Minireview

Manipulation of Cytokine Production Profiles as a Therapeutic Approach for Immunologic Pregnancy Loss

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Pregnancy is not as successful as one might think; it can be compromised by several complications such as recurrent spontaneous miscarriage, pre-term delivery, pre-eclampsia etc. Much attention has been paid to the possibility of the maternal immune system mediating deleterious effects on pregnancy. Research conducted during the last two decades has shed much light on cell-mediated immunologic effectors that might underlie these pregnancy complications. Of particular interest are the effects that pro-inflammatory and anti-inflammatory cytokines have on the foetus and placenta, and thus on the success and failure of pregnancy. This review presents evidences that certain cytokine profiles are associated with recurrent miscarriage and pre-term delivery and discusses possible pathways of effector function of cytokines in pregnancy loss and the redirection of cytokine profiles from one that is antagonistic to pregnancy towards one that is conducive to the success of pregnancy. Among the promising agents for the modulation of the Th1/Th2 balance are progestogens like progesterone and dydrogesterone; this review also discusses recent evidence that progestogens are capable of modulating cytokine production patterns in pregnancy loss.

Keywords: Recurrent miscarriage, Pre-term delivery, Cytokines

Introduction

Recurrent spontaneous miscarriage (RSM) and pre-term delivery (PTD) are two of the most common and challenging complications of pregnancy faced by obstetricians. Spontaneous miscarriage is defined as clinically detectable 3 or more pregnancy losses prior to 20 weeks of gestation; one of every four pregnant women suffers from one or more pregnancy losses. Only about 40-50% of RSMs is attributable to the “known” causes such as chromosomal anomalies, endocrinologic abnormalities, infections, anatomic problems and humoral factors, but as much as 60% relegated to “unknown” or “unexplained” etiology. Thus, the causes of RSM remain “unexplained” in the majority of women.

Pre-term delivery is a significant cause of perinatal morbidity and mortality and a condition for which there is a dearth of treatment modalities; it occurs at a rate of 12.5%. Pre-term labor is initiated by inappropriately early activation of elements that initiate normal parturition at term. Factors that predispose to pre-term labor and delivery include premature rupture of fetal membranes, pregnancy-induced hypertension, amniotic fluid infection, faulty implantation, serious maternal disease and genetic factors. Several factors such as changes in the levels of progesterone, oxytocin, relaxin, prostaglandins, cortisol, and corticosteroids have been studied in relation to the onset of pre-term delivery; however, the etiology of many cases of pre-term delivery is due to unexplained causes.

The existence of such a large proportion of cases of RSM and PTD with unidentified etiologies has in part fuelled great interest in the investigation of possible immunologic etiologies. Both humoral and cell-mediated etiologic factors have been investigated; the fetus appears to be impervious to attack by humoral immunity, except for anti-phospholipid antibodies. This led to greater focus on the possibility of cell-mediated immune effectors as possible etiologic agents. Research on the role of maternal cells and cytokines about a decade ago has shed light on subsets of T helper (Th) cells which has provided an excellent framework for understanding how the immune system directs responses to different types of pathogens and stimuli.

Th1, Th2 cells and cytokines

Th1 and Th2 cells are the major subsets of Th cells with different patterns of cytokine production and different roles in immune responses. Th1 cells
secrete the cytokines interferon (IFN)-γ, tumor necrosis factor α (TNF)-α, TNF-β and interleukin (IL)-2; these Th1-type cytokines activate cell-mediated reactions such as cytotoxic and delayed-type hypersensitivity (DTH) reactions. In general, Th1 cytokines mediate strong cellular immunity and inflammatory reactions and are implicated in graft rejection reactions, autoimmune reactions and cytotoxic immunity. Th2 cells, on the other hand, secrete IL-4, IL-5, IL-6, IL-10 and IL-13; these Th2-type cytokines encourage vigorous antibody production. Th1 and Th2 cells are mutually antagonistic to each other; thus, an individual who produces a strong Th1 response usually tends to have a low Th2 response and vice versa.

Cytokine profiles in RSM

Humoral immune responses are enhanced during pregnancy, while cell-mediated immune responses and the course of cell-mediated autoimmune disorders are downregulated. Clinical evidence indicates that pregnant women undergo immunological changes consistent with weakening of Th1 and strengthening of Th2 responses (reviewed in 7), leading to the contention that successful pregnancy is correlated with, and perhaps even depends on the preferential stimulation of Th2 cytokine-producing T cells.

