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A Correlation between the Expression of CD 8 Antigen and Specific Cytotoxicity of Tumor-infiltrating Lymphocytes

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Tumor-infiltrating lymphocytes (TIL) from six gynecologic malignant tumors (two uterine cervical cancers, two ovarian serous cystadenocarcinomas, and two uterine corpus cancers), cultured in the presence of recombinant interleukin 2, were assayed for their cytotoxic activities against various fresh tumor cells including autologous tumors. A clear correlation between phenotype and cytotoxic activity of TIL was observed. Four of six TIL preparations exhibited strong cytotoxic activity against autologous fresh tumor target cells, and were all $CD8^+$. In contrast, cytotoxic activity was not detected in any of the $CD4^+$ TIL preparations. The cytotoxic activities of the $CD8^+$ TIL preparations were highly specific; only autologous fresh tumor cells were lysed. This result is consistent with the notion that TIL are of a different cell lineage from lymphokine-activated killer cells which are antigen-nonspecific and $CD8^-$. Instead, TIL appear to be of cytotoxic T cell lineage that is highly antigen-specific and $CD8^+$. To explore the potential for clinical use, we have attempted to augment the cytotoxic activities of these $CD8^+$ TIL by treatment of the target tumor cells with gamma interferon (IFN) *in vitro*, hoping that elevated expression of MHC class I gene products on the cell surface would enhance their recognition. It was observed that brief treatment of freshly prepared tumor cells *in vitro* with gamma-IFN resulted in augmentation of the expression of MHC class I gene products, and the treated tumor cells were more susceptible to lysis by TIL than untreated cells.

Key words: Major histocompatibility complex — CD8 — Tumor-infiltrating lymphocytes — Immunotherapy

The prime goal of cancer immunotherapy is the identification and adoptive transfer of selected immune cells with specific antitumor activity into a tumor-bearing patient to mediate cancer regression. Recently, it has been demonstrated experimentally that the adoptive transfer of lymphokine-activated killer (LAK) cells plus interleukin 2 (IL-2) can mediate tumor regression in a variety of animal and human tumors.[1-6] However, this approach requires a large number of lymphocytes for transfer into humans along with the systemic administration of a high dose of IL-2, which always causes serious side effects. It is known that many human tumors are infiltrated with chronic inflammatory cells, including lymphocytes. Rosenberg *et al.* recently identified a population of lymphoid cells infiltrating murine tumors that could be expanded *in vitro* in the presence of IL-2 and were capable of eliminating established pulmonary metastases.[7] In the present study we have examined the immunobiology of IL-2-expanded tumor-infiltrating lymphocytes (TIL) obtained from 6 freshly resected human gynecologic cancer specimens and have tested the effect of treatment of tumor cells with gamma interferon (IFN) on the effectiveness of TIL in mediating antitumor activity in short-term ^{51}Cr release assays.

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MATERIALS AND METHODS

Preparation of tumor cell suspensions Tumor cell suspensions were prepared by mincing freshly resected tumor nodules into pieces of about 5 mm^3, then digesting them for 6 h in RPMI 1640 containing 0.01% hyaluronidase type V (1500 U/g), 0.1% collagenase type IV (163 to 230 U/g), and 0.002% deoxyribonuclease type I (100 U/g) (Sigma Chemical Co., St. Louis, MO). The cells were washed twice with Hanks' balanced salt solution without Ca^{2+} and Mg^{2+}, then viable cells were counted and placed in 24-well tissue culture plates in complete culture medium, or frozen in 90% human type AB serum with 10% dimethyl sulfoxide. Approximately 20 to 40% of the cells from the tumor digest appeared to be lymphocytes by microscopic examination.

Culture conditions Complete culture medium was RPMI 1640 containing 2% human AB serum, 2 m*M* glutamine, 50 U/ml penicillin, 50 μg/ml streptomycin, 50 μg/ml gentamicin (M.A. Bioproducts, Walkersville, MD), and 200 U/ml of recombinant IL-2 (Shionogi & Co., Ltd.). Cells were cultured for 7 to 13 days at 1.25 to 2.5×10^5 cells/ml in a humidified atmosphere containing 5% CO_2 at 37°C.

LAK cell induction The techniques for induction of LAK cells have been described in detail.[1-6] In brief,

lymphocytes were separated using Ficoll-Hypaque density gradients, washed and suspended in RPMI 1640 medium containing antibiotics, 2% heat-inactivated human AB serum and 1,000 U/ml recombinant IL-2 (rIL-2). Lymphocyte culture was set up in 75 cm^2 flasks at the cell concentration of 1×10^6 cells/ml and the flasks were incubated at 37°C in an atmosphere containing 5% CO_2 for four days.

