HHS Public Access

Author manuscript

Clin Sci (Lond). Author manuscript; available in PMC 2019 February 14.

Published in final edited form as:

Clin Sci (Lond). 2018 February 14; 132(3): 419–436. doi:10.1042/CS20171059.

Elevated Vasopressin in Pregnant Mice Induces T Helper Subset Alterations Consistent with Human Preeclampsia

Sabrina M. Scroggins, PhD[1,2], **Donna A. Santillan, PhD**[1,2,3], **Jenna M. Lund, BS**[4], **Jeremy A. Sandgren, BS**[4,5], **Lindsay K. Krotz, BS**[1], **Wendy S. Hamilton, BS**[1], **Eric J. Devor, PhD**[1], **Heather A. Davis, MS**[6], **Gary L. Pierce, PhD**[3,7], **Katherine N. Gibson-Corley, DVM, PhD**[8], **Curt D. Sigmund, PhD**[3,5], **Justin L. Grobe, PhD**[*,3,5,9,10], and **Mark K. Santillan, MD, PhD**[*,1,2,3]

[1]Department of Obstetrics and Gynecology, University of Iowa, Iowa City, Iowa 52242

[2]Center for Immunology and Immune Based Diseases, University of Iowa, Iowa City, Iowa 52242

[3]Center for Hypertension Research, University of Iowa, Iowa City, Iowa 52242

[4]Carver College of Medicine, University of Iowa, Iowa City, Iowa 52242

[5]Department of Pharmacology, University of Iowa, Iowa City, Iowa 52242

[6]Institute for Clinical and Translational Science, University of Iowa, Iowa City, Iowa 52242

[7]Department of Health and Human Physiology, University of Iowa, Iowa City, Iowa 52242

[8]Department of Pathology, University of Iowa, Iowa City, Iowa 52242

[9]Obesity Research and Education Initiative, University of Iowa, Iowa City, Iowa 52242

[10]Iowa Neuroscience Institute, University of Iowa, Iowa City, Iowa 52242

Abstract

The pathogenesis of preeclampsia, a hypertensive disorder of pregnancy, involves imbalanced T helper (T_H) cell populations and resultant changes in pro- and anti-inflammatory cytokine release. Elevated copeptin (an inert biomarker of arginine vasopressin (AVP)), secretion precedes the development of symptoms in preeclampsia in humans, and infusion of AVP proximal to and throughout gestation is sufficient to initiate cardiovascular and renal phenotypes of preeclampsia in wild-type C57BL/6J mice. We hypothesize that AVP infusion in wild-type mice is sufficient to induce the immune changes observed in human preeclampsia. AVP infusion throughout gestation

Corresponding Author: Mark K. Santillan, Department of Obstetrics & Gynecology, University of Iowa, 200 Hawkins Drive, PFP 31330, Iowa City, IA 52242, Tel: (319) 356-3180, Fax: (319) 384-8663, mark-santillan@uiowa.edu, Justin L. Grobe, Department of Pharmacology, University of Iowa, 200 Hawkins Drive, 2-307 BSB, Iowa City, IA 52242, Tel: (319) 353-5789, justin-grobe@uiowa.edu.

*Co-corresponding authors

Disclosure Statement: The authors report no conflict of interest.

Author Contribution

S.M.S, D.A.S., W.S.H., E.J.D., H.A.D., C.D.S., J.L.G., and M.K.S. designed the experiments, experiments and data collection were performed by S.M.S., D.A.S., J.M.L., J.A.S., L.K.K., W.S.H., E.J.D., H.A.D., K.N.G-C., J.L.G., and M.K.S., data analysis was performed by S.M.S., D.A.S., E.J.D., and M.K.S., data interpretation was conducted by S.M.S., D.A.S., E.J.D., G.L.P., C.D.S., J.L.G., and M.K.S., and all authors contributed to and approved the final manuscript.

*The funding sources had no involvement in study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

in mice resulted in increased pro-inflammatory interferon gamma (IFNg) (T_H1) in the maternal plasma. The T_H17-associated cytokine IL-17 was elevated in the maternal plasma, amniotic fluid, and placenta following AVP infusion. Conversely, the T_H2-associated anti-inflammatory cytokine interleukin (IL)-4 was decreased in the maternal and fetal kidneys from AVP-infused dams while IL-10 was decreased in the maternal kidney and all fetal tissues. Collectively, these results demonstrate the sufficiency of AVP to induce the immune changes typical of preeclampsia. We investigated if T cells can respond directly to AVP by evaluating the expression of AVP receptors (AVPRs) on mouse and human CD4+ T cells. Mouse and human T cells expressed AVPR1a, AVPR1b, and AVPR2. The expression of AVPR1a was decreased in CD4+ T cells obtained from preeclampsia-affected women. In total, our data are consistent with a potential initiating role for AVP in the immune dysfunction typical of preeclampsia and identifies putative signaling mechanism(s) for future investigation.

Keywords

APCs= antigen presenting cells; AVP= arginine vasopressin; AVPR= arginine vasopressin receptor; BLD= below the limit of detection; CLII= major histocompatibility complex II; Ct= cycle threshold; DC= dendritic cell; ICS= intracellular staining; IFNg= interferon gamma; IL-= interleukin; LAP= latency associated peptide; MFTB= maternal fetal tissue bank; MNC= mononuclear cell; PD-L1= programmed death ligand 1; pg/g= picogram per gram; PIR B= paired immunoglobulin receptor B; PreE= preeclampsia; TGFb= transforming growth factor beta; T_H= T helper cell; Treg= T regulatory cell

Introduction

Preeclampsia, a hypertensive disorder in pregnancy, affects 5–7% of all U.S. pregnancies and yet, disproportionately comprises 15% of all maternal-fetal morbidity and mortality (1). Preeclampsia is known to cause immediate and long-term maternal and fetal morbidities (2–7). Current methods of early diagnosis and prevention of preeclampsia are limited and provide at best a few months of lead time before clinical symptoms appear (8). Aspirin can be used as a preventative agent with varied effectiveness (9, 10). This prevention is partially thought to be due to an early modulation of immune responses. If we can unravel the early immune pathogenic mechanism(s) involved in the development of preeclampsia, there is potential for improved prevention.

Human maternal plasma copeptin, a stable biomarker of arginine vasopressin (AVP) secretion, is elevated mid- to late-pregnancy in women who develop preeclampsia (11–16). Importantly, copeptin is a robust early-pregnancy (as early as the 6th week of gestation) predictor of the development of preeclampsia (17). Further, chronic infusion of AVP in wild-type, pregnant C57BL/6J females is sufficient to model human preeclampsia by inducing pregnancy-specific hypertension, proteinuria, glomerular endotheliosis, and fetal growth restriction (17). These data support a role for AVP in the early pathogenesis of preeclampsia, and provide key insight toward the future development of therapeutic interventions for preeclampsia

While the development of preeclampsia is multifactorial and involves many systems and processes including inadequate trophoblastic invasion and poor spiral artery remodeling, an altered inflammatory response is thought to be involved in the early pathogenesis of preeclampsia (18–23). In healthy pregnancies, pro-inflammatory CD4+ T helper (T_H) 1 related activity is dominant early and later shifts to a more anti-inflammatory T_H2 type of immune response. An imbalance of these T_H1 and T_H2 cells occurs during preeclampsia (24–27). This paradigm has expanded to include an increase in pro-inflammatory T_H17 cells (24, 28–30). Although the precise mechanisms involved in the development of preeclampsia are complex and poorly understood, an aberrant pro-inflammatory T_H cell response is clearly involved (18, 21, 31–34).

Roles for AVP in the regulation of blood pressure and fluid homeostasis are generally accepted, however, a role for AVP in immune system function is less well appreciated. AVP secretion stimulates pro-inflammatory cytokine secretion and activation of lymphocytes (35–38). The immune system is vital to the development of vascular dysfunction in hypertensive diseases. McMaster *et al.* review the evidence demonstrating T cells and T cell-derived cytokines, such as interferon gamma (IFNg) and interleukin-17 (IL-17), play a role in the renal and vascular dysfunction present in hypertensive diseases (39). As in preeclampsia, hypertension is thought to skew T cells towards pro-inflammatory T_H1 (IFNg) and T_H17 (IL-17) dominant phenotypes (40). T_H IFNg production is a dynamic interaction that requires the presence of IL-2. Suppressor cells absorb IL-2, preventing induction of IFNg secretion and inhibiting T_H1-associated responses (36, 37, 41). As reviewed by Chikanza *et al.*, AVP has been shown to boost T_H1 responses *in vitro* and *in vivo* via enhancement of IFNg (42, 43). Further, AVP has been shown to replace the requirement of IL-2 for the production of IFNg, thus inhibiting appropriate down-regulation of T_H1 responses. Further, Johnson *et al.* show that AVP induces proliferation of C57BL/6 thymocytes in culture (36, 37, 44). Taken together, these data support the potential for AVP to induce increased IFNg (and thus a T_H1 response) in an IL-2 independent manner to circumvent appropriate conversion from an inflammatory T_H1 response needed for placentation and spiral artery remodeling to a more anti-inflammatory T_H2 environment. To our knowledge, the potential role of T_H17 and IL-17 in the regulation of AVP production has not been investigated. Further, this immunologic role of AVP has not been assessed in pregnancy. Thus, AVP is uniquely positioned to potentially initiate known mechanisms of preeclampsia, and may therefore represent an early cause of preeclampsia. The objective of this study is to test the hypothesis that AVP infusion during pregnancy in wild-type C57BL/6J mice is sufficient to induce the immunologic alterations observed in human preeclampsia. These data may provide novel insights into immune mechanisms mediated by AVP in preeclampsia.

Materials and Methods

Animal Studies

The animal procedures used were approved by the University of Iowa Institutional Animal Care and Use Committee. Wild-type 12–16 week old C57BL/6J male and female mice were obtained from Jackson Laboratories and maintained on standard chow under standard care conditions. Virgin females were subcutaneously implanted with osmotic mini-pumps (Alzet

Model #1004, Cupertine, CA) infusing either saline or AVP (24 ng/hr, Sigma, St. Louis, MO) as previously described (17). Three days after implantation, dams were individually mated for a single overnight period. Pregnancies were timed by post-coital vaginal plugs indicative of gestational day (GD) 0.5. Maternal and fetal tissues were harvested on GD 18. Data were collected from multiple pregnancies from independent experiments. A subset of the cohort presented in the current study was previously published by Santillan *et al.* (17) and subsequent experiments have confirmed the preeclampsia phenotype (pregnancy-specific hypertension, proteinuria, fetal growth restriction, and kidney glomerular endotheliosis). Each pregnancy was considered N=1 for maternal tissues. Due to fetal tissue mass, 5 pairs of fetal kidneys and 5 fetal livers were pooled for an N=1 from a single pregnancy. Maternal tissues (plasma, kidney, and liver) and fetal tissues (amniotic fluid, kidney, liver, and placenta) had $N \geq 5$ per group from at least two independent experiments. All whole tissues (except spleen) and plasma were stored at −80°C until protein extraction and analysis. Maternal spleen was kept on ice in phosphate buffered saline supplemented with 2% fetal bovine serum for immediate dissociation of cells.