Conversely, the activation of some forms of maternal cellular immunity is potentially hazardous for fetal development (reviewed in ref. 8). Cellular immunity mediated by effector cells and/or cytokines released by them have shown significant deleterious effects on the foetus. The injection of TNFα, IFNγ and IL-2 into pregnant mice causes abortions while the injection of anti-TNFα antibodies results in a reduction in resorption rates in a murine model of natural, immunologically mediated abortion. TNFα and IFNγ inhibit the outgrowth of human trophoblast cells in vitro and synergistically stimulate apoptosis of human primary villous trophoblast cells. The stimulation of maternal spleen cells in vitro with placentas of mice prone to immunologically-mediated spontaneous fetal resorption results in the secretion of high levels of TNFα, IFNγ and IL-2; interestingly, these cytokines together fit the Th1 cytokine profile.

Most studies to date suggest that women with RSM have a greater Th1-type or pro-inflammatory cytokine bias as compared to normal pregnant women. Hill and colleagues have shown that peripheral blood mononuclear cells (PBMC) of women with a history of RSM, when stimulated with a human trophoblast antigen extract produce higher levels of the Th1 cytokines and embryotoxic activity as compared to normal pregnancy. They concluded that Th1 immunity to trophoblast antigens is associated with recurrent miscarriage and may play a role in reproductive failure while Th2 immunity may be the natural response to the trophoblast, in contributing to normal, successful pregnancy.

A series of studies in our laboratory has presented convincing evidence for a clear Th1 cytokine bias in RSM. Peripheral blood lymphocytes from normal pregnant women at the end of first trimester and at delivery, and from recurrent aborters at the time of abortion were stimulated with a mitogen and the supernatants tested for Th1 and Th2 cytokines. Levels of IL-4, IL-5, IL-6 and IL-10 were higher at the end of first trimester and at delivery in normal pregnancy than in RSM, while the levels of IL-2, IFNγ and TNFα were uniformly higher in RSM than in normal pregnancy. This was substantiated by studies on specific maternal immunity to placental antigens assessed by co-culturing maternal lymphocytes with autologous placental cells and exposing maternal lymphocytes to a trophoblast antigen preparation.

Since the absolute concentrations of secreted cytokines may not reveal a Th1 or Th2 bias per se, we analyzed the ratios of Th1 to Th2 cytokines in the various permutations. In every combination of Th1 to Th2 cytokines, the ratios were higher in the RSM group, as compared to the normal pregnancy group, indicating a greater Th1 bias in RSM and a greater Th2 bias in normal pregnancy.

A laboratory study by Clerici’s group on 40 women with normal pregnancy and 5 women with spontaneous miscarriage showed decreased production of Th2 cytokines and increased production of Th1 cytokines by antigen-stimulated lymphocytes from women with RSM as opposed to those from normal pregnancy. Significantly higher levels of Th2 cytokine-producing T cell clones were demonstrated from the decidua of women with normal pregnancy than those with unexplained RSM. Most reports thus support the contention that women with recurrent miscarriage exhibit primarily Th1 cytokines, whereas healthy women show decreased Th1 cytokines and increased Th2 cytokines; there is thus an increased pro-inflammatory cytokine bias in recurrent miscarriage.
Effect of Th1 immunity on pregnancy

Increased blood and uterine NK activity has been linked to miscarriage, and increased NK activity in the blood has been shown to be predictive of RSM. While direct cell-mediated lysis of trophoblast cells has not been demonstrated, it has been proposed that NK cells, like activated Th1 cells could release cytokines deleterious to the trophoblast. Hill and Choi suggest that cells in the decidua respond to trophoblast invasion by generating a Th1-dominated response which could be detrimental to early placental differentiation and growth and may be toxic to embryonic development. Alternatively or additionally, Th1 cytokines may convert NK cells to lymphokine-activated killer (LAK) cells that have been shown to lyse trophoblast cells, and systemic levels of LAK-like cells correlate with high miscarriage rates. Decidual NK cells are not cytolytic, but produce IFNγ which activates decidual macrophages; these researchers suggest that activated NK cells produce cytokines that activate decidual macrophages which then secrete toxic levels of nitric oxide. For their part, activated macrophages may bring about damage to the conceptus not by direct lysis of trophoblast cells, but via the production of nitric oxide and TNFα. The role of Th1 cytokines in this scenario would be to activate such cellular effectors and to also cause apoptotic damage to the placenta.

