

# The hidden maternal-fetal interface: events involving the lymphoid organs in maternal-fetal tolerance

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**ABSTRACT** The genetic disparity between the mother and fetus has long enticed immunologists to search for mechanisms of maternal tolerance to fetal antigens. The study of antigen-specific tolerance in murine and human pregnancy has gained new momentum in recent years through the focus on antigen-presenting cells, uterine lymphatics and fetal antigen-specific maternal T cell responses. In mice, we now know that these responses occur within the secondary lymphoid structures as they can be conveniently tracked through the use of defined, often transgenic fetal antigens and maternal T cell receptors. Although the secondary lymphoid organs are sites of both immunization and tolerization to antigens, the immunological processes that occur in response to fetal antigens during the healthy pregnancy must invariably lead to tolerance. The molecular properties of these maternal-fetal tolerogenic interactions are still being unraveled, and are likely to be greatly influenced by tissue-specific microenvironments and the hormonal milieu of pregnancy. In this article, we discuss the events leading to antigen-specific maternal tolerance, including the trafficking of fetal antigens to secondary lymphoid organs, the properties of the antigen-presenting cells that display them to maternal T lymphocytes, and the nature of the ensuing tolerogenic response. Experimental data generated from human biological specimens as well as murine transgenic models are considered.

**KEY WORDS:** *dendritic cell, fetal antigen, spleen, lymph node, maternal-fetal interface, maternal-fetal tolerance, placenta, regulatory T cells, trophoblast*

## The multifaceted maternal-fetal immunological interface

The presence of fetal alloantigens during gestation necessitates immunologic adaptations by the mother to ensure successful reproduction while maintaining maternal immunocompetence to infection. Studies of maternal-fetal tolerance have focused on defining mechanisms for establishment of 'immunological privilege' within the uterus during pregnancy. These reports, together with studies in other immunologically privileged sites (e.g. eye, gonads and central nervous system) have created a foundation upon which to investigate mechanisms of tolerance to tissue-specific antigens (Simpson, 2006). In each case, immune tolerance that is established in the periphery depends on characteristic surface-associated and secreted factors produced by parenchymal and resident immune cells. Cell-cell interactions together with locally produced cytokines and hormones set up an environment that ensures that circulating and resident immune cells will regard unique tissue antigens as "self". In some tissues, there

may also be a physical barrier that restricts access of circulating leukocytes to the tissue and limits drainage of tissue-specific antigens to secondary lymphoid organs.

The fetal-placental unit represents an unparalleled physiological situation in which the pregnant female is confronted with tissue-specific antigens deriving from the fetus and placenta, as well as foreign antigens deriving from the paternal genome. The mother is immunologically naïve to these fetal, placental and paternal antigens. Many properties that are exhibited by other immunologically privileged sites are shared by the placenta, with the exception that there are no physical barriers to immune cells in the placenta. This is particularly true in species with the hemochorial placental arrangement.

In fact, the anatomical arrangement of the hemochorial placenta introduces multiple possibilities for exposure of maternal

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*Abbreviations used in this paper:* DC, dendritic cell; MHC, major histocompatibility complex.

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immune cells to trophoblast cells, fetal cells and their respective antigens. Virtually all points of contact between the mother and fetus are replete with immune cells. In women, villous placental syncytiotrophoblast cells are in direct contact with maternal blood, and thus great exposure of the syncytiotrophoblast to maternal peripheral blood leukocytes occurs (Benirschke and Kaufmann, 2000). This situation is mirrored in the labyrinth portion of the mature murine placenta, in which the outermost mononuclear trophoblast cells of a trilaminar layer are directly apposed to maternal blood (Simmons and Cross, 2005). In the decidua of both species, invading cytotrophoblast cells are also in close association with maternal leukocytes. These include uterine natural killer cells, but also macrophages, dendritic cells (DC), and some T cells (Hanna and Mandelboim, 2007).

The maintenance of maternal-fetal immune privilege is often attributed to the apposition between trophoblast and maternal immune cells, and the ability of these cells to communicate with each other via direct contact or through secreted immunomodulatory products. Although species differences do exist, there is general agreement that the trophoblast of mice and humans are well-equipped with potent immunomodulators including IDO, FasL, TRAIL, CD200, galectin-1, and B7-H1 (Lin *et al.*, 2005; Petroff, 2005; Vicovac *et al.*, 1998). Maternal cells also actively contribute to the immunological environment; both decidual cell-immune cell interactions and immune cell-immune cell interactions utilizing many of the same modulators have been proposed (Blois *et al.*, 2007a; Mackler *et al.*, 2003; Qiu *et al.*, 2005). Finally, the Class Ib HLA proteins that are expressed on extravillous trophoblast cells are of special interest. HLA-G, -E, and possibly -F have diverse functions as modulators of T cells, NK cells, macrophages and dendritic cells. The roles these proteins play in pregnancy have recently been reviewed (Hunt, 2006; Ishitani *et al.*, 2005).

While the roles of cells and their products within and around the placenta have been intensively studied, recent research has also implicated a second interface, the secondary (peripheral) lymphoid organs, in the generation of maternal-fetal tolerance. Local tissue-draining lymph nodes and the spleen are not only the settings for initiation of immune responses, but are also sites in which lymphocyte tolerance to peripheral tissue-specific antigens is established. We propose here that these secondary lymphoid structures, rather than the placenta itself, are the key locations for establishing antigen-specific maternal peripheral tolerance to the fetus in many, if not all eutherians. The question now lies not in whether or not the mother is "aware" (Tafari *et al.*, 1995) of fetal alloantigens, but in the cellular and molecular events surrounding the unequivocal acceptance of the fetus despite their expression. In this article, we review studies done with human and murine models to elucidate the nature of fetal alloantigens, how these antigens might reach peripheral lymphoid organs, in what context they are presented to T cells, and finally the quality of the ensuing response.

