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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 to 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 (iNOS). 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.

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Keywords

macrophages; T cells; iNos; IL-10; tolerance

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., 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 (M-CSF) 1 produced by fetal trophoblasts are important for appropriate differentiation of decidual macrophages and T regs (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-hypertension. 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 lab 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 previously described (Sones et al., 2016; Young et al., 2012).

2.3 Enzyme-linked Immunosorbent Assay (ELISA)

Homogenates of myometrium and decidua were prepared in PBS from three randomly selected e7.5 implantation sites from each of 8 C57BL/6 and 8 BPH/5 mice. IL-10 concentration per 500µL (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 supplemental table 1 for a list of antibodies used and their concentrations.

2.5 Immunofluorescence

10µm 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 $\pm$ 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 (Figure 1A and 1D). We quantitated dMØ on the antimesometrial and mesometrial sides of the implantation site (Figure 1G). While macrophages were present on the mesometrial side of the decidua, the site of placenta formation, in C57BL/6 implantation sites (Figure 1 B–C), they were noticeably diminished on the mesometrial side of BPH/5 decidua (Figure 1 E–F, and I). There was also a decrease in macrophage number on the antimesometrial side (Figure 1H), indicating an overall decrease in macrophages throughout the BPH/5 decidua.

3.2 BPH/5 decidua have altered CD14+ macrophage populations

Macrophages can be identified by the expression of surface markers such as CD14 and CD11b (Houser et al., 2011; Zhao et al., 2015). Houser et al. used CD14, CD11b, and CD11c to characterize macrophages in first trimester human decidua. We used two gating strategies to confirm the identity of macrophages in the decidua by flow cytometry; the Houser et al gating strategy and an additional strategy in which these same samples were gated based on F4/80 and CD64 (Supplemental Figure 2). We obtained the same results regardless of gating strategy used; however, F4/80 and CD64 more accurately identified macrophages populations in the mouse. After identifying F4/80+/CD64+ macrophages, we compared CD11c expression on the CD11b+ population of macrophages in BPH/5 and C57BL/6 decidua. We identified two populations of macrophages, CD11c^{hi} (upper right quadrant) and CD11c^{low} (lower right quadrant) in both C57BL/6 and BPH/5 mice (Figure 2 A–B; FMOs and Backgating can be found in Supplemental Figure 1 and 2). The percent of CD11c^{hi} cells was significantly reduced in the BPH/5 decidua (Figure 2C), while the percent of CD11c^{low} cells was increased (Figure 2D). Thus we conclude that macrophages within the BPH/5 decidua are skewed toward more CD11c^{low} macrophages.

3.3 IL-10 is reduced in BPH/5 myometrium and decidua

As Houser et al. found that CD11c^{hi} dMØ are the primary producers of IL-10 in human first-trimester decidua, we hypothesized that the reduction in the proportion of CD11c^{hi} cells seen in BPH/5 decidua may correlate with lower decidual IL-10 concentrations (Houser et al., 2011). Alternatively, IL-10, which is also produced by decidual stromal cells, is important for the appropriate differentiation of dMØ and reduced IL-10 may account for the reduction in dMØ seen in BPH/5 mice (Svensson et al., 2011). IL-10 was significantly reduced in BPH/5 myometrium and decidua compared to C57BL/6 controls (Figure 3A). Furthermore, IL-10 was significantly higher in normal C57BL/6 decidua compared to C57BL/6 myometrium; however, this difference was not observed in BPH/5 (Figure 3A). Thus, the reduction of total dMØ and specifically CD11c^{hi} macrophages is associated with decreased IL-10 production in BPH/5 implantation sites, which may play a role in the increased inflammation seen in BPH/5 decidua. Decreased IL-10 is associated with several pregnancy complications, including preeclampsia (Chatterjee et al., 2014; Lai et al., 2011).

3.4 Regulatory T and regulatory NK cells are similar in BPH/5 and C57BL/6 decidua

In addition to macrophages, Tregs and NKr1 (CD25⁺) cells can also produce IL-10 (Gagliani et al., 2013; Higuma-Myojo et al., 2005). Among Tregs, the Tr1 subset (Foxp3⁻/CD49b⁺/LAG-3⁺) produces significantly more IL-10 than the Foxp3⁺/CD25⁺ subset (Gagliani et al., 2013). Gating was determined based on FMO staining (Supplemental Figure 3) There was no significant difference in the percent of Foxp3⁺ Treg cells in the decidua of BPH/5 compared to C57BL/6 controls at e7.5 (Supplemental Figure 4). Furthermore, we did not detect any Tr1 or NKr1 cells in either the C57BL/6 or BPH/5 decidua (Supplemental Figure 2). Thus, these cell types are not a significant source of IL-10 production in BPH/5 or C57BL/6 implantation sites at this time point.

