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Lymphocyte Immunotherapy (LI) Increases Serum Levels of Progesterone Induced Blocking Factor (PIBF)

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PROBLEM: To determine if allogenic stimulation from leukocyte immunization (LI) can increase the production of an immunomodulatory protein called progesterone induced blocking factor (PIBF) by CD8+ T-lymphocytes.

METHOD: The study group consisted of 35 women, 29 who failed to conceive after repeated embryo transfers (ETs) and six with recurrent spontaneous abortion (RSA). The women underwent LI using the male partner's blood as the source of leukocytes. Progesterone induced blocking factor was measured pre- and post-LI with an immunocytochemistry method using a PIBF-specific polyclonal antibody.

RESULTS: The mean percentage of lymphocytes expressing PIBF, as well as the percentage of cases whose PIBF level increased to 1% or more, was significantly higher post-LI. Similarly post-LI, there was a significantly lower percentage of zero PIBF levels.

CONCLUSIONS: Leukocyte immunization causes an increase in PIBF in many cases. Possibly the improved pregnancy outcome in immunized patients with RSA or previous failure to conceive with in vitro fertilization may be partially or possibly completely explained by its stimulatory effect on PIBF.

Key words:
Immunomodulatory protein, IVF, leukocyte immunization, NK cells, progesterone receptor, spontaneous abortion

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Leukocyte immunization of women with male partner's lymphocytes resulted in increased lymphocyte expression of an immunomodulatory protein that increases humoral and decreases cellular immunity.

INTRODUCTION

Progesterone (P) is a critical hormone for maintenance of a normal pregnancy.[1] One of the functions of P is to act as an immunosuppressant.[2] There are some controversial data suggesting that pregnancy lymphocytes have nuclear P receptors while non-pregnancy lymphocytes do not.[3] There is evidence that lymphocytes of pregnant women develop specific P receptors after exposure to an allogeneic source.[4–6]

The immunomodulatory effects of P may be through the stimulation of a 34 kD protein known as progesterone induced blocking factor (PIBF), which results from the interaction of a high concentration of P with P receptors on certain CD8+ lymphocytes.[4,7,8] Progesterone induced blocking factor inhibits natural killer (NK) activity.[8] Natural killer activity seems to play a role in spontaneous pregnancy termination; a decrease in CD56+ CD16- NK cells and an increase in CD56+ CD16+ NK cells is frequently found.[9]

During pregnancy, the maternal immune response is modulated: There is a decrease in cellular immune response and an increase in hormonal immunity.[10] The change in immune parameters may be mediated by the secretion of cytokines by T-helper (TH cells) cells.[11] In normal pregnancies, there is a shift in the decidua from the production of TH1 to the production of TH2 cytokines, leading to a decrease in cell-mediated responses and an increase in immunoglobulin synthesis.[12] Progesterone induced blocking factor may exert immunomodulation through its effect on cytokines, especially influencing a shift to TH2 cytokines which inhibit NK activity.[13] Also, PIBF has been found to increase IL-10 (TH2 cytokine) production by murine spleen cells and to decrease IL-12 (TH1 cytokine) production.[13] Progesterone induced blocking factor expression on lymphocytes is inversely related to serum concentration of TH2 cytokines.[13]

Allogeneic stimuli other than paternal antigens (e.g., blood transfusions or liver transplants) have been shown to increase the expression of P receptors in lymphocytes.[14] To our knowledge, these data have never been corroborated.

If the theory of induction of P receptors on CD8+ T-lymphocytes by allogeneic stimuli (e.g., blood transfusion) is correct, then LI may be expected to do the same. Induction of PIBF expression on lymphocytes should then hypothetically occur following exposure to P.

The study presented herein evaluated PIBF levels before and after LI. The rationale for performing this study was based on preliminary data (but not statistically significant) showing a delivery rate twice as high (33.3% vs. 16.7%) in couples who had failed to conceive despite at least two previous IVF-ET cycles where the female partner was given paternal leukocyte immunization.[15]

MATERIALS AND METHODS

Study Group

Thirty-five women received LI using male partners' blood as the source of lymphocytes. Included were 29 women who had failed to conceive after at least two attempts of embryo transfers (ET) (fresh or frozen) and six women with recurrent SAB (at least three); one woman was used twice in the study after two LI's.

To prepare the lymphocytes for immunization, 400 milliliters of whole blood was obtained from the male partner and the plasma and erythrocytes were removed. The remainder was resuspended with an equal volume by weight of sterile saline. The cells were then layered over sodium metrizoate/Ficoll and centrifuged. The mononuclear cell layer was then aspirated and washed three to five times with sterile saline with a final slow spin to remove platelets and then resuspended with 5 mL of sterile saline. The lymphocyte suspension was then aspirated into five syringes (Nos. 1–4 with 0.5 mL and No. 5 with 3.0 mL). The patients then received two intramuscular and two subcutaneous injections with the .5 mL suspensions and the 3 mL suspension was given by intravenous saline drip.

