

Lysis of Fresh and Cultured Autologous Tumor by Human Lymphocytes Cultured in T-Cell Growth Factor¹

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ABSTRACT

Human lymphocytes derived from the peripheral blood of patients with a variety of cancers were grown in T-cell growth factor (TCGF) and tested in a 4-hr ⁵¹Cr microcytotoxicity assay against fresh and cultured autologous tumor, autologous cultured skin fibroblasts, and autologous fresh peripheral blood lymphocytes. Lymphocytes grown in TCGF caused significant lysis of autologous cultured tumor and fibroblasts but caused little lysis of fresh autologous peripheral blood lymphocytes in all of seven patients tested. This lytic activity against autologous cultured cells was not dependent on the source of serum used in culturing the lymphoid cells or the targets.

Lymphoid cells grown in TCGF also were capable of causing selective lysis of fresh autologous tumor cells that had never been in culture in five of nine patients. Lymphoid cells growing in lectin-free TCGF caused selective lysis of autologous tumor in five of seven patients.

These observations demonstrate that peripheral lymphoid cells grown in TCGF can be lytic for autologous cultured and autologous fresh tumor compared to the lysis of fresh autologous peripheral blood lymphocytes. The fact that these autoreactive cells, lytic for tumor, may be expanded to large numbers in TCGF suggests a possible role for these cells in studies of the control of the cytotoxic response of activated cells to tumor and possibly in the immunotherapy of tumors as well.

INTRODUCTION

The specificity of the T-cell cytotoxic response has been demonstrated in human lymphocytes sensitized to alloantigens (37), virus-infected cells (3), chemically modified cells (29), and minor histocompatibility antigens (7). The search for a specific cytotoxic response to putative tumor antigens on autologous human tumor cells, however, has been complicated by a number of factors. Major problems have included the need to use cultured tumor targets for most assays and the low levels of spontaneous cytotoxicity in both normal and tumor-bearing individuals against these cultured lines (23).

We have been interested in the use of TCGF² to expand functional cytotoxic T-cells to large numbers and have demonstrated that specific reactivity to alloantigens in mass culture (25, 30) and in cultured clones (15, 26) is maintained in both the mouse and human. In murine studies, we found that normal splenocytes cultured in TCGF were lytic for freshly harvested

in vivo-passaged syngeneic tumor cells (35). In this report, we demonstrate that human peripheral lymphoid cells from cancer patients exhibit lysis for autologous fresh cultured tumor cells when cultured and expanded in C-TCGF or LF-TCGF.

MATERIALS AND METHODS

PBL. PBL from cancer patients were separated from venous blood on a Ficoll-Hypaque gradient, as described previously (30). The cells were resuspended in Roswell Park Memorial Institute Tissue Culture Medium 1640 (Grand Island Biological Co., Grand Island, N. Y.) containing 10% heat-inactivated ABS (North American Biological, Miami, Fla.), penicillin (100 units/ml) and streptomycin (100 µg/ml) (NIH Media Unit). This is referred to as CM in this paper. Cells were used fresh or frozen and subsequently thawed as needed as described previously (30). A ¹³⁷Cs source (Isomedex, Inc., Parsippany, N. J.) was used for irradiation of cells.

TCGF-containing Preparations. C-TCGF preparations used in experiments described in this paper were either purchased from Associated Biomedic System, Inc. (Buffalo, N. Y.), or made by us (30). These represent the 2- to 3-day supernatants of pooled O-positive donor mononuclear cells cultured in media (10⁶ cells/ml) containing 1% autologous plasma and 0.08% PHA-P (Difco Laboratories, Inc., Detroit, Mich.). Individual units of approximately 800 ml were received frozen and thawed as needed, filtered through Whatman No. 1 filter paper (H. Reeve Angel and Co., Inc., Clifton, N. J.), and concentrated 5:1 over an Amicon PM-10 membrane (Amicon Corp., Lexington, Mass.). All preparations were sterilized by passage through Swinnex 25-mm 0.45-µm filter units (Millipore Corp., Bedford, Mass.) and tested for their ability to maintain long-term cultures of T-lymphocytes prior to use.

Partial Purification and Removal of Lectin from TCGF Preparations. The full details of this procedure and an analysis of the activity of these lectin-free preparations are reported elsewhere (14). In brief, the C-TCGF was brought to 50% saturation with ammonium sulfate and the precipitate was discarded. The supernatant was then brought to 75% ammonium sulfate saturation, the precipitate resuspended in phosphate-buffered saline (NIH Media Unit), dialyzed, and passed over an affinity column (14 x 0.9 cm) of Sepharose 4B (Pharmacia, Uppsala, Sweden), coupled to a rabbit anti-PHA serum. By following ¹²⁵I-radio-labeled purified PHA throughout the purification process (labeled PHA was the kind gift of Dr. R. Falsted, National Cancer Institute, Baltimore, Md.), the column eluate was found to be 98 to 99.9% free of lectin. Also, no PHA could be detected in these eluates when tested in a mitogenic assay on fresh human lymphocytes. The partially purified and LF-TCGF was assayed for TCGF activity in parallel with the nonpurified material using cultured T-cells addicted to TCGF. The LF-TCGF was used at a titer equal to that of the nonpurified material.

