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Effects of PSK on Interleukin-2 Production by Peripheral Lymphocytes of Patients with Advanced Ovarian Carcinoma during Chemotherapy

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The effects of PSK on OKT 4/OKT 8 cell ratio, interleukin-2 (IL-2) production and expression of IL-2 receptor were examined in peripheral blood lymphocytes (PBL) from patients with advanced ovarian cancer during the course of chemotherapy. Preoperative levels of OKT 4/OKT 8 cell ratio and IL-2 production in PBL from patients with advanced ovarian cancer were significantly lower than those in cases of benign ovarian tumor. However, the expression of IL-2 receptor did not show any significant difference between ovarian cancer and benign ovarian tumor patients. When a combination chemotherapy of cisplatin, adriamycin and cyclophosphamide was given, the OKT 4/OKT 8 cell ratio was significantly increased with a significant decrease of the absolute number of the OKT 8 cell subset, while the expression of IL-2 receptor and the absolute number of the OKT 4 cell subset remained unchanged. In contrast, the IL-2 production was markedly depressed after the first course of chemotherapy. When PSK was combined with combination chemotherapy, the degree of inhibition of IL-2 production was reduced (though the effect was not statistically significant). If treatment with PSK was initiated after completion of combination chemotherapy, in addition to a significant elevation of OKT 4/OKT 8 cell ratio the depressed IL-2 production was restored to benign control levels. On the other hand, the expression of IL-2 receptor remained unchanged even if PSK was given after completion of chemotherapy.

Key words: PSK — Interleukin-2 — Human ovarian carcinoma — Combination chemotherapy

Debilitation of cell-mediated immune responses during tumor growth is associated with a concomitant decline in the functional capacity of T lymphocytes.[1-3] It is well established that T lymphocytes release a variety of lymphokines upon antigenic or mitogenic stimulation. It has been reported that the slowdown or cessation of cytotoxic activity of lymphocytes against tumor cells may be due to the absence/perturbation of IL-2.[4] A significant decline of IL-2 production during tumor growth was also demonstrated.[5] The cellular requirements for IL-2 production have been extensively investigated. Recently, it has been reported that 50–60% of peripheral T lymphocytes were IL-2 producing cells and about 75% of OKT 4 cells had IL-2-producing capacity whereas about 15% of OKT 8 cells exhibited this functional potential.[6] In most patients with advanced ovarian cancer, cisplatin-based combination chemotherapy has been given after surgery and the survival time has been improved.[7-9] We have already reported that such combination chemotherapy inhibited the NK activity in all cases.[10] In addition, the depressed NK activity was demonstrated to be restored by treatment with PSK. It is well known that IL-2 is one of the most important immunoregulatory lymphokines elaborated by helper T-lymphocytes and NK cells. IL-2 is also a potent augmentor of NK cell tumoricidal activity.[11, 12] These results prompted us to examine the effects of PSK on IL-2 production, expression of IL-2 receptor and OKT 4/OKT 8 cell ratio in PBL of ovarian cancer patients receiving combination chemotherapy. We now report inhibitory effects of combination chemotherapy on IL-2 production and restorative effects of PSK on the depressed IL-2 production.

Abbreviations used: IL-2, interleukin-2; NK, natural killer; PBL, peripheral blood lymphocytes; FCS, fetal calf serum; PHA, phytohemagglutinin; BSA, bovine serum albumin.

Materials and Methods

Subjects The study comprised 32 patients (median age, 48) with benign ovarian tumor and 45 patients (median age, 52) with advanced ovarian carcinoma (5 stage IIc patients, 32 stage III patients and 8 stage IV patients). All patients received 6 courses of combination chemotherapy from 2 weeks after surgery. Blood samples before chemotherapy were taken about 10 days after surgery. One course of combination chemotherapy consisted of cisplatin (30 mg/m^2/day intravenously, on day 1), adriamycin (7 mg/m^2/day intravenously, on days 1–5) and cyclophosphamide (140 mg/m^2/day intravenously, on days 1–5). To examine the effect of combination chemotherapy on immune functions, OKT 4/OKT 8 cell ratio, IL-2 production and expression of IL-2 in PBL before chemotherapy were compared to those 3–5 days after the first course of combination chemotherapy. The effects of the combination of PSK with chemotherapy on the IL-2 production and expression of IL-2 receptor were also compared to those in the case of chemotherapy alone. In addition, to determine the effects of PSK on OKT 4/OKT 8 cell ratio, IL-2 production and expression of IL-2 receptor after completion of combination chemotherapy, PSK was administered po for more than 60 days after completion of chemotherapy and blood samples were collected before, 30 days and 60 days after treatment with PSK.

