

Expression of an Immunomodulatory Protein Known as Progesterone Induced Blocking Factor (PIBF) Does Not Correlate With First Trimester Spontaneous Abortions in Progesterone Supplemented Women

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PROBLEM: An immunomodulatory protein known as the progesterone induced blocking factor (PIBF) has been found to positively correlate with early pregnancy beta human chorionic gonadotropin (B-hCG) levels. The study presented herein evaluated PIBF levels from conception to the end of the first trimester to determine if lower levels will correlate with first trimester spontaneous abortions (SAB).

METHOD: Progesterone induced blocking factor expression by lymphocytes measured using an immunocytochemistry method was compared in pregnant women with ongoing vs. failed pregnancies.

RESULTS: There were no differences in the proportion of women having lymphocytes expressing PIBF or in the median numbers when comparing ongoing vs. failed pregnancies. There was no B-hCG interval where failed pregnancies were found to have lower frequency of PIBF expressing lymphocytes.

CONCLUSION: Inadequate PIBF expression independent of low P levels does not appear to be an etiologic factor for first trimester SABs; thus measuring this protein in pregnant women lacks practical usefulness.

Key words:

Natural killer cells, progesterone receptors, T-lymphocytes

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INTRODUCTION

It is well known that the continued production of progesterone (P) is essential for the maintenance of the early pregnancy as evidenced by evaluating the effect of lutectomy on subsequent pregnancy outcome.¹ This role of P in maintaining pregnancy was further supported by studies with oocyte donation where spontaneous abortion (SAB) occurred if there was no P supplementation.² One of the mechanisms by which P allows continuation of pregnancy may be by influencing the secretion of certain immunomodulatory proteins.

Data has been presented showing that some factor may be secreted by lymphocytes of pregnant women that is able to suppress natural lymphocyte cytotoxicity.³

This factor is found in the supernatants of the lymphocytes, and by using the protein synthesis inhibitor cycloheximide inhibited the release of the suppressor factor.³ By a series of partial purification procedures using Amicon filters, a 34 kDa protein was identified and labelled the progesterone induced blocking factor (PIBF).³

The cell responsible for PIBF secretion is the CD8+ T-lymphocyte.⁴ The supernatant of P-treated lymphocytes, which contains PIBF, has been found to strongly block natural killer (NK) cell mediated lysis of k562 cells.⁴ A comparable level of inhibition of cell lysis was achieved by the supernatant of CD4+ depleted P-treated pregnancy lymphocytes.⁴ However, no significant inhibition could be observed with the supernatant of P-treated pregnancy lymphocytes depleted of CD8+ cells.⁴ Furthermore, the supernatants of P-treated lymphocytes from healthy pregnancy lymphocytes produced in the presence of the anti-progesterone receptor blocker RU486 exerted no detectable effect on lymphocyte cytotoxicity.⁴ Thus, the CD8+ lymphocytes not only produce PIBF, but progesterone-progesterone receptor interaction is required for its secretion. Further evidence that the P receptor was needed rather than the glucocorticoid receptor (since RU486 can also bind to glucocorticoid receptors) was provided in a study where the supernatants were produced in the presence of the glucocorticoid receptor blocker RU43044, and there was no subsequent reduction in blocking factor effect.⁵

P receptors have not been demonstrated in normal T-lymphocytes. However, data were presented where P binding sites in lymphocytes from pregnant women were found.⁶ These binding sites appeared to be at a lower density than other P-receptor containing tissues.⁷ The presence of nuclear reactivity in lymphocytes from pregnant but not non-pregnant women was demonstrated after staining with a panel of P receptor specific monoclonal antibodies.⁷ Chiu et al., confirmed the existence of P receptors in lymphocytes from pregnant women using indirect immunofluorescence flow cytometry.⁸

Allogenic stimulation of maternal lymphocytes by paternal-type antigens to produce P receptors is a real possibility during pregnancy.⁹⁻¹² However, there are data suggesting that any allogeneic stimulus, not just paternal antigens, can stimulate the induction of P receptors in maternal T-lymphocytes. In fact, P receptor induction on lymphocytes was also found in patients who had liver transplants or blood transfusions.¹³