Growth of Lymphocytes in C-TCGF or LF-TCGF. Cultures were initiated by placing 5 x 10⁴ to 1 x 10⁵ cells/ml in 2-ml wells of a 24-well cluster plate (Costar, Cambridge, Mass.) in CM and 20% TCGF. Cells were diluted into fresh CM and 20% TCGF every 5 to 7 days or when total cell numbers reached 5 x 10⁵/ml to 1 x 10⁶/ml. Cells cultured in C-TCGF were generally expanded over 2 to 3 weeks prior to testing in the microcytotoxicity assay.

Cultures in LF-TCGF were initiated at 5 x 10⁵ to 1 x 10⁶ PBL per ml and then cultured for 7 days.

¹ This paper is the fourth in a series on the topic; see also Refs. 14, 15, and 30.

² The abbreviations used are: TCGF, T-cell growth factor; C-TCGF, crude T-cell growth factor; LF-TCGF, lectin-free T-cell growth factor; PBL, peripheral blood lymphocytes; ABS, human AB serum; CM, complete media; PHA, phytohemagglutinin; HBSS, Hanks' balanced salt solution; FCS, fetal calf serum; NK, natural killer; IF, interferon.

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In Vitro Sensitization and Measurement of Cytotoxicity. Primary *in vitro* sensitization was performed as described previously in either the 2-ml wells of 24-well cluster plates or in 8 ml in Falcon 3013 flasks incubated vertically (30). Pool sensitization was carried out in Costar 3050 flasks using 4×10^6 responder lymphocytes and a pool of 5×10^5 irradiated stimulator lymphocytes from each of 12 separate normal individuals in a total volume of 10 ml of CM. Cultures were maintained at 37°, 5% CO₂, and high humidity for 7 days, harvested, washed, and used immediately or frozen for subsequent use. A 4-hr ⁵¹Cr release microcytotoxicity assay as described previously (30) was utilized. Target cells were either thawed the evening prior to the assay and incubated in CM at 37°, 5% CO₂, and high humidity overnight or thawed on the morning of the assay and labeled directly. Cultured tumor and skin fibroblasts were harvested on the morning of the assay by brief (5 to 10 min) trypsinization of the flask and 3 subsequent washes with CM. All targets were labeled with 400 µCi Na⁵¹Cr (Amersham Corp., Arlington Heights, Ill.) for 90 to 120 min in 0.5 ml of CM. These were washed 4 times and added at 1×10^3 to 5×10^3 cells/well to various numbers of effector lymphocytes in round-bottomed microtiter plates (Limbro Scientific Co., Hamden, Conn.). Supernatants were harvested with the Skatron-Titertek system (Skatron AS, Lierbyen, Norway) and counted in a γ-counter. Maximum release was produced by incubation of the targets with 0.1 N HCl. Spontaneous release was produced by incubation of targets with media alone. The percentage of lysis was calculated by:

$$\frac{\text{Experimental cpm} - \text{spontaneous cpm}}{\text{Maximal cpm} - \text{spontaneous cpm}} \times 100\%$$

All determinations were made in triplicate, and data are reported as the mean ± S.E.

Preparation of Tumor and Skin Target Cells. All patients from whom lymphocytes, tumor, or skin was obtained were being treated at the Clinical Center, NIH. Solid tumor and skin were obtained directly from surgery, washed with HBSS and established in culture as described in detail previously (24). Tumor and skin explant cultures were initiated in 175-sq cm Falcon tissue culture flasks (Becton Dickinson Labware, Oxnard, Calif.) in 5 ml of CM or Roswell Park Memorial Institute Tissue Culture Medium 1640 supplemented with 10% FCS. Malignant ascites and pleural effusions were obtained by withdrawing the fluid into heparinized syringes (100 units/ml). The malignant fluid was washed twice with HBSS and resuspended in CM. Cultures from these preparations were similarly initiated by placing 1×10^6 tumor cells into 5 ml of medium in tissue culture flasks.

Single-cell suspensions of human solid tumors were obtained by finely mincing the tumor into 5-cu mm fragments in HBSS or in HBSS containing hyaluronidase (5 units/ml; Sigma Chemical Co., St. Louis, Mo.), washing once with 0.25% trypsin in Dulbecco's phosphate-buffered saline containing DNase (0.2 mg/ml; NIH Media Unit) and then trypsinized at room temperature for 10 min. The supernatant containing the cells was decanted and mixed with an equal volume of CM or 100% ABS. An equal volume of fresh trypsin was added to the trypsinization flask, and the procedure was repeated once. The pooled cell suspensions were washed 3 times with HBSS, resuspended in CM, and then counted. In some cases, the tumor cell suspension was further purified by centrifugation on a Percoll (Pharmacia) step gradient of 10-15-25% Percoll for 7 min at 250 rpm according to the method of Kedar.³ Single-cell suspensions from solid tumors and from malignant effusions were either used immediately or cryopreserved for future use. All single-cell suspensions used as targets were evaluated in the Cytology Department, Clinical Center, NIH, and assessed as malignant. All preparations were composed of 50 to 100% tumor cells. One individual (Patient 10) with acute lymphocytic leukemia and >90% circulating lymphoblasts had blood drawn and separated on a Ficoll-Hypaque gradient. These cells and remission lymphocytes were the

kind gift of Dr. David Poplak, Pediatric Oncology Branch, National Cancer Institute. The patient material available for these studies is presented in Table 1.

