Outlook

T cells in normal pregnancy and recurrent pregnancy loss

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Abstract

This review focuses on the possible role of T cells in successful pregnancy and in unexplained recurrent abortion. The functions exhibited by Th1 and Th2 cells have suggested, perhaps in a simplistic way, that Th1-type cytokines, which promote allograft rejection, may compromise pregnancy, whereas the Th2-type cytokines, by inhibiting Th1 responses, promote allograft tolerance and therefore may improve fetal survival. However, Th1 cytokines are not always detrimental for pregnancy development. Th1 cytokines, depending on their time of expression, stage of gestation and relative concentrations, could have a positive role in successful pregnancy. Other cytokines (LIF, M-CSF) produced by T cells seem to be important for the maintenance of pregnancy. Hormones present in the microenvironment of the decidual T cells could be responsible, at least in part, for the cytokine profile of the T cells. Indeed, progesterone is a potent inducer of Th2-type cytokines (e.g. IL-4 and IL-5), LIF and M-CSF production by T cells, whereas relaxin induces T cells to produce IFN-γ. Of course, the success of pregnancy depends on many mechanisms induced by different type of cells. Th2 cells could be one of these.

Keywords: abortion, cytokines, pregnancy, T lymphocytes, Th1, Th2

Introduction

The mechanisms by which the fetus is protected from the maternal immune system during pregnancy are not fully understood. The presence of a specialized immune system within the maternal part of the placenta (decidua) could have a role in this process. During pregnancy, uterine mucosa is characterized by a large number of maternal immune cells found in close contact with trophoblast. Natural killer (NK) cells CD56+, CD38+, CD2−−, CD3−−, and CD16− macrophages and CD3+ T cells are the most abundant immunocytes present in the decidua, whereas B cells are virtually absent. It is apparent that immunological mechanisms may play a role in pregnancy. This review focuses on the possible roles of T cells in successful pregnancy and in unexplained recurrent pregnancy loss.

T cells in normal pregnancy and unexplained recurrent abortion

Repeated pregnancy loss, which affects approximately 1% of the population, is defined as the loss of three or more consecutive pregnancies in the first trimester of pregnancy. The cause of spontaneous abortion is multifactorial, and can be divided into embryologically driven causes due to abnormal karyotype, and maternally driven causes that affect the endometrium and/or placental development. The known causes of maternal defects are coagulation disorders, autoimmune defects, endocrine disorders and endometrial defects. The aetiology in approximately 50% of cases is unknown. A proportion of repeated pregnancy losses without known cause may be due to immune factors. This has led researchers to study the role of immune cells, and in particular T cells, in unexplained recurrent abortion (URA).
In mice, the role of T cells in pregnancy has been demonstrated by transfer and depletion experiments. T cell depletion prevents placenta growth, transfer of T cells prevents abortion in a conventional murine abortion model (Wegmann, 1984), and CD4+ cell depletion in normal mice results in hypotrophic fetoplacental units (Chaouat et al., 1988).

T cells can be classified according to protein components of the T cell receptor (TCR), which consists of two polypeptide chains, α and β, found in the majority of the T cells, and also γ and δ chains. In mice, decidual αβ T cells seem to be important immediately after implantation, whereas decidual γδ T cells seem to be important in preventing recurrent abortions. The production of IL-10 and TGFβ2 by decidual γδ T cells is lower than 5–10% of γδ T cells in decidua (Vassiladou and Bulmer, 1996), which has less evidence of the important of this mechanism.

T cells are 32% around the expected time of implantation (LH + 7 days) and fewer (around 20%) in early pregnancy (Vassiladou et al., 1996). Apparently there is no change prior to pregnancy and during pregnancy in the number of CD3+ T cells present in the endometrium of women suffering from URA, whereas during pregnancy, the number of peripheral blood T cells in these women, compared with fertile women, seems to decrease (Kwak et al., 1995). In the endometrium, a higher CD4+CD8+ ratio in women with URA compared with fertile women has been found, due to the decreased number of CD8+ cells or to the increased number of CD4+ cells (Lachappelle et al., 1996; Quenby et al., 1999). The expression of CD25 (IL-2 receptor alpha chain) by T cells indicates that these cells are activated. The increased number of CD25+CD4+ T cells in the first-trimester decidua of women undergoing spontaneous abortion, and in first-trimester decidua of women with URA (with chromosomally normal fetuses), compared with decidua from elective terminations and miscarriage with chromosomally abnormal fetuses (Quack et al., 2001) suggests that the decidual T cells are activated during spontaneous abortion and in particular in URA with chromosomally normal fetuses. The activated CD4+ cells, which exhibit low levels of CD25 expression, are different from the CD4+CD25+ regulatory T cells (T reg). The CD4+CD25+ cells inhibit proliferation of convidial T cells through cell–cell contact or immunosuppressive cytokines IL-10 and TGFβ. CD4+CD25+ regulatory T cells up-regulate IDO (indoleamine 2,3 dioxygenase) in APC and extravillous trophoblast, which has a role in tolerance and maintenance of allogeneic pregnancy (Munn et al., 1998). Recently, it has been reported that the absence of CD25- cells led to a failure of gestation, suggesting that CD25+ and perhaps CD4+CD25+ T cells mediate maternal tolerance to the fetus (Aluvihare et al., 2004). The role of T reg in the maintenance of pregnancy (Zenclussen et al., 2006) remains the subject of controversy. T reg constitute 20% of the decidual CD4+ cells in early pregnancy and seem to decrease to 6% in decidua of spontaneous abortions (Saito et al., 2004).

