From the Decidual Cell Internet: Trophoblast-Recognizing T Cells

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ABSTRACT
The mammalian fetus has been perceived, paradoxically, as a successful allograft, a successful tumor, and a successful parasite. Success depends on fetal trophoblast cells, which form the interface with the mother. The maternal immune system is involved in the success of pregnancy and its failure. The discovery that maternal γδ T cells may recognize and react to the fetal trophoblast and the definition of a vascular mechanism whereby their Th1 and Th2/3-type cytokines may abort embryos replaces confusion and debate with a new and simple clarity that enables further research.

INTRODUCTION
The mammalian fetus has been perceived, paradoxically, as a successful allograft, a successful tumor, and a successful parasite [1–3]. The maternal immune system is involved in the success of pregnancy or its failure (as in spontaneous abortions). The role of conventional decidual T cells expressing αβ receptors is still controversial; they do not seem to recognize or react to trophoblast cells, and the acquisition of maternal T cells to a transient state of tolerance specific for paternal alloantigens has been proposed [4–7]. Neither T cells nor natural killer (NK) cells and macrophages involved in abortions can kill trophoblast cells, and anti-aborptive immune responses involving CD8+ T cells and sometimes antibody production against antigens on trophoblast have been unexplained [2, 8]. The discovery that γδ T (and γδ T/NK) cells may recognize and react to trophoblast cells and the definition of a vascular mechanism whereby their Th1 and Th2/3-type cytokines may abort embryos [9] ends an era of confusion and debate with a new and simple clarity, and leads to three questions for further research: What do these cells recognize? What determines the Th cytokine phenotype? And how do distinct trophoblast-reactive cell populations communicate with each other at the feto-maternal interface and with the systemic immune system?

Shifting Paradigms in the Feto-Maternal Relationship
For a long time, successful pregnancy has been compared to the transplantation of an allograft that is tolerated by the recipient and not rejected [1]. The pregnant mother will reject allografts bearing major histocompatibility complex (MHC) antigens of her husband, but the intrauterine fetus bearing these antigens is protected [2]. This protection is attributable to an outer layer of fetal trophoblast cells that form the placenta and membranous chorionic sac enclosing the fetus in a cocoon [3]. Because human trophoblast cells do not express classical major histocompatibility antigens (human leukocyte antigen [HLA]-A, -B, or -D) [10–13], doubt has been cast increasingly upon the “transplantation model” of pregnancy. Indeed, when such MHC antigens are expressed on trophoblast cells by transplantation, or in vitro in response to interferon-γ (IFN-γ), or due to malignant transformation, the trophoblast remains resistant to lysis by antibody-dependent cellular cytotoxicity and specific cytotoxic T lymphocytes (CTL) [8, 14–17]. Indeed, the trophoblast is also resistant to killing by conventional NK cells and macrophages [18]. It has been suggested that early embryo loss is a nonspecific event mediated by the triggering of cytotoxic production by primed decidual macrophages [18]. However, NK cells transformed by interleukin (IL)-2 into lymphokine-activated killer cells may kill trophoblast cells, and in vivo depletion of NK-lineage cells using anti-asialoGM1 antibody abrogates recurrent spontaneous abortions in CBA/J × DBA/2 mice and mouse abortions elicited by an injection of bacterial lipopolysaccharide or the NK cell activator poly I:C [19, 20]. The resistance of trophoblast cells to all effector cells except cytokine-activated NK-type cells is reminiscent of cancer cells [21]. For this reason, the fetal trophoblast-maternal relationship has been suggested to be analogous to a tumor-host interaction [2, 22].

More recently discovered, a previously unknown MHC class I molecule called HLA-G is exclusively expressed on human invasive cytotrophoblast cells [10, 11, 13, 15], and there is now evidence for expression of HLA-C, HLA-E, and HLA-F on trophoblast cell lines [14, 23, 24]. HLA-E and HLA-F have been proposed to be of great importance in HLA-G-induced NK cell regulation [25]. Also, a novel 80-kDa alloantigen is expressed on both the syncytiotrophoblast, to which antibodies are commonly made during human pregnancy [26].

