

A study to determine if estrogen (E) is needed to induce de novo progesterone (P) receptors on gamma/delta T cells as evidenced by determining the degree of rise of progesterone induced blocking factor (PIBF) following P exposure in males

J.H. Check^{1,2,*}, A. DiAntonio², D.L. Check², M.P. Dougherty³, G. Diantonio²

¹ Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology & Infertility,
Cooper Medical School of Rowan University, Camden, New Jersey (USA)

² Cooper Institute for Reproductive Hormonal Disorders, P.C., Marlton, New Jersey (USA)

³ Department Of Obstetrics, Gynecology And Reproductive Sciences, Rutgers Robert Wood Johnson Medical School,
Robert Wood Johnson University Hospital, New Brunswick, NJ (USA)

Summary

Purpose: To determine if exposure to progesterone (P) alone without prior estrogen (E) exposure can cause a rise in serum progesterone induced blocking factor (PIBF). **Materials and Methods:** Male volunteers received IM progesterone 100 mg for seven days. Baseline and day seven levels of serum P and PIBF were taken. PIBF was measured using a non-commercial newly developed ELISA assay. **Results:** A significant rise in PIBF was found in male volunteers on day seven. **Conclusions:** The precipitous rise in serum PIBF in males despite a short exposure to progesterone shows that a person does not need E to induce PIBF secretion by gamma/delta T cells. The possibility still exists pending future studies that E may improve PIBF secretion.

Key words: Immunomodulatory proteins; Progesterone; Progesterone induced blocking factor (PIBF); Males; Estrogen.

Introduction

Cell mediated immune reactivity has been shown to have significant deleterious effects on the fetus [1]. Cytotoxic lymphocytes seem to exert their action via cytokines [2]. Successful pregnancy seems to be associated with a shift of thymic helper (TH) 1 to TH 2 cytokines which seem to inhibit inflammatory responses [3].

Progesterone (P) receptors have been demonstrated on peripheral blood gamma/delta T cells of pregnant women [4]. It is known that women can produce a precipitous rise in serum progesterone induced blocking factor (PIBF) by merely being exposed to P without the pregnancy state and without an allogeneic stimulus [5]. There is evidence that one of the main functions of P in women is to stimulate a rise in serum PIBF or intracellular PIBF to suppress NK cell cytotoxicity by stabilizing perforin granules, and by causing a shift from TH1 to TH2 cytokines, thus helping the fetal semiallograft to escape immune surveillance [6].

Though a small amount of P is detected in the sera of women even in the follicular phase, the serum P rises precipitously after ovulation [5, 6]. Thus, it is not surprising that some PIBF is detected in the serum even in the follicular phase, but it rises greatly in the luteal phase [5, 6]. It

is well known that estrogen (E) upregulates the induction of P receptors in the endometrium [6]. The question arises as to whether E is needed to induce the P receptors in gamma/delta T cells or for that matter is a female karyotype needed to cause a rise in serum PIBF. To help answer this question, a study was performed where male volunteers were injected with P to see if one could elicit a rise in serum PIBF from P exposure without E.

Materials and Methods

To determine whether an allogeneic exposure and/or E is needed to develop or upregulate P receptors on gamma/delta T cells, thus leading to PIBF expression when exposed to P, we injected two male volunteers with P for seven days (100 mg I.M. daily). Serum PIBF levels were then measured by a new non-commercial ELISA assay on the 7th day of P injection. One male received an injection of 100 mg P the morning of the blood test and the other male received his last injection the night before.

The PIBF assay was performed as follows: a non-commercial enzyme linked immunoabsorbent assay (ELISA) was used to measure PIBF in serum. Serum specimens were stored at -20 °C. Fifty microliters of recombinant PIBF standard were added to each pre-coated goat anti-rabbit antibody well in duplicate.