Role of cytokines produced by T cells in normal pregnancy and recurrent pregnancy loss - M-P Piccinni

Inasmuch as many T-cell effects are mediated via the production of cytokines, the type of cytokines produced could influence the maintenance of the fetoplacental unit. Human CD4+ T cells can be classified into Th1 and Th2 cells on the basis of their pattern of cytokine production (Mossman et al., 1989; Romagnani et al., 1991). Human type 1 CD4+ T cells (Th1) produce interleukin (IL)-2, tumour necrosis factor (TNF)β and interferon (IFN)γ, and are the main effectors of phagocyte-mediated host defence, which is highly protective against infections sustained by intracellular parasites. On the other hand, human type 2 CD4+ T cells (Th2) produce IL-4, IL-5, IL-13 and IL-10, which together with IL-4 inhibit several macrophage functions. The Th2 cell is mainly responsible for phagocyte-independent host defence, e.g. against certain pathogens. There also exists a Th3 subpopulation that produce TGFβ, which seems to be reduced in decidua near the placenta attachment site in 59% of patients with URA (Lea et al., 1995).

In 1993, it was suggested that successful pregnancy in mice was associated with a predominant Th2 cytokine profile and that Th1 cytokines were detrimental to pregnancy (Wegmann et al., 1993). A deleterious role was attributed to Th1 cells in pregnancy because some Th1-dependent effector mechanisms play a central role in acute allograft rejection (Erdmann et al., 2004; Burns et al., 2005). In fact, proteins and/or transcripts for intra graft IL-2, IFNγ and the CTL-specific marker, granzyme B, have consistently been detected in rejecting allografts. However, the production of Th2-type cytokines seems to be central for the induction and the maintenance of allograft tolerance (Nickerson et al., 1994; Strom et al., 1996; Li et al., 1998). Changes in the pattern of cytokines produced by T cells may play an important role in the immunological tolerance or rejection of the conceptus, which could be considered, because of the presence of paternal MHC antigens, as an allograft. Therefore, it has been suggested, perhaps in a simplistic way, that Th1-type cytokines, which promote allograft rejection, may compromise pregnancy, whereas the Th2-type cytokines, through inhibiting the Th1 responses, promote allograft tolerance and therefore may improve fetal survival. Accordingly, more TNF and IFNγ and less IL-4 and IL-10 have been found in the uterus of aborting matings of CBA/J female mice with DBA/2 than in CBA/J × BALB/c resulting in normal pregnancy outcome (Chaouat et al., 1995). Injection of TNFα, IFNγ or IL-2 in CBA/J × BALB/c resulted in increased abortion rates and injection of IL-10 in CBA/J × BalbC decreased abortion rates. Some mice strains (C57Bl/6) infected with Leishmania major show a Th1 anti-infectious response spontaneously, which is associated with increased fetal resorption and implantation failure rates, whereas BALB/c mice characterized by a Th2 response to Leishmania infection were unaffected (Krishnan et al., 1996).

Against the Th1/Th2 paradigm in pregnancy, there are some knock-out (KO) mouse models. IL-10 KO mice did not show alteration of pregnancy, and IL-4–, IL-5–, IL-9 and IL-13 KO did not affect pregnancy rates and litter size (Fallon et al., 2002). There is a tendency to extrapolate directly from animal models (particularly rodents) to humans, and this has led to assumptions of mechanisms for which the evidence is incomplete. The redundancy of different cytokines having the same function can sometimes lead to rapid conclusions.