The role of these trophoblast antigens still needs to be identified [13, 26]. In humans, it is argued that anti-HLA antibodies are stimulated by fetal cells that cross the trophoblast into the mother; but in the mouse, only the placenta is required to induce antibodies [3]. Anti-paternal antigen antibody responses to paternal antigens have been noted on trophoblast in the rat and noted in the horse, where the paternal alloantigen is expressed on invading tropho-
that forms cups (islands) in the uterine lining [27]. In all species that have been studied, such antibodies appear harmless [28]. The main role currently postulated for MHC expression on trophoblast is the inhibition of cytotoxic effector cells such as NK cells and lymphokine-activated killer cells via killer-inhibitory receptors [29].

The development of IgG of antibody to non-MHC paternal alloantigens expressed on trophoblast implies generation of Th2 helper cells, as IgG responses are usually T cell-dependent; antibody may also arise as a result of allogeneic pregnancy in rodents [30, 31]. Further, it has been shown in the CBA/J × DBA/2 mouse model of recurrent abortions that pregnancy losses can be prevented by immunization against the MHC alloantigens and background genes of the male [32, 33]. By contrast to protective anti-abortive immune responses, Raghupathy [34] has shown that placentas from resorption-prone CBA × DBA/2 matings activate CD8+ T cells, which, on adoptive transfer, cause abortions, whereas placentas from non-resorption-prone matings do not stimulate CD8+ T cells to become abortive. On the other hand, Chaouat et al. [35] have shown that CD8+ T cells are essential for immunization to prevent abortions, and in vivo injection of anti-CD8 antibody into abortion-prone CBA/J × DBA/2 pregnancies either has no effect on the abortion rate (if injected after Day 8.5 of gestation) or boosts the abortion rate (if injected earlier). The apparent contradictory effects of CD8+ T cells may in part be explained by the Th1/Th2 paradigm of Raghupathy [34] and Wegmann and coworkers [36, 37]; CD8+ T cells can potentially belong to either phenotype. Th1-type cytokines stimulate abortions and Th2-type cytokines prevent abortions. In pregnancy a Th1 → Th2 shift is postulated, and data from both mice and humans appear to support this hypothesis [20]. Indeed, in pregnant C57Bl/6 mice infected with Leishmania major (a parasite that is rejected via a Th1 response), if the parasite is rejected (by a shift to Th1), abortions occur, whereas if the pregnancies succeed, so also does the infection [38, 39]. These and related features of the fetomaternal interaction have given rise to the new paradigm that pregnancy is a host-parasite relationship, and one that can be terminated if necessary to preserve the life of the mother for the ultimate benefit of the species. Th1-type cytokines stimulate the NK-macrophage system that is involved in abortions, whereas Th2-type cytokines (and CD8+ T cells) suppress the NK-macrophage system [35]. Interestingly, psychic stress may cause abortions in mice and humans via substance P-mediated activation of mast cells and macrophages in the uterus and by inhibition of CD8+ suppressor cells [40–42]. Endotoxin, another potent abortogen, stimulates macrophages; these cells then release tumor necrosis factor α (TNFα) in the same way they would if activated by Th1-derived IFN-γ. Immunization to paternal antigens prevents stress-triggered abortions. Therefore, several different pathways may lead to activation of NK cells and macrophages involved in rejection of the embryo/parasite in the uterus, as illustrated in Figure 1, and a Th2-type immune response appears to be protective. However, it has yet to be resolved whether conventional circulating T cells that bear T cell receptor (TcR) αβ recognize and react to antigens expressed by the trophoblast [29, 43]. Recently, γδ T lymphocytes have been identified in murine and human decidua, and these cells may be able to react with antigens expressed by trophoblast and may interact with TcR αβ+ T cells [43–50]. Indeed, cells of the innate resistance system such as NK cells, macrophages, and γδ T cells appear to program the conventional immune system to mount an immune response [51]. It is proposed that γδ and αβ T cells communicate with each other in determining the nature and magnitude of the response to be generated.

