J. Perinat. Med. 37 (2009) 338–342 • Copyright © by Walter de Gruyter • Berlin • New York. DOI 10.1515/JPM.2009.061

Progesterone – induced blocking factor (PIBF) and Th_1/Th_2 cytokine in women with threatened spontaneous abortion

Igor Hudić* and Zlatan Fatušić

Clinic for Gynecology and Obstetrics, University Clinical Center, Tuzla, Bosnia and Herzegovina

Abstract

Objective: The aim of this prospective study was to compare serum and urine concentrations of progesterone-induced blocking factor (PIBF) and serum concentrations of anti-inflammatory (IL10) and pro-inflammatory (IL6, TNFα, IFNγ) cytokines of women with threatened spontaneous abortion with normal pregnancy and to evaluate the impact of PIBF on outcome of pregnancy.
Methods: A sample of 30 women with threatened spontaneous abortion (study group) and 20 healthy pregnant women (control group) between 6th and 24th gestational weeks was studied. Serum and urine PIBF, IL10 and IL6, TNFα, IFNγ cytokine concentrations were measured by enzyme-linked immunosorbent assay (ELISA).
Results: Five (16.7%) pregnancies in the study group ended missed abortion vs. none in the control group ($P < 0.05$). Five (20%) threatened aborters delivered between 24th and 37th weeks of gestation, whereas two (10%) preterm deliveries occurred in the controls ($P > 0.05$). PIBF concentrations in urine (19.5 ± 12.9 ng/mL) and serum (214.4 ± 120.6 of patients with threatened abortion were significantly lower than in healthy pregnant women (45.3 ± 33.7 ng/mL and 357.3 ± 159.9 ng/mL, respectively). Women with threatened abortion had significantly lower serum levels of anti-inflammatory cytokine, but levels of proinflammatory cytokines were higher in this group compared with healthy controls.
Conclusions: Determination of progesteron-induced blocking factor level in body fluids in early pregnancy might be used for the diagnosis and prognosis of threatened abortion.

*Corresponding author:
Igor Hudić
University Clinical Center Tuzla
Clinic for Gynecology and Obstetrics
Trnovac bb, 75 000 Tuzla
Bosna i Herzegovina
Tel.: +387 35 303 402, +387 61 858 825
E-mail: i.hudic@bih.net.ba

Keywords: Anti-inflammatory cytokines; progesterone-induced blocking factor (PIBF); pro-inflammatory cytokines; threatened spontaneous abortion.

Introduction

Pregnancy can be compromised by a number of complications such as threatened abortion, recurrent spontaneous miscarriage, and preterm delivery [20]. Spontaneous abortion is a common problem affecting 15–20% of all recognized pregnancies. Spontaneous miscarriages have a range of possible causes including genetic, anatomical, endocrine, immune, infective, thrombophilic, and unexplained [13]. A substantial part of unexplained spontaneous abortion might be attributable to a deleterious immune response of a mother toward the fetus. A growing body of evidence suggests that progesterone might play a role in establishing an adequate immune environment during the early stages of pregnancy [12, 25]. Particularly interesting are the effects of pro-inflammatory and anti-inflammatory cytokines on the conceptus and thus, on the success or failure of pregnancy [20]. In the presence of progesterone, lymphocytes of pregnant women release a protein named the progesterone-induced blocking factor (PIBF) [26] which mediates the immunomodulatory and anti-abortive effects of progesterone [28, 30, 31]. Immunologic recognition of pregnancy and subsequent activation of maternal immune system results in an upregulation of progesterone receptors on activated lymphocytes among placental cells and CD8+ cells. In the presence of sufficient progesterone levels, these cells synthesize PIBF [32]. Patients at risk of spontaneous abortion have increased pro-inflammatory and low PIBF and IL-10 expressions on lymphocytes [35]. PIBF alters the profile of cytokine secretion of activated lymphocytes shifting the balance toward Th_2 dominance [33]. During normal uneventful pregnancy the concentration of PIBF continuously increased from the 6th to the 37th gestational week. After the 41st week, PIBF concentrations dramatically decrease. In patients with threatened spontaneous abortion PIBF levels failed to increase during pregnancy [19]. Therapeutic regimens to prevent spontaneous abortion are limited in efficacy. Still, there is a considerable controversy about the use of progestogens for the treatment of threatened spontaneous abortion. There are four main approaches towards progestogen therapy: 1. Do nothing; 2. No routine therapy, but once or twice weekly

measurements of progesterone levels to suggest whether support with progestogens is or is not necessary (the cut-off level is 300 nmol/L); 3. Routine pharmacological "support of the pregnancy". This is frequently advocated empirically in all pregnancies following recurrent losses because "it can do no harm". In our view, this is an unacceptable argument which has no place in ethical treatment regimens; 4. Treat only within properly designed prospective trials (with adequate follow-up). This is our preferred option [6].

