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REVIEW

Mother's little helpers: mechanisms of maternal-fetal tolerance

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The evolutionary adaptation in mammals that allows implantation of their embryos in the mother's womb creates an immunological problem. Although it ensures optimal nourishment and protection of the fetus throughout its early development, intimate contact with the mother's uterine tissue makes the fetus a potential target for her immune system. As half the fetal genes are derived from the father, the developing embryo and placenta must be considered a 'semi-allograft'. Such a mismatched organ transplant would be readily rejected without powerful immune suppression. During pregnancy, however, the semi-allogeneic fetus is protected from assault by the maternal immune system over an extended period of time. The mother's immune system seems to recognize the fetus as 'temporary self'. How this feat is managed is key to understanding immunological tolerance and intervention in treating disease.

Hemolytic disease of the newborn was the first recognized immunological complication in pregnancy[1]. In this condition, immunization of the mother by fetal erythrocyte antigens (rhesus) through exposure to fetal blood during birth leads indirectly to antibody-mediated hemolysis in the fetus of a consecutive pregnancy. The idea of maternal-fetal tolerance itself, however, was first recognized and discussed in depth by Sir Peter Medawar[2].

Billingham, Brent and Medawar[3] extended Owen's discovery that dizygotic cattle twins are born with and long retain blood cells of the other twin[4] by demonstrating that most cattle twins at birth, and for a long time after, are fully tolerant of grafts of each other's skin. That observation, along with other experiments in mice, led them to formulate the idea of immunological tolerance[3]. Although most of Medawar's work thereafter was concerned with the mechanisms of actively acquired tolerance, he was also the first to recognize the complex and far-reaching implications of maternal-fetal tolerance. In his monolog "Some immunological and endocrinological problems raised by the evolution of viviparity in vertebrates," he proposed three explanations for maternal immunological tolerance[2]: physical separation of maternal and fetal tissues; the antigenic immaturity of the fetal tissues; and immunological inertness of the mother. Although none of those explanations has wholly held up, they had a profound influence on research concerned with maternal-fetal tolerance and mechanisms of fetal immune evasion.

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Potential for antifetal immune responses

Medawar and his colleagues were concerned with the response of the maternal immune system directed against paternal alloantigen. This is the most obvious of the targets against which a detrimental maternal immune response could be launched. There are others. These include self antigens, such as carcinoembryonic antigen[5,6], whose expression is restricted to a small 'window' of time during embryonic development. It is likely that T cells expressing receptors specific for these 'oncofetal' antigens escape clonal deletion. These antigens have been considered for therapeutic exploitation, as they should allow the immune system to distinguish between neoplastic and normal tissue. Another legitimate target is infected tissue. Acute or chronic infection of the uterus or the fetus during pregnancy might necessitate activation of the immune system[7,8,9]. In this situation, rejection of the fetus could become an essential sacrifice for the mother to protect herself from the infection and to prevent a waste of resources by sustaining a child likely to be damaged by the infection at a critical early stage of its life.

There are hints that the adaptive immune system recognizes paternal alloantigens. These include anecdotal reports of females going through many miscarriages, only to find themselves happily pregnant after a change of partners[10]. Indeed, anti-HLA antisera, used for many years in tissue typing, were derived from multiparous women[11]. Infertility without a detectable physiological cause was long suspected to have its roots in faults of toleration of the fetus by the maternal immune system[12]. The first experimental proof can be found in Medawar's skin-transplantation experiments. Medawar and Sparrow commented at the time that "repeated heterospecific pregnancies cause no immunization of the mother of a kind that shows itself by tissue transplantation immunity."[13] A closer look at their data actually shows prolongation of skin graft survival in those female mice that had been through multiple matched heterospecific (allogeneic) pregnancies, suggesting that rather than immunizing, the pregnancies had a tolerizing effect.

