A balancing act: mechanisms by which the fetus avoids rejection by the maternal immune system

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Abstract

Successful pregnancy requires strict temporal regulation of maternal immune function to accommodate the growing fetus. Early implantation is facilitated by inflammatory processes that ensure adequate vascular remodeling and placental invasion. To prevent rejection of the fetus, this inflammation must be curtailed; reproductive immunologists are discovering that this process is orchestrated by the fetal unit and, in particular, the extravillous trophoblast. Soluble and particulate factors produced by the trophoblast regulate maternal immune cells within the decidua, as well as in the periphery. The aim of this review is to discuss the action of recently discovered immunomodulatory factors and mechanisms, and the potential effects of dysregulation of such mechanisms on the maternal immune response that may result in pregnancy loss or preeclampsia.

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Introduction

Throughout human pregnancy, the genetically ‘foreign’ semi-allogeneic fetus and placenta evade maternal immune responses. Fetal survival requires significant regulation of the maternal immune system but not such as to compromise maternal immune protection. The mechanisms that regulate maternal ‘tolerance’ have been the subject of burgeoning interest since the seminal paper by Medawar (1953) published over 50 years ago. How these mechanisms are coordinated remains somewhat elusive, hindered by assumptions that, in their attempt to provide lucid explanations, have adopted simplified paradigms. For many years, it was posited that pregnancy is purely a Th2 phenomenon (Wegmann *et al.* 1993), i.e. maternal CD4⁺T helper type 1 (Th1) cells that mediate allospecific cytotoxic responses being inhibited during pregnancy to prevent rejection of the fetal allograft, while the number of Th2 cells that mediate antibody responses are increased, preventing complete immunosuppression. Th1 cytokine responses are abrogated through downregulation of nuclear factor κ B (NF κ B) during pregnancy (McCracken *et al.* 2003). This suppression of T-cell-mediated cytotoxic (Th1) responses is reflected in the frequent amelioration of cell-mediated autoimmune disorders such as rheumatoid arthritis. However, recent data indicate that success in early pregnancy requires augmentation of cytokines traditionally associated with Th1 responses. The maternal

immune system must therefore be subject to strict control that is temporally regulated in response to the changing needs of the developing fetus.

Over the past 20 years, the role of cells of the innate arm of the maternal immune system and the immunologic function of fetal-derived trophoblast have assumed importance for the development and protection of the fetoplacental unit. During pregnancy, fetal-derived trophoblast and membranes are in direct contact with decidual maternal immune effector cells, the majority of which are from the innate arm, predominantly decidual natural killer (dNKs) cells and macrophages. This indicates that the maternal–fetal interface is not an immune-privileged site, and multiple mechanisms originating from both mother and fetus contribute to the development and maintenance of tolerance (Fig. 1).

We discuss recent discoveries of mechanisms employed by fetal-derived trophoblast that indicate communication between fetus and maternal immune cells is necessary for pregnancy success. In contrast to the notion of simple maternal immune suppression, successful pregnancy is achieved through the fetoplacental unit not only inducing maternal tolerance but also enabling certain provoked inflammatory responses that facilitate trophoblast invasion. Understanding the phenomenon of the maternal–fetal allograft may assist in the understanding of pregnancy success and complications of pregnancy, and lead to the discovery of predictive biomarkers and novel therapeutic targets.

Figure 1 Trophoblast promotes vascular remodeling and maternal tolerance both locally at the maternal–fetal interface and in the periphery.

(A) Trophoblast shed microparticles such as exosomes (x) resulting in systemic paternal antigen exposure, driving T_{reg} proliferation. Exosomes express MIC and ULBP, both ligands for the NK receptor NKG2D, inhibiting maternal NK cytotoxicity when bound. Exosomes also express FASL, promoting apoptosis of peripheral activated maternal lymphocytes. (B) Extravillous trophoblast (EVT) invades uterine spiral arteries and replaces endothelium, aiding vascular remodeling critical for sufficient blood supply to the fetus. EVT expresses HLA-C. Upon binding the NK receptor, KIR, HLA-C inhibits cytotoxicity, and stimulates secretion of angiogenic and growth factors. This facilitates spiral artery remodeling and promotes trophoblast invasion. In preeclampsia, the combination of maternal KIRAA and fetal HLA-C2 is more common, inhibiting the production of angiogenic factors. EVT expresses FASL, promoting apoptosis of FAS-bearing activated $CD3^{+}$ T cells and endothelial cells (EC), protecting against maternal immune activation, while enabling placental invasion and spiral artery remodeling. The expression of FASL on EVT may be reduced in preeclampsia.

