Brain, Behavior, and Immunity 25 (2011) 863–871

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

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

Maternal immune stimulation during pregnancy affects adaptive immunity in offspring to promote development of TH17 cells

Mili Mandal [a], Atara C. Marzouk [b], Robert Donnelly [a,b], Nicholas M. Ponzio [a,b,*]

[a] UMDNJ – Graduate School of Biomedical Sciences, NJ, USA
[b] UMDNJ – New Jersey Medical School, NJ, USA

ARTICLE INFO

Article history:
Available online 18 September 2010

Keywords:
Immune stimulation
TH17 cells
Pregnancy
Cytokines
IL-6
Autism
Schizophrenia

ABSTRACT

Behavioral abnormalities in offspring of murine dams that receive immune stimulation with (poly)I:C during pregnancy are well-documented. In this prenatal model, (poly)I:C-induced maternal cytokines, particularly IL-6, appear involved in the etiology of the behavioral abnormalities. While much has been published on the abnormal behaviors of offspring in this model, much less is known about how maternal immune stimulation affects the adaptive immune system of the offspring, and its possible role in the observed pathophysiology. In the present study, pregnant dams were stimulated with (poly)I:C at E12, and 24 h later cytokine levels were measured in maternal sera and amniotic fluids. Lymphocytes from offspring were also analyzed for T Helper (TH) cell subsets. The results demonstrate that lymphocytes from offspring of pregnant dams stimulated with (poly)I:C develop into TH17 cells upon in vitro activation. This preferential TH17 cell differentiation occurs in offspring of pregnant dams with an immunological "memory" phenotype, but not in offspring of immunologically "naive" dams. Comparable levels of IL-6 were found in the sera of immune and naïve pregnant dams, however, there was a disparity between levels of IL-6 in maternal sera and amniotic fluids of (poly)I:C-injected dams. In matings between IL-6 KO dams (IL-$6^{-/-}$) and wild-type males (IL-$6^{+/+}$) there was no IL-6 in sera from (poly)I:C-injected dams, but there were high levels of IL-6 in their amniotic fluids. Analysis of supernatants of cultured placental cell preparations from these IL-6 KO dams confirmed that the IL-6 was produced from the fetal (IL-$6^{+/-}$) component, and heterozygous IL-$6^{+/-}$ offspring could also produce IL-6.

1. Introduction

The clinical and epidemiological data showing that children of mothers who are exposed to certain infectious micro-organisms during pregnancy have significantly higher frequencies of neurological disorders, including schizophrenia and autism are compelling (Croonenberghs et al., 2002; Deykin and MacMahon, 1979; Hagberg and Mallard, 2005; Hornig et al., 1999; Lipkin and Hornig, 2003; Malek-Ahmadi, 2001; Pardo and Eberhart, 2007; Patterson, 2002). In these instances, cytokines produced by maternal inflammatory and immune responses (rather than the infectious organisms themselves) have been linked to the observed pathologies, and are thought to be part of their etiology (reviewed in Jonakait (2007). While the design of animal experiments cannot fully replicate human conditions, rodent studies in which the maternal immune system is stimulated during pregnancy provide validated experimental models to investigate the developmental, behavioral, and immunological abnormalities seen in these human disorders (Bell and Hallenbeck, 2002; Carvey et al., 2003; Fatemi et al., 2002; Hagberg and Mallard, 2005; Hornig et al., 2001, 1999; Ito et al., 2010; Lancaster et al., 2007; Meyer et al., 2009; Patterson, 2002; Pletnikov et al., 2001, 1999; Ponzio et al., 2007; Rousset et al., 2006; Shi et al., 2003; Smith et al., 2007; Weissenbock et al., 2000).

