Brief communication

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

Robert L. Elliott, Jonathan F. Head and James L. McCoy
Mastology Research Institute, 1770 Physicians Park Drive, Baton Rouge, LA 70816, USA; Immuquest Laboratories, 362-C Christopher Avenue, Gaithersburg, MD 20879, USA

Key words: breast, cancer, immunity, TAA, estrogen receptors, progesterone receptors

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.

Address for offprints: R.L. Elliott, Mastology Research Institute, 1770 Physicians Park Drive, Baton Rouge, LA 70816, USA
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 × 5 × 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 × 10⁵) 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% CO₂ atmosphere.

Eighteen hours before termination of cultures, 0.05 ml of ³H-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 ³H-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.