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Role of progesterone and progestin therapy in threatened abortion and preterm labour

Julia Szekeres-Bartho[1], Jacek R. Wilczynski[2], Pawel Basta [3], Jaroslaw Kalinka[4]

[1] Department of Medical Microbiology and Immunology,Medical School, Pecs University, H-7643 Pecs, Hungary, [2] Dept. of Gynecological Surgery, Polish Mother's Health Center Research Institute, Lodz, Poland, 3rd Chair and Dept. of Obstetrics and Gynecology, Medical University, Lodz, Poland, [3] Department of Gynecology, Obstetrics and Oncology of the Jagiellonian University, 23 Kopernika Str, 31-501 Krakow, Poland, [4] Medical and Environmental Pregnancy Health Hazards Unit, Department of Perinatology, 1st Chair of Gynecology and Obstetrics, Medical University of Lodz, Poland, Wilenska 37, 94-029 Lodz, Poland

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1. ABSTRACT

Progesterone (P) has been widely used in an attempt to prevent threatened miscarriage, recurrent miscarriage and pre-term labour. Successful pregnancy depends on maternal tolerance of the fetal "semi-allograft". Along with its endocrine effects, P also acts as an "immunosteroid", by controlling the bias towards a pregnancy protective immune milieu. A protein called progesterone-induced blocking factor (PIBF), by inducing a Th2 dominant cytokine production mediates the immunological effects of progesterone. Progesterone plays a role in uterine homing of NK cells and up-regulates HLA-G gene expression, the ligand for various NK inhibitory receptors. At high concentrations, progesterone is a potent inducer of Th2-type cytokines as well as of LIF and M-CSF production by T cells. The possible mechanisms by which progesterone contributes to the maintenance of early and late pregnancy are discussed.

2. INTRODUCTION

Progesterone is the only hormone that needs to be replaced to maintain pregnancy. Progesterone has been used in attempt to prevent threatened miscarriage, recurrent miscarriage and pre-term labour. However, the mechanisms by which this hormone contributes to the maintenance of gestation might be different in the first, second and third trimesters. Besides its endocrine effects progesterone plays an immuno-modulating role. Several studies have demonstrated that progesterone blocks mitogen-stimulated lymphocyte proliferation, prolongs allograft survival (1), modulates antibody production, decreases the oxidative burst of monocytes, reduces the production of proinflammatory cytokines by macrophages in response to bacterial products and alters cytokine secretion of T-cell clones to favor IL-10 production (reviewed in 2).

This paper aims to review available evidence on the role, progesterone plays at different stages of gestation.

3. THE EFFECTS OF PROGESTERONE DURING PREGNANCY

Progesterone is required for both the establishment and the maintenance of pregnancy. This hormone is needed for preparing the endometrium for implantation, and further, for decidual transformation after implantation has taken place. Later, progesterone plays a role in controlling myometrial contractility, and via its immuno-modulating property regulates the feto- maternal immunological relationship throughout pregnancy.

3.1 Endocrine effects of progesterone

During the luteal phase of the menstrual cycle, progesterone is produced by the corpus luteum and once pregnancy is established, the trophoblast becomes the main source of this hormone. Progesterone induces the proliferation and differentiation of stromal cells (3) and prepares the endometrium for implantation. Progesterone acts on the endometrium via specific receptors that are members of the nuclear receptor superfamily and are regulated by estrogens. The intracellular receptors, progesterone receptor A (PR-A) and progesterone receptor B (PR-B) are the products of the same gene transcribed under control of two distinct promoters. The difference between the two isoforms is that PR-B contains an additional N-terminal stretch of about 165 amino acids. Spatial and temporal expression of PR-A and PR-B vary in reproductive tissues as a consequence of the developmental and the hormonal status. The mechanism by which progesterone controls decidualization, is not well understood. Emerging evidence indicates that locally expressed factors and activation of the cAMP second messenger pathway integrate hormonal inputs and confer cellular specificity to progesterone action through the induction of diverse transcription factors capable of modulating PR function (4).