Immunotherapy PSK (Kureha Chemical Co., Tokyo) was administered po (3.0 g/day) during the course of chemotherapy or after completion of chemotherapy.

Peripheral Blood Lymphocytes (PBL) Blood was collected by venipuncture into heparinized (10 U/ml of blood) tubes. Lymphocytes were separated by centrifugation on a Ficoll Hypaque gradient,[13] washed three times with phosphate-buffered saline (0.15*M*, pH 7.2), counted and resuspended in RPMI 1640 medium supplemented with 10% FCS (Gibco Laboratories, New York, NY) and 2m*M* glutamine.

Determination of OKT 4 and OKT 8 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_1 or fluorescein-conjugated goat anti-mouse IgG_2 in the second step. For staining, reagents were first centrifuged at 100,000*g* before use, and 10^6 target lymphocytes/well in microtiter plates were reacted with saturation levels of first and second step reagents. Cells were stained on ice in the presence of 0.01% NaN_3.

IL-2 Production PBL separated from heparinized blood by the Ficoll-Hypaque density gradient centrifugation method were suspended in PRMI 1640 medium supplemented with 5% FCS and 1% PHA to make 10^6 cells/ml, and cultured for 48 hr at 37° under a humidified 5% CO_2 atmosphere. The culture supernatant was collected and cryopreserved at −80°. The IL-2 content of the supernatants was estimated as described by Gillis *et al.*[14] using the IL-2-dependent cell line CTLL-2.[15] CTLL-2 cells are highly sensitive and specific to IL-2. IL-2 units were calculated using probit analysis by reference to one batch of standard IL-2.

Measurement of IL-2 Receptor Radiolabeled IL-2 binding to whole cells was performed as described in detail previously.[16] All cells were prepared for the assay by centrifugation, followed by incubation at 37° in IL-2-free RPMI 1640 medium (50 ml/10^7 cells) for two 1-hr intervals to promote dissociation and/or degradation of endogenously 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).[16] Serial dilutions of [^{3}H]-leu, lys-IL-2 were incubated with cells (10^6 cells/0.2 ml) in RPMI 1640 medium containing 1 mg/ml bovine serum albumin (BSA) at 37°. After a 20-min incubation, cold (4°) RPMI 1640-BSA (1 ml) was added and the cells were centrifuged (9,000*g*, 15 sec). The supernatant containing the unbound fraction was removed and counted via liquid scintillation. The cell pellet was resuspended in 100 μl of cold RPMI 1640-BSA and centrifuged (9,000*g*, 90 sec) through a 200-μl layer of a mixture of 85% 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 subtraction of the nonsaturable binding determined in the presence of a 150-fold molar excess of unlabeled IL-2. The lower limit of detection of receptor sites per cell was 200.

Results

Absolute numbers of OKT 4- and OKT 8-positive cells, IL-2 production and expression of IL-2 receptor in PBL from patients with advanced ovarian carcinoma were evaluated before surgery and after the course of chemotherapy. Preoperative levels of the OKT 4/OKT 8 cell ratio and IL-2 production in patients with advanced ovarian carcinoma who had an increase of OKT 8-positive cells were significantly lower than those in patients with benign ovarian tumor, while no significant

Table I. Preoperative Levels of OKT 4/OKT 8 Cell Ratio, IL-2 Production and Expression of IL-2 Receptor by PBL from Patients with Benign or Malignant Ovarian Tumor