**Antigen Recognition by γδ T Cells**

Classical αβ T cells in the mammalian species have not been considered able to recognize trophoblast because of the atypical expression of MHC class I and II molecules on human trophoblast cells or MHC expression on murine spongiotrophoblast [52–54]. The nature of antigens recognized by murine or human T cells, in particular by γδ TcR-bearing T cells, and the mode of their presentation are still being defined [55, 56]. γδ T cells, however, can recognize atypical class I MHC antigens, as typified by HLA-G on human trophoblast cells [55]. From experiments in mice we know that γδ T cells provide a first line of defense against infectious agents such as bacteria, viruses, and parasites because of their preferential location in epithelium and underlying stroma at mucosal surfaces [57]. In humans, γδ T cells are not predominantly associated with epithelia but are present in most tissues and in the same areas of lymphoid organs as αβ T cells [58]. Fujihashi et al. [58] have demonstrated that human gut γδ T cells are a potent source of Th1 and Th2 cytokines, and one third of the γδ T cells are producers of both Th1 and Th2 type cytokines, i.e., have a Th0 phenotype. A significant proportion of γδ T cells are thymus-independent and may have a TcR with a germ line-determined specificity (called canonical, as in ordained), lack CD4 and/or CD8, and recognize heat shock proteins (HSP) as well as specific antigens. HSP has been linked to a proposed role in homeostasis of epithelial surface, as damaged epithelial cells up-regulate HSP expression. Antigens unassociated with MHC, soluble antigen, and carbohydrates may also be recognized. On this basis, it has been suggested that γδ T cell recognition is more comparable to antibody recognition than αβ T cell recognition of antigen [56].

**Trophoblast Recognition by Murine γδ T Cells**

γδ T cells have been cloned from the decidua of syngenically pregnant C57Bl/6 mice using hybridoma methodology [44]. In 1994, Heyborne et al. [45] reported that the Vγ1+ subset of γδ T cells, a population that has been shown to be associated with HSP-60 reactivity, reacts to mouse and human trophoblast in a non-MHC-restricted manner by producing the cytokine IL-2. The assay used could not exclude IL-4, but using their 69BAS-122 hybridoma, we have confirmed production of IL-2 using CTLL2 cells, and intracellular cytokine staining also detected intra-
cellular TNFα (unpublished results). The Vγ1+ cells described by Heyborne et al. [44] would appear to have a Th1 phenotype, although it might be unlikely that the Th phenotype of γδ T cell hybridomas reflects the Th phenotype of the normal cells that gave rise to it. By contrast, Suzuki et al. [59] isolated uterine intraepithelial lymphocytes and found an increase in γδ T cells in allogeneic murine pregnancy, expression of the CD69 activation marker, and production of the immunosuppressive cytokine transforming growth factor (TGF)β, which appeared to be bioactive, TGFβ1>TGFβ2/3. In decidual stroma of pregnant CBA × DBA/2 mice, however, Clark et al. [60] found as much or more immunosuppressive activity due to bioactive TGFβ2 released by CD8− γδ T cells, and no activity could be ascribed to TGFβ1 or TGFβ3 [61].

Interestingly, these TGFβ2+ cells could also coexpress IL-10 consistent with a Th2/3 phenotype, and they are known to be dependent upon the presence of fetal trophoblast cells [38, 39]. Conversely, anti-IL-10 increases the re-absorption rate, but only in CBA × DBA/2 matings [62]. The importance of TGFβ2 and IL-10 in preventing abortions in CBA/J × DBA/2 mice is evident from the in vivo effect of neutralizing antibodies to these cytokines [62, 63].

