

**THE PARADOX OF PREGNANCY  
A TRIBUTE TO DESIGN**

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**KEY WORDS**

major histocompatibility complex (MHC)	antigen presenting cells (APC)	interleukin (IL)
interferon (IFN)	tumor necrosis factor (TNF)	cytokine
colony stimulating factor (CSF)	low density lipoprotein (LDL)	natural killer cell (NK)
isotope switch	ligand	polymorphic
metalloproteins	trophoblast	syncytium
transforming growth factor (TGF)	monomorphic	allograft
apoptosis	adhesion factor	transferrin
cytotrophoblast	trophectoderm	fibronectin
epidermal growth factor (EGF)	trophoblast-lymphocyte cross reactive antigen (TLX)	
integrin		

**ABSTRACT**

The advent of molecular science has opened research into the role of the immune system in regulating growth and development. The primary functions of the immune system are to maintain the integrity of the organism and to eliminate any foreign invaders. In the case of mammals, pregnancy produces a paradox. The developing embryo is a foreign body; yet, it is nurtured rather than being rejected. The immune system is fundamental to the operation of this unique interaction of maternal and fetal cells. The ultimate paradox is that if maternal and paternal immunity are too similar, pregnancy fails. Cytokines, recently discovered chemical messengers, are intimately associated with the immune system, functioning as regulators of development according to specific time and space. Our knowledge regarding the number and nature of growth factors and cytokines is increasing. Regulation of these factors is critical to proper growth and development. Interruption of any part of this complex system causes pregnancy to fail. Mammalian pregnancy is a unique, intricately designed system, a tribute to a Master Designer.

**INTRODUCTION**

During the Fifties and Sixties, with the advent of molecular science, much new information about immunity was gained. At this time transplant science was a new and growing field. This development opened many questions about fetal development and how an embryo can survive within an environment in which it is a foreign tissue. The study of tumor survival and rejection increased the number of questions regarding fetal development. In the Eighties, a new branch of science arose: embryo transplant. This made the pregnancy paradox even more striking. How can a totally foreign embryo survive in an apparently hostile environment? Not only does it survive; but also, mechanisms are in place which allow it to grow and develop. Contrary to being rejected, it is nurtured by the mother's body. The immune system plays a vital role in this development, paradoxically controlling systematic development and preventing rejection.

The main function of the immune system is the recognition of self. A complex self-recognition system of cells and soluble factors is present within all living organisms and is most highly developed in mammals, especially humans. These products recognize foreign invaders and remove them by a variety of means.

The unique ability of an individual's immune system to recognize self is based on the molecular specificity of a cell membrane protein entity called the Major Histocompatibility complex (MHC). In

humans several MHC moieties have been described: HLA-A, B, C, D, and a unique monomorphic type found on the placenta called HLA-G. MHC is produced by highly polymorphic genes at several loci. Class II MHC is expressed on specialized cells which serve as antigen presenting cells. Two classes of MHC are universally recognized. Individuals possess a unique class I MHC which is expressed on almost all nucleated cells except sperm and placental cells. Class II MHC is expressed on specialized cells which serve as antigen presenting cells. The polymorphic nature of MHC accounts for the ability of the immune system to distinguish self from non-self, since a vast number of combinations are possible. T-lymphocytes produce specific unique membrane receptors which recognize antigen associated with MHC. This leads to proliferation of T cells and effector cells. T cells produce glycoproteins, or cytokines, identified as CD-1,2,3,4, and 8. They also produce a substance known as integrin. Three subgroups of T cells have been identified. One group, T-Helper Cells, (TH cells) manufacture and secrete CD-4 which activates cells of the immune system. Under the influence of TH, a second subgroup, Cytotoxic T-Cells (TC) proliferate into Cytotoxic T Lymphocytes (CTL). TH cells are activated only when antigen is displayed on specific Antigen Presenting Cells (APC). These are macrophages, B lymphocytes, and dendritic cells. The third subgroup is identified as T Suppressor Cells (TS). At the present time there is some controversy as to the existence of this subset.