The aim of this prospective study was to compare serum and urine concentrations of PIBF and serum concentrations of anti-inflammatory (IL10) and pro-inflammatory (IL6, TNFα, IFNγ) cytokines of women with threatened spontaneous abortion with normal pregnancy and to evaluate impact of PIBF on outcome of pregnancy as well as the possible underlying mechanisms.

Patients and methods

A prospective study was performed at the Clinics for Gynecology and Obstetrics, University-Clinical Center Tuzla and Counseling for pregnant women at the department for health protection of women at the Public Medical Center Tuzla. A study and control group of pregnant women were formed, as a consecutive sample, with a total of 50 examinations. Pregnant women aged 18–35 years, who had no risk factors for spontaneous abortion, were included in this research. The study group consisted of 30 pregnant women, who were hospitalized at the Department of Pathology of Pregnancy (Clinics for Gynecology and Obstetrics in Tuzla), but only those who were hospitalized due to threatened spontaneous abortion (between 6th and 24th weeks' gestation). Threatened spontaneous abortion was defined as bleeding, spotting and uterine cramps. A gestational age was determined in all patients by ultrasound examination. Exclusion criteria were: chronic diseases, e.g., hypertension, diabetes, renal or cardiac diseases, uncertain gestational age, previous spontaneous abortion, history of urinary infections in actual pregnancy, history of pregnancy loss, uterine anomalies and previous cesarean section. Only singleton pregnancies qualified for the study. The control group consisted of 20 healthy pregnant women from 6th to 24th gestation weeks who had no risk factors for spontaneous abortion and who underwent regular gynecological examinations. Serum and urine samples were collected from all subjects and PIBF concentration was measured by enzyme-linked immunosorbent assay (ELISA) [19]. The analysis was conducted at the Department of Microbiology and Immunology at Medical Faculty, University of Pecs, Hungary.

PIBF determination

Briefly, during overnight incubation at 4°C, 96-well microtiter plates were coated either with ani-human recombinant PIBF IgG (100 μL/well of 2 μg/mL) in 50 mm carbonate buffer pH 9.6 (plate 1), or with human recombinant PIBF (100 μL of 0.5 μg/mL) in 0.5 m Tris buffer pH 6.5 (plate 2). For generating a standard curve, recombinat PIBF (1000–0.1 ng/mL) in logarithmic dilutions in 0.5 m phosphate buffer (pH 7.3–7.4) was incubated with a standard amount of biotin-labeled anti-recombinant PIBF IgG (400 ng) for 60 min at 37°C. Serum and urine samples were diluted 1/2.5 and 1/5 and incubated with 400 ng biotin-labeled anti-recombinant PIBF IgG in 0.5 m phosphate-buffered saline (PBS) for 60 min at 37°C before being added to ELISA plate 1. During the 1 h incubation at 37°C, non-specific binding sites on plate 2 (coated with human recombinant PIBF) were blocked with 200 μL of 0.1% bovine serum albumin (BSA), 0.5% gelatin in PBS-Tween. After this incubation step, 100 μL standard solutions of the serum and urine samples were transferred from plate 1 to plate 2 and incubated for 1 h at 37°C. After washing the plates three times with PBS-Tween, 100 μL of 1:1000 diluted horseradish peroxidase (HRPO)-conjugated streptavidine (AP Hungary Ltd, Budapest, Hungary) in 0.1% BSA PBS-Tween was added and plates were incubated for 30 min at 37°C. The reaction was developed by adding the substrate orthophenylene-diamine (OPD; Sigma, Budapest, Hungary) and measured at 495 nm.

