

# How your Mother Tolerated you for Nine Months

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## Introduction

The human immune system is very adept at distinguishing between “self” and “non-self” antigens that are presented by the major histocompatibility complex (MHC) surface proteins on cells. The MHC molecules and their bound antigens are continually monitored by lymphocytes to ensure that the correct self-antigens are presented; the presence of any non-self antigens triggers an immune response.

Mammalian mothers are faced with a problem. The genome of the fetus they carry within their wombs is half maternal and half paternal. Thus, antigens presented by the fetus that are paternal in origin would be considered foreign by the mother’s immune system. Logic would dictate that the fetus would trigger an immune response and therefore would be eliminated. However, the presence of billions of humans on the Earth and the continued growth of the human population contradicts this prediction. How does the fetus evade the maternal immune system and survive for nine months in an environment that considers it foreign?

In 1953, Billingham and Medawar proposed a few hypotheses to address this conundrum. They proposed that perhaps the fetus was immunogenically immature, that the mother’s immune system was temporarily weakened during pregnancy, that the uterus is an immunoprivileged site, and that there is a unique and complex immune barrier at the placenta<sup>1</sup>. The first three of these hypotheses have generally been abandoned because several studies have disproved them. Studies by Hoskin and Murgita have shown that fetal cells can elicit an immune response and therefore are not immunogenically immature<sup>2</sup>. When the immune status of pregnant women and non-pregnant women are compared, no differences are observed<sup>3</sup>. Finally, the existence of ectopic pregnancies (though very

dangerous to the health of the mother) shows that the uterus is not an immunoprivileged site<sup>3</sup>.

The final hypothesis is still to be considered. A successful pregnancy may be mediated in two ways: the maternal immune system may be prevented from recognizing the fetal tissue as foreign and/or the cells of the maternal immune system may be prevented from mounting an immune response<sup>3</sup>.

## Escaping Detection or Suppressing Mom’s Response?

Intuitively, if a fetal cell does not want to be recognized as a foreign body, then hiding its antigens by not presenting them on the surface seems to be the most effective way of escaping detection. T lymphocytes cannot detect the presence of a pathogen or a foreign body if antigens are not presented to them via MHC molecules and therefore, no T-cell mediated response can be mounted. However, there are lymphoid cells that will destroy a cell that does not have any MHC molecules on its surface. These lymphoid cells are called natural killer (NK) cells. Thus, fetal cells devoid of antigens presented by MHC molecules would be eliminated by NK cells.

To prevent cytotoxic destruction by NK cells, fetal tissue displays a unique type of MHC class I molecule encoded by the gene HLA-G. This molecule is considered to be a non-classical MHC molecule because of its low allelic variation (only two polymorphs are known) and restricted tissue distribution (found only in the placenta)<sup>4</sup>. A study by Kovats et al. localized the expression of HLA-G to the trophoblast cells, the embryonic contribution to the placenta<sup>5</sup>. Its function in the placenta may be inhibitory as it may prevent the activation of decidual cytotoxic T cells and NK cells. (The decidua is the maternal contribution to the placenta). Studies have shown that cells expressing HLA-G are

unable to activate T cells<sup>6</sup> and inhibit cytotoxic lysis mediated by NK cells<sup>7</sup>. It has also been shown that NK cells have receptors called killer inhibitory receptors (KIRs) and that upon binding to MHC molecules, NK cell cytotoxic activities are inhibited.

Another lymphoid cell seems to play an important role in a successful pregnancy: the macrophage. Classically activated macrophages mediate proinflammatory responses, which involve  $T_H1$  cell activation and cytokine secretion. Cytokines such as IL-1, IL-6 and IL-12 promote inflammation by increasing the permeability of endothelial tissue. The increased permeability increases the access of effector cells, thereby increasing lymphocyte and NK cell activation, respectively. In contrast, “alternatively” activated macrophages promote anti-inflammatory responses by secreting cytokines, such as IL-10 and IL-1-R-antagonist, that downregulate the inflammatory response and inhibit the activation of  $T_H1$  lymphocytes<sup>8</sup>.

