The "Miracle" fetus: an excellent model for apoptotic cell-induced immune tolerance?

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1. ABSTRACT

For several decades, people have wondered why pregnant mothers do not reject fetuses bearing allogeneic paternal antigens. Several hypotheses have been proposed, including a physical barrier between fetuses and mothers, immaturity of fetal antigens and temporary dormancy of the maternal immune system. Based on the "cell death immune recognition model," the author proposes a hypothesis that pregnancy tolerance is an active immune response of the maternal immune system, which is induced by apoptotic cells bearing paternal antigens. The primary steps in the induction of pregnancy tolerance are apoptosis of fetal cells as well as spermatozoa; phagocytosis of those dying/dead cells by maternal antigen presenting cells (APC), migration of APC to local lymph nodes, antigen presentation and induction of regulatory T cells in local lymph nodes. The hypothesis outlined below will not only help us to understand how pregnancy tolerance is induced, but also provide novel strategies to develop clinical measures for patients with infertility or pregnancy-related disorders.

2. INTRODUCTION

The mechanisms by which the immune system tolerates some antigens while initiating aggressive immune responses to others are of paramount importance for an organism. The study of immune tolerance has been the focus of immunology and transplantation for more than half a century. Unfortunately, although many hypotheses have been proposed, there has been no consensus on the principles, as well as the mechanisms, underlying tolerance induction by the immune system.

Except for the experimental inbred strains, half of fetal antigens in wild animals come from genetically allogeneic fathers. According to the classic "clonal selection theory" proposed by Burnet and Medawar half a century ago, antigens of allogeneic nature should be recognized as "nonself" and eliminated by the mother's immune system. Surprisingly, fetal cells bearing allogeneic paternal antigens are neither destroyed nor eliminated, but instead they proliferate, differentiate, incorporate and assemble into a new individual, the fetus. Although

physical barriers between the fetuses and the mothers, lack of immunogenicity in fetuses and/or inertness of the maternal immune system have long been argued as the main mechanisms for pregnancy tolerance, none of those assumptions have been verified both experimentally and clinically.

In fact, pregnancy serves as a good model for how the immune system tolerates genetically foreign antigens without affecting maternal immune responses against dangerous germ or virus infection. Recently, the author proposed a novel immune recognition hypothesis, "cell death immune recognition model" to explain various immunological phenomena and stressed the importance of apoptotic cells and their phagocytes in tolerance induction (1, 2). Here, the author argues that the "cell death immune recognition model" can also explain how the maternal immune system tolerates paternal antigens during pregnancy. The author believes that apoptotic cells (especially apoptotic fetal extravillous trophoblasts and spermatozoas), maternal antigen presenting cells (APC, mainly macrophages and dendritic cells), as well as regulatory T cells, play crucial roles in pregnancy tolerance.

3. THE CHALLENGES TO MEDAWAR'S HYPOTHESIS FOR PREGNANCY TOLERANCE

In 1945, Owen found in cattle that each of a set of dizygotic twins retained cells of different blood types indefinitely after birth (3). Eight years later, Medawar reported that most cattle twins did not reject a skin graft from the other twin. Furthermore, adult animals did not reject skin grafts from allogeneic donors if the recipients had been injected with splenocytes from the same donors during their fetal life (4). Therefore, Burnet and Medawar established the classical immune recognition model, called the "clonal selection model" or "self and non-self discrimination model." According to that model, the immune system in adult animals only accepts those antigens that that have been encountered during the animals' fetal or newborn period (called self-antigens). The model states that the immune system in adult animals will reject those antigens that they have not encountered during the fetal or newborn period (called foreign antigens). Unexpectedly, although fetuses generally possess allogeneic paternal antigens (foreign antigens), they are not rejected by the maternal immune system. To explain why fetuses are not rejected during pregnancy, Medawar proposed three mechanisms, namely: the barrier between the maternal-fetal interface; the reduced immunogenicity of fetuses; and the temporarily suppressed maternal immune system. Although those assumptions have been repeatedly tested during the past several decades, they have never been proven. On the contrary, those ideas have been challenged by recent findings.

