Outlook

T cells in normal pregnancy and recurrent pregnancy loss

Dr Marie-Pierre Piccinni received a PhD in 1988 from the University Joseph Fourier of Grenoble, France and a second PhD from the University of Bari, Italy in 1999. She is Assistant Professor in the Faculty of Medicine and Surgery, University of Florence. She has published 55 papers in international per-review journals; her global impact factor is over 320 and her mean impact factor per paper is 5.8. Since joining the University of Florence in 1990 she has led research in the field of feto-maternal immune interactions, focusing the immunological mechanisms induced by maternal T cells. Dr Piccinni is a member of the European Network of Excellence Embryo Implantation Control group (EMBIC).

Dr Marie-Pierre Piccinni

Marie-Pierre Piccinni
Department of Internal Medicine, Immunoallergology Unit, Centre of Excellence of the University of Florence, Viale Morgagni 85, 50134 Florence, Italy
Correspondence: Dipartimento di Medicina Interna, Sezione di Immunoallergologia, Viale Morgagni 85, Firenze 50134, Italy. Tel: +39 55 4271499; Fax: +39 55 4271500; e-mail: mppiccinni@hotmail.com

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 IFNγ. 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 Vγ 1.1δ 6.3 T cells prevents miscarriage (Arck *et al.*, 1999). In humans, where less than 5–10% of γδ T cells are present in the decidua (Vassiladou and Bulmer, 1996), there is less evidence of the importance of this mechanism.

T cells are 32% around the expected time of implantation (LH + 7 days) and fewer (around 20%) in early pregnancy (Vassiladiou *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 (Lachapelle *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 $CD4^+CD25^{+high}$ regulatory T cells (T reg). The $CD4^+CD25^{+high}$ inhibit proliferation of conventional T cells through cell–cell contact or immunosuppressive cytokines IL-10 and TGFβ. $CD4^+CD25^{+high}$ 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 URA

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 nematodes. 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 intragraft 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 uteri 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 × Balb/C decreased abortion rates. Some mice strains (C57BI/6) infected by *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, IFN γ, is essential for 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 deciduas (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 deciduas 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; Knofler *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 cytotrophoblast cells into syncytium (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 (Chaouat *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 (Chaouat, 2003, 2004; Chaouat *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.

References

Adachi H, Takakuwa K, Mitsui T *et al*. 2003 Results of immunotherapy for patients with unexplained secondary recurrent abortions. *Clinical Immunology* **106**, 175–180.

Aluvihare VR, Kallikourdis M, Betz AG 2004 Regulatory T cells mediate maternal tolerance to the fetus. *Nature Immunology* **5**, 266–271.

Arck PC, Ferrick DA, Steele-Norwood D *et al*.1999 Murine T cell determination of pregnancy outcome. 1999 *Cellular Immunology* **196**, 129–140.

Ashkar AA, Di Santo JP, Croy BA 1998 Interferon gamma contributes to initiation of uterine vascular modification, decidual integrity, and uterine natural killer cell maturation during normal murine pregnancy. *Journal of Experimental Medicine* **187**, 217–223.

Burns WR, Wang Y, Tang PC *et al*. 2005 Recruitment of $CXCR3^+$ and $CCR5^+$ T cells and production of interferon-gamma-inducible chemokines in rejecting human arteries. *American Journal of Transplantation* **5**, 1226–1236.

Chaouat G 2004 A response to 'Shall we properly re-examine the status of allogeneic lymphocyte therapy for recurrent early pregnancy failure?', Clark D A. *American Journal of Reproductive Immunology* 2004, **51**, 7–15. *American Journal of Reproductive Immunology*. **52**, 337–339.

Chaouat G 2003 Should we re-examine the status of lymphocyte alloimmunization therapy for recurrent spontaneous abortion? *American Journal of Reproductive Immunology* **50**, 433–438.

Chaouat G, Ledee-Bataille N, Dubanchet S 2004 Is there a place for immunomodulation in assisted reproduction techniques? *Journal of Reproductive Immunology* **62**, 29–39.

