

Progesterone Induced Blocking Factor Seen in Pregnancy Lymphocytes Soon After Implantation

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PROBLEM: The immunomodulatory effect of progesterone (P) in pregnancy manifested via a protein named the P-induced blocking factor (PIBF) was previously reported. The goal of this study was to measure and compare the PIBF expression on lymphocytes between pregnant and non-pregnant women especially in early pregnancy.

METHODS: PIBF expression was determined by immunocytochemistry using a PIBF-specific polyclonal antibody. Levels were assessed during the mid-cycle, luteal phase, and first trimester of pregnancy.

RESULTS: PIBF expression was found in 24.9% of mid-cycle sera, 49% of luteal phase sera of women who failed to conceive, and 75% of luteal phase sera of women who conceived.

CONCLUSIONS: These data indicate that the percentage of PIBF expressing lymphocytes increases as a result of pregnancy and that the stimulus for PIBF induction occurs soon after implantation. These data support the concept that PIBF may play an important role in early implantation possibly by inhibiting the destructive function of natural killer lymphocytes.

Key words:

Immune surveillance, implantation, natural killer cells

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INTRODUCTION

There are data suggesting a depression of cell-mediated immunity during pregnancy.¹⁻³ This suppression of cellular immunity can be partially explained by the action of immunosuppressive factors found in the sera of pregnant women.⁴⁻⁹ There are data suggesting that specific elimination of progesterone (P) resulted in a decreased blocking activity of pregnancy serum.¹⁰ A 34 kDa protein has been isolated that may be at least partially responsible for the blocking effect of pregnancy serum and has been named progesterone-induced blocking factor (PIBF).³

An enzyme-linked immunosorbent assay (ELISA) was designed to detect PIBF in the serum.¹¹ The serum PIBF concentration was found to be similar from weeks 6-40 but dropped in women who aborted.¹¹

The study presented herein was designed to determine if PIBF expression on peripheral lymphocytes occurs soon after conception.

MATERIALS AND METHODS

The measurement of PIBF expression was determined by immunocytochemistry 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 blocking agent and then incubated overnight with anti-progesterone induced blocking factor (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. The slides were coverslipped and read under oil immersion (100 \times 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.

Sera samples were drawn at mid-cycle, in the luteal phase, and during the first trimester of pregnancy. The mean levels of PIBF expressing lymphocytes as well as the proportion of patients demonstrating presence of PIBF (levels >0) were compared at the following times: 1) mid-cycle; 2) luteal phase in non-pregnant patients; 3) luteal phase of pregnant patients; 4) first trimester when serum beta-human chorionic gonadotropin (hCG) ranged from 25 to 99 mIU/mL; 5) first trimester when beta-hCG ranged from 100 to 999 mIU/mL; 6) first trimester when beta-hCG ranged from 1,000 to 9,999 mIU/mL; 7) first trimester beta-hCG >10,000 mIU/mL. Additionally, PIBF expression was compared between women with ongoing pregnancy and those suffering spontaneous abortions (SAB).

Chi-square analysis and analysis of variance were used as indicated. A *P* value of .05 was used.

RESULTS

PIBF expression (>0%) was demonstrated in the lymphocytes in only 23.9% of patients' blood drawn at mid-cycle as compared to 49% in luteal phase of non-pregnant patients and 75% in luteal phase of pregnant patients (*P*<.01,

chi-square). The mean \pm SE of PIBF expressing lymphocytes was $1.0 \pm .4\%$ at mid cycle, $6.7 \pm 2.5\%$ in luteal phase of non-pregnant patients, and $4.8 \pm 2.1\%$ in luteal phase of pregnant patients (Table I).

During the first trimester, PIBF expression was demonstrated in 65.2% of group 4, 48.7% of group 5, 62.2% of group 6, and 74.7% of group 7. The mean \pm SE of PIBF expression at these times were $17.4 \pm 6.8\%$, $4.3 \pm 1.7\%$, $8.6 \pm 2.6\%$, and $16.8 \pm 3.2\%$, respectively (Table II).

There were only six patients who had an SAB, so no statistical inference about the data comparing ongoing pregnancies to SABs was possible at this time. Comparison of descriptive statistics for PIBF expression at the four beta-hCG ranges demonstrated that for the ongoing pregnancies the mean PIBF expressions were 27%, 3.6%, 8.8%, and 27.2%, respectively, whereas, the aborters had mean PIBF expressions of .4%, 1.8%, and 2.5% in the first three beta-hCG groups; none of the aborters reached a beta-hCG level of 10,000 mIU/mL. The maximum PIBF levels reached in the first trimester of ongoing pregnancies at the four beta-hCG levels were 99.3%, 8.5%, 66.7% and 100% as compared to .7%, 3.6% and 4.3% for aborters.

