Cytokine imbalance in pregnancy complications and its modulation

Raj Raghupathy[1], Jaroslaw Kalinka[2]

[1]Department of Microbiology, Faculty of Medicine, Kuwait University, Safat 13110, Kuwait, [2]Medical and Environmental Pregnancy Health Hazards Unit, Department of Perinatology, I Chair of Gynecology and Obstetrics, Medical University of Lodz, Poland, Wilenska 37, 94-029 Lodz, Poland

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1. ABSTRACT

The phenomenon of pregnancy can be compromised by a number of complications, such as threatened abortion, recurrent spontaneous miscarriage, preeclampsia, and preterm delivery. Research conducted during the last decade has opened up the possibility that cellular immune effectors may underlie such pregnancy complications. Particularly interesting are the effects of pro-inflammatory and anti-inflammatory cytokines on the conceptus and thus on the success or failure of pregnancy. This review focuses on the association between cytokines and the different complications of pregnancy as well as on the possible pathways of the effector function of cytokines in pregnancy loss. This review also goes on to discuss the redirection of the cytokine profile towards one that is more conducive to pregnancy. Among the most promising agents for the modulation of the Th1/Th2 balance are progestogens such as progesterone and dydrogesterone. Recently published studies lead us to propose that a therapeutic approach worth pursuing would be to assess the individual cytokine profiles of women with pregnancy complications and then to adjust individual therapy using the most effective progestogen.

2. INTRODUCTION

Even a cursory glance at the numbers suffices to indicate that pregnancy is not nearly as successful a phenomenon as one might think; as many as 20% of pregnancies result in miscarriage within two weeks of fertilization, and another 15% of conceptions fail within the first 14 weeks of gestation (1). Pregnancy is indeed besieged by a barrage of potential complications ranging from threatened abortion, spontaneous miscarriage, and preeclampsia to preterm rupture of membranes and preterm labor and delivery.

Only about 40-50% of recurrent spontaneous miscarriages (RSM), defined as the occurrence of 3 or more pregnancy losses before the 20th week of gestation, are attributable to so called "known" causes, such as chromosomal anomalies, endocrinologic abnormalities, infections, anatomic problems, and humoral factors, with as many as 60% of such miscarriages relegated to "unknown" or "unexplained" etiology (2). Preterm delivery, defined as birth before 37 weeks gestation, is a leading cause of perinatal morbidity and mortality and is a condition for which there are few treatment modalities; it occurs at a rate

of 6-12.5% (3). Preterm labor is conjectured to be stimulated by the inappropriately early activation of the elements that initiate normal, at term parturition. While several factors that predispose a woman to preterm labor and preterm delivery have been identified and described, the exact etiology of many cases of preterm delivery remains unexplained (4).

3. CYTOKINES AND PREGNANCY

Besides research on endocrinologic and infectious etiologies, a great deal of attention has been focused on the possible immunological causes of these pregnancy complications. Several humoral immunologic etiologic factors have been investigated, but the conceptus appears to be quite resistant to attack by humoral immunity, with the exception of anti-phospholipid antibodies. On the other hand, cellular immunity mediated by effector cells and/or the cytokines released by them has been shown to be detrimental to the conceptus.

Cytokines, as extremely potent, versatile, pluripotent mediators of an immense array of reactions ranging from induction of normal immune responses, rejection of allografts, autoimmune diseases, and hypersensitivity, have received much attention from reproductive immunologists. Both normal pregnancy and pregnancy with complications have been viewed from the perspective of the Th1/Th2 paradigm. Th1 and Th2 cells are the major subsets of T-helper (Th) cells with different patterns of cytokine production and different roles in immune responses (5, 6). Th1 cells secrete the cytokines interferon (IFN)-gamma, tumor necrosis factor (TNF)-beta, interleukin (IL)-2, and TNF-alpha; these so called Th1-type cytokines activate macrophages and cell-mediated reactions that play critical roles in resistance to infection by intracellular pathogens and in 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 against intracellular infections. Th2 cells secrete the cytokines IL-4, IL-5, IL-6, IL-10, and IL-13 which induce antibody production and are commonly found in association with strong humoral immunity. Some of these Th2 cytokines are anti-inflammatory. Furthermore, 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.

