INTERLEUKIN 2 ACTIVITY IN PERIPHERAL BLOOD MONONUCLEAR CELLS OF PATIENTS WITH GYNECOLOGIC MALIGNANCIES

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We studied production of, absorption of and response to interleukin 2 (IL-2) by peripheral blood mononuclear cells (PBMC) from 66 patients with gynecologic malignancies, in addition to measurement of the OKT 4/OKT 8 cell ratio. Patients with benign tumor served as controls. The OKT 4/OKT 8 cell ratio in patients with advanced (but not early) gynecologic malignancies was significantly lower than that in patients with benign tumor. PBMC from advanced cancer patients activated with phytohemagglutinin (PHA) had significantly lower IL-2 production compared to that from patients with benign tumors, while significant changes in their ability to respond to IL-2 and to absorb IL-2 were not observed. Absolute numbers of OKT 8 positive cells in PBMC of patients with good prognosis were significantly decreased after surgery and chemotherapy, while those of OKT 4 positive cells remained unchanged. Although IL-2 production in PBMC of patients with good prognosis was significantly elevated after chemotherapy, that in PBMC of patients with poor prognosis declined to about a half of pre-operative levels. The ability of PBMC to respond to IL-2 was significantly elevated not only in patients with good prognosis but also in patients with poor prognosis after termination of chemotherapy. On the other hand, the ability of PBMC to absorb IL-2 remained unchanged during the course of treatment.

These findings may contribute to the understanding of tumor-induced immune suppression.

Key words: Gynecologic malignancies, Interleukin 2, OKT 4 and OKT 8 positive cells.

INTRODUCTION

Aberration of cell-mediated immune response with increasing tumor burden is associated with a concomitant decline in the functional capacity of T-lymphocytes. In fact, many investigators have demonstrated declined response of T-cells to mitogens and depressed natural killer (NK) activity of peripheral blood mononuclear cells (PBMC) in cancer patients. The development of immune responses against mitogens is dependent upon a complex interaction of cells belonging to the immune system. These immune responses have been reported as being controlled by a series of soluble growth factors or activating agents designated lymphokines. Many recent studies have revealed that interleukin 2 (IL-2) molecules have a central role in immunity by their capacity to sustain growth of helper and cytotoxic T-lymphocytes. Human T-cells have been separated into two major subsets. One subset bears the differentiation antigen OKT 4 whereas the other has the surface antigen OKT 8. Thus, OKT 4 antibody detects a subset of approximately 65% of peripheral T-cells which contains the helper and inducer T-cells, whereas OKT 8 monoclonal antibody recognizes approximately 35% of peripheral T-lymphocytes and identifies a subset comprising the suppressor and cytotoxic T-cells. Recent studies indicate that IL-2 is important in supporting the immune regulatory functions of T-lymphocytes. It has also been reported that the slow-down or cessation of cytotoxic activity against tumor cells may be due to the absence or perturbation of IL-2.

In the present study, we report low IL-2 production by PBMC in patients with advanced cancer, in addition to reversion of OKT 4/OKT 8 cell ratio and changes of IL-2 production, response to IL-2 and IL-2 absorption during the course of treatment.

MATERIALS AND METHODS

Subjects

The study comprised 8 patients (median age, 47) with stage 0-I cervical cancer, 10 patients (median...
age, 58) with stage IIb-IV cervical cancer, 6 patients (median age, 56) with endometrial cancer, 8 patients (median age, 47) with stage I ovarian cancer, 22 patients (median age, 47) with stage II-IV ovarian cancer and 12 patients (median age, 46) with metastatic ovarian cancer. Twenty-five patients (median age, 46) with benign gynecologic tumor served as controls. All blood samples taken before surgery were used for assays of OKT 4/OKT 8 cell ratio and IL-2 activity. In addition, 14 patients with ovarian cancer received six courses of combination chemotherapy consisting of cisplatin, adriamycin and cyclophosphamide, so-called ‘CAP’. Out of these 14 patients, nine had complete remission for more than 3 months after treatment with the CAP, while the other five patients were dead from recurrent disease by 3–6 months after termination of treatment with CAP. Blood from nine patients with complete remission and five patients with poor prognosis was taken before surgery, 10 days after surgery and 30 days after termination of chemotherapy.