### Major and minor players: the nature of fetal alloantigens

Alloantigens can be broadly classified into those encoded within the major histocompatibility complex (MHC) and those encoded outside the MHC (minor histocompatibility antigens).

Each elicits characteristic immune responses in the host of an allograft. The highly polymorphic class Ia and class II MHC proteins are the principal hurdle to successful transplantation, in large part because these antigens on donor cells can be directly recognized by recipient T cells, without the requirement for proteolytic processing and presentation by antigen presenting cells. The frequency of T cells that can recognize unprocessed MHC is unexpectedly high – several orders of magnitude higher than T cells specific for other nominal, processed antigens (Lindahl and Wilson, 1977). Together, these characteristics of MHC antigens contribute to acute allograft failure in non-MHC identical partners.

The discovery of the dangers of disparate MHC in transplantation launched the field of reproductive immunology, since it was realized that expression of paternal MHC on the surface of trophoblast cells could, at least in theory, predispose the placenta to serve as a target of an anti-fetal alloresponse. It was soon learned that in human placentas, the highly polymorphic class Ia MHC molecules are unexpressed by normal villous trophoblast cells, even under the influence of interferon ( $\gamma$ ) *in vitro* (Hunt *et al.*, 1987; Sunderland *et al.*, 1981). Thus, most investigators agree that the villous trophoblast and syncytiotrophoblast are exonerated as an ostensible threat for eliciting an anti-fetal response because of their lack of HLA-A, -B, and -C.

Despite the lack of expression of class I HLA in the trophoblast, numerous studies (reviewed later in this article) have now shown that pregnancy in both women and mice can elicit lymphocyte responses to both major and minor histocompatibility antigens from the fetus. So, what are the antigens, and from whence do they originate?

### Major histocompatibility antigens in the trophoblast and fetus

As mentioned previously, extravillous trophoblast cells also display the class Ib HLA proteins; however, due to their low level of polymorphism and the likely induction of central tolerance to them in the thymus, these proteins are unlikely to elicit an allogeneic response. However, extravillous trophoblast cells do express the polymorphic class Ia protein, HLA-C (King *et al.*, 2000). The reduced polymorphism as compared to other class Ia proteins and its low expression on the surface of cells have been hypothesized to explain a lack of threat of HLA-C to allorecognition (Snary *et al.*, 1977; Trundley and Moffett, 2004). Instead, HLA-C appears to influence the function of decidual NK cells, either promoting or hindering proper placentation in a manner dependent upon allelic variation between paternally-derived HLA-C and maternally-derived killer inhibitory receptors (KIR) on resident decidual immune cells (Hiby *et al.*, 2008; Hiby *et al.*, 2004). Maternal allospecific T cells to fetal HLA-C have not been demonstrated, though theoretically could occur.

The murine placenta differs somewhat from the human placenta in its expression of MHC. Class Ia mRNA expression occurs as early as gestation day 7.5, with increasing levels thereafter (Hedley *et al.*, 1989). MHC class I protein expression is detectable in the mature placenta at midgestation, but levels are substantially lower than in adult tissues (Hedley *et al.*, 1989; Ozato *et al.*, 1985). Trophoblast giant cells are the first to express the protein, followed by the interstitial and spongiotrophoblast cells, but never

in the labyrinth placenta that is in contact with maternal blood (Jaffe *et al.*, 1991; Redline and Lu, 1989). Similarly to the placenta, the embryo itself does not express MHC class I until midgestation, and then only at much reduced levels in comparison to adult tissues (Hedley *et al.*, 1989; Ozato *et al.*, 1985). Embryonic class I expression appears to be highest in the liver (Jaffe *et al.*, 1991; Ozato *et al.*, 1985).

According to most reports, MHC class II molecules are absent from both mouse and human trophoblast cells (Murphy *et al.*, 2004). Aberrant expression of class II molecules on human trophoblasts has been linked to villitis, recurrent spontaneous abortion, and pemphigoid gestationis (Athanasakis *et al.*, 1985; Borthwick *et al.*, 1988; Labarrere and Faulk, 1990). The absence of class II expression on normal trophoblasts has been explained by a failure to express its major regulator, CIITA (Murphy and Tomasi, 1998). However, total lack of class II on these cells has recently been challenged by Ranella *et al.*, who report constitutive intracellular expression of HLA-DR as well as HLA-DO, which negatively regulates surface expression of -DR (Ranella *et al.*, 2005). In light of these new findings, class II expression in the placenta may need to be further reevaluated.

While expression of antigens in the human fetus is difficult to ascertain, the pattern for class I and II MHC proteins is known for the inner cell mass-derived villous stroma of the placenta. W6/32, a pan-class I HLA antibody, identifies cells in the villous stroma as early as 8 weeks of gestation, and this stromal expression of class I persists through term (Sunderland *et al.*, 1981). Hofbauer cells also exhibit MHC class II protein; expression on these cells is low or absent in early pregnancy but increases with advancing gestation (Bulmer *et al.*, 1988; Goldstein *et al.*, 1988; Sutton *et al.*, 1986).

### Minor histocompatibility antigens in the trophoblast and fetus

Minor alloantigens can also pose a barrier to transplantation despite their much lower allelic variation than MHC antigens. Historically, the first recognized minor histocompatibility antigens turned out to be encoded on the Y chromosome (Goulmy *et al.*, 1976). In all, there are now nearly 20 known minor antigens in humans, with about equal genetic distribution between autosomes and the Y chromosome. Minor antigens can be presented by either class I or class II MHC proteins, and they constitute a variety of proteins, including both intracellular and surface-bound proteins (Goulmy, 2006; Roopenian *et al.*, 2002). Expression of minor histocompatibility proteins, to our knowledge, has not been systematically examined in the placenta, although one such protein has been identified in the syncytiotrophoblast (Collier *et al.*, 2002). Given their often broad tissue distribution, however, expression of these minor antigens in the placenta would not be unsurprising and would perhaps be expected.

