

IMMUNOLOGY OF NORMAL PREGNANCY

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Reproductive immunology has become a mature discipline. It had its birth in the field of transplantation immunology. Scientists assumed that the fetus was a transplant and would follow the laws of rejection or acceptance, but many of these theories and hypotheses were difficult to validate and had to be discarded. Today, sophisticated immunological methods have been used by scientists to define more clearly the mechanisms of immunoregulation during a normal pregnancy, a failing pregnancy, and during implantation failure in women with idiopathic infertility undergoing in vitro fertilization. It is becoming clear that the embryonic cell, which attaches the embryo to the uterine lining and eventually forms the placenta, initiates responses that stimulate a T helper 2 (Th-2) suppressive response (Fig. 1). Human leukocyte antigen (HLA) DQ A1 compatibility between the mother and the fetus results in the destruction of the embryo during the early stages of pregnancy.¹⁰¹ In this circumstance, compatibility seems to breed autoimmune cellular and humoral immune contempt that becomes more aggressive the greater the number of pregnancy failures the woman experiences. Figures 1 and 2 outline in simple form how the trophoblast chooses the immune responses that protect versus those that are cytotoxic and result in failure.

KEY CONCEPTS

The Immune System and the Trophoblast

1. The trophoblast expresses (1) a unique major histocompatibility complex (MHC) antigen, HLA-G, which is polymorphic,⁹⁸ and

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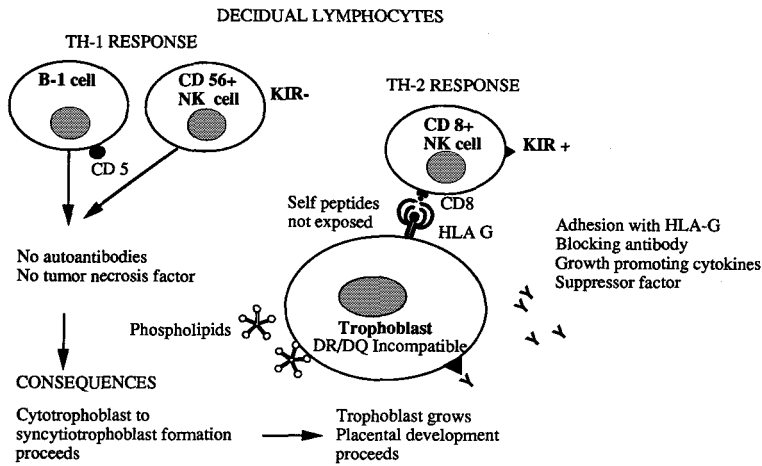


Figure 1. Maternal–fetal immunogenetic determinants of an alloimmune response during successful early pregnancy (T helper 2 response).

(2) other class I antigens that orchestrate many immune functions, including immune suppression and production of growth-promoting cytokines. This result renders the placenta a privileged organ, resistant to immune damage by lymphocytes, cytotoxic antibodies, and antigen–antibody complexes in most circumstances.

2. The trophoblast forms a physical barrier to most immune ef-

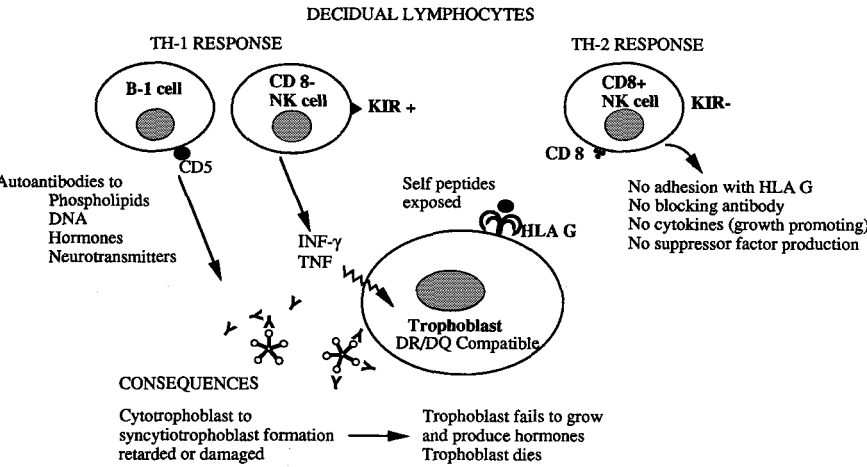


Figure 2. Maternal–fetal immunogenetic determinants of an autoimmune response during unsuccessful early pregnancy (T helper 1 response).

factors except IgG antibodies. These antibodies attach to Fc receptors on the placenta and are transported to the infant from the earliest stages of pregnancy onwards.

3. The trophoblast signals and recruits the migration of a family of lymphocytes to the uterine decidua. These serve a variety of growth-supporting functions. It also draws lymphocytes that are capable of liberating suppressive factors that restrain the activities of cytotoxic lymphocytes.
4. The trophoblast produces steroidal and protein hormones and a variety of proteins locally that are apoptotic, anti-inflammatory, immunosuppressive, immuno-directive and regulate gene expression of trophoblast and uterine cells.
5. The trophoblast induces a variety of antibodies in the mother that are capable of directing maternal immune reactivity to paternal alloantigens. These antibodies down regulate cytotoxic natural killer (NK) cells through Fab receptor binding to killer inhibitory (KIR) or activatory receptor (KAR).
6. The trophoblast also acts as an immunosorbent to bind antibodies that act as growth regulators and to establish an immune camouflage that blocks the efferent limb of the immunologic reflex arc.
7. In women whose pregnancies fail repeatedly and in couples with intractable infertility and in vitro fertilization failures, the critical features for the immune protection of the trophoblast are not fully established, and cellular and humoral autoimmune processes are initiated. As a result, the trophoblast is not allowed to heal in and to take over the alien maternal environment for the duration of pregnancy.