Patients	N[a]	Age (range)	OKT 4/OKT 8 cell ratio[b]	IL-2 production (units/ml)	Expression of IL-2 receptor (%)[c]
Benign tumor	32	48 (29–77)	2.29±0.8[d] (1031/450)[e]	20.4±8.1	33.8±12.0
Ovarian cancer	45	52 (33–78)	1.61±0.4[f] (1055/564)	10.6±8.4[f]	27.3±17.1

a) Number of cases.
b) OKT 4/OKT 8 cell ratio was calculated as the absolute number of OKT 4 cells/absolute number of OKT 8 cells.
c) Percent of IL-2 receptor-positive cells.
d) Mean±SD.
e) Upper and lower figures in parenthesis show the mean absolute numbers per μl of OKT 4-positive cells and OKT 8-positive cells, respectively.
f) $P<0.05$ (Student's *t*-test), compared to benign tumor.

Table II. Effects of Combination Chemotherapy and PSK on T Cell Subsets

Treatment group	Before chemotherapy[a]		After chemotherapy[b]	
	OKT 4	OKT 8	OKT 4	OKT 8
Chemotherapy alone	796±412[c]	510±344	854±251	397±172[d]
Chemotherapy plus PSK	837±317	481±242	1034±516[d]	361±195[d]

a) Ten days after surgery. Each group consisted of 7 patients.
b) Three to 5 days after the first course of combination chemotherapy.
c) Absolute number of cells per μl (mean±SD).
d) $P<0.05$ (paired *t*-test), compared to before chemotherapy.

change in the expression of IL-2 receptor was observed (Table I). The patients with advanced ovarian carcinoma have been treated with combination chemotherapy after surgery. The combination chemotherapy has shown to suppress profoundly the NK activity.[10] Thus, we attempted to examine the effects of combination chemotherapy on immune functions. Absolute numbers of the OKT 8 cells were significantly decreased, while those of the OKT 4 cells were unchanged 3–5 days after the first course of combination chemotherapy, as compared with those before chemotherapy. If PSK was administered with chemotherapy, in addition to a significant decrease of absolute numbers of OKT 8 cells, those of OKT 4 cells were significantly increased (Table II). On the other hand, the IL-2 production was markedly inhibited after chemotherapy, though the inhibitory effect was reduced by treatment with PSK (Table III). Expression of IL-2 receptor was only slightly (not significant) inhibited after the first course of combination chemotherapy but if PSK was combined with the chemotherapy, expression of IL-2 receptor was not affected (Table III).

Next, to determine the adjuvant effects of PSK with the chemotherapy on the OKT 4/OKT 8 cell ratio, IL-2 production and IL-2 receptor we administered PSK to patients after completion of the chemotherapy. PSK administered after completion of the chemotherapy resulted in a significant elevation of the OKT 4/OKT 8 cell ratio 30 days and 60 days after treatment with PSK (Table IV). Although the IL-2 production in a group not treated with PSK remained unchanged either

Table III. Effects of Combination Chemotherapy and PSK on IL-2 Production and Expression of IL-2 Receptor

Treatment group	Before chemotherapy[a]		After chemotherapy[b]	
	IL-2 production[c]	IL-2 receptor[d]	IL-2 production	IL-2 receptor
Chemotherapy alone	25.6±11.7[e]	35.5±14.2	4.3±1.8[f]	26.2±15.0
Chemotherapy plus PSK	26.3±19.0	48.7±3.6	7.0±3.4[f]	37.5±12.0

a) Ten days after surgery. Each group consisted of 7 patients.
b) Three to 5 days after the first course of combination chemotherapy.
c) Units/ml.
d) Percent of IL-2 receptor-positive cells.
e) Mean±SD.
f) $P<0.001$ (paired *t*-test), compared to before chemotherapy.