Cellular interactions between TH cells, APC's, and B cells are required for generation of an immune response. Antigen must be presented with a MHC molecule on the membrane of an appropriate APC, particularly macrophages, which secrete interleukin and interferon. The antigen specific TH cell binds to antigen-MHC complex and secretes cytokines. These activate B cells, which then undergo cell division to produce more B cells. Three cytokines are secreted. Interleukin -1 (IL-1) induces the production of B cells. IL-II and interferon  $\gamma$  activate macrophages to become Natural Killer Cells (NKs). MHC functions to present non-self antigen. A class III MHC has been identified, with possibly a regulatory function. Three interferon factors have been identified, IFN  $\alpha$ ,  $\beta$ , and  $\gamma$ . These and Tumor Necrosis Factor (TNF) increase the expression of MHC class I and II and regulate phagocytes and monocytes. IFN  $\gamma$  augments expression of adhesion factors and suppresses the action of NK cells. IL-4 increases the expression of Class II MHC on resting B cells, leading to and increased production of B cells and increased membrane receptiveness. It favors the growth of TH cells, the source of IL-4, and inhibits the production of IFN  $\gamma$ . It also plays a role in creating an isotope switch from the production of IgG-2 to the manufacture of IgE and Ig G-1. This switches antibody production away from cellular components and toward humoral components. IFN  $\gamma$  and IL-4 have opposite effects and antagonize each other. There is a well developed time-space regulation of the immune response. This is accomplished by the following mechanisms. First B cells have a very short life span, so the proteins spontaneously decay rapidly. Second, there is a destabilization and inhibition of activated factors. Third, activated factors are destroyed by proteolytic cleavage.

### **HORMONES, CYTOKINES, AND OTHER MOIETIES**

Within the last ten years, many new chemical messengers have been discovered. Our understanding of the role these moieties play in cellular processes is still in its infancy. Gaining information in this area is difficult because these messengers exist for a very limited time, and tracking them requires intact whole organisms; although inoculating cell cultures has added a great deal of knowledge about how these chemicals work.

In early pregnancy, estrogen stimulates the release of several cytokines which prepare the uterus for the implanting embryo. These include Epidermal Growth Factor (EGF) Transforming Growth Factor (TGF), Leukemia Inhibiting Factor (LIF), Interleukin (IL), Interferon (IFN), and Colony Stimulating Hormone (CSF). As implantation proceeds, the level of estrogen decreases and the level of progesterone increases. This changes the physiology from one favoring implantation to one favoring growth and maintenance. Prostaglandins inhibit the production and action of interleukin, which serves to regulate inflammation and provide immunosuppression by inhibiting the production of Cytotoxic Lymphocytes. Prostaglandins also stimulate vascular development of the uterine wall, known as decidualization. Insulin Like Growth Factors (IGF) are secreted by the placenta during placental differentiation and influence growth and development.

Early embryo growth and uterine invasion have been compared to tumor and graft immunology, although the maternal-fetal relationship is far more complicated than a tumor or allograft reaction. The term "allograft" implies that the fetal environment is hostile and must be overcome. There are

similarities between the immunity of tumors and the immunity of pregnancy, but the environment of the uterus is very specific and the comparison to graft or tumor immunology is weak. A new term for the special fetal environment recognizes the beneficial interaction between the fetus and the mother. This term is "constructive or programmed symbiosis." Even this term is inadequate, since the fetus does not benefit the mother in a symbiotic relationship. While comparison of embryonic tissues with tumors has led to an understanding of the process of invasion and implantation, comparison with graft immunology has led to an understanding of fetal survival. The MHC plays an important role in graft survival or rejection. In order to elicit an immune response, the target cells must express both class I and class II MHC, which are recognized as foreign, setting up an immune response leading to rejection of the graft. Activated TH cells secrete IL-2, inducing the production of cytotoxic T lymphocytes. These cells react with the class I MHC of the graft, destroying it. Concurrently, IL-2 and IL-4 are produced by B lymphocytes to produce antibodies. Cells recognized by these antibodies are destroyed. A second graft is then rejected more quickly because of antibody memory. These elements of the immune system play a vital role in the development of the placenta, which is the lifeline between the growing infant and the endometrium. Immunosuppression is a factor in the survival of the fetus, but this is not the only factor. It is well known that the mother does not exhibit any compromised immune capability during pregnancy. Furthermore, the immune system plays a role in growth and organization of the mature organism. Undifferentiated embryonic cells implanted into a kidney, for instance, develop in a random manner and produce a jumbled mass of cell types. In the proper time-space relationship, the immune system is part of an intricate communication network which is necessary for organized growth and development. In the uterus, no class II MHC is expressed; but rather, only a unique monomorphic class I. In the uterus, IL-4 stimulates an isotope switch from IgG-2 to IgG- 1. By binding complement, T cells are inhibited from becoming NK cells. This favors the growth of TH-2 cells over TH- 1 cells and stimulates TH-2 proliferation. A coordinated production of these various factors controls the time-space invasion of trophoblast into the decidua.