During pregnancy, the maternal immune response is modulated; there is a decrease in cellular immune response and an increase in humoral immunity.¹⁴ The change in immune parameters may be mediated by the secretion of cytokines by T-helper cells (TH cells).¹⁵ In normal pregnancies, there is a shift in the decidua from the production of TH1 to TH2 cytokines leading to a decrease in cell mediated responses and an increase in immune globulin synthesis.¹⁶ Progesterone induced blocking factor may exert immuno-

modulation through its effects on cytokines, especially influencing a shift to TH2 cytokines, which inhibit NK cell activity. Progesterone induced blocking factor has been found to increase IL-10 (TH2 cytokine) production by murine spleen cells and to decrease IL-12 (TH1 cytokine) production.¹⁷

There are data demonstrating that PIBF expression of maternal T-lymphocytes is more likely to be present in the luteal phase in those who eventually have positive pregnancy tests.¹⁸ Furthermore, it was found that replacing embryos in the uterine cavity did not result in higher PIBF expression by lymphocytes than non-in vitro fertilization (IVF) cases when the pregnancy test was negative suggesting that implantation rather than mere fertilization may be the main factor correlated with PIBF expression.¹⁹

The study presented herein was designed to determine if PIBF expression in patients establishing a positive test for beta-human chorionic gonadotropin (B-hCG) will correlate with pregnancy outcome. If a correlation could be found, then the data would be analyzed as to whether low levels of PIBF in early pregnancy are predictive of later subsequent SABs or whether the level drops shortly before the pregnancy loss.

MATERIALS AND METHODS

Patient Selection

The patients included in the study all had initially presented with infertility or recurrent SAB during the time interval of 1/95 to 8/96. Patients having B-hCG levels also had blood drawn to measure PIBF expression by peripheral blood lymphocytes.

Pregnancies were considered as viable-ongoing if there was viability seen on ultrasound at the end of the first trimester. Failed pregnancies included chemical pregnancies (i.e., rising B-hCG levels without ultrasound evidence of a gestational sac), ectopic pregnancies, non-viable pregnancies despite a gestational sac, and SAB after establishing viability earlier in the first trimester.

The percentage of women demonstrating >1% of peripheral blood lymphocytes expressing PIBF was analyzed according to various ranges of B-hCG levels. Chi-square analysis was used to test the association of PIBF expression and pregnancy outcome. The *P* value of 0.05 was considered significant.

Method of Assay

The measurement of PIBF expression was determined by an immunocytochemistry method using a PIBF-specific polyclonal antibody. Mononuclear cells were removed using Isoprep (Robbins Scientific, Sunnyvale, CA) and cold centrifugation and were adjusted to a concentration of 2×10^6 /mL; 100 μ l aliquots of cell suspension were added to sample chambers and air dried then fixed in cold acetone. The cells were first incubated with protein block-

ing agent and then incubated overnight with anti-PIBF. The cells were washed in PBS (Gibco, Grand Islands, NY) and then covered with anti-rabbit peroxidase. Following a second PBS wash, fresh chromogen solution was added and the cells incubated; the reaction was then stopped with distilled water and the cells counterstained with hematoxylin and the slides were read under oil immersion (100× objective). A positive reaction was indicated by a reddish precipitate at sites of specific cellular antigen localization; 300 cells were counted. The percent of the cells positive was then determined. A test was considered positive if there were at least four lymphocytes of the 300 counted demonstrating the reddish precipitate. This cut-off level of 1% was chosen based on previous unpublished data using this immunocytochemistry technique in which the large majority of non-pregnant women showed <1% of the lymphocytes, demonstrating a positive reaction.

RESULTS

Progesterone induced blocking factor expression was observed in 78.4% of the 292 women with ongoing pregnancies compared to 79.1% of 91 women with failed pregnancies in the early first trimester as seen in Table I ($P=NS$).

Within each B-hCG level range, there were no differences found in the proportion of women having lymphocytes expressing PIBF except for the range of 500–1,000 mIU/mL; in this range there were more failed pregnancies with PIBF expression than ongoing pregnancies (76.2% vs. 48%, $P<.05$, chi-square) as seen in Table I.

