

The immunology of successful pregnancy

A.L.Veenstra van Nieuwenhoven¹, M.J.Heineman¹ and M.M.Faas^{2,3}

¹Department of Obstetrics and Gynaecology, University Hospital, Groningen and ²Reproductive Immunology, Division of Medical Biology, Department of Pathology and Laboratory Medicine, University of Groningen, The Netherlands

³To whom correspondence should be addressed at: Reproductive Immunology, Division of Medical Biology, Dept of Pathology and Laboratory Medicine, University of Groningen, P.O. Box 30.001, 9700 RB Groningen, The Netherlands.
E-mail: m.m.faas@med.rug.nl

Immune responses play an important role in various reproductive processes, including ovulation, menstruation and parturition. Clearly, during pregnancy, when the mother must accept a semi-allogeneic fetus, immune responses also play a very important role. This was first recognized by Medawar in 1953, when the concept of the fetal allograft was presented in order to explain the immunological relationship between mother and fetus. Since then, the immunology of pregnancy has been the leading subject within reproductive immunology research. Yet, the question of why the semi-allogeneic fetus is not rejected by the mother remains unresolved. The present review provides an update of current knowledge on the subject of the so-called ‘immunological paradox of pregnancy’.

Key words: cytokines/decidua/leukocytes/pregnancy/trophoblast

Introduction

In 1953, Medawar was the first to propose the concept of the fetal allograft (Medawar, 1953). In his paper, he suggested that the semi-allogeneic fetus is able to survive because the immunological interaction between the mother and fetus is suppressed. Medawar suggested that this was due to a lack of fetal antigen expression, resulting from an anatomical separation between the mother and the fetus, or from a functional suppression of maternal lymphocytes. Despite the fact that the mechanisms that induce immunological tolerance of the fetus are not completely understood, several features of the immunological state of pregnancy are clear. For example, it is now known that there is no anatomical separation between the mother and the fetus, as various fetal cells (e.g. trophoblasts) are in close contact with maternal (immune) cells. There is, however, a lack of antigen stimulation of maternal lymphocytes, since the fetal trophoblast cells do not express major histocompatibility complex (MHC) Ia antigens, which are responsible for the rapid rejection of allografts in humans. Moreover, Medawar's other suggestions cannot be completely discarded, because lymphocyte function indeed changes during pregnancy; this, however, is not a general suppression. In the present review, attention is first focused on current knowledge of the effects of pregnancy on the immune response, both peripherally and in the decidua, and this is followed by a discussion on fetal mechanisms to escape maternal immune attack.

Fetal–maternal contact

The present review deals with the placenta as an immunological barrier. As there is no vascular continuity between the mother and fetus, the placenta must play an important role in acceptance of the fetus. Trophoblast cells are the most important fetal cells coming in contact with maternal cells, and three different trophoblast populations that are exposed to different maternal elements can be distinguished. The first population is the villous cytotrophoblast; these form a pool of actively dividing trophoblast cells that remain in the villi. The second trophoblast population covers the first population and is called the syncytiotrophoblast; these float in the maternal blood. The third trophoblast population is the non-villous cytotrophoblast; these are proliferating precursor trophoblast cells that migrate into the decidua and myometrium. During the 20th week of human pregnancy, the surface area of the total trophoblast is about 15 m².

The effect of pregnancy on peripheral immune responses

Villous syncytiotrophoblast is floating in maternal blood, and is therefore in close contact with maternal peripheral leukocytes. Hence, it can be expected that the peripheral immune response is adapted to the presence of the semi-allogeneic syncytiotrophoblast cells. One of the first recognized changes in the peripheral maternal immune system during pregnancy was an increase in peripheral white blood

cell count (Siegel and Gleicher 1981; Kuhnert *et al.*, 1998; Minagawa *et al.*, 1999; Veenstra van Nieuwenhoven *et al.*, 2002). Clinical evidence for changes in the immune response during pregnancy is seen in rheumatoid arthritis and lupus erythematosus patients that respectively remit or flare-up during pregnancy (Ostensen *et al.*, 1983; Varner, 1991). In the following sections, the present point of view on the adaptation of peripheral immune responses during pregnancy will be highlighted.

T lymphocytes

The best-studied peripheral immune cells in human pregnancy are T lymphocytes. Within the T-lymphocyte population, helper T lymphocytes (Th) and cytotoxic T lymphocytes (Tc) can be distinguished. Th lymphocytes provide help to other immune cells by producing cytokines, whereas Tc lymphocytes can directly kill foreign or infected cells. The numbers of Tc lymphocytes and Th lymphocytes may (Matthiesen *et al.*, 1996; Luppi *et al.*, 2002b) or may not (Coulam *et al.*, 1983; Veenstra van Nieuwenhoven *et al.*, 2002) differ in pregnant women versus non-pregnant women. T lymphocytes can also be classified into different functional subsets based on their profile of cytokine production. Type 1 T cells produce, for example, interferon- γ (IFN- γ), interleukin-2 (IL-2) and tumour necrosis factor- α (TNF- α), which promote cellular immune responses, whereas type 2 T cells produce IL-4, IL-5, IL-9, IL-10 and IL-13 that provide optimal help for humoral immune responses (Mosmann *et al.*, 1986).

Based on various experimental findings (e.g. Athanassakis *et al.*, 1987; Armstrong and Chaouat, 1989), Wegmann was the first to propose the concept that pregnancy is a Th2 phenomenon (Wegmann *et al.*, 1993). The shift away from type 1 cytokine production during pregnancy is beneficial for pregnancy, since type 1 cytokines (e.g. IFN- γ and TNF- α) are harmful for pregnancy because they inhibit embryonic and fetal development (Chaouat *et al.*, 1990; Haimovici *et al.*, 1991) and terminate pregnancy when injected into pregnant mice (Chaouat *et al.*, 1990). Now, various groups have shown that—especially in the third trimester of human pregnancy—the ratio of type 1/type 2 cytokine production of peripheral T lymphocytes is decreased as compared with non-pregnant women (Sabahi *et al.*, 1995; Ekerfelt *et al.*, 1997; Reinhard *et al.*, 1998; Saito *et al.*, 1999; Veenstra van Nieuwenhoven *et al.*, 2002). However, there is no consensus as to whether this decreased type 1/type 2 ratio of cytokine production is due to a decreased production of type 1 cytokines (Saito *et al.*, 1999; Veenstra van Nieuwenhoven *et al.*, 2002) or to an increased production of type 2 cytokines (Ekerfelt *et al.*, 1997). In line with the view that a shift towards a type 2 immune response is important for the continuation of pregnancy is the fact that, in women with unexplained recurrent spontaneous miscarriage, a dominance of type 1 cytokine production by lymphocytes is seen as compared with uncomplicated term human pregnancies (Makhseed *et al.*, 1999; Raghupathy *et al.*, 2000). However, this is disputed by others (Bates *et al.*, 2002), who found a shift towards type 2 cytokine production in recurrent pregnancy loss.

The decreased ratio of type 1/type 2 cytokine production during pregnancy may be explained in different ways. First, some authors claim that the dramatic increase in pregnancy hormones (e.g. progesterone and estrogen) may directly affect lymphocytes by shifting their cytokine production towards type 2 (Piccinni *et al.*, 1995), while others dispute this suggestion (Faas *et al.*, 2000; A.Bouman *et al.*, unpublished data). An indirect role of progesterone has also been suggested as being due to the induction of a progesterone-induced blocking factor in lymphocytes (Szekeres-Bartho and Wegmann, 1996; Check *et al.*, 1997; Szereday *et al.*, 1997). Second, the placenta may interfere with lymphocyte cytokine production. *In vitro*, it has been shown that placental and trophoblast cells produce factors which inhibit cytotoxic T-lymphocyte activity (Djian *et al.*, 1996; Aarli *et al.*, 1997). Moreover, trophoblast cells also produce cytokines (mainly type 2) which may direct the maternal immune response towards a type 2 immune response (Roth *et al.*, 1996; Chaouat *et al.*, 1999; Agarwal *et al.*, 2000; Griesinger *et al.*, 2001; Sacks *et al.*, 2001). Another placental mechanism to inhibit T-cell responses has also been described. Villous syncytiotrophoblast express indoleamine diogenase (IDO), an enzyme which functions in the catabolism of tryptophan and indirectly suppresses maternal T-cell activity by tryptophan deprivation (Schrocksadel *et al.*, 1996; Munn *et al.*, 1998; Kudo *et al.*, 2001).

Natural killer (NK) cells

Surprisingly little is known about peripheral NK cells in pregnancy. The number of peripheral NK cells is decreased in pregnant women as compared with non-pregnant women (Watanabe *et al.*, 1997; Kuhnert *et al.*, 1998; Veenstra van Nieuwenhoven *et al.*, 2002). In pregnant women it is also known that, besides a decrease in the number of NK cells, their production of IFN- γ is also decreased (Veenstra van Nieuwenhoven *et al.*, 2002). These changes in NK cell number and activity during pregnancy are also consistent with a shift from a cellular to a humoral immune response during pregnancy. In pregnant women, NK cells appear to be embryotoxic, and one group (Beer *et al.*, 1996) showed that, in an IVF population, no live infants were born when the proportion of maternal peripheral NK cells was >18%. Moreover, in women with a history of spontaneous miscarriage, T lymphocytes and NK cells were embryotoxic *in vitro* (Polgar and Hill, 2002).

Monocytes and granulocytes

Although many investigations have been carried out on lymphocytes during pregnancy, little attention was paid to granulocytes and monocytes, the innate immune cells. Despite the fact that some years ago a variety of studies were performed to evaluate the function of the innate immune cells during pregnancy, the results were not consistent and the investigations were mostly performed with isolated monocytes or granulocytes (e.g. Persellin and Thoi, 1979; Siegel and Gleicher, 1981; Krause *et al.*, 1987). Moreover, results from isolated cells are difficult to extrapolate to the in-vivo situation.

At present, with the introduction of new techniques (most importantly, flow cytometry), monocyte and granulocyte function can be examined in whole blood.

In applying these new techniques, one group (Sacks *et al.*, 1998) first suggested a modified monocyte and granulocyte function during pregnancy. These authors observed that granulocytes and monocytes from pregnant women showed a significant increased expression of various activation-associated adhesion molecules. These results have been confirmed by others (Davis *et al.*, 1998; Naccasha *et al.*, 2001; Luppi *et al.*, 2002b). The innate immune cells are also functionally activated in pregnant women, and this has been demonstrated by measuring the production of oxygen free radicals (Sacks *et al.*, 1998) or of cytokines (Naccasha *et al.*, 2001; Luppi *et al.*, 2002a; Sakai *et al.*, 2002; Veenstra van Nieuwenhoven *et al.*, 2003). All the above-mentioned changes in peripheral monocytes and granulocytes are comparable with changes seen in patients with sepsis, which is a typical situation of activation of the innate immune response (Sacks *et al.*, 1998; Veenstra van Nieuwenhoven *et al.*, 2003). Therefore, it is now accepted that the innate immune system is activated during pregnancy.

