Variations in the Number of Regulatory T Cells ($CD4^+CD25^+$) in Patients with Breast Cancer during Herceptin Therapy

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We studied the effect of Herceptin therapy on the population composition of lymphocytes and percentage of $CD4^+CD25^+$ cells (regulatory T cells) in breast cancer patients. Herceptin treatment decreased the number of "professional" T suppressors ($CD4^+CD25^+$ cells and regulatory T cells) in the peripheral blood.

Key Words: *breast cancer; Herceptin; regulatory T lymphocytes*

T lymphocytes expressing both membrane marker CD4 and CD25 receptor for interleukin-2 ($CD4^+CD25^+$ cells) and called regulatory T cells (Treg) or "professional" T suppressor cells are responsible for the maintenance of immune homeostasis and prevent the development of autoimmune diseases [11]. They were isolated from the spleen of experimental animals [11], and then from human peripheral blood [3]. *In vitro* experiments showed that these weakly proliferating cells produce cytokines inhibiting immunoreactivity of TGF-β and interleukin-10. Animal studies showed that Treg suppress the immune response to tumor antigens. Elimination of Treg with monoclonal antibodies was followed by rejection of transplanted syngeneic tumors in mice [12,13]. Passive transplantation of these cells from animals with growing tumors impaired the antitumor immune response in recipients. In mice immunized with human ErbB-2 antigen (HER-2) administration of monoclonal antibodies for elimination of Treg cells caused regression of D2F2/E2 breast tumor, if tumor cells were transfected with the pCMV/E2 gene encoding human ErbB-2 (Her-2) [14]. In animals not subjected to elimination of these cells (compared to nonimmunized mice) tumor growth was only decelerated. Elimination of $CD4^+CD25^+$ cells with monoclonal antibodies improved the efficiency of antitumor vaccination with dendrite cells against melanoma B16-F10 antigen in mice [10].

The number of $CD4^+CD25^+$ cells increases in patients with tumors of lungs [15], pancreas, and mammary gland [5] and hepatocellular carcinoma [8]. Previous studies showed that the ratio of these cells increases under various pathological conditions. The influence of therapeutic procedures on the number and activity of $CD4^+CD25^+$ cells remains unknown.

Here we studied the effect of Herceptin therapy on the population composition of lymphocytes and number of $CD4^+CD25^+$ cells in breast cancer (BC) patients.

MATERIALS AND METHODS

We examined 19 patients with early stages of BC characterized by overexpression of HER-2/neu (stages I-IIIa). These patients were included in the International Clinical Trial BIG 01-01 BO 16348. Treatment included surgical therapy (radical mastectomy with preservation of the pectoral muscles, radical resection), adjuvant chemotherapy with anthracycline drugs, and radiotherapy (if required).

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Hormone therapy was prescribed taking into account the receptor status (tamoxifen, 5 years) after completion of adjuvant chemotherapy. According to the protocol of BIG 01-01 BO 16348, the patients were randomized into the control (n=8) and main groups (n=11, Herceptin therapy) after completion of standard adjuvant chemotherapy. Herceptin was infused intravenously over 90 min. The loading dose of Herceptin was 8 mg/kg. Herceptin in a dose of 6 mg/kg was given every 3 weeks for 1-2 years. The average age of patients was 52 years (40-63 years). The menstrual cycle was observed in 8 women.

The receptor status was determined by biochemical and/or immunohistochemical studies. Receptors for estrogens and/or progesterone were identified in 8 women. Overexpression of HER-2/neu was estimated by standard criteria in an immunohistochemical and/or FISH study.

To study the immunological status, the blood was taken immediately after randomization (before the first infusion of Herceptin in treated patients) and during the follow-up period at 2-month intervals.

Lymphocytes were isolated from heparinized venous blood by centrifugation in a Ficoll-Verografin density gradient (d=1.076 g/liter) [2]. The population composition of lymphocytes was studied by the cytofluorometric method using monoclonal antibodies against human lymphocyte differentiation antigens. The study was performed on a FACSCalibur flow cytofluorometer (Becton Dickinson) using commercial CellQuest software for the collection and analysis of data. The ratio of main subpopulations was determined by indirect immunofluorescence with unstained antibodies and subsequent staining with FITC-labeled Fab'-fragment of rabbit antibodies against human immunoglobulins. The ratio of $CD4^+CD25^+$ cells was measured by the method of direct immunofluorescence (double fluorescence labeling) with phycoerythrin-labeled anti-CD4 monoclonal antibodies (red staining) and FITC-labeled anti-CD25 antibodies (green staining). Activity of natural killer cells (NK cells) against K-562 erythroleukemia cells was studied in the cytotoxic test with modifications [4] using a semiautomatic spectrophotometric MTT assay [7]. Plasma immunoglobulin concentration was measured as described elsewhere [6]. The results were analyzed by Student's t test (SigmaPlot for Windows, Jandel Corporation).