The maternal immune system: preconceptionally prepared?

Epidemiological evidence suggests that tolerance to paternal antigens is facilitated by preconceptional exposure (Dekker & Robillard 2007). In murine models, seminal fluid primes the maternal immune response, resulting in expansion of paternal antigen-specific immune-tolerant cells (Moldenhauer *et al.* 2009), and has a favorable cytokine composition, postulated to be mediated by transforming growth factor- β (TGFB), to promote tolerance (Robertson *et al.* 2009). In humans, prior exposure may also occur, potentially protecting against pregnancy-induced hypertension (Robillard *et al.* 1994). Preeclampsia, a multi-system human pregnancy-specific disorder characterized by *de novo* hypertension and proteinuria, is a condition that involves insufficient invasion of the maternal uterine vasculature by extravillous trophoblast (EVT). Epidemiological observations that preeclampsia is more likely with first pregnancies, and in women undergoing IVF with donor sperm, compared with those using partner sperm (Kyrou *et al.* 2010), support the view that risk is attributed to the assumed minimal pre-exposure to paternal antigens (Saito *et al.* 2007). While the clinical symptoms of preeclampsia do not appear prior to 20 weeks gestation,

placental histology indicates impaired trophoblast invasion and spiral artery remodeling in early pregnancy. Vascular remodeling depends on inflammatory mediators, indicating that preeclampsia is a result of maternal immune dysfunction. It remains to be established whether this dysfunction arises out of aberrant paternal antigen presentation.

The maternal–fetal interface: trophoblast triggers tolerance?

Following implantation, immune recognition of the fetus occurs primarily at the maternal–fetal interface in the first trimester, due to direct invasion of EVT into the decidualized endometrium and inner third of the myometrium. The establishment of placentation is facilitated through early interactions between EVT and maternal cells, predominantly of the innate immune arm.

Innate immune mechanisms

In the first trimester of pregnancy, 30–40% of the decidua consists of maternal immune cells, of which most are innate dNK cells (70%), with macrophages (<30%), T cells (<20%) and dendritic cells (DC; <2%) also present (Loke *et al.* 1995).

NK cells are cytotoxic, instrumental in clearing virus-infected cells and tumor cells. Their activity is determined by engagement of their activating or inhibitory receptors. dNKs differ from their peripheral counterpart, not only in phenotype but also in apparent function. dNKs are typically CD56^{bright}/CD16[−], while peripheral NKs are CD56^{dim}/CD16⁺ (King *et al.* 1996). CD16 is directly involved in triggering the lysis of target cells. The absence of CD16 expression on dNKs results in reduced cytotoxicity in these cells, switching function to cytokine production. The vast majority of dNKs are located at the site of trophoblast invasion – the decidua basalis. Their growth factors and chemokines augment invading trophoblast and promote vascular remodeling (Hiby *et al.* 2004), while inhibition of dNK cells increases the likelihood of preeclampsia. A reduction in dNK cell numbers is associated with intrauterine growth restriction (IUGR; Williams *et al.* 2009); IUGR is indicative of limited trophoblast invasion.

dNK cells can be activated or inhibited by ligands expressed by invading EVT, the predominant one being paternal HLA-C, the only polymorphic major histocompatibility complex (MHC)-Class I molecule expressed on the surface of trophoblast. HLA-C molecules are recognized by the killer Ig-like receptor (KIR) family on the surface of NK cells. The proportion of dNKs expressing KIR is higher than their peripheral blood counterpart, indicating that dNKs have an increased capacity for communicating with invading EVT. KIR can be divided into two haplotypes: haplotype A contains only inhibitory KIR, while haplotype B contains inhibitory and activating KIR. These KIR distinguish HLA-C via two polymorphisms, and as such, all HLA-C allotypes can be classed as either C1 or C2. Particular HLA-C and KIR combinations were recently discovered to be associated with preeclampsia (Hiby *et al.* 2004, Moffett & Hiby 2007). Women with preeclampsia were more likely to have the inhibitory KIR AA haplotype combined with an HLA-C2 infant; HLA-C2 on trophoblast will only engage the inhibitory KIR2DL1 receptor.

