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# Suppressor cells in the effector phase of autologous cytotoxic reactions in cancer patients

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**Summary.** Cytotoxicity was induced in lymphocytes (CL) from 10 out of 15 patients by autologous mixed lymphocyte tumor cell culture and further cultivation with recombinant interleukin-2. In cells from 3 of the 10 patients, cytotoxicity was suppressed by more than 50% when autologous peripheral blood mononuclear cells (PBMC) from the patients with large tumors were added to the autologous killing system. The cells responsible for suppressing the cytotoxicity in the effector phase were adherent or nonadherent to plastic depending on the patient examined. The T cell fraction from 1 patient significantly suppressed the cytotoxic activity, and this suppression was seen only in the autologous system. On the other hand, plastic adherent cells but not T cells from PBMC of 2 subjects suppressed the cytotoxic activity of CL. The reason why the main cell population suppressing the CL activity differed among the patients is unclear. However, the findings that the suppression was mostly abrogated following resection of the tumor mass suggested that suppressor cells, either of macrophage lineage or T cells, are induced in patients with a large tumor mass. This speculation is supported by the finding that the PBMC from a patient with tumor recurrence regained the suppressive activity.

## Introduction

Several studies have revealed the presence of suppressor cells that inhibit the immune response against tumors in various phases [4, 10, 15, 16, 17, 18, 21, 26, 27]. The cell populations responsible for the suppression were identified as T lymphocytes and suppressor leukocytes belonging to the monocyte-macrophage lineage. Fujimoto et al. reported the suppression acting in the effector phase in mice. However, suppression in the effector phase (cytolytic phase) of the reaction with human cancer and autologous cytotoxic lymphoid cells has not been reported.

Vanky et al. [22] and Vose and Bonnard [24] found that autologous mixed tumor cell and lymphoid cell culture (MLTC) induced specific killing activity of lymphoid cells against autologous tumors in humans, and further cultivation of the cytotoxic lymphoid cells (CL) with culture medium containing T cell growth factor potentiated the activity and propagated the effector cells. We also succeeded in the induction of CL against autologous cancer cells by MLTC and interleukin-2 (IL-2) culture [8, 9].

In the present study, we observed the direct effect of autologous mononuclear cells on the inhibition of the killing activity in the cytolysis system.

## Materials and methods

### *Patients*

Cancer cells from 15 patients suffering from maxillary (4 cases), nasal (1 case), lingual (1 case), pharyngeal (1 case), laryngeal (5 cases), and parotid (2 cases) tumors (Table 1) were surgically removed and used in the experimental study. In each case, malignancy was diagnosed by histological examination; 14 of the tumors were squamous cell carcinomas and 1 was an adenocarcinoma. Of these patients, 11 were males and 4 were females, and they were between 52 and 92 years old. The patients, except 1 with a recurrent tumor, had received no anticancer drugs or radiation therapy at the time of biopsy or blood sampling.

### *Preparation and cryopreservation of fresh tumor cells*

Fresh tumor tissue was obtained by surgery and transferred to Dulbecco's phosphate-buffered saline (PBS) without calcium or magnesium. After removal of connective tissue, the tumor mass was cut with scissors into pieces 1–2 $mm^3$ in size within 2 h after excision. The pieces were digested in RPMI 1640 medium (M. A. Bioproduct, Walkersville, Md.) containing 800–1000 units/ml Dispase (Godo Susei Co., Tokyo) with gentle agitation by a magnetic stirrer.

