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Immunology of implantation

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Pregnancy represents an allograft

Cases of recurrent abortions, preeclampsia, or infants born with hemolytic diseases are puzzling. It is unknown why the mother's body rejected the baby. Considering the complexity of the maternal–fetal immune interaction and the number of successful pregnancies, it may be more apt to question why the mother's body didn't reject the infant.

During the early 1950s, Medawar recognized the unique immunology of the maternal–fetal interface and its potential relevance for transplantation. In his original work, he described the “fetal allograft analogy” in which the fetus is viewed as a semiallogeneic conceptus that evades rejection. The approaches over the next 50 years have followed the methodology and development of transplantation immunity, and, more recently, of tumor immunity, unveiling new hypotheses and redefining old concepts.

In 1991, Colbern and Main redefined the conceptual framework of reproductive immunology as maternal–placental tolerance instead of maternal–fetal tolerance, focusing on the interaction between the maternal immune system and the placenta rather than the fetus [1]. In early development, the embryo divides into two groups of cells: (1) an inner cell mass that gives rise to the embryo (the internal group) and (2) an embryonic trophoblast that becomes trophoblast cells and later the placenta (the external group). The cells from the placenta are the only part of the fetus to directly interact with the mother's uterine cells and maternal immune system, preventing immune rejection. The fetus itself has no direct contact with maternal cells. It also is known to express paternal

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major histocompatibility complex (MHC) antigens and is rejected as allograft if removed from its cocoon of trophoblast and transplanted to the thigh muscle or kidney capsule of the mother. This article focuses on the interaction between trophoblast cells and the maternal immune system.

General concepts of immunology

Types of immune responses

The immune system can be divided into two systems: (1) the natural or innate immune system and (2) the adaptive or acquired immune system. Natural or innate immunity produces a relatively unsophisticated response that prevents access of pathogens to the body. This primitive evolutionary response, which is described in more detail later, occurs without the need of previous exposure to similar pathogens. Adaptive immunity is an additional, more sophisticated response that is found in higher forms, such as humans. Cells of the innate immune system aid the acquired system by processing phagocytosed foreign material and presenting antigenic peptides to cells of the adaptive immune system. This immune response is highly specific and normally is potentiated by repeated antigenic encounters.

Adaptive immunity is highly specific and consists of two types of immune responses: (1) humoral immunity, in which antibodies are produced, and (2) cellular immunity, which involves cell lysis by cytolytic T lymphocytes. The adaptive immune system is characterized by an anamnestic response that enables the immune cells to acquire memory of the foreign antigenic encounter and to react faster and more vigorously to subsequent exposures to the same antigen. Cytokines produced by the adaptive immune system can communicate and regulate the innate immune response.

T_H1 and T_H2 type cytokines

Immune cells mediate their effects by releasing cytokines and thus establishing particular microenvironments. T helper lymphocytes (Th) that originate from the thymus (Fig. 1A) have a major role in creating a specific microenvironment for a particular organ or tissue. Following immunologic challenge, immune cells produce cytokines, the type of which determines their differentiation into T helper-1 (T_H1) or T_H2 lymphocytes. T_H1 lymphocytes secrete interleukin-2 (IL-2) and interferon- γ (IFN- γ), setting the basis for a proinflammatory environment. Conversely, T_H2 lymphocytes secrete IL-4 and IL-10 (see Fig. 1), which predominately are involved in antibody production following an antigenic challenge. The actions of these two types of lymphocytes are intertwined closely, both acting in concert and responding to counteract regulatory effects of their cytokines. T_H1 cytokine cells produce proinflammatory cytokine that, while reinforcing the cytolytic immune response, also down-regulate the production of

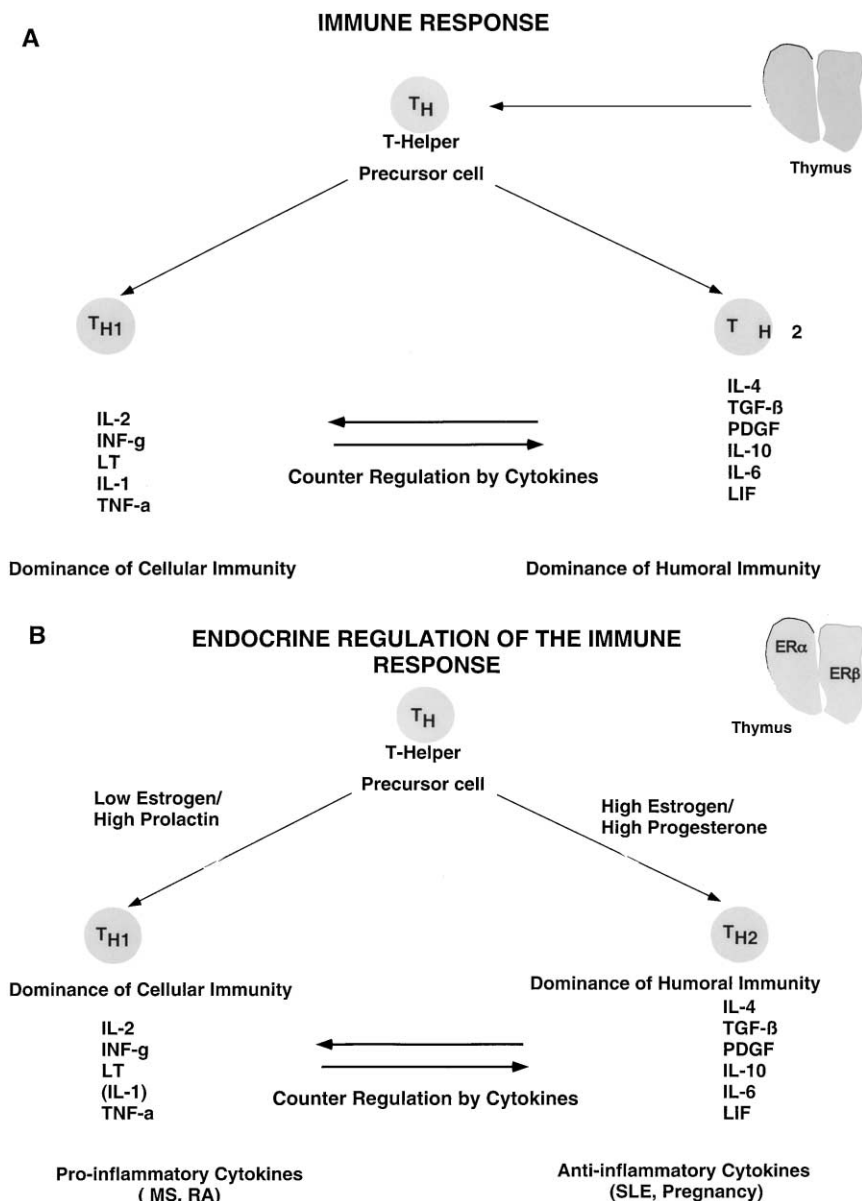


Fig. 1. (A) The classic immune response. Two types of immune response, T_H1 and T_H2 , are determined by the cytokine profile. (B) Immune-endocrine system. Interaction between cytokines and hormones.

T_H2 cytokines. The powerful effect of hormones on this action is shown (Fig. 1B; for review, see references [2,3]). Women during the reproductive years are more likely to develop a T_H1 response after challenge with an infectious agent or

antigen, except during pregnancy, which is when a T_H2 environment prevails (see Fig. 1B; also discussed later) [4].