Fetal cells are in close contact with maternal cells. Three main reasons explain why intimate contact between fetal and maternal cells is indispensable for fetuses. First, fetal tissues should be anchored on the uterine wall; second, fetuses depend on maternal nutrients and oxygen supply for growth; and third fetuses utilize the maternal excretion system to eliminate fetal metabolites. As human placenta is a type of hemochorial placenta in which maternal cells and fetal cells are not physically separated by a basement membrane, fetal trophoblasts are able to maintain close contact with the maternal immune-competent cells. Indeed, the fetal extravillous trophoblasts (EVT) invade the maternal uterine mucosa (called "decidua") during pregnancy. Furthermore, intimate contact between fetal extravillous trophoblasts and maternal decidual leukocytes has been demonstrated by electron microscopy and immunohistochemistry (5). At the beginning of the 2nd trimester, maternal blood starts to perfuse the intervillous space, providing more chances for fetal cells to access the maternal immune system (6-8). Therefore, it is not surprising that maternal cells are present in the offspring long after delivery; fetal cells can also be found in mothers (9). These findings clearly demonstrate the existence of intimate contact and a mutual exchange of cells between fetuses and mothers, indicating that the assumption of a "placental barrier" between them is not valid. Actually, since a pregnant woman usually needs 3 months to establish a functional placenta, one can easily imagine that her powerful immune system would have destroyed fetal tissues if there were no other "tolerogenic" measures to protect the fetus. Moreover, the occurrence of ectopic pregnancies, such as tubal pregnancies, also supports the notion that the uterus is not the unique site for embryo implantation. The only difference between uterine pregnancy and tubal pregnancy is that the uterine wall can prevent EVT cells from over-invasion and protect the uterus, while the tube cannot stop the invasion of EVT, which may result in tubal rupture and life-threatening bleeding. Therefore, cohabitation and exchange of fetal and maternal materials is a common practice during pregnancy; the maternal immune system is "friendly" in encounters with "non-self" fetal cells.

Fetal tissue transplants robustly elicit strong rejections in pregnant females without affecting pregnancy. In rat and rabbit models, immunizing the females with male skin allografts prior to mating did not affect the incidence of pregnancy. Fetal tissues transplanted into the respective pregnant mother, either un-sensitized or sensitized with skin grafts from the fathers before mating, typically evoked rejection of the first or second allograft (10). *In vitro* studies revealed that splenocytes from pregnant mice could readily react with fetal cells, leading to maternal lymphocyte proliferation (11). The results indicate that fetal tissues are antigenetically competent in eliciting maternal immune responses, and that the pregnant mothers are fully capable of immune responses similar to those of un-pregnant animals. Clinically, the immune responses of pregnant women are not significantly reduced as compared to those of non-pregnant women (12). Indeed, HLA antisera that are widely used in HLA typing in clinical transplantation are from multiparous women (13). Fetuses depend on the mother's immune system to deal with infections; maternal antibodies are transferred through the placenta into the uterus for dealing with pathogens. This even extends beyond birth, as the newborns rely on passive immunization supplied by maternal antibodies passed through the colostrum (14).

4. "CELL DEATH IMMUNE RECOGNITION MODEL" AND ITS SIGNIFICANCE IN PREGNANCY TOLERANCE

An appropriate theory explaining the principles of the immune system is critical in that it will not only provide reasonable explanations for various immune phenomena and direct future immunological research, but also lead to the development of more effective clinical measures or drugs. Therefore, over the past 50 years, researchers have continually tried to establish an immunological theory to explain the complicated effects and mechanisms of the immune system. The "self/non-self discrimination" model and other later proposed models, such as the "danger" model and "infectious non-self model," could not easily assimilate accumulating evidence regarding autoimmunity, tumorigenesis, liver immunity, or the role of regulatory T cells. Therefore the author proposed a "cell death immune recognition model" (1, 2, 15, 16).

