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Serum Interleukin 2 (IL-2) Levels and Prolactin Bioactive/Radioimmunoassay Ratios of Women at Risk for Breast Cancer*

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Abstract—*In a cohort of women at high risk for developing breast cancer we have observed that* 74% *of the women with prolactin BA/RIA ratios over* 1.4 *had detectable levels of IL-2 in their serum* (990 ± 400 mU/ml) *and the IL-2 levels were correlated with the prolactin BA/RIA ratio* (R = 0.79; P > 0.004). *Women with BA/RIA ratios were either approximately* 1.0 *or less than* 0.55 *had lower levels of serum IL-2* (177 ± 70 *and* 130 ± 40 mU/ml, *respectively*). *Detectable levels of serum IL-2 were found in* 58% *of those women with BA/RIA ratios of* 1.0 *and* 55% *of those with BA/RIA ratios less than* 0.55.

INTRODUCTION

WOMEN at risk for breast cancer have been reported to have high serum prolactin bioactive/radioimmunoactive (BA/RIA) ratios when bioactivity was determined by the Nb_2 lymphoma cell bioassay [1]. The Nb_2 lymphoma cell has been reported to be very sensitive and specific for hormones containing lactogenic activity such as prolactin, human growth hormone and human placental lactogens [2]. Recent reports, however, indicated that these cells also respond to the lymphokine, interleukin-2 [3–5]. It is thus important to evaluate the serum interleukin-2 levels of women with high prolactin BA/RIA ratios to determine whether this factor contributes to the high bioactivity of the serum as measured by the Nb_2 lymphoma cells.

MATERIALS AND METHODS

Serum samples were obtained from population of women (79) who are part of a breast cancer prevention research project, and who are judged to be at high risk of developing breast cancer. High risk is defined in this population by at least one first degree relative (mother/sister/daughter) with breast cancer or recent (within 1 year) mammogram showing P_2 or D_y Wolfe pattern. Serum samples were drawn after a 12 h overnight fast both in post-menopausal women and in pre-menopausal women. Samples were obtained from pre-menopausal women during days 17–24 of their menstrual cycle and in post-menopausal women in a random fashion. Women taking medications that might interfere with hormonal level determinations were excluded. Serum RIA prolactin levels were determined at one dilution (1:10) in triplicate using a commercially prepared kit supplied by Pantex (Santa Monica, CA, U.S.A.). A highly purified human prolactin preparation (NIAMDD hPRL, Batch #5-AFP 1582C; 30 IU/mg) was substituted for the prolactin standard supplied with the kit. The serum prolactin bioactivity was determined using the Nb_2 lymphoma cell bioassay. The details of the procedure have been previously published [6]. The serum was assayed in duplicate using 2.5 and 5.0 μl of serum and the assay was made specific for prolactin by absorbing out the growth hormone in the sample using a specific growth hormone antiserum (NIAMMD Anti hGH serum #31, 9-22-79). The amount of growth hormone antiserum added to the culture system was adequate to neutralize the equivalent of 80 ng of growth hormone/ml of serum. Determination of growth hormone levels by radioimmunoassay indicated that no serum evaluated had over 25 ng/ml of growth hormone and most sera had less than 3 ng/ml. The prolactin standard used in the BA was identical to the one used in the RIA. IL-2 levels in sera were determined by the Elisa method using a commercially prepared

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kit supplied by Genzyme, Inc. (Boston, MA, U.S.A.). The IL-2 standard was recombinant human IL-2. Serum IL-2 levels were determined in three groups of women: those women with a serum prolactin BA/RIA ratio greater than 1.4; those with a ratio of approximately 1.0; and those with a ratio of less than 0.55. Differences among serum prolactin BA/RIA ratios and serum IL-2 levels were determined statistically by analysis of variance followed by Duncans multiple range test. The IL-2 values were transformed to natural logarithms prior to statistical analysis to reduce variability. The correlation analysis was performed between the high BA/RIA ratio group and serum IL-2 levels. Correlation analyses were also performed between serum RIA and BA prolactin levels and IL-2 values. In all statistical analyses a P value of less than 0.05 was considered statistically significant.