The range of percentage of lymphocytes expressing PIBF in individual patients according to B-hCG range is seen in Table I. There were no differences in the median levels by conception outcome.

When PIBF expression was compared to type of failed pregnancy, no difference was found in the proportion of women demonstrating PIBF expression or the median PIBF levels measured (Table II).

DISCUSSION

One mechanism to explain the survival of the fetal semi-allograft is the following hypothesis: paternal antigens of the invading embryo stimulate the induction of P receptors on some maternal T-lymphocytes. The corpus luteum secretes P. The interaction of P with the receptor on the maternal T-lymphocyte leads to the secretion of PIBF. This immunomodulatory protein in turn inhibits NK cell cytolytic action and causes a shift from embryo-toxic TH1 cytokines to embryo protective TH2 cytokines and helps shift the balance from cellular immunity to humoral immunity. This allows the embryo to continue invasion into the decidua and helps inhibit subsequent immunological rejection. This hypothesis is based especially on previous experimentation by Dr. Szekeres-Bartho and her collaborators.²⁰

A theoretical problem with this hypothesis is that it does not allow a mechanism of specific immunological tolerance to the fetal semi-allograft but might suggest that the increased circulating levels of P during pregnancy will inhibit NK cell activity throughout the body. If this were true, NK cell paralysis would expose the pregnant woman to a significantly greater risk of infection and tumor formation, which is known not to be present to any great degree. Thus one additional corollary to the hypothesis, not as yet supported by experimental data, would be that the P receptor on the T-lymphocyte are relatively insensitive to P and that only very high levels, e.g., the levels generated at the maternal-fetal interface, are sufficient to cause PIBF secretion. This mechanism would thus allow PIBF to inhibit only decidual NK cells while sparing the rest in the body.

Szekeres-Bartho et al., by using an enzyme linked immunosorbent assay, measured PIBF levels in normal pregnancies, at the termination of pregnancy (at onset of labor, at time of SAB, and at time of preterm deliveries) and in the 16th week of women who subsequently spontaneously aborted.²¹ All women at pregnancy termination had sera PIBF levels lower than those of healthy pregnant women.²¹ They also found that using a cut-off value of 197.5 $\mu\text{g}/\text{mL}$ for PIBF, that 52 of 87 women who would eventually abort either immediately or up to 12 weeks later had low PIBF levels.²¹

By using a similar immunocytochemistry method as the one presented in this manuscript, Szekeres-Bartho et al. compared PIBF expression in pregnant women between the 9th and 40th week of gestation (only seven were tested in the first trimester).²² They found that the percentage of PIBF-expressing lymphocytes in the peripheral blood of 96 healthy pregnant women was $67\pm 2.99\%$ vs. only $6.5\pm 1\%$ in 62 women with pathological pregnancies.

The data from Szekeres-Bartho et al. found that over 90% of pathological pregnancies had PIBF levels below the established cut-off of normal for these later pregnancies (13%).²² Interestingly, 90% of these same blood samples showed NK cell activity >40%, a level considered to be high.²²

The data from Szekeres-Bartho et al. evaluated mostly pregnancies beyond the first trimester.²² There were no ultrasound data or P data presented so it was not clear if the low levels were performed on non-viable pregnancies but where active expulsion of fetal products had not occurred. The possibility also existed that low serum P levels that may have preceded the spontaneous early pregnancy termination led to a decrease in PIBF levels. If these were the circumstances leading to the drop in PIBF, from a practical standpoint it would be better to merely measure the serum P or B-hCG level or perform an ultrasound.