Clinical evidence and experimental studies suggest that Th1-type (inflammatory) responses are weakened during pregnancy, while Th2 responses are augmented. Humoral immune responses are enhanced during normal pregnancy, while cell-mediated immune responses, such as delayed-type hypersensitivity, natural killer (NK) activity, responses to intracellular infections and the course of cell-mediated autoimmune disorders are down-regulated (reviewed in 7). Thus, successful pregnancy appears to be correlated with Th2-type maternal immunity. On the other hand, maternal Th1-type immunity can be hazardous to fetal development (reviewed in 8). When injected into pregnant mice, TNF-alpha, IFN-gamma, and IL-2 cause abortions while the injection of

anti-TNF-alpha antibodies results in a decrease in resorption rates in a murine model of natural, immunologically-mediated abortion (9). TNF-alpha and IFN-gamma inhibit the outgrowth of human trophoblast cells *in vitro* (10) and synergistically stimulate apoptosis of human primary villous trophoblast cells (11). The stimulation of maternal spleen cells *in vitro* with the placentas of mice prone to immunologically-mediated spontaneous fetal resorption results in the secretion of high levels of IL-2, TNF-alpha, and IFN-gamma (12).

4. CYTOKINES IN PREGNANCY COMPLICATIONS

4.1. Cytokines in Recurrent Spontaneous Miscarriage

Several studies suggest that women with recurrent spontaneous miscarriage have a greater bias towards a Th1-type or pro-inflammatory cytokine profile as compared to normal pregnant women.

Hill and colleagues showed that when stimulated with human trophoblast antigens, the peripheral blood cells from women with a history of recurrent spontaneous miscarriage (RSM) produce higher levels of Th1 cytokines with embryotoxic activity as compared to the same cells of women with normal pregnancies (13). Raghupathy and colleagues showed that significantly higher levels of IL-4, IL-5, IL-6, and IL-10 were produced by mitogen-stimulated peripheral lymphocytes from women with a history of normal pregnancy, while lymphocytes from women with a history of RSM secreted significantly greater levels of the pro-inflammatory cytokines IL-2, IFN-gamma, and TNF-alpha than their counterparts with normal pregnancies (14, 15). This has been 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 (16). Ratios of inflammatory cytokines to anti-inflammatory cytokines 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. Piccinni *et al* (17) demonstrated significantly higher levels of Th2-cytokine-producing T-cell clones from the decidua of women with normal pregnancies than from women with unexplained RSM. Clerici's group (18) tested cytokine production in pregnant women 1-2 weeks before any upcoming pathology could be detected. Their study on 40 women with normal pregnancies and 5 women with spontaneous miscarriages showed decreased production of IL-4 and IL-10 and increased production of IFN-gamma and IL-2 by antigen-stimulated lymphocytes from women with RSM as opposed to those from women with normal pregnancies. Jenkins *et al.* (19) reported that continuing pregnancies were associated with increased levels of IL-10 and reduced levels of IFN-gamma, supporting the idea that pregnancy is associated with a Th2-type response. Pregnancies in women in the recurrent miscarriage group had lower levels of IL-10 and increased levels of IFN-gamma, thus confirming the view that miscarriage is a Th1-type phenomenon. Lim and colleagues (20) determined peri-implantation endometrial Th1 and Th2 cytokine profiles during a non-conception cycle. They found that women

with recurrent miscarriage exhibited primarily Th1 cytokines, whereas healthy women exhibited decreased Th1 cytokines and increased Th2 cytokines. Most reports thus support the contention that women with recurrent miscarriages produce elevated levels of Th1 cytokines, while women with normal pregnancies have decreased Th1 cytokines and increased Th2 cytokines; there is thus an increased pro-inflammatory cytokine bias in unexplained recurrent miscarriage (13-21).

