Brain, Behavior, and Immunity 62 (2017) 11–23

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

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

Full-length Article

The placental interleukin-6 signaling controls fetal brain development and behavior

Wei-Li Wu [a,*], Elaine Y. Hsiao [a,b], Zihao Yan [a], Sarkis K. Mazmanian [a], Paul H. Patterson [a,1]

[a] Division of Biology and Biological Engineering, California Institute of Technology, 1200 E. California Boulevard, Pasadena, CA 91125, USA
[b] Department of Integrative Biology & Physiology, University of California, Los Angeles, 610 Charles E. Young Drive, Los Angeles, CA 90095, USA

ARTICLE INFO

Article history:
Received 27 July 2016
Received in revised form 20 October 2016
Accepted 8 November 2016
Available online 9 November 2016

Keywords:
Maternal immune activation (MIA)
Maternal-placental-fetal axis
Interleukin-6 (IL-6)
Placenta
Hindbrain development
Purkinje cells
Autism spectrum disorder (ASD)

ABSTRACT

Epidemiological studies show that maternal immune activation (MIA) during pregnancy is a risk factor for autism. However, mechanisms for how MIA affects brain development and behaviors in offspring remain poorly described. To determine whether placental interleukin-6 (IL-6) signaling is required for mediating MIA on the offspring, we generated mice with restricted deletion of the receptor for IL-6 (IL-6Rα) in placental trophoblasts (*Cyp19-Cre*$^{+}$;*Il6ra*$^{fl/fl}$), and tested offspring of *Cyp19-Cre*$^{+}$;*Il6ra*$^{fl/fl}$ mothers for immunological, pathological and behavioral abnormalities following induction of MIA. We reveal that MIA results in acute inflammatory responses in the fetal brain. Lack of IL-6 signaling in trophoblasts effectively blocks MIA-induced inflammatory responses in the placenta and the fetal brain. Furthermore, behavioral abnormalities and cerebellar neuropathologies observed in MIA control offspring are prevented in *Cyp19-Cre*$^{+}$;*Il6ra*$^{fl/fl}$ offspring. Our results demonstrate that IL-6 activation in placenta is required for relaying inflammatory signals to the fetal brain and impacting behaviors and neuropathologies relevant to neurodevelopmental disease.

1. Introduction

Autism spectrum disorder (ASD) is a range of complex neurodevelopmental disorders, characterized by difficulties in social communication, and repetitive and stereotyped behaviors (Association, 2013). To date, the prevalence for ASD in the United States is one in 68 children (Investigators, 2014) and diagnoses worldwide are 62 per 10,000 people (Elsabbagh et al., 2012). Monozygotic twins studies indicate the concordance rate for ASD ranges between 60 and 91% (Ronald and Hoekstra, 2011), which suggests both genetic and non-genetic factors (e.g. environment) could contribute to the etiology of ASD.

Epidemiological studies suggest that maternal infection is a principal non-genetic risk factor for ASD (Brown, 2012). Analyses of large cohorts of ASD patients found that inpatient diagnosis of severe infection during pregnancy are associated with increased ASD risk (Atladottir et al., 2010; Lee et al., 2014). The diversity of infections and the observation that many are not transmitted to the fetus (Fatemi et al., 2012; Shi et al., 2005) suggest that maternal immune activation (MIA), rather than microbial pathogenesis, is responsible for increasing the risk of ASD in the offspring. This emphasis on MIA rather than a specific pathogen is supported by animal models that involve injection of non-pathogenic antigens, such as polyinosinic-polycytidylic acid (poly(I:C)) (Boksa, 2010; Meyer, 2014). Stimulation of the maternal immune system in these cases causes offspring to develop behavioral and neuropathological features of ASD similar to those seen in maternal infection (Boksa, 2010; Knuesel et al., 2014; Meyer, 2014).

The molecular mechanisms underlying abnormal neurodevelopment and behavior in MIA are poorly understood, though cytokines appear to be critical (Boksa, 2010; Knuesel et al., 2014). The immune dysregulation in MIA offspring persists postnatally (Garay et al., 2013; Hsiao et al., 2012). MIA causes long-lasting and region specific changes of brain cytokines in the offspring that vary based on developmental time point (Garay et al., 2013). Furthermore, MIA leads to decreases in splenic and mesenteric regulatory T cells (Tregs) and increases in interleukin (IL)-6 and IL-17 production in splenic CD4^{+} T cells in adult offspring (Hsiao et al., 2012). IL-6 levels are elevated in fetal brain after MIA induction (Connor et al., 2012; Meyer et al., 2006; Wu et al., 2015). Blocking IL-6, but not IL-1β or interferon gamma (IFNγ), after induction of MIA in pregnant mice prevents behavioral abnormalities in the offspring (Smith et al., 2007). Furthermore, injection of recombinant

* Corresponding author.
E-mail addresses: wlwu@caltech.edu (W.-L. Wu), ehsiao@ucla.edu (E.Y. Hsiao), Zihao_Yan@hms.harvard.edu (Z. Yan), sarkis@caltech.edu (S.K. Mazmanian), php@caltech.edu (P.H. Patterson).

