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Mouse model of intrauterine inflammation: Sex-specific differences in long-term neurologic and immune sequelae

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ABSTRACT

Preterm infants, especially those that are exposed to prenatal intrauterine infection or inflammation, are at a major risk for adverse neurological outcomes, including cognitive, motor and behavioral disabilities. We have previously shown in a mouse model that there is an acute fetal brain insult associated with intrauterine inflammation. The objectives of this study were: (1) to elucidate long-term (into adolescence and adulthood) neurological outcomes by assessing neurobehavioral development, MRI, immunohistochemistry and flow cytometry of cells of immune origin and (2) to determine whether there are any sex-specific differences in brain development associated with intrauterine inflammation. Our results have shown that prenatal exposure appeared to lead to changes in MRI and behavior patterns throughout the neonatal period and during adulthood. Furthermore, we observed chronic brain inflammation in the offspring, with persistence of microglial activation and increased numbers of macrophages in the brain, ultimately resulting in neuronal loss. Moreover, our study highlights the sex-specific differences in long-term sequelae. This study, while extending the growing literature of adverse neurologic outcomes following exposure to inflammation during early development, presents novel findings in the context of intrauterine inflammation.

1. Introduction

Recent annual summary of vital statistics shows that the preterm birth rate (<37 weeks) is 11.7% in the United States, with more than 500,000 infants born preterm each year (Hamilton et al., 2013). Preterm children exposed to intrauterine infection or inflammation are at a greater risk for a spectrum of long-term adverse neurologic disorders (Wu and Colford, 2000; Yoon et al., 2000), emotional disorders and even autism (Johnson et al., 2010a,b).

Mechanisms of inflammation-induced fetal brain injury are most likely determined by proinflammatory chemokine and cytokine signaling (Hsiao and Patterson, 2011; Hsiao et al., 2012; Lieblein-Boff et al., 2013; Smith et al., 2007; Williamson et al., 2011). Intrauterine infectious pathogens activate toll-like receptors on the surface of cells in the decidua and placental membranes, resulting in the production of proinflammatory cytokines that can cross compromised blood–brain barrier into the fetal brain where they induce activation of microglia, which contribute to glutamate excitotoxicity and production of reactive oxygen species. Altogether, this chain of events leads to cerebral inflammatory response and subsequent brain injury. While chorioamnionitis and *in utero* inflammation as a host immune response may underlie a whole spectrum of long-term effects on the nervous system of neonates, fetal inflammatory responses, which might be initiated by intrauterine inflammation, are now thought to have a stronger association with adverse effects in the neurodevelopment of offspring (Ashdown et al., 2006; Beloosesky et al., 2006; Cai et al., 2003; Dammann and O'Shea, 2008; de Vries, 2009; Hagberg and Mallard, 2005; Malaeb and Dammann, 2009).

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Our prior studies utilized an established mouse model of localized inflammation (Burd et al., 2012; Elovitz et al., 2003) by an intrauterine injection of lipopolysaccharide (LPS). In contrast to models of systemic inflammation induced by poly[I:C] and LPS administered intraperitoneally or models of neonatal inflammation, this model mimics a local inflammatory response in the uterus in the absence of overt infection symptoms in the dam, similar to the most common clinical scenario of preterm birth in humans. Our previous studies indicate that intrauterine inflammation results in acute perinatal brain injury as evidenced by altered neuronal morphology, upregulation of pro-inflammatory cytokines and neurotoxicity, which seems to contribute to irreversible neuronal damage (Burd et al., 2009, 2010a, 2011). However, less is known if this inflammation-induced brain injury could have chronic inflammatory impact and adverse neurological sequelae.

Hence, for the current study, we hypothesize that intrauterine inflammation-induced fetal brain injury has long-term effect on murine brain development into adulthood. Also, these studies sought to assess whether these long-term neurodevelopment outcomes are different between sexes.

2. Materials and methods

2.1. Mouse model of intrauterine inflammation

All animal care and treatment procedures were approved by the Institutional Animal Care and Use Committee. Animals were handled according to the National Institutes of Health guidelines. An established model of intrauterine inflammation was utilized for these studies (Burd et al., 2009, 2010a,b, 2011, 2012; Elovitz et al., 2003, 2011). Timed pregnant CD-1 outbred mouse strain was obtained from Charles River Laboratories (Wilmington, MA).