Innate immunity

The innate immune system, in conventional terms, provides rapid defense against invading pathogens with a relatively low specificity and no capacity for long-term protection. Innate responses are critical for the development of an adaptive immune response. The innate immune system consists of cellular and soluble components. Soluble components include complement, acute-phase proteins, and cytokines. The main cellular element of innate immunity are phagocytic cells, which include mononuclear phagocytes (monocytes, macrophages) and granulocytes (polymorphonuclear neutrophils, eosinophils). These cells recognize, engulf, and kill invading microorganisms. Cell-surface molecules, known as pattern-recognition receptors, are critical to such innate function and are expressed by phagocytes. These receptors are important for the recognition of pathogens because of their interaction with conserved structural elements, known as pathogen-associated molecular patterns, on the surface of microorganisms [5]. Mononuclear phagocytes, and in some cases neutrophils [6], help power adaptive immune responses by processing and presenting antigen to T lymphocytes. Other innate cells include natural killer (NK) cells, which are important for killing cells infected by viruses and for regulation of some autoimmune responses; basophils; and mast cells, which are additional inflammatory cells. The cellular and soluble components of the innate immune system can actively direct the path of acquired immunity. The system seems to have a major role in the normal physiology of the human female reproductive tract. More specifically, the innate immune cells may have a significant role in the process of implantation and in certain complications that arise during pregnancy [7]. In this article, the role of innate immune cells in implantation and pre-eclampsia is discussed.

Maternal–trophoblast immune interaction

The pregnant uterus as an immune-privileged site

During implantation, the blastocyst becomes intimately connected with the maternal endometrium or decidua. During implantation, the semiallogeneic fetus is in direct contact with the maternal uterine and blood-borne cells, however, fetal rejection by the maternal immune system, in most cases, is prevented by as yet undefined mechanisms. A number of mechanisms have been proposed to account for the immune-privileged state of the decidua, including: (1) a mechanical barrier effect of the trophoblast, (2) suppression of the maternal immune system during pregnancy, (3) the absence of MHC class I molecules in the trophoblast, (4) cytokine shift, and (5) local immune suppression mediated by the Fas/FasL

system. These hypotheses and the role of cells of the innate immune system at the site of implantation are reviewed.

Mechanical barrier

The concept of a mechanical barrier was proposed to explain the lack of an immune response in organs such as the brain, cornea, testicles, and kidneys. The authors refer to these tissues as immune-privilege sites, where an immune response represents a dangerous condition for the tissue. Immune-privilege sites are also organs or tissues of the body that, when grafted to conventional (nonprivileged) body sites, experience extended or indefinite survival. Foreign grafts placed at nonprivileged sites, however, are rejected promptly. The pregnant uterus is an immune-privileged site.

The first reasonable explanation of immune privilege was proposed by Medawar in the late 1940s [8]. He proposed that organs such as the brain and the anterior chamber of the eye resided behind blood–tissue barriers. According to his theory, a mechanical barrier (eg, the blood–brain barrier) prevents the movement of immune cells in and out of the tissue [9]. This barrier creates a state of immunologic ignorance in which antigens within the system are not detected by immune cells outside the system. The pregnant uterus was proposed to have a mechanical barrier formed by the trophoblast and decidua, which prevented the movement of activated T cells from the periphery to the implantation site. Similarly, this barrier would isolate the fetus and prevent the escape of fetal cells to maternal circulation.

Studies that challenge the concept of mechanical barrier show that the trophoblast–decidual interface is less inert and less impermeable than first envisioned. Evidence for traffic in both directions across the maternal–fetal interface includes the migration of maternal cells into the fetus, presence of fetal cells in maternal circulation [10], and transport of maternal immunoglobulin across the placenta by the neonatal Fc receptor, FcRn [11]. This cellular migration occurs in almost all immune-privilege tissues, including the blood–brain barrier. Conclusive evidence has shown that immune cells circulate through all the parts of the brain [12], indicating that immune cells are not deterred by mechanical barriers [13].

Systemic immune suppression

The second theory postulates the existence of nonspecific immune suppression during pregnancy. Numerous factors produced and isolated from the maternal–placental interface or from the serum of pregnant women have been associated with immune-suppressive activity. Studies have suggested that human placental lactogen, human placental protein 14, and pregnancy-associated plasma protein-A may have immune-depressant activity on lymphocytes. Soluble suppressor activity also has been identified in supernatants and cytosol fractions from placental explants and uterine secretions (for review, see reference [14]). Although all of these studies reported immunologic effects of the factors, many

factors were only purified partially and their actions were tested using in vitro assays for lymphocyte or NK cell activity. These assays are sensitive to impurities, and after further purification, many of these factors may have lost their immune-suppressive effects.

The steroid sex hormone progesterone has been suggested to have immune-suppressive effects [15]. In vitro, progesterone was described as highly suppressive of mitogen activation and cytotoxic T-cell generation [16]. Similarly, progesterone was shown to blunt an inflammatory response in an in vivo rat model [17]. Other studies have shown that progesterone inhibits cytotoxic and NK cell activity and prostaglandin F 2α synthesis [18]. It has been shown that progesterone activates regulatory T cells of a suppressor phenotype by induction of a 34-kDa protein from lymphocytes [16,19,20].

The concept of systemic immune suppression during pregnancy has been studied by numerous investigators and for many years became an accepted explanation. As described earlier, a wide array of materials in human serum have been found to have profound in vitro immune-suppressive activity [21], however, from an evolutionary point of view, it is difficult to perceive pregnancy as a stage of immune suppression. In areas in which many pregnant women are exposed to harsh sanitary conditions, the case of a suppressed immune system would make fetus survival impossible. Studies have demonstrated that maternal antiviral immunity is not affected by pregnancy. The obvious observation that HIV-positive pregnant women do not suffer from AIDS-like disease argues against the existence of such nonspecific immune suppression.

Lack of expression of human leukocyte antigens

A more recently postulated theory is based on the fact that polymorphic class I and II molecules have not been detected on the trophoblast [22]. MHC class I antigens are expressed on the surface of most nucleated cells and serve as important recognition molecules. In humans, these antigens also are known as human leukocyte antigens (HLAs). HLA class I genes are located on the same chromosomal region (6p.21.3). They have been subdivided into two groups (HLA class Ia, HLA class Ib) according to their polymorphism, tissue distribution, and functions. HLA-A, HLA-B, and HLA-C class Ia genes exhibit high levels of polymorphism, are expressed among most somatic tissues, and have well-established immunologic functions. They modulate antiviral and antitumoral immune responses through their interaction with T- and NK cell receptors. In contrast, HLA-E, HLA-F, and HLA-G class Ib genes are characterized by their limited polymorphism and restricted tissue distribution; their roles are poorly understood. The statement that the human placenta does not express polymorphic MHC class I molecules is not entirely accurate. The human placenta does not express the polymorphic HLA-A and HLA-B class I antigens, but it does express HLA-C, HLA-G, and HLA-E molecules [21,23,24]. Using immunostaining with antibodies against the class I molecule, Loke and King divided the human trophoblast into two distinct populations: (1) the villous trophoblast in contact

with maternal blood at the intervillous interface, which is class I negative, and (2) the extravillous trophoblast invading the uterine decidua, which is class I positive [21]. This data suggest there are two fetal–maternal interfaces in human reproduction and that they differ immunologically: (1) a trophoblast population that has immunologically neutral contact with the systemic maternal immune system and (2) a local immunologically active population of trophoblast cells migrating into the decidua that can be stimulated by HLA class I molecules [21].

HLA-G originally was cloned in 1987 was demonstrated to be present in abundant amounts at the maternal–fetal interface [25]. Based on what was believed to be an almost exclusive expression of HLA-G at the maternal–fetal interface, it was suggested that this molecule maintains a specialized role in this environment. Another unique feature of the HLA-G genes, which was postulated to be a prerequisite for maintenance of maternal immune tolerance, is the lack of polymorphism of the HLA-G nucleotide sequence, however, data have shown that this is not the case. Alternative splicing of HLA-G mRNA yields different membrane-bound and soluble variants of the HLA-G protein and a limited number of variable sites in the DNA sequence of the HLA-G gene [26–28]; thus, the hypothesis that HLA-G is the mediator of fetal–maternal tolerance because of its monomorphism and immunologic neutrality needed to be revised [29]. In animal studies, it has been shown that murine trophoblast cells express MHC class I genes and alloantigens at high levels early in gestation age. During those times, MHC class I barely is detected on fetal tissues [30,31]. It has been suggested that it is highly unlikely that maternal T cells circulating through the murine maternal–fetal interface do not encounter cells of fetal origin. This pattern of MHC class I expression at the maternal–fetal interface is incompatible with the hypothesis that fetal tissues at the interface are antigenically immature and do not provoke a maternal T-cell responses [32].