The resulting cell suspension was filtered through a stainless steel mesh, washed twice with PBS, overlaid onto Ficoll-Hypaque, and centrifuged at 800 g for 15 min to remove erythrocytes and dead cells. The cells were adjusted to a concentration of $1 \times 10^6$/ml in RPMI 1640. Finally, the cell suspension was layered onto discontinuous gradients of 45% and 60% Percoll (Pharmacia AB, Uppsala, Sweden), and centrifuged at 400 g for 30 min. Lymphocyte-rich mononuclear cells were collected from the 45% interface, and tumor cells from the 60% interface. Over 85% of these cells excluded trypan blue, and 80% to 95% of the viable cells were identified as tumor cells under a light microscope. These tumor cells were resuspended in RPMI 1640 supplemented with 20% heat-inactivated fetal calf serum (FCS) (Kyoto Biken Laboratories, Inc., Kyoto), and

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cryopreserved until use as described elsewhere [8, 9]. Briefly the cells were cooled to 4°C, mixed with gentle shaking with an equal volume of cold 20% dimethyl sulfoxide (Wako Pure Chemical Industries, Ltd., Tokyo) and were then divided into 2 ml aliquots in screw-capped sterile plastic tubes (Sumitomo Bakelite Co., Tokyo). The tubes were then placed in a cooled ethanol bath and put into a deep freezer (−80°C). After 4 h, the tubes were quickly transferred to a liquid nitrogen storage tank where they were kept until required. When needed, the cells were thawed rapidly without shaking in a water bath at 37°C, and diluted dropwise over a 10-min period at room temperature with 10 volumes of RPMI 1640 supplemented with 25 m*M* HEPES (Wako Pure Chemical Industries Ltd., Tokyo), 2 m*M* L-glutamine, 100 unites/ml penicillin G (Meiji Pharmaceutical Co., Tokyo), and 100 μg/ml streptomycin (Meiji Pharmaceutical Co., Tokyo), referred to subsequently as experimental medium (EM), and 20% FCS. This was followed by washing with EM containing 10% heat-inactivated human male serum. In cases where the viability was under 85% after thawing, the cell suspension was overlaid onto Ficoll-Hypaque and centrifuged at 800 g for 15 min to remove dead cells in order to increase the viability to greater than 85%.

*Preparation of autologous peripheral blood lymphocytes*

Blood was taken from cancer patients both before beginning primary therapy and 2–4 weeks afterwards. Mononuclear cells (PBMC) were obtained from the peripheral blood by Ficoll-Hypaque density gradient centrifugation and stored in liquid nitrogen until use. When required, they were thawed in the same manner as the cryopreserved tumor cells. The viability of the thawed PBMC was over 93%.

*Recombinant IL-2*

Recombinant IL-2 (rIL-2) was kindly supplied by Takeda Pharmaceutical Co., Osaka, Japan. Its specific activity was $3.5 \times 10^4$ units/mg, which corresponded to $1.2 \times 10^7$ units/mg when measured against Biological Response Modifiers Program reference reagent human IL-2 (Jurkat) [6].

*In vitro generation of CL*

*(a) Autologous MLTC.* The CL against autologous fresh tumor were induced and propagated according to the procedure previously described [8, 9]. Briefly, tumor cells were treated with mitomycin C (Kyowa Hakko Co., Tokyo) for 45 min at 37°C. After being washed twice, the treated cells were adjusted to $1 \times 10^6$/ml with EM containing 10% heat-inactivated human serum. Fresh noncryopreserved PBMC taken from the patients at 2 weeks after surgery were cocultured with mitomycin C-treated tumor cells in a tissue culture flask (Falcon 3013, Falcon Plastic, Oxnard, Calif.) for 5 days at 37°C, in a 5% $CO_2$ atmosphere. The ratio of stimulating to responding cells was 1 to 10.

*(b) In vitro propagation of CL with rIL-2.* The MLTC-activated lymphocytes were further cultured in the presence of 2.5 units/ml rIL-2 at 37°C in a 5% $CO_2$ atmosphere for 12 days. For continuous culture, the cells were collected and resuspended in fresh medium containing 2.5 units/ml rIL-2 every 3 days.

