Tolerance Induction at the Early Maternal–Placental Interface Through Selective Cell Recruitment and Targeting by Immune Polypeptides

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Pregnancy challenges immune cells and immunomodulatory circuits of the mother and the developing fetus to dynamically adapt to each other in an homeostatic and tolerant environment for fetal growth. This entails the coordination of multiple cellular processes all devoted to accommodate and nourish the fetus while protecting the mother from endogenous and exogenous threatens. From the earliest stages of pregnancy, several strategies to efficiently communicate immune and trophoblast cells within the interface or at a distance were identified and chemokines might act at on different targets through direct or indirect mechanisms. Here, we briefly review some mechanisms of T regulatory cell recruitment to the early maternal–placental interfaces to accomplish immunotolerance and homeostatic control and we discuss evidence on two locally released polypeptides, RANTES (regulated on activation, normal, T-cell expressed, and secreted) and vasoactive intestinal peptide (VIP), as novel contributors to the multiplicity of immune tolerant responses and uterine quiescence requirements.

Introduction

From an immunological standpoint, pregnancy was proposed to follow a temporal sequence with a predominantly pro-inflammatory first stage, an immunologically more quiescent, fetal growth promoting second period, and a final cut to a prominent inflammatory environment that precedes labor and delivery.[1] In humans, between weeks 3 and 8 of gestation, a variety of cellular processes are encompassed to ensure proper trophoblast growth and invasion, uterine quiescence, vascularization, and tissue remodeling in an immunotolerant microenvironment. Two major transition points appear to fully evoke efficient immunoregulatory mechanisms, and the first one occurs at about 8 weeks of gestation in transit to the early placentation period. This is a crucial step because it marks the transition from histiotrophic to placental nutrition,[2–6] and meanwhile, the immune system has succeeded in trophoblast accommodation and prepares for accompanying fetal growth by turning off pro-inflammatory signals. The immune signature of this transition phase is the induction and maintenance of tolerance, which will long for the next whole period requiring redundant circuits of cell-to-cell interaction as well as local mediators targeting multiple cells to sustain suppressor/tolerant microenvironment. An integrative view on this stage may help to get more insight into the mechanisms arising early after implantation that can compromise

pregnancy in an all-or-none manner with pregnancy loss or that can impair its clinical outcome at later stages as in preeclampsia.[1,2,7–10]

It has been pointed out that there is no other adult organ or tissue undergoing such a profound remodeling and leukocyte invasion with pro-inflammatory mediator release over such a short time period as the early pregnant uterus.[11] As expected, a tight homeostatic control in the successive maternal–placental interfaces is mostly provided by maternal immune cells, some of them maintained at constant levels by continuous recruitment, while others recruited in 'waves' to the pregnant uterus. Selective recruitment and expansion of decidual NK $CD16^{-}CD56^{bright}$ cells and NK-derived cytokines provide guiding signals for trophoblast invasion.[12–14] Several mechanisms have been proposed to explain the inability of NK cells to kill the semi-allogeneic fetal cells, however, nowadays is clear that decidual NK are potent secretors of angiogenic factors that induce vasodilatation and spiral artery remodeling, through specific interactions between activating/inhibitory receptors and their ligands expressed at the fetal–maternal interface.[15,16]

Meanwhile, macrophages display a notable cellular plasticity to control divergent processes.[17–21] Macrophages bearing suppressor/regulatory markers manage the silent clearance of apoptotic cells and contribute to wound healing. In contrast, classically activated macrophages face endogenous and exogenous threatening signals and calibrate the immune response fate to protect the mother from infections and excessive tissue injury. Finally, regulatory T-cell (Tregs) population is essential for preventing a maternal immune response against paternal antigens released by trophoblast cells or fetal cells at the implantation site and the maternal circulation. Tregs belong to the T-lymphocyte population, and among them, there are cells with distinct phenotype, cytokine secretion profile, and tissue origin; all of them displaying suppressive and regulatory properties that contribute to maintaining the antigen-specific T-cell tolerance.[22–24] Tregs are stimulated through antigen-specific or non-specific pathways; thus, exerting their suppressive actions is critical during the peri-implantation phase of pregnancy. In fact, paternal antigen-specific Treg cells present at the draining lymph nodes quickly migrate to the pregnant uterus where these cells proliferate resulting in the induction of paternal antigen-specific tolerance at the early stages of pregnancy.[25–28]

Tolerogenic responses during early pregnancy are currently analyzed as a transient and context-dependent suppression of the immune response, whereas alternative and redundant immune evasion mechanisms are necessarily active throughout gestation.[29,30] Here, we focused on chemokine-mediated programs for Treg cell recruitment and tolerance induction to trophoblast antigens, and we discuss evidence on the contribution of locally released vasoactive intestinal peptide (VIP) to an immune tolerant and quiescent microenvironment.

Chemokines as immune regulators of the maternal response

The chemokine family (short for chemo-attractant cytokines) is responsible of several physiological processes such as the coordination of normal leukocyte trafficking, embryonic development, embryonic growth, wound healing and angiogenesis.

Chemokines and their receptors are classified according to their structure or expression. In the first classification, cysteins in conserved positions distinguish the CXC family (α-chemokines) and the CC family (β-chemokines), whereas according to their expression, they are *inflammatory*, *induced upon T-cell activation* and *constitutive* chemokines that fulfill housekeeping functions and/or participate in constitutive leukocyte trafficking.[31–33] The switch from receptors for constitutive chemokines to receptors for inflammatory chemokines changes the migratory properties of leukocyte populations.[34–36] The high promiscuity of chemokine network not only implies multiple receptor binding by a single chemokine but also that one chemokine lacking might disrupt the entire network at acute inflammation sites and isolated cell subpopulations.[37]

Chemokine receptors belong to the superfamily of G-protein-coupled receptors and are named according to chemokine structure (CXCR for CXC or CCR for CC chemokines). They can form homo/heterodimers or heterooligomers whose physiological consequences are under study.[37] A number of adaptor proteins interact with chemokine receptors and facilitate internalization and signal transduction by forming a dynamic 'chemosynapse' with spatial and temporal plasticity.[38–40] However, the characterization and functional impact of such associations at the maternal–placental interface were not evaluated so far.