3.5 iNOS mRNA expression is increased in BPH/5 myometrium and decidua

We investigated the expression of *iNos*, which is expressed by classical proinflammatory macrophages and associated with embryo resorptions (Haddad et al., 1997). Real-time qPCR analyses demonstrated that there was no significant difference in *iNos* mRNA expression in whole implantation sites when experimental and control animals were compared at early time points (e4.5pm and e5.5am), immediately after implantation (Supplemental Figure 5). By e7.5 a significant increase in *iNos* expression in BPH/5 implantation sites could be detected when compared to C57BL/6 controls (Supplemental Figure 5). Further comparison of *iNos* expression specifically in the decidua showed *iNos* was significantly upregulated in BPH/5 decidua at e7.5 when compared to C57BL/6 controls (Figure 3B). The increase in *iNos* mRNA expression in BPH/5 implantation sites was also greater in the decidua compared to the surrounding myometrium (Figure 3B). Immunofluorescence revealed that iNos is expressed throughout the decidua, especially around the embryo; however, iNos did not appear to colocalize with F4/80+ macrophages (Figure 3C–F). Thus, BPH/5 decidua has higher levels of iNos expression, which is produced by cells other than macrophages.

3.6 BPH/5 implantation sites exhibit increased numbers of activated T cells

Since IL-10 was reduced and *iNos* mRNA was increased in BPH/5 mice, we hypothesized that T cell infiltration into the decidua may be increased (Nancy et al., 2012). We found that the percent of CD3e⁺ T cells within the total CD45⁺ lymphocyte population was increased at e8.5 in both the myometrium and decidua of BPH/5 implantation sites compared to C57BL/6 controls (Figure 4A–B). Although no myometrial CD3e⁺ T cells expressed the activation marker CD69⁺ (Figure 4C), a higher percentage of CD3e⁺ T cells expressed CD69 in BPH/5 decidua compared to C57BL/6 controls (Figure 4D). It is unlikely that these CD4⁺/CD69⁺ T cells are Tregs, as we did not see a difference in Tregs based on Foxp3⁺/CD25⁺ staining (Supplemental Figure 4). The increase in the percentage of BPH/5 decidual CD3e⁺ T cells correlated with increases in the percentages of both CD4⁺ and CD8⁺ T cells (Figure 4F and 4H), while only the percentage of CD4⁺ T cells was increased in the myometrium (Figure 4E and G). Thus, both the percent and the activation status of T cells were altered in BPH/5 implantation sites, suggesting a shift toward a proinflammatory state. Since an increase in proinflammatory T cells is linked to poor pregnancy outcomes such as intrauterine growth restriction (Erlebacher, 2013a, 2013b), we measured whether there was an impact on the weight of implantation sites during peak decidualization. There was a significant increase in implantation site weight (Supplemental Figure 6A), which may be the result of increased infiltration of inflammatory cells. The percent of CD45⁺ (common leukocyte antigen), which is found on most immune cells, was compared between C57BL/6 and BPH/5 mice (Supplemental Figure 6B). There was no difference in the percentages of CD45⁺ cells. Embryo-to-fetus transition does not occur until e10.5 when organogenesis and formation of the functioning fetoplacental unit occurs. At this time point, we saw evidence of intrauterine growth restriction as indicated by decreased crown-rump measurements and fetal weights at e12.5 when compared to C57BL/6 controls (Sones et al., 2016).

4. Discussion

Successful pregnancy requires a delicate balance of pro- and anti-inflammatory mediators. Immune cells within the uterus must balance the need to suppress immune activation against fetal antigens with the need to protect the mother against harmful invading pathogens (Cotechini et al., 2014; Erlebacher, 2013a; 2012; Ziegler et al., 2016). An overactive/inappropriate immune response to pathogens, commensal organisms, or fetal allo-antigens is associated with several pregnancy complications including fetal growth restriction, spontaneous abortion, and preeclampsia (Borzychowski et al., 2005; Kwak-Kim and Gilman-Sachs, 2008; Pollard et al., 1991).