RESULTS

Blood tests were performed 2-5 times (2 times in 2 patients, Table 1). The ratio of various populations of T lymphocytes and number of B lymphocytes

TABLE 1. Population Composition (%) of Peripheral Blood Lymphocytes in Patients of the Control and Herceptin Group ($M\pm m$)

Lymphocytes marker	Control group				Main group			
	test 1	test 2	test 3	test 4	test 1	test 2	test 3	test 4
CD3	70.5±3.7	72.6±3.1	70.8±3.1	72.3±3.3	69.30±3.53	61.77±5.70	57.6±3.9*	54.4±7.2*
CD5	72.6±4.2	70.9±6.3	74.0±3.9	73.8±0.1	67.40±2.47	68.44±3.90	60.9±4.0	54.8±6.7
CD7	69.8±4.2	81.7±2.1*	81.4±4.7	83.0±0.3	69.50±2.73	71.77±4.90	73.1±2.7	65.7±5.5
CD4	36.7±4.1	31.9±4.3	38.3±3.3	38.3±9.2	33.50±3.16	31.09±3.60	34.1±3.8	29.2±2.9
CD8	28.1±4.0	32.0±4.8	26.7±6.1	35.3±12.5	32.00±4.02	34.62±6.20	23.8±3.5*	25.4±3.9*
CD4/CD8	1.7±0.4	1.30±0.43	1.75±0.40	1.3±0.7	1.6±0.6	1.01±0.10	1.8±0.5	1.3±0.3
HLA-DR	12.7±1.5	10.4±1.4	21.3±1.9	8.4±2.8	15.50±3.14	12.21±1.50	13.0±2.1	8.5±1.4
CD38	44.7±3.9	48.5±3.5	36.2±6.1	37.1±3.2	44.5±5.2	44.77±7.80	39.5±6.0	32.3±3.6
CD25	4.6±1.6	3.6±1.6	3.42±2.08	2.25±0.05	6.30±1.32	3.97±2.10	2.8±1.6	1.1±0.4*
CD50	93.8±3.2	95.1±1.5	95.9±1.9	97.4±0.3	94.90±1.17	95.44±1.10	93.2±2.4	88.2±6.6
CD16	18.6±3.8	14.5±2.4	26.2±1.8	14.20±7.05	19.10±3.11	16.47±3.50	20.2±3.6	20.8±5.2
CD20	3.3±0.9	7.0±1.9	6.5±1.3	4.2±0.5	7.90±1.44	8.9±1.5	9.2±1.6	9.1±2.2
CD11b	30.3±4.4	23.9±4.4	19.3±5.0	17.5±4.7	23.50±3.72	26.7±5.5	24.4±5.3	16.9±5.0
CD95	34.8±5.8	52.6±4.0*	46.2±5.0	49.4±14.4	40.80±4.85	58.6±4.3*	46.1±7.3	38.5±7.0
CD45RA	46.1±3.9	59.5±1.9*	54.0±3.1	67.80±3.75*	47.10±3.44	63.66±4.30*	58.1±6.8	53.0±3.4
CD71	1.9±0.6	1.7±0.4	1.3±0.4	1.8±0.6	6.10±4.27	10.67±4.60	1.9±0.7	0.8±0.4
CD4CD25	2.24±0.73	1.9±0.5	1.02±0.31	1.2±0.2	3.98±0.96	0.74±0.20*	0.67±0.20*	0.6±0.2*

Note. *p<0.05 compared to the corresponding parameter before therapy.

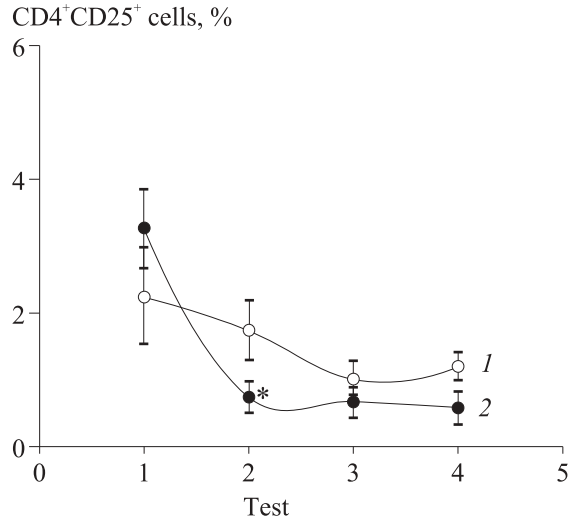

Fig. 1. Ratio of $CD4^+CD25^+$ cells in the control (*1*) and Herceptin groups (*2*). *$p<0.05$ compared to this parameter before Herceptin therapy.

($CD20^+$) and NK cells ($CD16^+$) in patients of the control group remained practically unchanged. The exceptions were apoptosis marker-expressing cells ($CD95^+$) and naive T cells ($CD45RA^+$). The ratio of these cells progressively increased after the 2nd test (Table 1). These changes were statistically significant. The test parameters remained within the normal range. Similar changes were revealed in treated patients (Table 1). Activity of NK cells and serum concentration of IgG, IgA, and IgM remained unchanged in patients of both groups. Significant variations in the number of regulatory T suppressor cells with the $CD4^+CD25^+$ phenotype were revealed in patients of the Herceptin group. The ratio of these cells in the 2nd test was 5.4 times lower than before therapy (Table 1, Fig. 1). The decrease in the ratio of cells was statistically significant. Cell number remained low until the end of the study. The count of these cells also decreased in patients of the control group. The maximum decrease was observed in the 3rd test (by 2.2 times). However, these changes were statistically insignificant and less pronounced than in patients of the Herceptin group. The count of T lymphocytes ($CD3^+$ cells) significantly decreased in Herceptin-treated patients. The ratio of $CD4^+CD25^+$ cells in all patients of the main group decreased by 2-10 times after the first infusion of Herceptin. In the control group the ratio of $CD4^+CD25^+$ cells decreases in 4 patients (by 2-5 times), remains unchanged in 2 patients, and increases in 2 patients (by 1.5 and 14.7 times, respectively).

Our results show that Herceptin therapy decreases the ratio of "professional" T suppressor cells ($CD4^+CD25^+$ cells, Treg lymphocytes) in the population of peripheral blood T lymphocytes from patients. Herceptin is the first drug producing this effect in patients with malignant tumors.

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