Th1 cytokines, such as TNFα and IFNγ, may directly damage the foetus by apoptosis of trophoblast cells and by inhibiting the secretion of the growth-stimulating GM-CSF from the uterine epithelium. Clark et al proposed that maternal “rejection” of the implanted conceptus might be due to the process of what they termed “cytokine-triggered vascular autoamputation” which involved activation of coagulation mechanisms, leading to vasculitis affecting maternal blood supply to the implanted embryo.

Cytokine profiles in PTD

Several lines of evidence suggest a role for pro-inflammatory cytokines in the sequence of events, leading to pre-term labour and delivery associated with intrauterine infection. Increased concentrations of the pro-inflammatory cytokines IL-1, TNFα, IL-6 and IL-8 have been found in the amniotic fluid of women with infection-associated pre-term labor. Studies have also reported higher levels of IFNγ in cervicovaginal fluid, IL-1, TNFα and IL-6 in placental cells and IL-1β, IL-6 and IL-8 in amniotic and chorionic-decidual tissues and in cervical secretions in PTD as compared to normal term delivery. Indeed, Romero et al proposed that pre-term labor in the setting of infection results from the actions of pro-inflammatory cytokines secreted as part of the fetal and/or maternal host response to microbial invasions and suggest that a fetal pro-inflammatory cytokine response is followed by the onset of spontaneous pre-term parturition.

Dudley suggests that pre-term labor associated with sub-clinical infection may trigger a dysregulation of a local inflammatory response, leading to a so-called “intra-uterine inflammatory response syndrome”, leading to pre-term labor and delivery. Even in the absence of intrauterine infection, pre-term labor has been shown to be associated with enhanced placental cytokine production; elevated levels of IL-1, IL-6 and IL-8 have been demonstrated in premature parturition with no signs of infection.

In laboratory studies, we have demonstrated that elevated levels of the Th1 cytokines IL-2 and IFNγ are produced by women with unexplained PTD, while higher levels of the Th2 cytokines IL-4, IL-5 and IL-10 are produced by mitogen-stimulated peripheral blood lymphocytes from women with normal pregnancy. Furthermore, the ratios of Th1 to Th2 cytokines are indicative of a bias toward stronger pro-inflammatory cytokine reactivity in PTD. These observations support the existence of ‘intra-uterine inflammatory response syndrome’ which may account for pre-term labor with both infectious and non-infectious etiologies, suggesting that production of inflammatory cytokines may be a mechanism that could form the pathophysiologic basis for this association.

Based on these observations, therapies that downregulate Th1 cytokine reactivity may well be valuable in the clinical management of recurrent aborters and women who go into premature labour with a predominant Th1 cytokine bias. Various strategies are worth pursuing; these include the downregulation of Th1 cytokines, the neutralization of Th1 cytokines and the upregulation of Th2 cytokines.

Can we redirect cytokine responses towards a pregnancy-conducive profile?

The demonstration of a possible association between RSM and PTD on the one hand and maternal Th1
cytokine bias on the other, has led to research on manipulating the cytokine balance, so as to downregulate pro-inflammatory cytokines such as IFNγ and TNFα, thereby creating a milieu that is more conducive to the success of pregnancy. One approach would be to use a hormone such as progesterone, which has shown anti-inflammatory and immunosuppressive properties. Piccinni and colleagues demonstrated that progesterone favors the development of human T cells producing Th2 cytokines; they argue that the Th1 cytokines IFNγ and TNFα promote allograft rejection and may compromise pregnancy, thus the production of Th1-inhibitory Th2-type cytokines may allow allograft tolerance and fetus survival. They suggest that progesterone-mediated immunosuppression is naturally needed for the maintenance of normal gestation.

These leads prompted us to investigate dydrogesterone (6-dehydro-9β, 10α-progesterone) for potential immunomodulatory properties. Dydrogesterone is a potent orally administered progestogen, similar to endogenous progesterone in its molecular structure and pharmacological effects, with a high affinity for the progesterone receptor. Our laboratory studies centered basically on exposing the lymphocytes from women with RSM or with PTD to the progestogens, dydrogesterone and progesterone. Thirty women with a history of unexplained RSM and 18 with a history of unexplained PTD were inducted into the study and their PBMC were stimulated with a mitogen phytohemagglutinin (PHA), in the presence of dydrogesterone, progesterone or tissue-culture medium alone. Initial standardization experiments helped determine the optimal concentrations of dydrogesterone and progesterone to be used in the experiments. PBMC were exposed to medium (as control) and progestogens for 4 days, thereafter culture supernatants were aspirated and tested for cytokines by ELISA.