*Cytotoxicity assay*

A 4-h $^{51}Cr$ release assay was used to estimate cytolysis of fresh tumor cells. Fresh tumor cells were incubated with 100 μCi of $Na^{51}CrO_4$ (Daiichi Radioisotope Laboratories, Tokyo) for 60 min in 0.5 ml EM and then washed 4 times at 4°C. The $^{51}Cr$-labeled cells at a concentration of $1 \times 10^4$/well were mixed with $4 \times 10^5$ effector cells/well in round-bottomed wells of multiwell microtiter plates (Linbro, Flow Laboratories, McLean, Va.).

The plates were centrifuged at 200 *g* for 5 min and incubated for 4 h at 37°C in a 5% $CO_2$ atmosphere. The supernatant was harvested using the Titertek Collecting System (Flow Laboratories) and radioactivity was counted in a gamma scintillation counter. Spontaneous release (SR) was estimated by incubation of labeled target cells with EM alone. Maximum release (MR) was measured by incubation of fresh tumor target cells with 1% Triton X-100. The SR from the fresh tumor targets was in the range of 23% to 37%. Percent cytotoxicity was calculated as follows:

$$\% \text{ Cytotoxicity} = \frac{E - SR}{MR - SR} \times 100$$

In this expression, E referred to counts per minute of the tested supernatant. All determinations were made in triplicate, and the results expressed as the mean ± SD. The statistical significance of differences in cytotoxicity was calculated by means of Student's *t*-test. Lymphocytes cultured in EM containing 10% heat inactivated human male serum alone served as the control. Control lymphocytes always showed less than 5% cytotoxicity. A value greater than 11% was considered to be significant ($P < 0.01$).

*Assays for suppression of cytotoxicity*

To assay the suppressive activity of PBMC from cancer patients, $4 \times 10^5$ PBMC, $8 \times 10^4$ plastic adherent cells or $4 \times 10^5$ plastic nonadherent, and nylon wool-passed cells were added to a mixture of $4 \times 10^5$ activated lymphoid cells and $1 \times 10^4$ $^{51}Cr$-labeled target cells in a final volume of 0.2 ml in the microtiter plates. The mixture was incubated at 37°C as described in the cytotoxicity assays, and the radioactivity released from the target cells measured. Plastic adherent cells were isolated according to the method described by Kumagai et al. [12]. Briefly PBMC suspended at a concentration of $2 \times 10^6$/ml in medium containing 10% FCS were placed in 100 × 20-mm plastic Petri dishes (Falcon, Becton, Dickinson and Co., Oxnard, Calif.) which had been coated overnight with heat-inactivated FCS, and incubated for 1 h at 37°C in a 5% $CO_2$ atmosphere. After incubation, medium containing nonadherent cells was decanted and the dish rinsed five times with RPMI 1640/10% FCS.

Adherent cells were then removed from the dish by a 15-min incubation at 4°C with PBS containing 0.2% ethylenediaminetetraacetic acid and 5% FCS. Cells recovered by this technique were determined to be 71%–85% monocyte-macrophage lineage as judged by phagocytosis assays of Baker's yeast [13].

Nylon wool fractionation was carried out as described by Julius et al. [11]. Briefly 0.3 g of sterile nylon wool (Wako pure Chemical Industries, Ltd., Tokyo) was put into the barrel of a 5 ml plastic syringe fitted with tweezers. The column was moistened with 5% FCS-containing EM and incubated at 37°C for 60 min. Plastic nonadherent cells (2