Taken together, the studies described above reveal that potential alloantigens deriving from the fetal-placental unit include:

- Class Ia and II MHC from the fetus and/or villous stromal cells;
- HLA-C from extravillous trophoblast cells or fetus;
- Minor antigens from trophoblast, villous stromal cells, or the fetus.

In the mouse, class I MHC exposure to the mother could occur from the fetus or trophoblast during the second half of gestation,

while minor antigen exposure could originate from either placental or fetal sources.

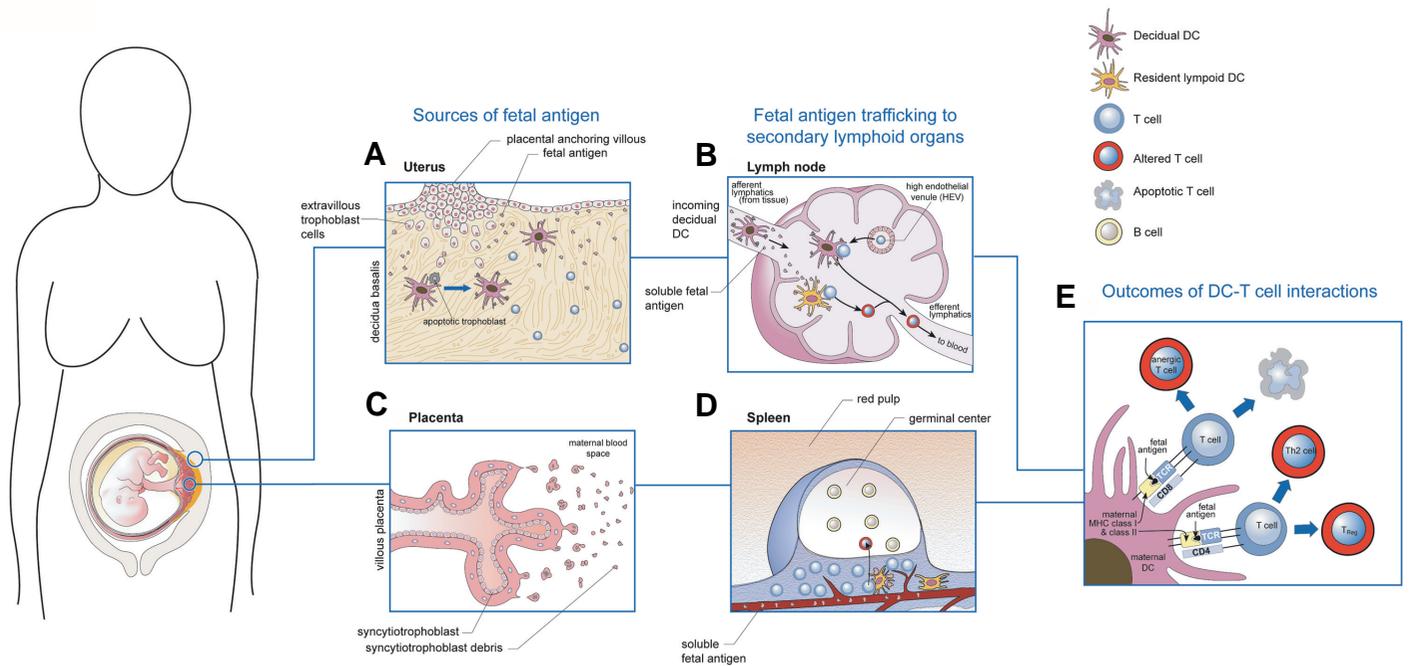
### How can fetal antigens traffic to secondary lymphoid organs?

Sequestration of organ-specific antigens from lymphatics and blood is considered an important mechanism for immune privilege in some organs (Caspi, 2006; Simpson, 2006). This concept was also put forth to explain maternal-fetal tolerance, and initially seemed to be supported by observations of the absence or paucity of lymphatic vessels within the secretory phase endometrium functionalis (Donoghue *et al.*, 2007; Koukourakis *et al.*, 2005; Medawar, 1954; Red-Horse *et al.*, 2006). However, recent immunohistochemical studies of lymphatic endothelium show that the human decidua does in fact contain lymphatic vessels, with which trophoblast cells reside in close proximity (Red-Horse *et al.*, 2006). Surprisingly, cytotrophoblasts were found to stimulate lymphangiogenesis *in vitro* in this study. In the mouse, the situation seems a bit less straightforward, since uterus-associated lymphatic vessels are restricted to the myometrium. Nonetheless, using a model fetal antigen, Erlebacher *et al.* (2007) provided definitive proof that fetal antigens can accumulate in murine uterus-draining lymph nodes, suggesting that a lymphatic pathway can serve as a conduit for fetal antigens in this species.

The abovementioned studies suggest an anatomical pathway for antigen drainage from the maternal-fetal interface into local lymph nodes. The route by which antigens access lymph nodes is intertwined with the trafficking patterns of dendritic cells (DC), which can be either highly motile cells, ferrying antigen from tissues to lymphoid organs, or alternatively, reside within the lymphoid organs awaiting the arrival of antigen via lymphatics (Itano *et al.*, 2003; Ueno *et al.*, 2007).