[1] This study is dedicated to Dr. Paul H. Patterson, who conceived and led the project prior to his passing in 2014.

http://dx.doi.org/10.1016/j.bbi.2016.11.007
0889-1591/

IL-6 alone into mice is sufficient to promote similar behavioral phenotypes seen in the MIA model (Hsiao and Patterson, 2011), arguing for a causal role for IL-6 signaling in this context. The importance of IL-6 in mediating MIA effects on brain and behavior in rodents is also supported by the studies shown in human ASD subjects, wherein IL-6 is increased in ASD subjects (Ashwood et al., 2011; Li et al., 2009; Masi et al., 2014; Vargas et al., 2005; Wei et al., 2011). IL-6 is both necessary and sufficient for mediating the effects of MIA on the development of ASD-related behavioral abnormalities, suggesting that tracing the pathways of MIA-induced IL-6 signaling may reveal novel mechanisms by which maternal insults disrupt fetal neurodevelopment.

The significance of the placenta in the occurrence of psychiatric disorders has been suggested by several studies. The concordance rate of monochorionic twins for schizophrenia is 60%, while dichorionic twins is only 10.7% (Davis et al., 1995). Examining the histology of the placenta from individuals with ASD reveals that about 3–8-fold increased odds of having trophoblast inclusion in the ASD groups compared to controls (Anderson et al., 2007; Walker et al., 2013). These correlations indicate that the uterine environment should also be considered when evaluating potential etiologies for psychiatric disorders.

During infection, increased cytokine levels in the maternal environment might directly transmit signals to the fetus through the placenta (Dahlgren et al., 2006; Zaretsky et al., 2004). Placenta is of fetal origin, juxtaposed against the maternal decidua (D) layer, and represents the primary molecular connection between the mother and its developing fetus. IL-6 production signaling in the placenta, particularly in the spongiotrophoblast (SP) layer, following induction of MIA has been reported (Hsiao and Patterson, 2011). Furthermore, the MIA-induced alterations in IL-6 signaling pathways in the placenta, and placental hormone production following MIA are prevented upon immune-activation of *Il6*$^{-/-}$ pregnant mice (Hsiao and Patterson, 2011), revealing dependence on placental IL-6. Whether placental IL-6 signaling is involved in relaying the detrimental effects of MIA to the developing embryo is unknown.

To understand whether IL-6 signaling in the placenta plays a role in modulating the MIA response, we crossed placental trophoblast specific Cre mice (*Cyp19-Cre*) (Wenzel and Leone, 2007) with IL-6Rα loxp-flanked mice (*Il6ra*$^{fl/fl}$) (McFarland-Mancini et al., 2010) to generate trophoblast-specific IL-6Rα knockout mice (*Cyp19-Cre*$^{+}$;*Il6ra*$^{fl/fl}$). The *Cyp19* gene encodes aromatase cytochrome P450 converting androgens to estrogens, which plays an important role in uterine and placental growth and differentiation (Furbass et al., 2008). Different promoter regions of the *Cyp19* gene drive its expression into different tissues (Rawn and Cross, 2008). The *Cyp19-Cre* 5912 founder line specifically expresses Cre recombinase at placental trophoblast precursor cells during the early stage of embryogenesis and shows minimal expression of Cre recombinase in fetal tissues (Wenzel and Leone, 2007), which allows us to examine the functionality of IL-6 in MIA model specifically in placental trophoblast population. Herein, we reveal that immune activation in the placenta perturbs fetal brain development during gestation, resulting in ASD-like behavioral symptoms in offspring. These findings support a growing appreciation of environmental risk factors for mental disorders.