Csapo postulated that myometrial contractility is controlled by the balance of two major endogenous regulators that exert opposing effects. Contractility is increased by prostaglandins, and inhibited by progesterone (5). Progesterone binds calcium and consequently raises the threshold of excitability of the myometrium, whereas prostaglandin exerts the opposite effect, when progesterone is absent.

3.2. Immunological effects of progesterone

Pregnancy is a natural model of an optimal immune regulation in a graft-host relation. In order to satisfy contradictory interests of mother and fetus, a balance is established to protect the mother from infections and tumours and at the same time to prevent an immunological attack towards the semi-allogeneic fetus. During normal pregnancy immunoglobulin synthesis is increased (6-9), whereas cell-mediated responses are decreased (10-12) thus the maternal immune response is biased towards humoral immunity. Th1 cytokines that promote strong cell-mediated responses have been shown to exert a detrimental effect on pregnancy in mice.

IFN gamma activates cytotoxic T cells and NK cells, which, - may than damage the trophoblast. IFN gamma also inhibits GM-CSF production as well as proliferation of Th2 cells and consequently B cell maturation and immunoglobulin synthesis. Low doses of IFN gamma retard intrauterine development in mice, whereas administration of high doses results in abortion (13). TNF-alpha inhibits trophoblast cell proliferation *in vitro*. Injection of recombinant murine or human TNF-alpha to pregnant mice results in miscarriage, while anti TNF antibodies or TNF antagonists normalize the high resorption rates in abortion-prone matings (13).

Th2 cytokines play a potential protective role in the feto-maternal relationship. IL-10 inhibits the production of Th1 cytokines and stimulates the proliferation of B cells. The lack of IL-10 results in pregnancy failure. IL-10 gene knock out mice are born with significantly lower birth weight than their heterozygous siblings. This can be prevented by administering rIL-10 or anti TNF treatment (14). Granulocyte-macrophage colony-stimulating factor (GM-CSF) has been identified as a potentially important mediator of intercellular communication in the female reproductive tract, with principal target cells being the preimplantation embryo, and trophoblast cells of the developing placenta (15,16).

In humans there is a well-established relationship between peripheral cytokine pattern and the outcome of pregnancy (17). It has been suggested that significantly increased Thl cytokine expression might represent the underlying phenomenon leading to reproductive failure (18). Further, the activation of peripheral blood mononuclear cells with trophoblast antigens (19) or mitogens (20) confirmed that women with idiopathic recurrent spontaneous pregnancy loss display a Thl-type cytokine profile, characterized by production of IL-2, TNF and interferon gamma (19).

A growing body of evidence suggests that progesterone might play a significant role in establishing an adequate immune environment for the early stages of pregnancy (21-24).

Progesterone at concentrations comparable to those present at the materno-foetal interface during pregnancy, is a potent inducer of Th2-type cytokines (i.e. IL-4 and IL-5) (21), and also of LIF and M-CSF production by T lymphocytes (25, 26), thus progesterone present in the microenvironment of the decidual T cells could be responsible, at least in part, for the Th2-biased cytokine production by these cells (27-29).

In addition to its direct effect on decidual T cells, progesterone also acts on the cytokine profile via a mediator. Following recognition of fetal antigens, activated maternal peripheral gamma/delta T cells express progesterone receptors (30), and upon P binding –produce a mediator; named PIBF (31-33), which induces a Th2 dominant cytokine production (34) and through altered cytokine production inhibits NK mediated killing in an indirect way (35). Neutralization of endogenous PIBF