In humans, various approaches have been adopted to underline the role of Th1 in the aetiology of URA and the role of Th2 cells in successful pregnancy using as study material peripheral blood before and at time of miscarriage in women suffering from URA (Hill et al., 1995; Raghupathy et al., 2000).
However, cytokines act locally and the measurements of T cell cytokine amounts at the fetomaternal interface are of greater significance than measurements in the peripheral blood or in the endometrium prior to implantation. Thus, the study of T cells in the decidual tissue appears to provide the best approach. A defect in IL-4 production by both decidual CD4+ and CD8+ T cells has been found, and there is also a defect in IL-10 and M-CSF by decidual CD4+ T cells of women suffering from URA undergoing spontaneous abortion (with normal chromosomal content), in comparison with the decidual T cells of women with a normal pregnancy undergoing a voluntary abortion (Piccinni et al., 1998). Therefore, in humans at the fetomaternal interface, the success of pregnancy seems to be associated with the production of IL-4, IL-10 and M-CSF by T cells. Accordingly, in women suffering from URA, decreased expression of CRTH2 (marker of IL-4-producing cells) by CD4+ and CD8+ T cells at the site of implantation has been reported (Michimata et al., 2003). Interestingly, the concentrations of IFNγ produced by decidual T cells of women with URA and normal pregnancy did not differ. No increased production of IFNγ by decidual T cells was found in URA, as could be expected because of the potential role of Th1-type cytokines on allograft rejection. In 1996, Vince and Johnson posed the provocative question: ‘Is there a Th2 bias in human pregnancy?’ Guilbert (1996) answered ‘There is a bias against type 1 cytokine expression and function in pregnancy’. Type 2 cytokines may not be essential to pregnancy per se, but they may provide a bias away from type 1 cytokines. Recently it has been shown that IFNγ can be beneficial for pregnancy; indeed, IFNγ is essential for the remodelling of spiral arteries (Ashkar et al., 2000). Surely Th1 cytokines, depending on their time of expression, the stage of gestation and their relative concentrations, could have a positive role in successful pregnancy.

Leukaemia inhibitory factor (LIF) is an endometrial requirement for implantation and embryo development (Stewart et al., 1992). LIF, known to be produced by endometrial epithelial cells and NK cells, is also produced by Th2-like cells (Piccinni et al., 1998). Defective production of LIF by decidual T cells in women suffering from URA was found (Piccinni et al., 1998). The relative contribution of LIF produced by T cells, compared with the contribution of LIF produced by endometrium epithelial cells or NK cells, is not clear. LIF mRNA expression by glandular epithelium is dramatically down-regulated after implantation, whereas expression by leukocytes is up-regulated in the decidua (Sharkey et al., 1999). As NK cells represent 70% of leukocytes present in decidua, Sharkey et al. (1999) suggested that LIF mRNA in decidua was expressed by NK cells. However, when Sharkey et al. (1999) purified decidual NK cells and cultured them alone or with exogenous IL-2, IL-1β or IFNγ, they did not produce LIF. Therefore, it seems that the production of the LIF protein in the decidua could be predominantly assigned to the T cells.

Factors responsible for Th1 and Th2 cell development in the decidua

Contrary to reports in other papers (Hill et al., 1995; Raghupathy et al., 2000), no change was found in the production of IFNγ, Th2-type cytokines, LIF and M-CSF in the peripheral blood of women suffering from URA compared with normal pregnancy (Piccinni et al., 1998), suggesting that this is not an inherent feature of T cells, but rather a microenvironmentally oriented alteration. One can speculate on which factors are present in the microenvironment of the T cells that could be responsible for the cytokine profile of the T cells in URA and in successful pregnancy. Some candidates could be hormones. Progesterone, which, at concentrations comparable with those present at the maternofetal interface during pregnancy, is a potent inducer of IL-4, IL-5, LIF and M-CSF production by T cells (Piccinni et al., 1995, 1998, 2001). Progesterone, present at high concentrations at the fetomaternal interface, may be at least in part responsible for a Th2 switch at the fetomaternal interface. IL-4 produced by the Th2 cells can in turn promote the development of T cells producing LIF and M-CSF, which seems to be important for embryo implantation and development. Both IL-4 and IL-10 can inhibit the development and function of Th1 cells and macrophages, thus preventing the allograft rejection. Moreover, relaxin, a polypeptide hormone predominantly produced by the corpus luteum and decidua during pregnancy, favours IFNγ production by T cells (Piccinni et al., 1999), which could in part increase the remodelling of spiral arteries (Monk et al., 2005). 17β-oestradiol and human chorionic gonadotrophin (HCG) have no effect on T cell differentiation into Th1 or Th2 cells (Piccinni et al., 1995). Thus, hormonal influences seem to play a critical role in determining the T cell cytokine pattern at the fetomaternal interface.

