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Serum levels of the immunomodulatory protein, the progesterone induced blocking factor (PIBF) which is found in high levels during pregnancy is not higher in women with progesterone (P) receptor (R) positive *vs.* negative breast cancer

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Summary

Purpose: To determine if serum levels of the immunomodulatory protein, the progesterone induced blocking factor (PIBF), which is present in high levels during normal pregnancy, is present in higher levels in women with breast cancer positive for progesterone receptors. The study would also determine whether the presence or absence of the estrogen receptor in any way modifies PIBF expression. *Materials and Methods:* PIBF using a research ELISA was evaluated in the follicular phase in 21 women with receptor status as follows: seven with estrogen receptor (ER)+ and progesterone receptor (PR)+, seven with ER- and PR+, and seven with ER+ and PR. *Results:* The results showed no differences in serum PIBF in the three groups. The serum PIBF levels were no different than historical controls in the follicular phase. *Conclusions:* Measurement of serum PIBF does not seem to be an important marker to use to either detect women with breast cancer or to help determine tumor virulence or potential specific therapies. If PIBF plays a role in helping cancer cells to escape immune surveillance, it seems that the intracytoplasmic PIBF would be the form most likely operative.

Key words: Natural killer cells; Intracellular splice variant; Hormonal receptor status; Breast cancer.

Introduction

An excellent review has been published concerning the possible role that progesterone (P) receptors play in breast cancer [1]. Despite the comprehensive review by Daniel *et al*. [1], one potential mechanism not mentioned was the role of P interacting with P receptors causing the secretion of an immunomodulatory protein known as the progesterone induced blocking factor (PIBF), which may aid breast cancer cells (and other malignancies) to escape immune surveillance [2].

The parent compound of PIBF is a 90 kDa protein consisting of 757 amino acid residues of which the 48 kDa terminal part is biologically active [3]. The protein seems to be unique sharing no significant amino acid sequence homology with any known protein [3].

The PIBF gene has been identified on chromosome 13. The 90 kDa isoform with the nuclear location seems to be the dominant form present in most rapidly growing cells as evidenced by western blot analysis using PIBF specific antibodies [4]. RNA expression analysis has shown that centrosomal PIBF is over-expressed in rapidly proliferating cells irrespective of whether they have been found to be positive or not for P receptors [4]. There has been identification of the exon 1-5+17-18 transcript encoding for a 35 kDa intracytoplasmic protein. The deletion observed in this transcript preserves the open reading frame for the full length PIBF protein [4]. Translation of the transcript results in a 35 kDa isoform of PIBF containing the N-terminal 223 and C-terminal 75 amino acids [4].

An isoform of similar molecular size to the intracytoplasmic protein dramatically rises in the serum following exposure to P [5-10]. Circulating PIBF has been found to inhibit cellular immunity by causing a shift from TH1 to TH2 cytokine dominance and by inhibiting natural killer (NK) cell cytotoxicity by inhibiting degranulation of NK cells and thus suppressing the release of perforin [11, 12].

Though it seems likely that the fetal semi-allograft escapes immune surveillance probably via intracytoplasmic and circulating PIBF, it is not clear if circulating PIBF plays a role in the escape of immune surveillance by cancer cells [2, 13]. If, in fact, circulating PIBF helps breast cancer cells to escape immune surveillance, the question arises as to

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Table 1 — *Mean serum PIBF according to estrogen and progesterone receptors in women with breast cancer.*

	Receptor status		
	E neg P pos	E pos P neg	E pos P pos
Mean PIBF ng/ml ±SD	24.6±28	31.0±49.7	24.8±37.46
Range (ng/ml)	6-86.9	2.1-92.5	7.4-108.32

whether higher serum PIBF levels are more likely to be present in those women whose breast cancer is P receptor positive?

The objective of the present study was to determine if serum PIBF levels are higher in women with breast cancer not exposed to exogenous or endogenous P according to whether the tumor is P receptor positive or not. If such a relationship exists, the next question would be whether the concomitant presence of estrogen (E) receptors in any way modifies the expression of serum PIBF.

Materials and Methods

From a group of sera samples from women with breast cancer obtained prior to surgery, 21 were selected for measurement of PIBF. The selection was random but there would be: seven with E receptor negative and P receptor positive tumors, seven with E receptor positive and P receptor negative tumors, seven with E receptor positive, and P receptor positive tumors. The serum PIBF was evaluated by a research non-commercial ELISA assay using a monoclonal anti-PIBF antibody as previously described [14].

Results

The sera levels of PIBF in 21 women according to E and P receptor status are shown in Table I. There were no significant differences (ANOVA) in sera PIBF levels in women with breast cancer whether the tumor was P receptor positive or not. The sera PIBF levels in women with breast cancer are not higher than in historical controls of women without cancer in the follicular phase.

Discussion

Some oncologists are under the impression that there will be a resumed interest in the use of P receptor antagonists for the treatment of breast cancer [15]. Of course, the thought of using anti-progestins by oncologists is based on the concept regarding breast cancer treatment with anti-estrogens for breast cancer positive for the E receptor, that somehow the P receptor aids some tumors to proliferate, and thus blocking the receptor can cause tumor regression [1,15].

The failure to demonstrate any increase in serum PIBF in women with breast cancer, even those with tumors that were positive for the P receptor, suggests that the P receptor aids tumor proliferation in some other way than by enhancing the production by gamma/delta T cells of a circulating immunosuppressive PIBF protein [2, 16, 17].

The P receptor antagonist mifepristone has been demonstrated to provide significant palliation in men and women with cancers not known to be P receptor positive [14, 18-20]. Thus, the present data could support the argument that the presence of the P receptor in certain cancers, e.g., breast, enables proliferation of the cancer by some other mechanism not involving PIBF [1]. One must consider, however, that the possibility still exists that cancer cells, through a P receptor mechanism, produce intracytoplasmic PIBF which confers immune protection. Indeed, mifepristone was able to suppress intracytoplasmic PIBF expression in leukemia cells lines [21].

Taking mifepristone by a pregnant woman in early pregnancy for just one day has a high rate of causing pregnancy termination [22]. Mifepristone has been demonstrated to modify NK cell activity from pregnant women [23]. PIBF has been found to inhibit NK cell activity and to reverse the shift of TH1 to TH2 cytokines during mammalian pregnancy [23]. Thus, it seemed logical that, most likely, once a good assay for PIBF was developed, one would probably demonstrate that mifepristone will markedly lower serum PIBF. However, in contrast, a study recently presented at the Pacific Coast Reproductive Society meeting by DiAntonio *et al.* found serum PIBF to progressively increase in a woman supplemented by P despite daily ingestion of 200 mg of mifepristone for several days [24]. Thus, it still seems quite possible that at least part of the pregnancy or cancer aborting effect of P receptor modulators could still be via suppressing PIBF, but only the intracytoplasmic form.

The data from the present study suggest that measuring the serum level of PIBF will not be useful in either identifying breast cancer aggressiveness or be used to determine which patients may be candidates for P receptor antagonist therapy. The caveat is that it is the authors' belief that these data should not negate the interest for using P receptor modulators for various cancers, whether they are known to be P receptor positive or not [15, 18-20].

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