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Full-length Article

Intrauterine inflammation induces sex-specific effects on neuroinflammation, white matter, and behavior

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ABSTRACT

Exposure to inflammation during pregnancy has been linked to adverse neurodevelopmental consequences for the offspring. One common route through which a developing fetus is exposed to inflammation is with intrauterine inflammation. To that end, we utilized an animal model of intrauterine inflammation (IUI; intrauterine lipopolysaccharide (LPS) administration, 50 μg, E15) to assess placental and fetal brain inflammatory responses, white matter integrity, anxiety-related behaviors (elevated zero maze, light dark box, open field), microglial counts, and the CNS cytokine response to an acute injection of LPS in both males and females. These studies revealed that for multiple endpoints (fetal brain cytokine levels, cytokine response to adult LPS challenge) male IUI offspring were uniquely affected by intrauterine inflammation, while for other endpoints (behavior, microglial number) both sexes were similarly affected. These data advance our understanding of sex-specific effects of early life exposure to inflammation in a translationally- relevant model.

1. Introduction

Maternal bacterial or viral infections during pregnancy increase the risk for development of certain mental health disorders including schizophrenia (Buka and et al., 2001; Brown and et al., 2004), autism (Fatemi and et al., 2005), and intellectual disabilities (Rantakallio and von Wendt, 1985). The most widely used models of prenatal infection and inflammation involves systemic (intraperitoneal (IP) or subcutaneous (SC)) administration of bacterial (lipopolysaccharide, LPS) or viral (poly I:C) mimetics to a pregnant dam (Arsenault and et al., 2014). These animal models of systemic prenatal inflammation have been shown to induce behavioral and neurochemical endophenotypes reminiscent of schizophrenia, mood/anxiety disorders, and autism. Specifically, dopaminergic dysfunction (Baharnoori et al., 2013; Aguilar-Valles and et al., 2012), deficits in prepulse inhibition, exaggerated locomotor response to amphetamine (Aguilar-Valles and et al., 2012), decreased "cognitive flexibility" (Bitanihirwe and et al., 2010), social behavior deficits (Machado and et al., 2015), hyperactivity and difficulty with sustained attention (Vuillermot and et al., 2012) and anxiety (Arsenault and et al., 2014; Penteado and et al., 2014) have all been documented in offspring exposed to inflammation during pregnancy. The present work was designed to examine the long term consequences of offspring exposure to local inflammation during pregnancy.

Clinically, a fetus is exposed to localized intrauterine inflammation in a spontaneous preterm birth or with the diagnosis of chorioamnionitis at term (inflammation of the fetal membranes). Chorioamnionitis is diagnosed with clinical criteria (maternal fever, tachycardia) or histological endpoints (presence of infiltrating leukocytes in placental tissues and fetal membranes) and occurs in approximately 10–15% of term births (Mueller-Heubach et al., 1990). However, in spontaneous preterm births, most women do not mount a fever or leukocytosis that would be indicative of chorioamnionitis. Yet, most women who have a spontaneous preterm birth will have evidence of *local* inflammation. This inflammation is noted by histological examination of the placenta, termed histological chorioamnionitis. In fact, in babies born at less than 28 weeks, approximately 85% will have histological chorioamnionitis, suggesting that intrauterine inflammation is present (Yoon and et al., 2000). Other studies have demonstrated

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elevated cytokines in the amniotic fluid of women who ultimately have a spontaneous preterm birth despite negative bacterial cultures (Romero et al., 2015), again suggesting the presence of intrauterine inflammation in a preterm birth. Since the spontaneous preterm birth rate ranges from 10 to 15% in the United States (Statistics and N.C.f.H., 2017), the number of children exposed to intrauterine inflammation is of great clinical significance.

Considering these clinical scenarios, the goal of the present work was to use an animal model to specifically address whether local (intrauterine) inflammation would adversely affect brain development, through evaluation of behavior, white matter integrity, and CNS monocyte and cytokine responses. Additionally, as there are well-documented sex differences in the prevalence of many neurodevelopmental and psychiatric disorders, such as autism, ADHD, anxiety/mood disorders and schizophrenia, we sought to determine if there were sex-specific differences in the response to intrauterine inflammation.

2. Methods

2.1. Intrauterine inflammation (IUI)

All animals were cared for according to the guidelines of both the University of Cincinnati and the University of Pennsylvania Institutional Animal Care and Use Committees and all procedures were in compliance with the NIH guidelines for the Care and Use of Animals. Details of the intrauterine inflammation model have been published (Elovitz and et al., 2011), and will be briefly summarized here. CD-1 out-bred mice (Charles River, Wilmington, MA) were used in the present study. These mice have a gestational period of 19 days, and the preterm period is defined as ~70% of gestation which would be embryonic day 15 (E15). To induce intrauterine inflammation, timed-pregnant CD-1 dams were placed under isoflurane anesthesia on E15. A mini-laparotomy was performed and the lower right uterine horn was identified. In separate groups of dams, lipopolysaccharide (LPS; 50 μg/100 μl/-mouse, serotype 055:B5, Sigma-Aldrich, St. Louis, MO) or sterile saline (100 μl) was infused between the first two gestational sacs, with care not to inject into the amniotic cavity. Dams were exposed to LPS or saline on E15 and allowed to deliver at term (any dams that delivered prior to E18 were not used). Litters were culled to equal sizes on postnatal day 2 (PN2). At weaning (P21), animals were weighed, and then group housed (4–5/cage) with house chow and water available *ad libitum*. Only one animal/sex/litter was used in any one experiment.