PLACENTAL COMPARTMENT AND CELL TYPES

To understand the immunology of pregnancy requires familiarity with the structure of the placenta and its cell types (Fig. 3). There are many trophoblast cell types in the placenta that initiate immune homeostasis or immune pathology with the mother. The first is the syncytiotrophoblast. It develops from the cytotrophoblast (an earlier cell type) by aggregation of these cells and then fusion into a syncytium. The same process occurs when myoblasts fuse to form myoblastic syncytium in the development of cardiac muscle.^{15, 100, 109, 110} This cell fusion process involves phospholipid adhesion molecules.¹ The syncytiotrophoblast is a membrane directly exposed to maternal blood and all of the immune effectors therein. This membrane completely surrounds the fetus and functions as a biologic dialysis membrane involved in two-way exchanges of all molecules entering or leaving the fetal circulation. Its surface area is huge, approaching 25 square meters. Not only is this tissue capable of self-renewal, it must achieve an immunological privileged status to camouflage and shield the developing fetus from cytotoxic and autoimmune effector mechanisms.

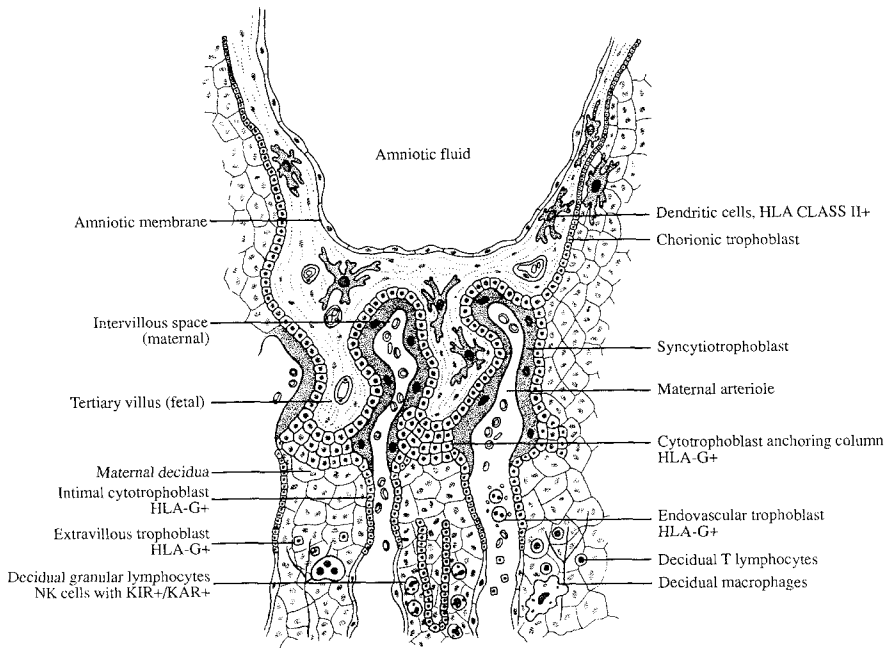


Figure 3. Placental compartments, cell types, and antigen expression.

Other cells and tissues within the placenta include the following types: (1) The cytotrophoblast anchoring cells form columns, which are at the terminal portions of the mature fetal villi. These cells attach the placenta to the maternal decidua and are in direct contact and union with this tissue; (2) Extravillous cytotrophoblasts migrate into and reside in the maternal uterine tissue. These invading cytotrophoblasts express HLA-G⁹²; (3) Endovascular trophoblasts erode into and alter the structure of the maternal arterioles feeding the placenta. These trophoblasts also express HLA-G.^{18, 69} They render these vessels noncontractile aneurysms by replacing the endogenous tunica intima and media. The large granular lymphocytes (LGLs) in the area synthesize nitric oxide (NO), which induces relaxation of the vascular smooth muscle⁶¹; and (4) Chorionic trophoblasts are present in vast expanse of tissue that forms the amniochorion.²² These cells serve to attach the amniotic membrane containing the amniotic fluid to the uterine lining.

The decidua is a maternally derived tissue with diverse biologic, nutritive, structural, and immunologic functions as interesting as those of the trophoblast tissue. The decidua, like the epithelial stroma of the thymus or the bone marrow, is a site of immigration, development, and functioning of unique sets and subsets of lymphocytes and natural killer (NK) cells. As in the thymus, many immigrant cells are induced to

undergo apoptosis (programmed cell death). These lymphocytes serve immunosuppressive, growth-stimulating, debris-clearing, antigen-processing and natural killer cell functions. At the implantation site, lymphatics become sparse,⁸⁴ and, unlike other cells of malignant or near malignant potential, trophoblasts rarely enter lymphatic channels, a feature not well understood. Scanty amounts of dendritic cells are found in the villous stroma of placenta.⁴⁹ These cells function to present antigen to CD4+ T cells, like the dendritic cells in the integument. These fetal cells bear HLA class II DQ A1 alleles. When the DQ A1 alleles of the mother and the infant are the same, an autoimmune response is initiated within the placenta, and the mother and the pregnancy both suffer.⁹⁶ Finally, emboli of trophoblasts are shed into the maternal circulation from the early stages of pregnancy.² Endovascular trophoblasts lodge in the capillary beds of the lungs where they reside for the duration of pregnancy and then undergo dissolution by enzymatic or other processes. The immune reactions to these cell emboli in the lung as well as their cell surface antigenic profiles are completely unexplored.