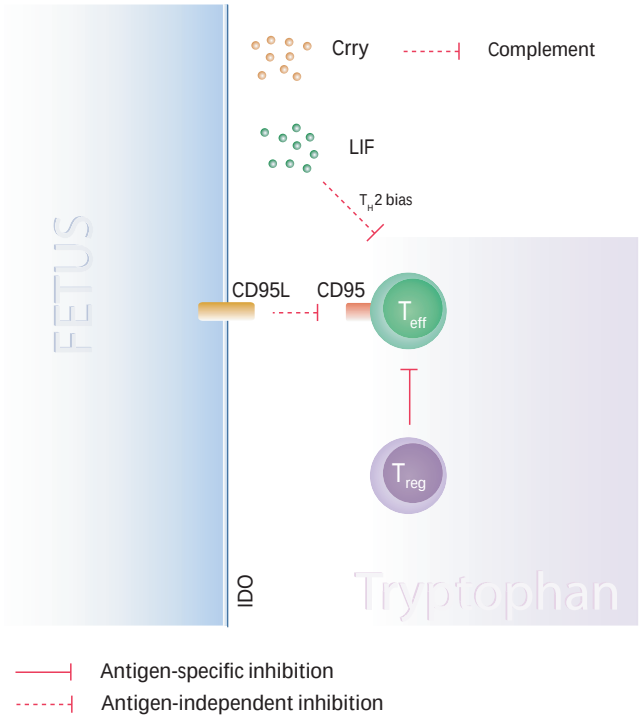

Figure 1 Mechanisms proposed to regulate mouse maternal-fetal tolerance. Crry is thought to control complement deposition. Leukemia inhibitory factor (LIF) may influence the T_H1/T_H2 ratio. $CD95^+$ effector T cells (T_{eff}) are held in check by CD95L and by regulatory T cells (T_{reg}). IDO depletes tryptophan, thus dampening local T cell responses.

Over the years, many authors have described the suppression of anti-fetal responses by a variety of factors and suppressor effects in protocols such as mixed lymphocyte reactions using maternal lymphocytes and fetal or paternal cells[14,15]. In an elegant study, the fate of T cells reactive to paternal alloantigens *in vivo* was monitored using a mouse transgenic T cell receptor model[16]. That study demonstrated that female mice accept an allogeneic tumor graft while pregnant with a conceptus from a father matching the allograft, but not while pregnant by a 'third-party' allogeneic father. After delivery of the pups, the tumors are rejected[16]. Such experiments demonstrate the specificity of the suppression to paternal alloantigen and its temporal nature.

Those findings raise the issue of how an immune response directed against the paternal antigens is prevented. In human placentation, fetus-derived extravillous trophoblast cells infiltrate the decidua and replace the endothelium of the uterine spiral arteries to ensure the development of an adequate blood supply to the fetal-maternal unit[17]. The implantation site is richly populated by maternal leukocytes in humans, which comprise approximately 70% natural killer (NK) cells of a unique uterine type (uNK) in addition to approximately 10% T cells and approximately 20% myelomonocytic cells. As the placenta is intimately associated with the mother's decidua, it constitutes the main target for immunological attack, and many fetal immune evasion mechanisms have evolved that seem to generate a nonspecific dampening of both the innate and adaptive arms of the immune response in the uterine tissue during pregnancy. Many of these have been studied in detail in rodents. Because of differences in placental architecture[18], expression of nonclassical major histocompatibility complex (MHC) class I molecules, fast estrus cycle and short length of gestation, caution must be taken when extrapolating data obtained from such model systems to the situation in humans. However, it is likely that some key mechanisms of maternal-fetal tolerance are conserved throughout mammalian evolution.

Mechanisms of fetal immune evasion

Medawar's postulates laid the foundation for the idea that the gravid uterus might constitute an immune-privileged site. The testes, eyes and brain are examples of tissues that are thought to prevent access to cells of the immune system by setting up anatomical and physiological barriers. They express factors that promote 'immunological ignorance' by nonspecific suppression of the cells of both the innate and the adaptive immune system[19–21]. Many factors promoting immune evasion have been explored, some of which are discussed below (**Fig. 1**).