The "cell death immune recognition model" more clearly explains various immune phenomena, and may facilitate a better understanding of immune recognition to facilitate the implementation of novel strategies for the control of autoimmune disorders, tumors, infections and transplant rejections. Four principles are essential to the "cell death immune recognition model." First, only apoptotic cell- or necrotic cell-derived antigens can be presented by phagocytes to naïve T cells. Antigens in healthy cells are not presented, as healthy cells do not send "eat me" signals to attract phagocytes, but actively transmit "do not eat me" signals even when phagocytes happen to be nearby (17). Second, either apoptotic or necrotic cells, but not healthy cells, attract phagocytes, namely dendritic cells or macrophages (that are also APC) to scavenge dying /dead cells (18, 19). Third, only macrophages or dendritic cells residing in non-lymphoid tissues phagocytose dying/dead cells, then migrate to lymphoid tissues and present antigens to naïve T cells. Fourth, tolerance or adaptive immune response is not dependent on whether the antigens are self or non-self, but on the manner of cell death during antigen presentation. When cells die apoptotically, and are adequately phagocytosed and processed, tolerogenic signals are transmitted to APC, leading to the generation of regulatory T cells to induce tolerance (20)[1]. On the other hand, necrotic cells cause local tissues to release inflammatory cytokines or danger signals to activate APC and initiate adaptive immune responses. The final outcomes of immune responses to a specific antigen depend on the dynamic balance between the "apoptotic" and "necrotic" signals (1, 2).

The "cell death immune recognition model" adequately explains autoimmunity, liver immunity, tumor immunity and transplant rejection (1). In this paper, the author argues that the "cell death immune recognition model" very appropriately explains why fetal tissues are not rejected by the maternal immune system. Three elements are indispensable for the successful application of the "cell death immune recognition model" to pregnancy tolerance: apoptotic cells carrying target antigens; phagocytes or APC to process apoptotic cells; and induction of regulatory T cells to adjust maternal immune responses. In the following text, the author will provide evidence that there are abundant fetal apoptotic cells carrying fetal antigens, a large number of maternal phagocytes infiltrating the maternal-fetal interface and prevalent induction of regulatory T cells in pregnant females. The author believes that this evidence strongly substantiates the hypothesis: fetal apoptotic cells, phagocytosis by maternal phagocytes and the induction of regulatory immune cells in the maternal immune system contribute to the main mechanisms of pregnancy tolerance.

5. MASSIVE APOPTOSIS OF FETAL CELLS AT THE FETAL-MATERNAL INTERFACE

It is not difficult to develop the rationale for massive apoptosis at the fetal-maternal interface. First, at the site of implantation, the uterine intima (decidua) should undergo apoptosis to establish a local environment conducive to fetal attachment and growth. Second, normal fetal and placental development depends on the proliferation, differentiation and invasion of trophoblasts. As both the fetus and the placenta develop very rapidly, they undergo constant tissue remodeling in order to finely shape organs and tissues and improve function, remodeling which is characterized by trophoblast apoptosis. Third, the remodeling of arterial blood vessels, known as the spiral arteries, in the uterine wall is necessary to meet the increasing demand of fetal blood supply. Fourth, after proliferation and differentiation, aged trophoblasts should be removed without affecting the development of neighboring younger cells (25). And finally, there must be some mechanism in the uterine wall to limit the invasion of fetal cells in order to prevent uterine wall breakage, probably through the induction of trophoblast apoptosis.

A large number of apoptotic cells have been found in both the maternal and fetal compartments of the placenta during pregnancy. Apoptotic cells have been found in three important stages of pregnancy: apposition, adhesion and invasion. Apoptotic cells play an important role in trophoblast differentiation and turnover (26-28). The villous trophoblasts are undergoing constant cell renewal through a well-orchestrated process of tissue remodeling involving apoptosis. Morphologically, apoptotic cells have been detected in the villous trophoblasts of normal placentas (27, 29-31), in cytotrophoblast fusion and in the formation of the syncytial layer (26). The percentage of apoptotic trophoblast cells increases dramatically in villous placenta as gestation proceeds (29, 30). In addition, the transformation of spiral arteries is mediated by apoptosis induction in endothelial cells lining the arterial lumen. It seems that the pro-apoptotic signals for endothelial cells may be delivered by the invading trophoblast, as caspase inhibitors suppress trophoblast-induced endothelial cell apoptosis (32,33). Finally, apoptosis has also been observed in parturition (28, 29).