Various mechanisms may account for activation of the innate immune cells during pregnancy. Obviously, as for lymphocytes, the pregnancy hormones have been suggested to be one of the factors responsible for activation of the innate immune cells. No studies have been performed to investigate the influence of pregnancy hormones on adhesion molecule expression or oxygen free radical production in innate immune cells, while estrogen and progesterone may increase cytokine production by monocytes (Polan *et al.*, 1989; Loy *et al.*, 1992; Bouman *et al.*, 2001). Another suggestion is that the placenta activates innate immune cells during pregnancy. This notion is supported by the fact that granulocytes become activated during their passage through the placenta (Mellembakken *et al.*, 2002). Moreover, several soluble placental products, which are released directly into the maternal circulation, can activate monocytes (Sacks *et al.*, 1999). In addition, whole cells of fetal or trophoblastic origin as well as syncytiotrophoblast microfragments can be detected in the maternal blood (Sargent, 1993; Bianchi *et al.*, 1996; Knight *et al.*, 1998; Sacks *et al.*, 2000). Such cells or particles would be eliminated by phagocytes (i.e. monocytes and granulocytes), resulting in their activation.

Dendritic cells

Dendritic cells (DCs) are known to be the most potent antigen-presenting cells (Steinman, 1991). Additionally, there is emerging evidence that DCs may be involved in the regulation of type 1/type 2 cytokine balance (Nishioka *et al.*, 2001; Osada *et al.*, 2001; Bradley *et al.*, 2002). It can, therefore, be expected that DCs play an important role in the immunological paradox of pregnancy, though little research has focused on DC activity in pregnancy. Two studies have evaluated the number of DCs in peripheral blood in pregnant women, but unfortunately these produced opposing results (Germain *et al.*, 2002; Williams *et al.*, 2002).

From the above information it may be concluded that, during pregnancy, the cell-mediated immune response of the maternal specific immune system is relatively suppressed, and that this suppression seems to be compensated for by an activation of the innate immune response. Although the innate immune response is essential in the response to extracellular bacterial infection, it is less efficient in clearing viruses and other intracellular pathogens than the specific immune response; indeed, pregnant women are more sensitive to such infections (Brabin, 1985).

Immune cells in the decidua

The decidua is the maternal part of the placenta in which there is a close contact between maternal and fetal cells. Consequently, the decidual cells may play an important role in acceptance of the fetus and the control of trophoblast invasion. Hence, the decidua contains a diverse population of cells, including decidualized stroma cells, lymphocytes, uterine NK cells (uNK cells), monocytes and epithelial cells. There are significant variations in the number of leukocytes in endometrial tissue. Typically, less than 10% of the decidual cells are leukocytes in the proliferative phase, but this increases to ~20% in the late secretory phase and to >40% in early pregnancy (Bulmer *et al.*, 1991; Ozenci *et al.*, 2001). These increases are mainly due to a rise in the numbers of uNK cells, which comprise over 60% of the leukocytes (Bulmer *et al.*, 1991; Ozenci *et al.*, 2001). Granulocytes and B-cells are uncommon in endometrium and decidua

uNK cells

The uNK cells have a NK cell-like function, but they are specific for the uterus as they show a different phenotype compared with peripheral NK cells (Ritson and Bulmer, 1987; Starkey *et al.*, 1988; King *et al.*, 1989; Nagler *et al.*, 1989; Pace *et al.*, 1989; Bulmer *et al.*, 1991). Remarkably, the number of uNK cells which are normally present in the endometrium increases markedly during early pregnancy (Pace *et al.*, 1989; Ho *et al.*, 1996; Ozenci *et al.*, 2001). The presence of uNK cells in the decidua may be explained by two mechanisms. The first mechanism is that peripheral blood uNK cells are selectively homing to the uterine mucosa, because they can interact with adhesion molecules on the decidual blood vessels (Marzusch *et al.*, 1993; Ruck *et al.*, 1994). The second mechanism is in-situ proliferation, as uNK are actively dividing (Pace *et al.*, 1989; King *et al.*, 1991; Kammerer *et al.*, 1999). This uNK cell proliferation can be stimulated by either cytokines produced by other decidual cells, or by (steroid) hormones (Ferry *et al.*, 1990; Stewart *et al.*, 1998; King *et al.*, 1999; Muller *et al.*, 1999; Verma *et al.*, 2000; Jones *et al.*, 2001; Henderson *et al.*, 2003).

Although the uNK cells are present in the decidua in large amounts, they do not attack the semi-allogeneic non-villous cytotrophoblast. This is due to the fact that uNK cells express inhibitory receptors. These receptors bind to the MHC Ia and b (HLA-C, HLA-E and HLA-G) on trophoblast (see also section 'MHC Ib expression by trophoblast'); by binding to these MHC I antigens, the inhibitory receptors inhibit the lytic activity of

the uNK cells (Hiby *et al.*, 1997; Verma *et al.*, 1997; King *et al.*, 2000a). There are several types of inhibitory receptors on uNK cells, namely Ig-like killer cell inhibitory receptor (KIR) (e.g. KIR2D, KIR2DL4) and lectin-like KIRs (CD94/NKG2A) (Hiby *et al.*, 1997; Soderstrom *et al.*, 1997; Verma *et al.*, 1997; Davis *et al.*, 1999; Rajagopalan and Long, 1999; King *et al.*, 2000a).

Knowledge of the function of uNK cells is still limited, but because they do not share all membrane expression markers with peripheral NK cells it is not clear whether these cell types have the same function. However, because of the large number of uNK cells in the uterus during early pregnancy, it is suggested that they play an important role in protection against infections or in the regulation of immunity, whilst at the same time perhaps affecting implantation and placentation (Guimond *et al.*, 1999; King, 2000). The effect of uNK cells on implantation and placentation is shown by various studies comparing their number and activity in normal and complicated pregnancies. The number of uNK cells in pre-implantation endometrium was increased in women with a history of recurrent pregnancy loss compared with women without such a history (Lachapelle *et al.*, 1996b; Clifford *et al.*, 1999; Quenby *et al.*, 1999). On the other hand, decreased numbers of uNK cells were found in the decidua of women who were pregnant with a genetically abnormal fetus as compared with women pregnant with a normal fetus (Yamamoto *et al.*, 1999; Quack *et al.*, 2001), but uNK cells were not found in tubal pregnancies (Vassiliadou and Bulmer, 1998; Proll *et al.*, 2000; von Rango *et al.*, 2001). Also consistent with a role for uNK cells in implantation and placentation were the findings that high pre-conceptual NK activity was associated with significantly higher rates of miscarriage (Aoki *et al.*, 1995) and infertility (Matsubayashi *et al.*, 2001).

It is well recognized that one function of the uNK cells is the production of cytokines (Saito *et al.*, 1993; Jokhi *et al.*, 1994; 1995; Vince and Johnson, 2000). These uNK cell-derived cytokines influence placentation. Granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor (M-CSF) and leukaemia inhibitory factor (LIF) stimulate growth of the trophoblast; colony-stimulating factors also promote trophoblast cell proliferation and differentiation (Loke *et al.*, 1992; Garcia-Lloret *et al.*, 1994), while LIF stimulates implantation (Stewart *et al.*, 1992; Kojima *et al.*, 1995; Sawai *et al.*, 1995; Nachtigall *et al.*, 1996). Transforming growth factor β (TGF β), on the other hand, inhibits trophoblast proliferation and differentiation (Morrish *et al.*, 1991; Graham *et al.*, 1992; Karmakar and Das, 2002). uNK cells also produce type 1 cytokines, such as TNF- α and IFN- γ , which may have negative effects on implantation and trophoblast invasion (see next section on T lymphocytes)

T lymphocytes

Other cells which are present in the decidua and may play a role in the immune response are the maternal T lymphocytes. Although these T cells in the decidua are in close contact with

trophoblast, they do not attack the non-villous cytotrophoblast, because they do not recognize the MHC Ia-negative trophoblast as being foreign (see section 'MHC Ia expression by trophoblast'). The number of T cells present in the decidua and the endometrium surrounding the placenta decreases during pregnancy as compared with the non-pregnant situation (Maruyama *et al.*, 1992; Haller *et al.*, 1993; Ho *et al.*, 1996; Lachapelle *et al.*, 1996a). However, by producing cytokines, these T lymphocytes may affect acceptance of the fetus.

T lymphocytes in the decidua can produce a variety of type 1 and type 2 cytokines (Chaouat *et al.*, 1990; Lin *et al.*, 1993; Wegmann *et al.*, 1993). As suggested previously, type 1 cytokines are harmful for pregnancy. In the decidua, they promote miscarriage by inhibiting trophoblast invasion; TNF- α stimulates apoptosis of human trophoblast cells and IFN- γ increases this TNF- α -mediated killing of trophoblast (Yui *et al.*, 1994; Hill *et al.*, 1995). These cytokines also inhibit the out-growth of human trophoblast cells *in vitro* (Berkowitz *et al.*, 1988; Haimovici *et al.*, 1991) and stimulate macrophage activity in the decidua, resulting in the production of factors by these macrophages that may be harmful to the embryo (Baines *et al.*, 1997; Haddad *et al.*, 1997a; b). Moreover, TNF- α and IFN- γ can also influence fetal growth in other ways, as they can activate a prothrombinase which generates thrombin. Thrombin activation leads to clotting and the production of IL-8, which stimulates granulocytes and endothelial cells to terminate blood supply to the developing placenta (Clark *et al.*, 1998; 2001).

Type 2 cytokines in general stimulate trophoblast outgrowth and invasion (Chaouat *et al.*, 1995a; Saito *et al.*, 1996; Goodwin *et al.*, 1998; Das *et al.*, 2002). The most accepted view at this time is that in the decidua, as in the peripheral blood during pregnancy, Th2 cells predominate (Piccinni and Romagnani, 1996; Saito *et al.*, 1999; Saito, 2000; Ho *et al.*, 2001). The importance of this relative dominance of type 2 cytokines over type 1 cytokines may be stressed by the fact that pregnancy loss is associated with less type 2 cytokine production as compared with normal pregnancies (Piccinni *et al.*, 1998; 2001). More recent investigations have shown that this view may be an oversimplification, however (Chaouat *et al.*, 2002). These authors evaluated the expression of several cytokines in the uterus, peri-implantation embryo, decidua and placental tissue. Part of the cytokine expression pattern fitted nicely into the type 1/type 2 dichotomy, while other cytokines (e.g. IL-11, -12, -13, -15, -16, -17 and -18) did not.

Monocytes and macrophages

Innate immune cells, especially macrophages, are also found in the decidua. In humans, macrophages are spread through the pregnant uterus, including the decidua (Khong, 1987; Bulmer *et al.*, 1988a) and are also associated with trophoblast cells of the placenta and extraplacental membranes (Bulmer and Johnson, 1984). The number of macrophages in the endometrium increases premenstrually, whilst in the decidua the macrophages constitute about 20–30% of all leukocytes (Bulmer *et al.*, 1988b; Vince *et al.*, 1990; Ozenci *et al.*,

2001). The number of macrophages in the uterus may be affected by estrogens, as these hormones stimulate the influx of leukocytes (including macrophages) into the uterus (Zheng *et al.*, 1988; Kachkache *et al.*, 1991). Moreover, cytotrophoblast can attract macrophages into the decidua by producing monocytes inflammatory protein-1 alpha (Drake *et al.*, 2001). It seems clear that the number of uterine macrophages must be tightly controlled, since in pre-implantation endometrium of patients with recurrent miscarriage significantly more macrophages were seen than in patients without these miscarriages (Quenby *et al.*, 1999; Reister *et al.*, 2001).