Fetal antigens could reach the uterus-draining lymph nodes in at least two ways, as illustrated in Fig. 1. First, soluble antigen that is shed or secreted from trophoblast cells into the decidual interstitial space (Fig. 1A) might drain directly into regional lymph nodes via afferent lymphatics. Once antigen arrives, maternal DC residing within the lymph nodes could ingest, process and present these antigens to maternal T cells (Fig. 1B). Recent studies have shown that maternal dendritic cells fulfill this role by cross presentation of fetal antigen (Erlebacher *et al.*, 2007; Moldenhauer *et al.*, 2006; Seavey and Mosmann, 2006). A second pathway (Fig. 1B) might involve resident decidual DC that either ingest fetal antigens from the interstitial space or that phagocytize extravillous trophoblast cells undergoing apoptosis, which is a normal process that occurs during placentation (Huppertz *et al.*, 2006; Jurisicova *et al.*, 2005). These DC would subsequently transport the fetal antigen, also via afferent vessels, to the draining lymph nodes (Fig. 1B).

Trophoblast and/or fetal antigens are also likely to accumulate in the spleens of species displaying hemochorial placentation. Again using the transgenically-expressed fetal ovalbumin model, it was shown that fetal antigen enters the spleen following establishment of a maternal blood supply to the murine placenta (Erlebacher *et al.*, 2007). It would not be surprising if, in human pregnancy, fetal antigens associated with syncytiotrophoblast apoptotic debris that is shed into the maternal blood space were



**Fig. 1. Role of maternal dendritic cells in the induction of fetal tolerance.** Fetal antigens released from trophoblast cells as a consequence of apoptotic cell death (A) or by shedding of syncytiotrophoblast debris (C) traffic into the mother, where they may be taken up and displayed by maternal dendritic cells (DC) within the uterus, spleen or lymph node (A,B,D). Apoptotic debris or cells program the dendritic cells to take on a tolerogenic phenotype. Peripheral T cell tolerance would subsequently be induced through apoptosis, anergy, or conversion to  $T_R$  cells (E).

also carried directly to the spleen (Fig. 1C). In fact, this idea is consistent with normal placental biology and growth. Placental homeostasis is maintained by the continuous fusion of cytotrophoblast cells to the overlying syncytiotrophoblast layer with concomitant shedding of apoptotic syncytiotrophoblast into maternal blood (Huppertz *et al.*, 1998). Trophoblast particles that are shed from the placenta into the maternal blood stream may be trapped in the spleen, ingested by maternal dendritic cells and cross-presented to T cells (Fig. 1D). Large amounts of this shedding is thought to occur; in late pregnancy, cell-free fetal DNA, which likely originates from these shed particles, accounts for more than 6% of all DNA in maternal plasma (Flori *et al.*, 2004; Lo *et al.*, 2000).

Another source of fetal antigens could derive from embryonic and fetal cells that traverse the placenta during pregnancy, producing fetal microchimerism in the mother. This occurs in virtually all pregnancies, even prior to delivery (Khosrotehrani and Bianchi, 2005). The source of the fetal cells producing microchimerism is as yet uncertain, but hematopoietic and mesenchymal stem cells have been implicated (Khosrotehrani *et al.*, 2005; Khosrotehrani *et al.*, 2008). A role for these cells in maternal fetal tolerance is possible (Adams Waldorf and Nelson, 2008)

These lines of observation in the mouse model and human lead one to the conclusion that the trafficking of fetal antigens to maternal secondary lymphoid organs is a normal and perhaps even desired biological event. The question of how the maternal immune system might recognize and respond to fetal antigen likely depends on the context in which they are displayed to maternal lymphocytes.

## Dendritic cells and antigen presentation in pregnancy

Dendritic cells (DC) comprise a heterogeneous group of cells that arise from precursors in the bone marrow. Plasmacytoid DC and myeloid DC comprise the two main subsets of hematopoietic progenitor cells that differ in both lineage and function. Broadly, human plasmacytoid DC are thought to support type 1 (Th1) immune responses, whereas myeloid DC support type 2 (Th2) responses; these roles are reversed in mice. The complexity of DC subsets should not be underestimated though; for example, different subsets of myeloid DC in the human skin may skew immune responses toward either Th1 and Th2 immunity (Ueno *et al.*, 2007). Defining these subsets using cell surface markers is also helpful, but similarly, the rules are not steadfast.

Nonetheless, it is generally accepted that the functional outcome of interactions between a DC and a naïve T cell is determined by the activation state of the DC at the time of interaction. The steady-state condition of DC in tissues and lymphoid organs is immature; these cells are characterized by high phagocytic activity and low antigen presentation potential. When stimulated by pathogen-derived signals, however, DC down-modulate their phagocytic function and become instead highly efficient antigen presenters. Presentation of an antigen by activated or mature DC can result in T cell priming and induction of an immune response to the corresponding antigen, while presentation of the same antigen by immature, or steady-state DC can induce antigen-specific T cell tolerance (Bonifaz *et al.*, 2002; Steinman *et al.*, 2003). The ultimate outcome of antigen presentation by DC is also influenced by the nature and dose of antigen, as well as the

cytokine and hormonal environment.

Immature and mature DC can be characterized by established cell surface markers, and while the correlation between phenotype and function is not absolute (Reis e Sousa, 2006), it appears that in human pregnancy, decidual DC have an immature surface phenotype and physiologic functions that correlate with tolerogenic properties (Blois *et al.*, 2007b). In fact, this phenotype is not unexpected, since DC typically mature only in the presence of pathogenic stimuli. Interestingly, DC expressing CD83, a marker characteristic of mature antigen presenting cells, predominate during the secretory phase of the endometrial cycle while DC-SIGN<sup>+</sup> (CD209<sup>+</sup>) cells, which typify immature DC, are in the majority in first trimester decidua (Gardner and Moffett, 2003; Kammerer *et al.*, 2000; Rieger *et al.*, 2004). This pattern is also evident in the rhesus monkey, in which DC-SIGN<sup>+</sup> cells are situated in close proximity to the implantation site, whereas mature DC were absent (Brebuda *et al.*, 2006). In term placentas, the decidua basalis was found to lack phenotypically mature DC (DC-LAMP<sup>+</sup>CD163<sup>+</sup>) (Bockle *et al.*, 2007). In contrast, pathological pregnancies appear to be associated with an overabundance of DC, including those expressing the mature phenotype, in the decidua (Askelund *et al.*, 2004; Huang *et al.*, 2008).