T cells are uniquely sensitive to fluctuations in the local concentration of the amino acid tryptophan. A drop in tryptophan concentration results in substantial suppression of T cell proliferation. Some tissue macrophages have exploited this property by producing indoleamine 2,3-dioxygenase (IDO) in response to interferon-γ. IDO, in turn, causes a local depletion of tryptophan, thus disabling local T cell responses. IDO is also produced by the maternal uterine mucosa (decidua) and by the fetal syncytiotrophoblast, which invades the uterus[22,23]. Treatment of pregnant mice carrying a syngeneic or allogeneic fetus with a pharmacological inhibitor of IDO results in loss of only the allogeneic fetus[23]. However, a mouse lacking IDO has been shown to undergo normal pregnancy, a finding that remains to be fully resolved[24].

Another mechanism in the fetal trophoblast's repertoire of local immune evasion strategies is the expression of CD95L (Fas ligand), which promotes apoptosis of activated lymphocytes expressing CD95 (Fas). Pregnancy in *gld* mice, a natural mutant strain lacking functional CD95L, is characterized by extensive infiltration of lymphocytes and necrosis at the interface of the decidua with the placenta[25]. This mutant strain suffers from small litters and an increase in the resorption of fetuses even if the pregnancy is a result of a homozygous mating. In this scenario, the maternal immunity lacks an allogeneic target although this does not exclude the possibility of involvement of CD95L in the dampening of antigen-specific responses, it suggests that CD95L has a more basic function during pregnancy. On a more speculative note, expression of CD95L might be considered to be involved in the prevention of autoimmune activation due to rare embryonic self antigens.

Similarly ambiguous is the function of complement inhibitor Crry. Lack of this protein in mice leads to gestational failure due to complement deposition at the maternal-fetal interface. This situation is 'rescued' by lack of the C3 component of complement[26]. Resorption of fetuses in $Crry^{-/-}$ mice shows some of the features of immunological rejection, such as complement deposition and infiltration of the extraembryonic tissue with polymorphonuclear cells. However, as with CD95L, an effect on pregnancy can be found in syngeneic mating lacking paternal alloantigen. Thus, it remains to be determined to what degree the prevention of complement activation is involved in maternal-fetal tolerance. There is no direct counterpart to Crry in humans, although there are alternative mechanisms of complement suppression[27].

The expression and secretion of leukemia inhibitory factor by the maternal endometrium and later by the decidua has been suggested to contribute to a localized immune suppressive environment[28]. Leukemia inhibitory factor is essential in blastocyst implantation, but its involvement in promoting maternal-fetal tolerance by shifting the 'T helper type 1 (T_H1)–T_H2 balance' is less well understood. Complications of human pregnancy such as pre-eclampsia and spontaneous fetal loss have been associated with a shift in the mother's serum from a T_H2-biased to a T_H1-biased cytokine profile[29,30]. Based on that observation, an experimentally induced shift from a T_H2 to a T_H1 bias of cytokine expression might expected to have detrimental effects on pregnancy

outcome. However, mutant mice with double- and quadruple-gene deletion leading to a lack of some or all of the T_H2 cytokines interleukin 4 (IL-4), IL-5, IL-9 and IL-13 undergo allogeneic pregnancies with normal litter sizes. That finding is not consistent with a causative link[31]. Thus, these cytokine shifts may be irrelevant to pregnancy. The appearance of T_H1 cytokines may be simply a reflection of cellular immunity 'kicking in' as a response to pregnancy complications. A change in cytokine expression provides a useful 'readout' for the underlying cellular immune response that accompanies stress or infection.