Progesterone induced blocking factor on lymphocytes was determined by an immunocytochemistry method using a PIBF-specific polyclonal antibody.[16] Mononuclear cells were removed using Isoprep (Robbins Scientific, Sunnyvale, CA) and cold centrifugation and were adjusted to a concentration of 2 x $10.^{6}$/mL; 100 μl aliquots of cell suspension were added to sample chambers, air dried and then fixed in cold ethanol. The cells were incubated first with protein blocking agent and then incubated overnight with anti-progesterone-inducing blocking factor (anti-PIBF). The cells were then washed in PBS (Shandon-Lipshaw, Pittsburgh, PA) and covered with anti-rabbit peroxidase. Following a second PBS wash, fresh chromogen solution was added and the cells incubated; the reaction was stopped with distilled water and the cells counterstained with hematoxylin. The slides were then coverslipped and read under oil-immersion (100 x objective). A positive reaction was indicated by a reddish precipitate at sites of specific cellular antigen localization. Three hundred cells were counted and the percent of positive cells was determined. A PIBF level >1% was considered positive.

Progesterone induced blocking factor levels were measured for each woman one to three weeks before the LI during the mid-luteal phase and were also obtained one to four weeks post-LI during the mid-luteal phase. The mean levels pre- and post-LI were compared using paired T-test. The percentage of positive levels (>1%) pre- and post-LI were compared using McNemar's test for matched proportions. The 1% cut-off was based on the negative controls used in the laboratory. Further attempts at clinical correlation may indeed find a level that better defines a negative vs. a positive response. A P value of .05 was used.

RESULTS

The mean ± SD of lymphocytes expressing PIBF was 2.9±7.1% pre-LI versus 8.7 ± 14% post-LI ($P < .05$, paired T-test). The percentage of patients with PIBF expression >1% was 33.3% (12 of 36) pre-LI and 58% (21 of 36) post-LI ($P < .05$, McNemar test). The percentage of patients with zero levels of PIBF was 56% (20 of 36) pre-LI versus 22% (8 of 36) post-LI ($P < .05$, McNemar test).

The five largest increments of PIBF from pre- to post-LI were 0 to 50%, 0.5 to 48.6%, 0 to 38.3%, 0 to 20.3%, IVF, and 0 to 17.7%, non-IVF. The five greatest decrements of PIBF from pre- to post-LI were 13.7 to 6.4%, 11.0 to 3.7%, 8.0 to 0.7%, 12.7 to 6.3%, and 1.3 to 0.0%.

There were ten patients with PIBF levels <1% both before and after LI; four conceived but one had a clinical pregnancy only, two had spontaneous abortions, and one had a delivery (10%). Otherwise, the rest of the group had a PIBF level that was positive (n = 26) and there were one chemical, one ectopic, three spontaneous abortions, and seven (27.0%) had deliveries (Fisher's test, not significant). Power analysis showed that to find a significant difference with 80% power would require a sample size of at least 100 patients.

DISCUSSION

Progesterone induced blocking factor alterations early in pregnancy may lead to immunologic rejection related to a shift to cellular immune activation. A decrease in PIBF levels have been found in women undergoing SAB or showing symptoms of premature pregnancy termination (e.g., bleeding).[16] Progesterone induced blocking factor levels have been found to be higher in late luteal phase in pregnant vs. non-pregnant patients .[17] Higher late luteal phase PIBF levels are associated with successful implantation,[18] whereas lower late luteal phase PIBF levels are associated with a higher rate of first-trimester spontaneous abortions (clinical, not just chemical).[19]

This study demonstrated increased serum levels of PIBF following LI. Thus, these data are consistent with the possibility that the association of LI and reduced rates of spontaneous abortion in patients with recurrent SABs may at least be in part by the stimulation of PIBF. Preliminary data also suggests that LI may be beneficial for patients having embryo transfers who have failed in at least two prior attempts.[15,20]

TJ6, another immunomodulatory protein, has been recently discovered that has a different pattern of expression on normal vs. abnormal pregnancies.[21] With successful pregnancies, higher levels of TJ6 are seen on CD19+B cells and lower levels on CD56+ NK cells. In contrast with spontaneous abortions, lower levels of TJ6 are found on CD19+B cells and higher levels on CD56+ NK cells.[22] In contrast, PIBF is expressed on CD8+ T-lymphocytes. Preliminary data suggest that TJ6 alterations may occur after the initiating event causing SAB.[22], whereas low PIBF may cause a shift in immune parameters leading to eventual SAB.