Because of the relative absence of other immune defence systems within the placental tissue, decidual macrophages are thought to have an important role in non-specific host defence in the placenta (Klein and Remington, 1990). They also have a phagocytic role, as they may be involved in the removal of cell and tissue debris associated with trophoblast invasion (Bulmer and Johnson, 1984). Macrophages are also suggested to have a role in placentation (Hunt and Robertson, 1996) and to produce cytokines (TNF- α , TGF- β and colony stimulating factors) (Redline *et al.*, 1990). As indicated in the previous section, these cytokines are of major importance in successful pregnancy. Beside cytokines, macrophages can also produce immunosuppressive prostaglandins, which may block the function of Tc lymphocytes and uNK cells (Parhar *et al.*, 1989).

It is clear from the information relating to immune cells in the decidua that an abundance of knowledge is available on this subject. However, despite the many reports concerning lymphocytes and uNK cells, at present the only facts relating to these cells is their presence in the decidua and their production of all types of cytokines, whilst their exact function and inter-relationship remains largely unknown. The present observations suggest that at the maternal–fetal interface, a balanced production of cytokines by various immune cells (T lymphocytes, uNK cells, macrophages) is needed for a successful pregnancy, which in turn suggests the existence of a complex fine-tuning system for cytokine production (Chaouat *et al.*, 2002). Interestingly, the non-villous cytotrophoblast cells also produce various cytokines (Guilbert *et al.*, 1993; Chaouat *et al.*, 1995b; Sacks *et al.*, 2001), by which means they may influence the cytokine balance in the decidua. Pregnancy-hormones have also been suggested to affect cytokine balance at the maternal–fetal interface (Piccinni and Romagnani, 1996; Piccinni *et al.*, 2000; Saito, 2000).

Mechanisms of the trophoblast to escape a maternal immune attack

It is clear from the previous sections that immune cells are in close contact with the trophoblast cells but do not attack it, despite being activated. The mechanisms of the trophoblast to escape maternal immune attack will be discussed in the next sections.

The highly polymorphic MHC is responsible for the distinction of the immune system between self and non-self. The MHC can be divided into three classes: class Ia (HLA-A, HLA-B and HLA-C); class II (HLA-DP, HLA-DQ and HLA-DR); and class Ib (HLA-E, HLA-F and HLA-G). The MHC class Ia antigens are normally

expressed by all nucleated cells and are involved in presenting antigens to Tc lymphocytes and in the inhibition and activation of NK cells via inhibitory NK-cell receptors (KIR) and activating NK-cell receptors (KAR) (Colonna *et al.*, 1996). Foreign cells, expressing non-self MHC Ia, can be directly recognized by Tc lymphocytes and killed. The MHC class II molecules are also involved in immunorecognition, but are only expressed by B lymphocytes, antigen-presenting cells and some epithelial cells. They present foreign antigens to Th lymphocytes. Although the exact function of the MHC class Ib proteins is not exactly known, they seem to play a role in the immunological acceptance of the fetus, since HLA-G and HLA-E are expressed by some trophoblast populations (see ‘MHC Ib expression by trophoblast’).

MHC Ia expression by trophoblast

Clearly, the expression of MHC Ia molecules by trophoblast cells may put these cells at risk of an immunological attack by the maternal immune system. The expression of MHC Ia molecules by the trophoblast has been the focus of research for many years. An early finding of investigations into this subject was that mammalian blastocysts can survive in ectopic sites and in the presence of blastocyst antisera as long as its zona pellucida remains intact (Heyner *et al.*, 1969; Moskalewski and Koprowski, 1972; Ewoldsen *et al.*, 1987). This suggested that the zona pellucida is not recognized as foreign, and it was therefore concluded that the MHC I antigens are not expressed on the zona pellucida. When in humans the zona pellucida was shed from the blastocyst, the full complement of MHC I antigens was expressed on the blastocyst before implantation (Seigler and Metzgar, 1970; Mori *et al.*, 2000). One group (Simons and Russell, 1962) studied trophoblast and/or embryos of mice that had been transplanted into allogeneic hosts, and found that the trophoblast survived but embryos did not. These authors concluded that trophoblast cells did not express MHC Ia antigens. Indeed, it has now been shown that MHC Ia is not expressed on either trophoblast population (Sutton *et al.*, 1983; Coady *et al.*, 1999; van der Elsen *et al.*, 2001). However, some authors have demonstrated the expression of HLA-C, which belongs to the MHC Ia molecules, by non-villous cytotrophoblast (Loke *et al.*, 1995; King *et al.*, 1996; 2000b; Proll *et al.*, 1999).

MHC Ib expression by trophoblast: HLA-G

Although MHC Ia molecules are hardly expressed by trophoblast cells, one group (Ellis *et al.*, 1986) identified a MHC molecule belonging to the class MHC Ib, expressed on human non-villous cytotrophoblast, and referred to this as HLA-G. In recent years, investigations have focused on the distribution and the function of HLA-G in the placenta. The placental extra-villous cytotrophoblast strongly expressed HLA-G (Yelavarthi *et al.*, 1991; Chumbley *et al.*, 1993; McMaster *et al.*, 1995; Hutter *et al.*, 1996; Proll *et al.*, 1999; Carosella *et al.*, 2000), but no expression was found on villous cyto- or syncytiotrophoblast (Yelavarthi *et al.*, 1991; Chumbley *et al.*, 1993; Hunt *et al.*, 2000).

The exact function of HLA-G remains unknown, although many suggestions have been proposed. One suggestion is that

HLA-G is simply a left-over of evolution (Parham, 1995), but the fact that HLA-G is only expressed in some subsets of trophoblast (i.e. the non-villous cytotrophoblast) may imply that HLA-G is functional. Indeed, a more accepted view is that HLA-G plays a role in the resistance of non-villous trophoblast cells to lysis by uNK cells (Pazmany *et al.*, 1996; Munz *et al.*, 1997; Rouas-Freiss *et al.*, 1997; Soderstrom *et al.*, 1997; Moreau *et al.*, 1998; Rieger *et al.*, 2002), and that HLA-G inhibits the migration of uNK cells through the placenta (Dorling *et al.*, 2000). In the decidua, large amounts uNK cells are present and, by binding to inhibitory receptors on uNK cells, HLA-G is able to inhibit NK cell activity (see 'Immune cells in the decidua').

Other possible functions of HLA-G have also been suggested. For example, HLA-G can suppress proliferation of T lymphocytes (Riteau *et al.*, 1999; Bainbridge *et al.*, 2000), and also influence Tc lymphocytes and uNK cells by altering their secretion of cytokines, shifting the immune response from type 1 to type 2 (Clark, 1997; Kapasi *et al.*, 2000; Kanai *et al.*, 2001). Besides membrane-bound HLA-G, a soluble counterpart (sHLA-G) may play an important role in the immunological establishment of pregnancy by affecting peripheral immune cells and modulating their function for the benefit of pregnancy (Le Bouteiller *et al.*, 1999). For example, Tc lymphocytes can be suppressed by the soluble form of HLA-G (Fournel *et al.*, 2000; Solier *et al.*, 2003). Recently, it has been shown that sHLA-G may play a key role in implantation of the embryo, as plasma sHLA-G levels were reduced in early miscarriage as compared with normal pregnancy (Pfeiffer *et al.*, 2000). In addition, following an IVF procedure only those embryos which secreted sHLA-G gave rise to a successful pregnancy (Fuzzi *et al.*, 2002).

MHC Ib expression by trophoblast: HLA-E

Next to HLA-G, another MHC class Ib molecule, HLA-E, is also expressed by non-villous trophoblast (King *et al.*, 2000a; Blaschitz *et al.*, 2001). As with HLA-G, the exact function of HLA-E is unknown, but it has been suggested that HLA-E, rather than HLA-G, is the important uNK cell inhibitor at the maternal–fetal interface (Braud *et al.*, 1998; King *et al.*, 2000a). It has also been suggested that co-expression of HLA-G and HLA-E is needed for the inhibition of uNK cells (Mandelboim *et al.*, 1997).

Apoptosis-inducing mechanism of the trophoblast

Another mechanism by which the trophoblast may escape attack by maternal immune cells is via the expression of apoptosis-inducing ligands. Induction of apoptosis by Fas Ligand (FasL) in invading lymphocytes acts as a mechanism of immune privilege and is important in graft rejection (Griffith *et al.*, 1995). FasL expression has been observed in human placentas (Runic *et al.*, 1996; Bamberger *et al.*, 1997; Uckan *et al.*, 1997; Hammer *et al.*, 1999; Kauma *et al.*, 1999). It was observed in syncytiotrophoblast and in villous and non-villous cytotrophoblast (Runic *et al.*, 1996; Uckan *et al.*, 1997; Hammer *et al.*, 1999; Kauma *et al.*, 1999). Moreover,

expression of Fas was found on decidual leukocytes (Hammer *et al.*, 1999; Kauma *et al.*, 1999), suggesting that FasL expression on trophoblast may be a mechanism protecting the trophoblast against activated leukocytes (Hammer and Dohr, 1999; Kauma *et al.*, 1999). The expression of FasL does not appear to be mandatory for pregnancy success, however (Chaouat and Clark, 2001).

Other apoptosis-inducing pathways, such as binding of the TNF-related apoptosis-inducing ligand (TRAIL) to its receptor (TRAIL-R) may also play an immunoprotective role in the placenta, as TRAIL is expressed on trophoblast (especially syncytiotrophoblast) (Phillips *et al.*, 1999). More recently, other members of the death-inducing TNF superfamily ligands and their receptors have been shown to be expressed in the placenta (Phillips *et al.*, 2001). Thus, Fas-FasL and TRAIL-TRAIL-R apoptosis induction in maternal immune cells in the decidua might be important in maternal immunotolerance of the fetal allograft during pregnancy.

The trophoblast cells thus have various mechanisms to escape a maternal immune attack. First, they lack expression of most MHC Ia molecules; they cannot be recognized as foreign by maternal T cells, though this lack of MHC Ia may place the non-villous trophoblast at risk of lysis by uNK cells which dominate in the decidua. Therefore, non-villous cytotrophoblast cells express MHC Ib molecules such as HLA-G and HLA-E, both of which may be important at the maternal–fetal interface by inhibiting lysis of non-villous cytotrophoblast by uNK-cells, via direct binding with inhibitory receptors on the uNK cells. Moreover, HLA-G—and its counterpart sHLA-G—may also suppress the activity of other immune cells either in the decidua or in the peripheral circulation. Another way in which the trophoblast, both villous and non-villous, is able to escape immune rejection is by the expression of apoptosis-inducing ligands. With these ligands, trophoblast cells can induce apoptosis of activated immune cells.

