

Review Article

The paradox of pregnancy: an update on the immunology of early pregnancy

Mark Formosa

Abstract

Pregnancy is an altered physiological state where an organism essentially foreign to the individual carrying it, grows, develops and at an appropriate time probably initiates a series of signals which lead to its safe expulsion from the woman's body.

The immunological changes which allow this process are unique to pregnancy. Recent work in this field has led to a further understanding of the changes which operate to adapt the woman to the pregnant state.

The concept that has developed over the years is one where a number of factors exert their effect both at the systemic but mostly at the local uterine level to modulate the immune response which will then refrain from mounting an inflammatory response against the invading trophoblast. The main protagonists of this immunomodulation are embryonic factors, uterine (endometrial) NK cells and, of course, the hormone progesterone.

Progress has been made from the original observations of miscarriage rates in HLA sharing couples and with the possibility of research in couples undergoing IVF cycles, factors are being identified which initiate immunomodulation. Once implantation occurs the endometrial NK cells which are abundant from the late luteal phase are activated to control trophoblastic invasion and enhance the changes in blood vessels which allow for adequate feto-maternal perfusion.

Key words

Pregnancy, miscarriage, immunology

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The immune response is controlled by PIBF under the influence of progesterone to bias towards a humoral response and suppress a cytotoxic response. All these processes are prone to fail at times and the clinical manifestation of such a failure is miscarriage along with other obstetric complications such as intra-uterine growth retardation, pre-eclampsia and placental abruption.

Progress in the understanding of the immunological processes which protect pregnancy will help in elucidating the mechanisms whereby these processes fail.

A consequence of this should be the explanation of those cases as yet classified as unexplained recurrent miscarriage.

The literature indicates that the prognosis for this group of patients is not as encouraging as one would hope and that progress in this area is eagerly awaited by both patients and doctors working in this field.

Introduction

The establishment of a mammalian pregnancy is a significant development over lower vertebrates. The long gestation period ensures the birth of a well developed neonate who is born with a mother at hand and who immediately assumes further responsibility, feeding and nurturing the newborn to ensure it reaches adult life and reproductive potential.

This is in stark contrast to what happens in lower vertebrates where a large number of eggs are hatched so that few may survive to adulthood given the large loss of life during the early phase of life. Mammalian pregnancies are internalised, i.e. grow inside a uterus and require the exchange of substances across the placenta-uterine interface.

This is where the achievement essentially lies.¹ The hemochorial placenta found in humans (and mice) is associated with intimate interaction between the conceptus and the uterus. Given that the human embryo expresses its genomic features as early as the 2-cell stage, how does the embryo survive maternal immune surveillance?

Initially a mechanical protection is provided by the *zona pellucida* and a few layers of cells from the *cumulus ophorus*. This protects it during its journey from the ampullary end of the fallopian tube to the uterine cavity which takes between 2 to 5 days.

Once the embryo comes into contact with the endometrium, the maternal immune system is in a position to set in motion a host vs graft reaction which should result in the destruction of the embryo and the resultant clinical event - miscarriage.

Immunotolerance and successful implantation

In 1988 the concept that genetic similarity (HLA sharing) between the parents leads to a lack of maternal recognition and eventual miscarriage became popular.² It is however clear, that pregnancy is not a classical graft/host reaction as in a transplant but involves specific compounds derived from the conceptus and which modulate the immune response.

The occurrence of term abdominal pregnancies strongly suggests that maternal immuno-modulation is a systemic one and not localised to the uterus.

Also, the success rates of egg donation programmes indicates that sharing of materno-foetal genes is not necessary and favours the idea that embryo-derived signals initiate the protection process.³ It is also a fact that these same egg donation programmes are associated with a high incidence of pregnancy complications especially pre-eclampsia and intra-uterine growth restriction which indicates that gene sharing may result in more efficient immunotolerance and is therefore less likely to be associated with complications.⁴

Further evidence of an embryonic role in successful pregnancies is that defective pregnancies are rejected (miscarried) early – probably reflecting a sub-optimal or actual failure of the mechanisms that lead to immuno-tolerance.

A low-molecular weight peptide present only in pregnancy and in all mammalian species has been identified and has been designated Pre-implantation factor (PIF). It is an

embryo derived factor⁵ and seems to fit the role of a primary immuno-modulator which is present very early on, even before implantation, paving the way for a successful pregnancy.

Its presence is detected as early as fertilization i.e. before implantation. Its role has been studied by following a series of pregnancies after embryo transfer in IVF cycles and the presence of PIF activity in maternal sera has been reported to predict viable pregnancy with excellent sensitivity (88%), specificity (95%), and positive and negative predictive values (94% and 90% respectively).⁶ These results are strongly suggestive that PIF is essential for initiating maternal immuno-modulation (not suppression) and possibly to prime the endometrium and prepare it for implantation.