Functionally, these general concepts are further supported by the finding that decidual DC secrete lower levels of the proinflammatory, T helper 1 (Th1)-promoting cytokine IL-12 than do their peripheral blood counterparts, and can thus preferentially drive differentiation of T cells into a Th2 phenotype (Miyazaki *et al.*, 2003). Thus, in normal pregnancy, it appears that dendritic cells with a mature phenotype become less abundant, while those expressing markers consistent with an immature phenotype, which may promote tolerance to the fetal allograft, accumulate.

Two studies have also recently characterized DC in the murine decidua and lymphoid organs (Bizargity and Bonney, 2008; Zarnani *et al.*, 2007). The studies were largely in agreement: myeloid DC, which in mice support Th2 responses, predominated in the decidua during most of gestation. The exception was that the number of myeloid cells dropped, resulting in lymphoid DC predominance, during midgestation. Interestingly, this time-frame corresponds with the start of fetal antigen access to the maternal spleen (Erlebacher *et al.*, 2007).

Local microenvironments play an important role in dictating DC function and phenotype; dendritic cells resident to lymph nodes likely have a distinct role from those within tissues, and those indigenous to different tissues likely have distinct functions from each other. In addition, pregnancy itself may alter specific properties of DC within decidua or other tissues. For example, the early response to male antigens or other unique fetal antigens may be kept in check through alterations in the properties of uterine antigen presenting cells and/or T cells by hormones, especially high levels of estrogen and transforming growth factor beta (Robertson *et al.*, 2002; Seavey and Mosmann, 2006). Elevated levels of anti-inflammatory cytokines like interleukin (IL)-10 could further enhance the tolerogenic potential of DC during pregnancy (Holmes *et al.*, 2003; Steinbrink *et al.*, 2000). IL-10 as well as 'suppressor' CD8<sup>+</sup> T cells, a population of which may also play a role in pregnancy, induce a state of functional tolerance in DC (Chang *et al.*, 2002; Manavalan *et al.*, 2003; Shao *et al.*, 2005). Intriguingly, these IL-10 and CD8<sup>+</sup> T cell-induced DC are characterized in part by expression of the HLA-G receptor,

ILT4. In turn, therefore, HLA-G might further alter DC function directly by stimulating Th2 cytokines and suppressing the T cell stimulatory capacity of these cells (Apps *et al.*, 2007).

An additional mechanism of DC programming during pregnancy may involve ingestion and presentation of foreign antigens associated with dying cells. Phagocytosis of dying cells by dendritic cells induces long-lasting T cell tolerance and provide a mechanism to prevent autoimmunity, provided that inflammatory signals are absent (Liu *et al.*, 2002). We recently hypothesized that physiological events in pregnancy are designed to establish maternal peripheral T cell tolerance to fetal antigens (illustrated in Fig. 1) (Adams *et al.*, 2007). Apoptotic trophoblast cells and debris containing fetal antigens are regularly shed into the maternal circulation during pregnancy, providing exactly the right types of signals to immature maternal dendritic cells for induction of T cell tolerance. Fetal antigens associated with apoptotic trophoblast debris could include peptides derived from polymorphic fetal proteins, translating into the possibility that unique fetal proteins, to which the mother is immunologically naïve, are presented by maternal dendritic cells. As outlined above, candidate antigens include class I and class II fetal MHC proteins, as well as minor histocompatibility antigens. Presentation of these antigens by maternal dendritic cells might subsequently lead to interaction with maternal CD8<sup>+</sup> and CD4<sup>+</sup> T cells, respectively (Albert *et al.*, 1998).

## Responsiveness of maternal lymphocytes to fetal antigens

### Lymphocytes residing within secondary lymphoid organs

The occurrence of fetal alloantigen expression, trafficking and presentation by antigen presenting cells becomes significant only if maternal lymphocytes actually detect the antigens. That fetal antigens are in fact detected by maternal lymphocytes became evident over a half century ago, when the presence of antibodies that could agglutinate paternal leukocytes were found in the sera of multiparous women (Payne and Rolfs, 1958). Inter-breeding of congenic mouse strains that differ genetically only in MHC antigens confirmed experimentally that paternal MHC antigens could elicit antibody production (Herzenberg and Gonzales, 1962). Studies have also documented the production of fetal HLA-G-specific antibodies as well as T cell expansion to H-Y antigens in multiparous women (Hunt *et al.*, 2003; James *et al.*, 2003; Piper *et al.*, 2007). While antibodies formed as a result of pre-existing disease (for example, antiphospholipid antibodies) can jeopardize pregnancy (Salmon and de Groot, 2008), the relative frequency of anti-paternal HLA antibodies and expanded T cell populations in normal pregnancy suggests that these phenomena are harmless and can occur normally. Still, however, the timing of initial antigen exposure in these human studies deserves clarification, since maternal exposure to fetal antigen invariably occurs during parturition. The absence of trophoblast class Ia molecules in humans suggests that other cell types are involved in eliciting these antibodies; it is, however, possible that expansion of H-Y-specific (and perhaps other minor antigen-specific) T cells results from trophoblast antigens.