How might Th1-type cytokines affect the conceptus? It has been proposed that NK cells, such as activated Th1 cells, could release cytokines deleterious to the trophoblast (22). Hill and Choi (23) refer to the Th1-mediated mechanisms as "immunodystrophism"; in this model, 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 also be toxic to embryonic development. Furthermore, pro-inflammatory cytokines may convert NK cells to lymphokine-activated killer (LAK) cells that have been shown to kill trophoblast cells, and systemic levels of LAK-like cells correlate with high miscarriage rates (24). Another cell type that may effect damage is the decidual macrophage; Baines *et al.* (25) have demonstrated a correlation between the triggering of cytotoxin production by primed decidual macrophages and early embryo loss. Activated macrophages may bring about damage to the conceptus not by direct lysis of trophoblast cells, but via the production of nitric oxide and TNF-alpha (25).

Th1 cytokines, such as TNF-alpha and IFN-gamma, may directly damage the conceptus by apoptosis of trophoblast cells (11), by inhibiting the secretion of the growth-stimulating GM-CSF from the uterine epithelium and by upregulating the procoagulant fg12, a prothrombinase (26). Clark *et al.* (26) propose that maternal "rejection" of the implanted conceptus may be due to the process of what they term "cytokine-triggered vascular autoamputation" that involves activation of coagulation mechanisms, leading to vasculitis affecting maternal blood supply to the implanted embryo. What is the relationship between cytokine profiles seen in the periphery and the local network of cytokines in the vicinity of the conceptus? If pregnancy brings about changes both in the periphery and in the uterus, then Th2 cells in the periphery may further promote Th2 immunity in the uterus. Similarly, if maternal immunity shifts to a type 1-dominance in the periphery---either due to infections or hormonal changes or as yet unresolved genetic causes---then Th1 immunity in the periphery may cause a shift at the maternal-fetal interface towards Th1 dominance. It is suggested that T cells in the periphery and T cells in the uterus may communicate with each other in determining the nature and magnitude of the response to be generated and that peripheral T cells might actively alter the Th1/Th2 ratio among "local" T cells (27).

4.2. Cytokines in Threatened Abortion

Threatened abortion (TA), one of the most commonly recognized medical problems in early pregnancy, is manifested by vaginal bleeding and/or uterine cramps in the presence of a closed cervix. This stage of gestation may culminate in spontaneous abortion or, alternatively, the pregnancy may proceed normally. In contrast to spontaneus abortion, the data concerning cytokine profiles in women with threatened abortions is relatively scarce (28, 29). Paradisi *et al.* (28) measured the Th2-type cytokines IL-6, IL-10, and IL-13 in the sera of women with threatened abortions and missed abortions, as well as of normal pregnant and non-pregnant women. Serum IL-6, IL-10, and IL-13 concentrations in women with threatened abortion showed no significant differences from those of normal and non-pregnant women. Recent data presented by Kalinka and Radwan (29) is consistent with this result, but also indicated, for the first time, that the mean concentrations of Th2-type cytokines and Th1-type did not differ significantly between the threatened aborters and the healthy pregnant women. Moreover, this data confirmed the maintenance of a stable, similar Th1/Th2 immune balance during early pregnancy in both groups. The results of this prospective study (29) did not confirm the existence of Th2-type cytokine deficiency in threatened abortion and indicated that threatened abortion with good outcome immunologically resembles normal pregnancy with a non-enhanced Th1 reactivity. Gucer et al. (30) also found that TNF-alpha levels did not differ between patients with threatened abortion with good outcome as compared to those with normal pregnancies, but nevertheless were significantly lower among patients with pathologic pregnancies.