RESULTS

Lysis of Cultured Autologous Tumor and Fibroblasts by Lymphocytes Grown in C-TCGF. Lymphocytes from 7 cancer patients were expanded 100- to 1000-fold and tested in a 4-hr ⁵¹Cr release assay against cultured autologous tumor, skin, and fresh lymphocytes. Chart 1 presents the results of a characteristic experiment (Patient 1). Autologous lymphoid cells grown in C-TCGF caused significant lysis of cultured tumor and skin fibroblasts with minimal lysis of fresh lymphocytes. All 3 target cells were found to be equally lysable using an allosensitized effector from a pool sensitization. Fresh lymphocytes from this patient caused less than 5% lysis of any of the 3 targets at a 100:1 effector:target cell ratio.

The results obtained in all 7 patients when lymphocytes grown in C-TCGF were tested against autologous cultured skin and tumor are presented in Chart 2. Significant cytotoxicity against both tumor and skin cultured autologous targets was seen in all patients. This was not related to the presence of FCS components since the effector lymphocytes were cultured only in medium containing human serum and target cells cultured in FCS (Patients 1 to 3) or human serum (Patients 4 to 7) were similarly lysed. In Patient 5, an attempt was made to analyze the role of serum in the reactivity of cultured lymphocytes. Peripheral blood lymphocytes expanded in C-TCGF in 10% ABS were tested against autologous tumor (melanoma) cultured in autologous serum, human AB serum, or FCS. Fresh autologous lymphocytes and a cultured allogeneic melanoma were also used as targets in this experiment (Table 2). Lymphocytes cultured in TCGF lysed the autologous melanoma cells regardless of the serum used in the cultures. The fresh lymphocytes of this patient were tested as effectors and exhibited no natural cytotoxicity towards any of these targets. Therefore, the lysis observed is dependent upon growth of effector lymphocytes in TCGF and does not appear to be related to a FCS-induced artifact.

Patient Lymphocytes Cultured in C-TCGF are Lytic for Fresh Autologous Tumor. Autologous lymphocytes grown in C-TCGF were tested for lysis of fresh autologous tumor using cells obtained from 9 cancer patients. In Table 3, a characteristic experiment (Patient 5) is presented in detail. The lysis by both an allogeneic effector sensitized to lymphocytes of Patient 5 (to determine lysability of targets) and by autologous lymphocytes expanded in C-TCGF are presented. Approximately equal lysis of all tumor and normal targets by the allosensitized effector cell was demonstrated. In contrast, significant lysis of fresh tumor cells by autologous cells grown in C-TCGF was seen, although no significant lysis of fresh lymphocyte targets was observed. As demonstrated previously, autologous lymphocytes grown in C-TCGF also lysed cultured autologous tumor and skin fibroblasts. Chart 3 summarizes the data from all 9 patients. In 5 of the 9 patients (Patients 1, 5, 6, 8, and 11), autologous lymphocytes grown in C-TCGF exhibited significant lysis of autologous fresh tumor cells and little or no lysis of fresh lymphocytes. The other 4 patients showed no significant lysis of tumor (Patients 9 and 10) or lysis of lymphocytes greater than or equal to that of tumor (Patients 7 and

³ E. Kedar, personal communication.

Table 1
Patients with tumor examined

Patient	Sex	Age (yr)	Diagnosis	Source of tumor	Autologous target cells available			
					Fresh		Cultured	
					Tumor	Lymphocytes	Tumor	Skin Fibroblasts
1	M	24	Osteogenic sarcoma	Primary tumor and lung metastasis	+	+	+	+
2	M	27	Osteogenic sarcoma	Local recurrence and lung metastasis	-	+	+	+
3	M	24	Osteogenic sarcoma	Lung metastasis	-	+	+	+
4	F	68	Adenocarcinoma, colon	Ascites	+	+	+	-
5	M	54	Melanoma	Ascites	+	+	+	+
6	F	31	Stromal sarcoma of breast	Primary tumor	+	+	+	+
7	F	56	Adenocarcinoma, colon	Primary tumor and liver metastasis	+	+	+	+
8	F	69	Ovarian carcinoma	Ascites	+	+	-	-
9	F	33	Ovarian carcinoma	Metastasis, abdominal wall	+	+	-	-
10	M	17	Acute lymphocytic leukemia	Peripheral blood	+	+	-	-
11	F	54	Ovarian carcinoma	Pleural effusion	+	+	-	-
12	F	48	Breast carcinoma	Ascites	+	+	-	-
13	F	50	Ovarian carcinoma	Primary tumor	+	+	-	-
14	F	48	Breast carcinoma	Ascites	+	+	-	-
15	F	36	Osteogenic sarcoma	Primary tumor	+	+	-	-
16	F	26	Angiosarcoma, breast	Metastasis	+	+	-	-
17	M	68	Melanoma	Metastasis	+	+	-	-
18	F	17	Osteogenic sarcoma	Metastasis	+	+	-	-
19	M	27	Melanoma	Metastasis, spleen	+	+	-	-