Although the apparent lack of classic MHC gene expression suggests that the preimplantation embryo is protected from direct immunologic attack by MHC-restricted T cells, the preimplantation embryo still could be vulnerable to delayed-type hypersensitivity reactions and to adverse effects of antibodies and cytokines by non-MHC-restricted effector cells.

Cytokine shift

The proliferation, invasion, and differentiation of trophoblast cells during implantation are involved in a tightly controlled process that is coordinated by a system of intercellular signals mediated by cytokines, growth factors, and hormones [32,33]. An extensive array of cytokines is produced at the trophoblast–maternal interface and contributes to the well being of the fetoplacental unit. These cytokines also regulate maternal immune responses, which have an important role in successful pregnancy outcomes [34].

Cytokines have diverse biologic effects that may involve cell growth, differentiation, and function [35]. Their role in regulating development and implantation of the human placenta has been the topic of much discussion [36,37]. The

field of cytokines and implantation could be divided into two aspects: (1) the role of cytokines as regulators of the immune response and (2) the role of cytokines as factors that control growth and implantation of trophoblast cells.

Implantation and T_H1 -type and T_H2 -type immune responses

In general, the normal reproductive woman has a strong tendency to respond to foreign antigens by developing a T_H1 -type immune response and by expressing high levels of proinflammatory cytokines [38,39]. During pregnancy, there is a decrease in cellular immunity and an enhancement of humoral immunity, characterized by higher levels of anti-inflammatory cytokines [40]. Human and murine pregnancies, particularly at the maternal–fetal interface, have been shown to exhibit a strong shift in cytokine balance from T_H1 type to T_H2 type (see Fig. 1A) [41]. The balance between T_H1 proinflammatory cytokines and T_H2 anti-inflammatory cytokines is believed to be crucial for determining pregnancy outcome (reviewed in reference[14]). It currently is believed that for the continuous normal development of pregnancy, production of inflammatory and potentially harmful T_H1 cytokines such as, IL-2, tumor necrosis factor- α (TNF- α) and IFN- γ , is suppressed, whereas production of T_H2 anti-inflammatory cytokines, such as IL-4, IL-6, or IL-10, is enhanced [41]. Placental and decidual tissues from normal pregnancies have been shown to express pro- and anti-inflammatory cytokines [42–47]. In humans and mice, T_H1 cytokines are potentially harmful to pregnancy. Excess production of TNF- α [48] or IFN- γ [49] has been associated with preterm delivery.

For many years, the most compelling animal model of immunologically mediated spontaneous fetal loss have been the murine CBA/J x DBA/2 mating combination, in which between 20% and 50% of the fetus is reabsorbed by gestational day 13 [50]. The model demonstrates the importance of the immunologic component based on the female's ability to suppress fetal loss by preimmunization with peritoneal macrophages before mating with a BALB/cJ male. NK cells and macrophages have been implicated as cellular mediators of the syndrome. Excessive nitric oxide and TNF- α released by decidual mononuclear cells represent the effector mechanisms that become dysregulated in this strain combination [51]. An indirect role for granulocyte-macrophage colony-stimulating factor (GM-CSF) and a significant role for maternal CD8⁺ T cells in preventing NK cell-mediated fetal loss in this system has been suggested.

These CBA/J x DBA/2 mice have been shown to be deficient in IL-10, and intraperitoneal injection of IL-10 reduces fetal loss to normal levels [52]. Similarly, low levels of IL-4 and IL-10 were observed in decidual T cells of women who experienced unexplained recurrent abortions and a spontaneous abortion during the first trimester of pregnancy [53]. Reduced production of leukemia-inhibiting factor, IL-4, and IL-10 in women with unexplained recurrent abortions was not observed at the level of peripheral blood, suggesting that this reduction is not an inherent characteristic of T cells but rather a local regulation at the implantation site.

Another important aspect in the study of cytokines and implantation is the local regulatory effect of cytokines on the growth and invasion of trophoblast. A complete review of cytokines and their role in implantation is available elsewhere [14,54,55].

Local immune suppression

Another hypothesis is the specific antipaternal suppressor or regulatory mechanism observed during pregnancy. Rossant and colleagues first noted the importance of local immune regulation. They used the *Mus musculus*: *Mus caroli* system (for more details, see reference [56]). They showed that the transfer of *M musculus* eggs into *M caroli* is always successful. In contrast, there is almost a constant time schedule for failure of *M caroli* embryos that have been transferred to the *M musculus* uterus. In such a case, cotransferred, adjacent *M musculus* embryos do survive, whereas all the *M caroli* embryos die from almost the same program. A strong immune infiltrate consisting of cytotoxic T lymphocytes (CTL) and NK cells is observed around day 9.5. By day 13, the embryos are completely reabsorbed [57]. It was later shown that *M caroli* embryos can survive until delivery, provided that a *M musculus* placenta is used [58,59]. These results suggested that an important part of the *M caroli* placenta was responsible for provoking death and resorption of *M musculus* embryos [59].

This model first described these immunologically mediated abortions and revealed the immunologic element of the placenta. It emphasized the importance of local immune-regulatory events. Evidence has shown that specific immune suppression is directed toward the paternally encoded MHC antigens. With these antigens, the maternal T cells that recognize paternal antigens on the trophoblast are abrogated selectively. Animal studies have shown that pregnant mice have reduced numbers of T cells that recognize the paternally encoding MHC antigens. Following delivery, the immune system returns to normal. It seems that the mother's T cells assume a reversible tolerant state during pregnancy in which the cells no longer recognize paternal antigens [60]. In a study by Vacchio et al, T cells that were specific for fetal antigens were shown to decrease in number during pregnancy and remain low after delivery as the result of an encounter with fetal cells that expressed the appropriate MHC–peptide complex [61]. The authors and other investigators have proposed that this reversible tolerant state is caused directly by the induction of apoptosis of maternal activated T cells by the Fas/Fas ligand (FasL) system [62–66].

Fas/FasL system and immune privilege

Apoptosis is a continuous physiologic process that is associated with embryonic development, metamorphosis, maturation of the immune system, and cell turnover. In contrast to necrotic death, which results from a failure to control cellular homeostasis after injury, apoptosis is a highly regulated process that is

induced by specific stimuli [67]. The Fas/FasL system is a major pathway for the induction of apoptosis in cells and tissues [68]. CD95 (Fas), a type I membrane protein (45 kDa), belongs to the TNF receptor family. FasL, a type II membrane protein (37 kDa), belongs to the TNF and CD40 ligand family. Fas is widely expressed in many tissue types; in T and B lymphocytes, surface expression of Fas is constitutively low on resting cells and is enhanced following lymphocyte activation. In contrast to Fas expression, FasL expression is reported to be more restricted and often requires cell activation [68,69].

Fas/FasL interactions have been implicated as a mechanism for clonal deletion following *in vivo* exposure to super-antigen and peptides [70], thereby controlling T-cell expansion during immune responses [69] and killing by cytotoxic T cells [71]. Studies using either T-cell hybridomas or peripheral T cells have demonstrated that T-cell receptor-induced apoptosis of activated T cells occurs by way of a Fas/FasL-mediated autocrine or paracrine pathway. Such Fas/FasL-induced apoptosis has a central role in the clonal deletion of autoreactive T cells in the peripheral lymphoid organs and in the elimination of activated T cells following an immune response. Data obtained from rodents demonstrate that other tissues, such as the eye, brain, and testis, express FasL [13,72,73]. In these tissues, the Fas/FasL system induces apoptosis of activated lymphocytes and confers the characteristics of an immune-privileged site [13].