Chromium-release assay of cytotoxicity A 4-h chromium (^{51}Cr)-release assay was performed by using, as target cells, cryopreserved preparations of fresh human tumor cells obtained by enzymatic digestion that were thawed and cultured in RPMI 1640 containing 10% human AB serum with or without human recombinant gamma interferon (Shionogi & Co., Ltd.) for 24 h. Single cell suspensions were prepared from monolayers of tumor cells by brief incubation with trypsin at 37°C, and washed twice in the medium, then 200 μCi $Na_2O_2{}^{51}Cr$ was added to each target cell preparation. Incubation was performed for 60 to 90 min on a rocker platform at 37°C. The cells were then washed three times in RPMI 1640 plus 2% AB serum, incubated at 37°C for another 30 min, washed, filtered through a gauze, counted, and resuspended at 10^5 cells/ml. Targets (5×10^3) were added to various numbers of effector cells in round-bottomed 96-well plates. Target cells incubated in medium alone or 2% Triton X-100 were used to determine spontaneous and maximum release of chromium, respectively. After centrifugation at 57g for 5 min, the plates were incubated for 4 h at 37°C, then harvested with a Skatron-Titertek System (Skatron AS, Lierbyen, Norway) and counted in a gamma counter. The percentage of lysis was calculated as follows:

$$\frac{(\text{experimental cpm}) - (\text{spontaneous cpm})}{(\text{maximal cpm}) - (\text{spontaneous cpm})}\times 100.$$

All determinations were made in triplicate. In all experiments the spontaneous release did not exceed 38%. IL-2 and recombinant human gamma-interferon were supplied by Shionogi & Co., Ltd., Osaka.

Fluorescence-activated cell sorter (FACS) analysis Cells were washed in Hanks' balanced salt solution without phenol red containing 1% fetal calf serum and 0.2% sodium azide at 4°C. Cells (1 to 2×10^5/tube) were stained with the appropriate fluorescein isothiocyanate (FITC)-labeled monoclonal antibody (Becton Dickinson Japan, Tokyo), incubated at 4°C for 45 min, washed twice, and resuspended in 0.5 ml of medium for FACS analysis. The antibodies used, the mouse immunoglobulin isotype, type of cell recognized, and antigen cluster designation (if assigned) were as follows: Leu-4 (IgGl,

Table I TIL Isolated from Human Gynecological Cancer

Patient	Age	Clinical diagnosis	Histopathology	Source of TIL	PS[a)]	Total days in culture
1-C	48	Uterine cervical cancer IIa	Squamous cell carcinoma	Tumor	0	57
2-C	39	Uterine cervical cancer IIb	Squamous cell carcinoma	Tumor	0	48
3-C	48	Uterine cervical cancer IIb	Squamous cell carcinoma	Tumor	0	63
4-E	47	Uterine corpus cancer II	Adenocarcinoma	Tumor	0	52
12-E	78	Uterine corpus cancer Ia	Adenocarcinoma	Tumor	1	72
14-E	58	Uterine corpus cancer Ia	Adenocarcinoma	Tumor	1	39
15-O	63	Ovarian cancer Ia	Serous cystadenocarcinoma	Tumor	0	62
16-E	65	Uterine corpus cancer II	Adenocarcinoma	Tumor	0	34
17-E	57	Uterine corpus cancer Ib	Adenocarcinoma	Tumor	0	45
18-E	63	Uterine corpus cancer II	Adenocarcinoma	Tumor	0	50
20-O	55	Ovarian cancer II	Serous cystadenocarcinoma	Tumor	0	39
24-S	27	Uterine sarcoma III, recurrence	Endometrial stromal sarcoma	Tumor	1	51
31-O	69	Ovarian cancer IV, recurrence	Serous cystadenocarcinoma	Pleural effusion	3	62
32-C	34	Uterine cervical cancer Ib	Squamous cell carcinoma	Tumor	0	71
34-S	51	Uterine sarcoma IV, recurrence	Leiomyosarcoma	Tumor	2	55
35-O	36	Ovarian cancer Ic	Serous cystadenocarcinoma	Tumor	0	48
40-O	44	Ovarian cancer II	Serous cystadenocarcinoma	Tumor	0	87
42-O	79	Ovarian cancer III	Serous cystadenocarcinoma	Tumor	2	73
43-O	52	Ovarian cancer III	Serous cystadenocarcinoma	Tumor	1	58
47-O	74	Ovarian cancer IV	Serous cystadenocarcinoma	Ascites	3	51
50-O	20	Ovarian cancer Ib	Dysgerminoma	Tumor	0	54

a) Performance status.

mature T cells, CD3), Leu-3a (IgG2a, class II-restrictive T helper/inducer cells, CD4), Leu-2b (IgG2a, class I-restrictive T cytotoxic/suppressor cells, CD8), and HLA-DR (IgG2a, B cells, monocytes, macrophages, and activated T cells).

