Brain, Behavior, and Immunity 59 (2017) 300–312

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Full-length Article

Maternal viral infection during pregnancy elicits anti-social behavior in neonatal piglet offspring independent of postnatal microglial cell activation

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ARTICLE INFO

Article history:
Received 28 June 2016
Received in revised form 31 August 2016
Accepted 16 September 2016
Available online 17 September 2016

Keywords:
Prenatal
Maternal immune activation
Microglia
Cytokines
Social behavior
Piglet

ABSTRACT

Maternal infection during pregnancy increases risk for neurodevelopmental disorders and reduced stress resilience in offspring, but the mechanisms are not fully understood. We hypothesized that piglets born from gilts infected with a respiratory virus during late gestation would exhibit aberrant microglia activity, cognitive deficits and reduced sociability. Pregnant gilts were inoculated with porcine reproductive and respiratory syndrome virus (PRRSV; 5×10^5 $TCID_{50}$ of live PRRSV) or saline at gestational day 76. Gilts infected with PRRSV exhibited fever ($p < 0.01$) and reduced appetite ($p < 0.001$) for 2 weeks post-inoculation and were PRRSV-positive at parturition. Piglets born from infected and control gilts were weaned at postnatal day (PD) 1 and assigned to two groups. Group 1 was challenged with lipopolysaccharide (LPS, 5 μg/kg body weight i.p.) or saline on PD 14 and tissues were collected. Group 2 was tested in a T-maze task to assess spatial learning and in a 3-chamber arena with unfamiliar conspecifics to assess social behavior from PD 14–27. Microglia ($CD11b^+$ $CD45^{low}$) isolated from Group 2 piglets at PD 28 were challenged *ex vivo* with LPS; a subset of cells was analyzed for MHCII expression. Maternal infection did not affect offspring circulating TNFα, IL-10, or cortisol levels basally or 4 h post-LPS challenge. While performance in the T-maze task was not affected by maternal infection, both sociability and preference for social novelty were decreased in piglets from infected gilts. There was no effect of maternal infection on microglial MHCII expression or LPS-induced cytokine production. Taken together, these results suggest the reduced social behavior elicited by maternal infection is not due to aberrant microglia activity postnatally.

1. Introduction

Maternal infection during pregnancy is associated with increased risk for development of neuropsychiatric disorders, such as autism and schizophrenia, in offspring (Atladóttir et al., 2010; Brown and Derkits, 2010). Studies in animal models suggest that during maternal immune activation (MIA), maternally-derived cytokines cross the placenta and affect fetal brain development (Gayle et al., 2004; Meyer et al., 2009b, 2007). Pregnant mice administered the viral mimetic polyinosinic:polycytidylic acid (poly I:C) at mid gestation give birth to offspring that display reduced social behavior, disruptions in vocalizations, and stereotypic behavior (Malkova et al., 2012). Evidence of mediation by maternal cytokines comes from studies where pregnant dams were injected with recombinant IL-6 at E12.5, resulting in offspring with behavioral abnormalities similar to what is observed in viral infection, poly I:C or LPS models (Smith et al., 2007). Furthermore, immunoneutralization of IL-6 in pregnant mice administered poly I:C at E12.5 normalized behavior of offspring (Smith et al., 2007). Other investigations revealed that direct injection of IL-17a into the fetal brain was sufficient to produce abnormal cortical development and autism-like phenotypes, while IL-6 was not. Additionally, ASD-like phenotypes produced either from maternal IL-6 or Poly I:C administration could be prevented by maternal pretreatment

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http://dx.doi.org/10.1016/j.bbi.2016.09.019
0889-1591/

with IL-17a-blocking antibody, suggesting that MIA or maternal IL-6-induced abnormal development is dependent on IL-17a (Choi et al., 2016).

Exactly how maternal cytokines affect fetal brain development is still unclear but microglia, the resident immune cells of the brain, have been implicated in the neuroimmune pathogenesis of neurodevelopmental disorders (Onore et al., 2012). Microglia are derived from progenitor cells in the yolk sac (Alliot et al., 1999) and colonize proliferative zones of the neocortex during the first trimester of prenatal development (Cunningham et al., 2013). Fetal microglia display both ramified (resting) and amoeboid (activated or proliferating) morphologies and participate in brain development through phagocytosis of neural precursor cells, neurons, and neuronal synapses (Cunningham et al., 2013; Kettenmann et al., 2011, 2013). Fetal microglia express many of the typical macrophage markers and express inflammatory genes associated with both classical pro-inflammatory and alternative anti-inflammatory phenotypes (Cunningham et al., 2013). Fetal mice exposed to the LPS model of MIA display a pro-inflammatory microglia phenotype that results in a significant reduction in cortical neurons that persists postnatally (Cunningham et al., 2013). However, fetal mice exposed to the Poly I:C model of MIA do not display an activated microglia phenotype or increased microglia density in the cortex and hippocampus (Smolders et al., 2015). Thus, though it has been suggested that MIA activates fetal microglia and alters their phenotype long term, resulting in prolonged neuroinflammation (Cunningham et al., 2013; Knuesel et al., 2014), consistent evidence in support of this hypothesis is lacking.

