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POSSIBLE IMMUNOLOGICAL BASIS FOR RECURRENT SPONTANEOUS ABORTIONS: A REVIEW

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ABSTRACT

Background: In normal pregnancy, the pregnant mother paradoxically tolerates the semi-allogeneic foetus until term. Experimental and clinical data to explain such tolerance in man reflects the involvement of multiple mechanisms.

Objective: To review the data pertaining to the experimental and clinical efforts to explain why the mother immunologically tolerates a semi-allogeneic pregnancy to term.

Design, setting and methods: A review of the literature on state of the art thinking among researchers and clinicians on recurrent spontaneous abortions is summarised.

Results: A large body of recently published data strongly suggest that a breakdown in immunological maternal-foetal interactions may lead to occasional or recurrent foetal loss. Immunoregulatory activities involving blocking antibodies, regulatory factors, immunological cells, hormones, structural proteins and cytokines constitute the pregnancy-sustaining network.

Conclusion: The majority of the evidence reviewed points to the involvement of immunological factors in successful pregnancies. However, the underlying mechanisms are inadequately explained, are largely speculative and require more focused investigation. A complete understanding of the mechanisms involved would enhance our capacity to develop rational ways of addressing recurrent pregnancy losses.

INTRODUCTION

In mammalian reproduction, the pregnant mother tolerates the semi-allogeneic foetus till term. Several non-exclusive mechanisms based on concepts developed from transplantation immunology models suggest that prolonged exposure of the mother to foetal tissues may result in acquired immunological tolerance to the foetus and/or that the immune response to paternal histocompatibility antigens is actively suppressed. Concepts evolving from cancer immunology are based on the finding that the extravillous cytotrophoblast which is present at the foetal-maternal interphase expresses HLA-G only, which is monomorphic and is therefore unlikely to provoke a classical immunological response. No other HLA antigens are expressed. Both transplantation and cancer immunology models share the postulate that a breakdown of maternal-foetal tolerance may lead to immunological rejection resulting in recurrent foetal losses(1). Besides immunological rejection, recurrent pregnancy losses may be caused by genetic, anatomical, endocrine, alcoholic, environmental toxins and placental anomalies(2). The purpose of this communication is to evaluate the immunological data from experimental and clinical sources that purports to validate the immunological basis for recurrent pregnancy losses.

DEFINITIONS

Abortion: In humans, abortion is defined as the damage and death of an embryo accompanied by uterine contractions (labour) and its subsequent expulsion from the body. Spontaneous abortion is the loss of a pregnancy before twenty weeks of gestation whereas recurrent spontaneous abortion is the loss of three or more consecutive pregnancies before 20 weeks of gestation.

IMMUNOLOGICAL PROTECTION OF THE FOETUS DURING PREGNANCY

Several mechanisms have been proposed in an attempt to explain the protection of the foetus during pregnancy although it is unclear which one is the most effective. Some of the well documented mechanisms include: (i) restricted expression of the major Histocompatibility Complex Class 1 antigens (HLA-G) (3); (ii) down-regulation of the maternal immune responses(4); (iii) production of blocking antibodies(5); (iv) expression of complement regulatory proteins on the trophoblast(6) and; (v) presence of a placental barrier.

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RESTRICTED EXPRESSION OF MHC CLASS I HLA-G

Immunological studies have identified two components of the trophoblast on the basis of HLA Class-1 antigens. The villous trophoblast (cytotrophoblast and syncytiotrophoblast) which is HLA Class 1 negative and the extravillous trophoblast which is HLA Class 1 positive. Studies of placentae from women with a history of recurrent spontaneous abortions have established the expression of HLA-G by the extravillous trophoblast. This antigen has a specific sequence on its transmembrane region and the cytoplasmic region of this Class 1 antigen is truncated, making it 19 amino acids shorter than the classical HLA antigens. This probably accounts for its observed smaller size (heavy chain, 39 kDa). Similar HLA-G sequences could be found in normal term chorionic plate trophoblast DNA after amplification by the polymerase chain reaction (PCR). Analysis of a number of tissues for the presence of HLA-E, F and G transcripts has clearly shown that HLA-G is present only in extraembryonic tissues such as the placenta and its membranes. This restriction of HLA-G suggests that this antigen may play an important role in the maintenance of mammalian pregnancy(7). In addition, human natural killer (NK) cells have been shown to express several killer immunoglobulin-like receptors (KIRs) that inhibit their HLA Class 1 molecules on target cells. A recombinant KIR denoted KIR 2DL4 has been shown to bind to cells expressing HLA-G and not to cells expressing other NK cell receptors(8). Since HLA-G expression is restricted to foetal trophoblast cells, KIR 2DL4 may provide important signals to the maternal NK decidual cells at the maternal foetal interface during pregnancy. It has further been shown that transfection of negative human K562 cell lines leads to inhibition of NK cytotoxicity as observed in peripheral blood of 25 male and female donors. This protection is lost when the cytotrophoblast is treated with HLA-G specific monoclonal antibodies, adding credibility to the hypothesis that HLA-G may play a protective role in pregnancy (9).