Fas/FasL system and implantation

Soon after implantation, invasive populations of extravillous trophoblast cells attach to, and interdigitate into, the extracellular spaces of the endometrium [74]. The endpoint of this invasive behavior is the penetration of maternal spiral arteries within the uterus [75]. Abnormalities in this invasive process have been correlated with early and midtrimester pregnancy loss, preeclampsia, eclampsia, and intrauterine growth retardation [76]. As discussed earlier, the maternal immune system at this stage of implantation is not indifferent to the presence of paternal alloantigens in the fetus. Dietl and colleagues introduced the notion of intradecidual T-cell tolerance toward fetal antigens; they observed that during early normal pregnancy, intradecidual T cells lack immunohistochemically detectable amounts of T-cell-receptor molecules yet express normal amounts of CD3 molecules [77]. Tafuri and co-workers followed the fate of T cells reactive to paternal alloantigens using a mouse that was transgenic for a T-cell receptor that recognized the MHC antigen H-2kb [60]. Mice bearing a kb-positive conceptus had reduced numbers of kb-reactive T cells. T-cell responsiveness was restored after delivery. Jiang and Vacchio showed that the deletion of T-cell clones that recognize paternal antigens undergo clonal deletion in ways that resemble mechanisms of peripheral tolerance. They also showed that one of the mechanisms is mediated by Fas/FasL and takes place at the maternal–fetal interface [61].

Jiang and Vacchio showed that the placental trophoblast could induce Fas-mediated cell death of T cells that recognize fetal antigens in manner that is similar to the process described for peripheral tolerance. In their experiments,

trophoblast cells in mice induced cell death in Fas-positive (2B4.11) T-cell lines but not in Fas-negative (d11S) T-cell lines; however, 2B4.11 was not killed by trophoblast cells that were isolated from placentas homozygous for the generalized lymphoproliferative disease (*gld*) mutation and therefore expressed non-functional FasL. Similarly, in the authors' studies of the human placenta, they have described the presence of FasL in the villi cytotrophoblast and in the invasive or extravillous trophoblast; the surrounding immune cells were Fas positive and presented characteristics of apoptosis [62]. Human trophoblast cells in culture induces apoptosis of Fas-positive Jurkat cells [62]. These findings are consistent with the proposal of clonal deletion as a mechanism of tolerance induction during pregnancy, and they support a role for Fas-mediated death signals in this process [62,64–66,78–81].

FasL: A double-edged sword

Attempts to use FasL to delete inflammatory or tumor cells have generated surprising results. FasL can protect and damage self-tissues [66]. Although over-expression of FasL in inflamed joints, certain tumors, and transplants can confer immune privilege, FasL expression in the thyroid glands, pancreatic islets, and other types of tumors can cause tissue injury and inflammation [82–85]. These data suggest that FasL could have beneficial and detrimental effects on self-tissues. In humans, FasL is expressed in the trophoblast throughout the entire pregnancy [81,86]. Fas expression has been shown in the chorion, amnion, and decidua of fetal membranes at term [63,87,88]. The authors' studies in reproductive tissues have shown that the expression of Fas and FasL are related mainly to tissue remodeling and involution [89,90]. The appearance of Fas and FasL in the placenta and membranes suggest a role for Fas/FasL signaling in apoptosis and in remodeling of the fetal membrane and trophoblast cells. Whether the Fas/FasL system is protective or apoptotic is not known. The authors have shown that the microenvironment at the implantation site influences FasL expression [91] and can act on the trophoblast in two directions: (1) the promotion of cytotoxicity by FasL (immune protection) or (2) the regulation of Fas sensitivity on the trophoblast (apoptosis). The success of implantation depends on which pathway becomes activated [92].

Role of the innate immune system in pregnancy

During normal pregnancy, several cellular components of the innate immune system are found at the site of implantation. From the first trimester onward, circulating monocytes and granulocytes increase in number and acquire an activated phenotype. This evidence suggests that the innate immune system is not indifferent to the fetus and may have a role in host protection to infections and in fetomaternal immune adjustment. In the following section, the possible roles of cells of the innate immune system during pregnancy are discussed.

Natural killer cells and implantation

Implantation involves the invasion of modified uterine mucosa, known as the decidua, by fetal-derived trophoblast cells, which then form the placenta. In the human decidua, NK cells are seen in close proximity to the invading extravillous trophoblast [93]. For many years, the role of these cells at the implantation site has been a puzzle in the field of reproductive immunology. Animal studies have suggested a supportive role for NK cells in pregnancy through production of cytokines that may stimulate placental growth [94–97]. This finding can be seen in the deleterious effect that the depletion of NK cells has on implantation [95,98]. Uterine NK cells may have a key role in the immunology of implantation and in the regulation of decidualization; therefore, discerning their biology has become an important aspect for understanding the immunology of implantation.

Natural killer cells and trophoblast protection

As discussed previously, uterine invasion by genetically distinct trophoblast cells requires a lack of recognition by the maternal immune system. Several investigators have proposed that such allogeneic tolerance may be assisted by uterine NK cells [21]. Extravillous trophoblast cells have been shown to express HLA-C [93], HLA-G [99,100], and HLA-E [24]. Expression of these MHC molecules, particularly HLA-G, may protect the trophoblast against NK cell cytotoxicity and promote maternal immune-tolerance by suppressing local T-cell responses (reviewed in references[101,102]). Such protection of the trophoblast against NK cell-mediated lysis seems to occur through the ability of the HLA class I molecules expressed by the trophoblast to trigger more inhibitory, rather than stimulatory, NK cell signals. Uterine NK cells express subtypes of killer inhibitory receptors and killer activator receptors, which recognize and bind all HLA-C alleles. These killer inhibitory receptors also bind directly to HLA-G [103]. Uterine NK cells also express CD94 and NKG2, which, as a heterodimer, recognize and bind HLA-E; this interaction results in inhibition of NK cell cytotoxicity [21]. Expression of these HLA molecules may confer protection against NK cell-mediated lysis, allowing trophoblastic invasion of the decidua and maintaining cytotoxicity against invading pathogens.

A new aspect of the physiology of NK cells is their role in the support of normal pregnancy. Although current hypotheses of successful implantation and pregnancy in humans propose a bias toward a T_H2 cytokine microenvironment (discussed earlier; see also Fig. 1), studies in mice have implicated a role for uterine NK cell-derived $IFN-\gamma$ in the formation and maintenance of the decidua [104,105]. NK cell production of $IFN-\gamma$ can be maintained in the absence of lytic activity [106]. In the nonpregnant human endometrium, $IFN-\gamma$ is produced throughout the menstrual cycle, and its major source is neutrophils. Evidence of a positive role for $IFN-\gamma$ in human implantation is lacking, however, the production of other NK cell-derived cytokines may be important.

Abnormal functions of NK cells in women with recurrent spontaneous abortions have been postulated. Yamamoto and colleagues showed that, compared with normal pregnancies, recurrent spontaneous abortions have increased NK cell cytotoxicity and an abnormally high decidual CD56⁺:CD16[−] ratio [107]. Although the new understanding of NK cells at the implantation site has improved, many questions remain unanswered.

Neutrophils and pregnancy

Neutrophils are a crucial cellular component of the innate immune system. On activation, neutrophils undergo an intense respiratory burst and release degradative enzymes and inflammatory mediators by degranulation [108]. During the mid- to late secretory phase of the normal menstrual cycle, neutrophil numbers in the fallopian tubes and endometrium significantly increase [109,110], and these cells are believed to be involved in the endometrial breakdown associated with menses [111]. The role of neutrophils in normal pregnancy is still unclear, although the presence and activation of neutrophils has been associated with pathologic conditions of pregnancy, such as preeclampsia [112].

Preeclampsia is a pregnancy-associated disease that is characterized by hypertension, proteinuria, and edema, which can have fatal consequences. Although the cause and mechanism of pathogenesis is unknown, a strong immunologic element has been suggested [112]. Endothelial cell dysfunction has been observed in preeclampsia [113], and neutrophils have been implicated as an important mediator of excessive endothelial activation [114].