DISCUSSION

There have been previous data showing that lymphocytes of healthy pregnant women possess progesterone receptors while those with threatened SAB or premature pregnancy did not have them.^{12,13} Binding of P to these receptors initiated a series of events, resulting in the release of an immunomodulatory protein.^{14,15} In fact, in contrast to normal non-pregnancy lymphocytes, lymphocytes activated by in vitro mitogenic or alloantigenic stimulation resulted in the development of progesterone receptors in normal resting human lymphocytes¹² which produced PIBF in response to P.¹⁴ Since the serum concentration of the PIBF correlated with natural killer (NK) blocking effect of the respective sera, it seemed likely that the protein blocked NK activity in vivo and that the block of NK activity plays a role in the maintenance of pregnancy.

TABLE I. Mid-Cycle and Luteal Phase PIBF Levels (%)

	Group 1—Mid cycle group (n = 117)	Group 2—Luteal phase not pregnant (n = 60)	Group 3—Luteal phase pregnant (n = 8)
PIBF ^a			
absent (0)	89 (76.1%)	31 (51.2%)	2 (25.0%)
present (>0)	28 (23.9%)	29 (48.8%)	6 (75.0%)
Mean \pm SE ^b	$1.0 \pm .4$	6.7 ± 2.5	4.8 ± 2.1
Median ^c	0	0	2.8

^a*P*<.05, chi-square (mid cycle differs from both luteal phase groups).

^b*P*<.05, ANOVA (mid cycle differs from both luteal phase groups).

^c*P*<.05 Kruskal-Wallis (mid cycle differs from both luteal phase groups).

TABLE II. Comparison of PIBF by Stage of Pregnancy

	Group 4 β-hCG (25-99 mIU/mL) (n=23)	Group 5 β-hCG (100- 999 mIU/mL) (n=37)	Group 6 β-hCG (1,000- 9,999 mIU/mL) (n=45)	Group 7 β-hCG (≥10,000 mIU/mL) (n=95)
PIBF ^a				
absent (0)	8 (34.8%)	19 (51.3%)	17 (37.8%)	24 (25.3%)
present (>0)	15 (65.2%)	18 (48.7%)	28 (62.2%)	71 (74.7%)
Mean ± SE ^b	17.4 ± 6.8	4.3 ± 1.7	8.6 ± 2.6	16.8 ± 3.2
Median ^c	2.3	0	1.3	2.7

^aP<.05, chi-square (group 5 differs from others).

^bP<.05, ANOVA (groups 5 differs from others).

^cP<.05, Kruskal-Wallis (groups 5 differs from others).

At first this hypothesis may seem to dispute data suggesting that CD56+ lymphocytes present within the decidua at the site of implantation are associated with successful pregnancy outcome.¹⁶⁻¹⁸ This protection may be mediated by the cytokine transforming growth factor beta-2.¹⁹ These lymphocytes are CD56+ and are large granular lymphocytes similar to NK cells but differ in that they are CD16 negative. Spontaneous abortion may be associated with a decrease in CD56+ CD16- lymphocytes in the placental bed but an increase in CD56+ CD16+ NK cells.¹⁹ In fact there are data suggesting that finding an increased percentage (>12%) of CD56+ cells in the circulation may be associated with an increase in spontaneous abortion.²⁰ We would thus hypothesize that PIBF does not affect CD56+ CD16- lymphocytes but only the CD56+ CD16+ ones.

If the trend for higher spontaneous abortion rates with lower PIBF levels continues, it would be interesting to see if failure to induce PIBF may be associated with an increase in circulating NK cells of the CD56+ CD16+ type and in the placental bed. If this is found the possibility would exist that not only does PIBF inhibit NK cell activity, but it may prevent placental invasion and decrease the percentage of circulatory NK lymphocytes in addition to blocking the cytotoxic effect of NK cells:

The data presented herein suggests that PIBF is produced very early in pregnancy and may thus help with early implantation and early escape from maternal immune surveillance. Previously, the concentration of PIBF was found to be higher in the sera of healthy pregnant women than in those of non-pregnant individuals or pregnant women (at least 6 weeks) with symptoms of threatened abortion.¹¹ The present data support this previous study but demonstrate that the lower production of PIBF in the pregnancies with poor outcome may begin early after conception. Future studies will evaluate in a larger series whether spontaneous abortion correlates with failure to generate PIBF production. Furthermore, these studies will evaluate whether failure to generate PIBF is related to the percentage of circulating CD56+ lymphocytes or

major histocompatibility antigen sharing between husband and wife.

When lymphocyte immunotherapy results in a decrease in NK cell activity, an improved pregnancy outcome has been found.²¹ We are also planning to evaluate whether lymphocyte immunotherapy and even intravenous immunoglobulin (IVIg) may cause an increase in PIBF and possibly be the mechanism for reduction in NK cell activity following these treatments. If this correlation is indeed found, then failure to generate PIBF by husband's lymphocytes may indicate to the treating physician the use of lymphocytes from another source or the use of IVIg.

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