Selective recruitment of maternal Treg cells to the early interfaces

Considering the major role of Treg cells in tolerance induction during the first weeks of gestation, it is conceivable that the polarizing microenvironment will determine the chemokine receptor profile on Treg cells and therefore might direct them to appropriate tissue sites for immune suppression. Induced Treg cells specific for the antigen will upregulate the expression of chemokine receptors that are also expressed by effector T cells, and this overlap will allow Treg cells to localize with effector T cells and induce the suppression of diverse inflammatory conditions.[32] On the other hand, much evidence has accumulated on the role of β-chemokines as peripheral tolerance inducers. Two chemokine-mediated programs contributing to maternal–placental interface generation are discussed next: selective recruitment of Treg cells and maternal tolerance induction toward trophoblast antigens.

Depending on the cytokine milieu where Treg cells are activated, a differential chemokine profile expression is displayed. Hence, allorecognition of paternal or trophoblast antigens enhances maternal leukocyte recruitment and the production of pro-implantatory mediators. In the murine gestation, Treg cells expressing CCR5 are recruited to the uterus, playing an essential role in preventing fetal rejection by the maternal immune system. The accumulation of CCR5+ Treg cells with high suppressant ability occurs selectively in the uterus, in contrast to the systemic expansion of Treg cells that appears to be alloantigen independent.[41]

In human pregnancy, Mold et al. demonstrated that maternal alloantigens promote the development of tolerogenic fetal Treg cells *in utero*. Maternal cells cross the placenta to reside in fetal lymph nodes, inducing the development of fetal Tregs that suppress fetal antimaternal immunity and persist at least until early adulthood.[42–44] Consistently, Tilburgs et al.[45,46] presented evidence of a selective migration of fetus-specific CD4+CD25^{bright} Treg cells to decidua basalis and parietalis that suppress fetus-specific and non-specific responses.

Trophoblast cells not only contribute to iTreg cell differentiation, but also selectively recruit them. Migration assays performed in transwell systems with conditioned media from first trimester trophoblast HTR-8[47] or Swan71 cell lines[48] doubled Foxp3$^+$ cell recruitment compared to the positive control of human serum. In fact, the frequency of Foxp3$^+$ cells migrated toward trophoblast cells in the presence of a bacterial or viral stimulus increased and CCL4, CCL5, CXCL1, and CXCL8 secretion by trophoblast cells further recruited iTregs.[26] Regarding chemokine production to limit T-cell access to the maternal–placental interface, Nancy et al.[49] recently reported that genes encoding chemokines responsible for Th1 attraction and T cytotoxic profiles are subject of epigenetic silencing in decidual stromal cells.

RANTES contribution to the maternal tolerance response

Increasing evidence supports that β-chemokines are inducers of peripheral tolerance as extensively discussed.[50–53] During the maternal–placental cross-talk, RANTES (*regulated on activation, normal, T-cell expressed, and secreted*) contributes to immune homeostasis displaying different strategies at systemic and local levels. RANTES specifically and dose dependently suppressed the maternal allogeneic response to paternal antigens in human mixed lymphocyte cultures by inducing activated T-cell apoptosis and Bcl-2 modulation.[54] Likewise, an anti-RANTES antibody blocked the proliferative response induced by fertile women sera, supporting that RANTES acts as a novel suppressive factor of the allogeneic maternal response.[54]

RANTES is locally released by the pre-implantation endometrium, and of note, it has the potential to act in an autocrine manner by a differential expression of RANTES receptors CCR1, CCR3, and CCR5.[55] In addition, RANTES is produced by human endometrial T-infiltrated lymphocytes, CD4$^+$ and CD8$^+$, whose production increased in the presence of physiological progesterone concentrations.

At the maternal–placental interface, trophoblast cells constitutively secrete RANTES accompanied by pro-inflammatory cytokine production such as TNF-α, low levels of IFN-γ and IL-12, nitrite production related to uterine quiescence and angiogenesis, and LIF expression, characteristic of a pro-implantatory microenvironment.[12,56]

Using an *in vitro* model of maternal–placental cross-talk represented by coculture of trophoblast cell line Swan 71 and maternal PBMCs, RANTES modulated T effector/Treg balance.[57] On one hand, RANTES-induced apoptosis of potentially deleterious CD3+ lymphocytes correlating with a signifi-

cant decrease in the maternal T-cell proliferative response. Interestingly, Swan 71 cells did not express CCR5, making them potentially resistant to RANTES-induced apoptosis and suggesting a possible mechanism by which RANTES could selectively induce alloreactive maternal lymphocyte apoptosis to control an exacerbated alloresponse.[57] On the other hand, RANTES increased the frequency of Tregs (CD4+CD25+Foxp3+) during the maternal PBMC–trophoblast interaction and this effect was prevented by anti-RANTES-neutralizing Ab. Taken together, evidence support a role for RANTES during early implantation through increasing regulatory T lymphocytes in an adequate pro-inflammatory microenvironment, inducing apoptosis of maternal alloactivated T cells and favoring trophoblast survival and maternal tolerance to fetal antigens.

The association between RANTES and pregnancy complications was evidenced in human and animal models. In the CBA/J × DBA/2 murine model of pregnancy loss,[58] placentas produced high levels of RANTES, correlating with an exacerbated Th1 response. Furthermore, the deleterious effect was abrogated after multiple pregnancies, supporting that allorecognition may also confer beneficial effects.[58]

An example of this association in human pregnancy is the villitis of unknown etiology (VUE), a destructive inflammatory lesion of villous placenta characterized by the decidual macrophage activated in an inflammatory profile and T helper-1 effector profile.[59] The transcriptoma of VUE placentas revealed an increase in a subset of chemokines and their receptors, including CCL5 and CCR5, accompanied with a systemic derangement of CXC chemokines in maternal and fetal circulation.[59] On the other hand, cocultures of trophoblast cells and PBMCs from women with recurrent spontaneous abortions (RSA) displayed an altered temporal window of RANTES production, which correlated with a misbalance of the Teffector/Treg response.[57] RSA-PBMCs displayed an exacerbated pro-inflammatory and Th1 response after the interaction with trophoblast cells and a decrease in Treg frequency with lower levels of TGF-β and IL-10 secretion.[60] Interestingly, a high frequency of apoptotic trophoblast cells appeared after trophoblast cells interacts with maternal RSA-PBMCs and this increase correlated with low levels of apoptotic maternal CD3+ lymphocytes potentially deleterious to fetal survival.

