ventricles and a spatially-localized deficit in Purkinje cells, characteristic of SZ and ASD, respectively (Meyer et al., 2009; Piontkewitz et al., 2009; Shi et al., 2009). Related findings have also been reported in non-human primate models of maternal infection and poly(I:C) MIA (Bauman et al., 2011; Short et al., 2010).

Despite recent progress in developing and characterizing rodent MIA models, much remains to be studied about how MIA alters fetal brain development. Current evidence indicates that the maternal cytokine response is crucial (Patterson, 2009), which leads to immune activation and endocrine changes in the placenta (Hsiao and Patterson, 2011; Mandal et al., 2011). Interleukin (IL)-6 is necessary and sufficient to mediate these effects since the effects of MIA on neuropathology and behavior in the offspring are prevented by injection of pregnant dams with poly(I:C) combined with an anti-IL-6 antibody and are mimicked by a single maternal injection of IL-6 (Smith et al., 2007). Induction of maternal cytokines then alters cytokine expression in the fetal brain, including IL-1β, IL-6, IL-17, IL-13, MCP-1, and MIP1α, hours after MIA (Fatemi et al., 2008; Meyer et al., 2006b; Meyer et al., 2008), with only IL-1β remaining elevated in the fetal brain 24 h following poly(I:C) injection (Arrode-Bruses and Bruses, 2012). However, it is unknown if MIA causes chronic changes in brain cytokines and/or immune cell infiltration in offspring during postnatal development and/or in adult offspring.

Here, we test this possibility using proteomic analysis of cytokine levels in the postnatal brain. The levels of 23 cytokines were measured in the blood and three brain regions from offspring of poly(I:C)- and saline-injected mice at five ages (postnatal day 0 (P0), P7, P14, P30, and P60) using multiplex technology. Perhaps surprisingly, in control brains, most of the 23 cytokines examined are detectable in serum and in all three brain regions (frontal cortex (FC), cingulate cortex (CC), and hippocampus (HC)) from birth through adulthood. The levels of individual cytokines are modulated in age- and region-specific patterns that have not been previously described. Most important, MIA induces striking, long-lasting changes in cytokine levels in the brains of offspring, many of which are region- and age-specific and include widespread decreases as well as increases, compared to controls. Our results indicate that MIA leads to chronic changes in brain cytokine levels in offspring that may mediate MIA-induced changes in CNS development and behavior.

2. Methods and materials

2.1. MIA for cytokine measurements

Female C57BL/6 J mice (Charles River; Wilmington, MA) were obtained from the Caltech breeding facility and housed on a 12:12 h light:dark cycle at 29 ± 1 °C with food and water available *ad libitum*. Mice were mated overnight, and the presence of a vaginal plug on the following morning was noted as E0.5. Pregnant mice were injected intraperitoneally (i.p.) on E12.5 with saline or poly(I:C) potassium salt (Sigma Aldrich; St. Louis, MO). E12.5 was chosen since this stage of gestation correlates with the late first trimester in humans (Clancy et al., 2007)—the time that infections are most closely linked to increased incidence of SZ and ASD (Atladottir et al., 2010; Brown et al., 2004). Poly(I:C) was freshly dissolved in saline and administered i.p. at 20 mg/kg based on the weight of the poly(I:C) itself, not including the total weight of the potassium salts. Control mice were injected with saline alone at 5 μl per gram body weight. This concentration of poly(I:C) is higher than that used for intravenous injections (Meyer et al. 2006b) and was selected because it is the optimal i.p. dose that causes MIA, while preserving viability of offspring (Ito et al. 2010).

2.2. Blood collection and brain dissections

Control and poly(I:C) offspring were sacrificed at 5 ages: P0, P7, P14, P30, and P60. Both male and female offspring were used in this study. Mice were deeply anesthetized with nembutal (10 μl/g). At least 150 μl of blood/animal was first collected by cardiac puncture and transcardial perfusion was then performed using 10–30 ml of sterile PBS (by weight). P0 offspring were processed without perfusion. Whole brains were quickly removed and placed in ice-cold Earle's balanced salt solution for microdissection of the FC, CC, and HC. All tissues were snap-frozen in liquid nitrogen and stored at −80 °C. Both blood and brains were sent overnight on dry ice to U.C. Davis for processing.

2.3. Sample processing

Blood was centrifuged (12,000*g*, 4 °C, 20 min) to obtain serum, which was then stored at −80 °C. Frozen tissues were thawed and disrupted in Bioplex cell lysis buffer (BioRad) containing factors 1 and 2 (protease and phosphatase inhibitors, respectively; BioRad) and the protease inhibitor phenyl-methylsulfonyl fluoride (500 mM; Sigma–Aldrich). A small plastic pestle was used to homogenize the samples. Tissue was further homogenized by trituration using 200 μl pipette tips. The homogenate was then agitated for 30–40 min on ice and centrifuged at 4 °C and 6000*g* (Eppendorf centrifuge 5417R) for 20 min. The supernatant was removed and aliquots stored at −80 °C. The protein content of each sample was determined using the BioRad Protein Assay (BioRad), with bovine serum albumin as a standard, according to the manufacturer's protocol. Sample absorbances were read at 560 nm using a spectrophotometer (Perkin Elmer HTS7000).

2.4. Cytokine measurements

BioRad (Hercules, CA) 23-plex mouse kits were used for all assays. Assays were performed according to the manufacturer's instructions. Reagents were kept on ice until use, minimizing exposure of the beads to light. All samples were run in duplicate and were assayed with the BioRad cytokine reagent kit and either the diluent kit for serum samples or the cell lysis kit for tissue samples. All buffers and diluents were warmed to room temperature prior to use. Lyophilized cytokine standards (containing IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, Eotaxin, G-CSF, GM-CSF, IFNγ, KC, MCP-1, MIP 1α, MIP1β, RANTES, and TNF-α) were reconstituted first to a master standard stock using 500 μl of diluent. Nine concentrations of the standards were made by eight, threefold serial dilutions of the master standard stock in either cell lysis buffer (for brain tissue) or serum diluent. Samples were run at 200 μg protein/well for the mouse brain homogenates. Serum samples were diluted 1:3 prior to assay. All samples were held at 4 °C for 10 min before the start of the assay. Corresponding buffer blanks were run to determine the level of background. All the wash steps were performed on a Bio-Plex Pro