RNA blot analysis Poly(A)$^+$ RNA was isolated as previously described.[8] Aliquots of RNA were adjusted to 50% formamide, 20 m*M* morpholine-propanesulfonic acid (MOPS, pH 7.0), 5 m*M* sodium acetate, 1 m*M* EDTA and 2.2 *M* formaldehyde, heated at 60°C for 10 min, and subjected to electrophoresis on a 1.0% agarose gel containing 2.2 *M* formaldehyde. The running buffer was 20 m*M* MOPS (pH 7.0), 5 m*M* sodium acetate, 1 m*M* EDTA; and electrophoresis was performed at 35–40 mA for 4 h at 4°C. The RNA was transferred to a nitrocellulose filter as described.[8] The conditions for hybridization have been described.[8]

Table II. Phenotypic Flow Cytometry Analysis of Cultured TIL from Tumor Tissues or Ascites in Patients with Gynecological Cancer

Patient	% of positive TIL				Days in culture
	CD3$^+$	CD4$^+$	CD8$^+$	HLA-DR$^+$	
1-C	96.3	6.0	92.4	98.9	31
2-C	92.4	8.0	94.1	91.3	29
3-C	88.4	11.3	88.5	94.7	28
4-E	96.0	3.6	94.3	90.4	30
12-E	97.4	8.7	90.2	92.5	33
14-E	89.4	12.2	80.6	89.0	29
15-O	94.8	5.5	97.6	88.1	34
16-E	90.3	34.5	63.7	90.6	29
17-E	89.7	5.9	94.5	86.4	30
18-E	93.4	93.7	4.3	91.2	32
20-O	92.6	8.6	89.4	90.3	29
24-S	97.5	92.8	5.4	91.0	34
31-O	93.6	7.1	77.5	85.7	28
32-C	97.7	94.5	6.9	84.9	29
34-S	89.1	90.0	14.4	79.9	33
35-O	97.4	3.2	94.6	91.0	32
40-O	80.8	5.2	92.5	80.6	29
42-O	90.2	68.6	37.1	91.9	30
43-O	86.4	15.3	67.6	79.8	34
47-O	91.2	79.3	10.4	92.7	29
50-O	81.6	13.7	73.1	78.9	28

Phenotype of TIL was determined by FACS analysis performed with a 488-nm argon laser on a Becton-Dickinson FACS. FITC-labeled monoclonal antibodies were purchased from Becton-Dickinson Japan and used at the appropriate dilution. Leu-4 recognizes mature T cells (CD 3); Leu-3 recognizes class I restrictive cytotoxic/suppressor T cells (CD 8); Leu-2 recognizes class II restrictive helper/suppressor inducer T (CD 4) cells; Leu-DR recognizes B cells, monocytes, macrophages and activated T cells.

RESULTS

We have propagated lymphocytes in the presence of rIL-2 (TIL) from fresh tumor tissues of twenty-one patients with various gynecologic malignancies. Fifteen of twenty-one individuals were at stage I or II of the disease and six were at an advanced stage (stage III, IV or recurrence) (Table I).

First, to observe the changes in the cellular surface phenotype of TIL expanding in rIL-2, flow cytometry was performed on cultured TIL (Table II). In all TIL preparations, the CD3$^+$ T lymphocytes were clearly a major cell type and many of these were activated T lymphocytes as judged by the expression of the HLA DR antigen. However, fifteen preparations of TIL were CD3$^+$CD8$^+$ dominant and six were CD3$^+$CD4$^+$. The majority of TIL showed homogeneous populations as evidenced by a single fluorescent cell peak, though some were heterogeneous.

To examine the cytotoxic properties of TIL, preparations expressing homogeneous population from six patients at early stages of disease (stages I and II) were selected and tested in a 4-h ^{51}Cr release assay at 20–30 days after cultivation. After recovery from storage in liquid nitrogen, the majority of cell populations (from 70 to 85%) was confirmed to be tumor cells by morphological and histochemical analysis employing monoclonal antibodies to squamous cell carcinoma antigen (SCC)[9] in the cases of 1-C and 32-C and to CA125 antigen[10] in the cases of 15-O and 35-O (data not shown). TIL growing in cultures supplemented with rIL-2 were tested in a 4-h ^{51}Cr release cytotoxicity assay during the same time period as cell surface marker analysis (Table III). TIL from patient 1-C showed high cytotoxic activity against only autologous tumor cells, not allogeneic tumor cells, from patient 2-C or 3-C even though these three tissues shared the same pathological phenotype. In two out of six preparations (15-O and 17-E), the cytotoxicity against autologous normal fibroblasts was extremely low compared to that against tumor cells. The cytotoxicity of LAK cells from patient 17-E against autologous tumor cells was 26.3%, 17.1% and 5.3%, while that of TIL from the same patient was 49.4%, 55.7% and 20.4%, at E/T ratios of 50:1, 15:1 and 5:1, respectively. As LAK cells have been shown to be capable of lysing a broad panel of fresh tumor cells in short-term ^{51}Cr release assay,[1-6] LAK cells from PBL incubated for four days with IL-2 were employed as positive controls for the lysis of fresh tumors. However, cultured TIL from patients 18-E and 32-C showed weak cytotoxicity towards either autologous or allogeneic tumor cells. Thus, we examined the correlation between cytotoxicity against autologous tumor cells and the phenotype of cultured TIL. An analysis of cell surface markers employing monoclonal