This may lead to inhibition of angiogenic factors produced by dNK cells, resulting in the failure of vascular remodeling that precedes the onset of preeclampsia (Hanna *et al.* 2006).

NKG2D is an activating receptor on the surface of NKs, as well as cytotoxic CD8⁺ and $\gamma\delta$ T cells. NKG2D binds to MHC-I chain-related proteins (MIC) A and B, and to UL-16-binding protein (ULBP), leading to perforin-mediated cytotoxicity. Soluble MIC binds NKG2D and downregulates its expression on CTL and NK, leading to immunosuppression. Exosomes derived from early placenta explants express MIC and ULBP, and initiate internalization of NKG2D in dNKs, CD8⁺ and $\gamma\delta$ T cells, reducing cell surface expression and subsequent cytotoxic capacity (Hedlund *et al.* 2009). Interestingly, NKG2D is ubiquitously expressed on dNKs (Apps *et al.* 2008) and while ULBP is constitutively expressed by syncytiotrophoblast intracellularly, it is not detectable on the cell surface (Hedlund *et al.* 2009). Findings from a recent study indicate the ULBP expression on lymphoma cells render them susceptible to cytotoxic killing by $\gamma\delta$ T cells (Lanca *et al.* 2010), indicating that the internalization of this protein in trophoblast is a controlled mechanism inhibiting dNK-mediated cytolysis. Questions such as ‘What mechanism regulates the expression of this protein in trophoblast, and is ULBP expression on trophoblast upregulated in preeclampsia?’ are yet to be answered.

Thus, NK cells at the maternal–fetal interface are functionally active. Rather than being a threat to pregnancy, the dNK cell is recruited and harnessed by the trophoblast via the production of chemokines and specific ligands. Consequently, the dNK cell secretes important cytokines and growth factors (Hanna *et al.* 2006) such as vascular endothelial growth factor (VEGF) and interferon- γ (IFNG; see Box 1), promoting vascular remodeling and invasion of the trophoblast into the uterus in a highly regulated manner. Inhibition of the activity of dNK at the maternal–fetal interface

Box 1 IFNG: pregnancy friend or foe?

In early pregnancy, dNKs produce IFNG. This cytokine acts in an autocrine manner on dNKs, stimulating further production of IFNG, as well as VEGF, ANGPT2 (AngII), and PGF, creating a proangiogenic milieu. IFNG also activates nitric oxide synthase (NOS) that produces nitric oxide (NO), a compound that stimulates vasodilation and inhibits smooth muscle cell proliferation. Importantly, IFNG can have either proliferative or cytotoxic effects, determined primarily by the relative density of its two receptors: IFNGR1 and IFNGR2. These two receptors are expressed on the surface of trophoblast throughout pregnancy, while the expression of IFNG is only present in early pregnancy. Interestingly, IFNGR1 expression seems to be stable throughout pregnancy, while IFNGR2 expression is low in early pregnancy and increases in late pregnancy. The expression of IFNGR2 seems to be dysregulated in PET as it is similar to that of early pregnancy (Banerjee *et al.* 2005). This may be a result of the hypoxic placenta, as low oxygen tension and NO modulates IFNGR2 density on T cells. Chronic activation with IFNG can inhibit NOS, leading to vasoconstriction, hypertension, and IUGR. Increased IFNG production has been noted in decidual cells in preeclampsia (Wilczynski *et al.* 2003).

may lead to dysregulation of vascular remodeling, limiting the depth of trophoblast invasion as observed in preeclampsia.

Adaptive immune mechanisms

The close proximity between maternal cells and fetal trophoblast would also be expected to activate adaptive immune responses, comprising of B and T lymphocytes, which are highly specific and primarily delayed. Subsequent long-lasting memory enables lymphocytes to respond more rapidly and robustly upon repeated antigen exposure. Adaptive immunity is pivotal in allograft rejection, mediated mainly by CD8⁺ cytotoxic T cells. Adaptive immune function is necessarily restrained during pregnancy to protect the fetal allograft. This restraint is thought to be primarily mediated by abrogation of Th1 responses and augmentation of regulatory T (T_{reg}) cells.