**Function of T Cells during Murine Pregnancy**

The role of γδ T cells during pregnancy was addressed first. γδ T cells were depleted by injecting monoclonal antibody (mAb) against the TcR δ chain 1 day after implantation in the abortion-prone mouse model CBA/J × DBA/2 [48]. This led to a significant decrease in susceptibility to abortion in the pregnant CBA/J females, and cytotoxic studies of γδ T cells in control and δ TcR-depleted mice showed a decrease of TNFα−δ− cells, both systemically and locally in the decidua [64]. We found γδ T cells accumulating in decidua from Day 6.5 of gestation in CBA/J × DBA/2 matings accounted for up to 20% of total T cells on gestation Day 13.5, with a decrease in TcR αβ T cells. Anti-δ injected on Day 5.5 prevented the early rise in γδ cells, but a later wave of γδ cells beginning after Day 8.5 of gestation representing TGFβ2+ cells occurred normally. To determine whether either population might be Vγ1+, purified mAb against the Vγ1 subset was injected on Day 5.5 or 8.5. The Day 5.5 injection reduced the abortion rate and depleted δ− T cells with TNFα; the Day 8.5 injection boosted the abortion rate, depleted TGFβ2+ cells, and increased the TNFα− subset [65]. These data suggested two functionally distinct populations of Vγ1+ cells with the potential to respond to antigens on trophoblast cells. Immunization with Balb/c spleen cells, which prevents abortions in this system, had two interesting effects. First, the Th1-type δ− subset was greatly reduced, particularly cells that were IFN-γ−, and Th2/3 TGFβ2+ and IL-10− δ+ cell numbers were boosted [64, 65]. Second, the total number of δ+ cells in the deciduala decreased and the number of TcR β+ cells increased. This suggested that αβ T cells might actively alter the Th1/Th2 ratio among γδ T cells; whether these αβ T cells are the CD8+ subset required for immunization to prevent abortions remains to be determined. The current model of the interactions is illustrated in Figure 2.

The Th1/Th2 evolution in decidual γδ T cells is similar in many respects to the γδ response to other “parasites” in which the Th2 component suppresses the inflammatory response. Interestingly, the uterus is an immunoprivileged site, and during pregnancy the mother retains control. If the health of the mother is threatened, the parasite can be terminated, allowing other offspring needed for prolongation of the species to be conceived. But how do NK cells and macrophages cause pregnancy failure? The current focus of research emphasizes possible killing of trophoblast by activated NK cells or macrophage-produced nitric oxide (NO) [66–69]. Published data show that the cytokines TNFα and IFN-γ produced by macrophages and NK cells can abort more than 80% of the implanted embryos in CBA/J × DBA/2 where the NK cells have been depleted by anti-asialoGM1 or macrophages have been depleted by silicon dioxide treatment [9]. The abortions in spontaneously aborting and cytokine-boosted mice are blocked by antibody to the fgl2 prothrombinase molecule expressed by cytokine-activated vascular endothelial cells. Generation of thrombin stimulates IL-8, which recruits granulocytes, and anti-granulocyte antibody treatment is almost as effective as antibody to fgl2. Thus, the process of abortion may be characterized as a cytokine-triggered vascular autoamputation that requires conversion of prothrombin to thrombin [9].
The production of TGFβ-related suppressive activity was identified in decidual supernatants, whereby the levels in SCID mice were lower than in immunocompetent (non-SCID) controls. The lower levels of suppressor activity in T cell-deficient SCID mice are similar to the lower levels in human decidua. This may reflect the lower number of γδ T and T-NK cells in the human compared to the mouse. Did the TGFβ2 come from another source such as placental trophoblast or epithelium? The production of TGFβ2 from another source might be comparable to the occurrence in the TgfE26 T/NK cell-deficient mice, which lack NO-producing GMG cells in their decidual supernatants. In SCID mice, the NK cell population increases, and the kinetics of NK cell infiltration into decidua in CBA/J × DBA/2 closely parallels the accumulation of γδ T cells, with the initial infiltration beginning by Day 6.5 of pregnancy [70]. Further, both αβ and γδ T cells may be associated with NK cell markers such as NK1.1 or asialoGM1 [80, 81]. It has recently been shown that uterine NK cells appear to have critical functions in pregnancy that promote decidual health, the appropriate vascularization of implantation sites, and placental size [80]. Recent experiments containing γδ and asialoGM1 have confirmed the occurrence of double-positive cells in the CBA/J × DBA/2 decidua; using TNFα or IFN-γ as a reflection of Th1 and TGFβ2 as a reflection of Th2/3-type cytokine production, the double-positives and asialoGM1+ γδ− cells seem to be Th1 > Th2/3, whereas γδ-only cells are the opposite (Fig. 3) [81]. These data are based on intracellular cytokine detection, and the magnitude of secretion of either by the cell populations remains to be determined. As all of the TGFβ2-suppressive activity was dependent on γδ+ cells in decidua of immunocompetent CBA/J mice [58], release from asialoGM1+ γδ− NK cells may be normally suppressed; CD8+ T cells have been shown to inhibit NK cells [82]. The presence of different cell subsets in the decidua has added a new and challenging dimension to understanding responses to trophoblast signals and interactions among the various cell populations that may shift the balance between Th1 and Th2/3.