antibodies showed that anti-CD8 antibody reacted strongly with TIL from patients 1-C, 15-O, 17-E and 35-O, and anti-CD4 with TIL from patients 18-E and 32-O (Table II). This observation, together with the results of our cytotoxicity experiments, indicated a close correlation between the specific cytotoxic activity against fresh tumors and the expression of CD8 antigen.

To examine the effect of increased expression of MHC class I antigens on tumor cells upon specific killing by TIL, we treated tumor cells with 2000 units/ml of gamma-IFN for 24 h. There are two bodies of evidence in support of such an approach, including the fact that $CD8^+$ CTL recognize foreign antigens in the context of self MHC class I antigens,[11] and the finding that expression of MHC class I gene products increases the immunogenicity of tumors with greatly reduced levels of these antigens.[12–15] To determine whether gamma-IFN treatment can induce the expression of HLA class I genes in tumor cells, a human cDNA probe specific for HLA class I gene was used.[16] RNAs from gamma-IFN-treated or untreated tumor cells were analyzed by northern blot hybridization. All tumors expressed detectable but different levels of HLA class I specific transcripts (Fig. 1). The levels of HLA specific transcripts of tumor cells from patients 15-O, 17-E, 18-E were similar to that of normal tissue obtained from patient 15-O. However, the level of HLA specific transcripts of tumor cells from patient 1-C was marginal (Fig. 1). In gamma-IFN-treated cells, HLA specific transcripts were in each case at least fifty

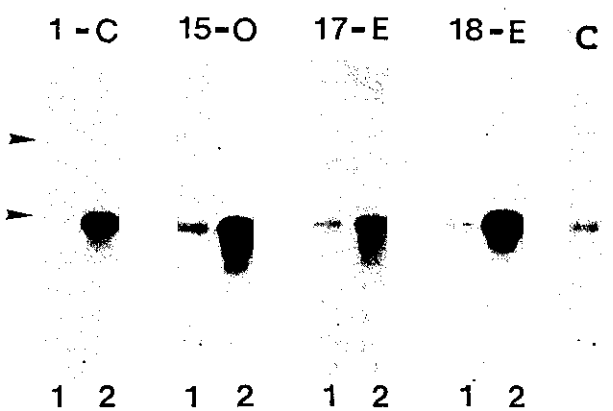

Fig. 1. Effect of interferon on the steady-state level of HLA class I mRNA. Total poly(A)$^+$ RNA obtained from untreated tumor cells (lane 1), tumor cells treated with gamma-IFN at 8000 IU/ml (lane 2), and untreated normal tissue from patient 15-O designated (C) were fractionated by electrophoresis in a 1.0% agarose gel and transferred to a nitrocellulose membrane. DNA probe specific to HLA class I transcripts was ^{32}P-labeled by nick translation. The top arrow indicates the position of the 28S ribosomal RNA and the bottom arrow that of 18S ribosomal RNA.