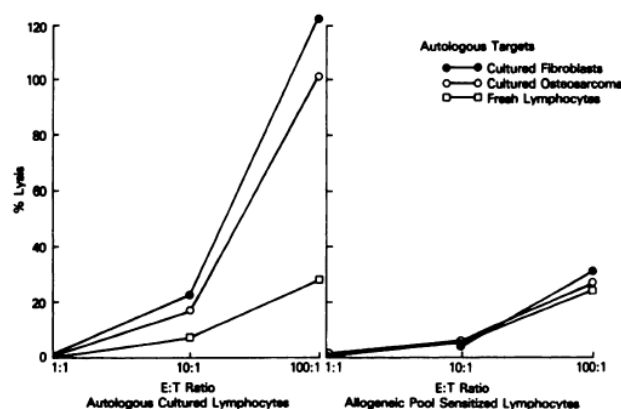


Chart 1. PBL were expanded from Patient 1 in TCGF and tested in a ^{51}Cr release assay against labeled autologous cultured fibroblasts, autologous cultured osteosarcoma, and autologous fresh lymphocytes. Lysis of autologous cultured targets and fresh lymphocytes is seen at left. These same targets were equivalently lysed by an allogeneic pool sensitized effector (right).

12). To determine whether the differential lysis of autologous fresh tumor and fresh lymphocytes seen in some patients was related to differences in intrinsic lysability of the target cells, pool-sensitized effectors were generated from a normal individual and tested against paired fresh tumor and fresh lymphocyte targets from Patients 5, 6, 9, 10, and 12. The results from these experiments are presented in Chart 4. Similar lysability of fresh tumor and lymphocytes was found in 4 pairs (Patients 5, 10, 11, and 12), while differences in lysability of targets were found in Patients 6 and 9. In Patient 6, the tumor was more lysable, and in Patient 9 the lymphocytes were more lysable. Therefore, while the differential lysis of tumor and lymphocytes by TCGF-expanded PBL from Patient 6 could be explained by differences in lysability, this does not appear to explain the selective lysis of tumor cells by PBL expanded in C-TCGF from Patients 5 and 11.

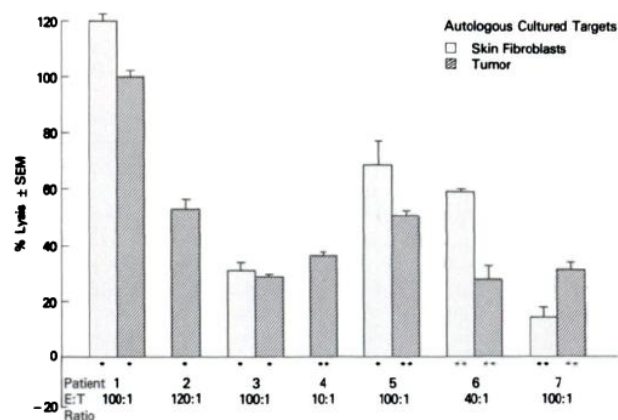


Chart 2. Lysis of cultured autologous skin fibroblasts and cultured autologous tumor by lymphocytes cultured in TCGF and 10% ABS. The effector:target cell (E:T) ratio shown is the highest tested in each case. All cultured cells were in early passage (passage 1 to 5). Targets were initiated and expanded in FCS (*) or human serum (**). Bars, S.E.

Patient Lymphocytes Cultured in Lectin-free TCGF are Lytic for Autologous Fresh Tumor. To rule out the possibility that residual PHA in our TCGF preparations was at least partially responsible for lysis of tumor, we performed a series of cultures of patients PBL in LF-TCGF and tested these cells for lysis of autologous tumor. Table 4 shows the results of 9 different cultures from 7 patients. In 7 of these 9 cultures (5 of 7 patients), significant autologous cytotoxicity versus the autologous tumor cell was generated and was greater than the lysis of autologous fresh lymphoid cell targets. The 2 patients who exhibited no lysis (Patients 17 and 19) were both found to be immunoincompetent in a parallel culture testing their ability to generate allogeneic cytotoxic lymphocytes. Both patients died within 2 weeks of donating their peripheral blood for assay. Lysis of autologous fresh PBL was not eliminated by the use of LF-TCGF, suggesting that in the occasional combina-

tions where such lysis is seen it is not due to the presence of lectin. Although not studied extensively, cytotoxic cells generated in LF-TCGF were also found to be lytic for other nonautologous cultured and fresh tumor cells.

DISCUSSION

In this report, we have demonstrated that lymphocytes obtained directly from peripheral blood of cancer patients and cultured in TCGF are lytic for autologous fresh tumor in a 4-hr ^{51}Cr release assay. The lytic activity generated in culture was dependent on the presence of TCGF and did not occur in cultures lacking this growth stimulus. To our knowledge, this is the first report of consistent lysis of fresh solid tumor cells by autologous human lymphocytes cultured in TCGF and is similar to our previous observations in the mouse (35).

It should be emphasized that, in our studies, neither the lymphoid cells nor the fresh target cells were ever exposed to xenogeneic serum, a known source of generating nonspecific cytotoxic effects (6, 21, 31, 34). Lysis was not due to the presence of PHA, since lymphoid cells cultured in LF-TCGF exhibited excellent lysis of tumor target cells.

Previous reports have demonstrated that fresh and cultured human lymphocytes manifest significant cytotoxicity against selected cultured allogeneic tumor targets (6, 19, 21, 31, 34). Our initial studies utilized both autologous tumor and paired autologous normal fibroblast lines grown in tissue culture as targets in short-term ^{51}Cr release assays. In all 7 patients tested, lymphocytes grown in TCGF were lytic for both tumor and normal autologous cultured lines.