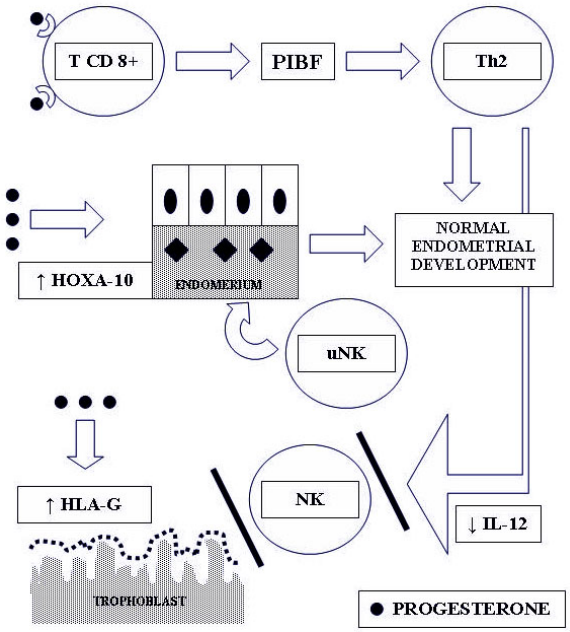

Figure 1. The role of progesterone in establishment and maintenance of pregnancy. Both the direct- and Hoxa-10 inducing effects of progesterone are needed for normal endometrial development. Progesterone-induced PIBF creates a favourable cytokine milieu for the developing embryo. Progesterone promotes NK cell trafficking from peripheral blood to the decidua, and up-regulates expression of HLA-G, which is the ligand of NK cell inhibitory receptors.

activity in pregnant mice by specific anti-PIBF antibody causes a significant reduction in the number of viable foetuses, and this is associated with an increased splenic NK activity, together with reduced IL-10 and increased interferon γ. production of the spleen cells (36). These effects are reversed by treatment of the pregnant animals with anti-NK antibodies (36). These data suggest that in mice PIBF contributes to the success of pregnancy and that the major part of its pregnancy-protective effect lies in controlling NK activity.

During pregnancy a unique NK cell population called uterine-, endometrial- or decidual NK cells appears in the endometrium. Decidual NK cells resemble the $CD56^{bright}$ peripheral NK subset in their phenotype but, unlike the former, they contain cytotoxic granules (37), and among the genes selectively over-expressed in decidual NK are secreted proteins with known immunosuppressive activity (38). Decidual NK cells secrete angiogenic factors and induce vascular growth in the decidua. These findings together, with their dynamics of the appearance suggest that one of their functions might be the control of placentation. A recent study (39) showed that decidual NK cells regulate trophoblast invasion by production of the interleukin-8- and interferon-inducible protein-10 chemokines. On the other hand, perforin and granzymes are also expressed by decidual NK (38, 40), suggesting that under certain conditions the otherwise peaceful decidual NK may become cytotoxic.

Among decidual immune cells dendritic cells (DCs) play a key role. Immature DCs reside in the early pregnancy human and mouse decidua (41-44) and exhibit a tolerogenic phenotype. In mice pregnancy loss is accompanied by increased presence of mature decidual DCs (42), which via IL-12 production might increase NK activity. Progesterone inhibits in a receptor-mediated fashion pro-inflammatory cytokine production by mature DCs (45).

Uterine NK cells are under hormonal control in rhodents, and also in humans. Progesterone plays a role in uterine homing of NK cells by promoting NK cell interactions with the endothelium (46). NK cell migration to the endometrium is also supported by sex hormone-induced specific endometrial production of chemokines (47). Furthermore, progesterone up-regulates HLA-G gene expression (48). HLA-G is the ligand for various NK inhibitory receptors, thus increased availability of HLA-G will keep NK activity at a low level. Finally, the expression of Hoxa-10, a homeobox transcription factor - which mediates the progesterone-stimulated proliferation of uterine stromal cells, and is crucial for natural killer (NK) cell differentiation - is also regulated by progesterone (49) (Figure 1).

The role of NK cell activity in the reproductive process has received much attention. NK activity was shown to display deleterious effects on fetal development, resulting in spontaneous abortion in mice (50). In mice there is direct evidence for the involvement of high NK activity in pregnancy termination. NK cell infiltration was demonstrated in damaged mouse fetuses and placentae. Transfer of high NK activity spleen cells to pregnant Balb/c mice induces abortion (51). Normal human pregnancy is characterized by low peripheral NK activity (52) and increased NK activity seems be an attribute of spontaneous abortions of unknown etiology. In human it is difficult to demonstrate a direct relationship between spontaneous pregnancy termination and NK activity, however, in several studies, increased NK activity was observed in association with different forms of spontaneous pregnancy termination.

Some argue that since there is no evidence for the association between the levels of NK cells in peripheral blood and in the uterine mucosa, testing peripheral NK cell count or activity has no relevance.

