The immunology of successful pregnancy

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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 (Sabuhi 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 cytokotoxic 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 (Schoocksnadel 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 monocytes and granulocytes, 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 Tho, 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; Balmer 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 placentaion (Guimond et al., 1999; King, 2000). The effect of uNK cells on implantation and placentaion 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 placentaion were the findings that high preconceptional 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 placentaion. 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 differentiaion (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 (TGFβ), on the other hand, inhibits trophoblast proliferation and differentiaion (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 eects 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-villus 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 outgrowth 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.,
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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 placentaion (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 (Chauvat et al., 2002). Interestingly, the non-villous cytotrophoblast cells also produce various cytokines (Guilbert et al., 1993; Chauvat 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). Moreover, 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,
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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 under-explored. 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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