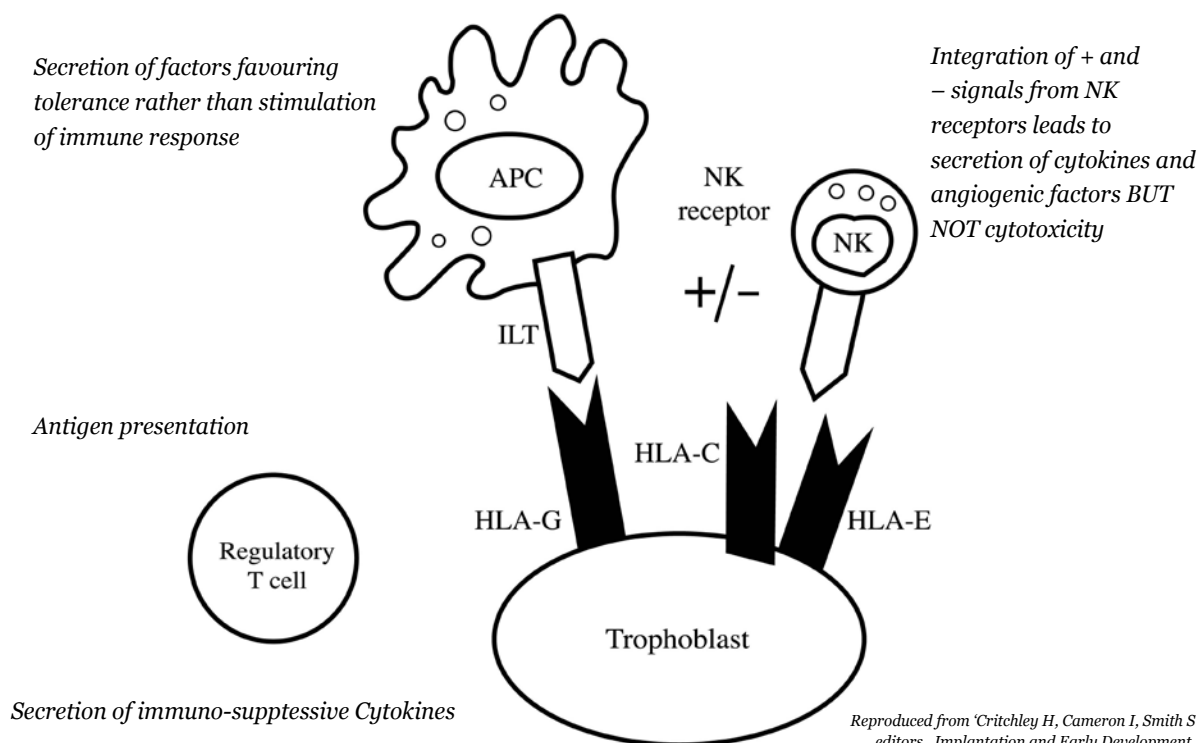
Uterine Natural Killer cells

The endometrium undergoes decidual change in the late luteal phase of the menstrual cycle. The dominant cell population in the decidua in the first trimester of pregnancy are Natural Killer (NK) cells.

There is evidence that, as the blastocyst implants, uterine NK cells become activated and through a series of molecules control cytotrophoblastic invasion through their cytotoxic activity and also initiate changes in the decidual arteries to increase the blood supply to the foetoplacental unit.⁷

Also through Th2 type cytokine expression (vide infra) and growth factors, NK cells participate in placental aggregation and local immunosuppression and modulation.^{8,9}

Figure 1: Trophoblast does not express HLA-A or B but cells of innate immunity have receptors to recognize trophoblast HLA ligands. Cytokine milieu favors implantation with little evidence of inflammation, adoptive immunity or cytotoxicity



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Decidual NK cells are also the main cell population involved in alloimmune miscarriage¹⁰ and the classical T and B cells of the adaptive immune system are sparse in these circumstances. Under the influence of Th1 cytokines (unfavourable for pregnancy) they become classical NK cells expressing CD16 which can damage the trophoblast either directly through cytolytic activity or indirectly by producing inflammatory cytokines.¹¹

NK cell activity seems to be especially important in achieving an optimal balanced trophoblast invasion. This means neither underinvasion resulting in underperfusion of the spiral arterioles which is the model for IUGR and pre-eclampsia¹⁴ nor overinvasion leading to morbid adherence of the placenta.

This control is achieved through foetal polymorphic HLA-C genes which are expressed by the trophoblast cells and modulated through the Killer Immunomodulating Receptors (KIR) found on NK cells.¹⁵ Trophoblasts do not express HLA-A or B genes (see Figure 1).