Tissue Protein Extraction and Protein Analysis

Total protein lysate was generated by homogenization of tissues in buffer containing 5M NaCl, 1M Tris, 0.5M EDTA, NP-40, protease inhibitor (Roche, Switzerland), and phosphatase inhibitor (Roche, Switzerland). A commercially available bicinchoninic acid (BCA) protein assay kit (Thermo Fisher Scientific, Waltham, MA) was utilized per protocol to assess total protein concentration. Plasma and tissue protein extracts were diluted where needed and commercially available ELISAs for mouse pro-inflammatory IFNg (T_H1) and IL-17 (T_H17) and anti-inflammatory IL-4 (T_H2), IL-10 (T_H2), and TGFb cytokines were performed per protocol in duplicate (eBioscience, San Diego, CA). Cytokine concentrations were normalized to total protein and are presented as picograms/gram of total protein (pg/g).

Cell Preparation and Flow Cytometric Analysis

Single cell suspensions were prepared by mechanical maceration from spleens of saline and AVP-infused dams followed by density gradient separation (FicoLite LM, Atlanta Biologicals, Lawrenceville, GA) to obtain MNCs. Cell suspensions were then stained with fluorochrome-conjugated or biotinylated antibodies (Supplemental Table 1), followed by streptavidin-conjugated fluorochromes. Fluorochrome-conjugated, purified rat immunoglobulins were used as isotype controls for background fluorescence. All cell samples were incubated with anti-CD16/32 (clone 2.4G2) and rat serum during staining to prevent background FcγR binding. Intracellular Fixation and Permeabilization Buffer Set (eBioscience, San Diego, CA) was used per protocol for intracellular staining (ICS) of cytokines. Following staining, cells were fixed with either 0.1% formaldehyde or intracellular fixation buffer (eBioscience, San Diego, CA) where appropriate. Flow cytometric data were obtained within 24 hours using a Becton Dickinson LSR II (San Jose, CA) and analyzed using FlowJo software (Treestar Inc., Ashland, OR). Dead cells were excluded by forward/orthogonal light scatter characteristics. Single cells were identified via forward scatter-area (FSC-A) versus side scatter-width (SSC-W). Gating strategies of CD4+ T cells and dendritic cells (DCs) are shown in Supplemental Figure 2.

Human Studies

Human mononuclear cells (MNCs) from control (n=27) and preeclampsia-affected (n=24) pregnancies were obtained from the University of Iowa Maternal Fetal Tissue Bank (MFTB, IRB# 200910784). The MFTB is a pregnancy focused human biorepository with clinically annotated biosamples collected throughout gestation with quality control measures for clinical data and biosample integrity. As a prospective, cross-sectionally collected biorepository and clinical data warehouse, early pregnancy samples are collected before the onset of diagnoses. Coded clinical data were obtained through the MFTB as previously published (45), which derives data from our Clinical Research Data Warehouse. The human control and preeclampsia-affected samples used in this study are a subset of a previously published cohort (17) and no differences in characteristics were observed between groups (Supplemental Table 2). The diagnosis and classification of PreE was based on the standard American College of Obstetrics and Gynecology (ACOG) definitions for analysis (46). PreE cases were identified by cross-referencing the MFTB database with the bioinformatics query of ICD-9 and ICD-10 codes of bank participants at the time of delivery. The electronic medical record of each potential case was evaluated by the MFTB to confirm the diagnosis of PreE by the ACOG definitions (46). Case-control verification training of the MFTB and verification of cases and controls was led by the senior author (MKS) who is a clinical maternal fetal medicine specialist. Maternal age-matched plasma samples and corresponding clinical data for the control population were obtained by querying the MFTB database. Control pregnancies were pregnant women who did not develop PreE. The gestational age at the time of sample collection was recorded. MNCs are all processed and stored for viability in liquid nitrogen as previously described (45).

CD4+ T cell isolation

Human MNCs obtained from the MFTB. Splenic murine mononuclear cells were prepared as described above (cell preparation). Human and mouse CD4+ T cells were negatively selected using species-specific EasySep CD4+ T Cell Enrichment Kits per protocol (Stemcell Technologies Inc., Vancouver, BC). Human and mouse CD4+ T cell purity was determined to be ≥ 90% via flow cytometry.

Quantitative PCR

Total cellular RNA was purified from cells using the mirVana miRNA isolation per protocol (ThermoFisher Scientific, Waltham, MA). RNA concentration and purity were determined using a NanoDrop 1000 spectrophotometer (ThermoFisher Scientific, Waltham, MA). Quantitative PCR (qPCR) gene expression assays of AVP receptors 1a (AVPR1a), 1b (AVPR1b), and 2 (AVPR2) were performed following SuperScript III (ThermoFisher Scientific, Waltham, MA) reverse transcription of fixed total RNA mass (500ng). Resulting cDNAs were amplified using primer sets shown in Table 1. All primer efficiencies were between 93–95%. Amplicons were detected in a PowerSYBR Green qPCR assay carried out on an Applied Biosystems 7900HT Real Time PCR System in the Genomics Division of the Iowa Institute of Human Genetics (IIHG). Raw cycle threshold (Ct) values were normalized (ΔCt) against the 18S rRNA endogenous control. The ΔΔCt and expression fold change were calculated as previously described (47, 48).

Statistical Analysis

For continuous variables, a two sided Student's t test with unequal variance was utilized (GraphPad, Prism 7, La Jolla, CA). In addition, chi square was utilized for categorical variables. Statistical significance was designated at $\alpha = 0.05$ or as determined by Bonferroni correction for multiple comparisons ANOVA.

Results

AVP infusion throughout gestation heavily skews maternal T_H immunity toward a pro-inflammatory T_H1 and T_H17 phenotype

We previously demonstrated infusion of AVP into pregnant mice results in the hallmark features of human preeclampsia including, pregnancy-specific hypertension, proteinuria, renal glomerular endotheliosis, and fetal growth restriction (17). In the current study, we utilize this established mouse model of preeclampsia to investigate immune alterations caused by elevated AVP in pregnancy. The T_H1- and T_H17- associated pro-inflammatory cytokines, IFNg and IL-17, were significantly increased in the plasma of AVP-infused dams and unaffected by AVP in the maternal kidney and liver (Figure 1). T cells are activated in secondary lymphoid organs (eg. spleen); and consistent with circulating plasma IFNg and IL-17 elevations, we observed increases in both T_H1 and T_H17 CD4+ T cells in the spleen of AVP-infused dams (Figure 1). These data suggest elevated AVP during pregnancy induces a pro-inflammatory T_H1 and T_H17 milieu in pregnant mice similar to that observed in human preeclampsia-affected women.

Elevated AVP in pregnancy decreases anti-inflammatory T_H2 and TGFb cytokines in pregnant dams

Although we did not observe an AVP-induced change in the frequency of splenic CD4+ T cells producing anti-inflammatory cytokines during pregnancy, there were tissue-specific changes in anti-inflammatory cytokines (Figure 2). The T_H2-associated cytokines, IL-4 and IL-10, as well as the anti-inflammatory cytokine TGFb, were all decreased in the maternal kidneys of AVP-infused dams. Additionally, there was a significant decrease in plasma TGFb in these animals. The levels of IL-4, IL-10, and TGFb were all comparable between the saline and AVP dams in the maternal liver (Figure 2). These data demonstrate AVP infusion during pregnancy results in an overall reduction in maternal anti-inflammatory cytokines.

AVP infusion in pregnancy decreases Treg and alters the ratio of Treg:T_H17 cells

It has been observed in both humans and in animal models that Tregs are reduced and the ratio of Treg to T_H17 cells is altered in preeclampsia (24, 28, 33). Given the changes observed in the anti-inflammatory cytokines IL-10 and TGFb (Figure 2), we next evaluated Treg. Elevated AVP resulted in a decrease in anti-inflammatory Treg cells (Figure 3A) as well as an altered Treg:T_H17 ratio (Figure 3B).

Dendritic cells from AVP-treated dams show enhanced co-stimulatory molecule expression

Dendritic cells (DCs) are potent antigen presenting cells (APCs) that activate and influence T_H cell differentiation. This process involves not only cytokine production by both cell types, but also surface molecule interactions. The expression of co-stimulatory and inhibitory molecules by DCs was evaluated to determine if elevations in AVP during pregnancy alters the activation phenotype of DCs. The co-stimulatory surface molecules major histocompatibility complex class II (CLII), CD80, and CD86 are highly expressed on CD11c+ DCs from AVP-treated versus saline-treated dams (Figure 4A). Concomitantly, the expression of the inhibitory surface molecules programmed death ligand 1 (PD-L1) and paired immunoglobulin receptor B (PIR B) were significantly decreased on DCs from AVP-treated dams (Figure 4B). These data show that AVP programs DCs toward an activated, stimulatory phenotype and thus may enhance antigen presentation and pro-inflammatory T cell activation in preeclampsia.

Fetal exposure to elevated AVP in utero results in enhanced T_H17 and impaired T_H2 cytokine profiles

Unlike maternal tissues, IFNg was not elevated in the fetal tissues from GD 18 AVP-infused dams (Figure 5A). Congruent with increased maternal IL-17, the amniotic fluid, placenta, and fetal kidney all showed increased IL-17 levels compared to tissues from saline infused dams, suggesting increased T_H17 cells (Figure 5B). In addition to increases in pro-inflammatory IL-17, fetal tissues had marked decreases in the anti-inflammatory cytokines IL-4 (T_H2), IL-10 (T_H2), and TGFb. IL-4 was significantly decreased in the amniotic fluid, placenta, and fetal kidney, while IL-10 was decreased in all fetal tissues, including fetal liver (Figure 6A and 6B). These data suggest a global alteration in T_H2 cells in fetal tissues when exposed to elevated AVP during gestation. Further contributing to an elevated pro-inflammatory over an anti-inflammatory cytokine balance, the anti-inflammatory cytokine TGFb was also decreased in the placenta and fetal kidney from AVP-infused pregnancies (Figure 6C). In humans, it is unknown if maternal AVP crosses the placenta. Radiolabeled I^{125}-AVP experiments in pregnant ewes suggest that AVP does not cross the placenta. Further, rat studies (49) suggest that the source of placental AVP is from the fetus. In total, our findings and others suggest that AVP is sufficient directly to cause preeclampsia related physiologic and immunologic phenotypes in the mother. Yet, the fetal physiologic and immunologic phenotypes may be due to a combination of direct stimulation at the maternal-fetal interface and/or a fetal response to the AVP induced maternal phenotypes.

Mouse and human CD4+ T cells express AVP receptors

As T_H cells are altered in response to AVP during pregnancy in mice, CD4+ T cells were isolated and analyzed for expression of AVP receptors to determine if CD4+ T cells may be able to respond directly to elevated AVP. Interestingly, mouse and human CD4+ T cells expressed AVP receptors 1a, 1b, and 2 (Table 2 and 3). Similar to mouse CD4+ T cells, human CD4+ T cells highly expressed AVPR2, but AVPR1a was also highly expressed (Table 3). To focus on potential early and late pregnancy changes in circulating CD4+ T cells, AVPR expression was determined in the first and third trimester of human

pregnancies. CD4+ T cells isolated from human preeclampsia-affected pregnancies expressed significantly lower AVPR1a in the first trimester, with a 6.3 fold decrease in expression (Table 3). Additionally, although AVPR1b was not as highly expressed AVPR1a and 2, there was a 3.7 fold decrease in expression by CD4+ T cells isolated from preeclampsia-affected pregnancies during the first trimester. Interestingly, the changes in AVPR1a and AVPR1b expression observed normalized by the third trimester. Although AVPR2 expression was not altered in the preeclampsia-affected group in the first trimester, it was 1.7 fold increased the third trimester. These data not only demonstrate that CD4+ T cells express AVP receptors, but also that expression of AVPRs is differentially regulated throughout pregnancy in human preeclampsia-affected CD4+ T cells.