Fas/Fas L, TNF-R1/ TNF-alpha, TRAIIL-R1/R2 and TRAIL systems are involved in apoptosis induction at the fetal-maternal interface. The Fas/FasL system,

important apoptosis-inducing machinery, has recently been implicated as one of the mechanisms by which trophoblasts induce smooth muscle cell apoptosis (34, 35). Trophoectoderm expresses FasL, while endometrial epithelial cells express Fas; thus Fas/Fas L interaction induces apoptosis of endometrial epithelial cells, which helps trophoblasts to break the epithelial barrier and to invade the decidua during implantation. Similarly, Fas has also been detected on endothelial cells in spiral arteries, suggesting that the Fas/FasL system might be involved in apoptosis of endothelial cells. A FasL-blocking antibody could abrogate trophoblast-induced endothelial cell apoptosis (33). Interestingly, although trophoblast cells express Fas, they are resistant to Fas-mediated apoptosis under normal conditions (36). In contrast, trophoblast cells during the first-trimester actually proliferate rather than undergo apoptosis after Fas stimulation (37). TNF-alpha is another potent apoptosis inducer that plays crucial roles in the induction of trophoblast apoptosis (38, 39). TNF-α and its receptor,TNF-R1, have been found in villous as well as in extravillous trophoblast cells (40-42). TRAIL, TNF-related apoptosis-inducing ligand (TRAIL), is another important apoptosis-inducer that binds TRAIL receptor 1 and receptor 2 TRAIL-R1/R2 . Syncytiotrophoblasts strongly express TRAIL, while villous cytotrophoblast cells express high levels of TRAIL-R1/R2, indicating that the interaction of TRAIL and TRAIL-R1/R2 mediates cytotrophoblast apoptosis (40, 43). Notably, some reports have already shown that caspases are important mediators of cytotrophoblast differentiation (44-46).

6. MACROPHAGES ARE THE MAJOR SCAVENGING CELLS FOR APOPTOTIC CELLS AT THE FETAL-MATERNAL INTERFACE

Macrophages are the most potent scavenging cells that deal with apoptotic cells during pregnancy. Macrophages constitute approximately 20-30% of the infiltrating cells at the implantation site and their numbers remain high throughout the pregnancy (47, 48). Generally, macrophages are in close contact with apoptotic extravillous trophoblast cells, able to remove apoptotic cells by phagocytosis and promote trophoblast survival as well as the transformation of spiral arteries (49-51). Normally, the removal of apoptotic cells is so rapid and efficient that dead cells are hardly detected *in vivo*, even where cell death is massive. However, the 'smoking gun', the massive accumulation of macrophages in areas of cell death, is easily found during embryonic development (52, 53). Mutations in the genes encoding the key macrophage growth factor, colony-stimulating factor (CSF-1) (*op/op*), or encoding the CSF-1 receptor (*c-fms*), result in both male and female infertility (54-56). Phagocytosis of apoptotic cells drives macrophages to secrete? VEGF and TGF-beta, therefore, I have hypothesized that apoptotic cells also promote cellular proliferation in fetuses as well as in tumors (1). Curiously, macrophages themselves can transform into vascular elements, including endothelial cells, myofibroblasts and smooth muscle (57-62). Selectively depleting macrophages prevents eye remodeling, which can be restored with treatment of bone marrow-derived macrophages, substantiating the notion

that phagocytosis of apoptotic cells is indispensable in tissue remodeling (63, 64). Although dendritic cells are also present at the decidua parietalis and basalis with a unique expression of DC-SIGN, their significance in scavenging apoptotic cells and in antigen presentation during pregnancy remains elusive (65, 66). It should also be remembered that phagocytosis is so pivotal during fetal development that, even in the absence of macrophages, apoptotic cells are phagocytosed by neighboring mesenchymal cells, although the latter might not be as efficient as macrophages (67, 68).

7. DOES SEMEN INDUCE TOLERANCE TO PATERNAL ANTIGENS THROUGH APOPTOTIC SPERMATOZOA?

There have been many findings indicating that semen induces maternal immune tolerance to paternal antigens, which subsequently facilitates embryo implantation and placental development. Semen exposure, either "acute" exposure at the initiation of *in vitro* fertilization (IVF) pregnancy, or ongoing "cumulative" exposure over a period of time prior to conception, promotes pregnancy success (69). Clinically, the use of barrier methods for contraception or limited sexual experience is associated with increased risk of implantation failure, recurrent miscarriage and preeclampsia (70). Similarly, the length of sexual cohabitation before conception was inversely related to the incidence of pregnancy-induced hypertension. The relative risk of pregnancy-induced hypertension was seven times higher in women who reported less than 4 months' sexual cohabitation with their male partners as compared to those with more than one year's sexual cohabitation (70). Treatment with exogenous seminal plasma may improve embryonic viability in women suffering from recurrent miscarriages (71). Experimentally, intra-uterus injection of semen can increase the litter size and weight in rats. Furthermore, substantially low rates of implantation have been found in animals impregnated by embryo transfer without previous exposure to seminal plasma or spermatozoa (72).