**Table 1.** Cytotoxicity of autologous CL against tumor cells and inhibitory effect of autologous PBMC on the cytotoxicity[a]

| Case | Age (years) | Diagnosis (histology) | NonPBMC added | PBMC added | |
|---|---|---|---|---|---|
| | | | % Cytotoxicity[b] | % Cytotoxicity | (% inhibition) |
| A | 66 | Maxillary cancer (Scc)[c] | 13.6±0.4 | −0.5±1.5 | (103) |
| B | 70 | Laryngeal cancer (Scc) | 25.4±2.2 | 2.9±0.8 | (88) |
| C | 92 | Parotid gland cancer (Scc) | 18.5±1.2 | 7.5±2.3 | (59) |
| D | 60 | Maxillary cancer (Scc) | 16.3±1.9 | 9.9±2.1 | (39) |
| E | 81 | Nasal cancer (Scc) | 31.0±2.5 | 20.8±2.0 | (32) |
| F | 61 | Lingual cancer (Scc) | 20.4±5.3 | 15.1±0.7 | (25) |
| G | 60 | Maxillary cancer (Scc) | 13.2±3.0 | 10.6±3.8 | (19) |
| H | 62 | Lingual cancer (Scc) | 32.2±2.5 | 26.4±2.4 | (18) |
| I | 52 | Pharyngeal cancer (Scc) | 35.4±2.7 | 29.0±3.1 | (18) |
| J | 72 | Parotid gland cancer (Ad.Ca)[d] | 16.3±4.3 | 15.3±2.4 | (6) |
| K | 66 | Laryngeal cancer (Scc) | 7.2±3.6 | | |
| L | 56 | Laryngeal cancer (Scc) | −0.3±2.5 | | |
| M | 72 | Laryngeal cancer (Scc) | 3.0±2.5 | | |
| N | 56 | Laryngeal cancer (Scc) | 6.3±3.7 | | |
| O | 67 | Laryngeal cancer (Scc) | −1.6±2.5 | | |

[a] Cytotoxic lymphocytes (CL) were induced by autologous mixed cell culture (MLTC) for 5 days and further cultivation with 2.5 units/ml of recombinant interleukin-2 (rIL-2) for 12 days

[b] Percentage specific $^{51}Cr$ release after a 4-h coincubation with CL at an effecter: target cell ratio of 40:1. Data are presented as mean ± SD of triplicate determinations. Positive cytotoxicity occurrend when percentage specific lysis was more than 11.0%.

[c] Squamous cell carcinoma

[d] Adenocarcinoma

$\times 10^7$ in lml of EM) were then added to the nylon wool and allowed to flow into it before subsequent addition of 0.5 ml of warm medium. The PBMC were incubated on the nylon wool for 30 min at 37° C and then eluted with warm medium at a flow rate of 2 ml/min, followed by two washes. OKT3-positive cells made up 92%–95% of the cells eluted, according to analysis with an Ortho Spectrum III laser Flow Cytometry System (Ortho Diagnostic System, Inc., Westwood, Mass.).

## Results

### *Cytotoxicity of autologous MLTC-activated lymphocytes cultured with rIL-2*

According to the results from preliminary experiments, subsequent cultures were taken with EM containing 10% heat-inactivated human male serum and 2.5 units/ml rIL-2. The PBMC from 15 cancer patients (Table 1) were cultured with mitomycin C-treated autologous tumor cells for 5 days and further cultured with rIL-2 for 12 days. We tested the PBMC for cytotoxicity against autologous tumor cells at various effector/target (E/T) ratios in 7 cases (Fig. 1). Since a 40:1 E/T ratio appeared adequate, we then tested 8 additional cases at a 40:1 E/T ratio. Table 1 shows that lymphocytes from 10 of the 15 patients (66%) were activated with autologous tumor cells and differentiated to CL. The CL from these 10 cases were used at a 40:1 E/T ratio for the following suppression experiments.

### *Suppressive effect of PBMC on cytotoxicity*

To examine the suppressive activity of PBMC from cancer patients, the same number of PBMC as already-present effector cells were added to the cytotoxic phase in the reaction of CL with autologous tumor cells. Suppression of the cytotoxicity was observed as shown in Table 1. Cases A, B, and C showed markedly high suppression (higher than 50%), and cases D, E, and F moderate suppression (higher than 25%).