2. Materials and methods

2.1. Mice

Wild-type C57BL/6N mice were obtained through Caltech's barrier animal facility (originally from Charles River, Wilmington, MA, USA). *Il6*$^{-/-}$ (002650; B6.129S2-*Il6tm1Kopf*/J) and *Il6ra*$^{fl/fl}$ (012944; B6;SJL-*Il6ratm1.1Drew*/J012944) mouse lines were obtained from Jackson Laboratory (Bar Harbor, ME, USA). *Cyp19-Cre* mouse (5912 line) was obtained by Dr. Gustavo Leone from Ohio State University (Wenzel and Leone, 2007). *ROSA::LSL-lacZ* mouse was kindly provided by Dr. David J. Anderson at Caltech. *Ate1*$^{-/-}$ mice were kindly provided by Dr. Alexander J. Varshavsky at Caltech (Brower and Varshavsky, 2009). Mice were maintained at Caltech's barrier animal facility and transferred to Caltech's Broad animal facility for experiments. All mice were group housed (2–5 mice per cage) with a 13 h light/11 h dark cycle (lights on at 06:00) at 21–23 °C and 45% relative humidity within a range of 30–70% in ventilated cages (Super Mouse 750™, Lab Products Inc, Seaford, DE, USA). Pregnant and lactating mice were fed a mix of half 5053 PicoLab Rodent Diet and half 5058 PicoLab Rodent Diet (5053, Lad Diet, St. Louis, MO, USA). All experiments were performed under the approval of the California Institute of Technology Institutional Animal Care and Use Committee (IACUC).

2.2. Generation and genotyping of placental trophoblast IL-6Rα knockout mice

Placental trophoblast IL-6Rα knockout mice, *Cyp19-Cre*$^{+}$; *Il6ra*$^{fl/fl}$, were generated by crossing two mouse lines- *Il6ra*$^{fl/fl}$ and *Cyp19-Cre* (5912 line). To yield a congenic strain of *Cyp19-Cre*$^{+}$; *Il6ra*$^{fl/fl}$ mice, the *Cyp19-Cre*$^{+}$ mouse line was backcrossed to C57BL/6N for at least 8 generations. After backcrossing, C57BL/6N *Cyp19-Cre*$^{+}$ mice were crossed with *Il6ra*$^{fl/fl}$ mice, which were originally derived and maintained on C57BL/6J background. F1 offspring were *Il6ra*$^{fl/+}$ and *Cyp19-Cre*$^{+}$;*Il6ra*$^{fl/+}$. These were then crossed to yield F2: *Il6ra*$^{+/+}$, *Il6ra*$^{fl/+}$, *Il6ra*$^{fl/fl}$, *Cyp19-Cre*$^{+}$; *Il6ra*$^{+/+}$, *Cyp19-Cre*$^{+}$; *Il6ra*$^{fl/+}$, and *Cyp19-Cre*$^{+}$; *Il6ra*$^{fl/fl}$. Offspring of *Il6ra*$^{fl/fl}$ and *Cyp19-Cre*$^{+}$; *Il6ra*$^{fl/fl}$ genotypes were maintained for experiments.

Mice were weaned at the age of 3 weeks. Then the mice were labeled by ear punch and tail snips were collected immediately after weaning. gDNA was extracted using a standard DNA extraction protocol. PCR was performed to amplify a fragment of *Il6* flox allele or wild-type allele. Cre was detected in a separate round of PCR. Primer sequences are listed below.

Il6ra flox allele: Forward 5′-GAA GGA GGA GCT TGA CCT TGG-3′; Reverse: 5′-AAC CAT GCC TAT CAT CCT TTG G-3′.

Cre: Forward 5′-GGC GTT TTC TGA GCA TAC CTG-3′; Reverse: 5′-CAT TCT CCC ACC GTC AGT ACG-3′.

For genotyping placentas and fetuses, a piece of tail from the fetus was processed using the standard gDNA extraction and PCR procedure, as described above. The genotype of the placenta can be determined by the genotype of the corresponding fetus.

2.3. Timed-mating for C57BL/6N wild-type, *Il6*$^{-/-}$, and *Cyp19-Cre*$^{+}$; *Il6a*$^{fl/fl}$ mutant mice

We adopted a trio timed-mating strategy (two female and one male) to minimize the number of sires and limit variation. The females were transferred to a clean cage one day before the introduced of the male into the cage. Timed-mating pairs were set up in the late phase of the light period. Vaginal plugs were checked the following morning. The day of vaginal plug presence was considered embryonic day 0.5 (E0.5). Three independent mouse lines were used in this study- C57BL/6N wild-type line, *Il6*$^{-/-}$ mutant line, and *Cyp19-Cre*$^{+}$;*Il6a*$^{fl/fl}$ mutant line. For the timed-mating of *Il6*$^{-/-}$ mutant line for Luminex cytokine array study, two kinds of breeding pairs were used- wild-type sire *x* *Il6*$^{-/-}$ dam and *Il6*$^{-/-}$ sire *x* wild-type dam. The genotype of offspring yielded from the two kinds of breeding pairs is *Il6*$^{+/-}$. For the timed-mating of *Cyp19-Cre*$^{+}$;*Il6a*$^{fl/fl}$ mutant line, the pair we used was *Il6ra*$^{fl/fl}$ sire x *Cyp19-Cre*$^{+}$;*Il6a*$^{fl/fl}$ dam. The genotype of offspring we yield from