Clinical studies have shown that patients who tend to abort have increased numbers of NK cells in the uterus as well as increased blood NK cell subsets and antibodies all of which have been associated with the miscarriage of a euploid (therefore presumably normal) embryo.

This knowledge led to an attempt to assess the risk of miscarriage using a serum assay of the level of NK cell activity and selecting women for immunotherapy (e.g. prednisolone) in those cases who exhibit a high level of NK cell activity.^{12,13}

Unfortunately a major flaw in this reasoning is that the NK cells in the decidua are a totally different cell populations from those measured in peripheral blood.

The association is therefore very tenuous, and this practice has largely been abandoned.

Progesterone

Progesterone is the hormone of pregnancy

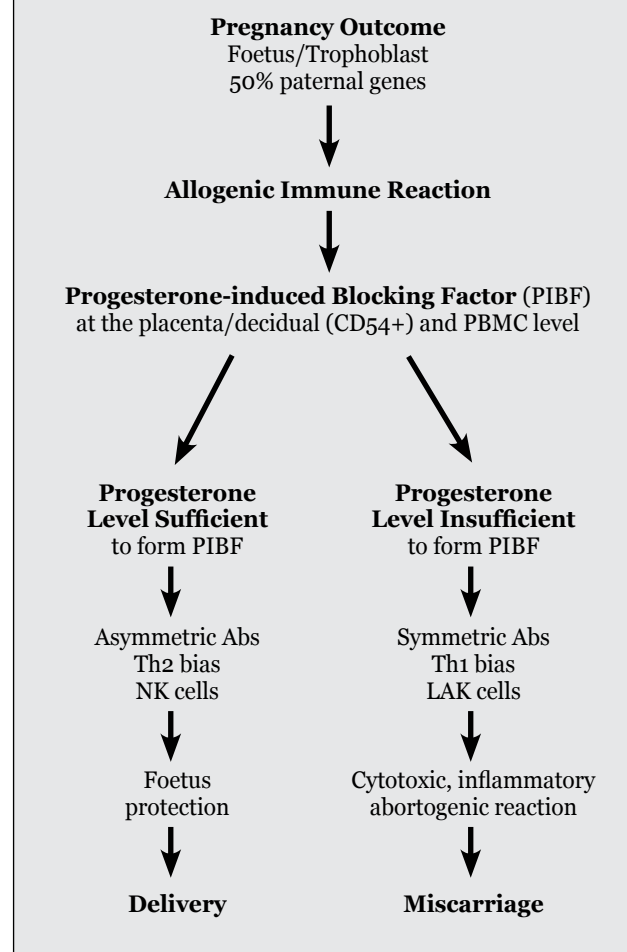
Inadequate levels of progesterone in early pregnancy will unequivocally lead to miscarriage.¹⁶ Recently this hormone has received considerable attention as it has been shown to be effective in preventing preterm labour.¹⁷ However, its role in preventing miscarriage has been bedevilled by contrasting results when it comes to achieving statistically significant outcomes.¹⁸

Breakthroughs in the way progesterone modulates the immune response have confirmed its essential and central function in maintaining pregnancy and re-inforced the belief workers in the field of miscarriage have had in progesterone supplementation.

Progesterone Induced Blocking Factor (PIBF)

PIBF is a 34 kDa protein that can block NK cell-mediated lysis of K562 tumour cells.¹⁹ The expression of this protein by CD8 T lymphocytes (gamma/delta T cells) requires progesterone for expression, and it has therefore been called progesterone induced blocking factor. PIBF is only synthesised at the maternal-foetal interface because that is where there

Figure 2: *The effect of PIBF on T-helper cell bias*



is an adequate progesterone concentration to stimulate this synthesis.

When there are sufficient levels of endogenous progesterone to form PIBF, this then safeguards the foetus by preventing inflammatory and thrombotic secondary reactions towards the trophoblast through three distinct actions²⁰ :

- by inducing asymmetric, protective 'blocking' antibodies²¹
- by blocking NK cell degranulation²²
- by inducing T-helper 2 (Th2) cell dependent cytokines, thereby shifting the balance towards a Th2-dominated, cytoprotective immune response.²³

The diagram (Figure 2) illustrates clearly the crucial role of progesterone through PIBF in the process of successful implantation and development of trophoblast and therefore the foeto-placental unit.

Progesterone receptors (PgR) have not been demonstrated in normal T-lymphocytes but are found in gamma/delta T lymphocytes and the high concentration of progesterone in the maternal-foetal interface induces the expression of PIBF which can then exert its influence on immunomodulation of pregnancy exactly at the foeto-maternal interface where it is most important. It is interesting to note that liver transplants, blood transfusions and paternal lymphocyte injections in the late

luteal phase increases PIBF expression in women.²⁴ Much less PIBF expression has been reported in women who miscarried compared to women who carried their pregnancy to term.^{25,26}

The difficulty in determining the role of progesterone in preventing recurrent pregnancy loss is due to the difficulty in determining who has progesterone deficiency. Luteal phase serum assays and endometrial biopsies lack both sensitivity and specificity.

