Immune interactions and tolerance between mother and embryo

An overview of mechanisms and related obstetric complications

Dr Gamal Matthias
FRANZCOG

The embryo in the first few weeks of its existence overcomes commonly known and established rules to adult life. The immune evasion of an embryo or fetus is a crucial element of normal reproduction.

Immunology of reproduction and tolerance of the genetically distinct embryo and its symbiotic existence with the mother is one of the most essential and intriguing paradoxes of life. This is an intriguing facet of reproduction which has interested me for over 16 years, looking after patients with immune disorders related to or associated with conception and pregnancy, and having collaborated and been trained with the pioneer in this field, the late Dr Alan Beer of Chicago. Understanding the processes that occur in normal conception will greatly enhance our understanding of pathological conditions and how we can manage them.

The trophoblast invasion

After the blastocyst attaches to the epithelial surface of endometrium, the trophoblast erodes into the endometrial stroma. The extravillous trophoblast invades endometrial capillaries forming venous sinusoids establishing uteroplacental circulation. That is followed by invading the endometrial spiral arteries tapping into it, destroying its walls and remodelling it.

In a normal pregnancy, the trophoblast-induced vascular changes extend all the way from the intervillous space to the origin of the spiral arterioles, from the radial arteries in the inner one third of the myometrium. It is suggested that these vascular changes are effected in two stages: the conversion of the decidual segments of the spiral arterioles by a wave of endovascular trophoblast migration in the first trimester; and the myometrial segments by a subsequent wave in the second trimester. This process was reportedly associated with fibrinoid formation and degeneration of the muscular layer in the arterial wall. These vascular changes result in the conversion of approximately 100 to 150 spiral arterioles into distended, tortuous and funnel-shaped vessels that communicate through multiple openings into the intervillous spaces.

This process of invasion of the extravillous trophoblast cells into the uterus is well balanced and finely controlled. There are delicate interactions at the implantation site with uterine natural killer (uNK) cells, macrophages and CD3-T cells. This results in production of the right balance of cytokines and chemokines, which controls the extent of trophoblast infiltration and ultimately determines reproductive success. Trophoblast over-invasion will endanger the mother. Trophoblast under-invasion and failure to modify the maternal spiral arteries leads to reproductive failure or complications. A fine boundary should, therefore, be correctly delineated between mother and fetus for pregnancy to succeed without rejection or parasitism occurring.

Figure 1.
Maternal-embryonic interface, Week 1

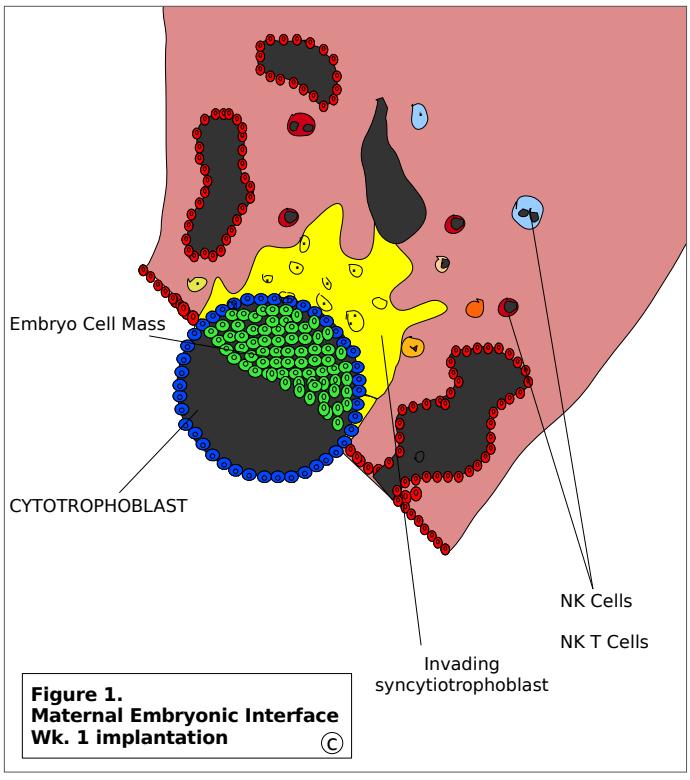

Figure 2.
Normal and abnormal implantation and placentation.