As the ejaculate is mostly confined at the cervix and only a small number of spermatozoa reach the uterus, it is important to elucidate whether local factors in the vagina contribute to semen-induced immune tolerance. The findings thus far suggest that phagocytosis of apoptotic spermatozoa by antigen-presenting cells in the vaginal wall as well as antigen presentation to lymphocytes in local lymph nodes play an important role.

Apoptosis is a normal and common practice in testicular germ cell apoptosis. It has been shown that in a normal state, Sertoli cells express Fas L to kill Fas-positive spermatozoa. As only a small number of spermatozoa penetrate the uterine cervix, most of them should die in the vaginal tract and this process should be mediated by apoptosis. In mice, mating initiates an influx and activation of macrophages and dendritic cells within hours (73). In women, macrophages also accumulate after sexual intercourse and have been shown to phagocytose post-

capacitated spermatozoa (74, 75). Removing the lymph nodes draining the uterus after exposure to semen results in an aggressive immune response that leads to reduced litter size, as well as decreased fetal and placental weights (76, 77).

As spermatozoa express many paternal antigens, including MHC I and MHC II, the author argues that phagocytosis of apoptotic spermatozoa by APC in the vaginal tract, migration of paternal antigen-primed APC to local lymph nodes and antigen presentation to local T cells help to establish semen-induced immune tolerance.

8. PREGNANCY TOLERANCE IS A DOMINANT PROCESS THAT INCLUDES THE INDUCTION OF REGULATORY T CELLS

Successful pregnancy has long been associated with a Th2-type cytokine profile that has been proposed to be the main mechanism for tolerance induction by the maternal immune system (78-80). As Th2 has been extensively reviewed, the author will mainly focus on the significance of a recently identified T cell subpopulation, CD4+CD25+ regulatory cells, during pregnancy (81).

Clinical observations indicate that many women experience considerable improvement in certain autoimmune diseases during pregnancy, implicating the role of regulatory immune cells. Early evidence came from experiments that splenocytes from pregnant mice prolonged tumor graft survival as well as inhibited MLR in comparison to splenocytes from virgin mice (82, 83). After the identification of CD4+CD25+ as a marker for regulatory T cells, accumulating experimental evidences proved that regulatory T cells are important in the induction of pregnancy tolerance. In mice, CD4+ regulatory T cells are induced in semi-allogeneic and syngeneic pregnancies, indicating that their induction is a normal part of the early pregnancy "program" and not specific to allogeneic antigens (84-86). A substantial increase in CD4+CD25+ T cells in the spleen and lymph nodes draining the urogenital tract can be noted as early as the second day of pregnancy. In humans, natural regulatory T cells were found in the 1st and 2nd trimesters, peaking in the 2nd trimester (87). Recently, the expression of Foxp3 has been identified as an important marker of regulatory T cells. Regulatory T cells (one third of total CD4+ T cells) exhibit high levels of Foxp3 mRNA accumulated in the uterus. Similar changes in CD4+CD25+Foxp3+ regulatory T cells have been observed in pregnant women (87-89).

It is interesting that the percentage of CD4+CD25+[bright] cells was higher in decidual tissue as compared to peripheral blood (84, 90). The population of regulatory T cells was reduced in women with spontaneous abortion compared to those undergoing elective termination of pregnancy (89, 90). Similarly, an absence of CD4+CD25+ T cells leads to early failure of gestation after implantation in allogeneic but not syngeneic pregnancies (86). Furthermore, adoptive transfer of the splenocytes from pregnant mice promoted the growth of tumor grafts (82). Notably, apart from CD4+CD25+ regulatory T cells,

other kinds of regulatory T cells might also exist during pregnancy, such as CD8+ regulatory T cells (91).