4.3. Cytokines in Preterm Delivery

Maternal inflammatory response to lower genital tract infection has been identified as an important link between maternal infection and preterm delivery. Substantial evidence seems to indicate that pro-inflammatory cytokines play a role in the sequence of events leading to preterm labor and delivery (PTD) associated with intrauterine infection. The majority of the early studies on the role of cytokines in the pathogenesis of preterm delivery employed amniotic fluid measurements (31-33). The elevation of pro-inflammatory cytokines in maternal serum or in amniotic fluid during infection and shortly before parturition has also been extensively described (34-36). Significantly higher levels of the pro-inflammatory cytokines IL-1, TNF-alpha, IL-6, and IL-8 have been found in the amniotic fluid of women with infection-associated preterm labor (37). Several researchers have suggested that the estimation of cytokine concentrations in cervicovaginal fluid could be of some value in predicting intrauterine infection and preterm birth, especially in the relatively short period immediately prior to delivery (38, 39), or even several weeks prior to it (40, 41). Higher levels of IFN-gamma in cervicovaginal fluid, IL-1, TNF-alpha, and IL-6 in placental cells and IL-1beta, IL-6, and IL-8 have been demonstrated in the amniotic and chorionic-decidual tissues and in the cervical secretions (42, 43) from women with PTD as compared to those from women with normal term delivery. Raghupathy *et al.* have demonstrated that higher levels of the Th1 cytokines IL-2 and IFN-gamma are produced by women with unexplained PTD, while greater concentrations of the Th2 cytokines IL-4, IL-5, and IL-10 are produced by mitogen-stimulated peripheral blood lymphocytes from women with normal

pregnancies (43). Furthermore, the ratios of Th1 to Th2 cytokines are indicative of a bias towards stronger pro-inflammatory cytokine reactivity in PTD. Similarly, analyses of the ratios of cytokines produced by autologous placenta-stimulated and trophoblast antigen-stimulated PBMC is suggestive of relatively greater production of Th1 cytokines in PTD as compared to normal pregnancy (43). Based on such observations, Romero *et al.* (44) propose that preterm labor in the case of infection results from the actions of pro-inflammatory cytokines secreted as part of the maternal host response to microbial invasions; they suggest that a fetal pro-inflammatory cytokine response is followed by the onset of spontaneous preterm parturition. Dudley (39) suggests that preterm 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," culminating in preterm labor and delivery. Even in the absence of intrauterine infection, preterm 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 (46).

4.4. Cytokines in Preeclampsia

A growing body of evidence suggests that cytokines play a role in the mechanisms underlying preeclampsia, a dangerous disease of human pregnancy that affects both the mother and the fetus and that remains a major cause of perinatal morbidity and mortality around the world. It is a rapidly progressive condition characterized by increased systemic blood pressure, edema, abnormal clotting, proteinuria, and liver and renal dysfunction (47). Elevated concentrations of TNF-alpha have been observed in the blood of women with preeclampsia (48); likewise, elevated levels of IL-6 and IL-8 have been reported in the plasma and amniotic fluid of such women (49). Saito *et al.* (50) reported the measurement of intracellular IFN-gamma and IL-4 by flow cytometry in peripheral blood mononuclear cells; they found increased levels of IFN-gamma and decreased levels of IL-4 in preeclamptic women as compared to women with normal pregnancies. Rein et al. (51) reported a shift to a predominantly Th1-type immunity using flow cytometric intracellular cytokine detection. Saito et al (50) also found significantly increased ratios of Th1 (IFN-positive) cells to Th2 (IL-4-positive) cells in preeclamptic women. In a previous study, this group reported increased production of IL-2, IFN-gamma, and TNF-alpha by PBMC in preeclampsia and, interestingly, a positive correlation between mean blood pressure and these Th1 cytokines (52). A predominance of Th1 cytokine expression in peripheral blood T cells and NK cells. has also been reported by Darmochwal-Kolarz et al. (53)