Concluding remarks

Since the first report of Medawar in the 1950s (Medawar, 1953), numerous possibilities have been suggested as to why the semi-allogeneic fetus is not rejected by the mother. The suggestion of Medawar—that there is a lack of fetal antigen expression to activate maternal cells—appears to be true. However, this lack of antigen stimulation of maternal cells is not due to an anatomical separation of fetal and maternal cells, because the maternal and fetal trophoblast cells are in close contact in both the decidua and the peripheral circulation. Rather, the trophoblast cells in contact with maternal (immune) cells do not express MHC Ia antigens and are therefore not recognized as 'non-self' by maternal T lymphocytes. To escape lysis by uNK cells, the trophoblast cells express the MHC Ib antigens, HLA-E and HLA-G. Moreover, if immune cells in the presence of the trophoblast cells still become activated, the trophoblast cells are able to induce apoptosis in these activated immune cells, since they express apoptosis-inducing ligands, such as FasL and TRAIL.

The suggestion of Medawar that the function of the maternal lymphocytes is also suppressed bears an element of truth. There is,

however, not a general suppression of the maternal lymphocytes, but just a suppression of cell-mediated immunity during pregnancy—that is, a suppression of type 1 cytokine production. In the decidua, this suppression appears to be necessary for implantation and invasion of trophoblasts, as in general type 2 cytokines promote implantation and trophoblast invasion and are thus protective for pregnancy, while type 1 cytokines have the opposite effect. A balanced production of cytokines in the decidua is therefore very important for successful pregnancy.

Interestingly, in the peripheral circulation there is no general suppression of maternal immune responses. On the contrary, the relative suppression of lymphocyte function—namely a suppression of cell-mediated immunology—is accompanied by activation of the innate immune system. This is most likely necessary to ensure the mother's immune integrity.

The immunological paradox of pregnancy appears therefore to be extremely complex, and despite the vast body of knowledge about the subject there remain very many questions. In particular, the role of the innate immune system—both in the periphery and in the decidua—in successful pregnancy has until now been underexposed. Future research should therefore focus on this innate immune system in pregnancy, especially as the activated innate immune system may also cause complications of pregnancy, as has been shown in pre-eclampsia which may be the result of an excessively activated innate immune response (Sacks *et al.*, 1998; Faas and Schuiling, 2001).

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References

- Aarli, A., Kristoffersen, E.K., Jensen, T.S., Ulvestad, E. and Matre, R. (1997) Suppressive effect on lymphoproliferation *in vitro* by soluble annexin II released from isolated placental membranes. *Am. J. Reprod. Immunol.*, **38**, 313–319.
- Agarwal, R., Loganath, A., Roy, A.C., Wong, Y.C. and Ng, S.C. (2000) Effect of T-helper 1 cytokines on secretion of T-helper 2 cytokines by term trophoblast cells in culture. *Gynecol. Endocrinol.*, **14**, 305–310.
- Aoki, K., Kajiyama, S., Matsumoto, Y., Ogasawara, M., Okada, S., Yagami, Y. and Gleicher, N. (1995) Preconceptional natural-killer-cell activity as a predictor of miscarriage. *Lancet*, **345**, 1340–1342.
- Armstrong, D.T. and Chaouat, G. (1989) Effects of lymphokines and immune complexes on murine placental cell growth *in vitro*. *Biol. Reprod.*, **40**, 466–474.
- Athanassakis, I., Bleackley, R.C., Paetkau, V., Guilbert, L., Barr, P.J. and Wegmann, T.G. (1987) The immunostimulatory effect of T cells and T cell lymphokines on murine fetally derived placental cells. *J. Immunol.*, **138**, 37–44.
- Bainbridge, D.R., Ellis, S.A. and Sargent, I.L. (2000) HLA-G suppresses proliferation of CD4(+) T-lymphocytes. *J. Reprod. Immunol.*, **48**, 17–26.
- Baines, M.G., Duclos, A.J., Anteckka, E. and Haddad, E.K. (1997) Decidual infiltration and activation of macrophages leads to early embryo loss. *Am. J. Reprod. Immunol.*, **37**, 471–477.
- Bamberger, A.M., Schulte, H.M., Thuncke, I., Erdmann, I., Mamberger, C.M. and Asa, S.L. (1997) Expression of the apoptosis-inducing Fas ligand (FasL) in human first trimester and third trimester placenta and choriocarcinoma cells. *J. Clin. Endocrinol. Metab.*, **82**, 3173–3175.
- Bates, M.D., Quenby, S., Takakuwa, K., Johnson, P.M. and Vince, G.S. (2002) Aberrant cytokine production by peripheral blood mononuclear cells in recurrent pregnancy loss? *Hum. Reprod.*, **17**, 2439–2444.
- Beer, A.E., Kwak, J.Y. and Ruiz, J.E. (1996) Immunophenotypic profiles of peripheral blood lymphocytes in women with recurrent pregnancy losses and in infertile women with multiple failed *in vitro* fertilization cycles. *Am. J. Reprod. Immunol.*, **35**, 376–382.
- Berkowitz, R.S., Hill, J.A., Kurtz, C.B. and Anderson, D.J. (1988) Effects of products of activated leukocytes (lymphokines and monokines) on the growth of malignant trophoblast cells *in vitro*. *Am. J. Obstet. Gynecol.*, **158**, 199–203.
- Bianchi, D.W., Zickwolf, G.K., Weil, G.J., Sylvester, S. and DeMaria, M.A. (1996) Male fetal progenitor cells persist in maternal blood for as long as 27 years postpartum. *Proc. Natl Acad. Sci. USA*, **93**, 705–708.
- Blaschitz, A., Hutter, H. and Dohr, G. (2001) HLA Class I protein expression in the human placenta. *Early Pregnancy*, **5**, 67–69.
- Bouman, A., Moes, H., Heineman, M.J., de Leij, L.F. and Faas, M.M. (2001) The immune response during the luteal phase of the ovarian cycle: increasing sensitivity of human monocytes to endotoxin. *Fertil. Steril.*, **76**, 555–559.
- Brabin, B.J. (1985) Epidemiology of infection in pregnancy. *Rev. Infect. Dis.*, **7**, 579–603.
- Bradley, L.M., Harbertson, J., Biederman, E., Zhang, Y., Bradley, S.M. and Linton, P.J. (2002) Availability of antigen-presenting cells can determine the extent of CD4 effector expansion and priming for secretion of Th2 cytokines *in vivo*. *Eur. J. Immunol.*, **32**, 2338–2346.
- Braud, V.M., Allan, D.S., O'Callaghan, C.A., Soderstrom, K., D'Andrea, A., Ogg, G.S., Lazetic, S., Young, N.T., Bell, J.I., Phillips, T.A. *et al.* (1998) HLA-E binds to natural killer cell receptors CD94/NKG2A, B and C. *Nature*, **391**, 795–799.
- Bulmer, J.N. and Johnson, P.M. (1984) Macrophage populations in the human placenta and amniochorion. *Clin. Exp. Immunol.*, **57**, 393–403.
- Bulmer, J.N., Smith, J., Morrison, L. and Wells, M. (1988a) Maternal and fetal cellular relationships in the human placental basal plate. *Placenta*, **9**, 237–246.
- Bulmer, J.N., Morrison, L. and Smith, J.C. (1988b) Expression of class II MHC gene products by macrophages in human uteroplacental tissue. *Immunology*, **63**, 707–714.
- Bulmer, J.N., Morrison, L., Longfellow, M., Ritson, A. and Pace, D. (1991) Granulated lymphocytes in human endometrium: histochemical and immunohistochemical studies. *Hum. Reprod.*, **6**, 791–798.
- Carosella, E.D., Paul, P., Moreau, P. and Rouas-Freiss, N. (2000) HLA-G and HLA-E: fundamental and pathophysiological aspects. *Immunol. Today*, **21**, 532–534.
- Chaouat, G. and Clark, D.A. (2001) FAS/FAS ligand interaction at the placental interface is not required for the success of allogeneic pregnancy in anti-paternal MHC preimmunized mice. *Am. J. Reprod. Immunol.*, **45**, 108–115.
- Chaouat, G., Menu, E., Clark, D.A., Dy, M., Minkowski, M. and Wegmann, T.G. (1990) Control of fetal survival in CBA x DBA/2 mice by lymphokine therapy. *J. Reprod. Fertil.*, **89**, 447–458.
- Chaouat, G., Assal Meliani, A., Martal, J., Raghupathy, R., Elliot, J., Mosmann, T. and Wegmann, T.G. (1995a) IL-10 prevents naturally occurring fetal loss in the CBA x DBA/2 mating combination, and local defect in IL-10 production in the abortion-prone combination is corrected by *in vivo* injection of IFN-tau. *J. Immunol.*, **154**, 4261–4268.
- Chaouat, G., Menu, E., Delage, G., Moreau, J.F., Krishnan, L., Hui, L., Meliani, A.A., Martal, J., Raghupathy, R. and Lelaidier, C. (1995b) Immuno-endocrine interactions in early pregnancy. *Hum. Reprod.*, **10** (Suppl. 2), 55–59.
- Chaouat, G., Cayol, V., Mairovitz, V. and Dubanchet, S. (1999) Localization of the Th2 cytokines IL-3, IL-4, IL-10 at the fetomaternal interface during human and murine pregnancy and lack of requirement for Fas/Fas ligand interaction for a successful allogeneic pregnancy. *Am. J. Reprod. Immunol.*, **42**, 1–13.
- Chaouat, G., Zourbas, S., Ostojic, S., Lappree-Delag, G., Dubanchet, S., Ledee, N. and Martal, J. (2002) A brief review of recent data on some cytokine expression at the materno-fetal interface which might challenge the classical Th1/Th2 dichotomy. *J. Reprod. Immunol.*, **53**, 241–256.
- Check, J.H., Arwitz, M., Gross, J., Szekeres-Bartho, J. and Wu, C.H. (1997) Evidence that the expression of progesterone-induced blocking factor by maternal T-lymphocytes is positively correlated with conception. *Am. J. Reprod. Immunol.*, **38**, 6–8.
- Chumbley, G., King, A., Holmes, N., and Loke, Y.W. (1993) *in situ* hybridization and northern blot demonstration of HLA-G mRNA in human trophoblast populations by locus-specific oligonucleotide. *Hum. Immunol.*, **37**, 17–22.
- Clark, D.A. (1997) HLA-G finally does something! *Am. J. Reprod. Immunol.*, **38**, 75–78.
- Clark, D.A., Chaouat, G., Arck, P.C., Mittrucker, H.W. and Levy, G.A.