Discussion

The early pathogenesis of preeclampsia involves the failure of the maternal immune system to normally tolerate the pregnancy (21). Previous studies have indicated that during a healthy human pregnancy, a shift occurs from a pro-inflammatory T_H1 (IFNg dominant) CD4+ T cell response required early for appropriate placentation to an anti-inflammatory T_H2 (IL-4 and IL-10 dominant) response required for fetal tolerance. The concept that persistent T_H1 related feto-placental intolerance is important in the pathogenesis of preeclampsia has been demonstrated by our lab and others (50–53). T_H1 responses and related cytokines (IFNg) directly inhibit the development of T_H2 responses and vice versa (31, 54, 55). IFNg is elevated in human preeclampsia with a concurrent decrease in IL-10 and IL-4 production, driving a T_H1 response (55–58). These T_H1 cells further increase IFNg production, resulting in increased recruitment and activation of antigen presenting cells and cytotoxic CD8+ T cells. Recently, the production of IL-17 and the percent of T_H17 cells have been shown to be increased in preeclampsia and potentially contribute to poor fetal tolerance (28, 33). Taken together, our data extend previous studies indicating there is both an increase in T_H1 and T_H17 cells, by identifying AVP as a potential, novel contributor to poor fetal tolerance and the development of preeclampsia.

For the first time, our data show that AVP-infusion during mouse pregnancy induces the T_H subset changes and pro-inflammatory milieu similar to that observed in human preeclampsia. In mouse maternal circulation, there is a pro-inflammatory T_H1 and T_H17 milieu with elevated IFNg and IL-17 and a concomitant decrease in anti-inflammatory TGFb. Correlating with previously observed AVP-induced renal changes (17), there were maternal kidney specific decreases in the T_H2-associated anti-inflammatory cytokines IL-4 and IL-10, as well as anti-inflammatory TGFb. Our observed differences in cytokines between the mouse maternal circulation and target tissues, underscores the concept that circulating immune responses may not completely represent tissue-specific immune responses. In addition to producing soluble proteins that are detectable in the circulation, T cells travel to the site of inflammation to exert their function. Although IFNg is elevated in the maternal plasma of our AVP-infused dams, but not in the tissues evaluated, it is likely IFNg is elevated in the spleen (given the observed increase in splenic CD4+ IFNg+ T cells). As T cells are activated in the secondary lymphoid organs and also influence other immune responses, the observed changes in maternal plasma IFNg may be acting at the level of the spleen. In regards to IL-17, the elevation observed in the circulation does correlate with a

known target organ in preeclampsia, the placenta. Lastly, immune cells in the target organs may also locally produce cytokines and further contribute to tissue inflammation and disease progression. Collectively, our data expands what other groups have observed in mouse models and in human preeclampsia (18, 25, 27, 28, 33, 56, 59, 60), in that we observed changes in $T_H1/T_H2/T_H17$ associated cytokines in our AVP-induced mouse model of preeclampsia, highlighting AVP as a novel, potential immunologic agent in the development of preeclampsia.

We contend that AVP is an important initiating pathway in the early immunologic phenotype of preeclampsia. T_H1 cells, seen in abundance in preeclampsia, are potent sources of IFNg which can induce AVP production (61). AVP secretion (via copeptin detection) is elevated very early in human preeclampsia (17) and is capable of inducing the early immunologic changes observed in the first trimester preeclampsia. Additionally, it has been demonstrated that the immune system, more specifically T_H1 and T_H17 cells, play a pivotal role in the development of vascular dysfunction (39, 40). We observed AVP-induced changes in both the maternal and fetal compartments. Although toward the end of mouse gestation, GD 18 mouse pups developmentally correspond to GD 58–60 in humans (62–64), which falls well within the first trimester of human gestation. Our mouse fetal immune findings may translate into early first trimester immunologic changes in the human fetus. This is consistent with the current understanding of a very early initiating cause of preeclampsia, as first trimester immunologic dysregulation lies upstream of the placental/vascular phenotype (21, 65). These observations also further the concept that AVP has immune effects beyond the canonical blood pressure/volume control. Together, these data suggest that AVP, whether via direct interaction with immune cells or indirectly via alterations in cardiovascular and renal function, may be an early cause of immune changes in preeclampsia.

Although the exact role of AVP in immune responses still requires further investigation, AVP is thought to stimulate immune cell activity in non-pregnant environments (35–38, 44, 66). As reviewed by Chikanza *et al.*, rats with chronic inflammatory disease also had elevated levels of plasma AVP. A reduction in circulating AVP decreased the pro-inflammatory response in these animals (67). *In vitro* experiments demonstrated a dose-dependent augmentation of autologous mixed lymphocyte reactions, including the requirement of IL-2 for IFNg production (36, 44). Further, addition of IL-1 beta (IL-1b) or IL-6 to *in vitro* cultures resulted in a dose-dependent increase in AVP production (68). Lastly, human cancer patients administered IL-6 had significantly higher circulating AVP levels within two hours of administration (69).

To our knowledge, the role of AVP in immune responses has not been investigated in pregnancy, and more specifically, not in preeclampsia-affected pregnancies. Here, we demonstrate that in pregnancy, mouse and human CD4+ T cells express AVP receptors, and that AVPR1a and AVPR2 are highest expressed. Interestingly, AVPR1a and 1b expression by human CD4+ T cells is significantly down-regulated in preeclampsia-affected pregnancies in the first trimester and this normalizes by the third trimester. AVPR1a is expressed in blood vessels and is known to play a role in baroreceptor reflexes and blood volume homeostasis (70–72). Although high placental AVPR1a expression has also been purposed as a potential mediator early in pregnancy for increased blood flow and appropriate placental growth (73),

these data suggest that AVPR1A may also mediate immune responses during preeclampsia and provide insight into potential therapeutic targets. In the third trimester, CD4+ T cells from preeclampsia-affected pregnancies increased expression of AVPR2 compared to cells from normotensive control pregnancies. AVPR2 is highly expressed in the kidney and is pivotal in renal water reabsorption and thus urine concentration (74, 75) and under conditions of elevated AVP, it effects renal vasoconstriction (76). T cells have been shown to play a role in hypertension and renal dysfunction. More specifically, Increased CD4+ IL-17 producing T cells are seen in hypertension and these cells traffic to the kidney and vasculature to cause dysfunction that leads to hypertension (77, 78). Renal dysfunction and hypertension are often observed in PreE in the third trimester. Our data showing an increase in inflammatory T_H17 cells with altered AVPR2 expression suggests this receptor may play a currently unknown role in immune responses and PreE toward the end of pregnancy. Although the function of the expression of AVPRs on CD4+ T cells in PreE is currently unknown, our data suggests these receptors may be a previously uncharacterized link between renal, cardiovascular, and immune dysfunction in PreE.

APCs are critical to the activation and programming of T_H cells. AVP in pregnancy is sufficient to induce the up-regulation of co-stimulatory (CD80, CD86, and CLII) and down-regulation of inhibitory (PD-L1 and PIR B) molecule expression on DC, resulting in the generation of more T_H1 and T_H17 cells, which are necessary in the early pathogenesis of preeclampsia. Increased expression of co-stimulatory molecule CD86 has been shown to correlate highly with poor maternal-fetal immune balance (79). Blockade of CD86 in pregnancy allows for expansion of protective Treg cells and promotes the differentiation of a more tolerant T_H2 environment (80). The expression of PD-L1 during pregnancy has been shown to be T cell dependent and required to confer fetal tolerance (81). Both, co-stimulatory and inhibitory molecules, play a key role in maternal-fetal tolerance. Our data show that elevated AVP during pregnancy results in the up-regulation of co-stimulatory and down-regulation of inhibitory molecules by DCs. These alterations in signals received by the DCs are a likely mechanism contributing to the altered T_H phenotype induced by elevated AVP during pregnancy and the development of preeclampsia. These alterations in the co-stimulatory phenotype of DCs in the spleen leads to a differentiation of CD4+ T cells toward a T_H1 and T_H17 phenotype. Peripheral T_H1 and T_H17 cells, as well as, decidual cell populations skew the placenta toward a milieu rich in pro-inflammatory cytokines that furthers contributes to the development and pathogenesis of preeclampsia (Figure 7).

Our data demonstrate that elevated AVP in mice during pregnancy induces maternal and fetal T_H related changes; these alterations mimic those previously observed in human preeclampsia-affected pregnancies. Additionally, the expression of AVP receptors by CD4+ T cells identifies a putative signaling mechanism and new therapeutic targets for future investigation. From our studies, we propose that manipulation of the AVP pathway may be a novel preventative and therapeutic target to address the vascular, renal, and immune causes of preeclampsia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank the staff of the following facilities and departments: the University of Iowa Department of Obstetrics and Gynecology, the University of Iowa Office of Animal Resources, the University of Iowa Women's Health Tissue Repository, the University of Iowa Institute for Clinical and Translational Science, and the Carver College of Medicine Flow Cytometry Facility.

Financial Support:

American Heart Association Innovative Research Grant (14IRG18710013)

American Heart Association Postdoctoral Fellowship (16POST30960016)

American Heart Association Strategically Focused Research Network (15 SFRN 23730000, 18679000, 18679001,18679002, 18679003)

Burroughs Wellcome Fund (1015358)

Clinical and Translational Science Award (NIH U54TR001356)

John Warner Maternal Health Grant, Shelly Bridgewater Dreams Foundation K99/R00 (NIH HL098276)

March of Dimes Foundation (4-FY15-415)

NIH R01 (NIH HL134850)

Program Project Grant (NIH HL084207)

Reproductive Scientist Development Program (NIH K12 HD000849) (NIH K12 HD000849-28)

University of Iowa Carver College of Medicine Collaborative Grant

University of Iowa Center for Hypertension Research

University of Iowa Immunology Postdoctoral Fellowship (NIH 5 T32 AI 7260-29)

University of Iowa Institute for Clinical and Translational Science (NIH KL2 RR024980-2)

References

1. Kuklina EV, Ayala C, Callaghan WM. Hypertensive disorders and severe obstetric morbidity in the United States. Obstetrics and gynecology. 2009; 113(6):1299–306. [PubMed: 19461426]
2. Garovic VD, Hayman SR. Hypertension in pregnancy: an emerging risk factor for cardiovascular disease. Nature clinical practice Nephrology. 2007; 3(11):613–22.
3. Lykke JA, Langhoff-Roos J, Sibai BM, Funai EF, Triche EW, Paidas MJ. Hypertensive pregnancy disorders and subsequent cardiovascular morbidity and type 2 diabetes mellitus in the mother. Hypertension. 2009; 53(6):944–51. [PubMed: 19433776]
4. Magnussen EB, Vatten LJ, Smith GD, Romundstad PR. Hypertensive disorders in pregnancy and subsequently measured cardiovascular risk factors. Obstetrics and gynecology. 2009; 114(5):961–70. [PubMed: 20168095]
5. Kajantie E, Eriksson JG, Osmond C, Thornburg K, Barker DJ. Pre-eclampsia is associated with increased risk of stroke in the adult offspring: the Helsinki birth cohort study. Stroke; a journal of cerebral circulation. 2009; 40(4):1176–80.
6. Wu CS, Sun Y, Vestergaard M, Christensen J, Ness RB, Haggerty CL, et al. Preeclampsia and risk for epilepsy in offspring. Pediatrics. 2008; 122(5):1072–8. [PubMed: 18977989]
7. Wu CS, Nohr EA, Bech BH, Vestergaard M, Catov JM, Olsen J. Health of children born to mothers who had preeclampsia: a population-based cohort study. American journal of obstetrics and gynecology. 2009; 201(3):269. e1 - e10. [PubMed: 19733276]