Chaouat G, Assal Meliani A, Martal J *et al*. 1995 IL-10 prevents naturally occurring fetal loss in the CBA × DBA/2 mating combination, and local defect in IL-10 production in this abortion-prone combination is corrected by in vivo injection of IFN-tau. *Journal of Immunology* **154**, 4261–4268.

Chaouat G, Menu E, Athanassakis I *et al*. 1988 Maternal T cells regulate placental size and fetal survival. *Regulatory Immunology* **1**, 143–148.

Christiansen OB, Nielsen HS, Pedersen B 2004 Active or passive immunization in an explained recurrent miscarriage. *Journal of Reproductive Immunology* **62**, 41–52.

Clark DA, Blois S, Kandil J *et al*. 2005 Reduced uterine indoleamine **2**,3-dioxygenase versus increased TH1/TH2 cytokine ratios as a basis for occult and clinical pregnancy failure in mice and humans. *American Journal of Reproductive Immunology* **54**, 203–216.

Erdmann AA, Jung U, Foley JE *et al*. 2004 Co-stimulated/Tc2 cells abrogate murine marrow graft rejection. *Biology of Blood Marrow Transplantation* **10**, 604–613.

Fallon PG, Jolin HE, Smith P *et al*. 2002 IL-4 induces characteristic Th2 responses even in the combined absence of IL-5, IL-9, and IL-13. *Immunity* **17**, 7–17.

Guilbert LG 1996 There is a bias against type 1 (inflammatory) cytokine expression and function in pregnancy. *Journal of Reproductive Immunology* **32**, 105–110.

Haddad EK, Duclos AJ, Antecka E *et al*. 1997 Role of interferon-gamma in the priming of decidual macrophages for nitric oxide production and early pregnancy loss. *Cellular Immunology* **181**, 68–75.

Hamilton GS, Lysiak JJ, Watson AJ *et al*. 1998 Effects of colony stimulating factor-1 on human extravillous trophoblast growth and invasion. *Journal of Endocrinology* **159**, 69–77.

Hayakawa S, Fujikawa T, Fukuoka H *et al*. 2000 Murine fetal resorption and experimental pre-eclampsia are induced by both excessive Th1 and Th2 activation. *Journal of Reproductive Immunology* **47**, 121–138.

Hill JA, Polgar K, Anderson DJ 1995 T-helper 1-type immunity to trophoblast in women with recurrent spontaneous abortion. *JAMA* **273**, 1933–1936.

Jokhi PP, King A, Boocock C *et al*. 1995 Secretion of colony stimulating factor-1 by human first trimester placental and decidual cell populations and the effect of this cytokine on trophoblast thymidine uptake in vitro. *Human Reproduction* **10**, 2800–2807.

Knackstedt M, Ding JW, Arck PC *et al*. 2001 Activation of the novel prothrombinase, fg12, as a basis for the pregnancy complications spontaneous abortion and pre-eclampsia. *American Journal of Reproductive Immunology* **46**, 196–210.

Knofler M, Mosl B, Bauer S *et al*. 2000 TNF-alpha/TNFRI in primary and immortalized first trimester cytotrophoblasts. *Placenta* **21**, 525–535.

Krishnan L, Guilbert LJ, Russell AS *et al*. 1996 Pregnancy impairs resistance of C57BL/6 mice to *Leishmania major* infection and causes decreased antigen-specific IFN-gamma response and increased production of T helper 2 cytokines. *Journal of*