## **IMPLANTATION AND INVASION**

The placenta confronts the maternal immune system at two times. The first time occurs when the peripheral trophoblastic cells of the placenta break through the uterine epithelial basement membrane and invade the uterine stroma, which contains lymphocytes and immigrant leucocytes. The second occurs at the cytotrophoblastic placenta during development of the hemochorial placenta. An immune response is generated by the mother at these times. This inflammatory response leads to decidualization of the endometrium. Interaction between the uterine endometrium and the blastocyst results in a regulatory system that allows blastocyst survival and growth.

Implantation requires an immune response by the maternal tissues. This takes place within a prescribed time-space relationship during which migration, adhesion, and apoptosis (or self-destruction) occur, without eliciting an immune response which would destroy the embryonic tissue. The placenta also generates an immune response. Several cellular types make up the placenta, collectively called the trophoblast. Cytotrophoblast and chorionic trophoblast invade the hormonally prepared uterine wall, called the decidua. First trimester trophoblast cells show many factors enabling invasion and growth. The list of factors is long and growing. Degradative enzymes secreted by early trophoblast include several extracellular matrix ligands and integrins. Cytotrophoblast cells which adhere to the basement membrane express  $\alpha$  and  $\gamma$  integrin. It also appears that these cells actually produce the basement membrane. Cytotrophoblast column cells express fibronectin rather than integrin. These factors are precisely regulated and produce opposite effects. Research shows that integrins, termed  $\alpha 5/\beta 1$ , interacting with fibronectin inhibit invasion, while integrins, termed  $\alpha 1/\beta 1$  and  $\alpha 6/\beta 1$ , interacting with laminin enhance invasion. Transition of integrin type is essential to pregnancy. If it is not normal, invasion is too shallow, leading to abortion.

An adhesion factor, E cadhedron, is important in maintaining cell-cell adhesion. Estrogens secreted by the blastocyst on days 1 and 2 of pregnancy induce the uterine epithelium to produce EGF, TGF, and Leukemia Inhibiting Factor (LIF). EGF is expressed only at the site of blastocyst adhesion. The function of EGF appears to be the induction of proliferation of epithelial cells, resulting in the formation of the decidua. LIF also inhibits differentiation of trophoctoderm and is necessary for implantation. It appears to be regulatory in function, co-ordinating cellular processes between the implanting blastocyst and the transforming uterine epithelium. The production of LIF is induced by maternal estrogen. This was shown by H. Blatt, and referred to by Stewart [13, p. 160]. The study used ovariectomized mice, which do not produce LIF. Estrogen induces the production of an adhesion factor, CAM 1, which is

responsible for adhesion of the blastocyst to the endometrium. By day 3, CAM 1 is inhibited by progesterone. CAM 1 and glycans secreted by the endometrium cause dissociation of endometrial cells, allowing implantation.