8. Rana S, Karumanchi SA, Lindheimer MD. Angiogenic factors in diagnosis, management, and research in preeclampsia. Hypertension. 2014; 63(2):198–202. [PubMed: 24166749]
9. Henderson JT, Whitlock EP, O'Connor E, Senger CA, Thompson JH, Rowland MG. Low-dose aspirin for prevention of morbidity and mortality from preeclampsia: a systematic evidence review for the U.S. Preventive Services Task Force. Ann Intern Med. 2014; 160(10):695–703. [PubMed: 24711050]
10. Rolnik DL, Wright D, Poon LC, O'Gorman N, Syngelaki A, de Paco Matallana C, et al. Aspirin versus Placebo in Pregnancies at High Risk for Preterm Preeclampsia. The New England journal of medicine. 2017; 377(7):613–22. [PubMed: 28657417]
11. Akinlade KS, Adediji IO, Rahamon SK, Fawole AO, Tongo OO. Serum copeptin and pregnancy outcome in preeclampsia. Niger Med J. 2015; 56(5):362–8. [PubMed: 26778890]
12. Foda AA, Abdel Aal IA. Maternal and Neonatal Copeptin Levels at Cesarean Section and Vaginal Delivery. European Journal of Obsterics & Gynecology and Reproductive Biology. 2012; 165:215–8.
13. Tuten A, Oncul M, Kucur M, Imamoglu M, Ekmekci OB, Acikgoz AS, et al. Maternal serum copeptin concentrations in early- and late-onset pre-eclampsia. Taiwan J Obstet Gynecol. 2015; 54(4):350–4. [PubMed: 26384049]
14. Yesil A, Kanawati A, Helvacioglu C, Kaya C, Ozgun CG, Cengiz H. Identification of patients at risk for preeclampsia with the use of uterine artery Doppler velocimetry and copeptin. J Matern Fetal Neonatal Med. 2016:1–6.
15. Yeung EH, Liu A, Mills JL, Zhang C, Männistö T, Lu Z, et al. Increased Levels of Copeptin Before Clinical Diagnosis of Preelcampsia. Hypertension. 2014; 64(6):1362–7. [PubMed: 25225209]
16. Zulfikaroglu E, Islimye M, Tonguc EA, Payasli A, Isman F, Var T, et al. Circulating levels of copeptin, a novel biomarker in pre-eclampsia. The journal of obstetrics and gynaecology research. 2011; 37(9):1198–202. [PubMed: 21501333]
17. Santillan MK, Santillan DA, Scroggins SM, Min JY, Sandgren JA, Pearson NA, et al. Vasopressin in preeclampsia: a novel very early human pregnancy biomarker and clinically relevant mouse model. Hypertension. 2014; 64(4):852–9. [PubMed: 25001273]
18. Laresgoiti-Servitje E, Gómez-López N, Olson DM. An immunological insight into the origins of pre-eclampsia. Human Reproduction Update. 2010; 16(5):510–24. [PubMed: 20388637]
19. Goswami D, Tannetta DS, Magee LA, Fuchisawa A, Redman CW, Sargent IL, et al. Excess syncytiotrophoblast microparticle shedding is a feature of early-onset pre-eclampsia, but not normotensive intrauterine growth restriction. Placenta. 2006; 27(1):56–61. [PubMed: 16310038]
20. Formigli L, Papucci L, Tani A, Schiavone N, Tempestini A, Orlandini GE, et al. Aponecrosis: morphological and biochemical exploration of a syncretic process of cell death sharing apoptosis and necrosis. Journal of cellular physiology. 2000; 182(1):41–9. [PubMed: 10567915]
21. Redman CW, Sargent IL. Immunology of pre-eclampsia. Am J Reprod Immunol. 2010; 63(6):534–43. [PubMed: 20331588]
22. DiFederico E, Genbacev O, Fisher SJ. Preeclampsia is associated with widespread apoptosis of placental cytotrophoblasts within the uterine wall. Am J Pathol. 1999; 155(1):293–301. [PubMed: 10393861]
23. Kadyrov M, Kingdom JC, Huppertz B. Divergent trophoblast invasion and apoptosis in placental bed spiral arteries from pregnancies complicated by maternal anemia and early-onset preeclampsia/intrauterine growth restriction. American journal of obstetrics and gynecology. 2006; 194(2):557–63. [PubMed: 16458661]
24. Mjösberg J, Berg G, Jenmalm MC, Ernerudh J. FOXP3+ Regulatory T Cells and T Helper 1, T Helper 2, and T Helper 17 Cells in Human Early Pregnancy Decidua. Biology of Reproduction. 2010; 82(4):698–705. [PubMed: 20018909]
25. Darmochwal-Kolarz D, Leszczynska-Gorzelak B, Rolinski J, Oleszczuk J. T helper 1- and T helper 2-type cytokine imbalance in pregnant women with pre-eclampsia. European Journal of Obstetrics & Gynecology and Reproductive Biology. 1999; 86(2):165–70. [PubMed: 10509785]
26. Wilczyński JR. Th1/Th2 cytokines balance—yin and yang of reproductive immunology. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2005; 122(2):136–43. [PubMed: 15893871]

27. Saito S, Sakai M, Sasaki Y, Tanebe K, Tsuda H, Michimata T. Quantitative analysis of peripheral blood Th0, Th1, Th2 and the Th1:Th2 cell ratio during normal human pregnancy and preeclampsia. Clinical and Experimental Immunology. 1999; 117(3):550–5. [PubMed: 10469061]
28. Darmochwal-Kolarz D, Kludka-Sternik M, Tabarkiewicz J, Kolarz B, Rolinski J, Leszczynska-Gorzelak B, et al. The predominance of Th17 lymphocytes and decreased number and function of Treg cells in preeclampsia. Journal of Reproductive Immunology. 2012; 93(2):75–81. [PubMed: 22370101]
29. Nakashima A, Ito M, Yoneda S, Shiozaki A, Hidaka T, Saito S. SHORT COMMUNICATION: Circulating and Decidual Th17 Cell Levels in Healthy Pregnancy. American Journal of Reproductive Immunology. 2010; 63(2):104–9. [PubMed: 20015328]
30. Santner-Nanan B, Peek MJ, Khanam R, Richarts L, Zhu E, Fazekas de St Groth B, et al. Systemic Increase in the Ratio between Foxp3+ and IL-17-Producing CD4+ T Cells in Healthy Pregnancy but Not in Preeclampsia. The Journal of Immunology. 2009; 183(11):7023–30. [PubMed: 19915051]
31. Wilczynski JR. Immunological analogy between allograft rejection, recurrent abortion and pre-eclampsia - the same basic mechanism? Human Immunology. 2006; 67(7):492–511. [PubMed: 16829304]
32. Redman CW, Sargent IL. Latest advances in understanding preeclampsia. Science. 2005; 308(5728):1592–4. [PubMed: 15947178]
33. Wallace K, Richards S, Dhillon P, Weimer A, Edholm ES, Bengten E, et al. CD4+ T-helper cells stimulated in response to placental ischemia mediate hypertension during pregnancy. Hypertension. 2011; 57(5):949–55. [PubMed: 21464392]
34. Wallace K, Morris R, Kyle PB, Cornelius D, Darby M, Scott J, et al. Hypertension, inflammation and T lymphocytes are increased in a rat model of HELLP syndrome. Hypertension in pregnancy : official journal of the International Society for the Study of Hypertension in Pregnancy. 2014; 33(1):41–54.
35. Andrianov IG, Dobkin AN, Kiselev OI, Okulov VB, Semiglazov VF. [Immunomodulating activity of natural killers in patients with breast tumors using vasopressin and interleukin 2 in vitro]. Voprosy onkologii. 1989; 35(10):1186–91. [PubMed: 2596062]
36. Johnson HM, Farrar WL, Torres BA. Vasopressin replacement of interleukin 2 requirement in gamma interferon production: lymphokine activity of a neuroendocrine hormone. The Journal of Immunology. 1982; 129(3):983–6. [PubMed: 6180008]
37. Johnson HM, Torres BA. Regulation of lymphokine production by arginine vasopressin and oxytocin: modulation of lymphocyte function by neurohypophyseal hormones. Journal of Immunology. 1985; 135(2 Suppl):773s–5s.
38. Kruimel JW, Pesman GJ, Sweep CG, van der Vliet JA, Liem T, Jansen JB, et al. Depression of plasma levels of cytokines and ex-vivo cytokine production in relation to the activity of the pituitary-adrenal axis, in patients undergoing major vascular surgery. Cytokine. 1999; 11(5):382–8. [PubMed: 10328878]
39. McMaster WG, Kirabo A, Madhur MS, Harrison DG. Inflammation, Immunity, and Hypertensive End-Organ Damage. Circulation research. 2015; 116(6):1022–33. [PubMed: 25767287]
40. Wenzel U, Turner JE, Krebs C, Kurts C, Harrison DG, Ehmke H. Immune Mechanisms in Arterial Hypertension. Journal of the American Society of Nephrology. 2015
41. Chikanza IC, Grossman AS. Hypothalamic-pituitary-mediated immunomodulation: arginine vasopressin is a neuroendocrine immune mediator. Rheumatology. 1998; 37(2):131–6.
42. Chikanza CI, Grossman BA. Neuroendocrine Immune Respinses to Inflammation: The Concept of the Neuroendocrine Immune Loop. Neuroendocrine Immune Loop. 1996; 10(2):199–225.
43. Chikanza IC, Grossman AB. Reciprocal Interactions Between the Neuroendocrine and Immune System During Inflammation. Rheumatic Disease Clinics of North America. 2000; 26(4):693–711. [PubMed: 11084940]
44. Torres BA, Johnson HM. Arginine vasopressin (AVP) replacement of helper cell requirement in IFN-gamma production. Evidence for a novel AVP receptor on mouse lymphocytes. The Journal of Immunology. 1988; 140(7):2179–83. [PubMed: 2965181]