LYMPHOID CELL TYPES AND FUNCTIONS IN MATERNAL DECIDUA

The maternal lymphocytes present in the human decidua during the early weeks of pregnancy are composed of CD56+ NK cells (80%), CD3+ T cells (10%), and CD14+ macrophages (10%).⁷³ These leukocytes accumulate in large numbers and density specifically in the decidua at the implantation site. Their roles are important in directing a TH-1 cytotoxic or a TH-2 suppressive and growth-promoting response toward the placenta.²⁵

Natural Killer Cells

The main populations of lymphocytes found in the human decidua are NK-like cells with a distinctive phenotype, CD56 bright CD16- CD3-. They have the characteristic morphology of LGLs.^{51, 72, 75} Unlike CD56+ NK cells in peripheral blood, decidual CD56+ cells lack type III Fc receptors (CD16) and do not express significant levels of either type I FcR (CD64) or type II FcR (CDw32).³¹ Almost all the decidual CD56bright++ LGLs express very late antigen 1 (VLA-1, CD49a), but virtually none of the peripheral blood CD56+ NK cells express this antigen.⁴⁵

Large granular lymphocytes are particularly abundant around the time of implantation and during early pregnancy. Their association with two HLA class I molecules, HLA-G and HLA-C, on the surface of extravillous trophoblast is well documented. There is considerable evidence to document the direct communication of HLA-G with these NK cells at the time of implantation.⁷¹ Indeed these cells seem to accompany

the invading trophoblast. It is clear in women with recurrent pregnancy losses and in those with repeated *in vitro* fertilization and embryo transfer (IVF-ET) failures that the trophoblast as well as the developing decidua are cytotoxic targets of certain of the NK cells resident there. Pregnancy fails repeatedly in these women and immune therapy dramatically improves reproductive outcome.^{8, 82} Functional assays indicate that uterine large granulated lymphocytes differ fundamentally from both peripheral blood lymphocytes and even from classical circulating NK cells.⁷⁵

Trophoblast cells are resistant to lysis by NK cells from both peripheral blood and decidua; however, decidual NK cells extracted by enzymatic desegregation do exhibit cytotoxicity against the NK-sensitive cell line K562.⁷⁰ Decidual NK cells display reduced lytic activity compared with autologous peripheral blood NK cells, but this difference in cytotoxicity is abolished by short-term culture. When decidual NK cells are activated by interleukin 2 (IL-2), these cells are capable of damaging the trophoblast.^{19, 43, 51} Questions have been raised whether IL-2 is essential for NK cell maturation and activation that potentially damages the placenta, characterized by granular morphology and phenotyping. It has been reported that IL-2 is not essential for the maturation of uterine NK cells or for normal placental development, although uterine NK cells contain mRNA for IL-2R.³⁶ Recently, expression of KIR/KAR and the CD94 receptor by decidual NK cells have been reported.⁷⁴ It is speculated that the expression of HLA class I antigens, HLA-G, and HLA-C by these trophoblast cells may permit maternal decidual NK cells to recognize and respond to the fetal trophoblast cells by activation of KAR or KIR. The recognition of HLA-G/HLA-C by KIR/KAR and CD94 could provide a mechanism by which decidual NK cells control trophoblast migration.⁷⁴ Thus, the maternal-fetal interaction, and hence reproductive success, may depend on an NK allorecognition system.⁷²

Decidual NK cells are always in very close association with the trophoblast cells, which invade into the uterus. It has been reported that syncytiotrophoblast plasma membranes also inhibit the cytolytic activities of cytotoxic T lymphocytes (CTL) and NK against their target cells.^{37, 38} Expression of a nonclassical Class I HLA molecule is also correlated with NK resistance in the same way as a classical Class I HLA molecule. HLA-G and HLA-A2 transfectants using LCL 721.221 HLA-null cells are resistant to NK effectors isolated from decidua or peripheral blood.³⁰ Early findings that have received very little follow-up demonstrate that peptides of HLA-G bind as adhesion molecules to CD8+ LGLs that have migrated to the decidua of the uterus during the preimplantation period of pregnancy.¹⁰⁸ In this way the trophoblast may select an army of suppressor cells that accompany it as it interfaces and interacts with the maternal host. In addition, CD3-CD8+CD56++ decidual granular lymphocyte clones are a functionally more inert subset of CD3-CD56++ cells with respect to cytokine production. CD3-CD8- decidual granular leukocyte clones produce significant amounts of transforming growth factor alpha (TNF- α), GM-CSF, and

IFN- γ ; in contrast CD3-CD8+ cells produce very little of any of these cytokines.⁶⁴ It appears that CD3-CD8+CD56+ decidual granular leukocytes can bind to trophoblast cells through HLA-G; however, these cells are functionally inert and produce very few TH-1 related cytokines. In addition, trophoblasts express specific low affinity receptors for these TH-1 related cytokines. As a consequence, TH-2 response is preferred at the implantation site.