Vasoactive intestinal peptide: an old actor with renewed characters at the maternal–placental interface

As pointed out before, the transition to and maintenance of an anti-inflammatory and immune tolerant second period clearly depends on redundancy of immune tolerance circuits where locally released mediators may have a prominent role. Among them, VIP is an interesting example that fulfills criteria for multiple cell target factors synthesized at the maternal interface with suppressant/tolerant activity.

Vasoactive intestinal peptide is a 28-amino acid peptide that is structurally related to secretin, pituitary adenylate cyclase–activating polypeptide (PACAP), glucagon and growth hormone–releasing factor, among others. It binds to class B members of the G-protein-coupled receptors superfamily.[61] Two subtypes of VIP receptors named VPAC1 and VPAC2 were described on the basis of their sequence, affinity, expression, and signaling profiles.[62] They recognize VIP and PACAP with similar affinity, whereas other members of class B GPCRs bind VIP with lower affinity.[62] Both VPACs are coupled to Gs/AMPc/PKA signaling, and they also signal through PLC, MAPK, and NF-κB inhibition.[63–65]

First described as a neurotransmitter by Sami Said and Viktor Mutt in 1970, VIP proved to have potent immunomodulatory and trophic effects through its action on VPACs on adult and embryonic tissues. It has direct and indirect neuromodulatory and neurotrophic effects[66] and elicits trophic, prosecretory, and vasodilator effects on exocrine gland cells.[67,68] VIP could contribute to post-implantation uterus quiescence because it induces smooth muscle relaxation of pregnant and nonpregnant uterus[69] where a reduction in prostaglandin synthesis and nitric oxide synthase stimulation was reported.[70]

Evidence on VIP anti-inflammatory and tolerogenic effects was provided by *in vitro* designs with human[71] and murine cells,[72] as well as from studies in animal models of viral disease[73] and chronic inflammation.[74–80] Due to its low bioavailability, dendritic cells transduced with lentiviral vectors expressing VIP were also used as a strategy to locally deliver the peptide in inflammation models.[81] Likewise, VIP modifies the inflammatory profile of patient cells in arthritis and osteoarthritis patients.[82,83] VIP induces IL-10 synthesis and reduces IL-12, TNF-α, and inducible nitric oxide synthase activity in human and murine

macrophages through both VPACs, thus proposed as a regulatory/suppressant macrophage phenotype inducer.[17,65,76] Dendritic cells are also targeted by VIP to differentiate into a tolerogenic profile that produce high levels of IL-10 and induce antigen-specific Treg cells.[80] Regarding T cells, VIP reduces Th17/Th1 and Th1/Th2 ratios,[84] induces Foxp3+ regulatory T cells[85] and, in the presence of TGF-β, VIP can differentiate murine CD4+T cells to a distinctive Th17 cell phenotype that generates IL-17 but not IL-6 or IL-21.[86] It has also emerged as a putative physiological inhibitor of the calcineurin–NFAT pathway, a master regulator of immune responses, and lack of the VIP gene results in inflammation and smooth muscle contraction.[87] VIP is not synthesized by human or murine macrophages, and a limited peptide expression was found in CD4+ T cells after antigen stimulation.[88,89]

Vasoactive intestinal peptide at the early maternal–placental interface

Vasoactive intestinal peptide levels raise in murine maternal–placental interface with higher expression in decidual tissue and a peak at gestational days 9–12 that mark the transition to the placentation period.[90–93] This period corresponds to murine pregnancy midgestation at the end of the most vulnerable period for intrauterine development when embryonic developmental events can be approximately compared with days 22–32 of human pregnancy.[90] VIP stimulates neural differentiation of mouse embryo: VIP from maternal sources enhanced post-implantation embryo growth at day E9 and its blockade in pregnant mice on days 9.5–11.5 but not afterward induced growth retardation and microcephaly.[91,92,94] The temporal window of maternal VIP expression precedes the appearance of embryonic VIP at day 14.5 in murine peripheral nervous system.[95] Thus, an apparent paradox arises: embryo growth regulation by VIP occurs at early post-implantation stage when VIP sources are only available in maternal tissues. Accordingly, offspring of VIP-deficient mothers exhibit developmental delays and lower birth weight than WT mice or offspring *vip (+/+), vip (+/−), or vip (−/−)* born to wild-type mothers, highlighting the role of maternal rather than fetal VIP in early neural development.[96]

In pregnant women, localization of VIP in decidual face of full-term placentas has been reported.[97] It was shown to localize in the trophoblast (syncytium and extravillous cytotrophoblast cells) of first trimester and term placenta.[98] Extravillous cytotrophoblastic cells and some decidual cells (negative for cytokeratin) were also weakly stained for VIP. Interestingly, VIP dose dependently stimulated progesterone secretion from human primary cultured trophoblast cells and JEK-3 cells, where it also stimulated hCG production.[98,99] Moreover, arterial and venous concentrations of VIP in human umbilical cord were more than twofold the concentration in peripheral venous blood pointing to a predominant local action profile.[100]

Cumulative evidence on human and murine pregnancy supports an immunomodulatory role of VIP at early stages: It increased CD4+CD25+Foxp3+ Treg cells and LIF expression in implantation site explants of normal mice at day 9.[101] Consistent with a trophic and suppressant effect of the peptide at these early stages, lower levels of decidual VIP expression were found in viable implantation sites of prediabetic non-obese diabetic (NOD) mice, a high resorption rate mouse strain related to an inflammatory background.[101] However, VPAC receptors were normally expressed in NOD mice implantation sites at gestational day 9 and VIP could induce Foxp3 and LIF expression pointing to the integrity of VPAC signaling.[101] A predominant suppressant phenotype was observed in peritoneal macrophages from early pregnant mice, and VIP contributes to this phenotype switch with reduced nitric oxide and enhanced IL-10 production.[102]

In experimental coculture designs with human cells, VIP showed trophic effects and modulated the immune/trophoblast cell interaction.[103,104] First trimester human Swan-71 trophoblast cells express VPACs and synthesize VIP, which stimulated trophoblast cell proliferation. Furthermore, VIP induced a tolerogenic phenotype in human peripheral blood T cells when they were cocultured with trophoblast cells.[103] It significantly decreased T-bet expression, reduced MCP-1 and nitrite production in cocultures of fertile women PBMCs with trophoblast cells, while it increased the frequency of CD4+CD25+Foxp3+ cells, TFGβ expression, and IL-10 secretion. VIP also induced LIF production. In fact, VIP and progesterone increased the frequency of CD4+ LIF+ cells from fertile women in response to paternal and trophoblast antigens *in vitro*.[104] PBMCs from these women also showed a significant frequency of VIP-producer CD4 lymphocytes after the interaction with trophoblast cells.[60] Finally, as reported in murine models, we observed human Treg cell induction by VIP in the presence of Swan-71 trophoblast cells through a

TGF-β-dependent pathway. Moreover, in this *in vitro* design, VIP enhanced inducible Treg cell migration toward trophoblast cells (manuscript in preparation).