Raghupathy and co-workers have demonstrated that significantly higher levels of the Th1 cytokines IFN-gamma and TNF-alpha are produced by peripheral lymphocytes from preeclamptic women than by lymphocytes from normal pregnant women, who on the contrary, show a significantly greater production of the Th2 cytokines, IL-4, IL-5, IL-6, and IL-10. A comparison of the ratios of Th2 to Th1 cytokines indicates a higher Th1 cytokine production in the presence of preeclampsia as compared to during normal pregnancy (54). A crucial

observation in preeclampsia is the generalized activation or injury of maternal vascular endothelial cells, leading to microthrombus formation and vasospasm (55). Given the rather dramatic effects of cytokines on endothelial cells, the increased propensity for maternal blood cells to produce these cytokines is likely to be important. If indeed, as proposed by several researchers, placental trophoblasts and maternal vascular endothelial cells are the targets of immune aggression in this condition (48, 56, 57), then maternal inflammatory cytokines are likely to be important effectors of this aggression. In fact, Redman *et al.* suggest that the clinical features of preeclampsia are best described as a cytokine-mediated excessive maternal inflammatory response (57). Taylor proposes that endothelial activation or an altered state of endothelial cell differentiation induced by cytokines could be an important component of preeclampsia (56) and suggests that cytokine action is one of the most attractive hypotheses of immunological dysfunction in this syndrome (56).

## 5. MODULATION OF CYTOKINE PROFILES

These observations on a pro-inflammatory cytokine profile associated with pregnancy complications naturally lead us to a rather provocative and intriguing question: Can we prevent or treat these conditions using therapies that down-regulate Th1 or pro-inflammatory cytokine reactivity? Researchers in the field of cytokines have been pursuing various strategies for modulating cytokine profiles; these include the down-regulation of Th1 cytokines, the neutralization of Th1 cytokines, and the upregulation of Th2 cytokines.

Can we redirect cytokine responses towards a pregnancy-conducive profile? Can we manipulate cytokine production patterns in such a manner as to down-regulate pro-inflammatory cytokines such as IFN-gamma and TNF-alpha, thereby creating an environment that is more conducive to the success of pregnancy? One approach would be to use a hormone, such as progesterone, which has been shown to have both anti-inflammatory and immunosuppressive properties (58). Indeed, several studies have demonstrated that progesterone blocks mitogen-stimulated lymphocyte proliferation, improves allograft survival time, modulates antibody production, decreases the oxidative burst of monocytes, reduces the production of pro-inflammatory cytokines by macrophages in response to bacterial products, and alters cytokine secretion of T-cell clones to favor IL-10 production (59). Piccinni and colleagues (60) have also demonstrated that progesterone favors the development of human T cells producing Th2 cytokines; their contention is that since the Th1 cytokines IFN-gamma and TNF-alpha promote allograft rejection and may compromise pregnancy, the production of Th2-type cytokines IL-4 and IL-10 that inhibit Th1 responses may promote allograft tolerance and fetus survival.