Check²⁷ argues that “if the need for extra progesterone supplementation occurred in only one-third of pregnancies, would it not be worthwhile to use such supplementation in every cycle to eliminate a 33% miscarriage rate.”

Two studies^{28,29} have shown that pregnancies presenting with low levels of progesterone (8 and 15ng/ml) could be salvaged with aggressive progesterone therapy. However, many of the studies on progesterone therapy in recurrent miscarriage which have been carried out, are small, lacked placebo controls or were not double-blinded. This fact is the view held by Professor Regan³⁰ of the St Mary’s group who argues that since it is not evidence-based practice, it should not be used routinely although it not associated with any foetal adverse effects.

Historical correlations

Unexplained recurrent miscarriage deserves special mention as this represents a particularly distressing aspect of the condition for both patients and physicians alike.

Attempts have been made in the past to classify these cases as immunological in nature. This was particularly so when the antiphospholipid antibody syndrome (APS)³¹ was first described.

The enthusiasm was such that Pattison *et al.*³² postulated at the time that other antiphospholipid antibodies (apart from lupus anticoagulant and anticardiolipin antibodies) may be responsible for unexplained recurrent miscarriage. In fact, approximately 30% of women with ‘unexplained recurrent miscarriage’ have increased serum levels of autoantibodies, especially antiphospholipid antibodies.³³

This observation had in fact, led to the definition of Reproductive Autoimmune Failure Syndrome (RAFS) already in 1988.³⁴ Later in 1999 the American Society of Reproductive Immunology, defined Reproductive Autoimmune Syndrome (RAS) as including not only RAFS but also APS (antiphospholipid syndrome).^{35,36} This approach was advocated as both drugs are very safe to use in pregnancy and have shown a significant benefit in the presence of antiphospholipid antibodies.^{37,38,39}

The development of an immunological aspect to the aetiology of recurrent miscarriage was reflected in the recommended pharmacological treatment of the time, from corticosteroids to immunoglobulins, paternal lymphocyte transfusions and subsequently a combination of heparin and aspirin.

In 1996 and 1997 Kutteh³⁷ and Rai *et al.*³⁸ published their studies confirming that a combination of heparin and aspirin was superior to prednisolone and aspirin and to all the other therapies attempted i.e. warfarin, azathiaprine, plasmapheresis and intravenous immunoglobulin in the management

of recurrent miscarriage associated with the presence of antiphospholipid antibodies. Also by then Cauchi *et al.*⁴⁰ had published their study concluding that there was no benefit from paternal lymphocyte infusion in recurrent miscarriage, a treatment based on the concept of immunotolerance and made popular following the original publication by Mowbray *et al.*⁴¹

Heparin, used clinically as an anticoagulant, also has anti-inflammatory properties and has been described to inhibit interferon-gamma (IFN-gamma) responses in endothelial cells. It has also been shown to be capable of binding to aPL and also of antagonising the action of Th1 cytokine IFN-gamma, thereby protecting the trophoblast and maternal vascular endothelium from damage in early pregnancy.⁴²

A number of papers have reported the effect of heparin on chemokines and Th1 cells. This has confirmed heparin to have anti-inflammatory properties rather than simply anti-coagulant ones.⁴³ An important fact which explains the role of heparin is the first trimester of pregnancy when there is no effective foeto-maternal circulation, but when if omitted in APS patients will lead to a high incidence of miscarriage. In fact, if one looks at the results of the Rai³⁸ study it becomes clear that the survival is not affected when treatment is started after the 13th week of gestation, suggesting that the effect of heparin and aspirin is mainly during the first trimester i.e. during the development of the placento-uterine circulation. This new feature of heparin is leading investigators to consider aspirin as being possibly unnecessary in the treatment of antibody-positive recurrent miscarriage, but because of the results achieved with aspirin in some early studies, it may be difficult to design studies without it altogether. Certainly as the molecular biology of recurrent miscarriage becomes more clear and the actions of known therapeutic agents on these molecules unravel, the possibility that unexplained recurrent miscarriage and its empirical treatment will become a thing of the past is a possibility. This will be very welcome to us physicians but especially to our patients who are at a loss to believe that they have suffered three pregnancy losses while no cause can be found.

The renaissance of progesterone following the important breakthroughs in the processes of immunomodulation coupled by years of clinical application and the knowledge of its safety (10.3 million babies exposed to dydrogesterone worldwide⁴⁴) lead me to ask IF we should be prescribing progesterone to all women who are trying to conceive? Not evidence based but there is a sound scientific basis to this idea.

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