Decidual CD56brightCD16- NK cells express mRNA for CSF-1, TNF- α , IFN- γ , TGF- β 1, and leukocyte inhibitory factor (LIF).⁶⁵ These CD56+ decidual NK cells but not CD56- cells secrete TGF- β 2, which suppresses the generation of cytotoxic cells.³¹ Decidual NK cells produce CSF-1, which promote differentiation and growth of trophoblast cells.⁴⁰ Appreciable amounts of GM-CSF is produced in the first trimester maternal decidua, and a significant component of this secretion is from decidual LGLs. Production of GM-CSF by LGLs in the decidua is considerably greater than that of freshly isolated peripheral blood leukocytes. GM-CSF secretion by decidual LGLs is enhanced by co-culture on a monolayer of decidual stromal cells and can also be increased in a dose-dependent manner by stimulation with IL-1 or IL-2. GM-CSF from uterine lymphocytes and from trophoblast itself may influence placental growth and development in both a paracrine and an autocrine manner.⁶⁵

A pregnancy that is successful represents a relatively immunocompromised state.⁴⁸ Maternal acceptance of the fetal-allograft is in part due to suppression of cell-mediated cytotoxicity. Peripheral blood NK activities are significantly lower in primigravidas than in nonpregnant women.^{48, 53} During early pregnancy, peripheral blood CD56+ NK cell levels are decreased very significantly as compared with nonpregnant status.⁷⁹ The target-binding capacity of the effector cells remains unimpaired up to 16 weeks, but a significant reduction in the post-binding lytic potential is observed, which parallels the drop in cytotoxicity.^{85, 86} Absolute NK activity decreases in the third trimester compared with the activity in normal nonpregnant controls and increases again postpartum.⁵²

Studies on peripheral blood NK cells in women with repeated pregnancy failures through miscarriage or IVF-ET failures document that women sharing HLA DQ A1 alleles with their spouses show an increase in the numbers as well as the activities of CD 56+ bright cells in the peripheral blood.⁸⁰ These cells are not down regulated as seen in women with normal pregnancies. Suppressing their cytotoxicity and their numbers prior to a cycle of conception and during the first 10 weeks of pregnancy dramatically improves reproductive outcome.⁶

T Cells

In the decidua, the majority of CD45+ cells are localized in large lymphoid cell clusters (LCC), near endometrial glands, or as intraepithelial lymphocytes (IEL) in glandular epithelium. The major cell popula-

tions in LCC are CD56+ T cell receptor (TCR)- $\gamma\delta$ + cells, CD56+ cells, TCR- α/β +CD4+ cells, and TCR- α/β +CD8+ cells. T cells derived from decidual tissue and endometrium are different from those derived from peripheral blood. In both endometrium and decidua, more T cells express activation markers (CD45RO, Kp43, CD69, CD71, CD38 antigens and/or HML-1) and MHC class II (HLA-DR, HLA-DP, and/or HLA-DQ) antigens than those in peripheral blood.^{45, 54, 94} LCC are centers of immune reactivity where T and NK cells become activated.⁹⁴

T cells are activated regionally in the first trimester as the result of the stimulation by fetal antigens. Although HLA DQ compatibility between the mother and the fetus is associated with this cytotoxic activation, HLA DQ A antigens are not present on the implanting placental trophoblast. To establish fetal tolerance, the endometrium maintains the proportion of CD8+ T lymphocyte and CD4:CD8 ratios. When this proportion is disrupted, a pregnancy suffers from autoimmune activation, which results in repetitive pregnancy losses. In recurrent aborters, the percentage of endometrial CD8+ T lymphocytes is significantly decreased, and the CD4:CD8 ratio increased.⁸³ DNA analysis of pregnancies that failed revealed a highly significant proportion of those that are HLA DQ A1 compatible with the mother when compared with DNA analysis of children that were born alive.⁹⁹

The lymphocyte differentiation marker CD8 acts as a coreceptor with TCR during recognition of peptides presented by MHC class I molecules, HLA-G. The CD8 α/α homodimer binds to the classical MHC class I classical molecules. A cell bearing CD8 can also bind to HLA-G; therefore CD8+ T cells may interact with HLA-G-positive trophoblast cells. The functions of CD8 in the TCR complex are thought to be signaling through the association of CD8 with the protein tyrosine kinase p56lck and adhesion to MHC class I through the $\alpha 3$ domain.¹⁰⁸ Because intradecidual CD3+ T cells express either TCR α/β or γ/δ , and the level of expression of the CD3/TCR complex is downregulated in normal pregnancy,²³ CD8 α/α homodimer is not the HLA-G binding site.

During pregnancy and early puerperium, peripheral blood T-cell subpopulations change. From the first trimester and throughout pregnancy, the absolute counts of T(CD3) and T-cell subsets (CD4, CD8) decrease. There is also a decrease in the absolute lymphocyte count and a decrease in the percentage of T helper-inducer (CD4) cells in the first trimester. In the postpartum period, the percentages and absolute counts of T cells (CD3) increase transiently. These changes in lymphocyte subsets may indicate suppression of immune activity during pregnancy and an increase in the postpartum period.⁶³ When T-cell subpopulations are investigated, T helper/suppressor (CD4+CD45RA+) and suppressor/effector cells (CD8+S6F1) are increased and T helper/inducer (CD4+CD29+), T helper/memory (CD4+CD45RO+), killer/effector T cells (CD8+S6F1+), and NK cells (CD56+) are decreased. The numbers of activated lymphocytes expressing IL-2 receptor (CD25+) and T cells that express HLA-DR (HLA-DR+CD3+) are decreased.⁹¹ Lymphocyte proliferation is also impaired in autologous serum with concanavalin A

(ConA), phytohemagglutinin (PHA), or IL-2, prostaglandin E2 (PGE2) mediated suppressor activity during pregnancy.⁹¹

Although a state of systemic immune suppression is present in the mother during pregnancy, a dynamic antigen-specific T-cell response exists in her. A large number of distinctly expanded T-cell clones are clearly detected as early as the ninth to tenth week after conception, and these changes reach a maximum during the second trimester. After the 30th week of gestation, nearly all expanded T-cell clones disappear before parturition and the degree of clonality reaches normal levels.⁵⁵ Other studies analyzing decidual T cells show the same cellular activation. These findings suggest a role in trophoblast growth and placental development.⁴⁵