- (1998) Cytokine-dependent abortion in CBA x DBA/2 mice is mediated by the procoagulant fgl2 prothrombinase. *J. Immunol.*, **160**, 545–549.
- Clark, D.A., Yu, G., Levy, G.A. and Gorczynski, R.M. (2001) Procoagulants in fetus rejection: the role of the OX-2 (CD200) tolerance signal. *Semin. Immunol.*, **13**, 255–263.
- Clifford, K., Flanagan, A.M. and Regan, L. (1999) Endometrial CD56+ natural killer cells in women with recurrent miscarriage: a histomorphometric study. *Hum. Reprod.*, **14**, 2727–2730.
- Coady, M.A., Mandapati, D., Arunachalam, B., Jensen, K., Maher, S.E., Bothwell, A.L. and Hammond, G.L. (1999) Dominant negative suppression of major histocompatibility complex genes occurs in trophoblasts. *Transplantation*, **67**, 1461–1467.
- Colonna, M., Dohring, C., Samaridis, J., Scheidegger, D., Dessing, M., Cella, M. and Campbell, K.S. (1996) Human killer inhibitory receptors: specificity for HLA-class I molecules and mechanisms of signal transduction. *Transplant. Proc.*, **28**, 3035.
- Coulam, C.B., Silverfield, J.C., Kazmar, R.E. and Fathman, C.G. (1983) T-lymphocyte subsets during pregnancy and the menstrual cycle. *Am. J. Reprod. Immunol.*, **4**, 88–90.
- Das, C., Kumar, V.S., Gupta, S. and Kumar, S. (2002) Network of cytokines, integrins and hormones in human trophoblast cells. *J. Reprod. Immunol.*, **53**, 257–268.
- Davis, D., Kaufmann, R. and Moticka, E.J. (1998) Nonspecific immunity in pregnancy: monocyte surface Fcγ receptor expression and function. *J. Reprod. Immunol.*, **40**, 119–128.
- Davis, D.M., Mandelboim, O., Luque, I., Baba, E., Boyson, J. and Strominger, J.L. (1999) The transmembrane sequence of human histocompatibility leukocyte antigen (HLA)-C as a determinant in inhibition of a subset of natural killer cells. *J. Exp. Med.*, **189**, 1265–1274.
- Djian, V., Menu, E., Thibault, G., Ropert, S. and Chaouat, G. (1996) Immunoactive products of placenta. V. Immunoregulatory properties of a low molecular weight compound obtained from human placental cultures. *Am. J. Reprod. Immunol.*, **36**, 11–24.
- Dorling, A., Monk, N. and Lechler, R. (2000) HLA-G inhibits the transendothelial cell migration of human NK cells: a strategy for inhibiting xenograft rejection. *Transplant. Proc.*, **32**, 938.
- Drake, P.M., Gunn, M.D., Charo, I.F., Tsou, C.L., Zhou, Y., Huang, L. and Fisher, S.J. (2001) Human placental cytotrophoblasts attract monocytes and CD56(bright) natural killer cells via the actions of monocyte inflammatory protein 1α. *J. Exp. Med.*, **193**, 1199–1212.
- Ekerfelt, C., Matthesen, L., Berg, G. and Ernerudh, J. (1997) Paternal leukocytes selectively increase secretion of IL-4 in peripheral blood during normal pregnancies: demonstrated by a novel one-way MLC measuring cytokine secretion. *Am. J. Reprod. Immunol.*, **38**, 320–326.
- Ellis, S.A., Sargent, I.L., Redman, C.W. and McMichael, A.J. (1986) Evidence for a novel HLA antigen found on human extravillous trophoblast and a choriocarcinoma cell line. *Immunology*, **59**, 595–601.
- Ewoldsen, M.A., Ostlie, N.S. and Warner, C.M. (1987) Killing of mouse blastocyst stage embryos by cytotoxic T lymphocytes directed to major histocompatibility complex antigens. *J. Immunol.*, **138**, 2764–2770.
- Faas, M.M. and Schuiling, G.A. (2001) Pre-eclampsia and the inflammatory response. *Eur. J. Obstet. Gynecol. Reprod. Biol.*, **95**, 213–217.
- Faas, M.M., Bouman, A., Moes, H., Heineman, M.J., de Leij, L. and Schuiling, G. (2000) The immune response during the luteal phase of the ovarian cycle: a Th2- type response? *Fertil. Steril.*, **74**, 1008–1013.
- Ferry, B.L., Starkey, P.M., Sargent, I.L., Watt, G.M., Jackson, M. and Redman, C.W. (1990) Cell populations in the human early pregnancy decidua: natural killer activity and response to interleukin-2 of CD56-positive large granular lymphocytes. *Immunology*, **70**, 446–452.
- Fournel, S., Aguerre-Girr, M., Huc, X., Lenfant, F., Alam, A., Toubert, A., Bensussan, A. and Le Bouteiller, P. (2000) Cutting edge: soluble HLA-G1 triggers CD95/CD95 ligand-mediated apoptosis in activated CD8+ cells by interacting with CD8. *J. Immunol.*, **164**, 6100–6104.
- Fuzzi, B., Rizzo, R., Criscuolo, L., Noci, I., Melchiorri, L., Scarselli, B., Bencini, E., Menicucci, A. and Baricordi, O.R. (2002) HLA-G expression in early embryos is a fundamental prerequisite for the obtainment of pregnancy. *Eur. J. Immunol.*, **32**, 311–315.
- Garcia-Lloret, M.I., Morrish, D.W., Wegmann, T.G., Honore, L., Turner, A.R. and Guilbert, L.J. (1994) Demonstration of functional cytokine-placental interactions: CSF-1 and GM-CSF stimulate human cytotrophoblast differentiation and peptide hormone secretion. *Exp. Cell Res.*, **214**, 46–54.
- Germain, S., Redman, C. and Sargent, I. (2002) Peripheral blood dendritic cells are decreased in pre-eclampsia. *Hypertension in Pregnancy*, **21**, 96.
- Goodwin, V.J., Sato, T.A., Mitchell, M.D. and Keelan, J.A. (1998) Anti-inflammatory effects of interleukin-4, interleukin-10, and transforming growth factor-beta on human placental cells *in vitro*. *Am. J. Reprod. Immunol.*, **40**, 319–325.
- Graham, C.H., Lysiak, J.J. and McCrae, K.R. (1992) Localization of transforming growth factor-β at the fetal-maternal interface: role in trophoblast growth and differentiation. *Biol. Reprod.*, **46**, 561–572.
- Griesinger, G., Saleh, L., Bauer, S., Husslein, P. and Knofler, M. (2001) Production of pro- and anti-inflammatory cytokines of human placental trophoblasts in response to pathogenic bacteria. *J. Soc. Gynecol. Invest.*, **8**, 334–340.
- Griffith, T.S., Brunner, T., Fletcher, S.M., Green, D.R. and Ferguson, T.A. (1995) Fas ligand-induced apoptosis as a mechanism of immune privilege. *Science*, **270**, 1189–1192.
- Guilbert, L., Robertson, S.A. and Wegmann, T.G. (1993) The trophoblast as an integral component of a macrophage-cytokine network. *Immunol. Cell. Biol.*, **71** (Pt 1), 49–57.
- Guimond, M., Wang, B. and Croy, B.A. (1999) Immune competence involving the natural killer cell lineage promotes placental growth. *Placenta*, **20**, 441–450.
- Haddad, E.K., Duclos, A.J., Anteck, E., Lapp, W.S. and Baines, M.G. (1997a) Role of interferon-gamma in the priming of decidual macrophages for nitric oxide production and early pregnancy loss. *Cell. Immunol.*, **181**, 68–75.
- Haddad, E.K., Duclos, A.J., Lapp, W.S. and Baines, M.G. (1997b) Early embryo loss is associated with the prior expression of macrophage activation markers in the decidua. *J. Immunol.*, **158**, 4886–4892.
- Haimovici, F., Hill, J.A. and Anderson, D.J. (1991) The effects of soluble products of activated lymphocytes and macrophages on blastocyst implantation events *in vitro*. *Biol. Reprod.*, **44**, 69–75.
- Haller, H., Radillo, O., Rukavina, D., Tedesco, F., Candussi, G., Petrovic, O. and Randic, L. (1993) An immunohistochemical study of leukocytes in human endometrium, first and third trimester basal decidua. *J. Reprod. Immunol.*, **23**, 41–49.
- Hammer, A. and Dohr, G. (1999) Apoptotic nuclei within the uterine decidua of first trimester pregnancy arise from CD45 positive leukocytes. *Am. J. Reprod. Immunol.*, **42**, 88–94.
- Hammer, A., Blaschitz, A., Daxböck, C., Walcher, W. and Dohr, G. (1999) Fas and Fas-ligand are expressed in the uteroplacental unit of first-trimester pregnancy. *Am. J. Reprod. Immunol.*, **41**, 41–51.
- Henderson, T.A., Saunders, P.T., Moffet-King, A., Groome, N.P. and Critchley, H.O. (2003) Steroid receptor expression in uterine natural killer cells. *J. Clin. Endocrinol. Metab.*, **88**, 440–449.
- Heyner, S., Brinster, R.L. and Palm, J. (1969) Effect of iso-antibody on pre-implantation mouse embryos. *Nature*, **222**, 783–784.
- Hiby, S.E., King, A., Sharkey, A.M. and Loke, Y.W. (1997) Human uterine NK cells have a similar repertoire of killer inhibitory and activatory receptors to those found in blood, as demonstrated by RT-PCR and sequencing. *Mol. Immunol.*, **34**, 419–430.
- Hill, J.A., Polgar, K. and Anderson, D.J. (1995) T-helper 1-type immunity to trophoblast in women with recurrent spontaneous abortion. *JAMA*, **273**, 1933–1936.
- Ho, H.N., Chao, K.H., Chen, C.K., Yang, Y.S. and Huang, S.C. (1996) Activation status of T and NK cells in the endometrium throughout menstrual cycle and normal and abnormal early pregnancy. *Hum. Immunol.*, **49**, 130–136.
- Ho, H.N., Chao, K.H., Chen, S.U., Wu, M.Y. and Yang, Y.S. (2001) Distribution of Th1 and Th2 cell populations in human peripheral and decidual T cells from normal and anembryonic pregnancies. *Fertil. Steril.*, **76**, 797–803.
- Hunt, J.S. and Robertson, S.A. (1996) Uterine macrophages and environmental programming for pregnancy success. *J. Reprod. Immunol.*, **32**, 1–25.
- Hunt, J.S., Petroff, M.G., Morales, P., Sedlmayr, P., Geraghty, D.E. and Ober, C. (2000) HLA-G in reproduction: studies on the maternal-fetal interface. *Hum. Immunol.*, **61**, 1113–1117.
- Hutter, H., Hammer, A., Blaschitz, A., Hartmann, M., Ebbesen, P., Dohr, G., Ziegler, A. and Uchanska-Ziegler, B. (1996) Expression of HLA class I molecules in human first trimester and term placenta trophoblast. *Cell. Tissue Res.*, **286**, 439–447.
- Jokhi, P.P., King, A. and Loke, Y.W. (1994) Production of granulocyte-macrophage colony-stimulating factor by human trophoblast cells and by decidual large granular lymphocytes. *Hum. Reprod.*, **9**, 1660–1669.
- Jokhi, P.P., King, A., Boocock, C. and Loke, Y.W. (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*. *Hum. Reprod.*, **10**, 2800–2807.