Finally, in the context of redundant circuits required for tolerance induction and maintenance, it is plausible that VIP could trigger additional tolerogenic programs. Noteworthy, VIP can selectively upregulate galectin-1 expression during the differentiation or maturation of mouse dendritic cells,[105] an observation that further supports the well-known central immunoregulatory role of galectins in the post-implantation period,[5,106–108] a subject that has been updated by Dr Rabinovich in this issue. Briefly, Gal1 is evolutionarily conserved and abundantly expressed in the placenta and female reproductive tract of various species.[109,110] Mice lacking Gal1 (Lgals1−/−) showed higher rates of fetal loss compared to their wild-type counterpart in allogeneic, and the administration of recombinant Gal1 prevents fetal loss and restores tolerance *in vivo*.[107] This lectin is mainly expressed in invasive extravillous trophoblast cells of human first trimester and term placenta, and it is regulated by progesterone and pro-inflammatory cytokines thus limiting T-cell viability, dampening the secretion of Th1-type cytokines, and favoring the expansion of CD4+CD25+FoxP3+ Treg cells. In addition, using coculture experiments without exogenous addition of rhGal1 that trophoblast cells negatively regulate T-cell survival via Gal1-mediated mechanisms. Pro-inflammatory cytokines such as TNF-α and IL-2 considerably upregulate Gal1 in the human JEG-3 choriocarcinoma cell line, as a homeostatic mechanism to favor the resolution of exacerbated T-cell responses.[106]

Conclusions and perspective

During the first weeks of pregnancy, embryo accommodation and nutrition occur within a strict homeostatic control. The endovascular route of trophoblast invasion requires constant vasodilator activity, and the more recently described trophoblast endoglandular route provides embryo nutrition by endometrial gland secretion, both entailing intense tissue remodeling pathways.[1–6] The encountering of specifically and timely recruited immune cells with locally released immunomodulatory factors in a trophoblast orchestrated manner underlies the control of maternal immune homeostasis at these early stages. Redundant circuits for tolerance induction and maintenance are mandatory, thus multiplicity in cells targeted by locally released immunomodulators seems to be crucial and VIP and RANTES may play that role. The contribution of various VIP sources namely neural, immune, smooth muscle, and trophoblastic at the maternal–placental interface may reflect its complementary immune tolerant, uterine quiescent, and trophic effects.

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References

1 Mor G, Cardenas I: The immune system in pregnancy: a unique complexity. *Am J Reprod Immunol* 2010; 63:425–433.
2 Redman CWG, Sargent IL: Immunology of pre-eclampsia. *Am J Reprod Immunol* 2010; 63:534–543.
3 Dekel N, Gnainsky Y, Granot I, Mor G: Inflammation and implantation. *Am J Reprod Immunol* 2010; 63:17–21.
4 Moser G, Gauster M, Orendi K, Glasner A, Theuerkauf R, Huppertz B: Endoglandular trophoblast, an alternative route of trophoblast invasion? Analysis with novel confrontation co-culture models. *Hum Reprod* 2010; 25:1127–1136.
5 Fitzgerald JS, Germeyer A, Huppertz B, Jeschke U, Knöfler M, Moser G, Scholz C, Sonderegger S, Toth B, Markert UR: Governing the invasive trophoblast: current aspects on intra- and extracellular regulation. *Am J Reprod Immunol* 2010; 63:492–505.
6 Burton GJ, Jauniaux E, Charnock-Jones DS: Human early placental development: potential roles of the endometrial glands. *Placenta* 2007; 28(Suppl A):S64–S69.
7 Daher S, de Arruda Geraldes Denardi K, Blotta MH, Mamoni RL, Reck AP, Camano L, Mattar R: Cytokines in recurrent pregnancy loss. *J Reprod Immunol* 2004; 62:151–157.
8 Clark DA, Yu G, Arck PC, Levy GA, Gorczynski RM: MD-1 is a critical part of the mechanism causing Th1-cytokine-triggered murine fetal loss syndrome. *Am J Reprod Immunol* 2003; 49: 297–307.
9 Kwak-Kim J, Yang KM, Gilman-Sachs A: Recurrent pregnancy loss: a disease of inflammation and coagulation. *J Obstet Gynaecol Res* 2009; 35:609–622.