Maternal T cells do recognize fetal antigens. This antigen-specific T-cell response to paternally inherited antigens results in proliferation and accumulation of certain T-cell clones. During pregnancy, cytotoxic T lymphocytes are primed *in vivo* by paternally driven pregnancy-induced HLA antibodies. Naive cytotoxic T lymphocyte responses are also detected against paternal antigens (inherited and noninherited). These responses do not result in antibody formation. In contrast, inherited paternal antigens that induce HLA-specific antibodies in the mother give rise to elevated cytotoxic T lymphocyte precursor frequencies, when compared with the responses to third-party antigens.¹⁴ We evaluated this status in children who received a mother's kidney versus a father's kidney. There was no difference in survival of renal allograft unless the mother breast-fed the child. There was significantly less rejection and prolonged survival of mother's kidney in the breast-fed child versus the father's kidney.²⁰

Macrophages

The uterus contains a large number of macrophages in the endometrium and myometrium.⁵⁹ Ovarian hormones regulate their concentrations. Macrophages express estrogen receptors.⁹³ Uterine endometrial epithelial and stromal cells with estrogen receptors respond to steroid hormones. The estrogen-stimulated uterine growth factors attract macrophages.⁶⁰ Bulmer demonstrated that macrophages in functionalis region near the implantation site increase by 45% between the proliferative and early secretory stages of the human menstrual cycle.¹⁷

At implantation, macrophages increase in numbers and redistribute into specific uterine compartments.¹⁷ Factors that contribute to macrophage chemotaxis are as follows: (1) the inflammatory responses induced by mating and invasion of the uterine epithelium by the blastocyst; (2) elevated levels of female steroid hormones, and (3) increased concentration of hormonally stimulated peptides, such as CSF-1, GM-CSF, TNF- α and IL-6.⁶⁰ Decidual lymphocytes produce GM-CSF spontaneously and after paternal antigen stimulation, whereas peripheral leukocytes do not.⁴¹ Although GM-CSF or CSF-1 serves as a chemoattractant, it is not

essential to the process by which macrophages are recruited to the implantation site.¹⁰² Macrophages are evenly distributed throughout the endometrium during the peri-implantation period. Subsequently, with implantation, macrophages are redistributed to the secondary decidua, distant to the implantation site throughout pregnancy in mice.¹⁶ Decidual macrophages associated with fetal membranes are uniformly HLA class II antigen positive at all stages of human pregnancy.⁸⁷ Macrophages express low levels of HLA-G mRNA and protein. INF- γ selectively enhances expression of this HLA class Ib gene relative to HLA class Ia. This enhancement could influence the repertoire of peptides presented to the mother during embryogenesis and implantation.¹¹⁶

Macrophage activation is initiated by inflammation resulting from tissue destruction and INF- γ , a cytokine supplied by NK cells.¹⁰⁵ Maximum activation is achieved upon encounter with LPS from gram-negative bacteria. During pregnancy, elevated levels of cytokines secreted by uteroplacental macrophages activated by either bacterial endotoxins or receptor-bound cytokines may compromise pregnancy. In particular, a family of cytokines has been implicated in the induction of preterm labor associated with infections.^{58, 106} Macrophage activation enhances production of a wide range of pleiotropic, multifunctional polypeptide growth factors, reactive oxygen intermediates, and bioactive lipids. Activated uterine hematopoietic cells are equipped to perform certain immunologic and nonimmunologic functions within their microenvironments that can have major influences on the course of pregnancy.⁵⁹ During pregnancy, macrophages inhibit T-cell mitogenic responses. INF- γ is prominent near the termination of mouse pregnancy, and this cytokine enhances NO output from uterine macrophages.¹¹ In contrast, TGF- β 1, the immunosuppressive cytokine, is less abundant as pregnancy approaches termination.²⁶

Certain cytokines such as IL-10 and TGF- β reduce macrophage activation as demonstrated by lower expression of class II MHC (Ia) antigen and diminished production of TNF- α .¹³ In addition, a CSF-1:GM-CSF equilibrium has a pivotal role in regulating the transition between immunosuppressive and immunostimulatory phenotypes. When this equilibrium is disrupted, implantation is prevented.¹¹² Ovarian steroids control effector molecule production from activated macrophages. Progesterone enhances PGE2 production by human placental macrophages and suppresses cytotoxicity of human mononuclear phagocytes. In a mouse model, progesterone suppresses the steady state levels of inducible NO mRNA and inhibits NO production from macrophages⁹³; however, progesterone exerts a direct control over factor production once their glucocorticoid receptors are engaged and mechanisms not involving specific hormone receptors are activated.⁶⁰ Perturbed cytokine levels have a negative impact on fertility and pregnancy maintenance,^{24, 112} and loss of regulation of macrophage activity results in poor reproductive outcome.