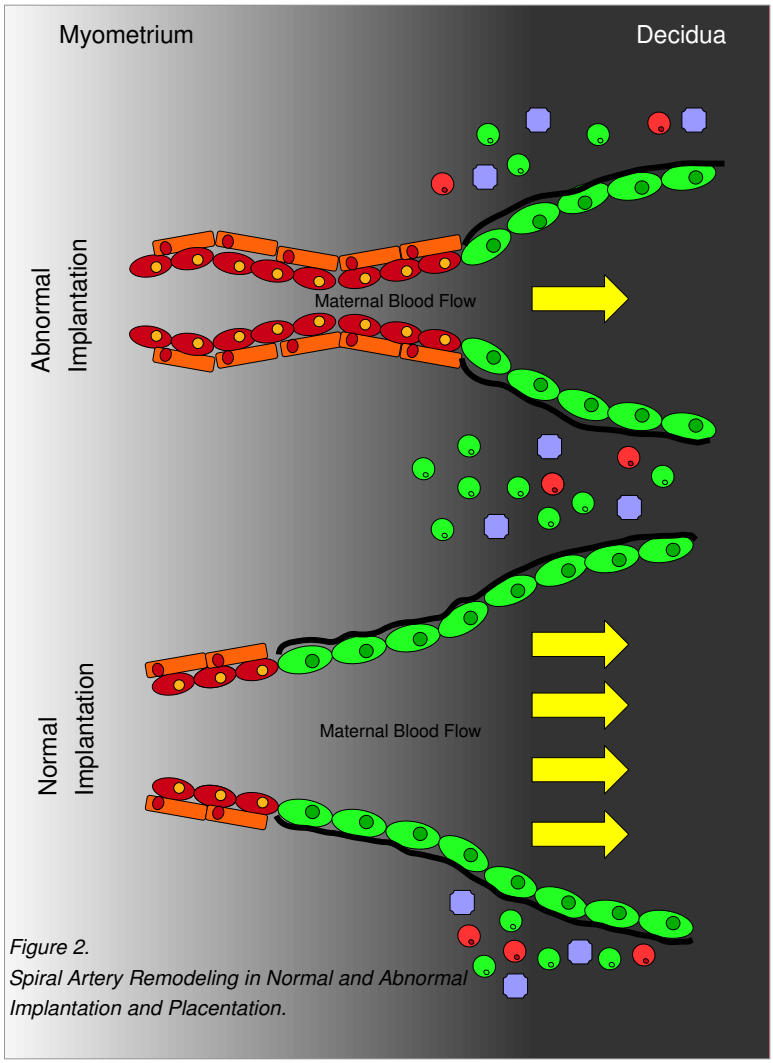

Figure 2.
Spiral Artery Remodeling in Normal and Abnormal Implantation and Placentation.

(Thanks to James E Matthias for preparation and production of Figures 1 and 2.)

Why isn't the embryo or fetus immunologically rejected by the mother?

Many hypotheses have been proposed to explain the mechanisms of maternal-fetal immune tolerance. It is still however 'work in progress' since the early works of Burnet, Medawar and his colleagues Brent, Billingham and later, Wegmann. It is difficult to cover all its aspects in detail in this setting.

The trophoblast and placenta constitute the main target for immunological attack, being in direct contact with the mother. Maternalfetal tolerance is promoted by the systemic shift in T Helper 1 and its cytokine profile to T Helper 2 with its Cytokine profile. TH2 response is an anti-inflammatory in nature.

However more recently, this view has been modified and a pathway for a controlled pro-inflammatory environment at the implantation site has been proposed to help attachment and invasion of the blastocyst. It is now considered that the conventional TH1/TH2 paradigm probably has limitations and is not applicable to all the various developmental stages of pregnancy.

The pregnant uterus and the site of a surgical tissue graft exhibit important dissimilarities. Both sites express pro-inflammatory cytokines. However the maternal-embryonic interface also expresses many anti-inflammatory cytokines and other substances that limit the maternal NK cell aggression and proliferation such as Interlukin10 (IL-10). It is now thought that the balance between the pro and anti-inflammatory cytokines at the implantation site is crucial for the success of the pregnancy. Uterine natural killer cells have unique phenotype compared to blood NK cells. They produce various cytokines and play an important role in vascular remodeling during implantation and vascular remodeling.

Leukaemia inhibitory factor (LIF), a cytokine of the interlukin-6 family, is essential in blastocyst implantation. It is secreted by the deciduas and contributes to localised immune suppressive environment. It also promotes the shift to TH2. The embryo, prior to implantation, sends signals to the endometrium. The expression of LIF and other cytokines in the uterine epithelium are gradually increased.

Another mechanism of immune evasion is that an enzyme, indoleamine 2,3-dioxygenase (IDO) is produced by the syncytiotrophoblast, destroys amino acid tryptophan needed by the mother's T-cells to respond to the fetus. A drop in tryptophan results in substantial suppression of T-cells. Thus, IDO activity protects the fetus by suppressing T-cell dependent inflammatory responses to fetal allo-antigens.

Apoptosis is critical for invasion of the developing embryo. Apoptotic cell clearance by maternal macrophages without excessive release of paternal allo-antigens from trophoblast cells is essential for controlled resolution of the inflammatory process at the implanation site. Fas/Fasligand systems play an important role in maintaining the balance at the implantation site as the mediators of programmed cell death. It is one of the pathways regulating T-cell proliferation and apoptosis and is sensitive to hormonal factors and cytokines.