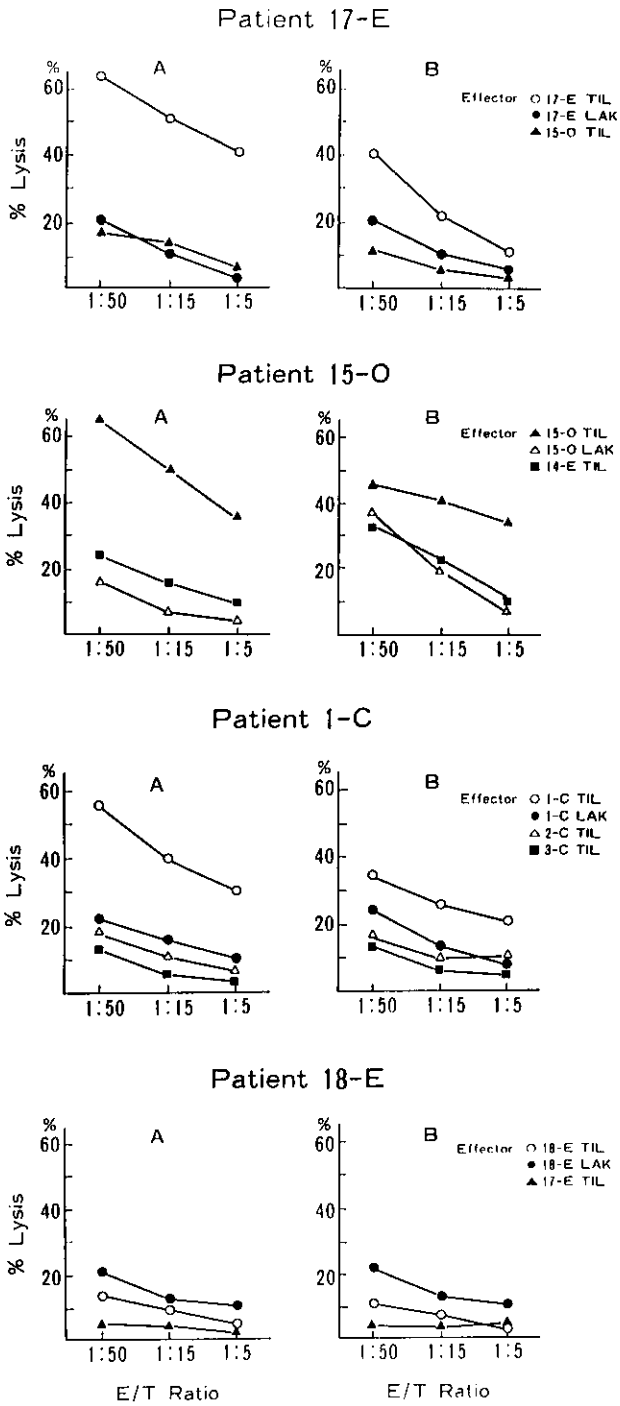

Fig. 2. Effect of gamma-IFN on the lytic specificity of IL-2-stimulated TIL derived from freshly excised gynecologic tumor nodules from patients 1-C, 15-O, 17-E, and 18-E, and cultured for 21 to 29 days. Panel A: tumor cells were cultured with 8000 units/ml of human gamma-IFN for 24 h. Panel B: tumor cells were cultured for 24 h in the absence of gamma-IFN.

Table III. Characteristics of Killing of Freah Human Tumors by IL-2 Activated Lymphocytes[a)]

Exp.	Effector cells	Target cells	E:T ratio[b)]		
			50:1	15:1	5:1
1	1-C TIL	1-C tumor cells	38.4	27.1	19.3
		2-C tumor cells	10.3	7.4	2.1
		3-C tumor cells	9.6	10.1	1.7
2	15-O TIL	15-O tumor cells	39.0	43.3	29.9
		15-O fibroblasts	10.1	8.2	4.2
		17-E tumor cells	9.2	8.0	8.3
3	17-E TIL	17-E tumor cells	49.4	55.7	20.4
		15-O tumor cells	14.7	8.2	2.1
		17-E fibroblasts	0.3	1.5	3.2
	17-E LAK	17-E tumor cells	26.3	17.1	5.3
		15-O tumor cells	34.3	19.5	9.1
		17-E fibroblasts	2.2	0.8	0.4
4	18-E TIL	18-E tumor cells	4.9	0.9	0.4
		23-E tumor cells	2.5	1.8	0.4
		18-E fibroblasts	2.0	−2.5	0.3
	18-E LAK	18-E tumor cells	22.2	11.9	7.3
		23-E tumor cells	19.9	17.2	3.5
5	32-C TIL	32-C tumor cells	6.7	6.1	3.5
		35-O tumor cells	11.5	16.5	5.7
	35-O TIL	32-C tumor cells	6.9	4.9	1.2
		35-O tumor cells	39.0	25.5	10.6

TIL from freshly excised tumor tissues from six patients were grown for 2 to 3 wk in RPMI 1640 medium containing 4% human AB serum with 200 units/ml of recombinant IL-2. These mononuclear cells lysed fresh autologous ^{51}Cr-labeled tumor cells, but not fresh allogeneic targets, when tested in a 4-h ^{51}Cr-release assay. All experiments were made in triplicate, and standard errors of the mean were less than 7%.
a) Values are percent of lysis. *b*) Effector-to-target ratio.

times more than that of the original tumor, and the induced levels were quite comparable among the four tumors (Fig. 1).

