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Brief communication

Comparison of estrogen and progesterone receptor status to lymphocyte immunity against tumor antigens in breast cancer patients

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Summary

Estrogen (ER) and progesterone receptor (PgR) content of tumors were determined by both the dextran-coated charcoal (DCC) cytosol and immunocytochemical assays (ICA), and these hormone receptor results were compared to lymphocyte immunity against tumor antigen(s) for 52 breast carcinoma patients. Hormone receptor analysis by both methods demonstrated that 60% of the patients' tumors had ERs, while 44% were positive for PgRs. The ICA procedure was more sensitive than the cytosol technique for determining PgR content of the tumors. This increased sensitivity was not observed for ER by ICA. Patient age, tumor size, and nodal status were not related to the ER and PgR receptor status. A total of 21/52 (40%) of the patients had positive lymphocyte immunity against tumor antigen. This immunity was independent of patient age, tumor size, and nodal status. There was no significant relationship between lymphocytic immunity against tumor antigen and ER or PgR content of tumors, suggesting that patient lymphocyte immunity against tumor is independent of hormone receptor status. This is further evidence that lymphocyte immunity against tumor antigen status is an independent prognostic indicator that may be useful in the selection of a subset of node negative patients for adjuvant chemotherapy.

Introduction

Several new prognostic indicators for breast cancer have been described over the past 15 years. These include estrogen and progesterone receptor analysis of tumor cells [1], S-phase and ploidy determination of tumor cells by flow cytometry [2, 3], HER-2/neu oncogene expression [4, 5], Cathepsin D content [6, 7], and epidermal growth factor receptor content [8]. Further, mitogen-induced lymphocyte proliferation [9] and lymphocyte immunity against autologous tumor antigen [10–12] also furnish additional prognostic information.

Some studies have attempted to determine if these new prognostic indicators are independent of each other and the more established clinical prognostic indicators. ER content is an accepted prognostic indicator in breast cancer, and has been shown to correlate with PgR content [5] and ploidy status by flow cytometry [2], and inversely with c-erbB-2 (HER-2/neu) concentrations [4].

When we first initiated studies on lymphocyte immunity against autologous tumor antigen in breast cancer patients, little was known about the potential prognostic value of estrogen and progesterone receptor levels, S-phase and ploidy analysis by flow cytometry, or any other of the newer prognostic indicators. Recently, we initiated a prognostic indica-

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tor evaluation program for our breast cancer patients using several tests, including the dextran-coated charcoal cytosol and immunocytochemical assays of estrogen and progesterone receptors, S-phase and ploidy analysis of tumor cells by flow cytometry, patient lymphocyte competence, and determination of immunity against autologous tumor antigens. This study will form the foundation for future chemotherapy treatment and potential immunotherapy regimens (including immunization of patients with autologous tumor antigen).

The present work describes our data with estrogen and progesterone receptor analysis, and lymphocyte immunity to tumor-associated antigen performed on a series of breast cancer patients. We have compared the results from the receptor and immunological assays with clinical pathological parameters of the patients (including age, tumor size, and nodal status) and more importantly with each other.

Materials and methods

Patients

This study includes 52 women, with histologically verified breast cancer. The studies were performed blind in that clinical information related to age, tumor size, and nodal involvement of the patients was not made available until tests were performed. Further, the estrogen and progesterone receptor analyses were performed at the Mastology Research Institute, while the testing of lymphocyte immunity to tumor antigen was conducted at ImmuQuest Laboratories. Whole heparinized blood specimens for lymphocyte studies were collected a few hours prior to surgical removal of tumor. Tumor tissue was immediately placed in tissue culture media for lymphocyte tumor antigen studies or frozen at – 80° C for receptor analysis. All tests were conducted before any chemotherapy was administered.

Preparation of autologous breast tumor antigen membranes

Surgically-removed breast cancer tissue was placed in sterile alpha-MEM containing gentamicin. The tumor was received in the laboratory within 24 hours of surgery and immediately processed. The tumor tissue (at least $5 \times 5 \times 5$ mm in size) was minced with sterile forceps and scissors, and a single cell suspension was prepared by expressing the fragments through a fine mesh screen.

Hypotonic salt treatment of these single cell suspensions of breast cancers produced intact or large fragments of surface membranes and resulted in a highly active tumor antigen preparation as described by Oren and Herberman. These preparations have been shown to be reactive in *in vitro* cellular immunity assays [10, 11].

Lymphocyte proliferative assay procedure

Mononuclear cells (2×10^5) in serum-free media (No. 56; Quality Biological Inc., Gaithersburg, MD) were added to wells of a 96-well flat bottom plate in 0.1 ml aliquots. Autologous tumor antigen (0.1 ml) at 1:10, 1:100, 1:1,000, and 1:10,000 dilutions was added to triplicate wells; 0.1 ml of medium was added to control wells. The cultures were incubated for seven days at 37° C in a humidified 5% CO_2 atmosphere.