Alternatively activated macrophages are preferentially found in healthy, normal placentas<sup>9</sup>. In vitro studies have shown that these macrophages inhibit the proliferation of cytotoxic and  $T_H1$  cells<sup>10</sup>. The secretion of anti-inflammatory cytokines and the inhibition of T cell activation and proliferation, strongly suggest that alternatively activated macrophages create an immunosuppressive environment in the placenta.

As mentioned above, alternatively activated macrophages produce the cytokine, IL-10. In addition to its functions already mentioned, IL-10 also upregulates the production and display of MHC class II molecules on the surface of macrophages. MHC class II molecules bind to  $T_H1$  and  $T_H2$  receptors to activate both kinds of T cells. However, the presence of IL-10 biases the activation towards  $T_H2$  because IL-10 has an inhibitory effect on  $T_H1$  cells. The significance of this bias lies in functions of  $T_H1$  and  $T_H2$  cells.  $T_H1$  lymphocytes function mainly as inflammatory T cells. They assist in classically activating macrophages, thereby promoting the inflammatory response.  $T_H1$  cells also help  $CD8^+$  T cells differentiate into cytolytic (or cytotoxic) lymphocytes (CTL). CTL-mediated immune response destroys target cells by releasing enzymes such as perforin and granzymes that puncture holes in the target membrane, thereby lysing the cell.  $T_H2$  lymphocytes function as helpers in activating B cells. Activated B cells can then differentiate into plasma cells that produce and secrete antibodies.

## Why Does Mom React The Way She Does?

Why should one type of immune response be favoured over another on the materno-fetal interface? The answer to this question is still uncertain. However, there are a few things known about each

type of immune response and their effect on the outcomes of pregnancy. Hill et al. conducted a study on women who experience recurrent spontaneous abortions (RSA)<sup>11</sup>. They took blood samples from women with RSA and fertile women, all of whom have been pregnant within a year of blood extraction. The blood supernatants were applied to mouse embryos. They observed severe embryotoxicity from the supernatant of the blood from women with RSA and no adverse effects from that of the fertile women. Researchers analyzed the cytokine profile of these supernatant samples. They found that the cytokine profile of the women with RSA was consistent with conditions of  $T_H1$  lymphocyte response: increased concentrations of IL-2 and TNF- $\gamma$ . In contrast, those of the fertile women were consistent with conditions of  $T_H2$  lymphocyte response: increased concentrations of IL-4, IL-10 and TNF- $\gamma$ . The correlation between a  $T_H1$  cytokine profile and recurrent miscarriages strongly suggest that a  $T_H1$  mediated immune response during pregnancy is detrimental to the fetus.

The main mechanism by which  $T_H1$  cytokines mediate the rejection of the fetus may be through the activation of cytotoxic T cells. These cytokines have been characterized to promote CTL proliferation. How is a CTL-mediated response different from a  $T_H2$ , B cell and antibody-mediated response? As mentioned above,  $T_H2$  cells activate B cells, which in turn produce antibodies. Should not the presence of antibodies be as detrimental to the fetus as cytotoxic T cells? Considering other conditions that are established by the immune cells in the placenta, antibodies probably do not harm the fetus. The main antibody found in the placenta is IgG. This is the only immunoglobulin isotype that can cross the placental barrier. IgG-covered cells are sensitized for killing by NK cells by binding to an Fc receptor on the surface of NK cells<sup>8</sup>. However, it was mentioned earlier that HLA-G MHC class I molecules inhibited the action of NK cells through their association with their inhibitory receptor called KIR. This inhibitory signal supercedes any activating signal the NK cell may receive from IgG-coated cells. Therefore, antibody-dependent cell-mediated cytotoxicity is essentially halted by the binding of HLA-G on the fetal cell to the KIR on the NK cell.