- Jones, C.J., Aplin, J.D. and Fazleabas, A.T. (2001) Decidual stromal cell-lymphocyte interactions in pregnancy. *Placenta*, **22**, 380–382.
- Kachkache, M., Acker, G.M., Chaouat, G., Noun, A. and Garabedian, M. (1991) Hormonal and local factors control the immunohistochemical distribution of immunocytes in the rat uterus before conceptus implantation: effects of ovariectomy, fallopian tube section, and injection. *Biol. Reprod.*, **45**, 860–868.
- Kammerer, U., Marzusch, K., Krober, S., Ruck, P., Handgretinger, R. and Dietl, J. (1999) A subset of CD56+ large granular lymphocytes in first-trimester human decidua are proliferating cells. *Fertil. Steril.*, **71**, 74–79.
- Kanai, T., Fujii, T., Unno, N., Yamashita, T., Hyodo, H., Miki, A., Hamai, Y., Kozuma, S. and Taketani, Y. (2001) Human leukocyte antigen-G-expressing cells differently modulate the release of cytokines from mononuclear cells present in the decidua versus peripheral blood. *Am. J. Reprod. Immunol.*, **45**, 94–99.
- Kapasi, K., Albert, S.E., Yie, S., Zavazava, N. and Librach, C.L. (2000) HLA-G has a concentration-dependent effect on the generation of an allo-CTL response. *Immunology*, **101**, 191–200.
- Karmakar, S. and Das, C. (2002) Regulation of trophoblast invasion by IL-1beta and TGF-beta1. *Am. J. Reprod. Immunol.*, **48**, 210–219.
- Kauma, S.W., Huff, T.F., Hayes, N. and Nilkaeo, A. (1999) Placental Fas ligand expression is a mechanism for maternal immune tolerance to the fetus. *J. Clin. Endocrinol. Metab.*, **84**, 2188–2194.
- Khong, T.Y. (1987) Immunohistologic study of the leukocytic infiltrate in maternal uterine tissues in normal and preeclamptic pregnancies at term. *Am. J. Reprod. Immunol. Microbiol.*, **15**, 1–8.
- King, A. (2000) Uterine leukocytes and decidualization. *Hum. Reprod. Update*, **6**, 28–36.
- King, A., Wellings, V., Gardner, L. and Loke, Y.W. (1989) Immunocytochemical characterization of the unusual large granular lymphocytes in human endometrium throughout the menstrual cycle. *Hum. Immunol.*, **24**, 195–205.
- King, A., Balendran, N., Wooding, P., Carter, N.P. and Loke, Y.W. (1991) CD3-leukocytes present in the human uterus during early placentation: phenotypic and morphologic characterization of the CD56++ population. *Dev. Immunol.*, **1**, 169–190.
- King, A., Boocock, C., Sharkey, A.M., Gardner, L., Beretta, A., Siccardi, A.G. and Loke, Y.W. (1996) Evidence for the expression of HLA-A-C class I mRNA and protein by human first trimester trophoblast. *J. Immunol.*, **156**, 2068–2076.
- King, A., Gardner, L. and Loke, Y.W. (1999) Co-stimulation of human decidual natural killer cells by interleukin-2 and stromal cells. *Hum. Reprod.*, **14**, 656–663.
- King, A., Allan, D.S., Bowen, M., Powis, S.J., Joseph, S., Verma, S., Hiby, S.E., McMichael, A.J., Loke, Y.W. and Braud, V.M. (2000a) HLA-E is expressed on trophoblast and interacts with CD94/NKG2 receptors on decidual NK cells. *Eur. J. Immunol.*, **30**, 1623–1631.
- King, A., Burrows, T.D., Hiby, S.E., Bowen, J.M., Joseph, S., Verma, S., Lim, P.B., Gardner, L., Le Bouteiller, P., Ziegler, A. et al. (2000b) Surface expression of HLA-C antigen by human extravillous trophoblast. *Placenta*, **21**, 376–387.
- Klein, J.O. and Remington, J.S. (1990) Current concepts of infections of the fetus and newborn infant. In: *Infectious Diseases of the Fetus and Newborn Infant*. W.B.Saunders Co., Philadelphia, pp. 3–5.
- Knight, M., Redman, C.W., Linton, E.A. and Sargent, I.L. (1998) Shedding of syncytiotrophoblast microvilli into the maternal circulation in preeclamptic pregnancies. *Br. J. Obstet. Gynecol.*, **105**, 632–640.
- Kojima, K., Kanzaki, H., Iwai, M., Hatayama, H., Fujimot, M., Narukawa, S., Higuchi, T., Kaneke, Y., Mori, T. and Fujita, J. (1995) Expression of leukemia inhibitory factor (LIF) receptor in human placenta: a possible role for LIF in growth and differentiation of trophoblast. *Hum. Reprod.*, **10**, 1907–1911.
- Krause, P.J., Ingardia, C.J., Pontius, L.T., Malech, H.L., LoBello, T.M. and Maderazo, E.G. (1987) Host defence during pregnancy: neutrophil chemotaxis and adherence. *Am. J. Obstet. Gynecol.*, **157**, 274–280.
- Kudo, Y., Boyd, C.A., Sargent, I.L. and Redman, C.W. (2001) Tryptophan degradation by human placental indoleamine 2,3-dioxygenase regulates lymphocyte proliferation. *J. Physiol.*, **535** (Pt 1), 207–215.
- Kuhnert, M., Strohmeier, R., Stegmuller, M. and Halberstadt, E. (1998) Changes in lymphocyte subsets during normal pregnancy. *Eur. J. Obstet. Gynecol. Reprod. Biol.*, **76**, 147–151.
- Lachapelle, M.H., Miron, P., Hemmings, R., Baron, C. and Roy, D.C. (1996a) Flow-cytometric characterization of hematopoietic cells in non-pregnant human endometrium. *Am. J. Reprod. Immunol.*, **35**, 5–13.
- Lachapelle, M.H., Miron, P., Hemmings, R. and Roy, D.C. (1996b) Endometrial T, B, and NK cells in patients with recurrent spontaneous abortion. Altered profile and pregnancy outcome. *J. Immunol.*, **156**, 4027–4034.
- LeBouteiller, P., Solier, C., Proll, J., Aguerre-Girr, M., Fournel, S. and Lenfant, F. (1999) Placental HLA-G protein expression in vivo: where and what for? *Hum. Reprod. Update*, **5**, 223–233.
- Lin, H., Mosmann, T.R., Guilbert, L., Tuntipopipat, S. and Wegmann, T.G. (1993) Synthesis of T helper 2-type cytokines at the maternal-fetal interface. *J. Immunol.*, **151**, 4562–4573.
- Loke, Y.W., King, A., Gardner, L. and Carter, N.P. (1992) Evidence for the expression of granulocyte-macrophage colony-stimulating factor receptors by human first trimester extravillous trophoblast and its response to this cytokine. *J. Reprod. Immunol.*, **22**, 33–45.
- Loke, Y.W., King, A. and Burrows, T.D. (1995) Decidua in human implantation. *Hum. Reprod.*, **10** (Suppl. 2), 14–21.
- Loy, R.A., Loukides, J.A. and Polan, M.L. (1992) Ovarian steroids modulate human monocyte tumor necrosis factor alpha messenger ribonucleic acid levels in cultured human peripheral monocytes. *Fertil. Steril.*, **58**, 733–739.
- Luppi, P., Haluszczak, C., Better, D., Richard, C.A., Trucco, M. and Deloia, J.A. (2002a) Monocytes are progressively activated in the circulation of pregnant women. *J. Leukoc. Biol.*, **72**, 874–884.
- Luppi, P., Haluszczak, C., Trucco, M. and Deloia, J.A. (2002b) Normal pregnancy is associated with peripheral leukocyte activation. *Am. J. Reprod. Immunol.*, **47**, 72–81.
- Makhseed, M., Raghupathy, R., Azizieh, F., Al Azemi, M.M., Hassan, N.A. and Bandar, A. (1999) Mitogen-induced cytokine responses of maternal peripheral blood lymphocytes indicate a differential Th-type bias in normal pregnancy and pregnancy failure. *Am. J. Reprod. Immunol.*, **42**, 273–281.
- Mandelboim, O., Pazmany, L., Davis, D.M., Vales-Gomez, M., Reyburn, H.T., Rybalov, B. and Strominger, J.L. (1997) Multiple receptors for HLA-G on human natural killer cells. *Proc. Natl Acad. Sci. USA*, **94**, 14666–14670.
- Maruyama, T., Makino, T., Sugi, T., Matsubayashi, H., Ozawa, N. and Nozawa, S. (1992) Flow-cytometric analysis of immune cell populations in human decidua from various types of first-trimester pregnancy. *Hum. Immunol.*, **34**, 212–218.
- Marzusch, K., Ruck, P., Geiselhart, A., Handgretinger, R., Dietl, J.A., Kaiserling, E., Horny, H.P., Vince, G., and Redman, C.W. (1993) Distribution of cell adhesion molecules on CD56++, CD3-. *Hum. Reprod.*, **8**, 1203–1208.
- Matsubayashi, H., Hosaka, T., Sugiyama, Y., Suzuki, T., Arai, T., Kondo, A., Sugi, T., Izumi, S. and Makino, T. (2001) Increased natural killer-cell activity is associated with infertile women. *Am. J. Reprod. Immunol.*, **46**, 318–322.
- Matthiesen, L., Berg, G., Ernerudh, J. and Hakansson, L. (1996) Lymphocyte subsets and mitogen stimulation of blood lymphocytes in normal pregnancy. *Am. J. Reprod. Immunol.*, **35**, 70–79.
- McMaster, M.T., Librach, C.L., Zhou, Y., Lim, K.H., Janatpour, M.J., DeMars, R., Kovats, S., Damsky, C. and Fisher, S.J. (1995) Human placental HLA-G expression is restricted to differentiated cytotrophoblasts. *J. Immunol.*, **154**, 3771–3778.
- Medawar, P.D. (1953) Some immunological and endocrinological problems raised by the evolution of viviparity in vertebrates. Symposium of the Society for Experimental Biology, p. 320.
- Mellembakken, J.R., Aukrust, P., Olafsen, M.K., Ueland, T., Hestdal, K. and Videm, V. (2002) Activation of leukocytes during the uteroplacental passage in preeclampsia. *Hypertension*, **39**, 155–160.
- Minagawa, M., Narita, J., Tada, T., Maruyama, S., Shimizu, T., Bannai, M., Oya, H., Hatakeyama, K. and Abo, T. (1999) Mechanisms underlying immunologic states during pregnancy: possible association of the sympathetic nervous system. *Cell. Immunol.*, **196**, 1–13.
- Moreau, P., Paul, P., Rouas-Freiss, N., Kirszenbaum, M., Dausset, J. and Carosella, E.D. (1998) Molecular and immunologic aspects of the nonclassical HLA class I antigen HLA-G: evidence for an important role in the maternal tolerance of the fetal allograft. *Am. J. Reprod. Immunol.*, **40**, 136–144.
- Mori, T., Guo, M.W., Sato, E., Baba, T., Takasaki, S. and Mori, E. (2000) Molecular and immunological approaches to mammalian fertilization. *J. Reprod. Immunol.*, **47**, 139–158.
- Morrish, D.W., Bhardwaj, D. and Paras, M.T. (1991) Transforming growth factor β 1 inhibits placental differentiation and human chorionic gonadotropin and human placental lactogen secretion. *Endocrinology*, **129**, 22–26.