Arguably the most important immune evasion strategy is the absence of classical MHC alloantigens on the trophoblast. MHC class II alloantigens are completely absent on extravillous trophoblast cells in both humans and rodents[32]. The lack of MHC class II antigen expression on trophoblasts has been postulated to be one of the essential mechanisms by which the semi-allogeneic fetus evades immune rejection by the maternal immune system. That line of argument is questionable, as constitutive MHC class II expression is restricted to B cells and 'professional' antigen-presenting cells such as dendritic cells and macrophages[33]. The fact that expression of MHC class II cannot be induced on the trophoblast by stimuli such as interferon-γ that are effective in other tissues is perhaps more notable, as it suggests that antigen presentation via MHC class II is prevented even in the context of inflammation[34]. The absence of classical MHC class I molecules[35], which are otherwise fairly ubiquitously expressed, is relevant, as their downregulation is exploited in viral immune escape strategies[36]. Human trophoblasts do not express classical MHC class I HLA-A and HLA-B molecules but do express classical HLA-C and nonclassical HLA-E and HLA-G MHC class I molecules (**Fig. 2**). HLA-G is mostly restricted in expression to the extravillous trophoblast, so it is generally assumed to be involved in relation to placental implantation, through modulation of maternal decidual leukocytes[37]. Some early studies suggested that HLA-G was polymorphic, but those were in error. It is now accepted that HLA-G is monomorphic, so variants do not account for individual differences in pregnancy outcome[38]. Loss of HLA-A and HLA-B, coupled with retention of HLA-C, by trophoblasts is an immune evasion strategy recapitulated by infection of cells with human immunodeficiency virus. It has been proposed that the virus selectively removes classical MHC class I molecules that would be targets for cytotoxic $CD8^+$ T cell responses. At the same time, HLA-C molecules are retained, as these are instrumental in downmodulating NK responses[39].

The uNK cells and placentation

The functions of uNK cells are poorly understood, but their activity, characteristics and abundance suggest that they participate in the 'decidualization' process, both as guardians of mucosal integrity and arterial function and as controllers of trophoblast invasion[17]. Decidualization induces uNK cells in each menstrual cycle. In peripheral circulation, NK cells are best known for their involvement in cytotoxicity against target cells and in cytokine production. The uNK cells are not cytotoxic and seem to have an altogether different function. Like a small subset of peripheral NK cells, uNK cells are $CD56^{hi}$, and they also differ from $CD56^{lo}$ peripheral NK cells in that they have considerable cytokine expression[17]. An activated and expanded set of uNK cells move into the decidua, where they are found at the site of vascular remodeling. Endothelial cells lining the arteries are replaced by extravillous trophoblast cells. Remodeling of the spiral arteries is exaggerated in humans, and the muscular vessels are converted into 'gullies' to meet the huge oxygen demands of the human fetus, with its exceptionally large brain. This process takes place during the first 16–20 weeks of pregnancy.

Findings in humans have invoked the engagement of an expanded set of maternal uNK cells with ligands on the invading trophoblast[40]. The recognition of paternal ligands and the resulting crosstalk could in principle control the extent of vascularization and placental invasion. The similarities of recognition of the invading paternal tissue by NK cells and immune recognition of cells affected by an invading organism have been noted, and attention has turned to the potential ligand-receptor interactions involved in these processes. HLA-C is of paramount importance for the interaction of human NK cells through killer Immunoglobulin receptors (KIRs), and the HLA-C–KIR interaction has been reported to influence pre-eclampsia, a condition resulting in inadequate blood supply to the fetus. That observation has led to the proposal that selection for reproductive success, rather than immune recognition of pathogens, may be responsible for driving the selection of balanced polymorphisms of the main HLA-C alleles and their KIR partners. Based on their specificity for KIR, HLA-C molecules are subdivided into two groups: HLA-C1 and HLA-C2. Increased risk of pre-eclampsia is associated with HLA-C2 in the fetus when the mother has only inhibitory KIR with specificity for HLA-C2. It has been suggested that the lack of activating KIR in these mothers leads to reduced vascularization. Data indicate that cells and molecules of the immune system are intimately associated with the reproductive process. In fact, the important HLA-C dimorphisms and associated KIR receptors emerged only in the evolution of higher primates[41]. Notably, both reproduction and immune recognition tend to differ considerably in different species. What replaces the HLA-C–KIR axis in species other than humans and do they also 'borrow' immune recognition to shape placental development?