HISTOCOMPATIBILITY GENE PRODUCTS AND THEIR ROLES IN IMMUNE RESPONSES

Alloantibody Production

Maternal anti-paternal alloantibody production during pregnancy is well documented. In healthy pregnancies, paternal antigen, maternal antipaternal antibody complexes, and maternal anti-paternal antibodies are detected in the maternal serum from the earliest stages of pregnancy onward.¹⁰³ The maternal alloimmune response is against some but not all of the mismatched HLA antigens of the fetus. These alloantibodies appear to be modulated by soluble HLA antigens and anti-idiotypic antibodies.¹⁰³ The role(s) of antipaternal antibody in the immune maintenance of the feto-placental unit is still debated. There is evidence that women who are HLA compatible with their spouses fail to produce alloantibody and experience recurrent pregnancy losses.¹⁰ Immunization of these women with paternal T and B cells with the subsequent production of maternal antipaternal antibody results in a restoring of reproductive competence and subsequent live births.¹⁰ The first recognizable maternal anti-paternal antibody is directed against Class II antigens and can be identified by flow cytometric analysis.⁴⁶ Later anti-class I cytotoxic and noncytotoxic antibodies are produced but not in all women. No firm data exists to document the antigens involved; however, HLA-G is the class I antigen most studied. It has been speculated that the HLA class II antigens stimulating the anti-HLA class II alloantibodies are the fetal dendritic cells in the placenta and the trophoblast emboli that live in the capillary beds of the lung throughout pregnancy. In recurrent aborting women lacking maternal-anti-paternal alloantibody, reproductive competence is restored by lymphocyte immune therapy (LIT) in those who produce antibody but not in those who fail to respond.^{10, 21} Metaanalyses of the effectiveness of this therapy applied to all recurrent aborting women regardless of cause show a 10% positive therapeutic effect over women receiving placebo therapy.³⁵ In women whose pregnancy losses evoke autoimmune responses to phospholipids and DNA antigens or in women with activation of NK cells, the lymphocyte immune therapy is far less effective.³⁵ These women fall into five categories of immune disorders listed in Table 1.

There is no evidence that the formation of anti-HLA antibodies evoked by normal pregnancy or lymphocyte immune therapy create immunopathology in the mother or in her infant unless they are directed against class I or class II paternal antigens on platelets, and this is a very rare occurrence. It is speculated that the production of anti-HLA antibodies is an indicator of a TH-2 immune response to paternal antigens. The absence of these antibodies in women with pregnancy losses or in vitro fertilization and embryo transfer failures is associated with a TH-1 autoimmune response that retards the development of or destroys placental cells. Restoration of the TH-1/TH-2 balance and treatment of the autoimmune condition restores reproductive competence in most

Table 1. FIVE CATEGORIES OF IMMUNE DISORDER IN WOMEN WITH RECURRENT SPONTANEOUS ABORTIONS

Type	Alloimmune Recognition	Autoimmune Abnormality		Cellular Immune Abnormality
		Antiphospholipid Antibody	Anti-DNA/Histone Antibody	
I	—	—	—	—
II	—	+	—	—
III	—	+	+	—
IV	—	—	+	—
V	—	+/-	+/-	+

women.⁸² DNA analysis of a large group of women with a history of recurrent pregnancy losses who deliver a live born infant or miscarry again with optimal therapy revealed that a significant deficit of HLA-DQA1 compatible live born children was present.⁹⁹ These results suggest that immune responses to paternal antigens or the lack of these contribute to the maintenance of histocompatibility polymorphisms in humans.⁴ This bias against HLA-DQA1 compatible embryos may also be a mechanism to prevent the induction of autoimmune diseases during pregnancy.⁹⁶

R80K, a 80-kDa single peptide chain on the trophoblast is also detected on B-lymphocytes and monocytes. IgG alloantibody can be eluted from this molecule on the placenta. R80K protein is also a target molecule for NK cell cytotoxicity. It has a polymorphic alloantigenic determinant that is completely bound by maternal antibody in all successful pregnancies. In murine NK cell-dependent models of abortion, a monoclonal antibody (mAb) against a monomorphic determinant present in human and murine R80K prevents abortion very effectively. The R80K molecule must be bound to an antibody against it to prevent an NK-mediated attack on trophoblast. The same molecule and antibody to it has been demonstrated in sheep.⁹⁵

No area of medicine or science has stimulated more controversy than the alloantibody responses discussed in this section. The following are documented functions of anti-paternal antibody: (1) suppression of cell mediated immunity and MLC reactivity that is paternal-specific⁹; (2) suppression of NK cell cytotoxicity (Kwak and Beer, unpublished data); (3) immunotrophism of trophoblast growth and development by production of GM-CSF and CSF-1¹¹⁵; (4) amelioration of autoimmune symptoms in women with rheumatoid arthritis when paternal lymphocyte immunization induces development of anti-paternal lymphocyte antibodies¹¹¹; and (5) resistance to HIV infection of T cells that are bound to maternal anti-paternal antibody during pregnancy.^{88, 101}

HLA-G Expression

HLA-G mRNA is identified in all extravillous trophoblast populations including the cytotrophoblast cell columns, interstitial trophoblast,

endovascular trophoblast, and placental bed giant cells (see Fig. 1). In addition, villous cytotrophoblast and villous mesenchymal cells contain HLA-G transcripts. Villous syncytiotrophoblast is always negative.²⁹ This polymorphic HLA class I gene has striking linkage disequilibrium with HLA-A alleles.⁹⁸ Although HLA-G is expressed on extravillous trophoblast,²⁸ amnion and fetal liver cells contain transcripts for HLA-G.^{56, 57} HLA-G transcript is found in adult peripheral lymphocytes and equally in B- and T-cell populations.⁷⁶ Invading trophoblast cells and cells of the basal plate express $\beta 2$ -microglobulin in association with HLA-G and HLA-C heavy chains. These class I heavy chains may also have been present as isolated molecules, although not in each of the cells. By contrast, cells of the chorion laeve exclusively express HLA-G and not HLA-A, B, or C antigens.⁶² This differential expression of classical versus nonclassical class I genes suggests tissue-specific regulation. A negative regulatory element (NRE) is identified in a murine embryonal carcinoma cell line that markedly inhibits class I gene expression.⁴⁴ Human trophoblasts have a tissue-specific nuclear binding factor that downregulates classical class I expression upon binding to the NRE sequence; however, the HLA-G gene does not have this NRE region, enabling its expression by these cells.²⁷ Both cell-associated and secreted HLA-G antigens are prominent in first trimester villous cytotrophoblasts and are greatly reduced in third trimester cytotrophoblasts.⁷⁸ Cytotrophoblasts from term placentas, which have reduced invasive capacity *in vitro*, also had decreased ability to upregulate HLA-G protein expression. HLA-G production is a critical component of cytotrophoblast differentiation and is critical for the success of its invasive pathway.⁹²