### *Effect of PBMC alone against autologous tumor cells*

Spontaneous release of radioisotope from labeled tumor cells both in the presence and absence of putative suppressor cells (PBMC) was examined and compared. We did not find anything significant (Table 2), which may suggest that protection or stabilization of tumor is not a possibility.

### *Nature of the suppressor cells*

Because the number of tumor cells and/or PBMC cryopreserved was limited, the experiments to determine the fraction responsible for the suppression were performed only in cases A, B, and F. As demonstrated in Table 3, the whole PBMC fraction of case A completely suppressed the CL activity against autologous tumor cells. We next fractionated the PBMC using plastic dishes and nylon wool columns, and each fraction was added to the cytotoxic phase. Adherent cells did not suppress, while both nonadherent cells and nonadherent T cells which had passed through nylon wool and contained 92%–95% OKT 3-positive cells were able to suppress. These cells were responsible for the suppressive effect in case A. When PBMC from case A were added to the cytolysis system of case D or I, the suppressive effect was not observed, suggesting that the suppressor T cell in this case specifically acted only in the autologous system (Table 4). On the other hand, the cells responsible for the suppression of autologous CL activity in case B were mainly plastic-adherent cells, while nylon wool-passed, nonadherent cells showed only minor suppression that was not significant. These results indicated that the suppressor activity in case B was due to monocyte-macrophages.

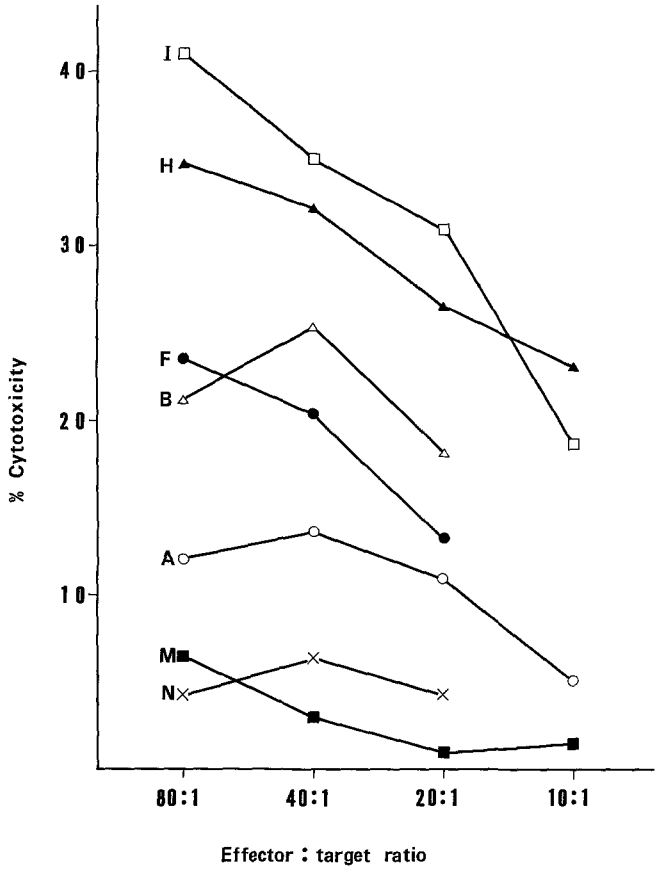


**Fig. 1.** Cytotoxicity of autologous MLTC-activated lymphocytes cultured with rIL-2. Cytotoxicity was measured by a 4-h $^{51}Cr$ release assay and each value is the mean of triplicate determinations

**Table 2.** Effect of PBMC alone against autologous tumor cells

| Case | Spontaneous $^{51}Cr$ release | | |
|---|---|---|---|
| | NonPBMC added | PBMC before treatment added | PBMC after treatment added |
| A | 880±50[a] (31)[b] | 876±37 (35) | 880±57 (37) |
| B | 401±24 (34) | 407±37 (35) | 433±54 (37) |
| C | 1,315±46 (23) | 1,317±59 (24) | 1,328±83 (23) |
| D | 440±28 (26) | 421±19 (25) | N. D |
| E | 304± 9 (27) | N. D[c] | N. D |
| F | 390±27 (34) | 410± 5 (35) | N. D |