We observed that dydrogesterone significantly reduced secretion of the Th1 cytokines IFNγ (P<0.001) and TNFα (P<0.01), similar to progesterone (P<0.05 for both cytokines) (Fig. 1). On the contrary, levels of the Th2 cytokines IL-4 and IL-6 were significantly elevated in the presence of dydrogesterone or progesterone (P<0.05 in all cases) (Fig. 2). Thus, the levels of Th1 cytokines decreased significantly, while those of Th2 cytokines increased significantly, when cells were exposed to dydrogesterone or progesterone.

As the relative levels of Th1 and Th2 cytokines are probably of greater importance than their absolute levels alone, we calculated the ratios of Th1 to Th2 cytokines. A marked reduction in these ratios is observed in cultures containing dydrogesterone (Fig. 3); for example, the IFNγ/IL-4 ratio is about 20-fold lower in the presence of dydrogesterone. Similarly, Th1/Th2 ratios are reduced in cultures containing progesterone (Fig. 4), indicating a decrease in Th1 cytokine bias.

In the PTD study, as in the RSM study, mitogen-stimulated PBMC were exposed to progesterone or dydrogesterone for 4 days, and thereafter culture supernatants were examined for levels of Th1 and Th2 cytokines. Th1 cytokine production was significantly inhibited by progesterone and
dydrogesterone. Levels of IFNγ were significantly lower in the presence of progesterone ($P<0.05$) and dydrogesterone ($P<0.001$) compared to IFNγ levels produced by mitogen-activated PBMC in the absence of these substances\textsuperscript{36}. Similarly, TNFα levels were lower in the presence of progesterone ($P<0.01$) or dydrogesterone ($P<0.05$) than in the absence of these substances.

In contrast to suppression of Th1 cytokine production, levels of the Th2 cytokine IL-4 were significantly higher in the presence of progesterone or dydrogesterone than in their absence ($P<0.05$). We found that Th1:Th2 ratio in every combination was lower in the cultures, where dydrogesterone or progesterone was added; for instance, the IFNγ:IL-4 ratio in the presence of dydrogesterone was 27-times lower and the IFNγ:IL-10 ratio was 7-fold lower\textsuperscript{36}. The general trend thus supports a decrease in pro-inflammatory cytokine bias in mitogen-activated PBMC from PTD patients in the presence of progesterone or dydrogesterone.

As dydrogesterone and progesterone act on lymphocytes to modulate cytokine production, we tried to ascertain whether these agents mediate their cytokine-modulating effects via the progesterone receptor; we tested the influence of progesterone-receptor antagonist RU486 on cytokine modulation by dydrogesterone and progesterone. Since RU486 competes with progesterone (and dydrogesterone) for progesterone-receptor, if its addition reverses the effects of these substances, it would indicate that dydrogesterone and progesterone have to interact with the progesterone-receptor to affect the cytokine production. When PBMC from subjects with unexplained RSM are cultured with unexplained RSM are cultured with progesterone or dydrogesterone in the presence or absence of RU486, we observed increased levels of IFNγ and TNFα, which otherwise are suppressed by dydrogesterone and progesterone.
progesterone and decreased levels of IL-4 and IL-6 which otherwise are upregulated by dydrogesterone and progesterone. Thus, RU486 reverses the effect of dydrogesterone, indicating that the effect of dydrogesterone and progesterone is mediated via the progesterone receptor35.

**Redirection of cytokines in RSM**

Our data based on laboratory studies indicate that significantly lower levels of Th1-type cytokines IFNγ and TNFα are produced by the lymphocytes exposed to dydrogesterone. These cytokines deter embryo development, implantation events and proliferation of the trophoblast10 and have apoptotic effects on human trophoblast cells11. In animals, these cytokines bring about fetal demise when injected during gestation9. Furthermore, IFNγ and TNFα may mediate placental/fetal damage via activation of NK activity and macrophages, both of which in their activated states have shown deleterious effects on the embryo. Thus, studies demonstrating an association between high levels of these Th1 cytokines and unexplained RSM indicate a potential benefit in downregulating their production.