More recently, the role of Toll-like receptors (TLR) expressed on the trophoblast and placenta have been suggested as key players in the activation of the innate immune system, increased production of pro-inflammatory chemokines and recruiting CD14+ monocytes to the implantation site.

One of the immune tolerance mechanisms expression of non-classical class 1 HLA-G and HLA-E, in addition to the classical HLA-C, on the extravillous trophoblast results in modulation of the maternal decidual NK cells. NK cells possess receptors as killer-cell immunoglobulin-like receptors (KIRs) and CD94, which bind to molecules HLA-G, C and E, and in doing so inhibit uterine NK target killing of trophoblast cells. The human KIR locus consists of 7 to 15 closely packed genes on chromosome 19q, which encode both inhibitory and activating KIRs.

Various immunogenetic factors have been studied including MHC genes or MHC-linked genetic regions. Some HLA haplotypes or loci in certain inbred communities could result in dysregulation of immune tolerance as it was proposed that a fetus recognising maternal tissue as non-self (a heterozygous compatible pregnancy) may have survival advantages over a homozygous compatible fetus, which would not recognise maternal tissues as non-self.

Another aspect of this hypothesis which was put forward was parental human leukocyte antigen (HLA) sharing especially with HLA-B, DQα, the locus showing the strongest association resulting in lack of blocking antibodies with subsequent exposure of the

embryo/fetus to maternal immune system attack. It suggests that maternal recognition of paternal allo-antigens, at least in early pregnancy, may be necessary for T-cell tolerance of pregnancy and establishment of the placenta.

The role of the male antigens in recruiting regulatory T-cells that migrate to the maternal fetal interface and induce a tolerant micro-environment characterised by high levels of protective molecules has been demonstrated. Moreover, some factors in seminal plasma contribute to immune endometrial preparation for implantation.

A protein called early pregnancy factor (EPF) may also have a role. By binding to a specific lymphocyte population, it recruits suppressor cells, which in turn release soluble suppressor factors that are believed to protect the pregnancy. In its association with dividing cells, EPF also has properties of a growth factor that regulates cell proliferation. Other immune-modulatory proteins such as B7 are expressed by the placenta and play a role in fetal allograft tolerance. Furthermore, Human chorionic gonadotrophin (HCG) and progesterone, both increase in early pregnancy and have immuno-suppressive properties. Additionally, HCG may be involved in maternal lymphocyte function.

'There is clearly active bi-directional dialogue between cells of trophoblast, embryo and mother to control implantation and establish vascular connections that are critical for sustaining fetal growth.'

Recurrent miscarriages

About 15 to 20 per cent of known pregnancies end in clinically recognised miscarriage. However, the total embryonic loss that is unrecognised prior to a diagnosis of pregnancy is much higher, probably 30 to 50 per cent. About 2 to 5 per cent of reproductive age women experience recurrent miscarriages, which is typically defined as three or more consecutive pregnancy losses.

Causes of recurrent miscarriages include genetic, hormonal, infectious, metabolic, anatomical (uterine and cervical), thrombophilia, environmental, occupational and immune disorders. Despite the many causes, the majority of these patients have no obvious aetiology. It can be postulated that a group of these patients have immunologic miscarriages. As there are various recognised pathways for immune evasion. Defects in these mechanisms can result in pregnancy loss.

There is evidence linking inappropriate Th1-type immunity to pregnancy loss. TH1 bias and increase in Th1 cytokines (for example, IL-2, IFN-γ,TNFα and TNFß) have been demonstrated in women suffering from unexplained recurrent miscarriages. Low Th2 cytokines (for example, IL-10 and IL-6) have also been shown in recurrent miscarriage patients.

It is possible that cytokine gene polymorphisms can influence the levels of cytokines, but that is an area that needs further evaluation. Dysregulation in the immune responses at the embryonic or fetal-maternal interface could result in exposure of the embryo or fetus to a maternal immune attack. That results in reproductive failure, manifesting as recurrent miscarriages or implantation failures and infertility. Various pathways and mechanisms are involved, encompassing TH1 bias, cytokine networks imbalances, NK cell and NK T-cell and T-cell abnormalities, immunogenetic factors and hormonal factors.

Defective deep placentation and late pregnancy obstetric complications

There is growing evidence that appropriate implantation and placentation in the early part of pregnancy ultimately influences the progress of pregnancy, fetal wellbeing and the development of obstetric complications in the latter part of pregnancy.

Intrauterine growth restriction

Poor placentation as a result of poor utero-placenal perfusion, due to defective trophoblast invasion in early pregnancy, has a major impact on fetal wellbeing in the latter part of pregnancy.The development of a degree of hypoxia in early pregnancy results in curtailing of the remodeling of the uterine spiral vessels by invading cytotrophoblast in the first and second trimesters. That could result in the fetal compromise and development of intrauterine growth restriction (IUGR).