The concentrations of IL10, IL6, TNFα, IFNγ were taken from blood samples and were measured manually with a quantitative sandwich enzyme immunoassay (ELISA) after freezing at –70°C, centrifugation and aliquation at the Department of Immunology and Microbiology in Tuzla. Cytokine concentrations were measured in serum manually with a high sensitive quantitative sandwich enzyme immunoassay (R&D Systems Inc., Minneapolis, USA). The minimum detectable concentrations of human pro-inflammatory and anti-inflammatory cytokines were as follows: IL10 – 3.9 pg/mL, IL6 – 0.7 pg/mL, IFNγ – 8 pg/mL and TNF-α – 1.6 pg/mL. The intra- and inter-assay precision of the all cytokine assays were $<$10%. In the serum of pregnant women from the examined and control group values of IL6, IFNγ, TNFα, IL10 were determined, and the analysis was conducted at the Department of Microbiology and Immunology, University Clinical Center Tuzla.

The study was approved by the Ethical Committee of the University Clinical Center Tuzla, Bosnia and Herzegovina (Decision No. 01/1-37-4-898/07). Every patient was provided with a written consent for participation.

SPSS V.11.5 software (SPSS Inc, Chicago, Illinois, USA), Arcus Quick Stat and Microsoft Excel XP Professional were used for statistical analysis of the study. Student's *t*-test was used to compare the mean values. The distribution of qualitative variables was compared by χ^2 or by Fisher's exact tests. A value of two-sided $P < 0.05$ was considered significant.

Results

Fifty patients were included in this study, without difference between clinical characteristics in women with preterm delivery and healthy pregnant controls in maternal age or gestational age at the time of enrollment. The mean gestational age at the time of blood sampling was also not different. Active smoking was declared by 20% in both groups (Table 1).

Five (16.7%) pregnancies with threatened abortion ended in missed abortion. No missed abortion was registered in the control group (difference was statistically significant). Five (20%) women with threatened abortion delivered between 24th and 37th weeks' gestation, where-

Table 1 Comparison of selected maternal characteristics in women with threatened spontaneous abortion and healthy pregnant controls.

	Threatened abortion (n=30)	Healthy controls (n=20)	P-value
Maternal age (years)			
Mean±SD	25.3±4.4	24.5±3.7	NS
BMI* (kg/m²)			
Mean±SD	24.7±3.7	25.0±3.9	NS
Gestational age at blood sampling			
Mean±SD	11.4±3.8	13.3±4.8	NS
Nulliparity (n%)	21 70.0	13 65.0	NS
Smoking (n%)	6 20.0	4 20.0	NS
Educational level (with secondary education; n%)	15 50.0	8 40.0	NS

*BMI=body mass index.

Table 2 Pregnancy outcome in study and control group.

	Threatened abortion (n=30)	Healthy controls (n=20)	P-value
Missed abortion (n%)	5 16.7	– –	P<0.05
Preterm delivery (n%)	5 20.0	2 10.0	NS

as two (10%) with preterm delivery were registered in the control group. There was no significant difference in preterm deliveries between the study and control group (Table 2).

PIBF concentrations in urine (19.5±12.9 ng/mL) and serum (214.4±120.6) samples of patients showing clinical symptoms of threatened abortion were significantly lower than in those of healthy pregnant women (45.3±33.7 ng/mL; 357.3±159.9 ng/mL, Table 3) Using a cut-off level of 20.7 ng/mL, PIBF urine concentrations predicted pregnancy termination with sensitivity 63%, specificity 90%, positive predictive value 91%, and negative predictive value 90%, whereas a cut-off level of 182.8 ng/mL PIBF serum concentrations predicted pregnancy termination with sensitivity 53%, specificity 95%, positive predictive value 94%, and negative predictive value 95%.

Women with symptoms of threatened spontaneous abortion had significantly lower serum levels of anti-inflammatory cytokine (IL10), but levels of proinflammatory (IL6, TNFα, IFNγ) cytokines were higher in this group compared to healthy controls (Table 4).

Discussion

This study shows that PIBF concentrations in body fluids from pregnant women reflects certain pathological events and is related to outcome of pregnacy. There is ample evidence that PIBF is relevant for maintenance of pregnacy and its actions are exerted through immunological mechanisms such as altered Th_1/Th_2 cytokine balance [29, 33]. All patients during hospitalization due to threatened miscarriage received gestagen therapy.