Our study also demonstrates that IL-4 and IL-6 are upregulated in the presence of dydrogesterone. The IL-4 is a quintessential Th2 cytokine with Th2-inducing capacity; and its increased production favors further Th2 bias which would affect the eventual outcome of Th1/Th2 dichotomy5,6. It is relevant to note that progesterone has been shown to promote the differentiation of T cells into Th2 effectors and is proposed to be responsible for a Th2 switch at the maternal-fetal interface during normal, successful gestation34. In our studies, progesterone was tested at a concentration (10⁻⁸ mol/L) similar to that achieved at maternal-fetal tissues. Thus, increased production of IL-4 and decreased production of IFNγ and TNFα together could well result in a substantial swing in Th1/Th2 reactivity towards the pregnancy-conducive Th2 profile and away from the potentially harmful Th1 profile. Indeed, Th1:Th2 cytokine ratios, such as the IFNγ/IL-4 ratio show a substantial reduction upon exposure to dydrogesterone.

Considering that inflammatory cytokines such as IFNγ and TNFα may have effects that are detrimental to pregnancy and may lead to miscarriage, a shift in cytokine production patterns away from a predominance of these cytokines may well lead to the prevention of pregnancy loss due to miscarriage.

**Redirection of cytokines in PTD**

A spate of recent publications from several researchers supports the idea that progesterone should be seriously considered for preventive therapy in women with a history of spontaneous pre-term delivery. Several promising clinical studies have been reported on the use of 17α-hydroxyprogesterone caproate (17P), a progestogen which is structurally related to progesterone and dydrogesterone and has been used to treat recurrent miscarriage and various menstrual disorders to women presenting with a history of spontaneous PTD. Administration of 17P in a clinical trial resulted in a significantly lower occurrence of PTD as well as a reduction in the risk of low birth weight37. A recent double-blind, placebo-controlled trial in women with a history of spontaneous PTD showed that weekly injections of 17P led to a substantial decrease in the rate of recurrent PTD as well as a reduction in the likelihood of perinatal mortality and very low birth weight infants38. It was demonstrated that the use of 17P not only reduced the overall risk of pre-term delivery, but also reduced the risk of pre-term birth in women with a history of more than one previous pre-term delivery39. The 17P therapy is also reported to be associated with a prolongation of pregnancy40. An analysis of randomized controlled trials concluded that patients treated with 17P had lower rates of PTD and reduced incidence of low-birth weight infants41. Prophylactic vaginal progesterone suppositories also reduced the frequency of PTD in women at risk for premature delivery; in fact, administration of 17P or progesterone suppositories in a clinical trial led to a significant protective effect against PTD in six out of seven published clinical trials (reviewed in ref. 39). Taken together, these results suggest that patients who have had a prior spontaneous pre-term birth may benefit from progesterone therapy.

Progesterone sustains uterine quiescence by reducing intracellular calcium levels, contractility42 and the concentration of phosphorylated myosin and promoting myometrial relaxation43. The relaxant effect of progesterone in the uterus, in addition to its ability to inhibit the oxytocin effect of prostaglandin and stimulation of alpha-adrenergic receptors34 may explain its ability to prevent pre-term labor and delivery; however, our studies described here suggest a possible additional, non-mutually-exclusive mechanism to elucidate the protective effect of
progestogens in pre-term delivery. Based on evidence that indicates an inflammatory bias in pre-term labour and delivery, shifting the cytokine production profile away from an inflammatory bias may lead to the prevention of pre-term labour and delivery\textsuperscript{36}.

Hill and colleagues\textsuperscript{43} suggest that potentially immunosuppressive doses of progesterone which has been termed “nature’s immunosuppressant”\textsuperscript{39} may benefit individuals in whom the etiology of RSM is related to maternal Th1 cytokine predominance. However, progesterone administered orally is poorly absorbed, is subject to first-pass mechanism, has a short biologic half-life\textsuperscript{46}, loses much of its bioactivity\textsuperscript{47} and is rapidly cleared\textsuperscript{48}. Therefore, the orally active dydrogesterone is quite attractive from this perspective.

Our data summarized here demonstrated that dydrogesterone, a progestogen currently indicated for progesterone-related pregnancy disorders has an immunomodulatory capability \textit{in vitro}; it appears to induce a maternal cytokine shift from Th1 cytokine dominance towards a Th2 bias, which has been described as being conducive to successful pregnancy. Our observations are based on \textit{in vitro} studies; if clinical trials confirm the immunomodulatory, cytokine-directing capability of dydrogesterone, then this molecule may well serve as an effective, safe and orally administered therapeutic intervention in unexplained RSM and pre-term delivery.

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References