Because of possible artifacts introduced by the use of cultured target cells, we then tested the lysis of fresh tumor cells

by autologous lymphoid cells grown in TCGF. In 7 of 9 patients, significant lysis of fresh tumor cells was seen although in 2 of these patients substantial lysis of fresh lymphocytes also occurred. When lymphoid cells were grown in LF-TCGF, 5 of 7 patients exhibited significant lysis of fresh autologous tumor. The 2 patients that did not lyse autologous tumor were immunoincompetent (based on the inability of their lymphoid cells to be allosensitized in *in vitro* sensitization cultures), and both patients died within 2 weeks of donating their PBL. The inability of these latter patients to mount an alloimmune response paralleled with their inability to generate autologous tumor killer cells suggests that TCGF-induced generation of killer cells does require an immunocompetent host and is an immunological response.

For some of our patients, we were able to show that the tumor and the PBL were equally lysable by allogeneic pool-sensitized cytotoxic lymphocytes. Presumably, this effector lymphocyte population contained killer cells with specificities for all histocompatibility antigens in the pool, and lysis of the tumor as well as the PBL was based on the presence of normal histocompatibility antigens. We would like to stress that inherent lysability of tumor *versus* control cells needs careful evaluation before claims of tumor specificity can be confirmed. In a number of our patients (Patients 5, 10, 11, and 12), these controls did not show approximately equal lysability at the effector:target cell ratios for which they were tested. In these cases, a significantly higher ^{51}Cr release value from the tumor

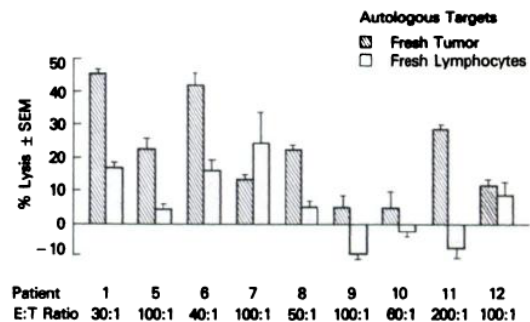


Chart 3. Lysis of fresh autologous targets. PBL from patients in which fresh, noncultured tumor targets were available were expanded 100-1000-fold in C-TCGF. Fresh autologous tumor and lymphocytes served as labeled targets in a 4-hr ^{51}Cr release assay. Results are presented at the highest effector:target cell (E:T) ratio tested. Significant difference between the lysis of tumor and lymphocyte targets ($p < 0.05$) was found in patients 1, 5, 6, 8, 9, and 11 (Student's *t* test). Bars, S.E.

Table 2
Effect of serum on TCGF associated lysis

Target cells	% of lysis by autologous lymphocytes ^a	
	Fresh	Cultured ^c
Cultured autologous melanoma in:		
Pooled ABS	0.2 ± 0.6 ^b	11.9 ± 1.7
FCS	-0.0 ± 0.4	13.1 ± 0.2
Autologous serum	0.4 ± 0.2	16.7 ± 1.5
Cultured allogeneic melanoma in FCS	1.0 ± 0.6	24.0 ± 0.4
Fresh autologous lymphocytes	-1.1 ± 1.1	1.7 ± 1.3

^a Effector:target ratio, 100:1.

^b Mean ± S.E.

^c Cultured in TCGF containing 10% ABS.

Table 3
Lysis of fresh and cultured autologous targets by lymphocytes cultured in TCGF (Patient 5)

Effector cell	E:T ratio	Fresh melanoma		Cultured melanoma (ABS)		Cultured skin (ABS)		Fresh lymphocytes	
		cpm	% of lysis	cpm	% of lysis	cpm	% of lysis	cpm	% of lysis
None		237 ± 7 ^b	0	1288 ± 54	0	429 ± 19	0	78 ± 3	0
HCl lysis		1,804 ± 52	100	12,411 ± 61	100	2,086 ± 389	100	267 ± 6	100
Cultured autologous lymphoid cells (TCGF)	100:1	635 ± 65	25.3 ± 4.1	6,933 ± 140	50.7 ± 1.2	1,560 ± 437	68.2 ± 26.3	86 ± 4	4.3 ± 1.1
	30:1	509 ± 31	19.0 ± 2.5	3,722 ± 131	21.8 ± 1.1	661 ± 25	14.0 ± 1.5	73 ± 6	-2.4 ± 3.0
	10:1	307 ± 5	4.4 ± 0.3	1,685 ± 39	3.5 ± 0.3	459 ± 18	1.8 ± 1.0	79 ± 6	0.5 ± 3.0
Allogeneic effector (sensitized to lymphocytes of patient)	100:1	520 ± 18	18.0 ± 1.1	3,426 ± 287	19.2 ± 2.5	566 ± 6	8.2 ± 0.0	109 ± 7	16.1 ± 3.8
	30:1	341 ± 34	6.5 ± 2.2	2,160 ± 46	7.8 ± 0.4	590 ± 23	9.7 ± 1.4	91 ± 4	7.0 ± 2.1
	10:1	346 ± 15	6.9 ± 0.9	1,579 ± 47	2.6 ± 0.4	472 ± 3	2.5 ± 0.1	78 ± 3	0.0 ± 1.6

^a E:T ratio, effector:target ratio. All targets at 10^5 cells/well. The cultured tumor target was cultured in flasks and trypsinized prior to labeling.