On encountering engaged antigen-presenting cells (APC) such as B cells or DC, CD4⁺T cells may differentiate into T helper (Th) lymphocytes or T_{reg} cells. The type of APC, coupled with the cytokine milieu and the dose of antigen, determines the differentiation pathway of the naïve T cell. During pregnancy, the dose of antigen is measured as the expression of non-maternal (i.e. paternally) inherited proteins on trophoblast at the maternal–fetal interface. The trophoblast thus influences the differentiation of maternal CD4⁺T lymphocytes into Th1, Th2, Th17 or T_{reg} cells.

Th1 cells, driven by the presence of interleukin 12 (IL12), promote cytotoxicity through the production of IL2 and IFNG, and tend to be inhibited in clinically normal pregnancies (McCracken *et al.* 2004). Th2 cells are predominant in peripheral blood in second and

third trimesters of healthy pregnancy (Saito *et al.* 1999). Th2 cells are driven by the presence of IL4 and IL10, promote antibody responses and produce IL4, 5, and 10, protecting against rejection of the allogeneic fetus by inhibiting the induction of Th1 cells (Fig. 2).

T_{reg} cells are a major class of CD4⁺T cells, driven to differentiate when binding their cognate antigen in the presence of TGFβ in the absence of IL6. T_{reg}s suppress activation of the immune system. Normal human pregnancy is characterized by increased numbers of T_{reg} in the periphery and at the maternal–fetal interface (Heikkinen *et al.* 2004, Sasaki *et al.* 2004, Somerset *et al.* 2004, Tilburgs *et al.* 2008), while in preeclampsia, T_{reg}s are reduced in number at the maternal–fetal interface (decidual FOXP3⁺/CD3⁺; Sasaki *et al.* 2007) and in the periphery (CD4⁺/CD25^{bright}; Darmochwal-Kolarz *et al.* 2007). Interestingly, the latter study also describe an increase in memory (CD4⁺/CD45RO⁺) T cells, indicating previous antigenic stimulation and activation of T cells, and an increase in CD8⁺ CD25⁺T cells in women with preeclampsia (Darmochwal-Kolarz *et al.* 2007). A recent study supports these data, and makes a strong case for the importance of maternal T_{reg} development in the maintenance of healthy pregnancy (Santner-Nanan *et al.* 2009). This importance was first posited by Aluvihare *et al.* (2004) in a murine model, where depletion of these cells led to pregnancy loss, and verified in a murine abortion model, where adoptive transfer of T_{reg}s from normal pregnant mice completely rescues fetal rejection in abortion-prone recipients (Zenclussen *et al.* 2005). Santner-Nanan *et al.* (2009) also discovered that healthy pregnancy is associated with a decrease in Th17 cells, while this pro-inflammatory CD4⁺ cell subset was increased in women with preeclampsia. This is not unexpected, as Th17 cells have been implicated in autoimmune and inflammatory pathologies such as

Figure 2 CD4⁺T cells can differentiate into type 1 (Th1) or type 2 (Th2) helper cells. In the presence of proinflammatory cytokine IL12 and increased quantity of antigen, CD4⁺T cells differentiate into Th1 cells, important in cell-mediated immunity and protecting against viral infection. Th1 cells mediate allograft rejection. In the presence of IL4 and IL10, and low level of antigen, CD4⁺T cells differentiate into Th2 cells, important in cell activation and mediate allergic responses. Th1 and Th2 cells mutually inhibit each other. Extravillous trophoblast secretes soluble HLA-G, inducing regulatory type 1 T cells (T_{reg}1) to produce IL10, promoting maternal tolerance.

Figure 3 The plasticity of T_{regs} and Th17 cells. TGFB drives the differentiation of T_{regs} in the absence of IL6 in clinically normal pregnancies. In pregnancies affected by preeclampsia, maternal monocytes produce both IL1B and IL6, driving the differentiation of Th17 cells and subsequent production of IL17. These cells are implicated in transplant rejection and autoimmune disease. T_{regs} are able to switch to a Th17 phenotype; however, this is inhibited by the presence of indoleamine-2,3-dioxygenase (IDO), a molecule expressed by extravillous trophoblast (EVT). EVT also expresses vasoactive intestinal peptide (VIP), stimulating the production of maternal dendritic cells (DC) to produce IL10, promoting T_{reg} differentiation.

multiple sclerosis (Matusevicius *et al.* 1999) and rheumatoid arthritis (Shen *et al.* 2009), and monocytes isolated from women with preeclampsia constitutively secrete higher levels of IL1B and IL6 – both these cytokines drive Th17 development (Luppi & Deloia 2006).