Immunology **156**, 644–652.
Kwak JY, Beaman KD, Gilman-Sachs A *et al*. 1995 Up-regulated expression of CD56$^+$, CD56$^+$/CD16$^+$, and CD19$^+$ cells in peripheral blood lymphocytes in pregnant women with recurrent pregnancy losses. *American Journal of Reproductive Immunology* **34**, 93–99.
Lachapelle MH, Miron P, Hemmings R *et al*. 1996 Endometrial T, B, and NK cells in patients with recurrent spontaneous abortion. Altered profile and pregnancy outcome. *Journal of Immunology* **156**, 4027–4034.
Laird SM, Tuckerman EM, Cork BA *et al*. 2003 A review of immune cells and molecules in women with recurrent miscarriage *Human Reproduction Update* **9**, 163–174.
Lea RG, Underwood J, Flanders KC *et al*. 1995 A subset of patients with recurrent spontaneous abortion is deficient in transforming growth factor beta-2-producing 'suppressor cells' in uterine tissue near the placental attachment site. *American Journal of Immunology* **34**, 52–64.
Ledee-Bataille N, Bonnet-Chea K, Hosny G *et al*. 2005 Role of the endometrial tripod interleukin-18, -15, and -12 in inadequate uterine receptivity in patients with a history of repeated in vitro fertilization–embryo transfer failure. *Fertility and Sterility* **83**, 598–605.
Ledee-Bataille N, Dubanchet S, Coulomb-L'hermine A *et al*. 2004. New role for natural killer cells, interleukin (IL)-12, and IL-18 in repeated implantation failure after in vitro fertilization. *Fertility and Sterility* **81**, 59–65.
Leonard S, Murrant C, Tayade C *et al*. 2006.Mechanisms regulating immune cell contributions to spiral artery modification – facts and hypotheses – a review. *Placenta*. **27** (Suppl. A) S40–46.
Li XC, Zand MS, Li Y *et al*. 1998 On histocompatibility barriers, Th1 to Th2 immune deviation, and the nature of the allograft responses. *Journal of Immunology* **161**, 2241–2247.
Lokki ML, Laitinen T 2001 Role of major histocompatibility complex class III genes in recurrent spontaneous abortions. *Frontiers in Bioscience* **6**, 23–29.
Michimata T, Sakai M, Miyazaki S *et al*. 2003 Decrease of T-helper 2 and T-cytotoxic 2 cells at implantation sites occurs in unexplained recurrent spontaneous abortion with normal chromosomal content. *Human Reproduction* **18**,1523–1528.
Mohamed OA, Jonnaert M, Labelle-Dumais C *et al*. 2005 Uterine Wnt/beta-catenin signaling is required for implantation. *Proceedings of the National Academy of Science of the USA* **102**, 8579–8584.
Monk JM, Leonard S, McBey BA *et al*. 2005 Induction of murine spiral artery modification by recombinant human interferon-gamma. *Placenta* **26**, 835–838.
Mosmann TR, Coffman RL 1989 Th1 and Th2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annual Review of Immunology* **7**, 145–173.
Munn DH, Zhou M, Attwood JT *et al*.1998 Prevention of allogeneic fetal rejection by tryptophan catabolism. *Science* **28**, 1191–1193.
Nickerson P, Steurer W, Steiger J *et al*. 1994 Cytokines and the Th1/Th2 paradigm in transplantation. *Current Opinion in Immunology* **6**, 757–764.
Perricone R, Di Muzio G, Perricone C *et al*. 2006 High levels of peripheral blood NK cells in women suffering from recurrent spontaneous abortion are reverted from high-dose intravenous immunoglobulins. *American Journal of Reproductive Immunology* **55**, 232–239.
Piccinni M-P, Scaletti C, Vultaggio A *et al*. 2001 Defective production of LIF, M-CSF and Th2-type cytokines by T cells at fetomaternal interface is associated with pregnancy loss. *Journal of Reproductive Immunology* **52**, 35–43.
Piccinni M-P, Bani D, Beloni L *et al*. 1999 Relaxin favors the development of activated human T cells into Th1-like effectors. *European Journal of Immunology* **29**, 2241–2247.
Piccinni M-P, Beloni L, Livi C *et al*. 1998 Defective production of both leukemia inhibitory factor and type 2 T-helper cytokines by decidual T cells in unexplained recurrent abortions. *Nature Medicine* **4**, 1020–1024.