Eighteen hours before termination of cultures, 0.05 ml of ^{3}H-thymidine (1 μCi) was added to each well. Cells were then harvested onto strips of fiberglass paper using a multiple sample harvester. The amount of incorporation in counts per minute (cpm) of ^{3}H-thymidine into the lymphocytes' DNA was determined by scintillation spectrometry.

A Stimulation Index (SI) was then calculated by dividing the average cpm incorporated into tumor antigen-stimulated mononuclear cell cultures by the average cpm incorporated into cell culture wells containing only mononuclear cells and medium. A test result was considered tumor antigen positive when the SI value of any one of the four dose levels of tumor antigen was greater than or equal to a value of 2.5.

DCC-cytosol and immunocytochemical procedures for estrogen and progesterone receptor analysis

The estrogen and progesterone receptors were quantitated by the dextran-coated charcoal cytosol method (Rianen™ Estrogen and Progesterone Receptor Assay Kits; New England Nuclear, North Billerica, MA) and the protein concentration of the supernatant was determined by the Waddell procedure. Receptor values are reported in femtomoles of estradiol or progesterone bound per mg of cytosolic protein, with tumors having values of 10 or more fmol/mg cytosolic protein being considered receptor positive. The immunological procedures were performed as described in the ER-ICA and PgR-ICA methods (Abbott Laboratories; Chicago, IL).

Statistical analysis method

All patient information and test results were stored in a SPSS/PC + database (SPSS Inc.; Chicago, IL). Cross tabulation analysis followed by the Pearson chi-square test for independence of non-linear variables was performed to test for associations between variables.

Results

Our results for cytosol and immunocytochemical analysis of estrogen and progesterone receptors in a small group of 52 breast carcinomas are in accord with those of other investigators [1, 13–15]. Overall, a total of 32/51 (63%) of the tumors were positive for either estrogen or progesterone receptors when the receptors were performed by both the cytosol and immunocytochemical assay procedures. A total of 27/45 (60%) of breast tumors were estrogen receptor positive by either the DCC cytosol or the immunocytochemical procedure: 21/45 (47%) were positive by both methods, an additional 6 (13%) were positive by only one of the methods and 18 (40%) were negative by both methods. Of the 27 tumors positive for estrogen receptors, 24 (89%) were positive by the cytosol method and 24 (89%) were positive by the immunocytochemical method. Thirty-one percent (14/45) of tumors were positive by both cytosol and immunocytochemistry for progesterone receptors, 13% (6/45) were positive by at least one progesterone receptor method, and 56% (25/45) were negative by both methods. A total of 44% (20/45) of the breast carcinomas were positive for the progesterone receptor. Of the 20 progesterone receptor positive tumors, 75% (15/20) were positive by the cytosol method as compared to 95% (19/20) being positive by the immunocytochemical technique.

We evaluated the distribution of estrogen and progesterone receptor-bearing tumors relative to patient age, tumor size, and nodal status. We found that 55% of the 52 patients that were less than 50 years of age had tumors positive for estrogen receptors as compared to 59% of patients ≥ 50 years of age having positive receptors. Fifty-five percent of the tumors from patients less than 50 years of age were progesterone receptor positive, while 37% of the patients ≥ 50 years of age were receptor positive. In analyzing estrogen and progesterone receptor content of these 52 breast tumors versus tumor size, the receptors are equally present on T1, T2, and T3 tumors. Further, node positive patients' tumors were no more likely to have estrogen receptors than node negative patients' tumors. This equal proportion of tumors positive for estrogen receptors in node negative and node positive patients was also found for the progesterone receptor.

Table 1 presents breast cancer patients' lymphocyte immunity to autologous tumor antigen relative to patient age, tumor size, and nodal status. Overall, approximately 40% of the 52 patients demonstrated lymphocyte immunity against tumor antigen. The proportions of tumor antigen positive patients to tumor antigen negative patients were not significantly different when comparing those < 50 years of age to those ≥ 50 years of age. Lymphocyte immunity to tumor antigen was equally distributed between small (T1), medium (T2), and large (T3) tumors. A similar proportion of node negative and node positive patients were tumor antigen positive.