2.1.1. Tissue collection: Early (E17)

To collect matched placenta and embryo tissue, dams were killed 48 h following intrauterine LPS (n = 10) or saline exposure (n = 5) (E17) (see Fig. 1 for experimental timeline). The frontal pole was dissected from the embryonic brain, and DNA and RNA were extracted using Qiagen AllPrep DNA/RNA Mini kit (Qiagen, Valencia, CA). Sex determination was done by assessment of the presence or absence of SRY (sex-determining region Y, expressed only on the Y chromosome) using embryo DNA.

2.1.2. Late (adult)-acute LPS challenge

Adult offspring (n = 5/group, 50 weeks of age) were given intraperitoneal injections (at 10 am) of either saline or LPS (10 μg/-mouse). Animals were killed 2 h later and brains were removed and placed immediately into RNA later. The prefrontal cortex (PFC), hypothalamus (HYP) and amygdala (AMYG) were dissected as previously described (Grissom et al., 2015; Grissom and et al., 2015) and RNA was extracted using Qiagen AllPrep DNA/RNA Mini kit (Qiagen, Valencia, CA).

2.2. Flow cytometry

Brains were removed from saline-perfused animals (28 weeks of age), weighed, minced, transferred to Medicon inserts, and ground in a MediMachine (BD Biosciences, San Jose, CA) for 20–30 s. The cell suspension was washed with HBSS, and cells were resuspended in 70% Percoll (GE Healthcare Life Sciences, Pittsburgh, PA) and overlaid with 30% Percoll. The gradient was centrifuged at 2250*g* for 30 min at 4 °C without brake. A total of 10^6 cells were incubated for 30 min on ice with saturating concentrations of labeled antibodies with 40 μg/ml unlabeled 2.4G2 mAb (BD Biosciences, San Jose, CA), to block binding to Fc receptors, and then washed 3 times with 1% BSA in PBS. After surface staining with antibodies against CD45, CD11b, and CD11c, cell suspensions were fixed and permeabilized by Cytofix/Cytoperm solution (BD Biosciences, San Jose, CA), followed by staining with anti– tumor necrosis factor alpha (TNF-α) antibodies. Fluorochrome-labeled antibodies against CD45 (fluorochrome: PerCP), CD11b (fluorochrome: phycoerythin) and appropriate isotype controls were purchased from BD. Fluorochrome-labeled antibody against CD11c (fluorochrome: FITC), and TNF-α (fluorochrome: APC) were purchased from eBioscience (San Dieg, CA). Isotype controls were the following: CD11b: PE Rat IgG2b, K Isotype Control Clone A95-1, CD45: PerCP Rat IgG2b, K Isotype Control Clone A95-1, CD11c: FITC Hamster IgG1, λ1 Isotype Control, TNF-α: APC Rat IgG1, K Isotype Control. Cell staining was acquired on a FACSCalibur or LSRII (BD Biosciences, San Jose, CA) and analyzed with FlowJo (Tree Star, Ashland, OR) software version 5.4.5.

2.3. Behavior

Behavior procedures were performed during the first 3 h of the light phase (7:00–10:00 am). The same animals were tested in open field, elevated zero maze, and light dark box (in this order) with 1 week between testing days. Testing occurred between 12 and 14 weeks of age. All tests were performed in dim light conditions.

2.3.1. Open field activity

Spontaneous activity in an open field was measured with a Photobeam Activity System (PAS)-Open Field (San Diego Instruments, San Diego, CA). Mice were individually placed in the arena (40.64 cm × 40.64 cm × 38.1 cm) for a single 10 min trial. The arena was fitted with a scaffold of infrared light emitting diodes and photo detectors that registered beam breaks. Horizontal and vertical beam breaks were collected to assess general locomotion and rearing activity.

2.3.2. Elevated zero maze

Mice were individually placed on a 6.35 cm wide circular track with an external diameter of 50.80 cm, raised 60.96 cm above the floor (San Diego Instruments, San Diego, CA). The track had two open and two walled quadrants of equal dimensions. Mice were placed in the center of a closed quadrant to begin a 5 min trial. A highly trained scorer, blind to group designation, graded the digitally recorded trials for time spent in the open quadrants. Mice were scored as within a segment when all four paws were within that segment. Transitions between quadrants were also noted as a measure of general locomotor activity.

2.3.3. Light-dark box

Mice were initially placed in the bright side of a 2-compartment light-dark box. Light intensity was 5 lux in the dark compartment

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