Romero and colleagues have shown that normal pregnancy is associated with phenotypic changes in peripheral blood monocytes and granulocytes and with increased intracellular reactive oxygen species under basal conditions and following stimulation with bacterial N-formyl-methionyl leucyl-phenylalanine (fMLP) [115,116]. They also demonstrated that acute maternal infections were associated with marked phenotypic and metabolic changes of these leukocytes compared with changes detected in leukocytes from normal pregnancies [112]. In other studies, mild neutrophilia and moderate systemic neutrophil activation were observed during normal pregnancy [117], however, increased neutrophilia is found in patients with preeclampsia, which correlates with disease severity [118–120]. Such high levels of circulating neutrophils may be explained by the observation that peripheral blood neutrophils from patients with preeclampsia are resistant to apoptosis [117]. In support of this finding, Fas and FasL expression by peripheral blood neutrophils is reduced in preeclamptic patients [121], and this in part may account for the elevated levels of circulating soluble Fas [88, 121–123].

Apoptosis, macrophages, cytokines, and the Fas/FasL system

During implantation, the uterine endometrium undergoes morphologic and physiologic changes to accommodate the embryo. This process of accommoda-

tion implies that the embryo has to degrade the endometrial extracellular matrix to invade the uterus in species with hemochorial placentation. Apoptosis has been observed in endometrial epithelial cells at the embryo implantation site, and it is believed to be caused by loss of contact with the endometrial extracellular matrix. Those apoptotic cells are removed by trophoblast or maternal macrophages.

Apoptotic cells express surface molecules that direct recognition, engulfment, and degradation by phagocytes [124]. This clearance process represents an active and coordinate event that sends specific signals to the remaining cells for either survival or death [125]. A signal from a macrophage to the wrong cell type may have profound consequences for the normal physiology of the tissue.

Macrophages are one of the major cell types in the maternal and fetal compartments of the uteroplacental unit [126]. During the first weeks of implantation in humans, macrophages are found in high numbers in the maternal decidua and in tissues in close proximity to the placenta [127]. Similarly in rodents, macrophages accumulate at or near the implantation site [128]. The dense macrophage infiltration at the maternal–fetal interface suggests that these cells are involved in specific pregnancy-associated functions that include functions other than their usual immunologic tasks [129]. Hunt and co-workers implied that maternal macrophages assist in the tissue remodeling that is necessary to accommodate expansion of extraembryonic tissue [130], however, macrophages are scavengers of dying cells and also actively orchestrate apoptosis of unwanted cells during tissue remodeling [124]. Macrophages synthesize and secrete cytokines and growth factors, which govern the local cellular and tissue interactions [127,130]. Numerous findings propose that the capacity for macrophages to influence cell death is regulated by the extent of uptake of apoptotic cells [124]. The cytolysis of tumor cells by activated macrophages is inhibited by the ingestion of apoptotic but not necrotic cells [131]. Similarly, during embryo implantation, uterine epithelial cells surrounding the blastocyst undergo apoptosis and may form an anti-inflammatory environment by increasing T_H2 -type cytokines. This action may explain the surprising cohabitation of macrophages and trophoblast cells at the implantation site. As stated earlier, the type of cytokines that is produced by a macrophage depends on its activation state [132]. The uptake of apoptotic cells suppresses activated macrophages from secreting proinflammatory cytokines, such as $TNF-\alpha$ and $IFN-\gamma$ [133] and promotes the release of T_H2 type, anti-inflammatory, and immunosuppressive cytokines [133].

This anti-inflammatory action of phagocytic clearance of apoptotic cells may be perturbed in disease processes. Antiphospholipid antibodies in apoptotic cells may bind to surface-expressed Fc receptors on macrophages, resulting in secretion of proinflammatory cytokines such as $TNF-\alpha$ [134]. During pregnancy, cytokine production by macrophages and other cells at the maternal–fetal interface may be altered drastically [135]. The results of the authors' studies are in concert with this concept and indicate that enhanced levels of proinflammatory macrophage products increase Fas expression and enhance trophoblast sensitivity to Fas-mediated apoptosis [92].

Summary

Important reproductive events, including implantation, trophoblast invasion, placental development, and immune protection, are regulated by the immune system at the maternal–fetal interface. This maternal–fetal immune interaction is complex, and it is difficult to perceive the whole process based on one mechanism of action. There are multiple mechanisms for the induction of peripheral and local tolerance during pregnancy that prevent fetal rejection while maintaining a strong and active immune surveillance against viral or bacterial infections, which may endanger the successful outcome and survival of the infant.

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References

- [1] Colbern GT, Main EK. Immunology of the maternal-placental interface in normal pregnancy. *Semin Perinatol* 1991;15:196–205.
- [2] Mor G, Naftolin F. Oestrogen, menopause and the immune system. *The Journal of the British Menopause Society* 1998;4:4–8.
- [3] Aschkenazi S, Naftolin F, Mor G. Menopause, sex hormones and the immune system. *Menopause Management* 2000;9:6–13.
- [4] Weetman AP. The immunology of pregnancy. *Thyroid* 1999;9:643–6.
- [5] Medzhitov R, Janeway C Jr. Innate immunity. *N Engl J Med* 2000;343:338–44.
- [6] Mushegian A, Medzhitov R. Evolutionary perspective on innate immune recognition. *J Cell Biol* 2001;155:705–10.
- [7] Sacks G, Sargent I, Redman C. Innate immunity in pregnancy. *Immunol Today* 2000;21:200–1.
- [8] Medawar PB. Immunity to homologous grafted skin: III. The fate of skin homografts transplanted to the brain, to subcutaneous tissue, and to the anterior chamber of the eye, *Br J Exp Pathol* 1948;29:58–69.
- [9] Cserr HF, Knopf PM. Cervical lymphatics, the blood-brain barrier and the immunoreactivity of the brain: a new view. *Immunol Today* 1992;13:507–12.
- [10] Bonney E, Matzinger P. The maternal immune system's interaction with circulating fetal cells. *J Immunol* 1997;158:40–7.
- [11] Story CM, Mikulska JE, Simister NE. A major histocompatibility complex class I-like Fc receptor cloned from human placenta: possible role in transfer of immunoglobulin G from mother to fetus. *J Exp Med* 1994;180:2377–81.
- [12] Streilein J. New insights into immunologic tolerance. *Transplant Proc* 1996;28:2066–70.
- [13] Bechmann I, Mor G, Nilsen J, Eliza M, Nitsch R, Naftolin F. FasL (CD95L, Apo1L) is expressed in the normal rat and human brain—evidence for the existence of an immunological brain barrier. *Glia* 1999;27:62–74.
- [14] Formby B. Immunologic response in pregnancy: its role in endocrine disorders of pregnancy and influence on the course of maternal autoimmune diseases. *Endocrinol Metab Clin North Am* 1995;24:187–205.
- [15] Szekeres-Bartho J, Varga P, Kinsky R, Chaouat G. Progesterone-mediated immunosuppression and the maintenance of pregnancy. *Res Immunol* 1990;141:175–81.
- [16] Szekeres-Bartho J, Szabo J, Kovacs L. Alteration of lymphocyte reactivity in pregnant women