10 Girardi G, Yarilin D, Thurman JM, Holers VM, Salmon JE: Complement activation induces dysregulation of angiogenic factors and causes fetal rejection and growth restriction. *J Exp Med* 2006; 203:2165–2175.
11 Gomez-Lopez N, Guilbert LJ, Olson DM: Invasion of the leukocytes into the fetal-maternal interface during pregnancy. *J Leukoc Biol* 2010; 88:625–633.
12 Ashkar AA, Di Santo JP, Croy BA: Interferon gamma contributes to initiation of uterine vascular modification, decidual integrity, and uterine natural killer cell maturation during normal murine pregnancy. *J Exp Med* 2000; 192:259–270.
13 van den Heuvel MJ, Xie X, Tayade C, Peralta C, Fang Y, Leonard S, Paffaro Jr VA, Sheikhi AK, Murrant C, Croy BA: A review of trafficking and activation of uterine natural killer cells. *Am J Reprod Immunol* 2005; 54:322–331.
14 Harris LK: IFPA Gabor Than Award lecture: transformation of the spiral arteries in human pregnancy: key events in the remodelling timeline. *Placenta* 2011; 32(Suppl 2):S154–S158.
15 Moffett-King A: Natural killer cells and pregnancy. *Nat Rev Immunol* 2002; 2:656–663.
16 Hanna J, Goldman-Wohl D, Hamani Y, Avraham I, Greenfield C, Natanson-Yaron S, Prus D, Cohen-Daniel L, Arnon TI, Manaster I, Gazit R, Yutkin V, Benharroch D, Porgador A, Keshet E, Yagel S, Mandelboim O: Decidual NK cells regulate key developmental processes at the human fetal-maternal interface. *Nat Med* 2006; 12:1065–1074.
17 Mosser DM, Edwards JP: Exploring the full spectrum of macrophage activation. *Nat Rev Immunol* 2008; 8:958–969.
18 Strazewski Chavez SL, Abrahams VM, Mor G: The role of apoptosis in the regulation of trophoblast survival and differentiation during pregnancy. *Endocr Rev* 2005; 26:877–897.
19 Fest S, Aldo PB, Abrahams VM, Visintin I, Alvero A, Chen R, Chavez SL, Romero R, Mor G: Trophoblast-macrophage interactions: a regulatory network for the protection of pregnancy. *Am J Reprod Immunol* 2007; 57:55–66.
20 Abrahams VM, Kim YM, Straszewski SL, Romero R, Mor G: Macrophages and apoptotic cell clearance during pregnancy. *Am J Reprod Immunol* 2004; 51:275–282.
21 Nagamatsu T, Schust DJ: The contribution of macrophages to normal and pathological pregnancies. *Am J Reprod Immunol* 2010; 63:460–471.
22 Aluvihare VR, Kallikourdis M, Betz AG: Regulatory T cells mediate maternal tolerance to the fetus. *Nat Immunol* 2004; 5:266–271.
23 Terness P, Kallikourdis M, Betz AG, Rabinovich GA, Saito S, Clark DA: Tolerance signaling molecules and pregnancy: IDO, galectins, and the renaissance of regulatory T cells. *Am J Reprod Immunol* 2007; 58:238–254.
24 Leber A, Teles A, Zenclussen AC: Regulatory T cells and their role in pregnancy. *Am J Reprod Immunol* 2010; 63:445–459.
25 Zenclussen AC, Gerlof K, Zenclussen ML, Sollwedel A, Bertoja AZ, Ritter T, Kotsch K, Leber J, Volk HD: Abnormal T-cell reactivity against paternal antigens in spontaneous abortion: adoptive transfer of pregnancy-induced CD4+CD25+ T regulatory cells prevents fetal rejection in a murine abortion model. *Am J Pathol* 2005; 166:811–822.
26 Ramhorst R, Fraccaroli L, Aldo P, Alvero AB, Cardenas I, Perez Leirós C, Mor G: Modulation and recruitment of inducible regulatory T cells by first trimester trophoblast cells. *Am J Reprod Immunol* 2012; 67:17–27.
27 Saito S, Nakashima A, Shima T, Ito M: Th1/Th2/Th17 and regulatory T-cell paradigm in pregnancy. *Am J Reprod Immunol* 2010; 63:601–610.
28 Schumacher A, Wafula PO, Teles A, El-Mousleh T, Linzke N, Zenclussen ML, Langwisch S, Heinze K, Wollenberg I, Casalis PA, Volk HD, Fest S, Zenclussen AC: Blockage of heme oxygenase-1 abrogates the protective effect of regulatory T cells on murine pregnancy and promotes the maturation of dendritic cells. *PLoS ONE* 2012; 7:e42301.
29 Munoz-Suano A, Hamilton AB, Betz AG: Gimme shelter: the immune system during pregnancy. *Immunol Rev* 2011; 241:20–38.
30 Warning JC, McCracken SA, Morris JM: A balancing act: mechanisms by which the fetus avoids rejection by the maternal immune system. *Reproduction* 2011; 141:715–724.
31 Rossi D, Zlotnik A: The biology of chemokines and their receptors. *Annu Rev Immunol* 2000; 18:217–242.
32 Bromley SK, Mempel TR, Luster AD: Orchestrating the orchestrators: chemokines in control of T cell traffic. *Nat Immunol* 2008; 9:970–980.
33 Fraccaroli L, Alfieri J, Pérez Leiros C, Ramhorst R: Immunomodulatory effects of chemokines during the early implantation window. *Front Biosc* 2009; 1:288–298.
34 Lutther AJ, Cyster AC: Chemokines as regulators of T cell differentiation. *Nat Med* 2001; 2:102–107.
35 Moser B, Loetscher P: Lymphocyte traffic control by chemokines. *Nat Med* 2001; 2:123–128.
36 Sohy D, Yano H, de Nadai P, Urizar E, Guillabert A, Javitch JA, Parmentier M, Springael JY: Hetero-oligomerization of CCR2, CCR5, and CXCR4 and the protean effects of "selective" antagonists. *J Biol Chem* 2009; 284:31270–31279.
37 Raman D, Sobolik-Delmaire T, Richmond A: Chemokines in health and disease. *Exp Cell Res* 2011; 317:575–589.
38 Neel NF, Barzik M, Raman D, Sobolik-Delmaire T, Sai J, Ham AJ, Mernaugh RL, Gertler FB, Richmond A: VASP is a CXCR2-interacting protein that regulates CXCR2-mediated polarization and chemotaxis. *J Cell Sci* 2009; 122:1882–1894.
39 Raman D, Neel NF, Sai J, Mernaugh RL, Ham AJ, Richmond AJ: Characterization of chemokine receptor CXCR2 interacting proteins using a proteomics approach to define the CXCR2 "chemosynapse". *Methods Enzymol* 2009; 460:315–330.
40 Raman D, Sai J, Neel NF, Chew CS, Richmond A: LIM and SH3 protein-1 modulates CXCR2- mediated cell migration. *PLoS ONE* 2010; 5:e10050.
41 Kallikourdis M, Andersen KG, Welch KA, Betz AG: Alloantigen-enhanced accumulation of CCR5+ effector regulatory T cells in the gravid uterus. *PNAS* 2007; 104:594–599.
42 Mold JE, Michaëlsson J, Burt TD, Muench MO, Beckerman PK, Busch MP, Lee T, Nixon DF, McCune JM: Maternal alloantigens promote the development of tolerogenic fetal regulatory t cells in utero. *Science* 2008; 322:1562–1565.
43 Mjösberg J, Svensson J, Johansson E, Hellström L, Casas R, Jenmalm MC, Boij R, Matthiesen L, Jönsson JI, Berg G, Ernerudh J: Systemic reduction of functionally suppressive CD4dimCD25highFoxp3+ Tregs in human second trimester pregnancy is induced by progesterone and 17beta-estradiol. *J Immunol* 2009; 183:759–769.
44 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. *Biol Reprod* 2010; 82:698–705.