Table IV. Changes of OKT 4/OKT 8 Cell Ratio, IL-2 Production and IL-2 Receptor in PBL from Patients Treated with PSK after Completion of Chemotherapy

Treatment[a]	Time after chemotherapy (days)	IL-2 production (U/ml)	IL-2 receptor (%)[b]
Chemotherapy alone	7	7.0±6.4[c]	28.8±13.2
	30	7.7±5.4	34.7±14.5
	60	9.9±6.7	31.1±16.5
Chemotherapy plus PSK	7	7.9±8.4	38.3±11.0
	30	20.6±10.8[d,e]	39.8±13.2
	60	20.1±8.4[d,e]	39.5±12.7

a) Each treatment group consisted of 5 patients who received 6 courses of chemotherapy.
b) Percent of IL-2 receptor-positive cells.
c) Mean±SD.
d) $P<0.01$ (paired *t*-test), compared to 7 days after completion of chemotherapy.
e) $P<0.01$ (Mann-Whitney U test), compared to chemotherapy alone.

30 days or 60 days after completion of the chemotherapy, that in a group treated with PSK was significantly increased both 30 days and 60 days after treatment (Table IV). On the other hand, even if PSK was administered after completion of the chemotherapy the expression of IL-2 receptor did not significantly change either 30 days or 60 days after completion of the chemotherapy.

Discussion

In the present study, we have demonstrated that preoperative levels of OKT 4/OKT 8 cell ratio and IL-2 production in patients with advanced ovarian carcinoma were significantly lower than those in patients with benign ovarian tumor. Similarly, a significantly reduced OKT 4/OKT 8 cell ratio has been observed in patients with multiple myeloma.[17] In the majority of patients a significant decrease of OKT 4-positive cells (helper/inducer phenotype) was found, and this was more evident in patients with advanced clinical stage. Conversely, an increase in the proportion of OKT 8-positive cells (suppressor/cytotoxic phenotype), was found only in stage I patients, while a significant reduction in the absolute number was observed in stage II-III patients compared with stage I patients. In the present study, a significant increase in the absolute number of OKT 8-positive cells was observed in patients with advanced ovarian carcinoma.

In addition, the relationship between tumor growth and depressed IL-2 production was observed in patients with endometrial carcinoma[18] and in mouse with fibrosarcoma.[5] Earlier reports disclosed that IL-2 is involved in the development of cytotoxic T lymphocytes[19,20] and that a host compromised by a tumor has a defective cell-mediated immune response involving these cytotoxic T lymphocytes.[14] In previous work, the existence of tumor-induced suppressor T cells that could inhibit cellular proliferation and IL-2 synthesis has been demonstrated.[5,21] Therefore, IL-2 production in patients with established cancer was considered to be suppressed. A significant elevation of OKT 4/OKT 8 cell ratio after the first course of chemotherapy seemed to have resulted from a significant decrease of absolute number of OKT 8 cells, while the IL-2 production was profoundly suppressed. These results suggest that lymphocytes, which are viable but damaged by chemotherapy, might be losing their functional activities including the production of lymphokines and the response to them. Although the absolute number of OKT 4-positive cells was certainly unchanged, their functional activities were unknown. PSK resulted in only a slight reduction of the inhibition of IL-2 by chemotherapy. On the other hand, the expression of IL-2 receptor was not affected by chemotherapy, suggesting that cells capable of binding to IL-2 are relatively resistant to chemotherapy. In addition, we examined the effects of PSK administered after completion of combination chemotherapy on the OKT 4/OKT 8 cell ratio, IL-2 production and IL-2 receptor. Unless PSK was given after completion of chemotherapy, the OKT 4/OKT 8 cell ratio, IL-2 production and IL-2 receptor remained unchanged at any period after completion of chemotherapy. In contrast, when PSK was given after completion of chemotherapy, the OKT 4/OKT 8 cell ratio as well as the IL-2 production was significantly enhanced 30 days and 60 days after completion of chemotherapy. Although the mode of action of PSK on immunologic systems is not fully understood, it was reported that IL-2 production of spleen cells derived from tumor-bearing mice was enhanced by *in vivo* administration of PSK.[22] PSK administered after completion of chemotherapy seemed to restore the depressed IL-2 productivity. Similarly, previous reports demonstrated that PSK can restore the immune responses suppressed by chemotherapy.[23,24] These results suggest that PSK would be of clinical use as an immunostimulator for ovarian cancer patients with depressed IL-2 productivity receiving combination chemotherapy.

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