Table 1. — *The role of P without estrogen and without an allogeneic stimulus on PIBF expression in males*

	Baseline		7 days of P	
	P (ng/mL)	PIBF (ng/mL)	P (ng/mL)	PIBF (ng/mL)
Male 1	0.0	<2	53.2	169.5
Male 2	0.5	10.5	26.7	75.0

The concentrations of the PIBF standard were S0 – 0, S1 – 3.2, S2 – 11.2, S3 – 40, S4 – 160, and S5 – 802 ng/mL. The patient's serum was then added to each well. Next 50 microliters of horse radish peroxidase conjugated PIBF antigen were added to each well except the zero standard. Next, anti-PIBF IgG antibody was added to each well. The microtiter plate was then incubated in the dark for one hour at 37 °C. After one hour, the wells were washed with phosphate buffered saline and decanted three times. Next, 50 microliters of substrate A (carbamide peroxide) and 50 microliters of substrate B (tetramethyl-benzidine) were added. The microtiter trays were then incubated in the dark at 37 °C for 15 minutes. Next, 50 microliters of stop solution, whose main component is sulfuric acid (H₂SO₄), was added. The plates were read within ten minutes using a microplate reader at 450 nm. The results were calculated using a four-parameter logistic curve fit. It should be noted that this ELISA assay for PIBF is not commercially available. The present assay cannot accurately measure PIBF levels that exceed 800 ng/mL. A coefficient of variation has not yet been established.

Results

The PIBF levels in two males after seven days of 100 mg I.M. P is seen in Table 1. Male 1 received the IM P the morning of PIBF assay. Male 2 received the last IM injection the evening before. A significant rise of PIBF in these two males over baseline was found. Having seen that the first pilot study of two men showed a rise of PIBF, it was decided that it would not be necessary to enlist any more males into the study nor determine if the PIBF levels would increase further with more extended exposure as initially planned.

Discussion

These data clearly show that a significant upregulation of PIBF by gamma/delta T cells with P exposure does not require a prior allogeneic exposure, e.g., a fetus or oncofetal antigens of a tumor. Originally, the concept of the mechanism of a significant rise in serum PIBF hypothesized the induction of de novo P receptors in gamma/delta T cells by the allogeneic stimulus of the fetal semi-allograft [4].

These data also show that the presence of E is not a pre-

requisite for induction of P receptors on gamma/delta T cells, since PIBF increased significantly with mere P exposure in males whose serum E levels are low. The possibility still exists that E can enhance P receptor expression which allows a greater level of PIBF secretion by gamma/delta T cells. To determine this one would need to perform a study where males are given E alone followed by E and P and PIBF measured after seven days of P exposure vs. males given only the P injection. To see if women are intrinsically better able to produce more PIBF, one could compare PIBF levels in menopausal women given E then E and P vs. males given E then E and P. We have not performed this study yet, though it would serve academic curiosity, it has not been given a high priority to perform it because the results do not appear to have clinical importance.

Acknowledgments

Actavis (Parsippany, NJ) provided financial support for the purchase of reagents for the PIBF assay.

Conflict of Interest

The authors declare no competing interests.

Submitted: November 03, 2018

Accepted: November 17, 2018

Published: June 15, 2020

References

- [1] Szekeres-Bartho J., Barakonyi A., Polgar B., Par G., Faust Z., Palkovics T., Szereday L.: "The role of γ/δ T cells in progesterone-mediated immunomodulation during pregnancy: a review". *Am. J. Reprod. Immunol.*, 1999, 42, 44.
- [2] Wegmann T.G., Hui Lin, Guilbert L., Mosmann T.R.: "Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a Th2 phenomenon?" *Immunol. Today*, 1993, 14, 353.
- [3] Raghupathy R., Makhseed M., Azizieh F., Omu A., Gupta M., Farhat R.: "Cytokine production by maternal lymphocytes during normal human pregnancy and in unexplained recurrent spontaneous abortion". *Hum. Reprod.*, 2000, 15, 713.
- [4] Polgar B., Barakonyi A., Xinos I., Szekeres-Bartho J.: "The role of γ/δ TCR positive cells in pregnancy". *Am. J. Reprod. Immunol.*, 1999, 41, 239.
- [5] Check J.H., Cohen R., Jaffè A., Tran J., Sarumi M.: "An allogeneic stimulus is not a prerequisite for the expression of the immunomodulatory protein the progesterone induced blocking factor (PIBF)". *Am. J. Reprod. Immunol.*, ISIR, 2013, Boston, MA.
- [6] Check J.H., Cohen R.: "The role of progesterone and the progesterone receptor in human reproduction and cancer". *Expert Rev. Endocrinol. Metab.*, 2013, 8, 469.

Corresponding Author:
 JEROME H. CHECK, M.D., PhD
 7447 Old York Road
 Melrose Park, PA 19027 USA)
 e-mail: laurie@ccivf.com