- treated with the progesterone receptor inhibitor ZK 98734. *Am J Reprod Immunol* 1989;21:46–9.
- [17] Ehring GR, Kerschbaum HH, Eder C, Neben AL, Fanger CM, Khoury RM, et al. A non-genomic mechanism for progesterone-mediated immunosuppression: inhibition of K⁺ channels, Ca²⁺ signaling, and gene expression in T lymphocytes. *J Exp Med* 1998;188:1593–602.
- [18] Harrison FA, Heap RB, Poyser NL. Production, chemical composition and prostaglandin F-2 α content of uterine fluid in pregnant sheep. *J Reprod Fertil* 1976;48:61–7.
- [19] Szekeres-Bartho J, Reznikoff-Etievant MF, Varga P, Pichon MF, Varga Z, Chaouat G. Lymphocytic progesterone receptors in normal and pathological human pregnancy. *J Reprod Immunol* 1989;16:239–47.
- [20] Szekeres-Bartho J, Varga P, Pejtsik B. ELISA test for the detection of an immunological blocking factor in human pregnancy serum. *J Reprod Immunol* 1989;16:19–29.
- [21] Loke YW, King A. Immunology of implantation. *Baillieres Best Pract Res Clin Obstet Gynaecol* 2000;14:827–37.
- [22] Kovats S, Main E, Librach C. HLA-G expressed in human trophoblast. *Science* 1990;248:220–3.
- [23] Schmidt C, Orr H. Maternal/fetal interactions: the roles of the MHC class I molecule HLA-G. *Crit Rev Immunol* 1994;13:207–24.
- [24] King A, Allan DS, Bowen M, Powis SJ, Joseph S, Verma S, et al. HLA-E is expressed on trophoblast and interacts with CD94/NKG2 receptors on decidual NK cells. *Eur J Immunol* 2000;30:1623–31.
- [25] Ellis S. HLA G: at the interface. *Am J Reprod Immunol* 1990;23:84–6.
- [26] van der Ven K, Skrabin S, Ober C, Krebs D. HLA-G polymorphisms: ethnic differences and implications for potential molecule function. *Am J Reprod Immunol* 1998;40:145–57.
- [27] van der Ven K, Skrabin S, Engels G, Krebs D. HLA-G polymorphisms and allele frequencies in Caucasians. *Hum Immunol* 1998;59:302–12.
- [28] Le Bouteiller P, Solier C, Proll J, Aguerre-Girr M, Fournel S, Lenfant F. Placental HLA-G protein expression in vivo: where and what for? *Hum Reprod Update* 1999;5:223–33.
- [29] van der Ven K, Pfeiffer K, Skrabin S. HLA-G polymorphisms and molecule function—questions and more questions—a review. *Placenta* 2000;21(Suppl):S86–92.
- [30] Chatterjee-Hasrouni S, Lala PK. Localization of paternal H-2K antigens on murine trophoblast cells in vivo. *J Exp Med* 1982;155:1679–89.
- [31] Lala PK, Chatterjee-Hasrouni S, Kearns M, Montgomery B, Colavincenzo V. Immunobiology of the feto-maternal interface. *Immunol Rev* 1983;75:87–116.
- [32] Mellor AL, Munn DH. Immunology at the maternal-fetal interface: lessons for T cell tolerance and suppression. *Annu Rev Immunol* 2000;18:367–91.
- [33] Wegmann TG, Guilbert LJ. Immune signaling at the maternal-fetal interface and trophoblast differentiation. *Dev Comp Immunol* 1992;16:425–30.
- [34] Fisher SJ. The placenta dilemma. *Semin Reprod Med* 2000;18:321–6.
- [35] McMaster MT, Bass KE, Fisher SJ. Human trophoblast invasion: autocrine control and paracrine modulation. *Ann N Y Acad Sci* 1994;734:122–31.
- [36] Cross JC, Werb Z, Fisher SJ. Implantation and the placenta: key pieces of the development puzzle. *Science* 1994;266:1508–18.
- [37] Rice A, Chard T. Cytokines in implantation. *Cytokine Growth Factor Rev* 1998;9:287–96.
- [38] Wilder RL. Hormones, pregnancy, and autoimmune diseases. *Ann N Y Acad Sci* 1998;840:45–50.
- [39] Elenkov IJ, Hoffman J, Wilder RL. Does differential neuroendocrine control of cytokine production govern the expression of autoimmune diseases in pregnancy and the postpartum period? *Mol Med Today* 1997;3:379–83.
- [40] Chaouat G, Cayol V, Mairovitz V, Dubanchet S. 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* 1999;42:1–13.

- [41] Wegmann TG, Lin H, Guilbert L, Mosmann TR. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon? *Immunol Today* 1993;14:353–6.
- [42] Tranchot-Diallo J, Gras G, Parnet-Mathieu F, Benveniste O, Marce D, Roques P, et al. Modulations of cytokine expression in pregnant women. *Am J Reprod Immunol* 1997;37:215–26.
- [43] Bennett WA, Lagoo-Deenadayalan S, Stopple JA, Barber WH, Hale E, Brackin MN, et al. Cytokine expression by first-trimester human chorionic villi. *Am J Reprod Immunol* 1998;40: 309–18.
- [44] Bennett WA, Lagoo-Deenadayalan S, Brackin MN, Hale E, Cowan BD. Cytokine expression by models of human trophoblast as assessed by a semiquantitative reverse transcription-polymerase chain reaction technique. *Am J Reprod Immunol* 1996;36:285–94.
- [45] Lea RG, Riley SC, Antipatis C, Hannah L, Ashworth CJ, Clark DA, et al. Cytokines and the regulation of apoptosis in reproductive tissues: a review. *Am J Reprod Immunol* 1999;42:100–9.
- [46] Hanna N, Hanna I, Hleb M, Wagner E, Dougherty J, Balkundi D, et al. Gestational age-dependent expression of IL-10 and its receptor in human placental tissues and isolated cytotrophoblasts. *J Immunol* 2000;164:5721–8.
- [47] Jokhi PP, King A, Loke YW. Cytokine production and cytokine receptor expression by cells of the human first trimester placental-uterine interface. *Cytokine* 1997;9:126–37.
- [48] Casey ML, Cox SM, Beutler B, Milewich L, MacDonald PC. Cachectin/tumor necrosis factor- α formation in human decidua. Potential role of cytokines in infection-induced preterm labor. *J Clin Invest* 1989;83:430–6.
- [49] Casey ML, Cox SM, Word RA, MacDonald PC. Cytokines and infection-induced preterm labour. *Reprod Fertil Dev* 1990;2:499–509.
- [50] Bobe P, Kiger N. Immunogenetic studies of spontaneous abortion in mice: III. Non-H-2 antigens and gestation. *J Immunogenet* 1989;16:223–31.
- [51] Haddad EK, Duclos AJ, Lapp WS, Baines MG. Early embryo loss is associated with the prior expression of macrophage activation markers in the decidua. *J Immunol* 1997;158:4886–92.
- [52] Chaouat G, Menu E, de Smedt D, Khrihnan L, Hui L, Assal Meliani A, et al. The emerging role of IL-10 in pregnancy. *Am J Reprod Immunol* 1996;35:325–9.
- [53] Piccinni MP, Beloni L, Livi C, Maggi E, Scarselli G, Romagnani S. Defective production of both leukemia inhibitory factor and type 2 T-helper cytokines by decidual T cells in unexplained recurrent abortions. *Nat Med* 1998;4:1020–4.
- [54] Simon C, Moreno C, Remohi J, Pellicer A. Cytokines and embryo implantation. *J Reprod Immunol* 1998;39:117–31.
- [55] Simon C, Martin JC, Meseguer M, Caballero-Campo P, Valbuena D, Pellicer A. Embryonic regulation of endometrial molecules in human implantation. *J Reprod Fertil* 2000;55(Suppl): 43–53.
- [56] Chaouat G. Placental infiltration of resorbing CBAXDBA/2 embryos. *J Reprod Immunol* 1986;134:1–5.
- [57] Croy BA, Rossant J, Clark DA. Recruitment of cytotoxic cells by ectopic grafts of xenogeneic, but not allogeneic, trophoblast. *Transplantation* 1984;37:84–90.
- [58] Rossant J, Mauro V, Croy B. Importance of trophoblast genotype for survival of interspecific murine chimeras. *Journal of Embryology and Experimental Morphology* 1982;69:141–9.
- [59] Rossant J, Croy B, Clark D, Chapman V. Interspecific hybrids and chimeras in mice. *J Exp Zool* 1983;288:223–33.
- [60] Tafuri A, Alferink J, Moller P, Hammerling J, Arnold B. T cell awareness of paternal alloantigens during pregnancy. *Science* 1995;270:630–3.
- [61] Jiang SP, Vacchio MS. Multiple mechanisms of peripheral T cell tolerance to the fetal “allograft”. *J Immunol* 1998;160:3086–90.
- [62] Mor G, Gutierrez L, Eliza M, Kahyaoglu F, Arici A. Fas-Fas ligand system induced apoptosis in human placenta and gestational trophoblastic disease. *Am J Reprod Immunol* 1998;40: 89–95.
- [63] Bamberger A, Schulte H, Thuncke I, Erdmann I, Bamberger C, Asa S. Expression of the