These leads have encouraged research on dydrogesterone (6-dehydro-9β, 10α-progesterone) for potential immunomodulatory activity on lymphocytes from women with RSM and women with preterm delivery (PTD). Dydrogesterone is a potent orally-administered
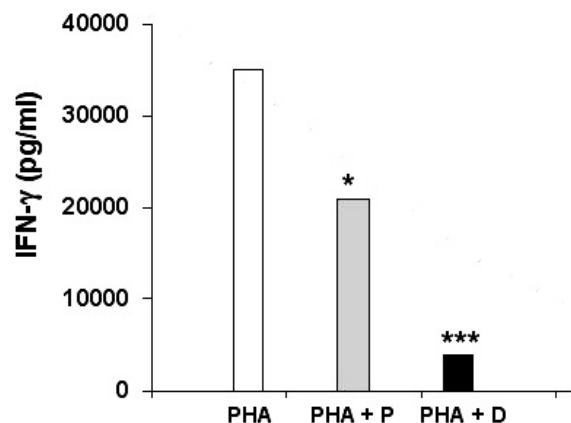
Figure 1. Effects of progesterone and dydrogesterone on the production of IFN-gamma by peripheral blood mononuclear cells from women with PTD. Levels of IFN-gamma produced by PHA-stimulated cells (PHA), by PHA-stimulated cells exposed to progesterone (PHA + P) and exposed to dydrogesterone (PHA + D) are depicted (* *P* < 0.05, *** P < 0.001). Reproduced with permission from 62.
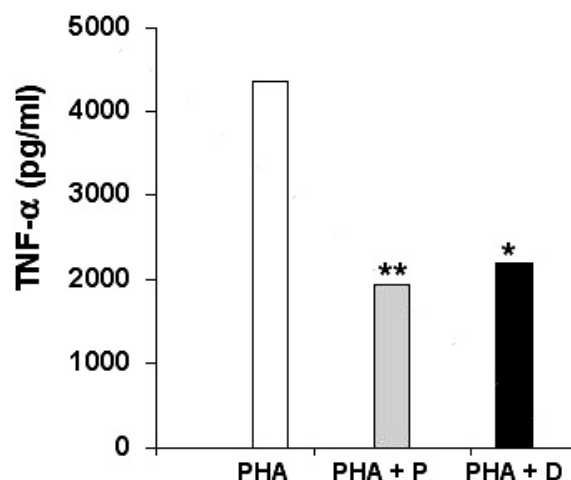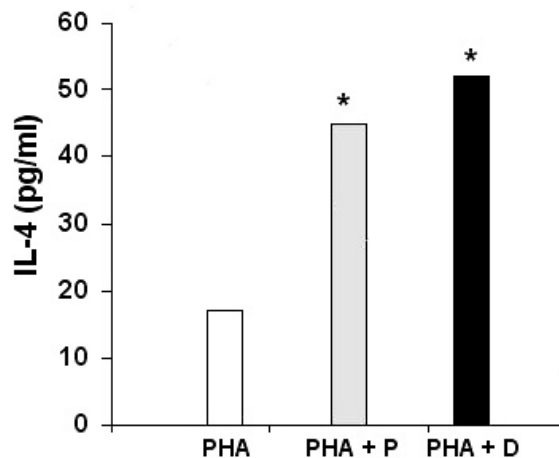

Figure 2. Effects of progesterone and dydrogesterone on the production of TNF-alpha by peripheral blood mononuclear cells from women with PTD. X-axes labels as in Figure 1 (* *P* < 0.05, ** P < 0.01).

progestogen, similar to endogenous progesterone in its molecular structure and pharmacological effects, with a high affinity for the progesterone receptor. Raghupathy *et al.* (61) found that when peripheral blood mononuclear cells (PBMC) from women with a history of unexplained RSM, obtained at the time of miscarriage, were stimulated with a mitogen in the presence of dydrogesterone, progesterone or tissue-culture medium alone, dydrogesterone brings about a significantly reduced secretion of the Th1 cytokines IFN-gamma and TNF-alpha, as does progesterone. On the contrary, levels of IL-4 and IL-6, both Th2 cytokines, are significantly elevated in the presence of dydrogesterone or progesterone (61). IL-4 is the quintessential Th2 cytokine with Th2-inducing

capacity; increased production of IL-4 has been shown to favor further Th2- bias which would affect the eventual outcome of Th1/Th2 balance (5, 6). These researchers also demonstrated a marked reduction in Th1/Th2 cytokine ratios in cultures containing dydrogesterone, indicating a decrease in Th1-cytokine bias. This study demonstrated also that significantly lower levels of the Th1-type cytokines IFN-gamma and TNF-alpha are produced by dydrogesterone-treated lymphocytes from women with RSM (61). Studies demonstrating an association between high levels of these Th1 cytokines and unexplained RSM (13-16, 18-21, 23, 26, 27) indicate a potential benefit in down-regulating their production. Thus the increased production of the Th2 cytokine IL-4 and together with the decreased production of the Th1 cytokines IFN-gamma and TNF-alpha could well result in a substantial swing in Th1/Th2 reactivity away from the potentially harmful Th1 profile and towards the pregnancy-conducive Th2 profile.