Studies using transgenic mice have established that antigenic exposure and lymphocytic responses in fact occur during first pregnancies, prior to delivery. In these models, maternal reac-

TABLE 1

MATERNAL RESPONSES AGAINST FETAL MHC ANTIGENS IN MICE<sup>1</sup>

| FETAL MHC ANTIGEN                |  | MATERNAL LYMPHOCYTES            |   |                                   | Major findings  | Reference                          |
|----------------------------------|--|---------------------------------|---|-----------------------------------|---|------------------------------------|
| Antigen source                   | Genetic control of antigen expression (Promoter) | Responding maternal lymphocytes | Lymphocyte receptor                         | Source of lymphocytes (GD)        |   |                                    |
| Fetus                            | Endogenous                                       | CD8+ T cells                    | Anti-Class I TCR (transgene)                | Spleen (GD9-11)                   | <ul style="list-style-type: none"> <li>• ↓ numbers of reactive T cells</li> <li>• ↓ expression of CD4 and CD8 on reactive T cells</li> <li>• Tolerance to Kb-bearing tumor grafts</li> <li>• Rebound of immunity and T cell phenotype postpartum</li> </ul>                                     | Tafari <i>et al.</i> , 1995        |
| Fetus                            | Endogenous                                       | B cells                         | Anti-Class I BCR (transgenic)               | Spleen, bone marrow, LN (GD10-18) | <ul style="list-style-type: none"> <li>• ↓ in reactive B cells after midgestation</li> <li>• Earliest response detected in bone marrow</li> <li>• Both hormone- and antigen-mediated ↓ of B cells</li> <li>• Cell numbers rebounded postpartum</li> <li>• No effect in LN</li> </ul>            | Ait-Azzouzene <i>et al.</i> , 1998 |
| Trophoblast giant cells<br>Fetus | Transgenic (PL II)<br>Endogenous                 | B cells                         | Anti-Class I BCR (transgene)                | Bone marrow                       | <ul style="list-style-type: none"> <li>• No effect of trophoblast MHC on litter size</li> <li>• No effect of pre-immunization of dams on litter size</li> <li>• Hormone- and antigen-mediated reduction of B cells</li> </ul>   | Ait-Azzouzene <i>et al.</i> , 2001 |
| Trophoblast giant cells          | Transgenic (PL II)                               | T cells                         | Anti-Class I TCR (transgenic or endogenous) | LN (GD13)                         | <ul style="list-style-type: none"> <li>• No effect of trophoblast MHC on litter size, even in absence of Fas signaling</li> <li>• No effect of pre-immunization or <i>ex vivo</i> activation of lymphocytes on litter size</li> <li>• No effect of trophoblast MHC on TCR expression</li> </ul> | Rogers <i>et al.</i> , 1998        |
| Trophoblast Fetus                | Transgenic (HLA-G)<br>Endogenous                 | CD8+ T cells                    | Anti-Class I TCR (transgene)                | Spleen, LN (GD10)                 | <ul style="list-style-type: none"> <li>• ↑ numbers of reactive T cells (Spleen and LN)</li> <li>• ↓ expression of CD8 and TCR</li> <li>• No change in activation markers</li> <li>• <i>In vitro</i> effector function not affected</li> </ul>   | Zhou and Mellor, 1998              |
| Fetus                            | Endogenous                                       | CD8+ T cells <sup>2</sup>       | Anti-Class I TCR (transgene)                | Spleen                            | <ul style="list-style-type: none"> <li>• No proliferation of reactive T cells</li> <li>• No change in of activation markers</li> </ul>  | Erlebacher <i>et al.</i> , 2007    |

<sup>1</sup> Abbreviations: BCR, B cell receptor; LN, lymph nodes; GD, gestation day on which lymphocytes were examined; PL II, placental lactogen type II; TCR, T cell receptor.

<sup>2</sup> Experiment in which responding lymphocytes were adoptively transferred.

tions to both major and minor histocompatibility antigens can be studied; findings of these studies are summarized in Tables 1 and 2. Both proliferation and accumulation have been demonstrated, albeit in separate model systems. In one model examining specific responses to minor fetal antigen, maternal endogenous antigen-specific T cells are found to increase at midgestation, followed by a drop later in pregnancy (Jiang and Vacchio, 1998). In a second system, adoptively transferred antigen-specific lymphocytes are directly shown to proliferate in response to antigen, but never proportionally accumulate (Erlebacher *et al.*, 2007). Together, these studies suggest that antigen-specific T cells proliferate in response to fetal antigen, but are soon thereafter deleted. Those T cells that avoid deletion appear to be hyporesponsive to subsequent antigenic stimulation *in vitro* and *in vivo* (James *et al.*, 2003; Jiang and Vacchio, 1998).

Interestingly, a memory phenotype occurs in fetal H-Y antigen-specific cells, which persists postpartum (James *et al.*, 2003; Vacchio and Hodes, 2003). In addition, tolerance to male (fetal) antigens may also persist (James *et al.*, 2003). However, the environmental conditions of initial antigen presentation may be critical to this long-lived tolerance, since immunization with H-Y-bearing splenocytes prior to pregnancy does not result in the same hyporesponsiveness during or after gestation (Bonney and Onyekwuluje, 2003). Possibly, antigen presenting cells of the dam are conditioned by pregnancy, as discussed above, such that responding lymphocytes exhibit long-term tolerance. Should antigen presentation occur in the absence of the gestational environment, the dam might be primed rather than tolerized to the exogenous fetal antigen bearing cells. Importantly, this pre-immunization does not compromise fetal viability in mice (Bonney and Onyekwuluje, 2003).