We used preeclamptic-like BPH/5 mice to study immune events that occur during the peak of murine decidualization (e7.5–e8.5). Early in pregnancy murine uterine stromal cells undergo radical growth and differentiation to regulate trophoblast invasion, maternal vascular remodeling, and placental formation (Bell and Searle, 1981). These processes are of particular interest in preeclampsia as abnormal placentation is thought to play a causal role in the maternal syndrome. Since macrophages play important roles in regulating the immune response to invading trophoblast cells and tissue remodeling, events required for normal placentation during human pregnancy (Nagamatsu and Schust, 2010; Yang et al., 2011), we sought to examine them in BPH/5 implantation sites early in pregnancy before the onset of hypertension and proteinuria. Although there are some caveats to the use of BPH/5 mice as a model of preeclampsia, it is the only mouse model that spontaneously develops late-gestation hypertension and proteinuria.

Our previous studies in the BPH/5 model showed increased inflammation, decreased trophoblast invasion, reduced spiral artery remodeling, and a reduction in NK cell number (Dokras et al., 2006; Gelber et al., 2015; Sones et al., 2016, 2014). Consistent with these findings, our current studies show a decrease in the number of macrophages in the decidua of BPH/5 implantation sites compared to C57BL/6 controls. Trophoblast invasion during placenta formation is directed toward the mesometrial side and fewer macrophages in BPH/5 decidua may adversely affect trophoblast invasion and placenta formation (Renaud and Graham, 2008). The reduction in decidual macrophages could be due to diminished macrophage migration into the decidua, perhaps due to changes in chemokine expression, or an inability of dMØ to proliferate once they enter the tissue. Svensson et al. demonstrated that IL-10 was important for the appropriate differentiation of macrophages in vitro (Svensson et al., 2011). Thus, lower IL-10 levels could impede macrophage differentiation, which could also account for lower numbers of macrophages in the decidua of BPH/5 mice. Additional investigation is necessary to determine which of these mechanisms is responsible for the reduction in macrophage number.

There are at least two macrophage populations within human pregnant decidua (Houser et al., 2011). CD11c^{hi} macrophages express more IL-10 than their CD11c^{low} counterparts. Furthermore, both populations of macrophages exhibit characteristics of M1 (proinflammatory) and M2 (tissue-remodeling) polarization (Houser et al., 2011; Xu et al., 2016). As mentioned earlier, we analyzed macrophages using two different gating strategies. We obtained the same results regardless of gating strategy used; however, F4/80 and CD64

more accurately identified macrophage populations in the mouse. While F4/80 and CD64 are commonly used to identify macrophages, we cannot exclude the possibility that our analysis may include some DCs. However, work characterizing mouse decidua shows that DCs make up 1–2% of immune cells while macrophages are 10–20% (Erlebacher, 2013a). We found a reduction in the percentage of CD11c^{hi} dMØ in BPH/5 e7.5 decidua, which was associated with decreased levels of immunosuppressive cytokine IL-10. IL-10 null-mice develop preeclampsia-like signs under hypoxic conditions and supplementation of IL-10 in the Reduced Uterine Perfusion Pressure (RUPP) model of preeclampsia reduces inflammation and endothelial dysfunction (Harmon et al., 2015; Lai et al., 2011). Unexpectedly, we did not identify any Tr1 or Nkr1 cells in either the BPH/5 or C57BL/6 control decidua. Since C57BL/6 decidua maintains IL-10 expression, the decreased IL-10 does not explain their absence in both C57BL/6 and BPH/5 mice. IL-10 is thought to play an important role in supporting tolerance to fetal antigens and women with preeclampsia have decreased circulating levels of IL-10 as well as a concomitant accumulation of activated T cells and decreased Tregs in the uterus (Couper et al., 2008; Harmon et al., 2015). Diminished IL-10 may result in loss of tolerance to fetal antigens, which is important for trophoblast invasion and spiral artery remodeling.

iNos is normally expressed during the first half of pregnancy, where production of NO plays a critical role in vasodilation and placental development. Excessive *iNos* expression in dMØ after exposure to proinflammatory stimuli is linked to embryo loss, which could be prevented by treatment with the *iNos* inhibitor, aminoguanidine (Haddad et al., 1997). Our study showed that the reduction in macrophage number and IL-10 production seen in BPH/5 decidua was associated with an increase in *iNos* mRNA expression; however, macrophages did not appear to be the source of *iNos*. This suggests that the upregulation of *iNos* in BPH/5 occurs in a cell type other than macrophages such as decidual NK cells or decidual stromal cells (Hunt et al., 1997; Ogando et al., 2003).