The availability of normal human placentae is restricted to the first trimester and labour. During an ongoing normal human pregnancy it is difficult to simultaneously test peripheral and decidual NK cells. There are only a few studies comparing peripheral and decidual NK cells from the same patient. Lewis *et al.* (53) showed that similarly to peripheral blood NK cells, decidual NK cells express the natural cytotoxicity receptors NKp30 and NKp46 but the significance of this will not become apparent until ligands for these molecules have been identified. Gulan *et al.* (54) demonstrated decreased perforin content of decidual lymphocytes from failed pregnancy as compared to those from normal pregnancy

deciduas, suggesting that an increased rate of degranulation had taken place in the former case. In early pregnancy peripheral blood IL-10-producing cells NK cells were significantly more frequent compared with those in non-pregnant women, and this cell population was decreased in women with miscarriage. Higuma Myojo et al., (55) identified the main populations of NK cells in normal decidua, as TGF-beta-producing NK3 type cells. This cell type was significantly reduced in deciduae from women with recurrent miscarriage. Data by Olivares et al. (56) support the hypothesis that activated decidual lymphocytes participate in human spontaneous abortion by inducing apoptosis but not necrosis of the trophoblast.

The value of findings by investigating placentae from spontaneous miscarriages or preterm delivery is questionable. These specimens are likely to contain artefacts and it is not clear whether the changes observed are the cause or the consequence of miscarriage. Results of animal experiments cannot be directly extrapolated to the human situation. Therefore, it is hard to tell, how the actually measured changes at the periphery relate to the local events.

4. PROGESTERONE AT EARLY PREGNANCY AND IN THREATENED ABORTION

Threatened abortion (TA), the most frequent clinical condition among women in early pregnancy, affects up to 15-20% of pregnant women. Threatened abortion is manifested by vaginal bleeding and/or uterine cramps while the cervix is closed. This stage may end up in spontaneous abortion in approximately 10-15% of the cases (57, 58) or, alternatively pregnancy may proceed normally. Women with threatened abortion are at increased risk of adverse pregnancy outcome (59, 60). A prospective multicenter study revealed, that first–trimester vaginal bleeding is an independent risk factor for intrauterine growth restriction (OR=2.6), preterm delivery (OR=3.0), preterm premature rupture of membranes (OR=3,2) and placental abruption (OR=3,6) (60). The risk of premature delivery (56) as well as delivering the baby of less than 1000 g after threatened abortion are also increased, OR=4,43 (57).

As far as the underlying pathology is concerned, threatened aborters form a very heterogeneous group. Age is a risk factor for miscarriage. A prospective study on women with threatened abortion reported that women older than 34 years had an odds ratio of 2.3 for miscarriage, however, the 95% confidence interval was wide (0.76 to 7.10), and the contribution of maternal age in regression analysis was not significant (P = 0.13) (61).

In a part of the cases there is a progesterone deficiency. Data from 358 women presenting with vaginal bleeding in the first 18 gestational weeks indicated that a single progesterone value of less than 45 nmol/l (14 ng/ml) is able to differentiate between abnormal and normal (ongoing) pregnancies, with a sensitivity of 87.6% and a specificity of 87.5% (62).

Data concerning cytokine profile in women with threatened abortion are relatively scarce. Paradisi *et al.* (63) reported on significantly lower serum concentrations of the T-helper 2-type cytokines IL-6, IL-10 and IL-13 in women with missed abortions, but not in those with threatened abortions, compared to normal pregnant and non-pregnant women. Another study (64) investigating the serum concentrations of the pro-inflammatory cytokines, IL-8 and IL-12 as well as that of the soluble interleukin-2 receptor (sIL-2R) showed that the cytokine patterns in threatened abortions with a favourable outcome resemble normal pregnancy. A recent prospective study failed to detect a Th2-type cytokine deficiency in threatened abortion, and indicated no difference between Th1- and Th2-type cytokine profiles of women with clinical symptoms of threatened abortion and those with normal pregnancies (65). In a similar vein, Gucer *et al.* (66) did not detect significantly increased IL-2 receptor and TNF-alpha levels in patients with threatened abortion with a favourable outcome. These data suggest that in women with threatened abortion cytokine profiles correlate with the outcome, rather than with the condition itself.