- Moskalewski, S. and Koprowski, H. (1972) Presence of egg antigen in immature oocytes and preimplantation embryos. *Nature*, **237**, 167–168.
- Mosmann, T.R., Cherwinski, H., Bond, M.W., Giedlin, M.A. and Coffman, R.L. (1986) Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J. Immunol.*, **136**, 2348–2357.
- Muller, H., Liu, B., Croy, B.A., Head, J.R., Hunt, J.S., Dai, G. and Soares, M.J. (1999) Uterine natural killer cells are targets for a trophoblast cell-specific cytokine, prolactin-like protein A. *Endocrinology*, **140**, 2711–2720.
- Munn, D.H., Zhou, M., Attwood, J.T., Bondarev, I., Conway, S.J., Marshall, B., Brown, C. and Mellor, A.L. (1998) Prevention of allogeneic fetal rejection by tryptophan catabolism. *Science*, **281**, 1191–1193.
- Munz, C., Holmes, N., King, A., Loke, Y.W., Colonna, M., Schild, H. and Rammensee, H.G. (1997) Human histocompatibility leukocyte antigen (HLA)-G molecules inhibit NKAT3 expressing natural killer cells. *J. Exp. Med.*, **185**, 385–391.
- Naccasha, N., Gervasi, M.T., Chaiworapongsa, T., Berman, S., Yoon, B.H., Maymon, E. and Romero, R. (2001) Phenotypic and metabolic characteristics of monocytes and granulocytes in normal pregnancy and maternal infection. *Am. J. Obstet. Gynecol.*, **185**, 1118–1123.
- Nachtigall, M.J., Kliman, H.J., Feinberg, R.F., Olive, D.L., Engin, O. and Arici, A. (1996) The effect of leukemia inhibitory factor (LIF) on trophoblast differentiation: a potential role in human implantation. *J. Clin. Endocrinol. Metab.*, **81**, 801–806.
- Nagler, A., Lanier, L.L., Cwirla, S. and Phillips, J.H. (1989) Comparative studies of human FcR3-positive and negative natural killer cells. *J. Immunol.*, **143**, 3183–3191.
- Nishioka, Y., Nishimura, N., Suzuki, Y. and Sone, S. (2001) Human monocyte-derived and CD83(+) blood dendritic cells enhance NK cell-mediated cytotoxicity. *Eur. J. Immunol.*, **31**, 2633–2641.
- Osada, T., Nagawa, H., Kitayama, J., Tsuno, N.H., Ishihara, S., Takamizawa, M. and Shibata, Y. (2001) Peripheral blood dendritic cells, but not monocyte-derived dendritic cells, can augment human NK cell function. *Cell. Immunol.*, **213**, 14–23.
- Ostensen, M., Aune, B. and Husby, G. (1983) Effect of pregnancy and hormonal changes on the activity of rheumatoid arthritis. *Scand. J. Rheumatol.*, **12**, 69–72.
- Ozenci, C.C., Korgun, E.T. and Demir, R. (2001) Immunohistochemical detection of CD45+, CD56+, and CD14+ cells in human decidua during early pregnancy. *Early Pregnancy*, **5**, 164–175.
- Pace, D., Morrison, L. and Bulmer, J.N. (1989) Proliferative activity in endometrial stromal granulocytes throughout menstrual cycle and early pregnancy. *J. Clin. Pathol.*, **42**, 35–39.
- Parham, P. (1995) Antigen presentation by class I major histocompatibility complex molecules: a context for thinking about HLA-G. *Am. J. Reprod. Immunol.*, **34**, 10–19.
- Parhar, R.S., Yagel, S. and Lala, P.K. (1989) PGE2-mediated immunosuppression by first trimester human decidua cells blocks activation of maternal leukocytes in the decidua with potential anti-trophoblast activity. *Cell. Immunol.*, **120**, 61–74.
- Pazmany, L., Mandelboim, O., Vales-Gomez, M., Davis, D.M., Reyburn, H.T. and Strominger, J.L. (1996) Protection from natural killer cell-mediated lysis by HLA-G expression on target cells. *Science*, **274**, 792–795.
- Persellin, R.H. and Thoi, L.L. (1979) Human polymorphonuclear leukocyte phagocytosis in pregnancy. Development of inhibition during gestation and recovery in the postpartum period. *Am. J. Obstet. Gynecol.*, **134**, 250–254.
- Pfeiffer, K.A., Rebmann, V., Passler, M., van der Ven, K., van der Ven, H., Krebs, D. and Grosse-Wilde, H. (2000) Soluble HLA levels in early pregnancy after *in vitro* fertilization. *Hum. Immunol.*, **61**, 559–564.
- Phillips, T.A., Ni, J., Pan, G., Ruben, S.M., Wei, Y.F., Pace, J.L. and Hunt, J.S. (1999) TRAIL (Apo-2L) and TRAIL receptors in human placentas: implications for immune privilege. *J. Immunol.*, **162**, 6053–6059.
- Phillips, T.A., Ni, J. and Hunt, J.S. (2001) Death-inducing tumour necrosis factor (TNF) superfamily ligands and receptors are transcribed in human placenta, cytotrophoblasts, placental macrophages and placental cell lines. *Placenta*, **22**, 663–672.
- Piccinni, M.P. and Romagnani, S. (1996) Regulation of fetal allograft survival by a hormone-controlled Th1- and Th2-type cytokines. *Immunol. Res.*, **15**, 141–150.
- Piccinni, M.P., Giudizi, M.G., Biagiotti, R., Beloni, L., Giannarini, L., Sampognaro, S., Parronchi, P., Manetti, R., Annunziato, F. and Livi, C. (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 cell clones. *J. Immunol.*, **155**, 128–133.
- Piccinni, M.P., Belone, L., Livi, C., Maggi, E., Scarselli, G. and Romagnani, S. (1998) Defective production of both leukemia inhibitory factor and type 2 T-helper cytokines by decidual T cells in unexplained recurrent abortions. *Nature Med.*, **4**, 1020–1024.
- Piccinni, M.P., Maggi, E. and Romagnani, S. (2000) Role of hormone-controlled T-cell cytokines in the maintenance of pregnancy. *Biochem. Soc. Trans.*, **28**, 212–215.
- Piccinni, M.P., Scaletti, C., Vultaggio, A., Maggi, E. and Romagnani, S. (2001) Defective production of LIF, M-CSF and Th2-type cytokines by T cells at fetomaternal interface is associated with pregnancy loss. *J. Reprod. Immunol.*, **52**, 35–43.
- Polan, M.L., Loukides, J., Nelson, P., Carding, S., Diamond, M., Walsh, A. and Bottomly, K. (1989) Progesterone and estradiol modulate interleukin-1 beta messenger ribonucleic acid levels in cultured human peripheral monocytes. *J. Clin. Endocrinol. Metab.*, **69**, 1200–1206.
- Polgar, K. and Hill, J.A. (2002) Identification of the white blood cell populations responsible for Th1 immunity to trophoblast and the timing of the response in women with recurrent pregnancy loss. *Gynecol. Obstet. Invest.*, **53**, 59–64.
- Proll, J., Blaschitz, A., Hutter, H. and Dohr, G. (1999) First trimester human endovascular trophoblast cells express both HLA-C and HLA-G. *Am. J. Reprod. Immunol.*, **42**, 30–36.
- Proll, J., Bensussan, A., Goffin, F., Foidart, J.M., Berrebi, A. and Le Bouteiller, P. (2000) Tubal versus uterine placentation: similar HLA-G expressing extravillous cytotrophoblast invasion but different maternal leukocyte recruitment. *Tissue Antigens*, **56**, 479–491.
- Quack, K.C., Vassiliadou, N., Pudney, J., Anderson, D.J. and Hill, J.A. (2001) Leukocyte activation in the decidua of chromosomally normal and abnormal fetuses from women with recurrent abortion. *Hum. Reprod.*, **16**, 949–955.
- Quenby, S., Bates, M., Doig, T., Brewster, J., Lewis-Jones, D.I., Johnson, P.M. and Vince, G. (1999) Pre-implantation endometrial leukocytes in women with recurrent miscarriage. *Hum. Reprod.*, **14**, 2386–2391.
- Raghupathy, R., Makhseed, M., Azizieh, F., Omu, A., Gupta, M. and Farhat, R. (2000) Cytokine production by maternal lymphocytes during normal human pregnancy and in unexplained recurrent spontaneous abortion. *Hum. Reprod.*, **15**, 713–718.
- Rajagopalan, S. and Long, E.O. (1999) A human histocompatibility leukocyte antigen (HLA)-G-specific receptor expressed on all natural killer cells. *J. Exp. Med.*, **189**, 1093–1100.
- Redline, R.W., McKay, D.B., Vazquez, M.A., Papaioannou, V.E. and Lu, C.Y. (1990) Macrophage functions are regulated by the substratum of murine decidua stromal cells. *J. Clin. Invest.*, **85**, 1951–1958.
- Reinhard, G., Noll, A., Schlebusch, H., Mallmann, P. and Ruecker, A.V. (1998) Shifts in the TH1/TH2 balance during human pregnancy correlate with apoptotic changes. *Biochem. Biophys. Res. Commun.*, **245**, 933–938.
- Reister, F., Frank, H.G., Kingdom, J.C., Heyl, W., Kaufmann, P., Rath, W. and Huppertz, B. (2001) Macrophage-induced apoptosis limits endovascular trophoblast invasion in the uterine wall of preeclamptic women. *Lab. Invest.*, **81**, 1143–1152.
- Rieger, L., Hofmeister, V., Probe, C., Dietl, J., Weiss, E.H., Steck, T. and Kammerer, U. (2002) Th1- and Th2-like cytokine production by first trimester decidua large granular lymphocytes is influenced by HLA-G and HLA-E. *Mol. Hum. Reprod.*, **8**, 255–261.
- Riteau, B., Menier, C., Khalil-Daher, I., Sedlik, C., Dausset, J., Rouas-Freiss, N. and Carosella, E.D. (1999) HLA-G inhibits the allogeneic proliferative response. *J. Reprod. Immunol.*, **43**, 203–211.
- Ritson, A. and Bulmer, J.N. (1987) Endometrial granulocytes in human decidua react with a natural-killer (NK) cell marker, NKH1. *Immunology*, **62**, 329–331.
- Roth, I., Corry, D.B., Locksley, R.M., Abrams, J.S., Litton, M.J. and Fisher, S.J. (1996) Human placental cytotrophoblast produce the immunosuppressive cytokine interleukin 10. *J. Exp. Med.*, **184**, 539–548.
- Rouas-Freiss, N., Goncalves, R.M., Menier, C., Dausset, J. and Carosella, E.D. (1997) Direct evidence to support the role of HLA-G in protecting the fetus from maternal uterine natural killer cytotoxicity. *Proc. Natl Acad. Sci. USA*, **94**, 11520–11525.
- Ruck, P., Marzusch, K., Kaiserling, E., Horny, H.P., Dietl, J., Geiselhart, A., Handgretinger, R. and Redman, C.W. (1994) Distribution of cell adhesion molecules in decidua of early human pregnancy. An immunohistochemical study. *Lab. Invest.*, **71**, 94–101.
- Runic, R., Lockwood, C.J., Ma, Y., Dipasquale, B. and Guller, S. (1996) Expression of Fas ligand by human cytotrophoblasts: implications in