DOWN REGULATION OF MATERNAL IMMUNE RESPONSE

Several immune-regulatory factors collectively called Blocking Factors, are thought to enhance the survival of the foetal allograft. These include among others: (i) serum factors that induce the generation of cell-mediated suppressor cells or products in *in vitro* cultures (10); (ii) factors that inhibit T-helper cells (TH) from releasing IL-2 after allogeneic stimulation(11); (iii) non-specific suppressors of lymphocyte mitogen stimulation(12) and; (iv) anti-idiotypic antibodies that block HLA receptors on maternal immunoblasts stimulated by paternal DR antigens(13).

PRODUCTION OF BLOCKING ANTIBODIES

A blocking factor was identified in early pregnancy serum and in sera from recurrently aborting women who carried successful pregnancies after immunisation with leukocytes. This factor suppressed the mixed lymphocyte reaction (MLR) of decidual and peripheral blood lymphocytes (14). It has been reported recently that pregnancy sera show a higher inhibition of NK cell activity than non-pregnant sera (15). Furthermore, a progesterone induced blocking factor, (PIBF) contributes to normal gestation in mice by blocking NK or natural cytotoxic (NC) cell activity, thus lowering the chances of foetal resorption (16). This blocking factor has been identified as an IgG molecule capable of modulating the maternal immune responses and that it can be absorbed by paternal lymphocytes. This IgG is therefore associated with normal protective mechanisms whose absence would permit immunological rejection of the foetus either by direct cell mediated immunity (CMI) or by inflammatory mediators. Two specific types of antibodies are known to down regulate maternal immune responses during pregnancy. These include asymmetric IgGs and anti-idiotypic antibodies. Asymmetric IgGs are a special class of antibodies described originally by Malan-Borel *et al*(17) in 1991. They are non-precipitating, characterised by a mannose-rich oligosaccharide in one of the two F(ab)2 arms, making them asymmetric, univalent and therefore having no effector functions. These antibodies cannot form large antibody-antigen complexes or trigger complement fixation, carry out phagocytic activity and antigen clearance and are therefore seen as antigen blockers. The proportion of asymmetric IgG in non-pregnant sera is usually about 12.5% which doubles to 24.4% in pregnant sera in humans(17). Asymmetric IgG has also been reported to rise during pregnancy in mice(18).

Anti-idiotypic antibodies are those raised against receptor molecules of other antibodies. These antibodies can and do regulate the effective concentration of the target antibody by reacting or complexing with it and removing it from circulation. In pregnancy, this means that a generalised immunosuppression may not be necessary because the response is specific to pregnancy-associated antigens. It is now well established that pregnant women make anti-idiotypic antibodies to maternally derived anti-paternal antibodies and that there are significant differences between the incidence of such antibodies in normal and abnormal pregnancies (19). In fact habitual abortion has been linked with failure of the mother to produce these antibodies. Treatment of these women with paternal leukocytes has led to the generation of such anti-idiotypic antibodies (20). Based on these and other studies leading to similar conclusions, it is hypothesised that blocking factors are a necessary component of successful pregnancy in mammals including man. Failure to produce blocking factors may thus lead to immunological pregnancy loss.

EXPRESSION OF COMPLEMENT REGULATORY PROTEINS IN THE TROPHOBLAST

Pregnancy loss associated with immunological reactions may be of two main types: Autoimmune reactions i.e. reaction to and with self tissues and Alloimmune reactions i.e. reaction to and with antigens from a genetically nonidentical individual of the same biological species.

Pregnancy loss due to autoimmune reactions: High titres of antiphospholipid antibodies have been repeatedly detected in sera from women with a history of recurrent spontaneous abortions. These antibodies are frequently associated with the antiphospholipid syndrome(22).