In peripheral blood of healthy pregnant women the percentage of PIBF positive lymphocytes was found to be significantly higher in all trimesters of pregnancy than in women showing clinical symptoms of threatened preterm pregnancy termination. (67). In urine samples of healthy pregnant women PIBF concentrations continuously increase until the 37th week of gestation, followed by a slow decrease until term. In pregnancies that end up in miscarriage or pre-term delivery, urinary PIBF levels fail to increase during pregnancy (68). Also, urine PIBF concentrations among threatened aborters were found to be significantly lower than in healthy controls at the same gestational age (23). Moreover, urine PIBF concentrations of threatened aborters whose pregnancies ended up in miscarriage were significantly decreased compared to those with ongoing pregnancies (23). Check *et al.* (69) determined PIBF expression in lymphocytes of 3 to 5 weeks pregnant women by immunocytochemistry. Though this study had insufficient power to reach statistical significance, it showed a trend for higher rates of miscarriage when PIBF was absent.

Salomon *et al.* (70) analyzed the expression rate of PIBF by peripheral lymphocytes in healthy pregnant women after administration of mifepristone for non-surgical termination of pregnancy at 5-8 weeks of gestation. In 17 out of 21 patients, the percentage of PIBF positive lymphocytes decreased after anti-progesterone administration. These data suggest that mifepristone-induced disturbances of progesterone-mediated immunosuppression might contribute to the termination of pregnancy.

Progesterone is prescribed in 15-40% of women with threatened miscarriage (71). Recently, in a randomized study Omar *et al.* (72) showed 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 women treated with dydrogesterone (95.9%) compared with women who received conservative treatment (86.3%). The odds ratio of the success rate between dydrogesterone treatment and non-treatment was 3.77 (CI: 1.009-14.108). In another prospective study (23) among threatened aborters undergoing dydrogesterone treatment the length of gestation did not significantly differ from that of healthy pregnant women, nor did the mean birth weight of the newborns. The incidence of pre-term delivery was still higher in the threatened abortion group, but the difference between the two groups did not reach statistical significance. Other studies (73-76) suggest that progesterone does not seem to improve outcome in women with threatened miscarriage.

The diverse etiological background of spontaneous abortion makes it difficult to prevent and manage the condition. A high percentage of the so-called 'threatened miscarriages' settle spontaneously as a result of bed rest and/or no treatment. By varying the inclusion or exclusion criteria for any given protocol, the results of a study treatment can be altered completely.

5. PROGESTERONE IN LATE PREGNANCY AND PRETERM DELIVERY

The incidence of preterm birth, which is the major cause of perinatal morbidity and mortality, has been increasing in the past decades (77). Prevention of preterm birth is therefore a major goal of obstetric care. Recent studies suggest that progestins might be the choice of treatment in preventing preterm labour. An NIH randomised controlled trial has shown that weekly administration of 17-α-hydroxyprogesterone caproate at 300 mg/day intramuscularly results in an almost 50% decrease in the incidence of subsequent preterm birth (78). Another randomized placebo-controlled trial in 142 women at risk for preterm birth revealed a significant effect in progesterone-treated patients (79). A recent meta-analysis of ten studies, including altogether 1339 patients showed that women treated with progestational agents had lower rates of preterm delivery (26.2% versus 35.9%; OR 0.45, 95% CI 0.25–0.80) (80).

The mechanism of progesterone action in preventing preterm birth is not clear. In animals, the onset of labour is preceded by a fall in progesterone levels (81) and recent data suggest altered function of progesterone receptors may also regulate the onset of labor (82).

Progesterone plays a role in the maintenance of uterine quiescence throughout pregnancy. It has been shown to possess a tocolytic action on the myometrium, (83) partly by inhibiting gap junction formation Gap junctions allow cell-to-cell communication between the smooth muscle cells. Labour is associated with an increase in the number of gap junctions, and this increase is regulated by oestrogen, progesterone, and prostaglandins (84).