Normal cells within a tissue adhere to each other and to the extracellular matrix. Integrins are cell surface molecules which anchor cells to the extracellular matrix. Cells lacking the necessary adhesion factors self-destruct. In order to survive, cells must have the proper adhesion molecules for their time and space. This property of cells is responsible for the death of abnormal cells which attempt to float away from their parent tissue and metastasize to other tissues. Enzymes classed as metalloproteinases are capable of dissolving the basement membrane, enabling invasion in a new site. Cancer cells, which contain metalloproteins, are capable of overcoming the need for adhesion factors. Then, at the site of metastasis, they produce adhesion molecules which recognize targets on cells at the new site. Tumor cells express transferrin and alkaline phosphatase which aid in the process of invasion by upgrading anabolic activities as well as the transfer of iron. Malignant cells produce an antigen known as CD-46. It is associated with MHC-I on the cell surface and is thought to play a role in preventing an immune reaction between the foreign cells and the host immune system. A similar antigen, CDX, is associated with placental trophoblast cells. Trophoblast also expresses transferrin and alkaline phosphatase. These enzymes control metabolism, so they are necessary for the growth of fetal tissues. The invading cytotrophoblast synthesizes several metalloproteinases and collagenases. These are all under the control of estrogen. The timing of the estrogen surge, the production of LIF and EGF plus the controlled presence of degradating enzymes allows adhesion and invasion in exactly the proper balance.

An immune response by the uterine epithelium is necessary for proper anchoring of the trophoblast to the decidua. The basal plate is a fibrin mesh of fetal and maternal fibrin. Thrombomodulin, an endothelial receptor, binds thrombin, preventing the conversion of fibrinogen to fibrin. IL-1 down-regulates thrombomodulin allowing the production of fibrin to proceed. IL-1 is stimulated by antigenic recognition which cause TH cells to release macrophage-activation factors that cause macrophages to release IL- 1.

Low Density Lipoproteins (LDL) have been discovered which affect implantation and placentation. Several lipoprotein particles have been discovered. All are similar in structure even though they are produced by the action of several different genes. They have a specific area of function, yet their functions appear to overlap. LDL receptors are found on tumor cells as well as on many other cells in the body. Studies of placental LDL receptors have shown them to be expressed in distinctive patterns which change with time and space. One, identified as Gp 330, is extensively expressed in pre-implantation trophoblast but not after implantation. Another, identified as LRP, is found on villous cytotrophoblast and syncytiotrophoblast at implantation sites. As pregnancy advances, it is found only on the syncytiotrophoblast. Various members of the LDL receptors control invasion, regulate general secondary carriers which change cell function and affect metabolism within the extracellular matrix. These are important functions during implantation. LDLs also affect the synthesis of steroids by the placenta.

As invasion progresses, trophoblast cells induce changes in the endometrium, resulting in the formation of the decidua. The endometrium also plays an active role in this process. Colony Stimulating Factor (CSF-1) receptors are found on trophoblast cells while their ligands are produced by uterine endothelial cells. CSF-1 expression is induced by an interaction of progesterone and estrogen. McMasters, [9,pp. 125-127], determined that essential growth factors, Epidermal Growth Factor and Transforming Growth Factor, which are produced by trophoblast cells only in the presence of receptors in maternal decidua, are the source of the ligand. EGF allows differentiation of the trophoblast into the syncytium. Cytotrophoblast produces Interleukin 1- $\beta$  and also IL 1- $\beta$  receptor. Uterine cells also produce and respond to IL-1 $\beta$ . This allows a coordinated development of both decidua and trophoblast. EGF increases the production of Human Chorionic Gonadotropin and hPL (Human Placental Lactogen). Anti-hPL antibodies are found on invading cytotrophoblasts which are in contact with the basement membrane of the uterine epithelium. Transcription factor OCT-4 is expressed by the blastocyst. This factor represses the activity of IFN- $\gamma$  and hCG. This is thought to be a control factor for trophoblast differentiation and growth.