Since NO is subsequently involved in T cell recruitment and activation, it is not surprising that BPH/5 decidua also showed an increase in activated T cells. Recognition of fetal antigens on trophoblast cells by activated T cells and subsequent trophoblast destruction may account for the decreased trophoblast invasion and reduced spiral artery remodeling seen in the BPH/5 mouse. Nancy et al. reported that treating pregnant mice with proinflammatory cytokines resulted in an increase in T cell migration into the decidua (Nancy et al., 2012). Therefore, the ability to induce an inflammatory response without activating fetal-specific T cells is an important factor in maintaining a successful pregnancy.

The phenotype seen in BPH/5 mice likely reflects a general perturbation of inflammatory cells including NK cells as shown in our previous work (Davisson et al., 2002; Sones and Davisson, 2016; Sones et al., 2016) and macrophages and T cells presented here. The reduction in macrophages in BPH/5 implantation sites during the peri-implantation period could reduce fetal tolerance and contribute directly to inflammation. Loss of macrophage-mediated homeostasis could result in the increased infiltration of inflammatory T cells seen here. The use of selective Cox-2 inhibitors or neutrophil depletion, as were carried out in previous studies using the BPH/5 mouse, may overcome the loss of macrophage-mediated immune suppression and restore the balance of regulatory mediators, resulting in improved

pregnancy outcomes (Gelber et al., 2015; Sones et al., 2016; Woods et al., 2011). Further work is necessary to determine the link between these early immune defects and the onset of hypertension late in pregnancy in these mice. Elucidation of defective immune processes before the onset of hypertension will help to identify potential therapeutic targets or genetic determinants that could aid in the earlier diagnosis and treatment of preeclampsia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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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
PGE₂	Prostaglandin E ₂
RUPP	Reduced Uterine Perfusion Pressure
Tr1	type 1 regulatory T cells
VEGF	vascular endothelial growth factor

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Highlights

- The BPH/5 preeclamptic-like mouse exhibits an overall reduction in decidual macrophages
- Loss of decidual macrophages is concurrent with a decrease in the immunosuppressive cytokine IL-10 and increased iNOS.
- Loss of immune suppression in BPH/5 decidua is associated with increases in activated T cells.

Figure 1. F4/80+ decidual macrophages are diminished in BPH/5 preeclamptic-like mice
Representative images of F4/80+ macrophages (green), and DAPI nuclear staining (blue) in e7.5 implantation sites from C57BL/6 (A, B, C) and BPH/5 (D, E, F) mice. Antimesometrial side is at the top of the image. (G) Schematic of an implantation site with the regions quantified by HALO software (Life Technologies) outlined in yellow (antimesometrial, H) and red (mesometrial, I). F4/80+ cells are normalized to the area (mm²). n = 10. Data shown as mean ± SEM, Antimesometrial side *p<0.05, Mesometrial side ***p< 0.001.

Figure 2. Macrophage populations are altered in BPH/5 decidua

Representative flow cytometry of CD14⁺ macrophages based on CD11c and CD11b staining of C57BL/6 (A) and BPH/5 (B) decidua at e7.5. Each flow experiment compared decidua pooled from all implantation sites in a pregnancy for 1 C57BL/6 and 1 BPH/5 mouse. Comparison of CD11c high (C) and CD11c low (D) populations pooled from 5 experiments. Data shown as mean $\pm$ SEM, * $p < 0.05$.

Figure 3. Implantation sites from BPH/5 preeclamptic-like mice are characterized by a shift from an anti- to pro-inflammatory environment

IL-10 protein in the myometrium and decidua was measured by ELISA (A), $n=8$. Data represent mean $\pm$ SEM, $*p<0.05$, $***p<0.001$. Expression of *iNos* mRNA was measured in the myometrium and decidua by quantitative real-time PCR (B). $n=9$, data shown as mean $\pm$ SEM, $**p<0.01$. Representative images from 5 experiments with F4/80 (green), iNos (red), and DAPI nuclear staining (blue) in C57BL/6 (C and D) and BPH/5 (E and F) implantation sites. Arrows indicate F4/80+ cells and arrowheads indicate iNos positive cells.

Figure 4. Activated T cells migrate into BPH/5 decidua

CD3⁺ T cells in individual myometrium (A) and decidua (B) from e8.5 C57BL/6 and BPH/5 implantation sites were quantified by flow cytometry. The CD69 activation marker was measured on CD3⁺ cells in the myometrium (C) and decidua (D). CD4⁺/CD3⁺ cells in the myometrium (E) and decidua (F). CD8⁺/CD3⁺ cells in the myometrium (G) and decidua (H). n = 7–15 for CD3⁺ n = 5–8 for CD4, CD8, and CD69. Data is shown as mean ± SEM *p < 0.05.