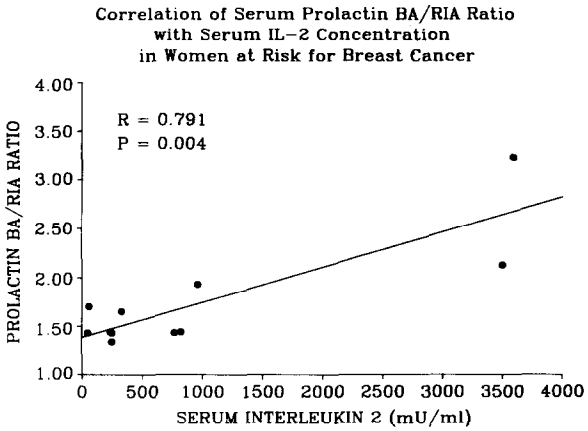

Fig. 1. Correlation of serum prolactin BA/RIA ratio with serum IL-2 levels in women at risk for breast cancer. Samples examined were from women who had a BA/RIA ratio greater than 1.4 and had detectable serum IL-2. A total of 11 samples was examined.

RESULTS

Seventy-nine women age 18–67 have had prolactin level measured and 85% of the women in this trial have at least one first degree relative with breast cancer and 15% had a P_2 or D_y mammogram pattern. Women with high serum prolactin BA/RIA ratios (>1.4) were observed to have elevated IL-2 levels ($P < 0.02$) when compared to women with BA/RIA ratios of approximately 1.0 or less than 0.55 (Table 1). The serum IL-2 levels of women with high BA/RIA ratios were positively correlated ($R = 0.791$; $P = 0.004$) with the BA/RIA ratios (Fig. 1). In the high BA/RIA ratio group the serum RIA prolactin levels were higher ($P < 0.05$) than in the other two groups (33.2 ± 7.4 vs. 16.0 ± 2.3 and 18.8 ± 1.4 ng/ml, respectively). However, a comparison of IL-2 levels with either serum RIA PRL or BA PRL levels did not reveal a statistically significant ($P > 0.05$) correlation suggesting no interrelationship between the level of serum prolactin and IL-2. There was also no association with the criteria for the assignment of breast cancer risk or menopausal status and the detection of serum IL-2.

DISCUSSION

These results suggest that the apparent high prolactin bioactivity in the serum of women at risk for breast cancer as measured by the Nb_2 lymphoma cell assay may not be due entirely to a hyperbioactive form of prolactin but may be due to an elevation in serum IL-2. However, not all individuals with high BA/RIA ratios had detectable levels (>50 mU/ml) of IL-2; the average BA/RIA ratio of this group of women (BA/RIA ratio > 1.40) without detectable serum IL-2 levels was 1.59 ± 0.14 (mean ± S.E.) while those with detectable IL-2 level had an average ratio of 1.84 ± 0.16. Thus, while IL-2 may contribute to the high ratios in some individuals, in other individuals there is some other factor, which may be a hyperbioactive prolactin or some other unidentified factor, that stimulates the

Table 1. Comparison of serum interleukin-2 (IL-2) levels with serum prolactin BA/RIA ratios in women at risk for breast cancer

	Serum prolactin		Serum interleukin-2		
Group	n	BA/RIA ratio[1]	n	mU/ml[1]	% subjects with detectable IL-2[2]
BA/RIA ratio > 1.4	15	1.77 ± 0.13*	11	990 ± 400**	74
BA/RIA ratio 1.0[3]	12	0.99 ± 0.02*	7	177 ± 70	58
BA/RIA ratio < 0.55	11	0.42 ± 0.03*	6	130 ± 40	55

[1]Mean ± S.E.
[2]IL-2 levels greater than 50 mU/ml.
[3]Range of BA/RIA ratio was 0.92–1.08.
*Significantly different from each other, $P < 0.01$.
**Significantly different from other groups, $P < 0.02$.

proliferation of Nb_2 lymphoma cells. It is noteworthy that in over half of the women with bioactivity equal to RIA activity or with low bioactivity, IL-2 levels were also detectable in the sera. The significance of these observations in relation to the etiology of breast cancer is not known at this time. We are at present evaluating sera from a large number of high risk women for serum IL-2 levels.

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