The HLA-G+ extravillous cytotrophoblasts, more than any other fetal or maternal tissue in the first trimester and at term, are very actively assembling MHC class I antigens with peptide transporter associated with antigen processing (TAP). This molecule is essential for peptide presentation and MHC class I assembly.⁴⁷ However, expression of MHC class I by the cytotrophoblast is not correspondingly elevated. This pattern may result from HLA-G being shed from the surface of the trophoblast, a process that may play a central role in protecting the fetus from maternal immune attack.³² HLA-G acts as a protective molecule against decidual NK lysis and allows trophoblast survival in the maternal tissues. CD3- decidual granular lymphocyte clones express MHC-nonrestricted cytolytic activity, notably against HLA-negative cells. HLA-G offers protection to target cells from cytolysis but not classical HLA class I antigens.³⁹ When HLA-G and HLA-A2 antigens were transfected to LCL 721.221 HLA-null cells, these antigens protect target cells from NK effectors isolated from decidua or peripheral blood. Expression of a nonclassical Class I HLA molecule is correlated with NK resistance.³⁰ These NK cells are always in close association with the trophoblast cells that invade into the uterus. The expression of HLA class I antigens, HLA-G and HLA-C, by these trophoblast cells raises the possibility that maternal NK cells can recognize and respond to the fetal trophoblast cells. Thus, the maternal-fetal interaction, and hence

reproductive success, may depend on an NK allorecognition system.⁷² HLA presents peptides that specifically activate KIR or specifically activate KAR and induce cytotoxicity. There are both inhibitory and activating NK cell receptors on large granulated decidual cells. These receptors are immunoglobulin members of super family. Peptides of HLA-G are recognized by these KIR, and cytotoxicity is prevented. The recognition of HLA-G by KIR or KAR and CD94 can provide the mechanism by which NK cells control whether the trophoblast grows or is rejected.⁷⁴

HLA-G is also identified on macrophages isolated from the placenta. Treatment of these cells with INF- γ increases HLA-G mRNA and levels of cell surface as well as intracellular expression. This production and expression may influence the repertoire of foreign peptides presented to the mother during placentation and also during inflammatory autoimmune conditions in the mother. Soluble HLA-G may dramatically influence both maternal and fetal immune responses.¹¹⁶ During trophoblast invasion, extravillous cytotrophoblasts (HLA-G positive) express the α -6 integrin subunit (high gelatinase and low fibronectin secretion). When expression of the α -5 integrin subunit is turned on, their invasive behavior ceases, and they secrete low amounts of gelatinases and high concentrations of fibronectin. HLA-G positive cells secrete significantly more gelatinases than HLA-G negative cells, but their HCG hormone secretion was similar.¹²

The attenuated expression of HLA-G protein on the extravillous trophoblasts has been reported in preeclamptic women.⁵⁰ This decrease in expression may be related to reduced numbers of trophoblasts in placental tissue examined at term from patients with primary preeclampsia.³³ This same lack of or attenuated expression seems likely in women with recurrent pregnancy losses or in vitro fertilization and embryo transfer failures. Approximately 40% of blastocysts express both HLA-G and β 2 microglobulin messenger RNA. Interestingly, sibling embryos from patients who became pregnant were significantly more likely to express HLA-G than embryos from patients that did not conceive as a result of their IVF cycles. HLA-G expression on the blastocyst is not associated with culture conditions, patient's age, or infertility diagnosis.⁶⁷ HLA-G mRNA was also detected in unfertilized oocytes and in early embryos but not in control cumulus oophorus cells from the ovulating follicle. β 2 microglobulin mRNA is also found in those embryos expressing HLA-G. Expression of HLA-G mRNA was associated with an increased cleavage rate compared with embryos lacking HLA-G transcript.⁶⁸

IMMUNOMODULATORY PROTEINS

TJ6 proteins have significant immunomodulatory roles in both pregnancy-induced tolerance as well as thymic tolerance.^{90, 97} TJ6 is normally expressed within the uterus. Its expression is far greater in the decidual lymph nodes draining the uterus during pregnancy. Anti-TJ6 binding antibody destroys early pregnancy in mice.¹⁰⁴ TJ6 has a definite role in

the maintenance of the fetal allograft. TJ6 is a protein whose membrane form is regularly expressed on the B lymphocytes of women during a successful pregnancy. Expression of TJ6 on circulating NK cells is associated with an unfavorable outcome of pregnancy. In addition, we show that this prognosticator can be reliably demonstrated early in the first trimester.³⁴ TJ6 secreted binds to receptors on cytotoxic NK cells at the implantation site and induces apoptosis and programmed cell death in these cells. Progesterone produced at high levels during early pregnancy stimulates the production of TJ6 to accomplish this mission.