Determination of OKT 4 and OKT 8 positive cells

Immunofluorescence staining of human lymphoid cells with the monoclonal antibodies OKT 4 and OKT 8 (Ortho Pharmaceutical, Raritan, NJ) was performed with an indirect system using monoclonal antibody-containing supernate in the first step and fluorescein-conjugated goat anti-mouse IgG or fluorescein-conjugated goat anti-mouse IgG in the second step. For staining, reagents were centrifuged at 100,000 g before use, and 10^6 target lymphocytes were reacted with saturation levels of first and second step reagents. Cells were stained on ice in the presence of 0.01% NaN3.

IL-2 PRODUCTION

PBMC separated from heparinized blood by the Ficoll-Hypaque density gradient centrifugation method were suspended in RPMI 1640 medium supplemented with 5% fetal calf serum and 1% phytohemagglutinin-P (PHA-P) (Difco Laboratories, Detroit, MI, U.S.A.) to make 10^6 cells ml^-1, and cultured for 48 h at 37°C under 5% CO₂ and humidified atmosphere. The culture supernatant was collected and cryopreserved at −80°C. The IL-2 content of the supernatants was estimated as described by Lotze et al., using the IL-2-dependent cell line CTLL-2. One unit of activity was defined as that quantity of IL-2 which gave half maximum activity in the bioassay using an 18 h incubation and 4 × 10^3 cells well^-1. The unit defined by the Biological Response Modifiers Program (BRMP) standard is approximately equal to 30–50 units in our assay. One BRMP unit is equal to approximately 2.3 Cetus units.

Lymphocyte proliferation assay

A suspension of 2 × 10^5 cells well^-1 of PBMC was delivered to each culture well in a 96-well flat-bottom microculture plate. Test wells received optimal concentrations of PHA (1 μg ml^-1). Either 0.1 ml of purified IL-2 (Cetus Corporation, Emeryville, CA) or medium was added to a final volume of 0.2 ml per culture well. After 96 h incubation (37°C, 5% CO₂), the cultures were pulsed with 1 μCi well^-1 ^3H-thymidine (spec. act. 6.7 Ci mmol^-1; New England Nuclear) for the last 7 h, harvested, and the radioactivity in the cells was counted in a liquid scintillation counter. The results were presented according to the following formula: true response to IL-2 (cpm in incorporated ^3H-thymidine) = response to combination of IL-2 and PHA (cpm) − [response to IL-2 alone (cpm) + response to PHA alone (cpm)].

Measurement of IL-2 receptors

Radiolabeled IL-2 binding to PBMC was performed as described in detail previously. All PBMC were prepared for the assay by centrifugation, followed by incubation at 37°C in IL-2-free RPMI 1640 medium (50 ml l^-1 × 10^7 cells) for two 1 h intervals, to promote dissociation and/or degradation of bound IL-2. These conditions were chosen based upon the dissociation rate constant previously determined for intact cells and isolated plasma membranes (t_{1/2} for dissociation is 25 min). Serial dilutions of [H]leu, lys-IL-2 (50 Ci mmol^-1, ICN, Irvine, CA) were incubated with cells (1 × 10^6 cells per 0.2 ml) in RPMI 1640 medium, 1 mg ml^-1 bovine serumalbumin (BSA) at 37°C. After a 20 min incubation, cold (4°C) RPMI 1640-BSA (1 ml) was added and the cells were centrifuged (9000 g, 15 sec). The supernatant containing the unbound fraction was removed and counted via liquid scintillation. The cell pellet was resuspended in 100 μl cold RPMI 1640-BSA and centrifuged (9000 g, 90 sec) through a 200 μl layer of a mixture of 84% silicone oil and 16% paraffin oil. The tips of the tubes containing the cell pellet were cut off and counted by liquid scintillation to determine the level of bound radioactivity. The calculated values of the number of binding sites per cell were obtained by Scatchard analysis of equilibrium binding data, after subtrac-