8. HYPOTHESIS AND PERSPECTIVE

From an evolutionary point of view, a constant challenge for organisms is the hostile environment that threatens the lives of organisms or their descendents. In order to keep the species alive throughout the long history of evolution, a key question for an organism is how it can safely pass genetic material on to descendents with superior reproductive fitness. Various methods have been developed, from the simple replication and division in virus and germs, oviparity in reptiles and birds, to viviparity in mammals. It should be remembered that the successful development of viviparity millions of years ago clearly illustrates the ability of the mammalian immune system to accept antigens of genetically foreign nature. Animals as well as plants co-exist with other genetically distinct organisms; humans harbor normal intestinal bacterial populations and plants utilize rhizobium for nitrogen fixation. Therefore, the immune system can be understood as an evolutionary achievement that helps organisms to keep infectious microbes from killing or damaging them.

Therefore, transplants are not good models for the study of the immune system, except for special instances. Nonetheless, transplants have been widely used in the study of immune responses due to convenience of the experimental design and relatively definite outcomes. Two issues that characterize transplantation do not happen in normal animals: first, ischemia and reperfusion injuries during donation, preservation and transplantation of the grafts; second, donor-derived APC are able to directly present donor-derived antigens to recipient T cells: direct recognition. These two factors dramatically activate both donor and recipient APC, greatly enhancing the frequency of donor antigen-specific T cells, leading to extraordinarily powerful immune responses against genetically distinct donor antigens. It is not surprising that theories established on the results of transplant models can hardly extrapolate to immune responses in other situations, such as pregnant immune tolerance, tumor immune tolerance, etc. As transplant is an artificial system, rather than an evolutionary achievement, we must be very cautious in explaining the findings from transplant models, especially when extrapolating to other immune responses.

On the other hand, the fetus itself represents an evolutionary attempt to create descendents, which is fundamentally different from an allograft, except for the commonality of allogeneically foreign antigens. One should not take for granted that the results from transplant models can apply to pregnancy tolerance. Perhaps, a more appropriate scientific question may be "why allografts are rejected," rather than "why fetuses are not rejected." Pregnancy serves as a good model for adult immune system acceptance of allogeneically "foreign" antigens without detrimental effects on maternal tissues. We have yet to determine how the maternal immune system tolerates paternal antigens, while preserving the capability to eliminate microbial pathogens.
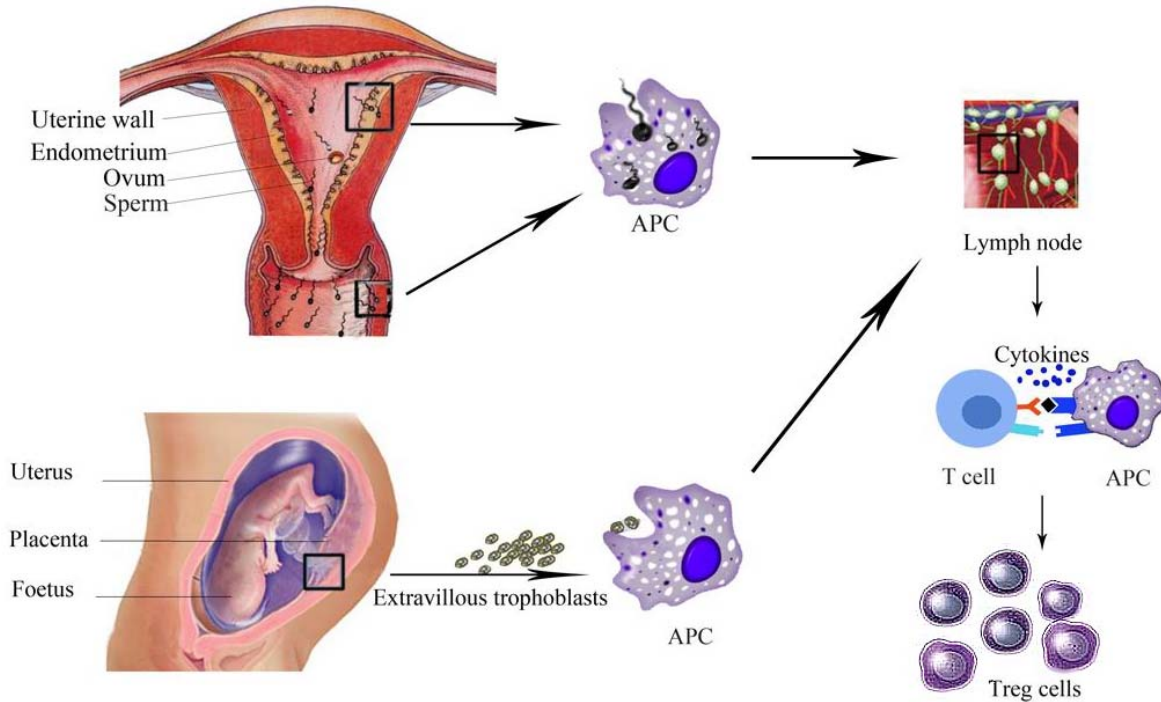
Figure 1. The mechanisms of tolerance induction of the maternal immune system to paternal antigens. Prior to pregnancy, large numbers of apoptotic spermatozoa accumulate after sexual intercourse in the vagina and cervix. During pregnancy, apoptotic cells, such as EVT, etc, accumulate at the fetal-maternal interface. APC are attracted to the vagina wall or fetal-maternal interface to scavenge those paternal antigen-bearing apoptotic cells. Phagocytosis of apoptotic cells by maternal APC, migration to local lymph nodes and the presentation of paternal antigens to maternal T cells are critical steps in paternal antigen priming. Finally, the induction of regulatory T cells in draining lymph nodes plays a crucial role in the genesis of the pregnancy tolerance exhibited by the maternal immune system when confronted with paternal antigens.