Csapo proposed that the relative loss of progesterone effect is implicated in the mechanism of human parturition (5). Prostaglandins increase during parturition and stimulate myometrial contractility. Progesterone prevents prostaglandin F2alpha synthesis and release, thereby promoting uterine quiescence. PIBF inhibits the synthesis of prostaglandin F2 alpha, and the above effects are abrogated in the presence of exogenous arachidonic acid, suggesting that PIBF interferes with either the release or the action of arachidonic acid (31). Indeed, PIBF inhibits phosholipase A2, which is needed for the liberation of arachidonic acid (85), thus PIBF reduces the availability of the precursor for PG synthesis. The lack of prostaglandins beside reducing the contractility of the uterine smooth muscle also inhibits the production of IL-12 (a cytokine which stimulates NK activity) and results in a lowered cytotoxic NK activity (86). Prostaglandin F2 alpha has been shown to cause a significant increase of peripheral NK activity *in vitro* and this effect was corrected by progesterone (87). Treatment of women at risk for preterm delivery with acetylsalicylic acid resulted in a decreased peripheral NK activity, compared to women receiving beta mimetics only, and with a lower rate of preterm delivery (1/9 and 9/13 in the treated and control groups respectively) (88).

Originally high progesterone sensitivity of pregnancy lymphocytes decreased while low sensitivity to prostaglandin E2 increased during labour. Except for prostaglandin E2 sensitivity all parameters of the lymphocytes obtained from women with threatened preterm delivery were similar to those of lymphocytes obtained during labor (89).

6. SUMMARY AND PERSPECTIVES

Because of the diverse aetiological background of threatened abortion, it is difficult to assess the efficacy of progesterone treatment in this condition. A high percentage of the cases settle spontaneously without treatment. Presently available data suggest that in unselected groups of women with threatened abortion progesterone does not improve the outcome of pregnancy. Cytokine profiles as well as lower than normal progesterone levels in these women correlate with the outcome, rather than with the condition itself. Moreover, urine PIBF concentrations of threatened aborters whose pregnancies ended up in miscarriage were shown to be significantly decreased compared to those with ongoing pregnancies. These data suggest that within the very heterogeneous group of threatened aborters there is a subgroup, which might respond to progesterone. Therefore, the use of various laboratory tests in well-designed randomized studies is needed to identify this population of patients.

There is increasing evidence that progestin treatment reduces the rate of pre-term delivery. The mechanism of protection could be a combination of an immunological effect and the action of progesterone on the myometrium. Prostaglandins increase during parturition and stimulate myometrial contractility. Progesterone counteracts this effect.

PIBF inhibits the liberation of arachidonic acid; consequently, the availability of the precursor for PG synthesis will be reduced. The lack of prostaglandins will not only result in lowered excitability of the smooth

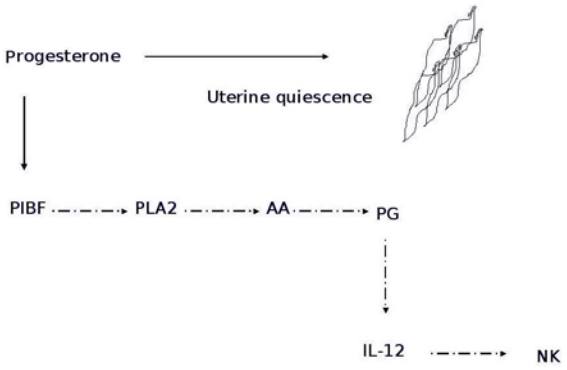

Figure 2. The role of progesterone in the maintenance of late pregnancy. Progesterone inhibits prostaglandin-induced uterine contractility. Progesterone induces PIBF, which inhibits phospholipase A2 activity. Since the latter is needed for the release of arachidonic acid, prostaglandin production will be inhibited in the presence of PIBF. Reduced prostaglandin concentrations result in inhibition of IL-12 production and consequently reduced NK activity as well as in uterine quiescence.

muscle, but also in reduced synthesis of the cytokine IL-12, which is needed for NK cell activation (Figure 2).

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Send correspondence to: Dr Julia Szekeres-Bartho, Department of Medical Microbiology and Immunology, Medical School, Pecs University, 12 Szigeti Str. H-7624 Pecs Hungary, Tel: 3672-536262, Fax: 3672-536253, E-mail: Julia.szekeres@aok.pte.hu

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