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The decidua of preeclamptic-like BPH/5 mice exhibits an exaggerated inflammatory response during early pregnancy

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ABSTRACT

Preeclampsia is a devastating complication of pregnancy characterized by late-gestation hypertension and proteinuria. Because the only definitive treatment is delivery of the fetus and placenta, preeclampsia contributes to increased morbidity and mortality of both mother and fetus. The BPH/5 mouse model, which spontaneously develops a syndrome strikingly similar to preeclampsia, displays excessive inflammation and suppression of inflammation improves pregnancy outcomes. During early pregnancy, decidual macrophages play an important role in promoting maternal tolerance to fetal antigens and regulating tissue remodeling, two functions that are critical for normal placental development. BPH/5 pregnancies are characterized by abnormal placentation; therefore, we hypothesized that macrophage localization and/or function is altered during early pregnancy at the site of placental formation (the decidua) compared to C57BL/6 controls. At early gestation time points, before the onset of maternal hypertension or proteinuria, there was a reduction in the number of macrophages in BPH/5 decidua and a concomitant increase in activated T cells compared with C57BL/6. BPH/5 decidua also exhibited decreased expression of the immunosuppressive cytokine, IL-10, and increased expression of pro-inflammatory, inducible nitric oxide synthase. Together, these data suggest that a reduction in decidual macrophages during pregnancy is associated with immune activation in BPH/5 mice, inadequate placental development and may contribute to adverse pregnancy outcomes in this model.

1. Introduction

Preeclampsia is a devastating syndrome of pregnancy that can result in increased morbidity or mortality in both mother and fetus (Leeman et al., 2016). The diagnosis of preeclampsia occurs after 20 weeks of gestation following the onset of increased maternal blood pressure and one of the following: proteinuria, thrombocytopenia, impaired liver function, pulmonary edema, or headache accompanied by visual disturbances (Leeman et al., 2016). The mechanisms underlying the development of preeclampsia are poorly understood, but may include defective placental formation early in pregnancy (Matsubara et al., 2015) and abnormal immune activation (Laresgoiti-Servitje, 2013). If left untreated, preeclampsia can progress to potentially fatal eclampsia (Valente and Economy, 2013); however, the only treatment for preeclampsia is delivery of the fetus and placenta, increasing the incidence of preterm birth and neonatal complications.

A balanced immune response is critical for a successful pregnancy and is mediated by natural killer (NK) cells, and macrophages (MØ), the first and second most common leukocyte populations at the maternal–fetal interface during normal pregnancy (Yang et al., 2011). NK cells and MØ secrete regulatory factors, including the immunosuppressive cytokine interleukin (IL)-10, which along with endocrine hormones signals the differentiation of uterine stromal cells into metabolically active decidual cells that promote normal placentation (Lai et al.,

Abbreviations: CSF-1, Colony stimulating factor; Cox-2, Cyclooxygenase-2; DAPI, 4′,6-diamidino-2-phenylindole; dMØ, Decidual macrophages; e, embryonic day; iNos, inducible nitric oxide synthase; NK, Natural Killer cells; NKr1, regulatory natural killer cells; PGE_2, Prostaglandin E_2; UPP, Reduced Uterine Perfusion Pressure; Tr1, type 1 regulatory T cells; VEGF, vascular endothelial growth factor

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2011). In first trimester human decidua, IL-10 production was primarily attributed to two populations of $CD14^+$ macrophages, with $CD11c^{hi}$ dMØ producing more IL-10 than $CD11c^{low}$ dMØ (Houser et al., 2011). Murine decidual macrophages produce the free radical nitric oxide (NO), which, at low concentrations, is important for implantation, decidualization, smooth muscle relaxation and vasodilation during normal pregnancy (Purcell et al., 1999). NK cells and MØ play a key role in regulating cytokine expression and the production of inflammatory mediators in the uterus. Mouse models lacking NK cells or MØ display profound defects in placental development and pregnancy progression (Kwak-Kim and Gilman-Sachs, 2008; Pollard et al., 1991). Regulatory NK (NKr1) cells, $Foxp3^+$ regulatory T (Tregs) cells, and $Foxp3^-$ type 1 T regulatory (Tr1) cells, as well as fetal trophoblast cells, also secrete immunosuppressive IL-10, which suppresses inflammatory responses directed toward the fetus (Higuma-Myojo et al., 2005; Svensson-Arvelund et al., 2015; Wang et al., 2009). In fact, several regulatory factors including IL-10, TGF-β, and macrophage colony stimulatory factory 1 produced by fetal trophoblasts are important for appropriate differentiation of decidual macrophages and Tregs (Svensson et al., 2011).