[a] Data are presented as mean ± SD of triplicate determinations
[b] Numbers in parenthesis indicate the percentage spontaneous $^{51}Cr$ release
[c] Not done

The PBMC from case F suppressed CL activity only 25.9%. However, the plastic-adherent cells of case F increased suppression of the CL activity (62.7%). The reason why PBMC from case F had a relatively weak suppressive effect may be that the monocyte population was smaller in the unfractionated PBMC ($6 \times 10^4/4 \times 10^5$), and the nonadherent cells of case F had no suppressive effect on the CL activity.

### *Tumor mass carried by patients as a factor inducing suppressor cells*

The PBMC used for the experiments described so far were obtained from patients who had not received any treatment for their cancer, indicating that the patients carried a

**Table 3.** Suppressive activity of adherent and nonadherent cells on cytolysis of autologous CL against tumor cells

| Cytolysis system[a] | | | Suppressor cells[b] (cell number/well) | | % Cytotoxicity[c] | % Inhibition |
|---|---|---|---|---|---|---|
| CL from | Target from | PBMC from | | | | |
| Case A | Case A | Case A | – | | 13.6±0.4 | – |
| | | | PBMC whole | ($4 \times 10^5$) | −0.5±1.5[g] | 103.6 |
| | | | Adherent[d] | ($8 \times 10^4$) | 12.0±0.2 | 11.7 |
| | | | Nonadherent[e] | ($4 \times 10^5$) | 2.8±3.9[g] | 79.4 |
| | | | Nylon wool[f]-passed | ($4 \times 10^5$) | −0.1±2.3[g] | 100.7 |
| Case B | Case B | Case B | – | | 25.4±2.2 | – |
| | | | PBMC whole | ($4 \times 10^5$) | 2.9±0.8[g] | 88.5 |
| | | | Adherent[d] | ($8 \times 10^4$) | 0.6±2.1[g] | 97.6 |
| | | | Nonadherent[e] | ($4 \times 10^5$) | 15.7±2.2 | 38.1 |
| | | | Nylon wool[f]-passed | ($4 \times 10^5$) | 15.7±3.8 | 38.1 |
| Case F | Case F | Case F | – | | 20.4±5.3 | – |
| | | | PBMC whole | ($4 \times 10^5$) | 15.1±0.7 | 25.9 |
| | | | Adherent[d] | ($8 \times 10^4$) | 7.6±1.0[h] | 62.7 |
| | | | Nonadherent[e] | ($4 \times 10^5$) | 21.4±1.6 | −4.9 |
| | | | Nylon wool[f]-passed | ($4 \times 10^5$) | 20.5±2.2 | −0.4 |

[a] $4 \times 10^5$ CL and $1 \times 10^4$ $^{51}Cr$-labeled autologous tumor cells were mixed together and incubated for 4 h
[b] PBMC obtained from each case before treatment
[c] Percentage cytotoxicity is presented as mean ± SD of triplicate determinations
[d] PBMC which adhered to a Petri dish during a 60-min incubation at 37°C
[e] PBMC which did not adhered to a Petri dish during a 60-min incubation at 37°C
[f] Nonadherent cells were further purified by passage through a nylon wool column after a 30-min incubation at 37°C
[g] $P < 0.01$ compared with controls (without suppressor cells)
[h] $P < 0.05$ compared with controls (without suppressor cells)