Upon confirmation that expression of the HLA class I gene could be induced, gamma-IFN-treated tumor cells were tested for their sensitivity to lysis by TIL. In lysis by LAK cells and by allogeneic TIL, no difference was observed between gamma-IFN-treated and untreated tumor cells in any of the four cases (Fig. 2). However, gamma-IFN treated tumor cells showed augmented susceptibility to lysis by $CD8^+$ TIL from patients 17-E, 15-O and 1-C. $CD4^+$ TIL from patient 18-E showed no cytotoxicity against either gamma-IFN-treated or untreated cells. In addition, in lysis by $CD8^+$ TIL, the antigenic specificity remained intact (Fig. 2). These results showed that the induction of HLA class I molecules on the cell surface rendered tumor cells more recognizable and resulted in increased sensitivity to lysis by $CD8^+$ TIL. Also, it appeared that this effect of gamma-IFN was the direct result of up-regulation of MHC class I gene expression rather than a consequence of modified expression of unique tumor antigens or increased expression of MHC class II molecules. At least in the case of patient 1-C, lysis of gamma-IFN-treated tumor cells by autologous TIL was reduced by 50% when tumor cells were preincubated with an excess of anti-HLA antibodies. Furthermore, treatment with gamma-IFN had no effect on the expression of MHC class II transcripts, including HLA DRα, DRβ, DPα, and DQβ (data not shown).

DISCUSSION

Several laboratories have begun to manipulate TIL as potential effector cells for adoptive immunotherapy.[17–20] It has been demonstrated that their presence correlates with the clinical prognosis.[21–23] Rosenberg *et al.* reported that TIL from a variety of murine tumors, when expanded in the presence of rIL-2, are from 50 to 100 times more effective than LAK cells in reducing established pulmonary metastasis and that TIL is relatively independent of the systemic administration of high-dose IL-2,

indicating the possibility of eliminating the serious side effects arising from the use of IL-2.[7] These murine studies raise the possibility that human TIL may also be of therapeutic value in the treatment of cancer. However, the issue of the specificity of TIL for autologous tumor cells has been controversial for a long time. Experiments in rodents performed by Brunner *et al.*[24] and Kikuchi *et al.*[25,26] indicated that tumor-specific immunity was mediated by Lyt-2-positive cytotoxic T cells (CTL) in preparations of freshly isolated TIL and that the efficiency of CTL precursors was higher in TIL than in PBL. In the past it has been difficult to isolate autologous tumor-specific CTL from humans bearing tumors, even though there have been suggestions that tumor-specific antigens and immune responses to them exist in humans. This could be related to technical problems of isolation and separation of TIL. Another difficulty relates to the small cell numbers which are rarely sufficient to allow functional studies with fresh TIL.

Mull *et al.* have reported that five of six preparations of TIL from patients with advanced malignant melanoma were derived from $CD4^+$ helper/inducer T cells.[17] With regard to cytotoxic capability, three of the six TIL preparations showed autologous tumor-specific cytotoxicity and the rest also had the ability to lyse allogeneic melanomas, sarcomas and mitogen-activated lymphoblasts in addition to autologous fresh tumors. Some of the discrepancies observed could be explained by whether advanced tumor tissue was used to generate TIL or not. It is interesting to note that in our study, thirteen of fifteen TIL from patients with an early stage of gynecologic malignancy (stages I and II), regardless of histologic type, were $CD8^+$ (Table II) and that four $CD8^+$ TIL expressed specific cytotoxicity against only fresh and autologous tumor cells. However, among six patients with advanced tumors, only two generated $CD8^+$ TIL, indicating that the proliferation and specific cytolytic response of TIL were inhibited by a suppressive factor, presumably a tumor-derived factor(s).

By using similar techniques, Herberman *et al.* recently obtained TIL from human ovarian tumors and reported that TIL exhibited cytotoxicity against autologous as well as allogeneic tumors.[19] In their experiment, the TIL population would have been relatively heterogeneous when the functional cytolytic experiment was performed because of culture with a high dose of IL-2 (1000 U/ml) compared to ours (200 U/ml) and the short period of culture in the presence of rIL-2. It is possible that more restricted reactivity would be detected with cells cultured in the presence of lower concentrations of IL-2. Cozzolino *et al.*, utilizing lower concentrations of IL-2, suggested that CTL specific to autologous tumor could be obtained from human TIL under carefully designed conditions of culture.[27] The difficulty in demonstrating the presence of specific TIL at the site might be more important in connection with the immunogenicity of human solid tumors,[28] although autologous specific TIL were reported for melanoma,[29,30] ovarian cancer[31] and glioblastoma.[32] In this report we have been able to demonstrate that tumor-specific cytotoxic T lymphocytes could be obtained from fresh tumor tissues, suggesting the existence of tumor-specific tumor antigen in human tumors.

Interestingly, we have observed in our clinical trials that a reduction of tumor load could be achieved only by the infusion of autologous $CD8^+$ TIL, but not of $CD4^+$ TIL. This would suggest that $CD8^+$ TIL could play an important role in adoptive immunotherapy.

The prospect of intervention by "immunotherapy" depends very much on the ability to render cancer cells more recognizable, as well as to potentiate the immune system. There is now direct evidence that augmentation of the expression of MHC class I antigens by gamma-IFN increased the sensitivity of tumor targets to lysis by TIL. These findings suggest a novel approach to tumor immunotherapy that involves using $CD8^+$ TIL as potential effector cells, in combination with the treatment of target tumor cells with gamma-IFN.