Similarly, when exposed to progesterone or dydrogesterone, mitogen-stimulated PBMC from women with unexplained preterm delivery showed significantly reduced levels of Th1 cytokines and increased levels of Th2 cytokines (62). Figures 1. and 2. show that the production of the Th1-type cytokines IFN-gamma and TNF-alpha are significantly inhibited by dydrogesterone and progesterone, while Figure 3. shows that the production of the Th2 cytokine IL-4 is significantly elevated by these progestogens. As seen in Figure 4, the production of IL-10 is not significantly affected by these agents. The calculated ratios of Th1 to Th2 cytokines in different combinations show a substantial shift towards a Th2 or anti-inflammatory bias (62). The increased production of the Th2 cytokine IL-4 and the decreased production of the Th1 cytokines IFN-gamma and TNF-alpha and the overall shift in cytokine ratios indicate the redirection of cytokine profiles from a predominantly pro-inflammatory bias towards an anti-inflammatory bias which is likely to help prevent the creation of a milieu that might lead to preterm labor.

In the presence of progesterone, the lymphocytes of pregnant women release a 35 kD protein named the progesterone-induced blocking factor (PIBF) (63) that mediates the immunomodulatory (64) and anti-abortive (65, 66) effects of progesterone. The immunologic recognition of pregnancy and the subsequent activation of the maternal immune system results in an up-regulation of progesterone receptors on activated lymphocytes among placental cells and CD8^{+} cells (67). In the presence of sufficient levels of progesterone, these cells synthesize PIBF. According to Szekeres-Bartho *et al.*, the immunological pregnancy protective effect of progesterone is, in part, manifested via controlling cytokine production (66). PIBF alters the profile of cytokine secretion of activated lymphocytes shifting the balance towards Th2 dominance (68). In vitro studies demonstrated that dydrogesterone induces the production of PIBF in a dose-dependent manner (61) and that dydrogesterone supplementation may be positively associated with an increased PIBF production in humans (69).

Figure 3. Effects of progesterone and dydrogesterone on the production of IL-4 by peripheral blood mononuclear cells from women with PTD. X-axes labels as in Figure 1 (* $P < 0.05$). Reproduced with permission from 62.
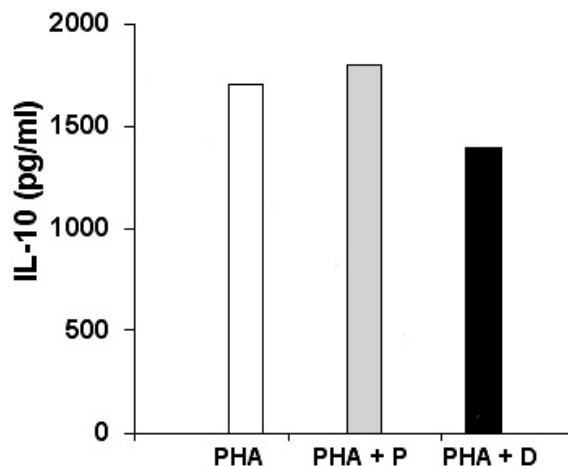
Figure 4. Effects of progesterone and dydrogesterone on the production of IL-10 by peripheral blood mononuclear cells from women with PTD. X-axes labels as in Figure 1 Reproduced with permission from 62.