45 Tilburgs T, Roelen DL, van der Mast BJ, de Groot-Swings GM, Kleijburg C, Scherjon SA, Claas FH: Evidence for a selective migration of fetus-specific CD4+CD25bright regulatory T cells from the peripheral blood to the decidua in human pregnancy. *J Immunol* 2008; 180:5737–5745.
46 Tilburgs T, Schonkeren D, Eikmans M, Nagtzaam NM, Datema G, Swings GM, Prins F, van Lith JM, van der Mast BJ, Roelen DL, Scherjon SA, Claas FH: Human decidual tissue contains differentiated CD8+ effector-memory T cells with unique properties. *J Immunol* 2010; 185:4470–4477.
47 Graham CH, Hawley TS, Hawley RG, MacDougall JR, Kerbel RS, Khoo N, Lala PK: Establishment and characterization of first trimester human trophoblast cells with extended lifespan. *Exp Cell Res* 1993; 206:204–211.
48 Straszewski-Chavez SL, Abrahams VM, Alvero AB, Aldo PB, Ma Y, Guller S, Romero R, Mor G: The isolation and characterization of a novel telomerase immortalized first trimester trophoblast cell line, Swan 71. *Placenta* 2009; 30:939–948.
49 Nancy P, Tagliani E, Tay CS, Asp P, Levy DE, Erlebacher A: Chemokine gene silencing in decidual stromal cells limits T cell access to the maternal-fetal interface. *Science* 2012; 336:1317–1321.
50 Cocchi F, DeVico AL, Garzino-Demo A, Arya SK, Gallo RC, Lusso P: Identification of RANTES, MIP-1αand MIP-1β as the major HIV-suppressive factors produced by CD8+ T cells. *Science* 1995; 270:1811–1815.
51 Hornung D, Klingel K, Dohrn K, Kandolf R, Wallwiener D, Tayler RN: Regulated on activation, normal T-cell expressed and –secreted mRNA expression in normal endometrium and endometriotic implants: assessment of autocrine/paracrine regulation by in situ hybridization. *Am J Pathol* 2001; 158:1949–1954.
52 Faunce D, Stein-Streilein J: NKT Cell-derived RANTES recruits APCs and CD8+ T cells to the spleen during the generation of regulatory T cells in tolerance. *J Immunol* 2002; 169:31–38.
53 Nakamura T, Sonoda K, Faunce D, Gumperz J, Yamamura T, Miyake S, Stein-Streilein T: CD4+ NKT cells, but not conventional CD4+ T cells are required to generate efferent CD8+ T regulatory cells following antigen inoculation in an immune-privileged site. *J Immunol* 2003; 171:1266–1271.
54 Rabinovich GA, Ramhorst RE, Rubinstein N, Corigliano A, Daroqui MC, Kier-Joffé EB, Fainboim L: Induction of allogenic T-cell hyporesponsiveness by galectin-1-mediated apoptotic and non-apoptotic mechanisms. *Cell Death Differ* 2002; 9:661–670.
55 Ramhorst R, Patel R, Corigliano A, Etchepareborda JJ, Fainboim L, Schust D: Induction of maternal tolerance to fetal alloantigens by RANTES production. *Am J Reprod Immunol* 2006; 56:302–311.
56 Paria B, Reese J, Das S, Dey S: Deciphering the cross talk of implantation: advances and challenges. *Science* 2002; 296:2185–2188.
57 Fraccaroli L, Alfieri J, Larocca L, Calafat M, Mor G, Pérez Leiros C, Ramhorst R: A potential tolerogenic immune mechanism in a trophoblast cell line through the activation of chemokine-induced T cell death and regulatory T cell modulation. *Hum Reprod* 2009; 24:166–175.
58 Ramhorst R, Gutiérrez G, Corigliano A, Junovich G, Fainboim L: Implication of RANTES in the modulation of alloimmune response by progesterone during pregnancy. *Am J Reprod Immunol* 2007; 57:147–152.
59 Kim MJ, Romero R, Kim CJ, Tarca AL, Chhauy S, LaJeunesse C, Lee DC, Draghici S, Gotsch F, Kusanovic JP, Hassan SS, Kim JS: Villitis of unknown etiology is associated with a distinct pattern of chemokine up-regulation in the feto-maternal and placental compartments: implications for conjoint maternal allograft rejection and maternal anti-fetal graft-versus-host disease. *J Immunol* 2009; 182:3919–3927.
60 Fraccaroli L, Grasso E, Hauk V, Cortelezzi M, Calo G, Pérez Leirós C, Ramhorst R: Defects in the vasoactive intestinal peptide (VIP)/VPAC system during early stages of the placental-maternal leucocyte interaction impair the maternal tolerogenic response. *Clin Exp Immunol* 2012; 170:310–320.
61 Said SI: The discovery of VIP: initially looked for in the lung, isolated from intestine, and identified as a neuropeptide. *Peptides* 2007; 28:1620–1621.
62 Couvineau A, Laburthe M: VPAC receptors: structure, molecular pharmacology and interaction with accessory proteins. *Br J Pharmacol* 2012; 166:42–50.
63 Onoue S, Misaka S, Yamada S: Structure-activity relationship of vasoactive intestinal peptide (VIP): potent agonists and potential clinical applications. *Naunyn-Schmiedeberg's Arch Pharmacol* 2008; 377:579–590.
64 Harmar AJ, Fahrenkrug J, Gozes I, Laburthe M, May V, Pisegna JR, Vaudry D, Vaudry H, Waschek JA, Said SI: Pharmacology and functions of receptors for vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide: IUPHAR review 1. *Br J Pharmacol* 2012; 166:4–17.
65 Delgado M, Munoz-Elias EJ, Gomariz RP, Ganea D: Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide prevent inducible nitric oxide synthase transcription in macrophages by inhibiting NF-kappa B and IFN regulatory factor 1 activation. *J Immunol* 1999; 162:4685–4696.
66 Gressens P, Marret S, Hill JM, Brenneman DE, Gozes I, Fridkin M, Evrard P: Vasoactive intestinal peptide prevents excitotoxic cell death in the murine developing brain. *J Clin Invest* 1997; 100:390–397.
67 Månsson B, Nilsson BO, Ekström J: Effects of repeated infusions of substance P and vasoactive intestinal peptide on the weights of salivary glands subjected to atrophying influences in rats. *Br J Pharmacol* 1990; 101:853–858.
68 Ekström J, Mansson B, Tobin G: Vasoactive intestinal peptide evokes secretion of fluid and protein from rat salivary glands and the development of supersensitivity. *Acta Physiol Scand* 1983; 119:169–175.
69 Jovanovic S, Grbovic L, Jovanovic A: Pregnancy does not alter the response of uterine arteries to vasoactive intestinal polypeptide. *Mol Hum Reprod* 2000; 6:361–368.
70 Roca V, Larocca L, Calafat M, Aisemberg J, Meiss R, Franchi A, Perez Leiros C: Reduced nitric oxide synthase and cyclo-oxygenase activity in the uterus of non-obese diabetic mice. *Reproduction* 2006; 32:931–938.
71 Lara-Marquez M, O'Dorisio M, O'Dorisio T, Shah M, Karacay B: Selective gene expression and activation-dependent regulation of vasoactive intestinal peptide receptor type 1 and type 2 in human T cells. *J Immunol* 2006; 166:2522–2530.
72 Martinez C, Delgado M, Abad C, Gomariz RP, Ganea D, Leceta J: Regulation of VIP production and secretion by murine lymphocytes. *J Neuroimmunol* 1999; 93:126–138.
73 Li JM, Southerland L, Hossain MS, Giver CR, Wang Y, Darlak K, Harris W, Waschek J, Waller EK: Absence of vasoactive intestinal peptide expression in hematopoietic cells enhances Th1 polarization and antiviral immunity in mice. *J Immunol* 2011; 187:1057–1065.
74 Lodde BM, Mineshiba F, Wang J, Cotrim P, Afione S, Tak PP, Baum BJ: Effect of human vasoactive intestinal peptide gene