In this paper, the author proposes a hypothesis that pregnancy tolerance is induced by apoptotic cells bearing paternal antigens, such as spermatozoa and EVT (Figure.1). The phagocytosis of apoptotic cells by maternal APC and the migration to local lymph nodes for the presentation of paternal antigens to maternal T cells are critical steps in antigen priming. The induction of regulatory T cells also plays an important role in the genesis of pregnancy tolerance. The detailed mechanisms remain to be elucidated, such as the role of different APC, macrophages and dendritic cells, as well as the detailed effects of different T cell subpopulations, such as Th2, CD4+CD25+ T cells, as well as other T cells with regulatory capability. Investigations to answer those questions will not only help us to more deeply understand the mechanisms of pregnancy tolerance, but also provide more clues to understand basic immune functions. Furthermore, the novel concept provides a more consolidated theoretical basis for clinicians to treat patients with infertility or other pregnancy-related complications that might be related to the inadequate induction of tolerance to paternal antigens.

10. ACKNOWLEDGEMENTS

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Footnote: Although the concept of regulatory T cells has been widely accepted, there has been no strict definition. Notably, this type of cell had been studied for many years under the name of "suppressor T cells." Due to the difficulty in identifying a specific marker and the inconsistent findings among different laboratories, that idea was abandoned. The finding that CD4/CD25 immunoreactivity serves as a good marker for a special subset of T cells with immunosuppressive activity revived the study of immunosuppressive T cells (21). As the authors coined a new name for those cells, regulatory T cells, people started to use "regulatory T cells" instead of "suppressor T cells" (22). In most cases, regulatory T cells refer to CD4+CD25+ T cells or CD4+CD25+Foxp3+ T cells. Other T cell subsets also have immunosuppressive activity, such as CD103+CD8+ T cells, CD8+CD28- T cells, etc (23,24). Here I use regulatory T cells to refer to any T cells that have immunosuppressive or regulatory activity, not just CD4+CD25+ T cells.

Abbreviations: APC: antigen-presenting cells; EVT: extravillous trophoblast cells; Fas L: Fas ligand; TNF: Tumor necrosis factor; TRAIL: TNF-related apoptosis-inducing ligand; TRAIL-R1/R2: TRAIL receptor 1 and receptor 2; CSF: colony-stimulating factor; VEGF: Vascular endothelial growth factor; TGF-beta: Transforming Growth Factor-beta; IVF: In vitro fertilization; MLR: mixed lymphocyte reaction; Foxp3: forkhead box P3;

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