The manipulation of a variety of tumor cells by biological response modifiers such as interferon, tumor necrosis factor, or interleukin 2, to super-induce the expression of MHC class I gene, offers an attractive approach for further investigation. One would expect, for the following reasons, that increased class I expression on tumor cells may render them more sensitive to TIL immunotherapy. (i) It is believed that tumor antigens are recognized in association with self MHC antigen to generate immune responses.[11,33] (ii) T cells expressing the Lyt-2 antigen in mice or CD8 antigen in human are MHC class I restricted.[11,33] (iii) Modulation of class I expression on murine tumor cells by gene transfer could potentiate immunogenicity, decrease tumorigenicity, and reduce metastatic properties.[12–15] In addition, in the case of human cervical carcinoma, we have observed that local administration of gamma-IFN at the junction between normal and tumor tissues resulted in an increase of infiltration of $CD8^+$ lymphocytes to tumor cells (our unpublished results).

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REFERENCES

1) Rosenberg, S. A. Adoptive immunotherapy of cancer using lymphokine activated killer cells and recombinant interleukin-2. *In* "Important Advances in Oncology," ed. V. T. Devita, S. Hellman and S. A. Rosenberg, pp. 55–91 (1986). J. B. Lippincott, New York.
2) Rosenberg, S. A. Lymphokine activated killer cells: a new approach to the immunotherapy of cancer. *J. Natl. Cancer Inst.*, **75,** 595–603 (1985).
3) Mule, J. J., Shu, S., Schwarz, S. L. and Rosenberg, S. A. Adoptive immunotherapy of established pulmonary metastases with LAK cells and recombinant interleukin-2. *Science,* **225,** 1487–1489 (1984).
4) Lafreniere, R. and Rosenberg, S. A. Successful immunotherapy of murine experimental hepatic metastases with lymphokine-activated killer cells and recombinant interleukin 2. *Cancer Res.*, **45,** 3735–3741 (1985).
5) Rosenberg, S. A., Lotze, M. T., Muul, L., Leitmann, S., Chang, A. E., Ettinghausen, S. E., Matory, Y. L., Skibber, J. M., Shiloni, E., Vetto, J. T., Seipp, C. A., Simpson, C. and Reichert, C. M. Observations on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2 to patients with metastatic cancer. *N. Engl. J. Med.*, **313,** 1485–1492 (1985).
6) Rosenberg, S. A. Adoptive immunotherapy of cancer: accomplishments and prospects. *Cancer Treat. Rep.*, **68,** 233–255 (1984).
7) Rosenberg, S. A., Spiess, P. and Lafreniere, R. A new approach to the adoptive immunotherapy of cancer with tumor-infiltrating lymphocytes. *Science,* **233,** 1318–1321 (1986).
8) Tanaka, K., Appella, E. and Jay, G. Developmental activation of the H-2K gene is correlated with an increase in DNA methylation. *Cell,* **35,** 457–483 (1983).
9) Kato, H. and Torigoe, H. Radioimmunoassay for tumor antigen of human cervical squamous cell carcinoma. *Cancer,* **40,** 1621–1629 (1977).
10) Bast, R. C., Feeney, M., Lazarus, H., Nadlar, L. M., Colvin, R. B. and Knapp, R. C. Reactivity of monoclonal antibody with human ovarian carcinoma. *J. Clin. Invest.*, **68,** 1331–1337 (1981).
11) Zinkernagel, R. M. and Doherty, P. C. MHC-restricted cytotoxic T cells; studies on the biological role of polymorphic major transplantation antigens determining T-cell restriction specificity, function and responsiveness. *Adv. Immunol.*, **27,** 51–72 (1979).
12) Hui, K., Grosveld, K. J. and Festenstein, H. Reversal of transplantable AKR leukemia cells following MHC DNA-mediated cell transformation. *Nature,* **311,** 750–753 (1985).
13) Tanaka, K., Isselbacher, K. J., Khoury, G. and Jay, G. Reversal of oncogenesis by the expression of a major histocompatibility class I gene. *Science,* **228,** 26–30 (1985).
14) Wallich, R., Bulbuc, N., Halmmerling, G. J., Katsav, S., Segal, S. and Feldman, M. Abrogation of metastatic properties of tumor cells by *de novo* expression of H-2K antigens following H-2 gene transfection. *Nature,* **315,** 301–305 (1985).
15) Tanaka, K., Hayashi, H., Hamada, C., Khoury, G. and Jay, G. Expression of major histocompatibility complex class I antigens as a strategy for the potentiation of immune recognition of tumor cells. *Proc. Natl. Acad. Sci. USA,* **83,** 8723–8726 (1986).
16) Kress, M., Liu, W-Y., Jay, E., Khoury, G. and Jay, G. Comparison of class I (H-2) gene sequences; derivation of unique probes for members of this multigene family. *J. Biol. Chem.*, **258,** 13929–13936 (1983).
17) Mull, L. M., Spiess, P. J., Director, E. P. and Rosenberg, S. A. Identification of specific cytolytic immune responses against autologous tumor in humans bearing malignant melanoma. *J. Immunol.*, **138,** 989–997 (1987).
18) Heo, D. S., Whiteside, T. L., Johnson, J. T., Chen, K., Barnes, E. L. and Herberman, R. B. Long-term interleukin 2-dependent growth and cytotoxic activity of tumor-infiltrating lymphocytes from human squamous cell carcinomas of the head and neck. *Cancer Res.*, **47,** 6353–6362 (1987).
19) Heo, D. S., Whiteside, T. L., Kanbour, A. and Herberman, R. B. Lymphocytes infiltrating human ovarian tumors. I. Role of leu-19 (NKH1)-positive recombinant IL-2-activated cultures of lymphocytes infiltrating human ovarian tumors. *J. Immunol.*, **140,** 4042–4049 (1988).
20) Belldegrun, A., Muul, L. M. and Rosenberg, A. Interleukin 2 expanded tumor-infiltrating lymphocytes in human renal cell cancer: isolation, characterization, and anti-tumor activity. *Cancer Res.*, **48,** 206–214 (1988).
21) Berg, J. W. Morphological evidence for immune response to breast cancer. A histological view. *Cancer,* **18,** 1453–1460 (1971).
22) Black, M. M., Freeman, C., Mork, T., Harvei, S. and Cutler, S. J. Prognostic significance of microscopic structure of gastric carcinomas and their regional lymph node. *Cancer,* **27,** 703–708 (1971).
23) Jondal, M., Svedmyer, E., Klein, E. and Sing, S. Killer T cells in Burkitt's lymphoma biopsy. *Nature,* **255,** 405–408 (1975).
24) Brunner, K. T., MacDonald, R. H. and Cerottini, J. C. Quantitation and clonal isolation of cytotoxic T lymphocyte precursors selectively infiltrating murine sarcoma virus-induced tumors. *J. Exp. Med.*, **154,** 362–374 (1981).
25) Kikuchi, K., Ishii, Y., Ueno, H. and Koshiha, H. Cell mediated immunity involved in autochthonous tumor rejection in rats. *Ann. N. Y. Acad. Sci.*, **276,** 188–206 (1976).
26) Ibarashi, Y., Ueda, T., Ueda, T. and Kikuchi, K. Functional analysis of mononuclear cells infiltrating into tumors: differential cytotoxicity of mononuclear cells from tumors of immune and non-immune rats. *J. Immunol.*, **134,** 648–653 (1985).
27) Cozzolino, F., Torcia, N., Caroccino, A. M., Giordani, R.,