The mechanisms for the pathophysiology of antiphospholipid-mediated pregnancy losses are closely associated with increased platelet aggregation, and endogenous anti-coagulant activity, increased thrombosis and decreased fibrinolysis culminating in uteroplacental thrombosis and vasoconstriction resulting from immunoglobulin binding to platelets and to endothelial membrane phospholipids(22). The observation that monoclonal antibodies to phosphatidyl serine inhibit intracellular fusion of human trophoblast cell lines *in vitro* and can impair both trophoblast hormone production and trophoblast invasion *in vitro* provide experimental evidence in support of the antiphosphatidylserine mediated pregnancy loss(23). However, whether or not the antiphospholipid antibodies are the cause or consequence of reproductive failure is unknown.

Autoimmunity to other self cellular components and tissues have been suggested as the cause of reproductive failure. Alternatively, reproductive failure has been interpreted as a manifestation of some undiagnosed autoimmune disorder(24). These suggestions are strengthened by the observation that a higher percentage of women with a history of recurrent spontaneous pregnancy losses have autoantibodies to various cell components and organ tissues than women with no history of reproductive failure(25).

Pregnancy loss due to alloimmune reactions: In about 35-50% of women with unexplained recurrent spontaneous abortions immune and inflammatory cell responsiveness to the trophoblast are activated. That Interferon Gamma (IFN) and Tumour Necrosis Factor (TNF) may limit trophoblast invasion, block embryo development and trophoblast growth are also well documented(26). Also, according to Mowbray *et al*(27), immunological damage to the trophoblast may involve a cytokine-dependent activation of NK cells or lymphokine activated killer (LAK) cells which may attach to the target antigen on the trophoblast. This pathway may be blocked by the binding of IgGs to the same targets on the trophoblast. In this model, an antigen of trophoblast origin would in the allogeneic pregnancy model stimulate macrophages, facilitate presentation to T lymphocytes and produce

IL-2 as well as activated NK or LAK cells. TNF may then activate NK cells to attack targets on the conceptus which are assumed to be external on the trophoblast.

CYTOKINE IMBALANCE IN RECURRENT PREGNANCY LOSS

T-helper 1 (Th1) and T-helper 2 (Th2) cytokine responses to the trophoblast have been associated with human pregnancy failure and success respectively(28). An imbalance of the delicate cytokine/growth factor ratio such as too much Interleukin-2 (IL-2), transforming growth factor (TGF- β) or interferon gamma (IFN- γ) may limit trophoblast invasion. In women with successful reproductive histories, absence of the Tumour Necrosis Factor (TNF) and lower levels of other Th1 cytokines has been observed(29). A detailed elucidation of the cytokine balance networks during pregnancy in health and disease needs to be carried out.

IS THE IMMUNOLOGICAL CONNECTION NECESSARY?

The concept of immunological aetiology for recurrent pregnancy loss is strongly supported by the evidence summarised in this review. However, the evidence has also, been subjected to considerable criticism. Thus, Regan *et al*(30) in an elaborate study reported that the respective antibodies are produced after 28 weeks of gestation and that they may disappear between normal pregnancies. Also, it has been shown that 40% of normal women with successful reproductive histories do not produce pregnancy-related antibodies(31). Another study(32) reported that females incapable of antibody secretion could still carry pregnancy to term. No further recent studies have confirmed these contradictory findings. A larger, better standardised controlled international study would probably help our understanding of the immunological basis for aetiology of recurrent pregnancy losses.

PLACENTAL BARRIER

One other hypothesis as to why the mother fails to reject the foetal-placental unit is based on the physical presence of the placental barrier and its attendant physico-chemical, biochemical and immunological properties of the trophoblast. The latter is an epithelial cell layer of foetal origin that forms a barrier between the mother and the developing conceptus. The placenta represents a foetally derived organ for the exchange of materials between the maternal and foetal circulations which do not mix but allow occasional cell traffic between them.

The trophoblast completely surrounds the foetus forming a selective barrier against entry into foetal circulation of harmful cellular and molecular entities

except maternal IgG. The trophoblast also expresses complement inhibitory proteins that are thought to protect the foetus from complement-mediated lysis(21). The mechanisms of such protective regulation remain unelucidated.

CONCLUSION

A large body of evidence supports the immunological aetiology of recurrent pregnancy losses in man although the immunological component is only part of a wider aetiological picture. That blocking factors are involved in the form of antibodies has been demonstrated. However, the specific roles of these antibodies remain largely speculative and require further investigations. Carefully designed studies into the autoimmune component as well as the involvement of an elaborate cytokine network would greatly enhance our understanding of human pregnancy.

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