Other studies examined maternal lymphocyte responses in MHC incompatibility between mother and fetus. Because incompatibility at these loci elicit the strongest and most rapid graft rejection, by way of extrapolation, maternal fetal MHC incompatibility might elicit the strongest maternal responses during pregnancy as well. Experiments in which the maternal CD8+ T cell repertoire that is engineered to almost exclusively recognize paternal MHC show virtually no deleterious effect on fetal viability (Tafari *et al.*, 1995; Zhou and Mellor, 1998). One explanation for these results is that MHC class I expression is absent in areas accessed by maternal blood, as discussed above. However, this conclusion was addressed separately in studies where paternal MHC class I molecules are ectopically over-expressed in the placenta. Surprisingly, these studies also revealed no immunologically mediated fetal demise (Ait-Azzouzene *et al.*, 2001; Rogers *et al.*, 1998; Zhou and Mellor, 1998). This is in direct contrast to the oft-cited critical nature of absence of MHC on trophoblast cells for successful pregnancy. Explanations such as lack of danger signals emanated by the fetus (Bonney and Matzinger, 1998) have been tested using pre-immunized mice (Ait-Azzouzene *et al.*, 2001; Rogers *et al.*, 1998) and inflammation-eliciting agents (in a minor histocompatibility model) (Erlebacher *et al.*, 2007). Lack of the maternal immunomodulators Fas also do not seem to endanger the pregnancies (Rogers *et al.*, 1998; Vacchio and Hodes, 2005). However, the role of regulatory T cells in these models, which appear to be required for fetal tolerance (Aluvihare *et al.*, 2004; Sasaki *et al.*, 2004), has not been addressed in these models. In addition, co-existing disparity in class II antigens, an important aspect of transplant rejection, may also be important; this hypothesis also has not been empirically tested in murine pregnancy.

Studies looking at major antigen differences have been partly beleaguered by inconsistencies in their findings. Tafuri *et al.* (1995) reported a transient state of tolerance to fetal MHC antigen bearing cells *in vivo*, in contrast to the longer-lived tolerance induced by minor fetal antigen (James *et al.*, 2003). These differences might be simply explained by the nature of the different antigen-TCR interactions. However, other observed differences are more difficult to account for. Changes in major antigen-reactive T cells reported include a drop in numbers (Tafuri *et al.*, 1995), an increase in number (Zhou and Mellor, 1998), or no change (Rogers *et al.*, 1998). The report by Erlebacher *et al.* (2007) was consistent with the latter of these, as neither proliferation nor upregulation of activation markers was observed during a short exposure of maternal cells to fetal MHC. The increase in T cell numbers could not be explained by the location of antigen expression alone, since Zhou and Mellor (1998) observed parallel changes caused by both endogenous MHC and transgene-driven expression of paternal MHC in the trophoblast. Nonetheless, avidity and affinity of TCR-MHC interactions should be kept in mind. Clearly, further experimentation is needed to clarify this controversy.

#### A role for central lymphoid organs?

Paradoxically, the expansion of lymph node and spleen is mirrored by a loss in cellularity of both the thymus and bone marrow during pregnancy. The physiological significance of pregnancy-induced changes in the thymus has been presumed to be in maintenance of maternal tolerance to the fetus (Clarke, 1979). Antigen specific B cells are lost in the bone marrow (Table 1) (Ait-Azzouzene *et al.*, 2001), and although deletion of T cells reactive to peripheral antigens can occur in the thymus, whether this happens in pregnancy has not been tested. However, major roles of both estrogen and progesterone in thymic involution have been described (Tibbetts *et al.*, 1999; Zoller and Kersh, 2006). Effects of pregnancy can largely be reproduced by injection of estradiol or progesterone into ovariectomized mice. Intriguingly, Tibbetts *et al.* (1999) showed that fertility is reduced when mice are grafted

with thymi that fail to regress during pregnancy due to the lack of progesterone receptor. Whether this effect was caused by T cells or was antigen-specific remains an exciting, yet open question.

#### Summary and perspectives

Many of the factors have documented roles in maintenance of self-tolerance have been suggested to have obligatory roles in maintenance of tolerance to the fetus, but their absolute requirement for pregnancy is still uncertain. Indeed, continued generation of species depends on an unfaltering state of immunological amity between the mother and fetus. It is not surprising, therefore, that few mechanisms have been robustly shown to be indispensable for curtailing a maternal anti-fetal immune reactions. Future studies examining maternal-fetal tolerance would benefit from examining networks of immunosuppressive pathways. That true tolerance of maternal T cells is required despite the absence of danger signals from the fetus is evidenced by multiple demonstrations of the strict requirement of CD4+CD25<sup>high</sup> regulator T cells for success of allogeneic pregnancy, as well as the recent report of the role of galectin-1 in maternal-fetal tolerance (Aluvihare *et al.*, 2004; Blois *et al.*, 2007a; Darrasse-Jeze *et al.*, 2006).

A host of questions pertaining to fetal antigen-specific tolerance in pregnancy remain unresolved (Box 1). Focusing on the interactions between fetal antigen and maternal cells that occur in the maternal secondary lymphoid organs will aid in understanding these tolerizing networks that may encompass the type and source of fetal antigen, the phenotype of maternal DC presenting that antigen, and subsequent responses of maternal T cells. (Fig. 1E). Characterization of the DC phenotype in secondary lymphoid organs, especially uterus-draining lymph nodes, warrants further exploration, as DC from lymph nodes draining mucosal immune sites often have different, and uniquely tolerogenic properties compared to systemic lymph nodes (Kraal *et al.*, 2006). Further investigation into the specific outcomes of maternal DC and T cell interactions would also be informative. MHC class I mediated responses to fetal antigen should result in deletion as well as