**Table 4.** Inhibitory effect of allogeneic PBMC on autologous cytotoxicity

| Cytolysis system[a] | | Added PBMC[b] from | % Cytotoxicity[c] | % Inhibition |
|---|---|---|---|---|
| CL from | Target from | | | |
| Case A | Case A | – | 13.6±0.4 | – |
| Case A | Case A | Case A | −0.5±1.5 | 103.6 |
| Case D | Case D | – | 16.3±1.9 | – |
| Case D | Case D | Case A | 15.1±3.2 | 7.3 |
| Case I | Case I | – | 35.4±2.7 | – |
| Case I | Case I | Case A | 32.3±2.2 | 8.7 |

[a] $4 \times 10^5$ CL and $1 \times 10^4$ $^{51}$Cr-labeled autologous tumor cells were mixed together and incubated for 4 h

[b] $4 \times 10^5$ PBMC obtained from case A before treatment were added to autologous cytotoxic reaction system of cases A, D, and I

[c] $\left(1 - \frac{\%\text{ specific lysis with PBMC}}{\%\text{ specific lysis without PBMC}}\right) \times 100$

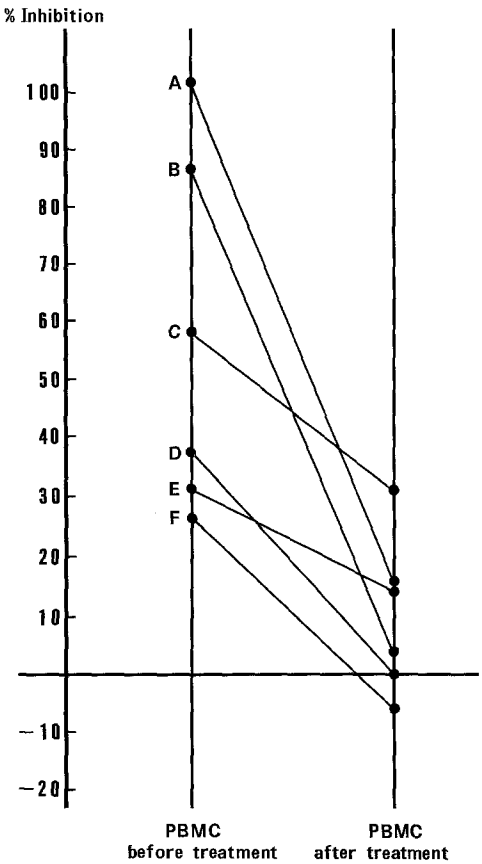


**Fig. 2.** Suppressive effect of peripheral blood mononuclear cells (PBMC) before and after removal of the tumor. $4 \times 10^5$ PBMC, $4 \times 10^5$ CL, and $1 \times 10^4$ $^{51}$Cr-labeled autologous tumor cells were mixed together and incubated for 4 h. Each value is the mean of triplicate determinations. Suppressive effects were reduced in all 6 cases after treatment

large tumor mass. In addition, the suppression experiments were performed using cryopreserved PBMC from the same patients at 2–4 weeks after surgical removal of the tumor mass, and these suppressive effects were compared. As shown in Fig. 2, suppressive effects were reduced in all 6 cases after treatment. In addition, the suppressive effect of

**Table 5.** Inhibitory effect of PBMC at the time of recurrent tumors in patient D[a]

| Added PBMC from | % Cytotoxicity[b] | % Inhibition |
|---|---|---|
| – | 16.3±1.9 | – |
| Before treatment | 9.9±2.1[c] | 39.2 |
| After treatment | 16.3±3.1 | 0 |
| At recurrence | 11.4±1.2[c] | 30.0 |

[a] $4 \times 10^5$ PBMC from patient D before treatment, 14 days after surgery, and at recurrence, were added to autologous $4 \times 10^5$ CL and $1 \times 10^4$ $^{51}$Cr-labeled autologous tumor cells, and incubated for 4 h

[b] Data are presented as mean ± SD of triplicate determinations

[c] $P < 0.01$ compared with controls (without PBMC)

PBMC when recurrence of cancer was found in case D was tested. We used the same autologous tumors obtained PBMC were the only cells obtained at different states (preoperation postoperation, and at recurrence). Recurrence of the suppressive effect was observed in the PBMC at recurrence (Table 5). These results suggested that clinically detectable tumor mass may be a factor inducing suppressor cells to CL activity in the effector phase.