All uterine NK cells express receptors for the nonclassical MHC class I molecules HLA-E and HLA-G, expressed by trophoblasts. Receptors for the human nonclassical MHC class I HLA-E molecule differ on uNK cells versus peripheral blood NK cells. All uNK cells have high expression of the inhibitory heterodimer CD94-NKG2A, a C-lectin-like receptor for HLA-E. HLA-E has a well understood function in immune recognition, but trophoblast HLA-E, in combination with a 'cargo' of peptide derived from HLA-G, could have a unique function in placentation, through interaction with lectin-like receptors. Initially it was proposed that HLA-G functions as a ligand that inhibits NK killing of trophoblasts. However, that finding may have been due to inhibition by CD94-NKG2A recognition of HLA-E, which is dependent on binding

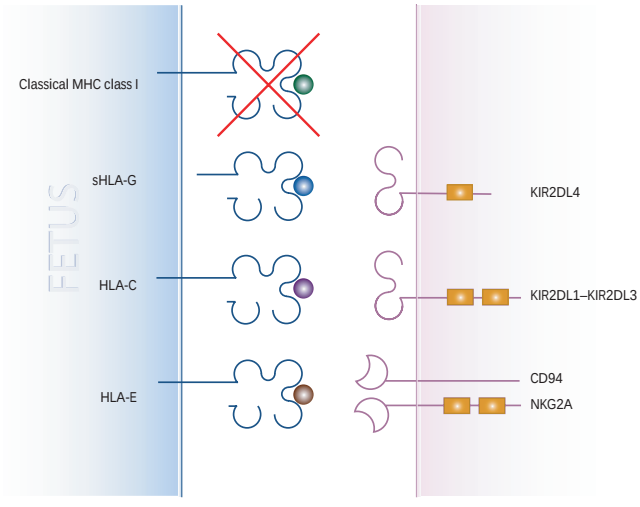

Figure 2 Some immune receptors proposed to regulate human maternal-fetal interaction. Classical MHC class I molecules are not expressed on trophoblasts. Nonclassical HLA-C and soluble HLA-G (sHLA-G) may interact with KIR2DL1–KIR2DL3 and KIR2DL4 receptors, respectively, to block NK cell recognition and to regulate vascularization.

of the leader peptide of HLA-G for surface expression[42]. Another proposed ligand for HLA-G is the unique KIR KIR2DL4, which is expressed by all NK cells[43]. That receptor-ligand association was not generally accepted in the past because of difficulties in demonstrating substantial cell surface KIR2DL4. The issue was clarified in a report showing that KIR2DL4 is unconventional in that it can function as a receptor inside the cell[44]. The KIR2DL4 molecule couples with soluble HLA-G at the cell surface and is fed into endosomal compartments postitive for the small GTPase Rab5, a molecule associated with the formation of early endosomes, by a dynamin-dependent process. This engagement of KIR2DL4 by soluble HLA-G in endosomes results in the secretion of inflammatory and angiogenic factors such as tumor necrosis factor, IL-1β, interferon-γ and IL-8. The data are consistent with the idea that the interaction of soluble HLA-G from the fetus with KIR2DL4 on uNK cells contributes to vascularization of the maternal decidua during pregnancy. Therefore, both HLA-C and HLA-G MHC class I–related ligand may be involved in implantation by shaping the vascular adaptation of maternal tissue by binding to KIR molecules. Some KIR-related leukocyte immunoglobulin-like receptors (also called ILT, LIR and CD85) are expressed on monomyelocytic and dendritic cells and also bind to HLA-G with a high affinity[45,46]. Dendritic cells that interact with HLA-G are unable to stimulate T cells, have reduced expression of the costimulatory molecules CD80 and CD86 and are associated with the generation of $CD4^+CD25^+$ regulatory T cells[47]. Thus, engagement of inhibitory leukocyte immunoglobulin-like receptors on decidual dendritic cells by placental HLA-G could act to downregulate maternal T cell–mediated responses during pregnancy.