Injection of polyclonal immune stimuli, [e.g., (poly)I:C, lipopolysaccharide (LPS)] or direct injection of the pro-inflammatory cytokines these stimuli induce (e.g., IL-1, IL-2, IL-6) to pregnant dams can also cause immune dysregulation and behavioral abnormalities in their offspring in comparison to the offspring of pregnant dams injected with a control substance, such as PBS (Conroy et al., 2004; Dammann and Leviton, 1997; Gilmore et al., 2004; Nawa and Takei, 2006; Ponzio et al., 2007; Samuelsson et al., 2006; Smith et al., 2007). The underlying mechanisms that mediate these abnormalities have not been completely defined, but there is general consensus that both genetic susceptibility and one or more environmental "triggers" are involved (Ashwood and Van de Water, 2004; Connolly et al., 2006; DiCicco-Bloom et al., 2006; Pardo et al., 2005).

Immunologically naïve females are used in the experimental design of these published studies. In the present study, we modified this design and also used pregnant dams that exhibited an immunological memory phenotype for comparison. This

* Corresponding author at: Department of Pathology and Laboratory Medicine, UMDNJ – New Jersey Medical School, 185 South Orange Avenue, Newark, NJ 07103, USA. Fax: +1 973 972 7293.
E-mail address: ponzio@umdnj.edu (N.M. Ponzio).

0889-1591/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbi.2010.09.011

experimental strategy more closely resembles the human condition, where women develop immunological memory resulting from immunizations and natural exposure to antigens prior to pregnancy.

Many publications have documented the developmental and behavioral abnormalities of offspring in these prenatal mouse models. However, less is known about how maternal immune stimulation during pregnancy affects the developing adaptive immune system of these offspring. Fetal and early life programming can affect the brain and subsequent behaviors that persist in later life (Bilbo and Schwarz, 2009; Schlotz and Phillips, 2009), and it is thought that cytokines also play a key role in such programming. Thus, it is possible that in addition to the influence on development of the brain, maternal immune stimulation during pregnancy also influences components of immune system of the developing fetus. These changes could also persist into later life, and have the potential to mediate diseases, including neurological disorders.

There is ample evidence that T cells are involved in normal brain development and cognition (Bilbo and Schwarz, 2009; Kipnis et al., 2004, 2008). T cells can also mediate autoimmune pathology in the central nervous system, (e.g., Experimental Autoimmune Encephalomyelitis – EAE), as well as in other organs and peripheral tissues. Early studies demonstrated that T Helper 1 (TH1) cells mediated pathology in these autoimmune models, but more recently, involvement of the TH17 subset has also been shown (Hirota et al., 2010; Langrish et al., 2005). TH17 cells are dependent on cytokines for their development, maintenance, and function, (Bettelli et al., 2006; Korn et al., 2009, 2007b; Mangan et al., 2006; Veldhoen et al., 2006) and have been implicated in modulating the incidence and/or progression of various inflammatory and autoimmune phenomena in experimental animals and humans, including EAE, multiple sclerosis, inflammatory bowel disease, psoriasis, collagen-induced and rheumatoid arthritis, and asthma (Bettelli et al., 2006; Furuzawa-Carballeda et al., 2007; Korn et al., 2009). Thus far, however, little is known about the involvement of Th17 cells in the developmental and behavioral abnormalities seen in prenatal models involving maternal immune stimulation during pregnancy.

The possibility that pro-inflammatory cytokines produced as a result of maternal immune stimulation during pregnancy can affect programming of developing TH cells was tested in the present study. The results show that T cells from offspring of (poly)I:C-injected pregnant dams with a pre-existing immunological memory phenotype exhibited a significantly higher tendency to differentiate into TH17 cells upon in vitro activation than T cells from offspring of (poly)I:C-injected immunologically naïve pregnant dams. Importantly, the effects on T cell development seen in offspring were independent of antigen-specific maternal immunological memory. The preferential polarization toward TH17 cell development in offspring of (poly)I:C-injected immune (vs. naïve) pregnant dams was not due to quantitative differences in maternal IL-6 production. However, investigation of disparity between IL-6 levels in maternal sera and amniotic fluid revealed a major placental/fetal contribution to cytokine production.