transfer in a murine model of Sjogren's syndrome. *Ann Rheum Dis* 2006; 65:195–200.
75 Delgado M, Abad C, Martinez C, Leceta J, Gomariz RP: Vasoactive intestinal peptide prevents experimental arthritis by downregulating both autoimmune and inflammatory components of the disease. *Nat Med* 2001; 7:563–568.
76 Gonzalez-Rey E, Delgado M: Vasoactive intestinal peptide and regulatory T-cell induction: a new mechanism and therapeutic potential for immune homeostasis. *Trends Mol Med* 2007; 13:241–251.
77 Yadav M, Goetzl EJ: Vasoactive intestinal peptide-mediated Th17 differentiation: an expanding spectrum of vasoactive intestinal peptide effects in immunity and autoimmunity. *Ann N Y Acad Sci* 2008; 1144:83–89.
78 Jimeno R, Gomariz RP, Gutiérrez-Cañas I, Martínez C, Juarranz Y, Leceta J: New insights into the role of VIP on the ratio of T-cell subsets during the development of autoimmune diabetes. *Immunol Cell Biol* 2010; 88:734–745.
79 Rosignoli F, Torroba M, Juarranz Y, Gomez IA, Martinez C, Gomariz RP, Pérez Leirós C, Leceta J: VIP and tolerance induction in autoimmunity. *Ann N Y Acad Sci* 2006; 6:1–6.
80 Chorny A, Gonzalez-Rey E, Fernandez Martin A, Ganea D, Delgado M: Vasointestinal peptide induces regulatory dendritic cells that prevent acute graft-host disease while maintaining the graft-versus tumor response. *Blood* 2006; 107:3787–3794.
81 Toscano MG, Delgado M, Kong W, Martin F, Skarica M, Ganea D: Dendritic cells transduced with lentiviral vectors expressing VIP differentiate into VIP-secreting tolerogenic-like DCs. *Mol Ther* 2010; 18:1035–1045.
82 Juarranz MG, Santiago B, Torroba M, Gutierrez-Cañas I, Palao G, Galindo M, Abad C, Martinez C, Leceta J, Pablos JL, Gomariz RP: Vasoactive intestinal peptide modulates proinflammatory mediator synthesis in osteoarthritic and rheumatoid synovial cells. *Rheumatology* 2004; 43:416–422.
83 Carrión M, Juarranz Y, Pérez-García S, Jimeno R, Pablos JL, Gomariz RP, Gutiérrez-Cañas I: RNA sensors in human osteoarthritis and rheumatoid arthritis synovial fibroblasts: immune regulation by vasoactive intestinal peptide. *Arthritis Rheum* 2011; 63:1626–1636.
84 Jimeno R, Leceta J, Martínez C, Gutiérrez-Cañas I, Pérez-García S, Carrión M, Gomariz RP, Juarranz Y: Effect of VIP on the balance between cytokines and master regulators of activated helper T cells. *Immunol Cell Biol* 2012; 90:178–186.
85 Pozo D, Anderson P, Gonzalez-Rey E: Induction of alloantigen-specific human T regulatory cells by vasoactive intestinal peptide. *J Immunol* 2009; 183:4346–4359.
86 Yadav M, Rosenbaum J, Goetzl EJ: Cutting edge: vasoactive intestinal peptide (VIP) induces differentiation of Th17 cells with a distinctive cytokine profile. *J Immunol* 2008; 180:2772–2776.
87 Said SI, Hamidi SA, Gonzalez Bosc L: Asthma and pulmonary arterial hypertension: do they share a key mechanism of pathogenesis? *Eur Respir J* 2010; 35:730–734.
88 Leceta J, Martínez C, Delgado M, Garrido E, Gomariz RP: Expression of vasoactive intestinal peptide in lymphocytes: a possible endogenous role in the regulation of the immune system. *Adv Neuroimmunol* 1996; 6:29–36.
89 Voice JK, Grinninger C, Kong Y, Bangale Y, Paul S, Goetzl EJ: Roles of vasoactive intestinal peptide (VIP) in the expression of different immune phenotypes by wild-type mice and T cell-targeted type II VIP receptor transgenic mice. *J Immunol* 2003; 170:308–314.
90 Hill JM, McCune SK, Alvero RJ, Glazner GW, Henins KA, Stanziale SF, Keimowitz JR, Brenneman DE: Maternal vasoactive intestinal peptide and the regulation of embryonic growth in the rodent. *J Clin Invest* 1996; 97:202–208.
91 Spong CY, Lee SJ, McCune SK, Gibney G, Abebe DT, Brenneman DE, Hill JM: Regulation of postimplantation mouse embryonic growth by maternal vasoactive intestinal peptide. *Ann N Y Acad Sci* 1999; 897:101–108.
92 Spong C, Lee S, Mcune S, Gibney G, Abebe D, Alvero R, Brenneman E, Hill G: Maternal regulation of embryonic growth: the role of vasoactive intestinal peptide. *Endocrinology* 1999; 140:917–924.
93 Hill JM, Hauser JM, Sheppard LM, Abebe D, Spivak-Pohis I, Kushnir M, Deitch I, Gozes I: Blockage of VIP during mouse embryogenesis modifies adult behavior and results in permanent changes in brain chemistry. *J Mol Neurosci* 2007; 31:183–200.
94 Gressens P, Paindaveine B, Hill JM, Evrard P, Brenneman DE: Vasoactive intestinal peptide shortens both G1 and S phases of neural cell cycle in whole postimplantation cultured mouse embryos. *Eur J Neurosci* 1994; 10:1734–1742.
95 Tyrrell S, Landis SC: The appearance of NPY and VIP in sympathetic neuroblasts and subsequent alterations in their expression. *J Neurosci* 1994; 14:4529–4547.
96 Lim MA, Stack CM, Cuasay K, Stone MM, McFarlane HG, Waschek JA, Hill JM: Regardless of genotype, offspring of VIP-deficient female mice exhibit developmental delays and deficits in social behavior. *Int J Dev Neurosci* 2008; 26:423–434.
97 Graf AH, Hütter W, Hacker GW, Steiner H, Anderson V, Staudach A, Dietze O: Localization and distribution of vasoactive neuropeptides in the human placenta. *Placenta* 1996; 17: 413–421.
98 Marzioni D, Fiore G, Giordano A, Nabissi M, Florio P, Verdenelli F, Petraglia F, Castellucci M: Placental expression of substance P and vasoactive intestinal peptide: evidence for a local effect on hormone release. *J Clin Endocrinol Metab* 2005; 90:2378–2383.
99 Deutsch PJ, Sun Y, Kroog GS: Vasoactive intestinal peptide increases intracellular cAMP and gonadotropin-alpha gene activity in JEG-3 syncytial trophoblasts. Constraints posed by desensitization. *J Biol Chem* 1990; 265:10274–10281.
100 Ottesen B, Ulrichsen H, Fahrenkrug J, Larsen JJ, Wagner G, Schierup L, Søndergaard F: Vasoactive intestinal polypeptide and the female genital tract: relationship to reproductive phase and delivery. *Am J Obstet Gynecol* 1982; 143:414–420.
101 Roca V, Calafat M, Larocca L, Ramhorst R, Farina M, Franchi AM, Pérez Leiros C: Potential immunomodulatory role of VIP in the implantation sites of prediabetic nonobese diabetic mice. *Reproduction* 2009; 138:733–742.
102 Larocca L, Hauk V, Calafat M, Roca V, Fraccaroli L, Franchi A, Ramhorst R, Pérez Leiros C: Modulation of macrophage inflammatory profile in pregnant nonobese diabetic (NOD) mice. *Mol Cell Endocrinol* 2011; 333:112–118.
103 Fraccaroli L, Alfieri J, Larocca L, Calafat M, Roca V, Lombardi E, Ramhorst R, Pérez Leirós C: VIP modulates the pro-inflammatory maternal response, inducing tolerance to trophoblast cells. *Br J Pharmacol* 2009; 156:116–126.
104 Fraccaroli L, Grasso E, Zeitler E, Lombardi E, Gogorza S, Etchepareborda JJ, Nagle C, Cortelezzi M, Pérez Leirós C, Ramhorst R: Modulation of maternal LIF producers T cells by trophoblast and paternal antigens. *Am J Reprod Immunol* 2011; 65:133–145.