Despite the evidence of NK cell involvement in placental recognition, pregnancy is not completely compromised in mice with substantially reduced NK cell numbers. Mature NK cells continually require IL-15 for survival and their numbers are reduced considerably in *Il15*$^{-/-}$ animals. Interpretation of those data are complicated by indications that uterine NK cell development and maturation are different from NK cell development in other tissues[48].

The adaptive immune response against paternal alloantigen

Despite all the various strategies of immune evasion, in fact the maternal adaptive immune system is 'aware of' paternal alloantigen. To explain that observation, it has been proposed that antiallogeneic T cells are somehow anergized during pregnancy[16]. In paternally matched tumor graft experiments, the allogeneic grafts are accepted until delivery, whereas more antigenic skin grafts are rejected even if the female is pregnant with a 'skin allotype-matched' conceptus. Thus, the maternal immune system retains the ability to react to paternal alloantigen throughout pregnancy. How does the maternal immune system detect the fetus, despite the immune evasion mechanisms described above? Although the trophoblast is the main contact of fetal tissue to the mother, there is extensive micro-chimerism between mother and fetus[49]. Fetal cells can be detected in the mother's blood throughout pregnancy and for a long time beyond. Conversely, maternal cells can be detected in the offspring long after delivery and survive into adulthood. Obviously the trophoblast is not the impenetrable physical barrier that prevents exchange of cells between mother and fetus, which Medawar had in mind. It is unlikely that this extent of sharing of cells between mother and fetus remains undetected by the immune system.

The trophoblast itself is thought to shed many buds and microparticles into the maternal blood circulation. Although the trophoblast might lack the ability to present paternal self antigen, there is no reason why the paternal antigens contained in its debris cannot be presented to the maternal immune system by its own antigen-presenting cells. Apoptotic cell death due to remodeling of fetal tissue might be another source of cellular debris, which will be disposed of by phagocytic cells, among them maternal antigen-presenting cells[50].

Uterine infections are likely to spread to the fetus and thereby lead to necrosis of fetal tissue. This scenario is particularly interesting, as infection during pregnancy can lead to spontaneous abortion, particularly in the early phases of pregnancy[8]. Perhaps the presentation of paternal alloantigen in the context of infection leads to a break in maternal-fetal tolerance, which in turn leads to rejection of the fetus. That could explain why the frequency of resorption of fetuses in 'abortion-prone' mouse models correlates with the health status of the animal facility in which the mice are kept[51–54]. The lower the health status of the facility, the greater the chance of infection in the females and the higher the frequency of resorption.

The function of regulatory T cells

The idea that suppressor cells might be involved in the prevention of rejection of the fetus was originally based on mixed lymphocyte reactions using splenocytes from either pregnant or virgin females[14]. Those simple coculture experiments showed suppressive, alloantigen-specific effects, which were best explained by evoking the presence of suppressor cells. Indeed, adoptive transfer of the splenocytes obtained from a pregnant mouse could promote the growth of a grafted tumor[55]. The engraftment only works if the tumor matches the allotype of the male used in the mating that led to pregnancy in the donor mouse. Again this observation invoked alloantigen specificity of the transferred suppressor cells. Early research in the field was hampered by technical limitations. In particular, the lack of markers for suppressor cells halted progress beyond a basic description of the effect. Overinterpretation of results in the field of suppressor T cells led to a slump in the credibility of the whole field[56,57]. However, suppressor T cells have undergone a phoenix-like revival under the guise of $CD4^+CD25^+$ regulatory T cells[58,59]. Doubts regarding the existence of an autonomous group of regulatory T cells have finally been laid to rest by the discovery of the regulatory T cell–specific genetic marker Foxp3 (refs. 60–62). Mice[63] and humans[64,65] lacking functional Foxp3 have no regulatory T cells and have severe autoimmune disease from birth. In contrast, ectopic expression of FoxP3 in 'normal' $CD4^+CD25^-$ T cells leads to gain of suppressor function[61].