- apoptosis-inducing Fas ligand (FasL) in human first and third trimester placenta and choriocarcinoma cells. *J Clin Endocrinol Metab* 1997;82:3173–5.
- [64] Rogers AM, Boime I, Connolly J, Cook JR, Russell JH. Maternal-fetal tolerance is maintained despite transgene-driven trophoblast expression of MHC class I, and defects in Fas and its ligand. *Eur J Immunol* 1998;28:3479–87.
- [65] Huppertz B, Frank HG, Kingdom JC, Reister F, Kaufmann P. Villous cytotrophoblast regulation of the syncytial apoptotic cascade in the human placenta. *Histochem Cell Biol* 1998;110:495–508.
- [66] Uckan D, Steele A, Cherry D, Wang BY, Chamizo W, Koutsonikolis A, et al. Trophoblasts express Fas ligand: a proposed mechanism for immune privilege in placenta and maternal invasion. *Mol Hum Reprod* 1997;3:655–62.
- [67] Wyllie A, Jf K, Currie A. Cell death: the significance of apoptosis. *Int Rev Cytol* 1980;68:251–306.
- [68] Nagata S. Fas and Fas ligand: a death factor and its receptor. *Adv Immunol* 1994;57:129–35.
- [69] Nagata S, Golstein P. The Fas death factor. *Science* 1995;267:1449–56.
- [70] Mogil R, Radvanyi L, Gonzalez-Quintal R, Miller R, Mills G, Theofilopoulos A, et al. Fas (CD95) participates in peripheral T cell deletion and associated apoptosis in vivo. *Int Immunol* 1995;7:1451–8.
- [71] Rouvier E, Luciani M, Golstein P. Fas involvement in Ca(2+)-independent T cell-mediated cytotoxicity. *J Exp Med* 1993;177:195–200.
- [72] Bellgrau D, Gold D, Selawry H, Moore J, Franzusoff A, Duke R. A role for CD95 ligand in preventing graft rejection. *Science* 1995;377:630–2.
- [73] Griffith T, Brunner T, Fletcher S, Gree D, Ferguson T. Fas ligand induces apoptosis as a mechanism of immune privilege. *Science* 1995;270:1187–92.
- [74] Kliman H. Trophoblast infiltration. *Reproductive Medicine Review* 1994;3:137–57.
- [75] Pijnenborg R, Robertson W, Brosens I, Dixon G. Trophoblast invasion and the establishment of haemochorial placentation in man and laboratory animals. *Placenta* 1981;2:71–91.
- [76] Pijnenborg R, Anthony J, Davey D, Rees A, Tiltman A, Vercruysse L, et al. Placental bed spiral arteries in the hypertensive disorders of pregnancy. *Br J Obstet Gynaecol* 1991;98:648–55.
- [77] Dietl J, Horny H, Ruck P, Marzusch K, Kaisersling E, Griesser H, et al. Intradecidual T lymphocytes lack immunohistochemical detectable T cell receptors. *Am J Reprod Immunol* 1990;24:33–6.
- [78] Guller S, LaChapelle L. The role of placental Fas ligand in maintaining immune privilege at maternal-fetal interfaces. *Semin Reprod Endocrinol* 1999;17:39–44.
- [79] Guller S. Role of Fas ligand in conferring immune privilege to non-lymphoid cells. *Ann N Y Acad Sci* 1997;828:268–72.
- [80] Kauma SW, Huff TF, Hayes N, Nilkaeo A. Placental Fas ligand expression is a mechanism for maternal immune tolerance to the fetus. *J Clin Endocrinol Metab* 1999;84:2188–94.
- [81] Hammer A, Dohr G. Expression of Fas-ligand in first trimester and term human placental villi. *J Reprod Immunol* 2000;46:83–90.
- [82] Giordano C, Stassi G, Demaria R, Todaro M, Richiusa P, Papoff G, et al. Potential involvement of Fas and its ligand in the pathogenesis of hashimotos thyroiditis. *Science* 1997;275:960–3.
- [83] Paolieri F, Salmaso C, Battifora M, Montagna P, Pesce G, Bagnasco M, et al. Possible pathogenetic relevance of interleukin-1 β in “destructive” organ-specific autoimmune disease (Hashimoto’s thyroiditis). *Ann N Y Acad Sci* 1999;876:221–8.
- [84] Xerri L, Devilard E, Hassoun J, Mawas C, Birg F. Fas ligand is not only expressed in immune privileged human organs but is also coexpressed with Fas in various epithelial tissues. *Mol Pathol* 1997;50:87–91.
- [85] Mitsiades N, Poulaki V, Tseleni-Balafouta S, Chrousos GP, Koutras DA. Fas ligand expression in thyroid follicular cells from patients with thionamide-treated Graves’ disease. *Thyroid* 2000;10:527–32.
- [86] Balkundi DR, Hanna N, Hileb M, Dougherty J, Sharma S. Labor-associated changes in Fas ligand expression and function in human placenta. *Pediatr Res* 2000;47:301–8.