Several protein molecules are reported to suppress TNF production from human NK cells that can damage the placenta. Spermine present in large amounts in the amnion counter-regulates the immune response by inhibiting the production of TNF and other proinflammatory cytokines by human mononuclear cells.¹¹⁷ Recently, it has been reported that a fetal plasma glycoprotein, fetuin, is required for the inhibition of TNF production by spermine. Both spermine and fetuin levels in amnion and fetus are high. Both are ideally poised to counter-regulate TNF production in pregnancy.¹¹⁴

Early pregnancy factor (EPF) is a possible immune modulatory protein.⁷⁷ EPF is a low-molecular weight protein that is produced by viable preimplantation embryos.¹⁰⁷ It appears in the serum of pregnant women 48 hours after fertilization.⁴² EPF has immune suppressive functions and is not present when the fertilized ovum is dead or aborted. It is a sensitive marker reflecting the survival of the embryo.⁸⁹

CONCLUSION

The following summarizes the immune response during normal pregnancy:

1. HLA-G, a polymorphic paternal antigen, is present on the implanting trophoblast cell and presents non-self-antigens (terminal peptides) to the mother (hidden self) (see Fig. 1).⁷⁸
2. The non-self-terminal peptides of HLA-G serve as adhesion molecules for CD8 antigen on decidual granular leukocytes that have already migrated to the decidua.¹⁰⁸ These CD3- CD8+ CD56+ NK cells are functionally more inert to produce TH-1 related cytokines such as TNF- α , INF- γ , compared with CD8- CD56+ decidual granular leukocytes. In addition, fetal trophoblast expresses specific low affinity receptors for TH-1-related cytokines such as TNF- α , INF- γ , and GM-CSF,⁶⁴ resulting in the preference of TH-2 responses at the maternal fetal interface.
3. There are counter-regulating molecules against TNF production such as fetuin and spermine in fetus and amnion. These molecules suppress TNF production and maintain an immunosuppression state.¹¹⁴

4. Decidual stromal cells and NK cells produce the cytokines GM-CSF, CSF-1, LIF, and TGF- β that are essential for trophoblast growth, proliferation, and differentiation.⁶⁶
5. This cytokine production results in trophoblast growth and increasing progesterone production. Progesterone in high doses stimulates the production locally of TJ6 and other placental and uterine proteins. TJ6m bound to decidual leukocytes, CD56+16-, induce apoptosis in these cells. They undergo programmed cell death.
6. The trophoblast proliferates, invades and departs itself into the maternal blood, and anti-paternal alloantibody is produced, which returns and binds to the placenta. Here it has immunotrophic functions and blocks the efferent limb of the immune response locally. The placenta has become a privileged tissue.
7. By 10 weeks the process is complete and not only has the fetus parasitized to the mother-to-be, it has established a lasting immunologic truce with her, which imprints itself on her immune system and lasts a lifetime.
8. Once established, there is essentially nothing that can be done immunologically to disrupt this relationship.
9. Hormonal and other events programmed near the end of pregnancy allow the immune repudiation of this union.
10. The uterus recycles and prepares itself for another successful experience if the father remains the same. Changing paternity can result in new and pathologic pregnancy experiences not seen with the first spouse.⁵

From the immunopathologic point of view, pregnancy in humans had to learn to deal with one of the oldest cells of the immune system, cytotoxic NK cells. NK cells inactivates cytotoxic NK cells locally via apoptotic processes and woos the attention of others for its survival. In pregnancies that fail, these cells are increased in numbers and cytotoxicity. They induce necrosis within the decidua housing and feed the trophoblast. Immune pathology confirms this result by identifying the process and the cells with monoclonal antibodies.¹¹³

The following items determine whether a pregnancy is vetoed (see Fig. 3): (1) HLA-G produced by the male partner, who is DQ A1 compatible, appears to be interpreted as self—perhaps HLA-G self-peptides are exposed. (2) There is no adhesion of HLA-G with CD8+ LGL NK cells in the decidua capable of inducing TH-2 cytokines or trophoblast growth-promoting molecules. (3) Progesterone production is low or absent by the trophoblast. (4) TJ6 apoptotic protein is not produced, and the cytotoxic NK cells capable of TNF secretion are not inactivated. (5) Decidual necrosis is induced and the pregnancy fails or withers on the vine. (6) The mother is left with expanded clones of cytotoxic NK cells and subsequent pregnancies fail earlier by the same immune mechanisms, which is confirmed by DNA analysis and by placental immunopathology using monoclonal antibodies that identify the implantation

site and the NK cell effectors in the decidua. (7) The greater the number of pregnancy failures, the more aggressive it is. Autoantibodies are produced against phospholipid molecules and DNA components. These are essential for uterine modeling for implantation and remodeling following the termination of pregnancy.⁸¹ Re-establishing the TH-1 and the TH-2 balance by lymphocyte immune therapy or intravenous immunoglobulin therapy and treating the autoimmune conditions before conception (before implantation) results in live born infants in 80% of women treated.^{81, 82}

In no field of medicine do we commit our patients to experience a malady three times over before we investigate the problem thoroughly. In the field of obstetrics and gynecology today, pregnancy losses are no national emergency to counseling physicians. We advise, "try again," "it's bad luck," "it wasn't a baby anyway," "it's meant to be," "we will try more progesterone," "it's God's will," or "we will look for a cause after you lose your third pregnancy." There is no science to underpin this counsel. When a pregnancy fails in utero early or late, something is wrong and it may be an immunologic problem. We are beginning to realize that there are circumstances in which pregnancy is immunologically rejected, and this is a common problem that occurs repeatedly in 1 out of 300 couples.³ Immunological and genetic testing including DNA analysis can reveal which couple is at risk and those that are affected with the problem.⁷ Analysis of HLA DQ alleles of both partners will also reveal those at risk to worsen their autoimmune disease during pregnancy and those who will remit their symptoms.⁹⁶

Understanding the immune dynamics of normal pregnancy and pregnancies that result in implantation failures or recurrent pregnancy losses will open doors to a new dimension of understanding autoimmune disorders. Why are they much more common in women than men? Why does pregnancy make these conditions worse in some and better in others? Why do some autoimmune disorders remit on their own and others have devastating courses? Understanding the immunology of pregnancy may also open new avenues of novel therapy for autoimmune disorders as well as retroviral diseases such as HIV infection.

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