Th17 cells produce IL17 (Fig. 3), a proinflammatory cytokine also produced by adaptive $CD4^{+}$ and $CD8^{+}$ T cells and innate monocytes and NKT cells (Cua & Tato 2010). In early pregnancy, IL17 production is restricted to $CD4^{+}$ T cells in both the periphery and decidua (Ito *et al.* 2010). The plasticity of immune responses is demonstrated by the discovery that T_{regs} can switch to a Th17 phenotype in the presence of inflammatory stimuli and cytokines (Koenen *et al.* 2008, Chaudhry *et al.* 2009), and this switch is suppressed by indoleamine-2,3-dioxygenase (IDO; Baban *et al.* 2009), a molecule expressed on EVT.

Fetal-derived factors

HLA-C

As discussed, HLA-C is the only classical HLA Class I molecule expressed on trophoblast. HLA-C is necessary for antigenic presentation to $CD8^{+}$ T cells. Interestingly, HLA-C-discordant pregnancies tend to have increased numbers of fetus-specific $CD4^{+}CD25^{\text{bright}}$ T_{reg} cells, perhaps to suppress T-cell activation driven by discordant HLA-C expression (Tilburgs *et al.* 2009), while HLA-concordant pregnancies are associated with an increased risk of spontaneous abortion (Ober *et al.* 1998), indicating a degree of maternal immune stimulation is necessary for successful pregnancy. HLA alloantigen recognition may also enhance

production of hormones and growth factors such as hCG (Harbour-McMenamin *et al.* 1986) and LIF (Nasef *et al.* 2008).

HLA-G

HLA-G, a non-classical Class I molecule, is also expressed on EVT. HLA-G modulates alloreactive T cells by downregulating CD4 and CD8 expression, increasing the proportion of HLA-DR $^{+}$ T cells, and generating suppressor T cells, termed induced IL10-producing type I T_{regs} cells (Tr1), in an IL10-dependent manner (Naji *et al.* 2007, Gregori *et al.* 2009). Tr1 cells secrete IL10 and TGFB that are able to suppress cytolytic responses without cell contact. This may provide a positive feedback loop in maintaining maternal tolerance, as membrane-bound HLA-G and its inhibitory receptor Ig-like transcript (ILT)-4 are upregulated in chronic antigen stimulation in the presence of IL10. Interestingly, an increase in soluble HLA-G in plasma correlates with an increase in IL10 and development of suppressor cells, and is strongly associated with graft survival (Naji *et al.* 2007). As the fetus can be considered an allograft, its production of membrane-bound and soluble HLA-G may be promoting maternal T-cell tolerance both locally and systemically.

HLA-G is both membrane-bound and secreted as soluble protein. Each can interact with its three inhibitory receptors: ILT-2, ILT-4, and KIR 2DL4 on the surface of NK cells, APC and T cells (see Hunt *et al.* (2005) for review). The interaction between HLA-G and its receptors protects the trophoblast against allogeneic NK and CTL-mediated cytotoxicity. Soluble HLA-G binds KIR2DL4, leading to the secretion of angiogenic factors

important in vascular remodeling during early pregnancy. Only a subset of dNKs expresses ILT-2, and this subset of NKs are inhibited by HLA-G, diminishing dNK production of growth factors IL8, IP-10, VEGF, and placental growth factor (PGF), providing a maternal mechanism to control trophoblast invasion (Hanna *et al.* 2006).

The role of FAS/FASL

Trophoblast cells in early healthy pregnancy also express both FAS and FASL, potentially rendering them susceptible to FAS-mediated apoptosis. The presence of FASL (CD95L) on trophoblast plays an active role in the deletion of FAS (CD95)-bearing activated T-cells. Trophoblast cells induce apoptosis of CD95⁺T-cells *in vitro*, and the *gld* mouse that lacks functional CD95 has increased T-cell infiltrates at the maternal–fetal interface and is associated with increased fetal loss (Hunt *et al.* 1997). It is of note that soluble HLA-G upregulates CD95L expression on activated CD8⁺T cells, triggering apoptosis *in vitro* (Fournel *et al.* 2000). The expression of FASL on trophoblast also facilitates apoptosis of endothelial cells, perhaps enabling the invasion and remodeling of maternal spiral arteries – limited data indicates reduced expression of FASL on trophoblast from pregnancies affected by preeclampsia (Eide *et al.* 2007).