^b Mean ± S.E. of triplicate determinations.

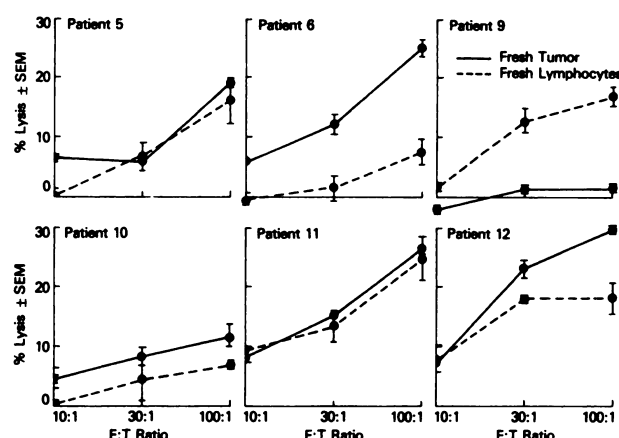


Chart 4. Lysability of paired fresh tumor and lymphocytes by allosensitized effector cells. Paired labeled targets were tested for lysis in a 4-hr ^{51}Cr release assay using cells all sensitized to a pool of 12 normal donor PBL (Patients 6, 9 to 12) or in primary mixed lymphocyte culture with PBL of the patient (Patient 5). E:T, effector:target cell ratio; bars, S.E.

Table 4
Lysis of autologous fresh tumor after 7 days of culture in LF-TCGF

Patient	E:T ^a	% of lysis		
		Fresh tumor	Fresh lymphocytes	Immunocompetence ^b
13	20:1	20.0 ± 2.0 ^c	NT	ND
14	20:1	32.6 ± 0.5	21.2 ± 2.1	+
15	40:1	36.0 ± 6.0	14 ± 1.0	—
16 ^d	40:1	1.8 ± 3.0	6.6 ± 0.9	—
17a ^d	20:1	49.8 ± 2.1	22.6 ± 4.0	+
17b	10:1	54.6 ± 2.2	12.5 ± 5.4	+
17c	10:1	11.6 ± 1.9	1.3 ± 0.9	+
18	40:1	27.2 ± 6.0	11.6 ± 2.4	+
19 ^d	40:1	1.9 ± 0.4	—7 ± 2.2	—

^a E:T, effector:target cell ratio; NT, not tested; ND, not done.

^b Assessed by the ability of lymphocytes from this patient to generate cytotoxic lymphocytes in *in vitro* sensitization cultures.

^c Mean ± S.E.

^d Both immunoincompetent deceased within 2 weeks of PBL donation.

^e Patient 17's lymphocytes were tested in 3 individual experiments.

cell preparation when tested with PBL cultured in TCGF can be regarded as a selective lysis of tumor.

The lysis of autologous fresh lymphocytes that is seen in several of our experiments is perplexing. Due to the inherent difficulties of using human cells, most experiments were done once; thus, the reproducibility of this finding for any one individual has not been determined. However, lysis of autologous lymphocytes was observed often. It is possible that lysis of autologous PBL is purely nonspecific during the 4-hr assay itself, such as would occur if PHA were causing a lectin-dependent killing reaction (2, 5, 16). We do not believe that this is the case, since some killing of fresh autologous PBL was exhibited by PBL cultured in LF-TCGF, and that the addition of TCGF to the ^{51}Cr release assay itself did not cause such a reaction (data not shown).

The basis of the killing of autologous tissue-cultured tumor and normal cell lines and of fresh autologous tumor by lymphoid cells grown in TCGF is unclear. A variety of nonspecific lytic interactions with tumor cells caused by human lymphoid cells

have been described. These phenomena include (1, 4, 11, 16, 18, 20, 31), anomalous cytotoxicity (28), activated cell killing (18), N-cell activation (12), polyclonal activation by lectins (10), and activation of human cells by *in vitro* "education" (17) pool sensitization (36).

Virtually all demonstrations of NK activity have used allogeneic tissue-cultured lines uniquely sensitive to putative NK effects (19, 21, 34). Although little has been studied concerning a direct comparison of the sensitivity of allogeneic and autologous cultured lines to putative NK phenomena, it has been suggested (19) that the lack of NK activity against autologous cultured tumor might be related to the circumvention of natural cytotoxic processes by tumor cells. We have demonstrated in this paper that, in most patients tested, cells grown in TCGF exhibit significant lysis of autologous cultured tumor and normal cells. The relationship of this phenomenon to reports of NK activity of cultured lines by others is unknown, nor is it known whether the cells growing in TCGF are of the same phenotype as those to which classical NK effects are attributed. Fresh tumor targets are poorly lysed by putative NK cells, although there is a report (20) that selected fresh tumor cells could serve as effective cold target inhibitors of lysis of an NK-sensitive target, K-562, by third-party effectors. Although these results indicate some common expression of determinants on fresh tumor and K-562, it is not clear that fresh tumor would be equivalently lysed by these cells.