105 Ilarregui JM, Croci DO, Bianco GA, Toscano MA, Salatino M, Vermeulen ME, Geffner JR, Rabinovich GA: Tolerogenic signals delivered by dendritic cells to T cells through a galectin-1-driven immunoregulatory circuit involving interleukin 27 and interleukin 10. *Nat Immunol* 2009; 10:981–991.

106 Ramhorst RE, Giribaldi L, Fraccaroli L, Toscano MA, Stupirski JC, Romero MD, Durand ES, Rubinstein N, Blaschitz A, Sedlmayr P, Genti-Raimondi S, Fainboim L, Rabinovich GA: Galectin-1 confers immune privilege to human trophoblast: implications in recurrent fetal loss. *Glycobiology* 2012; 22:1374–1386.

107 Blois SM, Ilarregui JM, Tometten M, Garcia M, Orsal AS, Cordo-Russo R, Toscano MA, Bianco GB, Kobelt P, Handjiski B, Tirado I, Markert UR, Klapp BF, Poirier F, Szekeres-Bartho J, Rabinovich GA, Arck PC: A pivotal role for galectin-1 in fetomaternal tolerance. *Nat Med* 2007; 13:1450–1457.

108 Rabinovich GA, Croci DO: Regulatory circuits mediated by lectin-glycan interactions in autoimmunity and cancer. *Immunity* 2012; 36:322–335.

109 Than NG, Romero R, Kim CJ, McGowen MR, Papp Z, Wildman DE: Galectins: guardians of eutherian pregnancy at the maternal-fetal interface. *Trends Endocrinol Metab* 2012; 23:23–31.

110 Than NG, Erez O, Wildman DE, Tarca AL, Edwin SS, Abbas A, Hotra J, Kusanovic JP, Gotsch F, Hassan SS, Espinoza J, Papp Z, Romero R: Severe preeclampsia is characterized by increased placental expression of galectin-1. *J Matern Fetal Neonatal Med* 2008; 21:429–442.