Piccinni M-P, Giudizi MG, Biagiotti R *et al*. 1995 Progesterone favors the development of human T helper cells producing Th2-type cytokines and promotes both IL-4 production and membrane CD30 expression in established Th1 cells clones. *Journal of Immunology* **155**, 128–133.
Quack KC, Vassiliadou N, *Pudney J et al*. 2001 Leukocyte activation in the decidua of chromosomally normal and abnormal fetuses from women with recurrent abortion. *Human Reproduction* **16**, 949–955.
Quenby S, Bates M, Doig T *et al*. 1999 Pre-implantation endometrial leukocytes in women with recurrent miscarriage. *Human Reproduction* **14**, 2386–2391.
Raghupathy R, Makhseed M, Azizieh F *et al*. 2000 Cytokine production by maternal lymphocytes during normal human pregnancy and in unexplained recurrent spontaneous abortion. *Human Reproduction* **15**, 713–718.
Romagnani S 1991 Human Th1 and Th2: doubt no more. *Immunology Today* **12**, 256–257.
Saito S, Sasaki Y, Sakai M *et al*. 2005 CD4$^+$CD25^{+high} regulatory T cells in human pregnancy. *Journal of Reproductive Immunology* **65**, 111–120.
Saito S, Harada N, Ishii N *et al*. 1997 Functional expression on human trophoblasts of interleukin 4 and interleukin 7 receptor complexes with a common gamma chain. *Biochemical Biophysical Research Community* **231**, 429–434.
Sharkey AM, King A, Clark DE *et al*. 1999 Localization of leukemia inhibitory factor and its receptor in human placenta throughout pregnancy. *Biology of Reproduction* **60**, 335–364.
Stewart CL, Kaspar P, Brunet LJ *et al*. 1992 Blastocyst implantation depends on maternal expression of leukemia inhibitory factor. *Nature* **359**, 76–79.
Strom TB, Roy-Chaudury R, Manfro R *et al*. 1996 The Th1/Th2 paradigm and allograft response. *Current Opinion in Immunology* **8**, 688–693.
Takeshita T 2004 Diagnosis and treatment of recurrent miscarriage associated with immunologic disorders: is paternal lymphocyte immunization a relic of the past? *Journal of Nippon Medical School* **71**, 308–313.
Tranguch S, Daikoku T, Guo Y *et al*. 2005 Molecular complexity in establishing uterine receptivity and implantation. *Cellular and Molecular Life Sciences* **62**,1964–1973.
Urman B, Yakin K, Balaban B 2005 Recurrent implantation failure in assisted reproduction: how to counsel and manage. Treatment options that have not been proven to benefit the couple. *Reproductive BioMedicine Online* **11**, 371–381.
Vassiliadou N, Bulmer JN 1996 Quantitative analysis of T lymphocyte subsets in pregnant and nonpregnant human endometrium. *Biology of Reproduction* **55**, 1017–1022.
Vince GS, Johnson PM 1996 Is there a Th2 bias in human pregnancy? *Journal of Reproductive Immunology* **32**, 101-104.
Wegmann TG 1984 Foetal protection against abortion: is it immunosuppression or immunostimulation? *Annales d' Immunologie (Paris)* 135D, 309–312.
Wegmann TG, Lin H, Guilbert L *et al*. 1993 Bidirectional cytokine interactions in the maternal–fetal relationship: is successful pregnancy a Th2 phenomenon? *Immunology Today* **14**, 353–356.
Xu C, Mao D, Holmers M *et al*. 2000 A critical role for complement regulatory Crry in feto maternal tolerance. *Science* **287**, 498–499.
Yui J, Garcia-Lloret M, Wegmann TG *et al*. 1994 Cytotoxicity of tumour necrosis factor-alpha and gamma-interferon against primary human placental trophoblasts. *Placenta* **15**, 819–835.
Zenclussen AC, Gerlof K, Zenclussen ML *et al*. 2006 Regulatory T cells induce a privileged tolerant microenvironment at the fetal–maternal interface. *European Journal of Immunology* **36**, 82–94.

Paper based on contribution presented at the International Serono Symposium 'Human implantation: the new frontiers of human assisted reproductive technologies' in Erice, Sicily, Italy, May 5–6, 2006.

Received 2 June 2006; refereed 19 June 2006; accepted 22 August 2006.