45. Santillan MK, Leslie KK, Hamilton WS, Boese BJ, Ahuja M, Hunter SK, et al. Collection of a lifetime: a practical approach to developing a longitudinal collection of women's healthcare biological samples. European journal of obstetrics, gynecology, and reproductive Biology. 2014; 179:94–9.
46. American College of O, Gynecologists, Task Force on Hypertension in P. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. Obstetrics and gynecology. 2013; 122(5):1122–31. [PubMed: 24150027]
47. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. Nat Protoc. 2008; 3(6):1101–8. [PubMed: 18546601]
48. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001; 25(4):402–8. [PubMed: 11846609]
49. Oosterbaan HP, Swaab DF, Boer GJ. Oxytocin and vasopressin in the rat do not readily pass from the mother to the amniotic fluid in late pregnancy. Journal of developmental physiology. 1985; 7(1):55–62. [PubMed: 3980926]
50. Santillan MK, Pelham CJ, Ketsawatsomkron P, Santillan DA, Davis DR, Devor EJ, et al. Pregnant mice lacking indoleamine 2,3-dioxygenase exhibit preeclampsia phenotypes. Physiol Rep. 2015; 3(1)
51. Munn DH, Zhou M, Attwood JT, Bondarev I, Conway SJ, Marshall B, et al. Prevention of allogeneic fetal rejection by tryptophan catabolism. Science. 1998; 281(5380):1191–3. [PubMed: 9712583]
52. Mellor AL, Munn DH. Tryptophan catabolism prevents maternal T cells from activating lethal anti-fetal immune responses. J Reprod Immunol. 2001; 52(1–2):5–13. [PubMed: 11600174]
53. Nishizawa H, Hasegawa K, Suzuki M, Achiwa Y, Kato T, Saito K, et al. Mouse model for allogeneic immune reaction against fetus recapitulates human pre-eclampsia. The journal of obstetrics and gynaecology research. 2008; 34(1):1–6. [PubMed: 18226121]
54. Moffett-King A. Natural killer cells and pregnancy. Nat Rev Immunol. 2002; 2(9):656–63. [PubMed: 12209134]
55. Wilczyński JR, Tchórzewski H, Banasik M, Głowacka E, Wieczorek A, Lewkowicz P, et al. Lymphocyte subset distribution and cytokine secretion in third trimester decidua in normal pregnancy and preeclampsia. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2003; 109(1):8–15. [PubMed: 12818436]
56. Hsu P, Nanan RKH. Innate and adaptive immune interactions at the fetal-maternal interface in healthy human pregnancy and preeclampsia. Frontiers in Immunology. 2014:5. [PubMed: 24478774]
57. Chatterjee P, Kopriva SE, Chiasson VL, Young KJ, Tobin RP, Newell-Rogers K, et al. Interleukin-4 deficiency induces mild preeclampsia in mice. Journal of Hypertension. 2013; 31(7):1414–23. discussion 23. [PubMed: 23552130]
58. Saito S, Sakai M. Th1/Th2 balance in preeclampsia. J Reprod Immunol. 2003; 59(2):161–73. [PubMed: 12896820]
59. LaMarca B, Cornelius D, Wallace K. Elucidating Immune Mechanisms Causing Hypertension During Pregnancy. physiology. 2013; 28(4):225–33. [PubMed: 23817797]
60. Li J, LaMarca B, Reckelhoff JF. A model of preeclampsia in rats: the reduced uterine perfusion pressure (RUPP) model. American Journal of Physiology - Heart and Circulatory physiology. 2012; 303(1):H1–H8. [PubMed: 22523250]
61. Dinan TG, Cryan J, Shanahan F, Keeling PW, Quigley EM. IBS: An epigenetic perspective. Nat Rev Gastroenterol Hepatol. 2010; 7(8):465–71. [PubMed: 20585338]
62. O'Rahilly R. Early human development and the chief sources of information on staged human embryos. European journal of obstetrics, gynecology, and reproductive Biology. 1979; 9(4):273–80.
63. O'Rahilly R, Muller F. Developmental stages in human embryos: revised and new measurements. Cells Tissues Organs. 2010; 192(2):73–84. [PubMed: 20185898]
64. Otis EM, Brent R. Equivalent ages in mouse and human embryos. Anat Rec. 1954; 120(1):33–63. [PubMed: 13207763]

65. Redman CWG, Staff AC. Preeclampsia, biomarkers, syncytiotrophoblast stress, and placental capacity. American Journal of Obstetrics & gynecology. 2015; 213(4) S9.e1-S9.e4.
66. Carr DJ, DeCosta BR, Jacobson AE, Rice KC, Blalock JE. Corticotropin-releasing hormone augments natural killer cell activity through a naloxone-sensitive pathway. J Neuroimmunol. 1990; 28(1):53–61. [PubMed: 2160475]
67. Chikanza IC, Petrou P, Chrousos G. Perturbations of arginine vasopressin secretion during inflammatory stress. Pathophysiologic implications. Annals of the New York Academy of Sciences. 2000; 917:825–34. [PubMed: 11268412]
68. Yasin SA, Costa A, Forsling ML, Grossman A. Interleukin-1 beta and interleukin-6 stimulate neurohypophysial hormone release in vitro. J Neuroendocrinol. 1994; 6(2):179–84. [PubMed: 8049716]
69. Mastorakos G, Weber JS, Magiakou MA, Gunn H, Chrousos GP. Hypothalamic-pituitary-adrenal axis activation and stimulation of systemic vasopressin secretion by recombinant interleukin-6 in humans: potential implications for the syndrome of inappropriate vasopressin secretion. The Journal of clinical endocrinology and metabolism. 1994; 79(4):934–9. [PubMed: 7962300]
70. Littlejohn NK, Siel RB Jr, Ketsawatsomkron P, Pelham CJ, Pearson NA, Hilzendeger AM, et al. Hypertension in mice with transgenic activation of the brain renin-angiotensin system is vasopressin dependent. American journal of physiology Regulatory, integrative and comparative physiology. 2013; 304(10):R818–28.
71. Aoyagi T, Koshimizu TA, Tanoue A. Vasopressin regulation of blood pressure and volume: findings from V1a receptor-deficient mice. Kidney international. 2009; 76(10):1035–9. [PubMed: 19693000]
72. Koshimizu TA, Nasa Y, Tanoue A, Oikawa R, Kawahara Y, Kiyono Y, et al. V1a vasopressin receptors maintain normal blood pressure by regulating circulating blood volume and baroreflex sensitivity. Proceedings of the National Academy of Sciences of the United States of America. 2006; 103(20):7807–12. [PubMed: 16682631]
73. Koukoulas I, Risvanis J, Douglas-Denton R, Burrell LM, Moritz KM, Wintour EM. Vasopressin receptor expression in the placenta. Biol Reprod. 2003; 69(2):679–86. [PubMed: 12724283]
74. Nakamura T, Sakamaki T, Kurashina T, Hoshino J, Sato K, Ono Z, et al. Effect of vasopressin V (OPC-21268) and V2 (OPC-31260) antagonists on renal hemodynamics and excretory function. Life sciences. 1994; 55(4):PL67–72. [PubMed: 8028441]
75. Schliebe N, Strotmann R, Busse K, Mitschke D, Biebermann H, Schomburg L, et al. V2 vasopressin receptor deficiency causes changes in expression and function of renal and hypothalamic components involved in electrolyte and water homeostasis. Am J Physiol Renal Physiol. 2008; 295(4):F1177–90. [PubMed: 18715941]
76. Rose CE Jr, Rose KY, Kinter LB. Effect of V1/V2-receptor antagonism on renal function and response to vasopressin in conscious dogs. The American journal of physiology. 1991; 260(2 Pt 2):F273–82. [PubMed: 1825453]
77. Harrison DG, Marvar PJ, Titze JM. Vascular inflammatory cells in hypertension. Front Physiol. 2012; 3:128. [PubMed: 22586409]
78. Itani HA, McMaster WG Jr, Saleh MA, Nazarewicz RR, Mikolajczyk TP, Kaszuba AM, et al. Activation of Human T Cells in Hypertension: Studies of Humanized Mice and Hypertensive Humans. Hypertension. 2016; 68(1):123–32. [PubMed: 27217403]
79. Jin LP, Fan DX, Zhang T, Guo PF, Li DJ. The costimulatory signal upregulation is associated with Th1 bias at the maternal-fetal interface in human miscarriage. Am J Reprod Immunol. 2011; 66(4): 270–8. [PubMed: 21481059]
80. Zhu X-Y, Zhou Y-H, Wang M-Y, Jin L-P, Yuan M-M, Li D-J. Blockade of CD86 Signaling Facilitates a Th2 Bias at the Maternal-Fetal Interface and Expands Peripheral CD4+CD25+ Regulatory T Cells to Rescue Abortion-Prone Fetuses. Biology of Reproduction. 2005; 72(2):338–45. [PubMed: 15456701]
81. Guleria I, Khosroshahi A, Ansari MJ, Habicht A, Azuma M, Yagita H, et al. A critical role for the programmed death ligand 1 in fetomaternal tolerance. The Journal of experimental medicine. 2005; 202(2):231–7. [PubMed: 16027236]

Clinical Perspectives

- AVP secretion during pregnancy, via copeptin detection, has been shown to be elevated very early in women who later develop preeclampsia. AVP infusion into pregnant mice recapitulates the physiological aspects of human preeclampsia. It is unknown if elevated AVP during pregnancy is sufficient to cause the maternal and fetal immune phenotypes consistent with human preeclampsia
- Elevated AVP in pregnant mice results in increased T_H1 and T_H17 associated cytokines and T cells with a concomitant decrease in the T_H2 associated cytokines. Additionally, CD4+ T cells express AVP receptors and expression of AVPR1a by human CD4+ T cells from preeclampsia-affected women is decreased.
- The data presented demonstrate a novel role for AVP in altering immune phenotypes in pregnancy. Additionally, the expression of AVP receptor expression by CD4+ T cells lends to the possibility of therapies utilizing AVP modulation to mitigate preeclampsia.