## THE PARADOX

Early thoughts that paternal antigens were not expressed, or were suppressed, have been proven inaccurate. Research with women who experience repeated abortions have shown that paternal antigen expression is actually important for successful pregnancy. The decidual response to paternal antigen stimulates the production of T cells. These T cells respond to class II antigen, and produce antibodies specific to paternal B lymphocytes. This blocks lymphocytotoxic activity of antipaternal antibodies by blocking the response to IL-2. A significant study done by Thomas, and noted by Beer, [1, p. 122] showed that couples sharing MHC types HLA A, B, or DR, were more likely to experience abortions. If maternal and paternal antigens are similar, a T cell proliferation does not occur, and the decidua does not produce suppressor cells, and pregnancy fails. Blocking of immunological responses makes the uterus a "privileged site". Grafts of fetal or paternal tissue are rejected if placed in extrauterine sites. Lymphocytic bone marrow-derived suppressor cells are found in the decidua of women carrying successful pregnancies. These suppressor cells block cytotoxic T activity against paternal antigens. IgG secreted by the endometrium, and responsible for the transfer of antibody to the fetus, expresses a moiety known as FcR. Fc is an active chain found on Ig. FcR is a receptor for Fc. FcR, expressed by trophoblast cells, neutralizes complement fixation, masks surface antigens, and prevents direct contact of the trophoblast with the decidua. This provides a unique escape mechanism for avoiding a harmful immune reaction between the invasive trophoblast and the decidua. It also provides a positive pathway for the transfer of essential products for fetal survival.

Women carrying successful pregnancies produce anti-B antibodies blocking reaction specifically to paternal antigens. Maternal response to tissue specific antigens of the trophoblast, Trophoblast-Lymphocyte Cross-Reactive Antigen, (TLX) is necessary to maintain pregnancy. When the maternal immune system comes in contact with paternal TLX on trophoblast cells, an anti-TLX response is mounted. This in turn stimulates an anti-anti-TLX response which blocks the first response. If mates are TLX compatible, neither response is stimulated and the pregnancy fails due to an uninhibited production of NK cells. Some women produce an anti TLX response, but not an anti-anti TLX response. In these cases pregnancy fails also: thus, both responses are essential.

The placenta is resistant to antibodies. Wood [15, pp. 235-248] noted that human cytotrophoblast cells express a unique monomorphic class I MHC, and no class II. In fact, trophoblast cells cannot be induced to produce polymorphic class I HLA. In addition, a suppressor factor, identified as Suppressor Inducer Factor (SIF) has been isolated from trophoblast cells. This is bound to IgG 1, an antibody also found in surviving tumors. IgG-1 is not complement fixing, and plays a role in antibody transfer to the fetus. IgG-2, a potential source of anti-fetal antibodies, is not produced by the mother. Prostaglandins produced by the decidua have been shown to enhance antibodies which inhibit the expression of IL-2 and IFN  $\gamma$  by NK lymphocytes. IL-2 is dependent on the presence of 2 signals: T cell Growth Factor and T Helper Factor. Antigen stimulates TCGF which in turn stimulates Helper cells to produce IL-2. This data has shown that, although TCGF is expressed, IL-2 is inhibited.

## CONCLUSION

The uterus has been called a "privileged site" because of the endometrium's uterine-specific immune factors which enable it to bypass rejection mechanisms. However, this is only part of the process. An immune difference between the two parents is actually necessary in order to establish a pregnancy. Merely accepting the foreign embryo, though, does not complete the process. The embryo must be nurtured. This is accomplished by a coordinated procession of immune factors and cytokines which enable the placenta, a tissue of fetal origin, to adhere, implant, and invade the uterine stroma. An intricate communication system is prerequisite if this process is to occur in an orderly fashion. The window for implantation is short. If the proper sequence factors is not available at exactly the right time, pregnancy will fail. Factors can not be deficient, or overproduced. Intricate balance according to time and space is essential. The embryo also contributes to this cytokine orchestration. Numerous fetal immune factors are produced early in gestation. These, interacting with immune factors produced by the mother, allow the placenta to develop smoothly with precise regulation which controls and changes the uterine physiology as needed throughout the pregnancy. Every moiety of this complex mechanism must be in place simultaneously. Any errors result in unsuccessful pregnancy. Mammalian pregnancy requires a vast amount of information, a system for dispensing and regulating this information, and physical plant in which this information is used. Since all meaningful information comes from an intelligent source, this complex precise system is a tribute to a Master Designer.

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