IDO and immune suppression

Amino acid deprivation as a regulator of lymphocyte responses has long been implicated in immunosuppression at the maternal–fetal interface (Munn *et al.* 1998). IDO depletes L-tryptophan, inhibiting T-cell proliferation, and is highly expressed on EVT (Honig *et al.* 2004). IDO may also be released by trophoblast, as conditioned media derived from trophoblast or villous explant cultures inhibit T-cell proliferation, while inhibiting IDO or adding tryptophan partly restored T-cell proliferation (Dong *et al.* 2008).

IDO also has physiological importance during pregnancy, as decreased levels of tryptophan also results in vasodilation, via decreased serotonin. This fact raised controversy as to which role is more relevant to pregnancy success, as T-cell-mediated pregnancy loss occurs when IDO is inhibited (Munn *et al.* 1998). Bonney & Matzinger (1998) state, it is their danger model that explains the immune activation when blocking IDO, as this increases reactive oxygen species intermediates, which activate NFκB and stimulate local macrophages, which in turn initiate the T-cell immune response. DC can also produce IDO, leading to the activation and maintenance of suppressor T_{regs} (Chen *et al.* 2008).

Decidual CD14⁺ myelomonocytic cells are found in close proximity to dNK cells. A recent study indicates that crosstalk between these two cell types at the

maternal–fetal interface may be responsible for the induction of T_{reg} cells (Vacca *et al.* 2010). Contact with dNK cells results in IFNG-mediated IDO expression in dCD14⁺ cells, which in conjunction with TGFB promotes the development of T_{reg} cells (Vacca *et al.* 2010). The peripheral counterparts of these cells were not able to similarly drive T_{reg}-cell development. This confirms findings of previous studies that NK cells found within the decidual compartment are functionally distinct from peripheral NK cells, and that dNK cells play an important role in mediating immune tolerance during pregnancy.

Anti-inflammatory VIP

Vasoactive intestinal peptide (VIP) is a neuropeptide expressed in trophoblast (Marzioni *et al.* 2005), and contributes to smooth muscle relaxation and vasodilation in the uterus. VIP also has anti-inflammatory effects and, like progesterone, promotes a Th2 shift through the stimulation of IL10. DCs differentiated in the presence of VIP induce T_{regs}, inhibiting allogeneic CD4 responses and increasing TGFB, suggesting a dual responsibility at the maternal–fetal interface (Fraccaroli *et al.* 2009).

Thus, maternal recognition of the fetal allograft seems to be necessary for the development and maintenance of a healthy pregnancy, with activation at least within maternal innate cells at the maternal–fetal interface facilitating vascular remodeling.

Systemic strategies

Changes in maternal peripheral blood indicate that pregnancy is associated with a mild yet sustained systemic inflammatory response (Sacks *et al.* 1999). Monocytes display increased activation, and this activation seems to be further exaggerated in preeclampsia (Redman *et al.* 1999, Redman & Sargent 2004, Luppi & Deloia 2006). It is of note that preeclampsia has recently been found to be associated with an increase in circulating placenta-derived microparticles (Goswami *et al.* 2006, Lok *et al.* 2008) and that these microparticles stimulate inflammatory responses in monocytes (Germain *et al.* 2007, Messerli *et al.* 2010).

Systemic release of paternal antigen throughout pregnancy may be necessary to maintain T_{reg} populations (Somerset *et al.* 2004, Zenclussen *et al.* 2010), mediating tolerance towards the semi-allogeneic fetus and limiting the maternal systemic inflammatory response. Interestingly, pregnant mice accept grafts expressing paternal-derived fetal antigen (Tafari *et al.* 1995); paternal antigen presentation may be required for the maintenance of paternal antigen-specific T_{regs}, as once pregnancy (and thus paternal antigen exposure) ceases, tolerance to paternal grafts also ceases (Tafari *et al.* 1995). Trophoblast shedding and secretion of

microparticles occur from second trimester, facilitating systemic exposure to paternal-derived antigen. Systemic exposure also occurs via fetal cell microchimerism, frequently described, though yet to be mechanistically explained. Fetal cells can persist in maternal blood and tissue, and in some studies, association of fetal cells with autoimmune disease have been reported, perhaps indicating a pathological tolerance in the maternal immune system (Lambert *et al.* 2002). However, recent data suggest that fetal cells may infiltrate injured maternal tissue as a repair mechanism (Khosrotehrani & Bianchi 2005, Sunami *et al.* 2010).