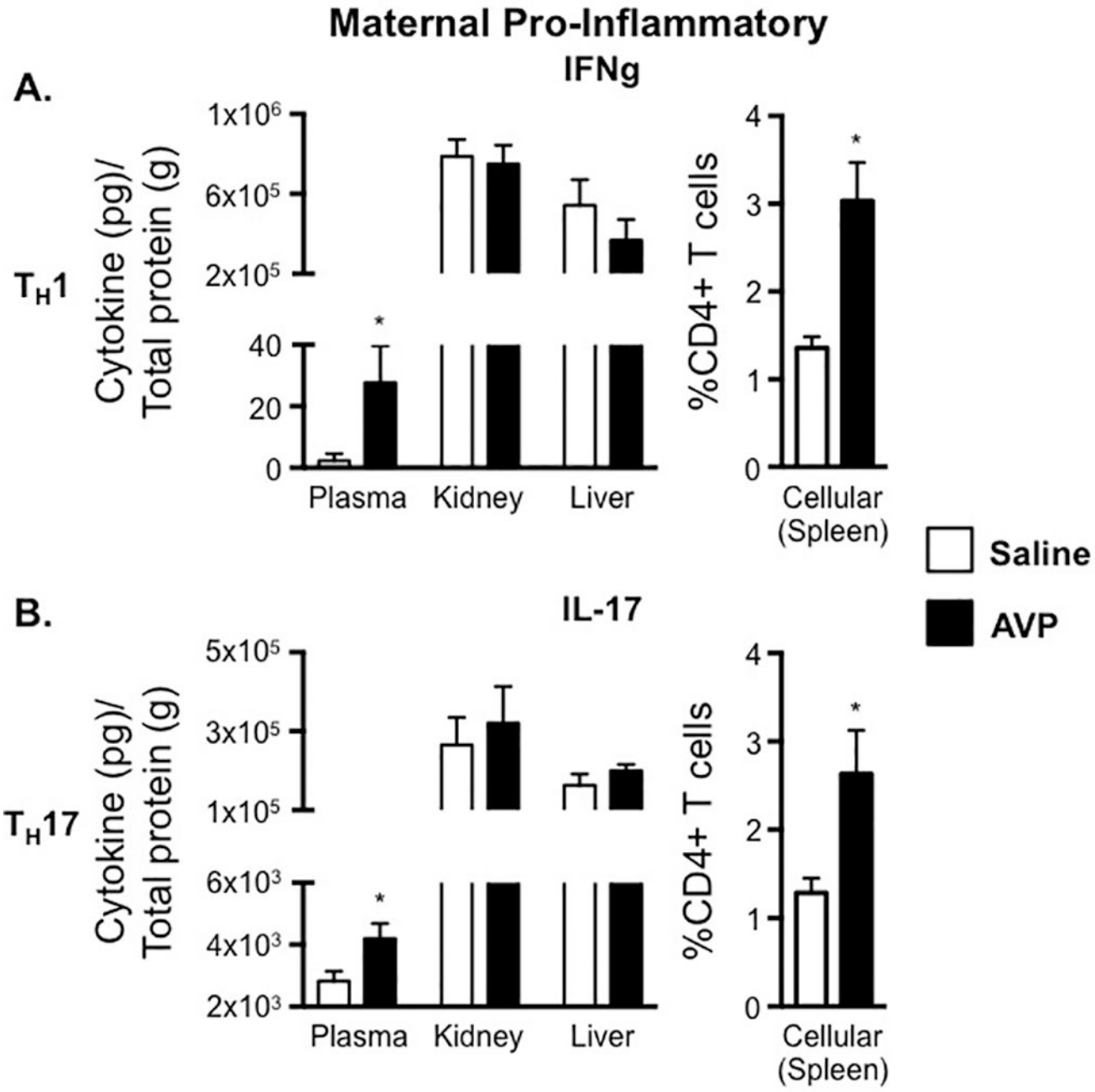

Figure 1.

AVP infusion is sufficient to induce T_H1 and T_H17 maternal immune alterations. IFNg (A) and IL-17 (B) concentrations in maternal plasma, maternal kidney, and maternal liver were normalized to total protein and are represented as pg of cytokine per g of total protein. Lymphocytes were isolated from the spleen of saline and AVP infused dams. Following intracellular cytokine staining, CD3+ CD4+ cells were gated as shown in Supplemental Figure 1A and the frequency of cells producing the pro-inflammatory cytokines IFNg (A) and IL-17 (B) was determined. Open bars= saline-infused; Solid bars= AVP-infused. N ≥ 5 per group from at least two independent experiments. Data are mean ± SEM. Statistical

significance was determined using a Student t test and the minimal level of confidence deemed statistically significant was a p value <0.05. *=p<0.05.

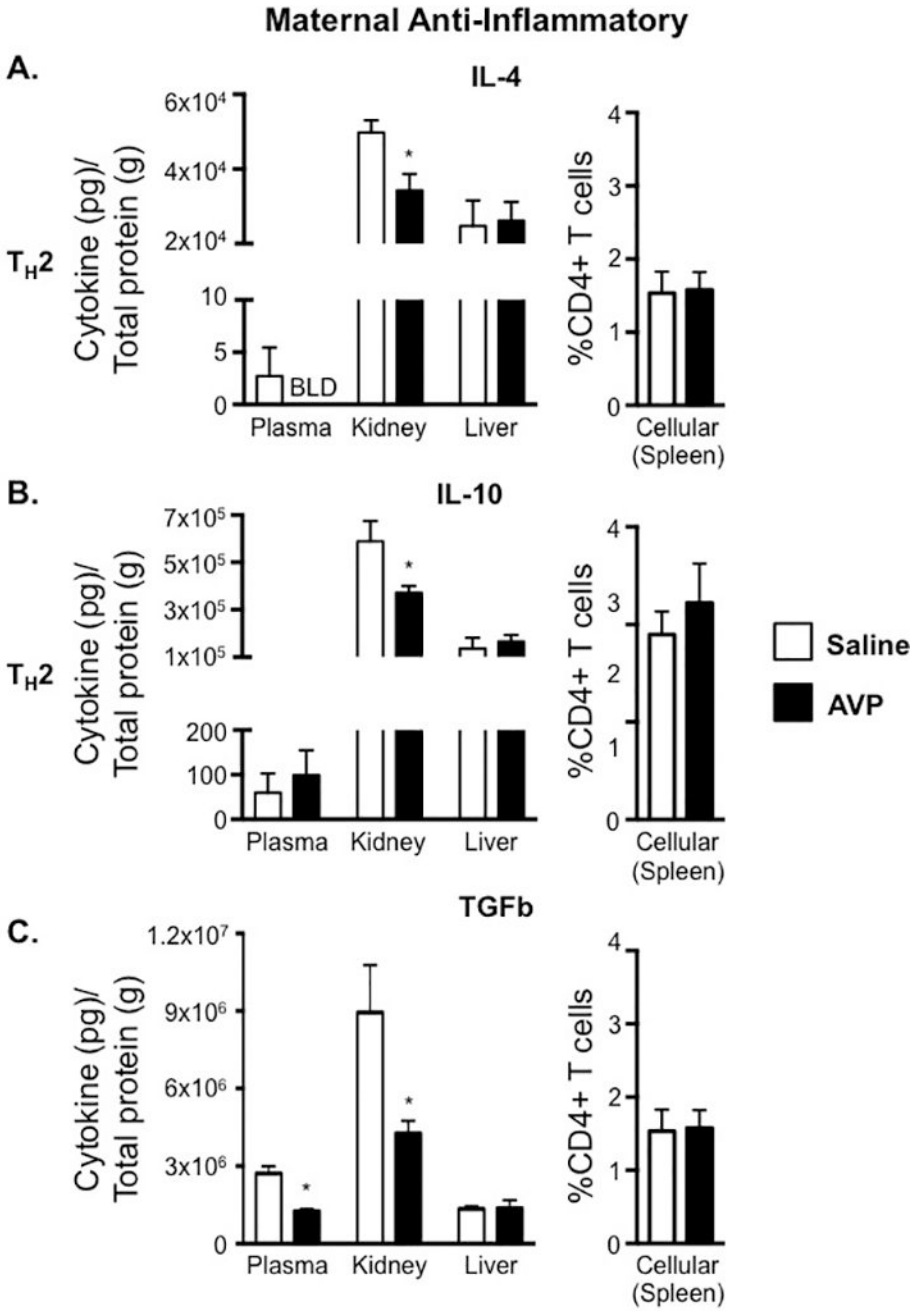

Figure 2.

Elevated AVP in pregnancy decreases anti-inflammatory cytokine production in maternal tissues. IL-4 (A), IL-10 (B), and TGFb (C) concentrations in maternal plasma, maternal kidney, and maternal liver were normalized to total protein and are represented as pg of cytokine per g of total protein. Lymphocytes were isolated from the spleen of saline and AVP infused dams. Following intracellular cytokine staining, CD3+ CD4+ cells were gated as shown in Supplemental Figure 1A and the frequency of cells producing the T_H2-associated cytokines IL-4 (A) and IL-10 (B) as well as LAP/TGFb (C) was determined. Open bars= saline-infused; Solid bars= AVP-infused. BLD is below the limit of detection. N

≥ 5 per group from at least two independent experiments. Data are mean ± SEM. Statistical significance was determined using a Student t test and the minimal level of confidence deemed statistically significant was a p value <0.05. *=p<0.05.

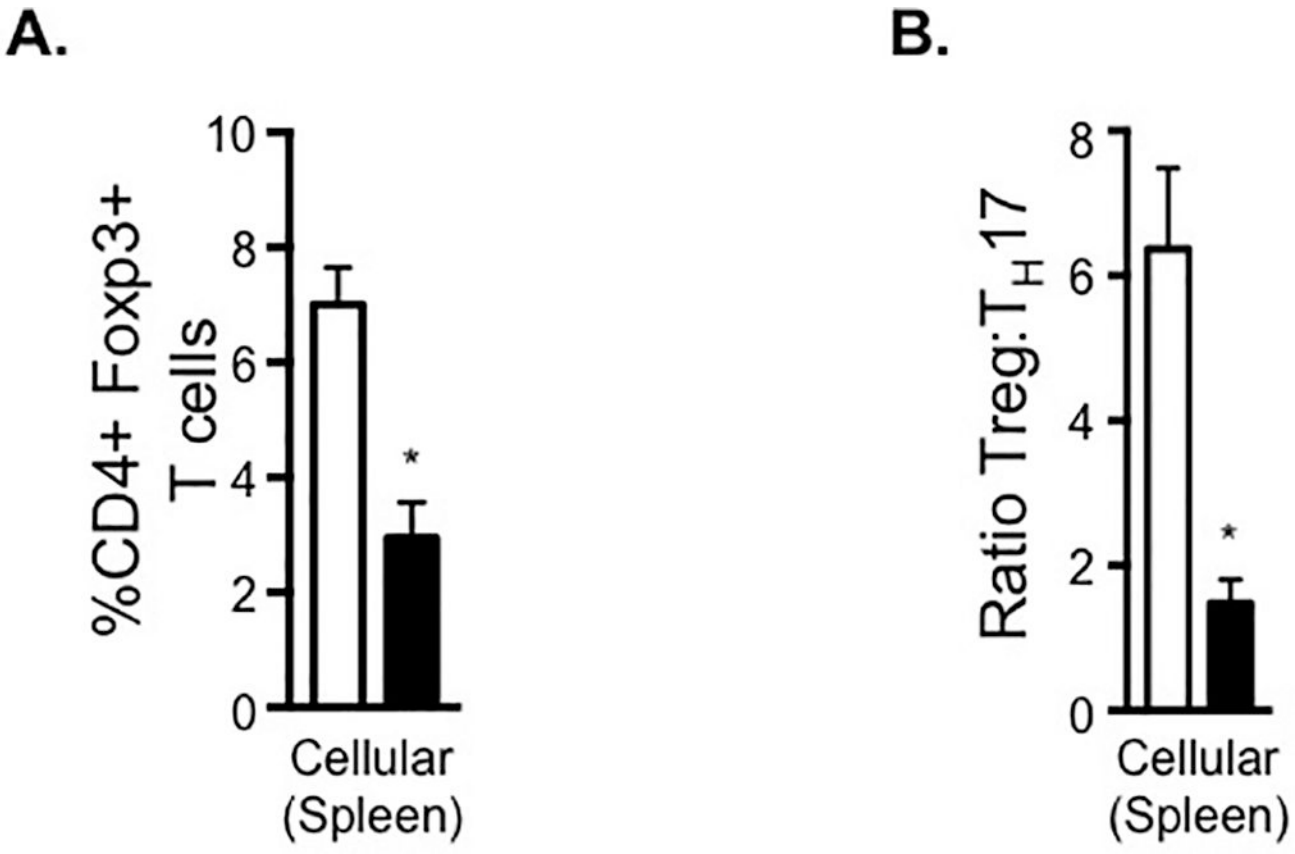

Figure 3.

AVP infusion in pregnancy alters the ratio of anti-inflammatory Tregs to pro-inflammatory T_H17 cells. Lymphocytes were isolated from the spleen of saline and AVP infused dams. Following intracellular cytokine staining, CD3+ CD4+ cells were gated as shown in Supplemental Figure 1A and the frequency of cells producing the Treg transcription factor Foxp3 (A) or the pro-inflammatory cytokine IL-17 was determined. The ratio of Treg to T_H17 cells was calculated (B). Open bars= saline-infused; Solid bars= AVP-infused. BLD is below the limit of detection. N ≥ 5 per group from at least two independent experiments. Data are mean ± SEM. Statistical significance was determined using a Student t test and the minimal level of confidence deemed statistically significant was a p value <0.05. *=p<0.05.