Failure to maintain a balanced immune response during the peri-implantation period leads to excessive inflammation and is thought to contribute to pregnancy complications including fetal growth restriction, spontaneous abortion, and preeclampsia (Borzychowski et al., 2005; Kwak-Kim and Gilman-Sachs, 2008; Pollard et al., 1991). Fetal growth restriction and the development of preeclamptic-like signs in mouse models have been linked to neutrophil and T cell infiltration into implantation sites and the production of excessive inflammatory cytokines and mediators, such as NO (Gelber et al., 2015; Haddad et al., 1997; Nancy et al., 2012). For example, elevated NO production after lipopolysaccharide injection restricted mouse embryo development and increased fetal resorption rates, both of which were reversed by treatment with iNOS inhibitor, aminoguanidine (Barroso et al., 1998; Ogando et al., 2003). These data underscore the importance of a tightly controlled immune response in successful pregnancy outcomes.

BPH/5 mice are genetically pre-hypertensive and spontaneously develop the hallmarks of preeclampsia including late-gestational hypertension and proteinuria (Davisson et al., 2002). While elevated blood pressure is a known risk factor for the disease (Sones and Davisson, 2016), the results obtained with the BPH/5 model should be interpreted in the context of the subset of preeclampsia patients with pre-hypertentsion. BPH/5 mice exhibit early deficiencies in placental development including decreased trophoblast invasion and reduced spiral artery remodeling (Dokras et al., 2006). Recent studies also showed excessive inflammation in the implantation site, which was associated with decreased litter size and decreased pup weight (Gelber et al., 2015; Sones et al., 2016). Neutrophil depletion or the use of pharmacological inhibition of complement or Cox-2 reduced excessive inflammation and improved pregnancy outcomes in this model (Gelber et al., 2015; Sones et al., 2016).

Previous work in the laboratory identified a decrease in CD122+/DBA-lectin+ decidual NK cells, suggesting that increased inflammation seen in the BPH/5 mouse model was associated with a perturbation in immune cell populations (Sones and Davisson, 2016). As macrophages are the second most prevalent immune cell type in the decidua, we hypothesized that BPH/5 implantation sites may exhibit alterations in macrophage location and/or function during early pregnancy. BPH/5 decidua displayed a significant reduction in macrophage number, which was associated with a concomitant increase in the percentage of activated T cells. The decidua also exhibited reduced IL-10 and increased *iNOS* expression. These data indicate a shift in the balance from immune suppression toward immune activation in the BPH/5 implantation site and may explain defects in trophoblast invasion, spiral artery remodeling, and placental development seen in BPH/5 mice.

2. Materials and Methods

A more detailed description of materials and methods can be found in the supplemental information.

2.1. Animals

BPH/5 and C57BL/6 mice were bred in-house as described (Davisson et al., 2002; Schlager, 1974). Virgin females (8–12 weeks old) were mated and pregnancy was confirmed by the detection of a copulation plug, designated embryonic day (e)0.5. All experiments were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals under the supervision of the Cornell University Center for Animal Resources and Education (CARE).

2.2. Quantitative real-time reverse transcription–polymerase chain reaction

Real-time qPCR was carried out as described previously (Sones et al., 2016; Young et al., 2012).

2.3. Enzyme-linked immunosorbent assay (ELISA)

Homogenates of myometrium and decidua were prepared in phosphate-buffered saline from three randomly selected e7.5 implantation sites from each of 8 C57BL/6 and 8 BPH/5 mice. IL-10 concentration per 500 μL (volume of homogenate) was determined using an IL-10 standard curve and expressed as pg of IL-10/g tissue.

2.4. Flow cytometry

Single cell suspensions from myometrium and decidua harvested at e7.5 or e8.5 were stained for viability using Fixable viability stain 510 (BD Pharmingen) and stained for the indicated surface and intracellular markers. See Supplementary Table 1 for a list of antibodies used and their concentrations.

2.5. Immunofluorescence

About 10 μm sections of implantation sites at e7.5 were stained for F4/80 and iNOS followed by appropriate fluorescently labeled secondary antibodies. Nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI), and slides were mounted with prolong gold prior to imaging on the Scanscope FL (Life Technologies).

2.6. Statistics

Results are expressed as mean ± SEM. Statistical analysis was carried out using Prism 5 (GraphPad). Comparison of two groups was carried out using an unpaired two-tailed Student's *t*-test. Analysis of data with more than two groups was carried out using a one-way ANOVA followed by Bonferroni's multiple comparisons post-test. Significance was set at $p < 0.05$.

3. Results

3.1. BPH/5 implantation sites have reduced numbers of $F4/80^+$ macrophages

Since macrophages within the decidua suppress inappropriate immune activation to fetal antigens, and BPH/5 implantation sites display excessive inflammation, we compared the number and localization of $F4/80^+$ macrophages present in implantation sites from BPH/5 and control C57BL/6 mice during the peak of decidualization (e7.5–8.5). Most macrophages in both strains were located in the surrounding muscle layer of the uterus or myometrium (Fig. 1A andD). We quantitated dMØ on the antimesometrial and mesometrial sides of

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