Although the TCGF preparations used to grow lymphocytes in this study are known (27) to contain significant amounts of IF, and IF seems to play an important regulatory function in the cytotoxicity of NK cells (9), a recent report (32) suggests that fresh autologous tumor is not sensitive to IF-induced cytotoxicity of human PBL. A significant increase in the specific ^{51}Cr release of allogeneic fresh tumor was demonstrated by IF; however, it was suggested that this might represent polyclonal activation of cells for alloantigenic determinants. The lysis of fresh tumor targets and autologous cultured lines by cells growing in TCGF suggests that this phenomenon is not mediated by the same cells causing NK effects, but this conclusion must remain tentative.

The phenomenon that we have described in this paper, *i.e.*, the lysis of fresh autologous tumor cells by unsensitized human lymphocytes grown in TCGF, may be related to several of the phenomena discussed above. It is not known whether the same cells that mediate the lysis we have observed are involved in the lysis of cultured cells described by others. It does appear, however, that the activation of human lymphoid cells by a variety of stimuli can lead to the appearance of a broad range of lytic properties. A unique feature of our observations is the generation of lysis of a variety of solid autologous human tumors. This observation raises the question of the possible use of these cells for adoptive immunotherapy. We have shown previously that these cells can be safely administered *i.v.* to humans (13).

An important question that remains to be answered is whether our cytotoxic populations contain any killer cells specific for autologous tumor or whether all killer cells in these populations express a heterogeneous killing distribution. We are currently using our previously described human cytotoxic T-lymphocytes cloning (15) and single cell lysis (8) techniques to attempt to distinguish among these activities.