Selli, C., Talini, G., Reali, E., Novelli, A., Pistoia, V. and Ferrarini, M. Characterization of cells from invaded lymph nodes in patients with solid tumors: lymphokine requirement for tumor-specific lymphoproliferative response. *J. Exp. Med.*, **166**, 303–321 (1987).

28) Drebbin, J. A., Perry, L. L., Carter, R. and Green, M. I. Regulation of the immune response to antigen on the malignant cell surface. *Springer Semin. Immunopathol.*, **5**, 175–198 (1982).

29) Kruth, A., Danowski, B., Oettgen, H. F. and Old, L. J. T-cell mediated cytotoxicity against autologous malignant melanoma: analysis with interleukin-2 dependent T cell cultures. *Proc. Natl. Acad. Sci. USA*, **81**, 3511–3515 (1984).

30) DeVries, L. E. and Spits, H. Cloned human cytotoxic T lymphocytes (CTL) lines reactive with autologous melanoma cells. I. *In vitro* generation, isolation and analysis of phenotype and specificity. *J. Immunol.*, **132**, 510–516 (1984).

31) Ferrini, S., Biassoni, R., Moretta, A., Buruzzone, M., Micoline, A. and Moretta, L. Clonal analysis of T lymhocytes isolated from ovarian carcinoma ascitic fluid: phenotypic and functional characterization of T cell clones capable of lysing autologous carcinoma cells. *Int. J. Cancer*, **36**, 337–341 (1985).

32) Whiteside, T. L., Miescher, S., Hurlimann, J., Moretta, L. and von Fliedner, V. Separation, phenotyping and limiting dilution analysis of lymphocytes infiltrating human solid tumors. *Int. J. Cancer*, **37**, 803–809 (1986).

33) Doherty, P. C., Knowles, B. B. and Wettstein, P. J. Immunological surveillance of tumors in the context of major histocompatibility complex restriction of T cell function. *Adv. Cancer Res.*, **42**, 1–65 (1984).