Possible mechanisms of pregnancy failure induced by T cells

There are different possible mechanisms of pregnancy failure induced by T cells. Cytokines produced by the T cells may affect trophoblast cell growth and function directly, or they may cause activation of macrophages which could attack the trophoblast. IFNγ activates decidual macrophages, that produce NO and TNFα. This process leads to abortion. IL-4 and IL-10 can inhibit Th1 cell and macrophage functions, preventing feto-allograft rejection. The direct effect of T cell cytokines on trophoblast cell growth and function could be mediated by receptors for IFNγ, IL-4, M-CSF, LIF, TNFα, TGFβ and IL-6, which are present on trophoblast. IFNγ and TNFα can inhibit human placental trophoblast cell growth and metabolic activity and stimulate apoptosis in vitro (Yui et al., 1994; Haddad et al., 1997; Knoftler et al., 2000). IL-4, IL-6 and LIF stimulate HCG secretion by the trophoblast (Saito, 1997). M-CSF stimulates trophoblast cell proliferation (Jokhi et al., 1995) and differentiation of cytrophoblast cells into syncytiotrophoblast cells (Hamilton et al., 1998). Thus, the abnormal production of any of these cytokines in women with URA may lead to abnormal placental growth and function and subsequent miscarriage. T cells could have a role in pregnancy failure through activation of thrombotic events. Th1 cytokines could bring about pregnancy loss via the up-regulation of a newly described pro-coagulant fg12. In fact, in mice anti-fg12 prevents spontaneous abortion. In humans, increased expression of fg12 in trophoblast cells from failing pregnancy with chromosomally normal embryos has been reported (Knackstedt, 2001). Fg12 could convert prothrombin into thrombin, which in turn leads to deposition of fibrin and activation of PMN that can destroy the vascular supply of the placenta. The Th2-type cytokines could antagonize this process, suppressing Th1 response.
Conclusion

Evidence obtained in humans and in rodent models shows that T cells producing Th2-type cytokines can have a role in the success of pregnancy (Chaoat et al., 1995; Piccinni et al., 1998; Raghupathy et al., 2000). Th1-type cytokines, in particular IFNγ, can be deleterious for pregnancy, at high concentrations (Leonard et al., 2006), but may not always be detrimental, since IFNγ has been shown to be responsible for the remodelling of spiral arteries (Monk et al., 2005). This illustrates the fact that immune cells and their products can have different effects according to the window in which they act.

T cells are not alone in the decidua; they are surrounded by other immune cells and non-immune cells able to produce different types of cytokines and/or regulators. T cells could work in parallel with these cells, restraining and/or increasing their effects, and these cells could restrain or increase the functional activities of the decidual T cells. For this reason, it is unlikely that a single mechanism covers all cases of early pregnancy loss. In fact, it is likely that there exist multiple regulators that could be linked to implantation failure. For example, IDO, whose role has been suggested in mice (Munn et al., 1998), though it is discussed by Clark et al. (2005); defects in complement regulation, seen in mice (Xu et al. 2000), are possibly involved in humans (Lokki and Laitinen, 2001); WNT partly regulates LIF production, and WNT defects lead to implantation (Mohamed et al., 2005). Other examples are possible, and the considerable degree of ‘molecular complexity’ in implantation was discussed by Trangush et al. (2005) and by Laird et al. (2003).

Although some attempts have been made to cure recurrent pregnancy loss (Adachi et al., 2003; Perricone et al., 2006) and repeated IVF–embryo transfer failure (Simon et al., 2005; Urman et al., 2005) by lymphocyte alloimmunization, or by intravenous immunoglobulin therapy (IVIG), which in some cases can be successful, the use of these therapies remains controversial (Chaoat, 2003, 2004; Chaoat et al., 2004; Christiansen et al., 2004; Toshiyuki, 2004). More importantly, recently the role of a lack of endometrial cytokines (IL-12, IL-15, IL-18) in repeated IVF–embryo transfer failure was revealed (Lédée-Bataille et al., 2004, 2005).

Thus, it must first be kept in mind that cytokines could be essential for the success of pregnancy at some stages and not so important, or even deleterious, at other stages, and that improper ‘activation’ of lymphocytes by high doses of IL-4 can lead to fetal demise (Hayakawa et al., 2000), suggesting that cytokine concentration is also important for a beneficial or detrimental effect on pregnancy.

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