Armed with molecular and genetic markers, research has provided evidence for the pivotal function of regulatory T cells in pregnancy. In mice, a substantial increase in $CD4^+CD25^+$ T cells in the spleen and in the lymph nodes that drain the urogenital tract can be noted from as early as day 2 of pregnancy[15]. Notably, expansion of the regulatory T cell population can be found in both allogeneic and syngeneic pregnancies, suggesting that it is independent of exposure to paternal alloantigen. As expansion of the regulatory T cell population seems to begin before implantation of the fetus, it may be driven by hormonal factors[15]. Indeed, pregnancy-associated hormones such as estrogen and progesterone have a variety of effects on the immune system. These hormones have been associated with an amelioration of autoimmune diseases such as rheumatoid arthritis[66–68]. Indeed, many women experience considerable improvement in certain autoimmune diseases during pregnancy. The pregnancy-induced increase in regulatory T cell numbers and function might provide an explanation for that effect. Despite the systemic effects that pregnancy has on the maternal immune system, it seems that the mother's immunosuppression is restricted to responses directed against the fetus and autoimmune targets. After all, both mother and developing fetus depend on the maternal immune system for the protection against pathogens. This even extends beyond birth, as the newborn relies on passive immunization by maternal antibodies transferred *in utero* through the placenta and later through the colostrum and milk for protection from diseases[69].

During pregnancy, regulatory T cells accumulate in the uterus, where they represent about a third of all $CD4^+$ T cells. Experimentally, this influx of regulatory T cells into the gravid uterus is reflected by a notable increase in Foxp3 transcripts among total uterine mRNA. An absence of $CD4^+CD25^+$ T cells leads to early failure of gestation after implantation in allogeneic but not syngeneic pregnancies[15]. In most cases, pregnancies fail very early into gestation. Sometimes, rejection of the fetus can be detected as late as midgestation. In those cases, extensive hemorrhaging at the maternal-fetal interface is accompanied by massive lymphocyte infiltration. The data are consistent with an analogous function for regulatory T cells in human pregnancy. Three independent studies have described both systemic and decidual expansion of $CD4^+CD25^+Foxp3^+$ regulatory T cell populations in the first two trimesters of pregnancy[70–72]. In mice, these cells suppress the proliferation of other T cells *ex vivo*. The finding that the proportion of regulatory T cells in the decidua of spontaneous abortions is much lower than that in elective abortions is consistent with a requirement for their presence at the maternal-fetal interface[71].

After Medawar, interest in placental tolerance decreased. For the past few decades, with a few exceptions, it has received scant attention. Part of the explanation for this has been a lack of appropriate molecular tools for studying this complex system, especially as some of the tolerance mechanisms, as explained above, are species specific. Given the spectacular successes of subjects such as immunoglobulin rearrangement and thymic T cell development, topics as important as innate immunity, regulatory T cells and adjuvant have been all but ignored[73]. Now that tools are available and regulatory T cells are accepted, perhaps it is time to take a fresh look at placental tolerance. A report of a previously unknown subset of $CD8^+$ regulatory T cells that are not MHC class I restricted but require costimulation through a member of the carcinoembryonic antigen family present on early gestation trophoblast indicates that understanding of this subject is only beginning[74].

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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