The study presented herein differed from that of Szekeres-Bartho et al., in that all pregnancies evaluated were first trimester and the levels were measured soon after conception. Furthermore, since the correlation of P and

TABLE I. Comparison of PIBF Expression by Pregnancy Outcome

| Status | PIBF >1% at least once in early pregnancy | Beta-hCG (mIU/mL) | | | | | |
|----------------|---|-------------------|------------|--------------|--------------|--------------|---------|
| | | <100 | 100-500 | 501-1000* | 1001-5000 | 5001-10,000 | >10,000 |
| Ongoing: n | 292 | 67 | 131 | 75 | 161 | 105 | 184 |
| Range (median) | 0-99.3 (1.3) | 0-98.4 (1.7) | 0-94 (1.0) | 0-98 (1.9) | 0-68.3 (2.0) | 0-100 (3.5) | |
| % positive | 78.40% | 50.80% | 55.70% | 48% | 57.80% | 61.90% | 65.80% |
| Failed: n | 91 | 50 | 38 | 21 | 39 | 16 | 21 |
| Range (median) | 0-68.3 (2.1) | 0-28.3 (2.3) | 0-17 (2.0) | 0-41.4 (3.0) | 0-28.3 (3.6) | 0-30.7 (3.3) | |
| % positive | 79.10% | 60.00% | 55.30% | 76.20% | 64.10% | 68.70% | 71.40% |

*Chi-square, P < 0.05.

TABLE II. Comparison of PIBF Levels by Pregnancy Outcome According to Type of Pregnancy

| Status | PIBF >1% at least once in early pregnancy | Beta-hCG (mIU/mL) | | | | | |
|------------------------|---|-------------------|--------------|--------------|---------------|----------------|---------|
| | | <100 | 100-500 | 501-1000 | 1001-5000 | 5001-10,000 | >10,000 |
| Chemical: n | 28 | 24 | 3 | 1 | | | |
| Range (median) | 0-38.3 (2.5) | 1.3-9.3 (7.1) | | | | | |
| % positive | 71.40% | 66.70% | 100.00% | | | | |
| Ectopic: n | 9 | 2 | 4 | 3 | 7 | 3 | 1 |
| Range (median) | .3-3.9 (2.1) | 0-2.7 (.5) | 0-14.9 (0) | 0-16.3 (2) | 2.3-3.3 (2.7) | | |
| % positive | 88.90% | 50.00% | 25.00% | 33.30% | 57.10% | 100.00% | |
| SAB after viability: n | 16 | 9 | 10 | 5 | 12 | 6 | 10 |
| Range (median) | 0-19.7 (2.6) | 0-28.3 (2.3) | 0-3.6 (.7) | 0-41.4 (5.1) | 0-28.3 (2.7) | 0-22 (2.6) | |
| % positive | 93.70% | 55.60% | 60.00% | 40.00% | 83.30% | 50.00% | 60.00% |
| Non-viable: n | 38 | 15 | 21 | 12 | 20 | 7 | 10 |
| Range (median) | 0-15.7 (2.0) | 0-10.3 (2.3) | 1.3-17 (2.3) | 0-32 (2) | 0-8.1 (4) | 0-7-30.7 (3.8) | |
| % positive | 76.30% | 53.30% | 52.40% | 100.00% | 55.00% | 71.40% | 90.00% |

PIBF was previously found, the patients were given P supplementation so that the serum P levels were maintained around 40 ng/mL so that low levels of PIBF could not be attributed to the lack of P. The percentage of lymphocytes expressing PIBF and especially the median levels, even in normal pregnancies, is much lower during the first trimester when compared to the levels reported by Szekeres-Bartho et al. during the second and third trimesters.

Progesterone induced blocking factor may be one of the main effectors of P action throughout pregnancy and may provide a mechanism by which the fetus escapes immune surveillance. However, based on the data presented herein, there does not appear to be any practical benefit in measuring this immunomodulatory protein once a woman is already pregnant. Inadequate PIBF expression by lymphocytes independent of low P levels does not appear to be an etiologic factor for first trimester SAB. Since previously, a correlation was found between PIBF and positive B-hCG levels,¹⁸ measurement of lymphocyte expression of this protein may prove more useful in evaluating cases of unexplained infertility. Repeated failure to conceive and produce PIBF may lead to the earlier trial of IVF; persistent failure to generate lymphocyte expression of PIBF despite embryo replacement would suggest an implantation abnormality, whereas the expression of PIBF may suggest that the use of assisted reproductive technology may have overcome a fertilization problem or tube-ovum pick-up abnormality.

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