- placentation and fetal survival. *J. Clin. Endocrinol. Metab.*, **81**, 3119–3122.
- Sabahi, F., Rola-Pleszczynski, M., O'Connell, S. and Frenkel, L.D. (1995) Qualitative and quantitative analysis of T lymphocytes during normal human pregnancy. *Am. J. Reprod. Immunol.*, **33**, 381–393.
- Sacks, G.P., Studena, K., Sargent, I.L. and Redman, C.W. (1998) Normal pregnancy and preeclampsia both produce inflammatory changes in peripheral blood leukocytes akin to those of sepsis. *Am. J. Obstet. Gynecol.*, **179**, 80–86.
- Sacks, G., Sargent, I. and Redman, C. (1999) An innate view of human pregnancy. *Immunol. Today*, **20**, 114–118.
- Sacks, G., Sargent, I. and Redman, C. (2000) Innate immunity in pregnancy. *Immunol. Today*, **21**, 200–201.
- Sacks, G.P., Clover, L.M., Bainbridge, D.R., Redman, C.W. and Sargent, I.L. (2001) Flow cytometric measurement of intracellular Th1 and Th2 cytokine production by human villous and extravillous cytotrophoblast. *Placenta*, **22**, 550–559.
- Saito, S. (2000) Cytokine network at the feto-maternal interface. *J. Reprod. Immunol.*, **47**, 87–103.
- Saito, S., Nishikawa, K., Morii, T., Enomoto, M., Narita, N., Motoyoshi, K. and Ichijo, M. (1993) Cytokine production by CD16-CD56^{bright} natural killer cells in the human early pregnancy decidua. *Int. Immunol.*, **5**, 559–563.
- Saito, S., Umekage, H., Nishikawa, K., Morii, T., Narita, N., Enomoto, M., Sakakura, S., Harada, N., Ichijo, M. and Morikawa, H. (1996) Interleukin 4 (IL-4) blocks the IL-2-induced increase in natural killer activity and DNA synthesis of decidual CD16-CD56^{bright}NK cells by inhibiting expression of the IL-2 receptor α , β , and γ . *Cell. Immunol.*, **170**, 71–77.
- Saito, S., Tsukaguchi, N., Hasegawa, T., Michimata, T., Tsuda, H. and Narita, N. (1999) Distribution of Th1, Th2, and Th0 and the Th1/Th2 cell ratios in human peripheral and endometrial T cells. *Am. J. Reprod. Immunol.*, **42**, 240–245.
- Sakai, M., Tsuda, H., Tanebe, K., Sasaki, Y. and Saito, S. (2002) Interleukin-12 secretion by peripheral blood mononuclear cells is decreased in normal pregnant subjects and increased in preeclamptic patients. *Am. J. Reprod. Immunol.*, **47**, 91–97.
- Sargent, I.L. (1993) Maternal and fetal immune responses during pregnancy. *Exp. Clin. Immunogenet.*, **10**, 85–102.
- Sawai, K., Matsuzaki, N., Kameda, T., Hashimoto, K., Okada, T., Shimoya, K., Nobunaga, T., Taga, T., Kishimoto, T. and Saji, F. (1995) Leukemia inhibitory factor produced at the fetomaternal interface stimulates chorionic gonadotrophin production: its possible implication during pregnancy, including implantation period. *J. Clin. Endocrinol. Metab.*, **80**, 1449–1456.
- Schrocksadel, H., Baier-Bitterlich, G., Dapunt, O., Wachter, H. and Fuchs, D. (1996) Decreased plasma tryptophan in pregnancy. *Obstet. Gynecol.*, **88**, 47–50.
- Seigler, H.F. and Metzgar, R.S. (1970) Embryonic development of human transplantation antigens. *Transplantation*, **9**, 478–486.
- Siegel, I. and Gleicher, N. (1981) Changes in peripheral mononuclear cells in pregnancy. *Am. J. Reprod. Immunol.*, **1**, 154–155.
- Simons, R.L. and Russell, P.S. (1962) Antigenicity of mouse trophoblast. *Ann. N. Y. Acad. Sci.*, **99**, 717.
- Soderstrom, K., Corliss, B., Lanier, L.L. and Phillips, J.H. (1997) CD94/NKG2 is the predominant inhibitory receptor involved in recognition of HLA-G by decidual and peripheral blood NK cells. *J. Immunol.*, **159**, 1072–1075.
- Solier, C., Aguerre-Girr, M., Lenfant, F., Campan, A., Berrebi, A., Rebmann, V., Grosse-Wilde, H. and Le Bouteiller, P. (2003) Secretion of pro-apoptotic intron 4-retaining soluble HLA-G1 by human villous trophoblast. *Eur. J. Immunol.*, **32**, 3576–3586.
- Starkey, P.M., Sargent, I.L. and Redman, C.W. (1988) Cell populations in human early pregnancy decidua: characterization and isolation of large granular lymphocytes by flow cytometry. *Immunology*, **65**, 129–134.
- Steinman, R.M. (1991) The dendritic cell system and its role in immunogenicity. *Annu. Rev. Immunol.*, **9**, 271–296.
- Stewart, C.L., Kaspar, P., Brunet, L.J., Bhatt, H., Gadi, I., Kontgen, F. and Abbondanzo, S.J. (1992) Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor. *Nature*, **359**, 76–79.
- Stewart, J.A., Bulmer, J.N. and Murdoch, A.P. (1998) Endometrial leucocytes: expression of steroid hormone receptors. *J. Clin. Pathol.*, **51**, 121–126.
- Sutton, L., Mason, D.Y. and Redman, C.W. (1983) HLA-DR positive cells in the human placenta. *Immunology*, **49**, 103–112.
- Szekeres-Bartho, J. and Wegmann, T.G. (1996) A progesterone-dependent immunomodulatory protein alters the Th1/Th2 balance. *J. Reprod. Immunol.*, **31**, 81–95.
- Szereday, L., Varga, P. and Szekeres-Bartho, J. (1997) Cytokine production by lymphocytes in pregnancy. *Am. J. Reprod. Immunol.*, **38**, 418–422.
- Uckan, D., Steele, A., Cherry, A., Wang, B.Y., Chamizo, W., Koutsonikolis, A., Gilbert-Barness, E. and Good, R.A. (1997) Trophoblasts express Fas ligand: a proposed mechanism for immune privilege in placenta and maternal invasion. *Mol. Hum. Reprod.*, **3**, 655–662.
- vanderElsen, P.J., Gobin, S.J., van der Stoep, G., Datema, G. and Viator, H.E. (2001) Transcriptional control of MHC genes in fetal trophoblast cells. *J. Reprod. Immunol.*, **52**, 129–145.
- Varner, M.W. (1991) Autoimmune disorders and pregnancy. *Semin. Perinatol.*, **15**, 238–250.
- Vassiliadou, N. and Bulmer, J.N. (1998) Characterization of tubal and decidual leukocyte populations in ectopic pregnancy: evidence that endometrial granulated lymphocytes are absent from the tubal implantation site. *Fertil. Steril.*, **69**, 760–767.
- VeenstravanNieuwenhoven, A.L., Bouman, A., Moes, H., Heineman, M.J., de Leij, L.F., Santema, J. and Faas, M.M. (2002) Cytokine production in natural killer cells and lymphocytes in pregnant women compared with women in the follicular phase of the ovarian cycle. *Fertil. Steril.*, **77**, 1032–1037.
- VeenstravanNieuwenhoven, A.L., Bouman, A., Moes, H., Heineman, M.J., de Leij, L.F.M.H., Santema, J. and Faas, M.M. (2003) Endotoxin-induced cytokine production of monocytes of third trimester pregnant women compared to women in the follicular phase of the menstrual cycle. *Am. J. Obstet. Gynecol.*, **188**, 1073–1077.
- Verma, S., King, A. and Loke, Y.W. (1997) Expression of killer cell inhibitory receptors on human uterine natural killer cells. *Eur. J. Immunol.*, **27**, 979–983.
- Verma, S., Hiby, S.E., Loke, Y.W. and King, A. (2000) Human decidual natural killer cells express the receptor for and respond to the cytokine interleukin 15. *Biol. Reprod.*, **62**, 959–968.
- Vince, G.S. and Johnson, P.M. (2000) Growth factors and cytokines at the maternal/fetal interface. *Biochem. Soc. Trans.*, **28**, 191–195.
- Vince, G.S., Starkey, P.M., Jackson, M.C., Sargent, I.L. and Redman, C.W. (1990) Flow cytometric characterisation of cell populations in human pregnancy decidua and isolation of decidual macrophages. *J. Immunol. Methods*, **132**, 181–189.
- vonRango, U., Classen-Linke, I., Kertschanska, S., Kemp, B. and Beier, H.M. (2001) Effects of trophoblast invasion on the distribution of leukocytes in uterine and tubal implantation sites. *Fertil. Steril.*, **76**, 116–124.
- Watanabe, M., Iwatani, Y., Kaneda, T., Hidaka, Y., Mitsuda, N., Morimoto, Y. and Amino, N. (1997) Changes in T, B, and NK lymphocyte subsets during and after normal pregnancy. *Am. J. Reprod. Immunol.*, **37**, 368–377.
- Wegmann, T.G., Lin, H., Guilbert, L. and Mosmann, T.R. (1993) Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon? *Immunol. Today*, **14**, 353–356.
- Williams, D.B., Bachy, V. and Ibrahim, M. (2002) Preeclampsia and activation of maternal innate immunity. *Hypertension in Pregnancy*, **21**, 104.
- Yamamoto, T., Takahashi, Y., Kase, N. and Mori, H. (1999) Role of decidual natural killer (NK) cells in patients with missed abortion: differences between cases with normal and abnormal chromosome. *Clin. Exp. Immunol.*, **116**, 449–452.
- Yelavarthi, K.K., Fishback, J.L. and Hunt, J.S. (1991) Analysis of HLA-G mRNA in human placental and extraplacental membrane cells by *in situ* hybridization. *J. Immunol.*, **146**, 2847–2854.
- Yui, J., Garcia-Lloret, M., Wegmann, T.G. and Guilbert, L.J. (1994) Cytotoxicity of tumour necrosis factor- α and gamma-interferon against primary human placental trophoblasts. *Placenta*, **15**, 819–835.
- Zheng, Y., Zhou, Z.Z., Lyttle, C.R. and Teuscher, C. (1988) Immunohistochemical characterization of the estrogen-stimulated leukocyte influx in the immature rat uterus. *J. Leukoc. Biol.*, **44**, 27–32.