- [87] Runic R, Lockwood CJ, LaChapelle L, Dipasquale B, Demopoulos RI, Kumar A, et al. Apoptosis and Fas expression in human fetal membranes. *J Clin Endocrinol Metab* 1998;83:660–6.
- [88] Hsu C, Harirah H, Basherra H, Mor G. Serum soluble Fas levels in preeclampsia. *Obstet Gynecol* 2001;97:530–2.
- [89] Song J, Sapi E, Brown W, Nilsen J, Tartaro K, Kacinski B, et al. Expression of Fas and Fas ligand during pregnancy, lactation and involution and its potential role during mammary gland remodeling. *J Clin Invest* 2000;106:1209–24.
- [90] Sapi E, Brown W, Aschkenazi S, Tartaro K, Lim C, Munoz A, et al. Regulation of Fas ligand expression by estrogen in normal ovary. *J Soc Gynecol Investig* 2002;9:187–94.
- [91] Garcia-Velasco J, Arici A, Naftolin F, Zreick T, Mor G. Macrophage-derived growth factors regulate FasL expression in endometrial stromal cells. *Mol Hum Reprod* 1998;5:642–50.
- [92] Aschkenazi S, Straszewski S, Verwer K, Foellmer H, Rutherford T, Mor G. Differential regulation and function of the Fas/Fas ligand system in human trophoblast cells. *Biol Reprod* 2002;66:1853–61.
- [93] King A, Loke YW, Chaouat G. NK cells and reproduction. *Immunol Today* 1997;18:64–6.
- [94] Croy BA, Guimond MJ, Luross J, Hahnel A, Wang B, van den Heuvel M. Uterine natural killer cells do not require interleukin-2 for their differentiation or maturation. *Am J Reprod Immunol* 1997;37:463–70.
- [95] Guimond M, Wang B, Croy BA. Immune competence involving the natural killer cell lineage promotes placental growth. *Placenta* 1999;20:441–50.
- [96] Greenwood JD, Minhas K, di Santo JP, Makita M, Kiso Y, Croy BA. Ultrastructural studies of implantation sites from mice deficient in uterine natural killer cells. *Placenta* 2000;21:693–702.
- [97] Croy BA, Di Santo JP, Greenwood JD, Chantakru S, Ashkar AA. Transplantation into genetically alymphoid mice as an approach to dissect the roles of uterine natural killer cells during pregnancy—a review. *Placenta* 2000;21(Suppl):S77–80.
- [98] Miller JS. The biology of natural killer cells in cancer, infection, and pregnancy. *Exp Hematol* 2001;29:1157–68.
- [99] King A, Hiby SE, Gardner L, Joseph S, Bowen JM, Verma S, et al. Recognition of trophoblast HLA class I molecules by decidual NK cell receptors. *Placenta* 2000;21(Suppl):S81–5.
- [100] Bainbridge D, Ellis S, Le Bouteiller P, Sargent I. HLA-G remains a mystery. *Trends Immunol* 2001;22:548–52.
- [101] Solier C, Mallet V, Lenfant F, Bertrand A, Huchencq A, Le Bouteiller P. HLA-G unique promoter region: functional implications. *Immunogenetics* 2001;53:617–25.
- [102] Le Bouteiller P, Solier C. Is antigen presentation the primary function of HLA-G? *Microbes Infect* 2001;3:323–32.
- [103] Pazmany L, Mandelboim O, Vales-Gomez M, Davis DM, Becker TC, Reyburn HT, et al. Human leucocyte antigen-G and its recognition by natural killer cells. *J Reprod Immunol* 1999;43:127–37.
- [104] Ashkar AA, Croy BA. Functions of uterine natural killer cells are mediated by interferon γ production during murine pregnancy. *Semin Immunol* 2001;13:235–41.
- [105] Ashkar AA, Di Santo JP, Croy BA. Interferon gamma contributes to initiation of uterine vascular modification, decidual integrity, and uterine natural killer cell maturation during normal murine pregnancy. *J Exp Med* 2000;192:259–70.
- [106] Smith KD, Kurago ZB, Lutz CT. Conformational changes in MHC class I molecules: antibody, T-cell receptor, and NK cell recognition in an HLA-B7 model system. *Immunol Res* 1997;16:243–59.
- [107] Yamamoto T, Takahashi Y, Kase N, Mori H. Proportion of CD56+3+ T cells in decidual and peripheral lymphocytes of normal pregnancy and spontaneous abortion with and without history of recurrent abortion. *Am J Reprod Immunol* 1999;42:355–60.
- [108] Witko-Sarsat V, Rieu P, Descamps-Latscha B, Lesavre P, Halbwachs-Mecarelli L. Neutrophils: molecules, functions and pathophysiological aspects. *Lab Invest* 2000;80:617–53.
- [109] Stern JE, Givan AL, Gonzalez JL, Harper DM, White HD, Wira CR. Leukocytes in the cervix: a quantitative evaluation of cervicitis. *Obstet Gynecol* 1998;91:987–92.

- [110] Mueller MD, Lebovic DI, Garrett E, Taylor RN. Neutrophils infiltrating the endometrium express vascular endothelial growth factor: potential role in endometrial angiogenesis. *Fertil Steril* 2000;74:107–12.
- [111] Lathbury LJ, Salamonsen LA. In-vitro studies of the potential role of neutrophils in the process of menstruation. *Mol Hum Reprod* 2000;6:899–906.
- [112] Naccasha N, Gervasi MT, Chaiworapongsa T, Berman S, Yoon BH, Maymon E, et al. Phenotypic and metabolic characteristics of monocytes and granulocytes in normal pregnancy and maternal infection. *Am J Obstet Gynecol* 2001;185:1118–23.
- [113] Roberts JM, Cooper DW. Pathogenesis and genetics of pre-eclampsia. *Lancet* 2001;357:53–6.
- [114] Clark P, Boswell F, Greer IA. The neutrophil and preeclampsia. *Semin Reprod Endocrinol* 1998;16:57–64.
- [115] Gervasi MT, Chaiworapongsa T, Naccasha N, Blackwell S, Yoon BH, Maymon E, et al. Phenotypic and metabolic characteristics of maternal monocytes and granulocytes in preterm labor with intact membranes. *Am J Obstet Gynecol* 2001;185:1124–9.
- [116] Maymon E, Romero R, Chaiworapongsa T, Kim JC, Berman S, Gomez R, et al. Value of amniotic fluid neutrophil collagenase concentrations in preterm premature rupture of membranes. *Am J Obstet Gynecol* 2001;185:1143–8.
- [117] von Dadelszen P, Watson RW, Noorwali F, Marshall JC, Parodo J, Farine D, et al. Maternal neutrophil apoptosis in normal pregnancy, preeclampsia, and normotensive intrauterine growth restriction. *Am J Obstet Gynecol* 1999;181:408–14.
- [118] Lurie S, Frenkel E, Tuvbin Y. Comparison of the differential distribution of leukocytes in preeclampsia versus uncomplicated pregnancy. *Gynecol Obstet Invest* 1998;45:229–31.
- [119] Sacks GP, Studena K, Sargent K, Redman CW. Normal pregnancy and preeclampsia both produce inflammatory changes in peripheral blood leukocytes akin to those of sepsis. *Am J Obstet Gynecol* 1998;179:80–6.
- [120] Redman CW, Sacks GP, Sargent IL. Preeclampsia: an excessive maternal inflammatory response to pregnancy. *Am J Obstet Gynecol* 1999;180:499–506.
- [121] Kuntz TB, Christensen RD, Stegner J, Duff P, Koenig JM. Fas and Fas ligand expression in maternal blood and in umbilical cord blood in preeclampsia. *Pediatr Res* 2001;50:743–9.
- [122] Darmochwal-Kolarz D, Leszczynska-Gorzelak B, Rolinski J, Oleszczuk J. The expression and concentrations of Fas/APO-1 (CD95) antigen in patients with severe pre-eclampsia. *J Reprod Immunol* 2001;49:153–64.
- [123] Allaire AD, Ballenger KA, Wells SR, McMahon MJ, Lessey BA. Placental apoptosis in preeclampsia. *Obstet Gynecol* 2000;96:271–6.
- [124] Savill J, Fadok V. Corpse clearance defines the meaning of cell death. *Nature* 2000;407:784–8.
- [125] Duvall E, Wyllie AH, Morris RG. Macrophage recognition of cells undergoing programmed cell death. *Immunology* 1985;56:351–8.
- [126] De M, Wood GW. Influence of oestrogen and progesterone on macrophage distribution in the mouse uterus. *J Endocrinol* 1990;126:417–24.
- [127] Miller L, Hunt J. Sex steroids hormones and macrophage function. *Life Sci* 1996;59:1–14.
- [128] Tachi C, Tachi S. Macrophages and implantation. *Ann N Y Acad Sci* 1986;476:158–82.
- [129] Ben-Hur H, Gurevich P, Berman V, Tchanyshv R, Gurevich E, Zusman I. The secretory immune system as part of the placental barrier in the second trimester of pregnancy in humans. *In Vivo* 2001;15:429–35.
- [130] Hunt J. Cytokine networks in the uteroplacental unit: macrophages as pivotal regulatory cells. *J Reprod Immunol* 1989;16:1–17.
- [131] Reiter I, Krammer B, Schwamberger G. Differential effect of apoptotic versus necrotic tumor cells on macrophage antitumor activities. *J Immunol* 1999;163:1730–2.
- [132] Cavaillon JM. Cytokines and macrophages. *Biomed Pharmacother* 1994;48:445–53.
- [133] Voll RE, Herrmann M, Roth EA, Stach C, Kalden JR, Girkontaite I. Immunosuppressive effects of apoptotic cells. *Nature* 1997;390:350–1.

- [134] Manfredi AA, Rovere P, Galati G, Heltai S, Bozzolo E, Soldini L, et al. Apoptotic cell clearance in systemic lupus erythematosus: I. Opsonization by antiphospholipid antibodies. *Arthritis Rheum* 1998;41:205–14.
- [135] Guleria I, Pollard JW. The trophoblast is a component of the innate immune system during pregnancy. *Nat Med* 2000;6:589–93.