Kalinka and Szekeres-Bartho (69) have evaluated the effect of dydrogesterone on urinary PIBF concentrations among threatened aborters. Initially, PIBF concentrations in the urine samples of patients showing clinical symptoms of threatened abortion were significantly lower than in those of healthy pregnant women (453.3 pg/ml vs. 1057.94, respectively; p=0.008). Following dydrogesterone treatment, the initially low PIBF concentrations of the threatened aborters significantly increased to reach the PIBF level found in the healthy controls. As the findings of other studies (22, 23) suggest that no Th1/Th2 cytokine imbalance is observed among women with threatened abortion, the immunological pregnancy protective effect of progesterone among threatened aborters is manifested via restoring PIBF concentration rather than via controlling cytokine production. The same mechanism has been previously

suggested by Joachim *et al.* (70) in a murine model. Stressed animals presented lower levels of progesterone and PIBF in their plasma and a reduced staining intensity of progesterone receptor at the feto-maternal interface. The injection of dydrogesterone abrogated the effect of stress on the abortion rate. Further, dydrogesterone increased levels of plasma PIBF in stressed mice and, interestingly, dramatically increased the percentage of IL-4 positive decidual immune cells in stressed mice. This data suggests that dydrogesterone abrogates stress-triggered abortion by inducing a Th2-biased local immune response. The research of Blois *et al.* (71), also based on a murine model, showed that progesterone substitution with dydrogesterone corrected the abortogenic effects of stress exposure by decreasing the frequency of Th1 cytokines via a CD8-dependent pathway.

Recently, Omar *et al.* (72) showed in a randomized study that therapy with dydrogesterone in threatened abortion during the first trimester of pregnancy could improve pregnancy outcome. The continuing pregnancy success rate was significantly higher (p=0.037) in the women treated with dydrogesterone (95.9%) compared with the women who received more conservative treatment (86.3%). The odds ratio of the success rate between dydrogesterone treatment and non-treatment was 3.773 (CI: 1.009-14.108).

Numerous recent publications from several researchers support the idea that progesterone should be seriously considered for preventive therapy in women with a history of spontaneous preterm delivery. 17α-hydroxyprogesterone caproate (17P) brings about a significantly lower occurrence of PTD as well as a reduction in the risk of low birth weight (73). 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 probability of perinatal mortality and very low birth weight infants (74). A secondary analysis of this data revealed that the use of 17P not only reduces the overall risk of preterm delivery, but also the risk of preterm birth in women with a history of more than one previous preterm delivery (75). A recent meta-analysis of randomized controlled trials concluded that patients treated with 17P had lower rates of PTD and reduced incidence of low-birth weight infants (76). Taken together, these results suggest that patients who have had a prior spontaneous preterm birth may benefit from progesterone therapy. Indeed, Hill and colleagues (77) further suggest that potentially immunosuppressive doses of progesterone, which has been termed "nature's immunosuppressant," 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 (78), loses much of its bioactivity (79), and is rapidly cleared from the blood (80). Therefore the orally-active progestogen dydrogesterone is quite attractive.

6. CONCLUDING PERSPECTIVES

It is now becoming abundantly clear that several cytokines likely play critical roles in the success or lack thereof of pregnancy. In complications such as recurrent miscarriage and preterm delivery where no clear etiologies have been identified, it may be useful to ascertain the levels of some pro-inflammatory and anti-inflammatory cytokines. It is tempting to speculate that the evaluation of "personal cytokine profiles" in women with pregnancy complications may point to an immunologic etiology. *In vitro* testing of cytokine levels could be accompanied by evaluating the effects of progesterone and dydrogesterone on the production of critical cytokines (61, 62, Figures 1-4). This could then be followed by modulation of cytokine profiles by therapeutic supplementation with progestogens as indicated by the "personal cytokine profile" of each patient. Research on this approach could also shed new light on the old debate on progesterone supplementation and the most effective method of its administration. The answer may well lie in the individual patient; her underlying immunologic etiology, and her "personal cytokine profile." Research in this area in the coming years is bound to be intriguing and may well lead to useful therapeutic and/or preventive modalities that can be extended to other medical problems as well.

7. ACKNOWLEDGMENTS:

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Send correspondence to: Dr Raj Raghupathy, Department of Microbiology, Faculty of Medicine, Kuwait University, P.O. Box 24923, Safat 13110, Kuwait, Tel: 965498-6527, Fax: 965533-2719, E-mail: raj@hsc.edu.kw