Table 3 Progesterone-induced blocking factor (PIBF) serum and urine concentrations of patients with threatened abortion and healthy pregnant women.

PIBF* (ng/mL)	Threatened abortion Mean±SD	Healthy controls Mean±SD	P-value
Urine	19.5±12.9	45.3±33.7	<0.05
Serum	214.4±120.6	357.3±159.9	<0.05

*PIBF=Progesterone-induced blocking factor.

Table 4 Serum concentrations of anti-inflammatory (IL10) and proinflammatory (IL6, TNFα, IFNγ) cytokines.

	Threatened abortion Mean±SD	Healthy controls Mean±SD	P-value
IL10 serum (pg/mL)	6.7±4.7	7.9±4.9	<0.05
IL6 serum (pg/mL)	3.7±1.4	1.3±1.0	<0.05
TNFα serum (pg/mL)	8.4±3.4	6.7±0.6	<0.05
IFNγ serum (pg/mL)	9.6±1.8	7.6±2.6	<0.05

IL=interleukin, TNF=tumor necrosis factor, IFN=interferon.

Premature delivery of 5 (20%) patients in our study took place after they were released from the clinics and stopped gestagen therapy. Sanches-Ramos et al. [21] published a randomized study that was conducted among 1339 patients to establish the efficiency of progesterone therapy on prevention of premature deliveries. They showed that gestagen (17-α hydroprogesteron and other types of progesterone) lowered the risk of premature delivery and delivery of newborns with low birth weight in patients with a history of early miscarriages and threatened miscarriage in the current pregnancy. Similar results found by Sfakianki and Norwitz [23], Szekeres-Bartho et al. [34] and Kalinka and Bartho [12], who determined the therapeutic effect of progesterone through PIBF in patients with symptoms of threatened miscarriage, noted that induction of production PIBF by Dydrogesteron significantly reduces the frequency of miscarriages. Similar conclusions were reached by other authors, in agreement with results of our study [3, 7].

We found significantly higher concentration of proinflammatory cytokines in patients with threatened spontaneous abortion, in accord with studies conducted by many authors [3, 7, 19, 36]. Most authors conclude that IL10 has the key anti-inflammatory immune response [2, 5, 19]. Palfi et al. [16] did not find a difference between healthy pregnant women and controls when the serum ratio of Th_1/Th_2 cytokines was measured in patients with habitual miscarriages, and suggested a crucial impact on outcome of pregnancy by a ratio between proinflammatory and anti-inflammatory cytokines. Sedlmayr [22] found higher IL6 concentration produced by macrophages in decidual cells in patients with repeated miscarriages, in accord with our research. Similar results were noted by other authors [9, 10]. These results are different from Wolff et al. [37] and Baek [1] who noted lower IL6 values in serum of patients with idiopathic miscarriages. TNFα belongs to the group of proinflammatory cytokines and it inhibits placental differentiation and invasion, laying down the extracellular matrix, hormone production, deactivation of macrophages and inhibits the production of cytokines in many tissues. These are actually pathophysiological mechanisms that lead to miscarriage [8, 15, 24]. Many authors noted a direct influence of higher TNFα values on development of miscarriages [2, 5, 14]. Interferon gamma belongs to the group of proinflammatory cytokines which was the subject of much research on immunological causes of miscarriages [4]. Most authors found direct connection between higher serum and local concentration (fetomaternal units) INFγ in patients with miscarriages, which was found in this study as well [5, 9, 11, 17, 27]. Contrary to our study, some studies did not find an influence of higher serum concentration of INFγ on development of miscarriage [16, 18].

We conclude that (1) concentrations of PIBF in urine and serum are significantly lower in pregnant women with threatened miscarriage; (2) concentration of anti-inflammatory cytokine (IL10) in serum is significantly lower, and concentration of proinflammatory cytokines (IL6, IFNγ, TNFα) is significantly higher in pregnant women with symptoms of threatened miscarriage; (3) determination of concentration PIBF in urine and in serum, as well as IL6, IFNγ, TNFα and IL10 cytokines in serum might be used as a diagnostic and prognostic parameter of threatened abortion.