Other circulating microvesicles include exosomes that are 30–100 nm, formed and released through the late endosomal compartment by many cell types, including syncytiotrophoblast. They carry membrane-bound and cytosolic proteins, mRNA, microRNA, and bioactive lipids, and are capable of transmitting pathogens. As a result, exosomes mimic actions of their parent cell; trophoblast-derived exosomes possess immune-suppressing capabilities. Placental-derived exosomes are found in the peripheral blood of pregnant women, a potential explanation on how pregnancy results in systemic immune suppression (Sabapatha *et al.* 2006). Indeed, such exosomes express FASL and programmed death-1 ligand (PD-L1), a pro-apoptotic protein that binds PD-1 on the surface of activated maternal lymphocytes, removing them from the maternal circulation. In addition, T-cell activation requires expression of the CD3 ζ chain of the T-cell receptor. Exosomes derived from ovarian carcinoma cells and pregnant serum suppress CD3 ζ expression in T-cell lines (Taylor & Gercel-Taylor 2005, Taylor *et al.* 2006), perhaps mediating the suppression of TCR/CD3 ζ expression seen in pregnancy (Lam *et al.* 2003).

During pregnancy, a woman's body is exposed to immense physiological stress. Under normal conditions, following cell stress or tissue damage, molecules usually located within the cell are released and can bind pattern recognition receptors (PRRs) on the surface of innate immune cells, triggering inflammation. These molecules are referred to as danger-associated molecular patterns (DAMP; Seong & Matzinger 2004). ATP is one such molecule, present in high concentrations within all cells and released rapidly from the cell following exposure to trauma or oxidants. Interestingly, moderate release of ATP has an immunosuppressive anti-inflammatory effect, activating the P2Y₁₁ receptor, leading to partial maturation of DC and differentiation towards a Th2 phenotype (Marteau *et al.* 2004). Increased levels of released ATP activate the low-affinity P2X₇ receptor, causing massive release of pro-inflammatory factors, including IL1B, IL18, IL6, and TNF, and reactive oxygen species, and can involve activation of the inflammasome. Non-apoptotic cell death (which may restrict trophoblast invasion in the presymptomatic stage of preeclampsia) results in higher levels of ATP in the

extracellular milieu. Extracellular ATP inhibits LPS-stimulated production of the immunosuppressive sHLA-G, via the P2X receptor (Rizzo *et al.* 2009). The P2X₇ receptor has been implicated in the production of tissue factor-expressing microparticles (Baroni *et al.* 2007), and MHC Class II-expressing exosomes (Qu *et al.* 2009). In rat models, P2X₇ receptor expression in myometrium is associated with delivery, both term and preterm (Urabe *et al.* 2009), while in glomeruli, its expression is associated with hypertension. Whether this receptor is present on decidual NKs or DCs during pregnancy and whether expression levels change in pregnancy-related pathology such as preeclampsia are unknown and deserving of investigation.

Conclusion

Pregnancy is characterized by mild systemic immunosuppression and inflammation. Traditionally, the fetal–maternal interface was likened to a graft–host interaction. It is becoming more apparent that pregnancy is now a co-dependent, regulatory interaction between the mother and developing fetus. The placenta actively produces and secretes various immunomodulatory factors to prevent rejection of the histoincompatible fetus by the innate and adaptive maternal immune system. Indeed, maternal immune recognition of the fetus occurs throughout uncomplicated pregnancies, and activated CD8⁺T cells are present at the maternal–fetal interface. Apoptosis is induced in these cells by the FASL-expressing EVT cells.

Immunological tolerance at the maternal–fetal interface requires tight temporal regulation between the immune and vascular networks, involving multiple mechanisms. These mechanisms perform a balancing act to regulate trophoblast invasion. Indeed, angiogenesis and trophoblast invasion are immunologically active processes; rather than ‘escaping’ maternal recognition, the fetus harnesses maternal immune surveillance mechanisms to facilitate survival and growth.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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