## Discussion

In the present study, we demonstrated that PBMC from cancer patients suppress the autologous CL activity against tumor cells, the cells responsible for suppressing CL cytotoxicity were adherent and nonadherent to plastic depending on the case examined. Suppressive activity was generated by monocyte-macrophages in cases B and F, and by T lymphocytes in case A. Suppressor macrophages have been observed in both human tumors [2, 10, 29] and various animal tumors [1, 7, 21]. In most cases, the suppressive activity appeared to be more pronounced against the induction phase of immune responses [14, 17, 21] and lymphoproliferative responses [7, 10, 23, 26]. In these studies, suppressor activities were analyzed in animal hosts bearing highly immunogenic tumors induced by viruses or chemicals, and the effector phase was generally not affected [5, 17, 20]. On the other hand, splenic macrophages of tumor-bearing mice exerting suppression cytotoxicity in the effector phase have been recently reported [3]. And, the monocyte-macrophage lineage observed in our studies clearly suppressed the effector limb of autologous tumor killing. This discrepancy may be caused by the differences of tumor antigens.

Suppressor T cells are also known to modulate T cell interaction in cancer patients and animal models [4, 15, 16, 19, 28, 29]. In animal models specific suppressor T cells at the effector limb have been reported. T lymphocytes from case A examined in our experiments suppressed the autologous CL activity. It is still unclear why the suppressor cell population induced in patients with a malignant tumor differs from case to case. Factors such as antigenic differences, size of tumor mass, produced substances of necrosis, and so on, may have a causal relationship with the character of the tumor. Furthermore, the physical condition of the patients, such as immune responsiveness, nutrition, and so on may also be responsible. Although the pri-

mary site of case A was maxillary while that of case B was the larynx, the histological findings in both cases were the same; namely squamous cell carcinoma. The stage of the malignancy was $T_3N_0M_0$ in case A and $T_3N_1M_0$ in case B, meaning that case B was in a more advanced state. On the other hand, clinical laboratory findings were not significantly different between the two cases, including percentage peripheral blood lymphocytes, serum immunoglobulin level, and percentage and absolute number of the T cell subset. There were also no marked differences in the general condition of the two patients. At present, we have not found any significant differences between the two cases except cervical lymph node metastasis which affected case B but not case A. However, the large tumor mass carried by the patient is probably a factor inducing suppressor cells, either monocyte-macrophage or T cell [25].

The effect of cryopreservation must be considered, since it is known to affect certain regulatory cells [17]. The PBMC obtained preoperatively and postoperatively were cryopreserved and thawed simultaneously when the suppressive study was performed. We therefore think that it is possible to compare the suppressive effect in both conditions.

It is still unclear whether the suppressive effects are directed to target cells or effectors. Spontaneous release of radioisotope from labeled tumor tagets both in the presence and absence of putative suppressor cells (PBMC) was not significant which leads us to believe that protection or stabilization of the tumor target cells was not a possibility. Rather, suppression took place in the effector region.

The CL induced by autologous MLTC and rIL-2 culture consisted of $OKT3^+8^+$ and $OKT3^+4^+$ cells [9]. The CL from the cases reported in this paper also probably consisted of at least 2 populations ($OKT3^+8^+$ and $OKT3^+4^+$). The significantly high suppressive activity (97.6% and 100.7% suppression) both in monocyte-macrophages and in T cells suggested that the suppression was effective on total cytolysis including cytotoxicity of $OKT3^+8^+$ and $OKT3^+4^+$ cells.

The suppression in the effector phase of the cytolytic activity of in vitro induced killer cells against the autologous tumor may be an important factor to be considered when using killer cells for adoptive immunotherapy in patients with large tumor masses.

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