Acknowledgements

The authors would like to acknowledge Prof. Julia Szekeres-Bartho and Doc. Beáta Polgar from Department of Medical Microbiology and Immunology, Pecs University, Medical School, Hungary for valuable advice and suggestions that improved methodology of our research.

References

[1] Baek H. Aberrant gene expression associated with recurrent pregnancy loss. Mol Hum Reprod. 2004;10:291–7.
[2] Chaouat G, Meliani A, Martal J, Raghupathy R, Elliot J, Mosmann T, et al. IL-10 prevents naturally occurring fetal loss in the CBA x DBA/2 mating combination and local defect in IL-10 production in this abortion-prone combination in corrected by *in vivo* injection of INF τ. J Immunol. 1995;154:4261–8.
[3] Choi BC, Polgar K, Xiao L, Hill JA. Progesteron inhibits in vitro embryotoxic Thl cytokine production to trophoblast in women with recurent pregnancy loss. Hum Reprod. 2000;15(Suppl 1):46–59.
[4] Coulam C. Understanding the immunobiology of pregnancy and applying it to treatment of recurrent pregnancy loss. Biology and Medicine. 2000;4:19–29.
[5] Dosiou C, Giudice L. Natural killer cells in pregnancy and recurrent pregnancy loss: endocrine and immunologic perspectives. Endocr Rev. 2005;26:44–62.
[6] Fatušic Z. Imunološki aspekti spontanog i habitualnog pobacaja. Med Arh. 2006;60:129–31.
[7] Faust Z, Laškarin G, Rukavina D, Szekeres-Bartho J. Progesterone induced blocking factor inhibits degranulation of NK cells. Am J Reprod Immunol. 1999;42:71–5.
[8] Guller S, Wozniak R, Kong L, Lockwood C. Opposing actions of transforming growth factor beta and glucocorticoids in the regulation of fibronectin expression in the human placenta. J Clin Endocr Metab. 1995;80:3273–8.
[9] Haddad E, Duclos A, Antecka E, Lapp W, Baines M. Role of interferon-gamma in the priming of decidual macrophages for nitric oxide production and early pregnancy loss. Cell Immnunol. 1997;181:68–75.
[10] Haddad E, Duclos A, Lapp W, Baines M. Early embryo loss is associated with the prior expression of macrophage activation markers in the decidua. J Immunol. 1997;158: 4886–92.
[11] Hill JA, Anderson D, Polgar K. T helper I-type cellular immunity to trophoblast in women with recurrent abortions. J Am Med Assoc. 1995;273:1933–8.
[12] Kalinka J, Szekeres-Bartho J. The impact of dydrogesterone supplementation on hormonal profile and progest-