Hopefully, these preliminary data will stimulate more worldwide interest in these very interesting immunomodulatory proteins. More extensive testing is needed to see if LI is only beneficial for women who fail to generate adequate PIBF levels despite exposure to the allogeneic fetus. Our preliminary data, though, showing a trend for higher rates of fetal rejection if PIBF was not generated, is far from conclusive since the small sample size did not come close to showing statistical significance. Possibly patients failing to generate sufficient PIBF despite immunization by the husband's leukocytes will respond better to donor leukocytes. Patients failing to respond to both sources of leukocyte preparations might be candidates for intravenous immunoglobulin therapy.[23]

REFERENCES

1. Check JH. The role of progesterone in supporting implantation and preventing early pregnancy loss. *In* Implantation and Early Pregnancy in Humans. Barnea ER, Check JH, Gedis Grudzinskas J, Maruo T (eds). Parthenon Publishing Group. 1994, chap. 11, pp 137–160.
2. Stites DP, Siiteri PK. Steroids as immunosuppressants in pregnancy. Immunol Rev 1983; 75:117–138.
3. Szekeres-Bartho J, Csernus V, Hadnagy J. The blocking effect of progesterone on lymphocyte responsiveness is receptor-mediated. Biol Immunol Reprod 1989; 15:36.
4. Szekeres-Bartho J, Szekeres GY, Debre P, Autran B, Chaouat G. Reactivity of lymphocytes to a progesterone receptor-specific monoclonal antibody. Cell Immunol 1990; 125:273–283.
5. Roussev RG, Higgins NG, McIntyre JA. Phenotypic characterization of normal human placental mononuclear cells. J Reprod Immunol 1993; 25:15–30.
6. Paldi A, d'Auriol L, Misrahi M, Bakos AM, Chaouat G, Szekeres-Bartho J. Expression of the gene coding for the progesterone receptor in activated human lymphocytes. Endoc J 1994; 2:317–319.
7. Szekeres-Bartho J, Kilar F, Falkay G, Csernus V, Torok A, Pacsa AS. Progesterone-treated lymphocytes of healthy pregnant women release a factor inhibiting cytotoxicity and prostaglandin synthesis. Am J Reprod Immunol Microbiol 1985; 9:15–18.
8. 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; 122:281–294.
9. De Fougerolles R, Baines M. Modulation of natural killer activity influences resorption rates in CBAxDBA/2 matings. J Reprod Immunol 1987; 11:147–153.
10. Wegmann TG, Hui Lin, Guilbert L, Mosmann TR. Bidirectional cytokine interactions in the maternal-fetal relationship: Is successful pregnancy a Th2 phenomenon? Immunology Today 1993; 14:353–356.
11. Mosmann TR, Coffman RL. Heterogeneity of cytokine secretion patterns and functions of helper T cells. Adv Immunol 1989; 46:111–147.
12. Lin H, Mosmann TR, Guilbert L, Tuntipopiat S, Wegmann TG. Synthesis of helper 2-type cytokines at the maternal-fetal interface. J Immunol 1993; 151:4562–4573.
13. Szekeres-Bartho J, Faust ZS, Varga P, Szereday L, Kelemen K. The immunological pregnancy protective effect of progesterone is manifested via controlling cytokine production. Am J Reprod Immunol 1996; 35:348–351.
14. Szekeres-Bartho J, Weill BJ, Mike G, Houssin D, Chaouat G. Progesterone receptors in lymphocytes of liver-transplanted and transfused patients. Immunology Letters 1989; 22:259–261.
15. Check JH, Kaplan N, Nazari A, Peymer M, Chuong J. Pregnancy and spontaneous abortion (SAB) rates following lymphocyte immunotherapy (LI) in patients undergoing in vitro fertilization-embryo transfer (IVF-ET). Abstracts of the 16th Annual Meeting of the American Society for Reproductive Immunology 1996; pp 457–458 (abs. #P204).
16. Szekeres-Bartho J, Faust ZS, Varga P. The expression of a progesterone induced immunomodulatory protein in pregnancy lymphocytes. Am J Reprod Immunol 1995; 34:342–348.

17. Check JH, Szekeres-Bartho J, O'Shaughnessy A. Progesterone induced blocking factor seen in pregnancy lymphocytes soon after implantation. Am J Reprod Immunol 1996; 35:277–280.
18. Check JH, Arwitz M, Gross J, Szekeres-Bartho J, Wu CH. Detection of progesterone induced blocking factor (PIBF) in very early pregnancy correlates more with successful implantation then mere conception. Abstracts of the 43rd Annual Meeting of the Society for Gynecologic Investigation 1996; p 201A (abs. #93).
19. Arwitz M, Gross J, Check JH, Szekeres-Bartho J. The association of progesterone induced blocking factor (PIBF) and first trimester spontaneous abortion (SAB). Abstracts of the 44th Annual Meeting of the Pacific Coast Fertility Society 1996; p A6 (abs. # O-11).
20. Carp HJA, Toder V, Mashiach S, Rabinovici J. Effect of paternal leukocyte immunization on implantation after biochemical pregnancies and repeated failure of embryo transfer. Am J Reprod Immunol 1994; 31:112–115.
21. Nichols TC, Kang JA, Angkachatchi V, Beer AE, Beaman KD. Expression of a membrane form of the pregnancy associated protein TJ6 on lymphocytes. Cell Immunol 1994; 155:219–229.
22. Coulam CB, Beaman KD. Reciprocal alteration in circulating TJ6+ CD19+ and TJ6+ leukocytes in early pregnancy predicts success or miscarriage. Am J Reprod Immunol 1995; 34:219–224
23. Coulam CB, Stephenson M, Stern JJ, Clark DA. Immunotherapy for recurrent pregnancy loss: Analysis of results from clinical trials. Am J Reprod Immunol 1996; 35:352–359.