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REFERENCES

- Bakacs, T., Gergely, P., and Klein, E. Characterization of cytotoxic human T lymphocyte subpopulations: the role of Fc receptor carrying cells. *Cell Immunol.*, 32: 317-328, 1977.
- Bevan, M. J., and Cohn, M. Cytotoxic effects of antigen- and mitogen-induced T cells on various targets. *J. Immunol.*, 114: 559-565, 1975.
- Biddison, W. E., and Shaw, S. Differences in HLA antigen recognition by human influenza virus-immune cytotoxic T cells. *J. Immunol.*, 122: 1705-1709, 1979.
- Bolhuis, R. L., Shunt, H. R. E., Nooy, A. M., and Ronteltap, C. P. M. Characterization of natural killer (NK) cells and killer (K) cells in human blood: discrimination between NK and K cell activities. *Eur. J. Immunol.*, 8: 731-740, 1978.
- Bonavida, B., and Bradley, T. P. Studies on the induction and expression of T cell-mediated immunity. V. Lectin-induced nonspecific cell mediated cytotoxicity by alloimmune lymphocytes. *Transplantation (Baltimore)*, 21: 94-102, 1976.
- Golub, S. H., Golightly, M. G., and Zielske, J. V. "NK-like" cytotoxicity of human lymphocytes cultured in media containing fetal bovine serum. *Int. J. Cancer*, 24: 273-283, 1979.
- Goulmy, E., Termijtelen, A., Bradley, B. A., and von Rood, J. J. Y-antigen killing by T cells of women is restricted by HLA. *Nature (Lond.)*, 266: 544-545, 1977.
- Grimm, E. A., and Bonavida, B. Mechanisms of cell-mediated cytotoxicity at the single cell level. I. Estimation of cytotoxic T lymphocyte frequency and relative lytic efficiency. *J. Immunol.*, 123: 2861-2869, 1979.
- Herberman, R. B., Ortaldo, J. R., Djou, J. Y., Holden, H. T., Jett, J., Lang, N. P., and Pestka, S. Role of interferon in regulation of cytotoxicity by natural killer cells and macrophages. *Ann. NY Acad. Sci.*, in press, 1981.
- Hunninghake, G. W., and Fauci, A. S. Lymphocyte-mediated cytotoxicity against human allogeneic and autologous lymphoid targets after concanavalin A-activation of cytotoxic effector cells. *J. Immunol.*, 119: 1122-1128, 1977.
- Kaplan, J., and Callevaret, D. Expression of human T-lymphocyte antigens by natural killer cells. *J. Natl. Cancer Inst.* 60: 961-964, 1978.
- Kolde, Y., and Takasugi, M. Augmentation of human natural cell-mediated cytotoxicity by a soluble factor. I. Production of N-cell-activating factor (NAF). *J. Immunol.*, 121: 872-879, 1978.
- Lotze, M. T., Line, B. R., Mathisen, D. J., and Rosenberg, S. A. The *in vivo* distribution of autologous human and murine lymphoid cells grown in T cell growth factor (TCGF): implications for the adoptive immunotherapy of tumors. *J. Immunol.*, 125: 1487-1493, 1980.
- Lotze, M. T., and Rosenberg, S. A. *In vitro* growth of cytotoxic human lymphocytes. III. The preparation of lectin free T cell growth factor (TCGF) and an analysis of its activity. *J. Immunol.*, 126: 2215-2220, 1981.
- Lotze, M. T., Strausser, J. L., and Rosenberg, S. A. *In vitro* growth of cytotoxic human lymphocytes. II. Use of T cell growth factor (TCGF) to clone human T cells. *J. Immunol.*, 124: 2972-2978, 1980.
- Lundgren, G., and Moller, G. Non-specific induction of cytotoxicity in normal human lymphocytes *in vitro*: Studies of mechanism and specificity of the reaction. *Clin. Exp. Immunol.*, 4: 435-452, 1969.
- Martin-Chandon, M. R., Vanky, F., Carnaud, C., and Klein, E. *In Vitro* "education on autologous human sarcoma generates non-specific killer cells. *Int. J. Cancer*, 15: 342-350, 1975.
- Masucci, M. G., Klein, E., and Argov, S. Disappearance of the NK effect after explanation of lymphocytes and generation of similar nonspecific cytotoxicity correlated to the level of blastogenesis in activated cultures. *J. Immunol.*, 124: 2458-2463, 1980.
- Mukherji, B., Flowers, A., Rothman, L., and Nathanson, L. Spontaneous *in vitro* cytotoxicity against autochthonous human melanoma cells. *J. Immunol.*, 124: 412-417, 1980.
- Ortaldo, J. R., Bonnard, G. D., and Herberman, R. B. Cytotoxic reactivity of human lymphocytes cultured *in vitro*. *J. Immunol.*, 119: 1351-1357, 1977.
- Ortaldo, J. R., Bonnard, G. D., Kind, P. D., and Herberman, R. B. Cytotoxicity by cultured human lymphocytes: characteristics of effector cells and specificity of cytotoxicity. *J. Immunol.*, 122: 1489-1494, 1979.
- Ortaldo, J. R., Oldham, R. K., Cannon, G. C., and Herberman, R. B. Specificity of natural cytotoxic reactivity of normal human lymphocytes against a myeloid leukemia cell line. *J. Natl. Cancer Inst.*, 59: 77-82, 1977.
- Pross, H. F., and Baines, M. G. Spontaneous human lymphocyte-mediated cytotoxicity against tumor target cells. VI. A brief review. *Cancer Immunol. Immunother.*, 3: 75-85, 1977.
- Rosenberg, S. A., Schwarz, S., Anding, H., Hyatt, C., and Williams, G. M. Comparison of multiple assays for detecting human antibodies directed against surface antigens on normal and malignant human tissue culture cells. *J. Immunol. Methods*, 17: 225-239, 1977.
- Rosenberg, S. A., Schwarz, S., and Spiess, P. J. *In vitro* growth of murine T cells. II. Growth of *in vitro* sensitized cells cytotoxic for alloantigens. *J. Immunol.*, 121: 1951-1955, 1978.
- Rosenberg, S. A., Spiess, P. J., and Schwarz, S. *In vitro* growth of murine T cells. IV. Use of T cell growth factor (TCGF) to clone lymphoid cells. *Cell Immunol.*, 54: 293-306, 1980.
- Ruscetti, F. W., Morgan, D. A., and Gallo, R. C. Functional and morphologic characterization of human T cells continuously grown *in vitro*. *J. Immunol.*, 119: 131-138, 1977.
- Seeley, J. K., and Golub, S. H. Studies on cytotoxicity generated in human mixed lymphocyte cultures. I. Time course and target spectrum of several distinct concomitant cytotoxic activities. *J. Immunol.*, 120: 1415-1422, 1978.
- Shaw, S., Nelson, D. L., and Shearer, G. M. Human cytotoxic response *in vitro* to trinitrophenyl-modified autologous cells. I. T cell shared antigens. *J. Immunol.*, 121: 281-289, 1978.
- Strausser, J. L., and Rosenberg, S. A. *In vitro* growth of cytotoxic human lymphocytes. I. Growth of cells sensitized *in vitro* to alloantigens. *J. Immunol.*, 121: 1491-1495, 1978.
- Treves, A. J., Heidelberger, E., Feldman, M., and Kaplan, H. S. *In vitro* sensitization of human lymphocytes against histiocytic lymphoma cell lines. II. Characterization of two different effector activities and of suppressor cells. *J. Immunol.*, 121: 86-96, 1978.
- Vanky, F. T., Argov, S. M., Einhorn, S. A., and Klein, E. Role of alloantigens in natural killing. Allogeneic but not autologous tumor biopsy cells are sensitive to interferon-induced cytotoxicity of human blood lymphocytes. *J. Exp. Med.*, 151: 1151-1165, 1980.
- Vanky, F., Klein, E., Stvernsward, J., Rodriguez, L., Peterffy, A., Steiner, L., and Nilsson, U. Human tumor-lymphocyte interaction *in vitro*. III. T lymphocytes in autologous tumor stimulation (ATS). *Int. J. Cancer*, 22: 679-686, 1978.
- West, W. H., Cannon, G. B., Kay, H. D., Bonnard, G. D., and Herberman, R. B. Natural cytotoxic reactivity of human lymphocytes against a myeloid cell line: characterization of effector cells. *J. Immunol.*, 118: 355-361, 1977.
- Yron, I., Wood, T. A., Jr., Spiess, P. J., and Rosenberg, S. A. *In vitro* growth of murine T cells. V. The isolation and growth of lymphoid cells infiltrating syngeneic solid tumors. *J. Immunol.*, 125: 238-245, 1980.
- Zarling, J. M., and Bach, F. H. Continuous culture of T cells cytotoxic for autologous human leukemia cells. *Nature (Lond.)*, 280: 685-688, 1979.
- Zier, K. S., and Bach, F. H. Secondary responses of human lymphocytes to alloantigens *in vitro*. *Scand. J. Immunol.*, 4: 607-611, 1977.