Pre-eclampsia

Pre-eclampsia is a disease of abnormal placentation. In pregnancies complicated by pre-eclampsia, there is inadequate maternal vascular response to placentation. In these pregnancies, the earlier mentioned vascular changes are usually found only in the decidual segments of the uteroplacental arteries. Hence, the myometrial segments of the spiral arterioles are left with their musculoelastic architecture, thereby rendering them responsive to hormonal influences. Furthermore, the number of well-developed arterioles is smaller than that found in normotensive pregnancies. It was postulated that this defective vascular response to placentation is due to inhibition of the second wave of endovascular trophoblast migration that normally occurs from about 16 weeks gestation onward. These pathological changes cause decreased uteroplacental blood flow seen in most cases of pre-eclampsia.

These vascular changes were also demonstrated in a significant proportion of normotensive pregnancies complicated by fetal growth restriction. However, there are other modifying factors involved in pre-eclampsia including various cytokines, proteins and growth factors. Furthermore, in pre-eclampsia, placental oxidative stress may lead to increased shedding of necrotic syncytiotrophoblast debris into the maternal circulation. These interact with the maternal NK cells producing pro-inflammatory cytokines. The maternal multisystem disease is later triggered secondary to endothelial dysfunction through multiple pathways involving many intermediary factors and mechanisms.

Premature labor

A subgroup of premature labor has been demonstrated to be related to dominant TH1 immune responses. Furthermore, innate immune responses to micro-organisms at the maternal-fetal interface through the expression of Toll-like receptors (TLR) could result in premature labor.

Adulthood diseases

There is growing evidence of a model for poor trophoblast invasion, defective re-modeling of uterine vessels by trophoblast and poor uteroplacental perfusion playing a role in the development of later adulthood diseases such as hypertension, diabetes and

coronary heart disease. This is as a result of altered fetal genetic programming by oxidative stress and inflammatory cytokines, and other mediators in very early pregnancy.

Conclusion

In a remarkable series of events, implantation and placental development occur connecting the embryo with the mother. The invading trophoblast expresses paternal proteins and interacts directly with the maternal immune system, but uses several mechanisms to avoid rejection. Trophoblast invasion anchors the placentas to the uterine wall. Trophoblasts are very invasive; they invade the decidua and inner third of myometrium and connect with the maternal circulation. There is clearly active bi-directional dialogue between cells of trophoblast, embryo and mother to control implantation and establish vascular connections that are critical for sustaining fetal growth. A well-controlled and balanced process between the inflammatory and anti-inflammatory forces at the implantation site is necessary for successful pregnancy.

Defective networks or disrupted pathways involved in implantation and placentation result in a wide spectrum of clinically important complications in early and late pregnancy. This could also have a role in the development of some adulthood diseases.

References and further reading

Billingham RE ,Beer AE : Reproductive Immunology: Past, present and future. *Perspect Biol Med*. 1984; 27:259-275.

Chaout G ,Ledee-Bataille N , Zourbas S , Dubanchat S ,Sandra O, Martal J, Ostojojic S, Frydman R. Can Immunological parameters of implantation failure be of interest for preclampsia? *J Reprod Immunol*. 2003; 59:205-217.

Medawar PB. Some immunological and endocrinological problems raised by the evolution of viviparity in vertebrates. *Symp Soc Exp Biol*. 1953; 7:320-338.

Murphy S, Fast L, Sharma S. IL10, uterine NK cells, inflammation and pregnancy. *Am J Reprod Immunol*. 2004; 51:434.

Metcalfe SM , Muthukumarana PA. Transplantation tolerance: gene expression profiles comparing allotolerance vs allorejection. *Int immunopharma*. 2005; 5:33-39.

Ng SC, Gilman-Sachs A, Thaker P, Beaman KD, Beer AE, Kwak-Kim J. Expression of intracellular Th1 and Th2 cytokines in women with recurrent spontaneous abortions. *Am J Reprod Immunol*. 2002; 48:77-86.

Ober C et al :Human Leukocyte antigen matching and fetal loss: results of a 10 – year prospective study. Human Reprod, 1998;13(1):33-38

Robertson SA, Bromfield J J, Tremellen KP. Seminal priming for protection from pre-eclampsia. *J Reprod Immunol*. 2003; 59(2): 253-265.

WegmannTG, Lin H, Guildert, Mossmann. Bidirectional cytokine interactions in the materno-fetal relationship. Successful allopregnancy is a TH2 phenomenon. *Immunol Today*.1993:14:353-355.

Zenclussen, AC. Regulatory T cells in pregnancy. *Semin Immun*. 2006; 28:31-39.