Proliferation and priming of microglia in response to pro-inflammatory stimuli can also be modulated by glucocorticoids (GCs), which can act as endogenous alarmins, or danger signals (Barrientos et al., 2015; Frank et al., 2014; Nair and Bonneau, 2006). Heightened hippocampal GC levels and GC receptor activation can lead to chronically sensitized microglia, like that seen in aging, a phenomenon that is also linked to learning and memory deficits (Barrientos et al., 2015). Rodent models of MIA have revealed alterations in hypothalamic-pituitaryadrenal (HPA) function (French et al., 2013; Reul et al., 1994) and increased anxiety-like behavior in adult offspring (Babri et al., 2014; Depino, 2015; Hsiao et al., 2012). The long-term negative effects of heightened maternal GCs on offspring HPA development and function, immune function, and behavior have garnered significant research interest (Couret et al., 2009; Howerton and Bale, 2012; Moisiadis and Matthews, 2014). As maternal GCs can cross the placenta (Klemcke, 1995) and blood-brain-barrier (Bellavance and Rivest, 2014), exposure to heightened levels of maternal GCs poses a direct threat to the developing fetus (Kapoor et al., 2008; Liu et al., 2001), and likely contributes to the fetal microglia response to MIA.

We developed a prenatal MIA paradigm in swine utilizing porcine reproductive and respiratory syndrome virus (PRRSV) during late gestation at a time when the fetal pig brain undergoes a dramatic growth spurt, similar to human neurodevelopment (Dickerson and Dobbing, 1967; Dobbing and Sands, 1979; Pond et al., 2000). The domestic pig is a precocious, gyrencephalic species whose brain anatomy, neurochemistry, and growth and development trajectories correspond closely to humans in prenatal and early postnatal life (Conrad and Johnson, 2015; Lind et al., 2007). Thus, we sought to extend current findings on MIA in a highly translatable pig model. As data demonstrating prolonged over-activation of microglia in offspring due to MIA are lacking, and recent evidence indicates that prolonged microglia anomalies may not occur in some MIA models (Giovanoli et al., 2015, 2016), we aimed to assess microglia activation status in prenatally challenged neonatal piglets. We hypothesized that maternal infection with PRRSV would lead to aberrant microglia activity in offspring, resulting in altered cognitive and social behaviors in early life. We further postulated that maternal infection would alter fetal HPA development and lead to GC resistance in microglia. Here, we show that piglets born from infected mothers display anti-social behaviors and a decreased preference for social novelty, in the absence of overt microglia activation and GC desensitization.

2. Materials and methods

2.1. Animals and experimental design

The experimental design is illustrated in Fig. 1. Crossbred pregnant gilts (PRRSV-free and not vaccinated), artificially inseminated with semen from the same boar (PIC 359 SS 6278, Birchwood Genetics, Inc., West Manchester, OH), were brought from the University of Illinois swine herd into the biomedical animal facility at gestational day (GD) 69. Gilts were individually housed in identical disease containment chambers kept at 22 °C and maintained on a 12 h light/dark cycle. Gilts were provided 2.3 kg of a standard corn-soybean meal-based gestation diet daily, with *ad libitum* access to water. Rectal temperature and food intake were monitored daily. If food intake decreased, gilts were provided supplemental corn syrup and/or tap water mixed in the diet until food intake returned to normal. Three identical trials were conducted, each consisting of one control gilt and two MIA gilts. One gilt in the MIA treatment group spontaneously aborted. Thus, the total number of gilts across the three trials was eight, three of which were control, five of which were MIA.

On GD 76, gilts in the MIA treatment group were inoculated intranasal with 5 mL of 1×10^5 50% tissue culture infectious dose (TCID 50) of live PRRSV (strain P-129-BV), obtained from the School of Veterinary Medicine at Purdue University (West Lafayette, Indiana). Control gilts received the same volume of sterile DMEM. PRRSV is an enveloped single-stranded RNA virus which causes interstitial pneumonia by infecting alveolar macrophages (Kim et al., 2002), and results in increased secretion of IL-1β, IL-6, IL-10, and TNFα (Conrad et al., 2015; Ji et al., 2016; Liu et al., 2009). Gilts were inoculated on GD 76 to coincide with the onset of accelerated fetal brain growth; this gestational day corresponds to approximately the beginning of the third trimester in humans (Andersen, 2003; Dobbing and Sands, 1973, 1979; Pond et al., 2000).

In a separate but identical study with this MIA model, blood was collected from the marginal ear vein of control and PRRSV gilts once weekly from GD 76 to GD 104 (Control: n = 9, PRRSV: n = 6). Heparinized blood was centrifuged (1300*g* at 4 °C for 15 min) and plasma was collected and stored at −80 °C until analyses of TNFα and IL-6. To avoid stress during pregnancy, for the present study saliva was collected from gilts once per week by allowing them to chew on cotton ropes, similar to what is described by Cook et al. (2013). Saliva was collected from the rope and frozen at −80 °C until analysis for PRRSV. On GD 104 gilts were moved into standard farrowing crates. On GD113, 10 mg of lutalyse (Pfizer, New York, NY) was given intramuscularly to induce parturition.

Approximately 24 h after birth [age postnatal day (PD) 1], all piglets were processed as follows: body weight and rectal temperature were recorded; blood was collected from the external jugular vein to confirm presence/absence of PRRSV; and intramuscular injections of iron dextran (100 mg/pig, Butler Schein Animal Health, Dublin, OH) and penicillin (60 kU/pig, Butler Schein Animal Health, Dublin, OH) were administered. Within 12 h of processing, piglets were moved to separate biomedical containment chambers and into individual cages. Housing, handling, and feeding of piglets was performed as previously described (Elmore et al., 2014), with some modifications. Briefly, piglets born from PRRSV-infected and