erone-induced blocking factor concentrations in women with threatened abortion. Am J Reprod Immunol. 2005; 53:166–71.
[13] Kavalier F. Investigation of recurrent miscarriages. Br Med J. 2005;331:121–2.
[14] Kwak-Kim J, Chung-Bang H, Ntrivalas E, Mangubat C, Beaman K, Beer A, et al. Increased T helper 1 cytokine responses by circulating T cells are present in women with RPL and in infertile women with multiple implantation failures afted IVF. Hum Reprod. 2003;18:767–73.
[15] Letterio J, Roberts A. TGF beta: a critical modulator of immune cell function. Clin Immunol Pathol. 1997;84: 244–50.
[16] Palfi M, Jablonowska B, Matthiesen L, Ernerudh J. Circulating interferon-gamma and interleukin-4-secreting cells in recurrent spontaneous abortions. Am J Reprod Immunol. 1999;41:257–63.
[17] Parant M. Possible mediators of endotoxin-induced abortions. Res Immunol. 1990;141:164–8.
[18] Piccinni MP. Role of T cell in pregnancy. In: Rukavina D, Chaouat G, editors. Embryo implantation: from basics to clinics. Rijeka. University of Rijeka. p. 113–7.
[19] Polgar B, Nagy E, Miko E, Varga P, Szekeres-Bartho J. Urinary progesterone-induced blocking factor concentration is related to pregnancy outcome. Biol Reprod. 2004; 71:1699–705.
[20] Raghupathy R, Kalinka J. Cytokine imbalance in pregnancy complications and its modulation. Front Biosci. 2008; 1:985–94.
[21] Sanchez-Ramos L, Kaunitz AM, Delke I. Progestational agents to prevent preterm birth: a meta-analysis of randomised controlled trials. Obstet Gynecol. 2005;105: 273–9.
[22] Sedlmayr P. Concepts of feto-maternal tolerance. In: Rukavina D, Chaouat G, editors. Embryo implantation: from basics to clinics. Rijeka: University of Rijeka; 2006. p. 95–102.
[23] Sfakianki A, Norwitz M. Mechanisms of progesterone action in inhibiting prematurity. J Matern Fetal Neonatal Med. 2006;19:763–72.
[24] Song Y, Keelan J, France J. Activin-A stimulates, while transforming growth factor beta 1 inhibits chorionic gonadotropin production and aromatase activity in cultured human placental trophoblasts. Placenta. 1996;17: 603–10.
[25] Stites DP, Bugbee S, Siiteri PK. Differential actions of progesterone and cortisol on lymphocyte and monocyte interaction during lymphocyte activation – relevance to immunosuppression in pregnancy. J Reprod Immunol. 1983;5:215–28.
[26] Szekeres-Bartho J, Kilar F, Falkay G, Csernus V, Totok A, Pacsa AS. The mechanism of the inhibitory effect of progesterone on lymphocyte cytotoxiciti: 1. Progesterone-treated lymphocytes release a substance inhibiting cytotoxicity and prostaglandin synthesis. Am J Reprod Immunol Microbiol. 1985;9:15–9.
[27] Szekeres-Bartho J, Kozma N, Halasz M, Polgar B, Milko E, Palkovics T, et al. Immuno-endocrine signals. In: Rukavina D, Chaouat G, editors. Embryo implantation: from basics to clinics. Rijeka. University of Rijeka; 2006. p. 105–7.
[28] Szekeres-Bartho J, Autran B, Debre P, Andreu G, Denver L, Chaouat G. Immunoregulatory effects of a suppressor factor from healthy pregnant women's lymphocytes after progesterone induction. Cell Immunol. 1989;122:281–94.
[29] Szekeres-Bartho J, Faust ZS, Varga P, Szereday L, Kelemen K. The immunological pregnancy protective effects of progesterone is manifested via controlling cytokine production. Am J Reprod Immunol. 1996;35:348–51.
[30] Szekeres-Bartho J, Par G, Dombay Gy, Smart YC, Volgyi Z. The anti-abortive effimice is manifested by modulating NK activity. Cell Immunol. 1997;177:194–9.
[31] Szekeres-Bartho J, Par G, Szereday L, Smart CY, Achacz I. Progesterone and non-specific immunological mechanisms in pregnancy. Am J Reprod Immunol. 1997;38: 176–82.
[32] Szekeres-Bartho J, Szekeres GY, Debre P, Autran B, Chaouat G. Reactivity of lymphocytes to a progesterone receptor-specific monoclonal antibody. Cell Immunol. 1990;125:273–83.
[33] Szekeres-Bartho J, Wegmann TG. A progesterone-dependent immunomodulatory protein alters the Th1/Th2 balance. J Reprod Immunol. 1996;31:81–95.
[34] Szekeres-Bartho J, Wilczynski JR, Basta P, Kalinka J. Role of progesterone and progestin therapy in threatened abortion and preterm labour. Front Biosci. 2008;13:1981–90.
[35] Szereday L, Varga P, Szekeres-Bartho J. Cytokine production by lymphocytes in pregnancy. Am J Reprod Immunol. 1997;38:418–22.
[36] Wegmann TG, Lin H, Guilbert L, Mossmann TR. Bidirectional cytokine interactions in the maternalfetal relationship: is successful pregnancy a Th2 phenomenon? Immunol Today. 1993;1014:353–6.
[37] Wolff M, Thaler C, Strowitzki T, Broome J, Stolz W, Tabibzedeh S. Regulated exspression of cytokines in human endometrium throughout the menstrual cycle: dysregulation in habitual abortion. Mol Hum Reprod. 2000;6:627–34.

The authors stated that there are no conflicts of interest regarding the publication of this article.

Received September 19, 2008. Revised November 14, 2008. Accepted November 24, 2008. Previously published online March 17, 2009.