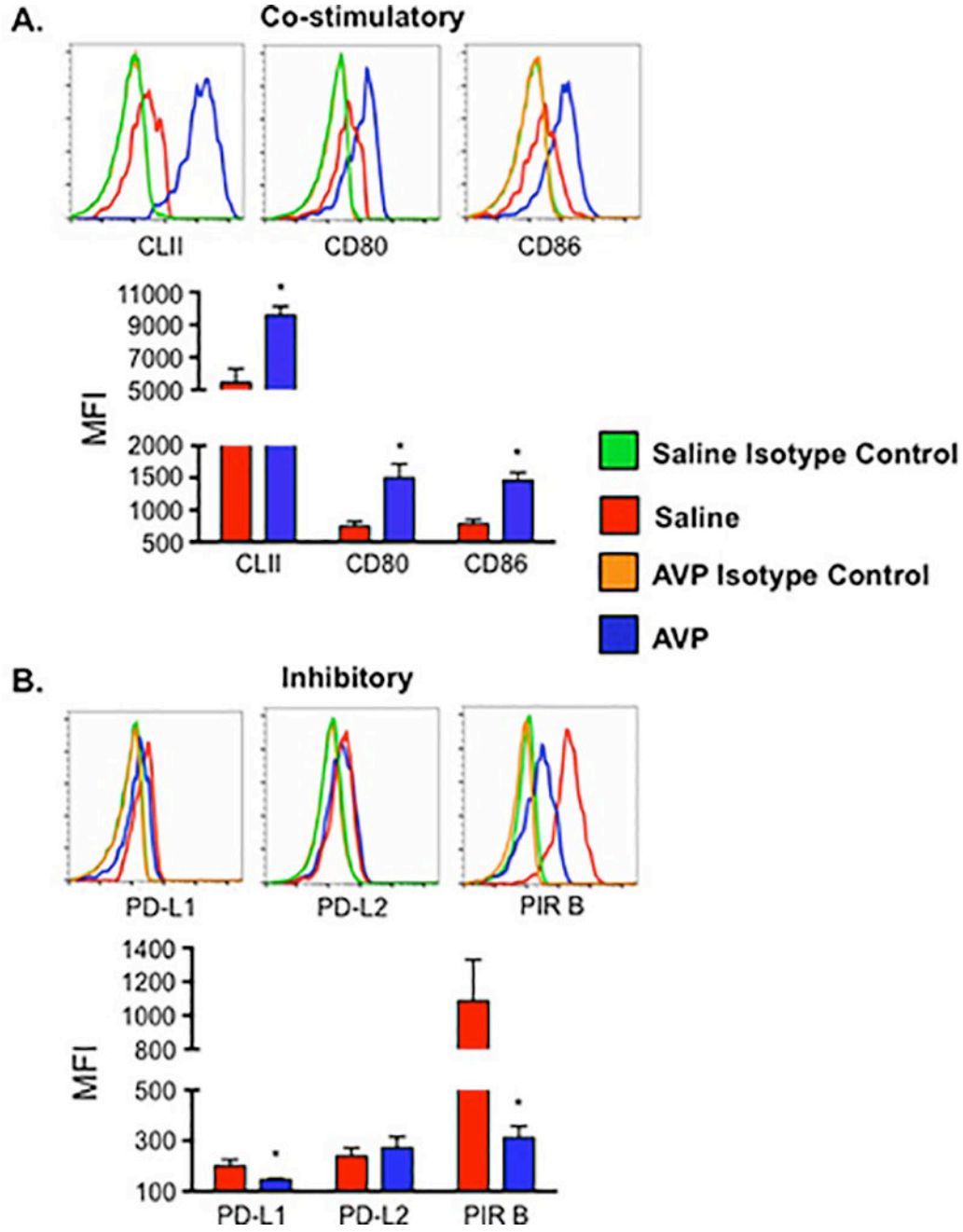

Figure 4.

AVP induces altered surface receptor expression on DC. Inhibitory molecules paired immunoglobulin-like receptor B (PIR B) and programmed death ligand 1 (PD-L1) are decreased while co-stimulatory molecules MHC Class II (CLII), CD80, and CD86 were increased on dendritic cells from AVP infused dams. Lymphocytes were isolated from the spleen of saline and AVP infused dams. Following fluorescent antibody staining, CD11c+ DCs were gated as shown in Supplemental Figure 1B. (A) Representative histograms and mean fluorescence intensity (MFI) of co-stimulatory cell surface molecules. (B) Representative histograms and MFI of inhibitory cell surface molecules. Background

fluorescence was determined by staining cells with corresponding fluorochrome-conjugated rat immunoglobulin isotype control antibodies. Isotype control subtracted MFIs are shown. $N \geq 5$ per group from at least two independent experiments. MFIs are mean ±SEM. Statistical significance was determined using a Student t test and the minimal level of confidence deemed statistically significant was a p value <0.05. *=$p<0.05$.

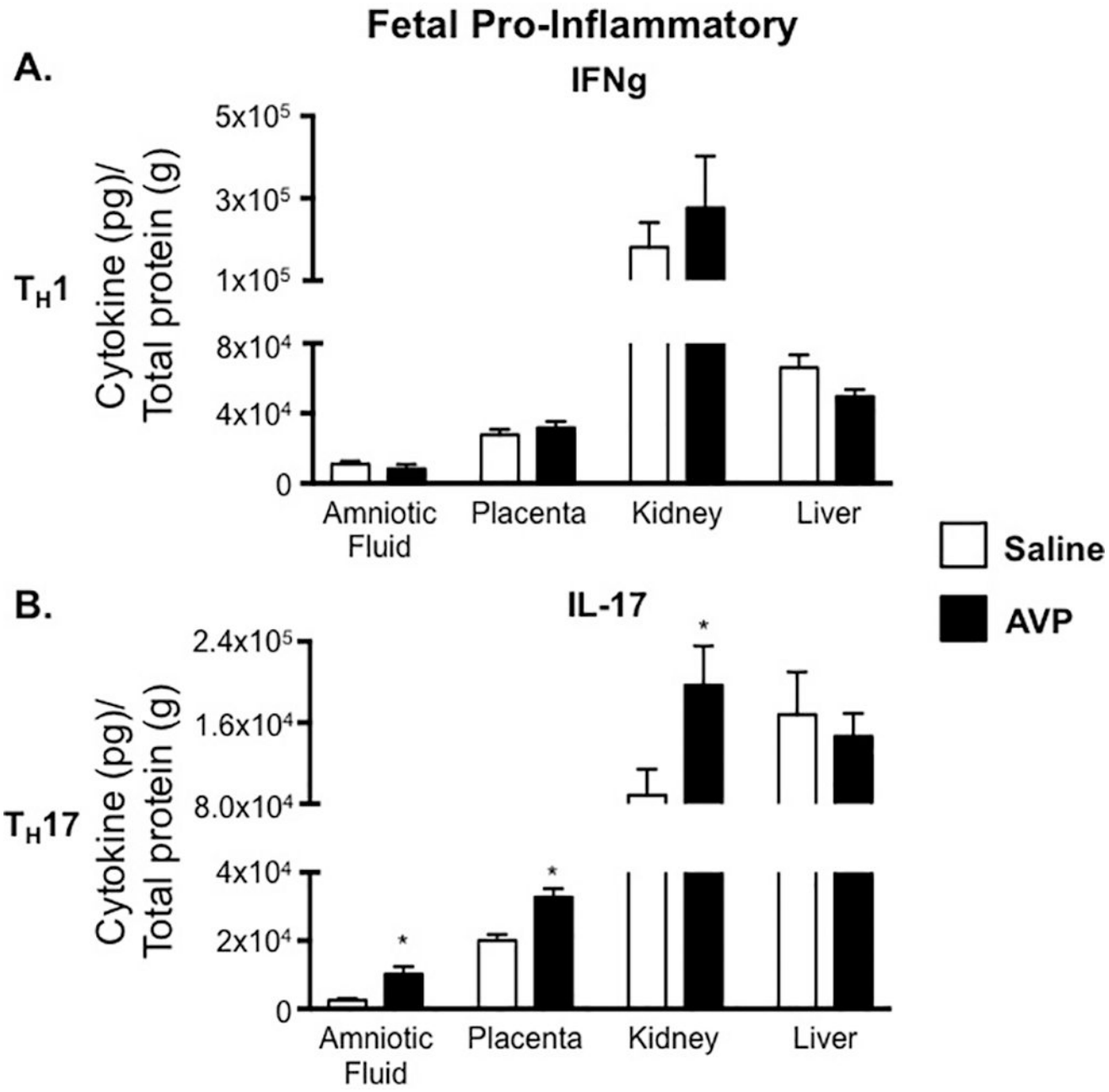

Figure 5.

Fetal tissue IL-17 cytokine production is altered by AVP. The T_H17-associated cytokine IL-17 is increased in fetal tissues obtained from AVP-infused dams. IFNg (A) and IL-17 (B) concentrations in amniotic fluid, placenta, fetal kidney, and fetal liver were normalized to total protein and are represented as pg of cytokine per g of total protein. Open bars= saline-infused; Solid bars= AVP-infused. Due to fetal tissue mass, 5 pairs of fetal kidneys and 5 fetal livers were pooled for an N=1 from a single pregnancy. N ≥ 5 per group from at least two independent experiments. Data are mean ±SEM. Statistical significance was determined using a Student t test and the minimal level of confidence deemed statistically significant was a p value <0.05. *=p<0.05.