2. Materials and methods

2.1. Mice

The following strains of mice were purchased from the Jackson Laboratory (Bar Harbor, ME): Balb/c, Wild-type (WT) C57BL/6 (B6), and IL-6 Knock-out (KO) B6.129S6-*Il6*^{tm1Kopf} (IL6$^{-/-}$). All mice were housed and bred in a pathogen-free animal facility at University of Medicine and Dentistry of New Jersey (UMDNJ, Newark). Experimental procedures using mice were approved by the Institutional Animal Care and Use Committee of UMDNJ, Newark.

2.2. Injection and immunization protocol

The experimental design for injections to immunize and stimulate pregnant dams is shown in Fig. 1. One group of B6 females received an intra-peritoneal (i.p.) injection with 2×10^7 γ-irradiated (3000R) allogeneic Balb/c spleen cells to establish immunological memory. One month after the immunization, these "immune" B6 females (and age-matched immunologically naïve B6 females) were mated with immunologically naïve B6 males. The appearance of a vaginal plug was considered embryonic day 0 (E0). Immune and naive pregnant B6 females received a single i.p. injection of (poly)I:C (20 mg/kg; Sigma; St. Louis, MO) or vehicle control injection of PBS on E12. This created four experimental groups for comparison based on immunological phenotype (immune or naïve) and immune stimulation during pregnancy ((poly)I:C or PBS) as shown in Fig. 1. Whole body weights of pregnant females were recorded before and 24 h after injections given on E12. Pregnant dams were divided into two groups: one group was allowed to give birth, and their offspring were used to measure selected immunological parameters. In the second group, pregnant dams were sacrificed 24 h after (poly)I:C or PBS injection for collection of sera and amniotic fluids.

2.3. Indirect immunofluorescence

To confirm that B6 (H-2^b) females immunized with Balb/c (H-2^d) spleen cells produced anti-(H-2^d) antibodies, their sera were incubated for 30 min with cells from the L1210 T lymphocyte tumor cell line, which also express H-2^d cell surface molecules. Cell preparations were then washed 2× to remove unbound primary antibody, and then incubated with fluorescein isothiocyanate-(FITC) conjugated goat anti-mouse IgG (secondary antibody) (Jackson ImmunoResearch, West Grove, PA). Thereafter, cells were washed 2×, and fixed in 2% buffered paraformaldehyde prior to analysis. Cell samples were acquired in the New Jersey Medical School (NJMS) Flow Cytometry Core, using FACSCalibur cytometer (Becton–Dickinson; Franklin Lakes, NJ), and data were analyzed using FloJo software (version 7.5; Ashland, OR).

2.4. In vitro activation for cytokine production

To confirm that IL-6 knock-out (KO) females did not produce IL-6, their spleen cells were activated in vitro with several different stimuli, including phorbol 12-myristate 13-acetate (PMA; 3 ng/ml; Sigma) and ionomycin (100 ng/ml; Sigma), LPS (100 μg/ml; Sigma), and (poly)I:C (100 μg/ml; Sigma). Similar cultures were established with spleen cells from WT B6 female mice for comparison. Parallel cultures were also established with placental cell preparations from individual embryos, and with spleen cells from offspring of (poly)I:C-injected IL-6 KO pregnant dams. Supernatants were collected 24 h after stimulation and tested for the presence of IL-6.

2.5. Cytotoxic T lymphocyte (CTL) assay

Responder (R) spleen cells from immune and naïve B6 (H-2^b) females were co-cultured for 5 days with γ-irradiated (3000R) allogeneic Balb/c (H-2^d) stimulator (S) spleen cells at responder to stimulator (R/S) ratios of 1:1, 2:1, and 4:1. Cells were then harvested, counted, and tested for cytotoxicity against ^{51}Cr labeled, L1210 (H-2^d) target cells. ^{51}Cr release from target cells incubated with medium alone (spontaneous release) and target cells incubated with 2% Nonidet P-40 (Sigma) (maximum release) were used to calculate the percent ^{51}Cr release due to CTL-mediated lysis