γδT Cells and γδ+ NK Cells in Humans

The differences in placentation and in reproductive physiology in the mouse make it important to ask whether findings derived from pregnant mice have any equivalent in the human. In contrast to experiments in mice, the documentation of γδ T cells in the human decidua has been controversial [82, 83]. CD3+ T cells are present, but the expression of αβ or γδ TcR appears to be down-regulated [82, 84]. A similar down-regulation of expression may occur in the mouse also, perhaps due to a low molecular weight trophoblast-derived factor that inhibits phosphorylation of TcR γ chains [85]. On the other hand, Mincheva-Nilsson et al. [86] have claimed that T cells expressing the γδ heterodimer are particularly abundant in the decidua, but the γδ epitope is very sensitive to fixation and freezing, leading to a failure of immunohistochemical detection techniques. Using freshly isolated, non-enzymatic treated cells, as well as electron microscopy detection, the authors demonstrated that γδ TcR+ T cells are enriched in the decidua throughout human pregnancy [81]. Some of these cells may arise from blood contamination [83], but Mincheva-Nilsson et al. [86] have documented that the majority of γδ T cell clones from human decidua express the Vδ1 gene segment in their TcR, but Vδ9 subset has a polymorphic (noncanonical) TcR. This suggests a continuous selective influx and retention of γδ T cells from peripheral blood—an early influx of mature γδ T cells that proliferate locally, rather than an expansion of the usual resident population. It has also been hypothesized that human early-pregnancy decidua is a transient site for extrathymic maturation, whereby the progenitors of TcR γδ+ cells are bone marrow-derived immature cells expressing the CD56 homing receptor [86]. CD56 is a marker for NK cells. CD56+ γδ+ cells in peripheral blood, when activated, manifest a Th1 phenotype by producing TNFα and IFN-γ; in decidua, CD56+ are abundant but lack the CD16 marker of blood-type NK cells, and reverse transcription-polymerase chain reaction suggests that such cytokines may be produced by at least some of these CD56+16- cells. On the other hand, a subset of CD56+16- cells can be stained for TGFβ2 [73]. Other sources of cytokine production, such as macrophages and mast cells, are certainly possible and are present at the feto-maternal interface [42, 89].

Intracellular Vγ chains have been found in a proportion of human decidual CD56+ cells, including Vγ1 [75].
magnitude of TGFβ2-immunosuppressive activity obtained from human decidual leukocytes is much lower than that routinely obtained from murine decidual tissues; but nevertheless, similarities to cytokine-producing T/NK populations in the mouse are striking. CD8+ and TcR αβ T cells are also present in human decidua, but again, there may be a down-regulation of expression of surface TcR [82]. Although αβ and γδ T cells with reduced surface TcR expression may be hyporeactive to antigen, normal reactivity would be expected prior to down-regulation, at the time of first contact with antigen; and once antigen activation has occurred, activation and cytokine synthesis might be maintained by autocrine and/or paracrine stimulation.

Study of women with recurrent spontaneous abortions also provides findings that can be compared to those obtained from abortion-prone mice. Up to 55% of human recurrent abortions may be due to chromosome abnormalities in the trophoblast; in mice, a 4–7% rate has been documented [90]. On this basis, one would expect about half of women with recurrent miscarriages to show features similar to those of the CBA/J × DBA/2 mouse. It is therefore notable that about 50% of women with unexplained recurrent miscarriages have an increased frequency of congenital miscarriages [90]. On this basis, one would expect about half of current abortions may be due to chromosome abnormalities in the trophoblast; in mice, a 4–7% rate has been documented [90]. On this basis, one would expect about half of women with recurrent miscarriages to show features similar to those of the CBA/J × DBA/2 mouse. 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