A comparison between estrogen or progesterone receptor content of breast carcinomas and lymphocyte immunity against tumor antigen is presented in

Table 1. Lymphocyte immunity to tumor antigen as a function of clinical/pathological parameters of breast cancer patients

	Lymphocyte immunity to TAA	
	Unreactive[a]	Reactive
Age		
< 50	11/50 (22.0%)[b]	12/50 (24.0%)
≥ 50	19/50 (38.0%)	8/50 (16.0%)
	30/50 (60.0%)	20/50 (40.0%)
p value	0.1049	
TUMOR SIZE		
T1	3/51 (5.9%)	4/51 (7.8%)
T2	20/51 (39.2%)	11/51 (21.6%)
T3	8/51 (15.7%)	5/51 (9.8%)
	31/51 (60.8%)	20/51 (39.2%)
p value	0.5689	
NODAL STATUS		
Negative	15/43 (34.9%)	7/43 (16.3%)
Positive	11/43 (25.6%)	10/43 (23.3%)
	26/43 (60.5%)	17/43 (39.5%)
p value	0.2895	

[a] When the SI is < 2.5 the patients' lymphocytes were considered unreactive.

[b] Number of patients per category/number tested (%).

Table 2. Of 21 patients who were tumor antigen positive, 13 (62%) had positive estrogen receptors on their tumors. Of the 21 patients who were TAA reactive and were tested for progesterone receptors, 10 (48%) had positive receptors. In tumor antigen unreactive patients, there was a similar distribution between estrogen and progesterone receptor content. There was no significant association between tumor antigen induced lymphocyte immunity in breast cancer patients and hormone receptor status.

Discussion

Assays of prognostic indicators, including estrogen and progesterone receptor content of tumors and lymphocyte immunity against tumor antigen(s), were performed on a small series of 52 breast cancer patients. Hormone receptor analysis was performed to define the proportion of patients or their tumors who had positive or negative results. The receptor and immunity data were then evaluated relative to patient clinical-pathologic parameters including age, tumor size, and nodal status. Finally and most importantly, a comparison was made between tumor hormone receptor status and the immunological status of the patients' lymphocytes to determine if the prognostic significance of lymphocyte immunity against tumor antigen is independent of receptor status (a known prognostic indicator in breast cancer patients).

Estrogen and progesterone receptors were positive in 60% and 44% of the patients' tumors, respectively. These data are similar to those earlier reported for these receptors in breast tumors [1, 13–15]. In this study the ICA technique appeared somewhat more sensitive than the DCC cytosol procedure for progesterone receptor determination, but not for estrogen receptor determination. The immunocytochemical procedure would more likely be positive than the cytosol procedure because a small percentage of positive tumor cells in a predominantly normal cell population, as is often the case with breast cancer tissue, will yield a positive result by ICA which will be negative in the cytosol method due to the dilution effect.

Table 2. Comparison of estrogen and progesterone receptor results in breast carcinomas to tumor antigen induced lymphocyte immunity in breast cancer patients

TAA Reactivity	Estrogen receptor		Progesterone receptor	
	Negative	Positive[a]	Negative	Positive[a]
Unreactive (SI < 2.5)	13/51 (25.5%)[b]	17/51 (33.3%)	17/51 (33.3%)	13/51 (25.5%)
Reactive (SI ≥ 2.5)	8/51 (15.7%)	13/51 (25.5%)	11/51 (21.6%)	10/51 (19.6%)
	21/51 (41.2%)	30/51 (58.8%)	28/51 (54.9%)	23/51 (45.1%)
p value	0.7083		0.7610	

[a] Assays performed by both DCC cytosol and immunocytochemical methods. If either one of these were positive, the result was considered positive for the patient.

[b] Number of patients per category/number tested (%).

Hormone receptor content did not differ significantly by patient age or tumor size. Further, both estrogen receptor and progesterone receptor content were similar in tumors from node negative and node positive patient tumors. Our results confirm the earlier report by Berger *et al.* [15], who demonstrated that tumor size and nodal status were not related to estrogen or progesterone receptor status.

The proportion (40%) of patients exhibiting lymphocyte immunity against autologous tumor antigen was somewhat higher than the 27% reported previously [10]. This may be due to the blood specimens in this study being collected pre-operatively as compared to 2–10 weeks postoperatively in the earlier study [10]. Also, our current lymphocyte tumor antigen test uses serum-free media compared to our previous use of media containing human serum [10], which may contain 'inhibitors' of the test and result in a less sensitive test. Our findings confirmed those of the earlier study [10] showing that lymphocyte immunity against autologous tumor antigen by the lymphocyte proliferation test was independent of patient age, tumor size, and nodal status of the patients.

A comparative analysis of patients exhibiting lymphocyte immunity against autologous tumor antigen and the estrogen/progesterone receptor content of their tumors demonstrated no significant difference in estrogen or progesterone receptor status in patients relative to lymphocyte immunity against tumor antigen. Thus, it appears that the prognostic significance of having lymphocyte immunity against tumor antigen is independent of the well established clinical (age, tumor size, nodal status) and biochemical (estrogen/progesterone receptor content of tumor cells) prognostic indicators in breast cancer. These results suggest that lymphocyte immunity against tumor antigen may be an independent prognostic indicator useful in selecting a subset of node negative breast cancer patients for adjuvant chemotherapy or active specific immunotherapy.

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