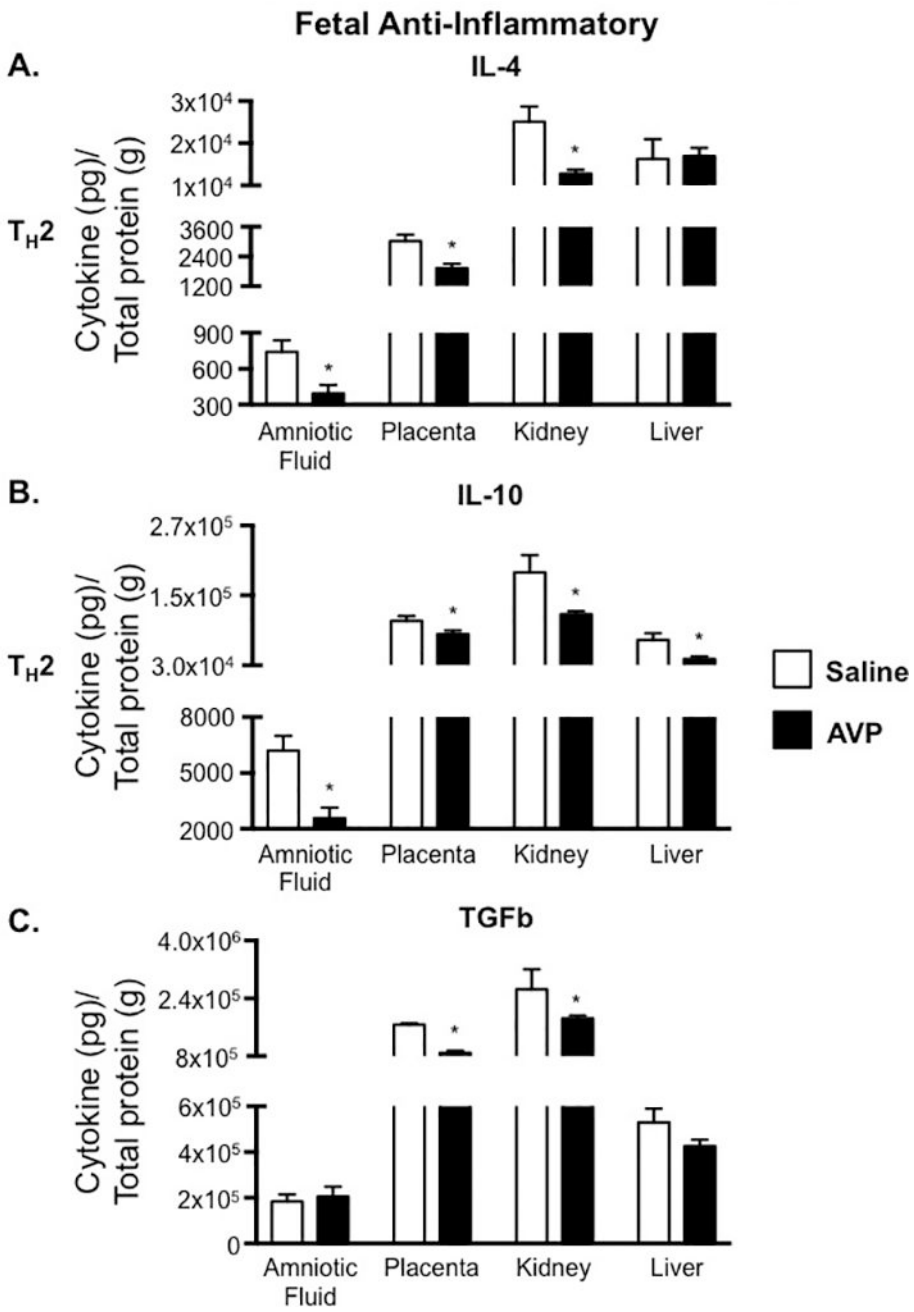

Figure 6.

Fetal concentrations of anti-inflammatory cytokines are decreased in the presence of elevated AVP during gestation. IL-4 (A), IL-10 (B), and TGFb (C) concentrations in amniotic fluid, placenta, fetal kidney, and fetal liver were normalized to total protein and are represented as pg of cytokine per g of total protein. Open bars= saline-infused; Solid bars= AVP-infused. Due to fetal tissue mass, 5 pairs of fetal kidneys and 5 fetal livers were pooled for an N=1 from a single pregnancy. N ≥ 5 per group from at least two independent experiments. Data are mean ± SEM. Statistical significance was determined using a Student

t test and the minimal level of confidence deemed statistically significant was a p value <0.05. *=p<0.05.

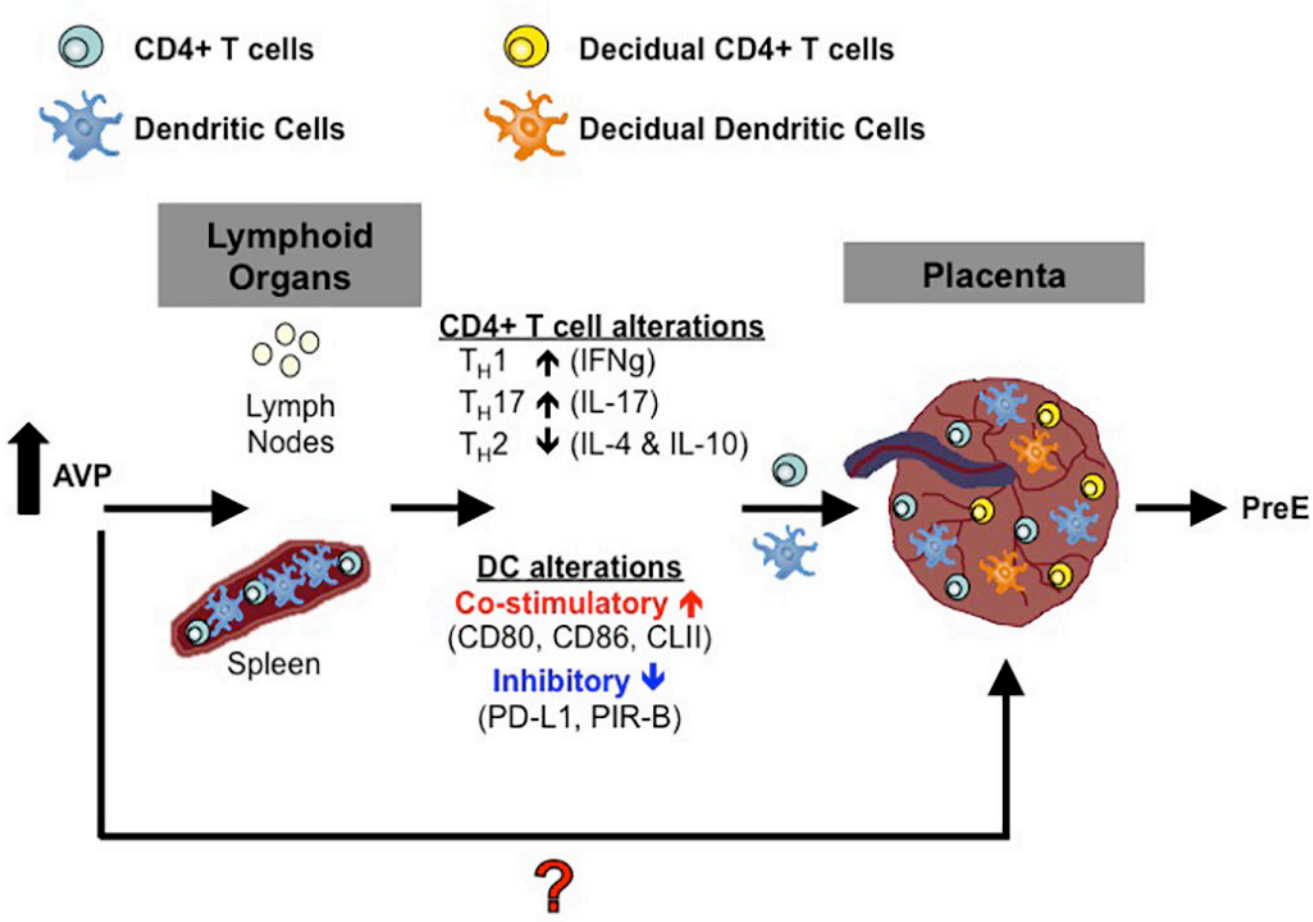

Figure 7.

Proposed model of AVP action in pregnancy. Elevated AVP during pregnancy induces DCs in secondary lymphoid organs to upregulate co-stimulatory molecules and down-regulate inhibitory molecules. These DCs activate T cells and the resultant helper T cells secrete more pro-inflammatory T_H1 and T_H17 and less anti-inflammatory T_H2 cytokines. These cells traffic to the placenta and along with resident decidual cell populations alter cytokine profiles in the placenta and fetal tissues. The red question mark indicates possibility of AVP acting directly on the placenta and tissue resident cell populations. PreE=preeclampsia.

Table 1

qPCR Primer Sequences *

Target#		Sequence	Tm	Amplicon
hAVPR1a	S	GTGCAGAGCAAGCGGGTGTG	61.8°C	438bp
	AS	CGAGTCCTTCCACATACCCGT	58.7°C	
mAVPR1a	S	CTCTGCTGGACACCTTTCTTC	55.5°C	218bp
	AS	GTTGGGCTTCGGTTGTTAGA	55.2°C	
hAVPR1b	S	CCAAGATCCGAACAGTGAAGAT	54.6°C	206bp
	AS	GCTGTTGAAGCCCATGTAGA	55.0°C	
mAVPR1b	S	AAGATCCGAACCGTGAAGATG	54.8°C	320bp
	AS	TGGGTCAGCAGTGTTGTG	55.4°C	
hAVPR2	S	GGCCAAGACTGTGAGGATGA	56.9°C	200bp
	S	ACACGCTGCTGCTGAAAGAT	57.5°C	
mAVPR2	S	AGGACACCGGACAGGAA	55.7°C	275bp
	AS	AAAGCAGGCTACGCAACT	54.8°C	
18S rRNA	S	AACTTTCGATGGTAGTCGCCG	57.3°C	104bp
	AS	CTTGGATGTGGTAGCCGTTT	57.6°C	

* qPCR primers were designed using the Integrated DNA Technologies PrimerQuest online tool (www.idtdna.com) against GenBank mRNA sequences.

#h = human-specific; m = mouse-specific; S = sense strand; AS = anti-sense strand; All primer efficiencies were between 93–95%.

Table 2

Murine CD4+ T cell expression of AVPRs

	Saline	AVP	Expression Fold Change	
	Average ΔCT			p-value
AVPR1a	20.7 ±0.9	21.0 ±0.5	−1.3	0.72
AVPR1b	20.6 ±0.7	21.6 ±0.3	−2.0	0.19
AVPR2	9.7 ±0.6	10.5 ±0.4	−1.7	0.26

Mouse splenic CD4+ T cells were negatively purified from saline and AVP-infused dams. Expression of AVP receptors was determined via qPCR. Raw cycle threshold (Ct) values were normalized (ΔCt) against the18S rRNA endogenous control. The lower the ΔCt value of a specific target, the higher the expression. The ΔΔCt and expression fold change of AVP versus saline and preeclampsia-affected versus control pregnancy were calculated as previously described (47, 48). Data are mean ±SEM. Statistical significance was determined using a Student's t test and the minimal level of confidence deemed statistically significant was a p value <0.05.

Table 3

CD4+ T cells isolated from human preeclampsia-affected women have trimester-specific alterations in AVPR expression

	1st Trimester				3rd Trimester			
	Control	PreE			Control	PreE		
	Average ΔCT		Fold Change	p-value	Average ΔCT		Fold Change	p-value
AVPR1a	**9.4±0.6**	**12.1±0.3**	**−6.3**	**0.0009**	10.2±0.4	10.5±0.3	−1.1	0.56
AVPR1b	**20.4±0.7**	**22.3±0.5**	**−3.7**	**0.04**	21.2±0.6	21.6±0.4	−1.3	0.62
AVPR2	8.0±0.3	8.7±0.3	−1.7	0.14	**8.8±0.2**	**8.0±0.1**	**1.7**	**0.01**

Human CD4+ T cells were negatively selected from peripheral blood mononuclear cells of control (1st trimester N=6; 3rd trimester N=15) and preeclampsia-affected (PreE; 1st trimester N=9; 3rd trimester N=12–14) pregnancies. Expression of AVP receptors was determined via qPCR. Raw cycle threshold (Ct) values were normalized (ΔCt) against the18S rRNA endogenous control. The lower the ΔCt value of a specific target, the higher the expression. The ΔΔCt and expression fold change of AVP versus saline and preeclampsia-affected versus control pregnancy were calculated as previously described (47, 48). Data are mean ±SEM. Statistical significance was determined using a Student's t test and the minimal level of confidence deemed statistically significant was a p value <0.05.