Similarly, IgG antibodies also recruit complement components to trigger a complement cascade<sup>8</sup>. The mechanism by which complements destroy cells is analogous to the way perforin and granzymes functions during CTL-mediated lysis; they converge on the surface of the target cell to create a hole, thereby lysing the cell. Recent studies have shown that the complement inhibitor, Crry, may be another way that the maternal immune system is regulated to promote a successful pregnancy. Crry is an inhibitor of the C3

and C4 components of the complement cascade<sup>12</sup>. Xu et al. showed that targeted disruption of *Crry* in the trophoblast and surrounding decidual tissue resulted in the progressive deterioration of mouse embryos and eventually fetal death. Evidence of C3 fixation on the embryos suggests that the complement cascade triggered fetal death. Supporting evidence shows that *Crry*-deficient embryos survive if the mother and the embryo are also deficient in C3, therefore, no complement-mediated lysis of fetal tissue occurs<sup>13</sup>.

## Another Mechanism For Keeping Mom Calm

Another mechanism for preventing fetal rejection that has received some attention from researchers is tryptophan catabolism. Tryptophan is an essential amino acid that humans cannot synthesize within the body. Even organisms that can synthesize their own tryptophan prefer to obtain it from outside sources because of its high energetic cost, for it is the most energetically expensive amino acid to synthesize<sup>14</sup>. Our immune system has taken advantage of that fact by utilizing an enzyme called indoleamine-2,3-dioxygenase (IDO) to catabolize tryptophan and deplete free tryptophan sources during a bacterial infection to curb or slow down the replication of bacteria.

Interestingly, it seems that tryptophan catabolism is employed by the immune system to accomplish another goal: to prevent rejection of the fetus. A study by Mellor et al. shows that IDO is secreted by cells located at the materno-fetal interface. The presence of IDO in the placenta results in a local depletion of the free tryptophan supply. It has been demonstrated that alternatively activated macrophages express IDO and can suppress T cell proliferation in vitro. Tryptophan deprivation may reduce or inhibit the immune response in two ways: it may inhibit lymphocyte proliferation or it may halt the manufacture of effector proteins<sup>14</sup>.

Mellor et al. investigated the role of IDO in sustaining a pregnancy. They administered 1-methyltryptophan, which is a pharmacological inhibitor of IDO, to pregnant mice carrying allogenic and syngenic fetuses. An allogenic fetus has a single MHC allele different from its mother, while a syngenic fetus has all the same MHC alleles as its mother. After administration of the IDO inhibitor, rapid rejection of the allogenic fetus was observed while the syngenic fetus remained unharmed.

The researchers then tested to see if the rejection of the allogenic fetus was mediated by maternal T cells. They took pregnant mice who suffer from severe combined immunodeficiency (SCID). SCID mice do not have either T cells or B cells due to a defect in the RAG enzyme, which catalyzes the rearrangement of the immunoglobulin genes. This defect

prevents the production of functional immunoglobulin receptors and therefore, these mice lack any mature T and B cells. When SCID mice carrying allogenic fetuses were exposed to the IDO inhibitor, the fetuses survive. Taken together, these results strongly suggest that tryptophan catabolism is necessary for sustaining a successful pregnancy.

## An Enigma And Reasons To Solve It

The immunosuppressive mechanisms discussed above probably all act in concert to prevent the rejection of the allogenic fetus. How the maternal immune system can tolerate such a large foreign body for nine months is truly an enigma. Immunosuppressive mechanisms are surely required and at the same time, the integrity of the mother's immune system must remain intact to protect the mother from pathogenic attack. Unraveling this puzzle is of great interest for its own sake. Subsequently, understanding these mechanisms may lead to treatments that will help women who experience recurrent spontaneous abortions maintain a pregnancy and carry a baby to term. Understanding these processes may also help us in preventing rejection of donor tissue grafts by the recipient, since the goals of a pregnancy and tissue grafts are relatively the same: to sustain a foreign tissue within an immunocompetent host. Discoveries from continued active research in this area are eagerly awaited by people interested in reproductive technologies and transplant technologies.

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