Intrauterine injections of LPS (from *Escherichia coli*, 055: B5, Sigma–Aldrich, St. Louis, MO) at a dose of 50 μg in 100 μL of phosphate-buffered saline (PBS) were administered on embryonic day 17 (E17) of a 19-day gestation in four independent experiments. Control dams for these experiments received the same volume of intrauterine injection of vehicle. In total, 11 dams were injected with PBS with all litters surviving and 43 dams were injected with LPS with 16 litters surviving. For survival surgery, pregnant mice were placed under a mask that kept a continuous flow of isoflurane/oxygen for adequate anesthesia. The mini-laparotomy was then performed in the lower abdomen, which was closed with suture and staples, and the dams were recovered in individual cages. Live pups were separated by sex for immunohistochemistry, behavioral, magnetic resonance imaging (MRI) studies as well as flow cytometry.

2.2. Behavioral evaluation

A developmental milestone scoring system (Hill et al., 2007) was used with modifications to evaluate pups. Ambulation and pivoting behavior was determined by the ability to move out of a circle 13 cm in diameter. The negative geotaxis test measured the ability to turn 180° when placed head down on a 45° inclined plane. The cliff aversion test measured the ability to turn and crawl away from an edge and the surface righting test determined the ability to right itself after being placed on its back. Open field evaluation, negative geotaxis, cliff aversion, and surface righting were performed on PND 5, 9 and 13 to assess preweaning neurodevelopment.

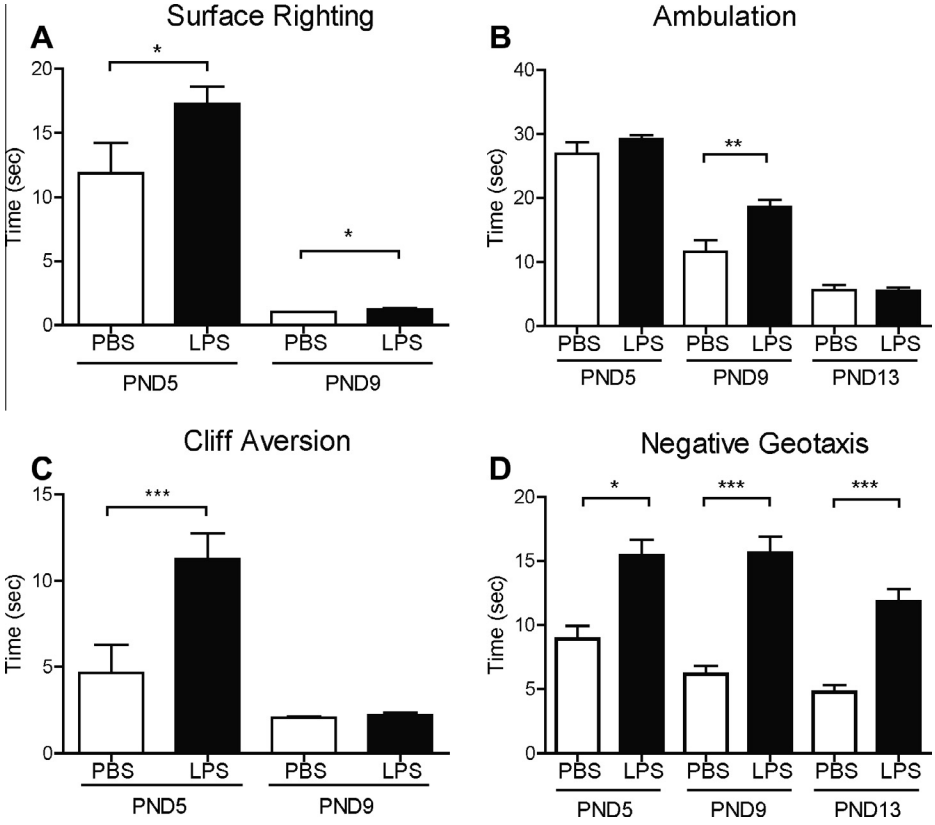

Fig. 1. *In utero* exposure to LPS resulted in decreased motor activity and increased time to perform tests in neonates. Surface righting (A), ambulation (B), cliff aversion (C) and negative geotaxis (D) tests were performed to assess neurodevelopment in normal saline PBS- and LPS-exposed pups. LPS-exposed pups had significantly slower completion times for (A) surface righting at PND 5 and PND 9 ($p < 0.05$), (B) ambulation at PND 9 ($p < 0.01$), (C) cliff aversion at PND 5 ($p < 0.001$), and (D) negative geotaxis at PND 5 ($p < 0.05$), and PND 9 and PND 13 ($p < 0.001$). Data bars represent means ± SEM. $n = 11$ PBS litters; $n = 16$ LPS litters; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.