TABLE 2

#### MATERNAL RESPONSES AGAINST FETAL MINOR ANTIGENS IN MICE<sup>1</sup>

| FETAL ANTIGEN |                             |                                       | MATERNAL LYMPHOCYTES   |                               |                               | Major findings  | Reference                       |
|---------------|-----------------------------|---------------------------------------|--|-------------------------------|-------------------------------|---|---------------------------------|
| Antigen       | Antigen Source <sup>2</sup> | Genetic control of antigen expression | Responding lymphocyte subset   | Lymphocyte receptor transgene | Source of lymphocytes (GD)    |   |                                 |
| H-Y           | Unspecified                 | Endogenous                            | CD8+ T cells   | Anti-H-Y TCR transgene        | Spleen, LN (GD7 - postpartum) | <ul style="list-style-type: none"> <li>• ↑, then ↓ in number of H-Y reactive T cells</li> <li>• Remaining H-Y-reactive T cells hyporesponsive <i>ex vivo</i></li> </ul>   | Jiang and Vacchio, 1998         |
| H-Y           | Unspecified                 | Endogenous                            | CD8+ T cells   | Anti-H-Y TCR transgene        | Spleen (GD18)                 | <ul style="list-style-type: none"> <li>• Maternal CD28 required for clonal deletion and hyporesponsiveness to H-Y<sup>+</sup> cells</li> <li>• ↑ in H-Y memory cells</li> </ul>   | Vacchio and Hodes, 2003         |
| H-Y           | Unspecified                 | Endogenous                            | CD8+ T cells   | Anti-H-Y TCR transgene        | Spleen (GD18)                 | <ul style="list-style-type: none"> <li>• Fetal FasL required for hypo-responsiveness to H-Y<sup>+</sup> cells</li> </ul>  | Vacchio and Hodes, 2005         |
| H-Y           | Unspecified                 | Endogenous                            | CD8+ T cells   | Endogenous TCR                | Soleen (postpartum)           | <ul style="list-style-type: none"> <li>• ↑ survival of male skin grafts in multiparous mice</li> <li>• ↑ in H-Y memory T cells</li> </ul>   | James <i>et al.</i> , 2003      |
| Ovalbumin     | Endovascular trophoblast    | Transgenic                            | <ul style="list-style-type: none"> <li>• CD8+ T cells<sup>3</sup></li> <li>• CD4+ T cells<sup>3</sup></li> </ul> | Anti-ovalbumin TCR transgene  | Spleen, LN (multiple GD)      | <ul style="list-style-type: none"> <li>• Proliferation but lack of accumulation of CD4+ and CD8+ ova-reactive T cells</li> <li>• Antigen exposure starting at midgestation</li> <li>• Cross-presentation of fetal antigen by maternal APC</li> <li>• ↓ naive phenotype</li> </ul> | Erlebacher <i>et al.</i> , 2007 |

<sup>1</sup> Abbreviations: TCR, T cell receptor; LN, lymph nodes; GD, gestation day on which lymphocytes were examined.

<sup>2</sup> Unspecified antigen source indicates that fetal antigen could derive from either trophoblast or fetal tissues.

<sup>3</sup> Experiments in which responding lymphocytes were adoptively transferred.

## Box 1

**Unresolved questions in maternal-fetal tolerance**

Many of these matters probably involve key events that occur within the secondary lymphoid structures. Use of transgenic models and defined fetal antigens may aid in illuminating these issues.

- Do specific properties of the uterus-draining lymph nodes contribute to the direction of the immune response?
- How does presentation of fetal antigens influence the development of regulatory T cells?
- What are the spatial and temporal patterns of expression of minor histocompatibility antigens in the fetus and placenta?
- Are lymphocyte responses to minor antigens a threat to pregnancy in humans?
- What are the differential roles of passage of fetal cells and antigen into the mother during pregnancy v. during parturition?
- Is there a role for fetal microchimerism in maternal-fetal tolerance?
- What are the ramifications of concomitant fetal antigen presentation to both CD4+ and CD8+ maternal T cells?
- Does co-presentation of fetal antigens and maternal peptides lead to transient remission of certain autoimmune diseases during pregnancy?
- Do pathogen-induced inflammatory signals (danger signals) elevate the risk of adverse fetal-antigen specific immunity by inducing maturation of dendritic cells that present fetal antigen?
- Does disruption of fetal antigen-specific tolerance contribute to pathologic pregnancies, such as IUGR, preeclampsia or preterm labor?

tolerization of maternal CD8<sup>+</sup> T cells (Fig. 1E), but the outcome of MHC class II-mediated antigen presentation to CD4<sup>+</sup> T cells are unknown. CD4<sup>+</sup> T cells have recently been shown to respond to fetal antigen when presented by maternal antigen presenting cells (Erlebacher *et al.*, 2007), but the details of this interaction remain unclear. In order to maintain tolerance against fetal tissues, likely outcomes include differentiation of CD4<sup>+</sup> Th cells into either Th2 or T<sub>R</sub> cells (Fig. 1E).

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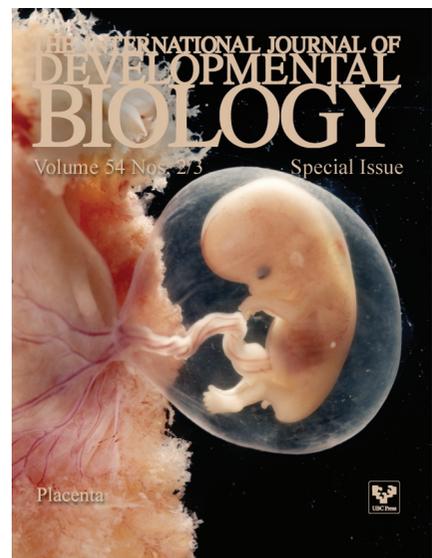
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