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Lysis by interleukin 2-stimulated tumor-infiltrating lymphocytes of autologous and allogeneic tumor target cells*

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Summary. Peripheral blood lymphocytes (PBL) and tumor-infiltrating lymphocytes (TIL) were isolated from six cancer patients and cultured in the presence of 100 units/ml of recombinant interleukin 2 (IL2). Both IL2-stimulated PBL (IL2-PBL) and IL2-stimulated TIL (IL2-TIL) lysed fresh and short-term cultured autologous tumor cells in four and six cases, respectively. In four out of six patients IL2-TIL showed a slightly higher tumor cytotoxicity than IL2-PBL without lysing autologous normal PBL or TIL. Like IL2-PBL, IL2-TIL also killed allogeneic fresh and cultured targets of different histotypes, suggesting a lack of autologous tumor cytotoxic specificity. TIL cultured for 3 weeks in IL2 maintained their killing activity against autologous and allogeneic tumor targets. Phenotypic analysis of uncultured TIL showed a predominance of $CD3^+$ T cells (~70%) with $CD4^+$ (~60%) and $CD8^+$ (20%) lymphocyte subsets, whereas ≤3% of $CD16^+$ natural killer cells were present. TIL but not PBL contained 12%–19% of lymphocytes which expressed activation markers such as DR and TAC. The culture of both TIL and PBL in IL2 for 2–3 weeks induced an increase in the percentage of $CD8^+$ and a decrease in $CD4^+$ and augmentation of Leu 19^+, DR^+, and TAC^+ cells. These results indicate that IL2-TIL can lyse autologous tumor cells slightly better than IL2-PBL, although such an effect was also evident against allogeneic neoplastic targets.

Introduction

Several reports have described the accumulation of lymphocytes at the tumor site [5, 9, 13, 32]. This observation has been taken as a manifestation of immunological recognition of tumor cells by the immune system of the host [28]. Analysis of the nature of the infiltrating lymphocytes has shown that cells with T phenotype are the predominant ones, whereas few natural killer (NK) or B cells are generally found [6, 10, 31]. Studies on the functional activities of tumor-infiltrating lymphocytes (TIL) have revealed that they generally express a weak lytic response against autologous tumor and a low natural killing activity as compared to that of peripheral blood lymphocytes (PBL) or noninfiltrated draining lymph node lymphocytes [12, 16, 26, 32, 33].

To explain this depressed antitumor reactivity of TIL, it has been suggested that tumor cells can induce suppressor lymphocytes or exert directly, or through soluble factors, an inhibitory activity on lymphocyte stimulation [8, 17, 22, 25, 31]. Moreover it has been reported that TIL show reduced immunological responses and poor cloning frequencies probably due to the effect of putative tumor-derived factors [14, 34].

However other studies, where interleukin 2 (IL2) was used to activate TIL (IL2-TIL), indicated that they can efficiently lyse autologous and allogeneic tumor cells [1, 6, 10, 18, 19, 29]. Moreover a higher spontaneous response to IL2 and a higher frequency of precursor lymphocytes specifically cytotoxic for autologous tumor have been reported in TIL from various cancer patients [29]. These latter data thus indicate that TIL contain immunocompetent cells which can be activated to proliferate and become cytotoxic for autologous tumor.

Many reports have shown that PBL from cancer patients, stimulated in vitro with IL2 (IL2-PBL), can kill autologous and allogeneic tumor cells but not autologous normal cells [20], whereas the killing specificity of IL2-TIL seems to be less clearly assessed. In fact a recent study [18] has suggested an autologous tumor-specific lysis of melanoma cells by IL2-TIL, whereas other reports have shown rather preferential or autologous tumor-nonspecific killing [1, 10, 19].

This issue of specificity requires further investigation since, in the mouse system, the antineoplastic effect of adoptive transfer of TIL is based on their tumor specificity and T cell nature [21, 24]. In fact it has been reported that IL2-TIL possess a therapeutic potency higher than that of lymphokine-activated killer (LAK) cells in the cure of metastases of experimental tumors [21, 24] and IL2-TIL have been used in an immunotherapeutic setting in human metastatic adenocarcinoma of the lung [11].

We report here the results of experiments undertaken to evaluate and compare the cytotoxic activity and specificity of in vitro IL2-TIL and IL2-PBL from six cancer patients. We found that (1) the killing of autologous tumor by IL2-TIL was somehow higher than that caused by IL2-PBL; (2) this lysis was not restricted to the autologous neoplasm since allogeneic tumor cells were also destroyed; and (3) autologous PBL or TIL were not killed by IL2-TIL.

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Materials and methods

Tumor cells and PBL. Tumor specimens and PBL were obtained from six patients: two with carcinoma (MaCa 5056: primary mammary carcinoma and lymph node metastasis; ReCa 1874: primary renal carcinoma) and four with malignant melanoma (Me 1346, Me 3190: lymph node metastasis; Me 9361, Me 665: subcutaneous metastasis). From patient 665, three different melanoma specimens were obtained: two metastases (665/1 and 665/2) and the recurrence 665/R. TIL were derived from 665/2, and tumor cells from this lesion only were employed in autologous experiments. Heparinized blood samples of patients were obtained at least 6 days after surgery and purified on Ficoll-Paque gradients (Pharmacia Fine Chemicals, Uppsala, Sweden) [4].

Isolation and culture of TIL and tumor cells. Tumor specimens were cleaned, minced in RPMI 1640 (MA Bioproducts, Walkersville, Md., USA), and dead cells were removed by treatment with trypsin and DNase for 2 min at room temperature. The relative amounts of lymphocytes and tumor cells in the original suspensions and purity of the various cell fractions were evaluated by microscopic examination after May-Grünwald-Giemsa staining. Cell suspension thus obtained contained 10%–95% tumor cells and 5%–90% lymphocytes. When possible, tumor and lymphoid cells were separated by discontinuous gradients (75%–100%) of Ficoll-Paque [31]. Lymphocyte-rich mononuclear cells (p-TIL) were collected from the 100% interface, tumor cells (F) were collected from the 75% interface, and frozen in 90% pooled human serum + 10% DMSO. Alternatively, the original cell suspensions were incubated in medium containing 100 units/ml of recombinant IL2 (Bioleukin), kindly provided by Glaxo Institute for Molecular Biology of Geneva, Switzerland (only recombinant IL2 was used throughout the study); in these conditions tumor cells were usually eliminated within 6–12 days and at that time only lymphocytes were recovered. Aliquots of the suspensions were incubated in IL2-free medium to permit the outgrowth of adherent tumor cells (C). In one case (MaCa 5056) TIL were obtained from the primary tumor (TIL pri) and from a metastatic lesion (TIL met). The PBL and TIL were cultured, at an initial concentration of $5-10 \times 10^5$ cells/ml, in RPMI 1640 supplemented with 2 m*M* glutamine (MA Bioproducts), 20 m*M* 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid buffer (MA Bioproducts), 100 units/ml penicillin, and 100 μg/ml streptomycin (Farmitalia-Carlo Erba, Milan, Italy) (complete medium) plus 10% human heat-inactivated serum, with or without 100 units/ml of IL2. Cells cultures were maintained in complete medium plus 10% fetal bovine serum (Flow Laboratories, Irvine, UK). All cell cultures were shown to be mycoplasma free by electron microscopic examination or the immunofluorescence Mycospec method (BRL, Gaithersburg, Mass, USA).

Determination of lymphocyte phenotype. The expression of T, NK, and activation surface markers on lymphocytes was evaluated by indirect immunofluorescence; percentages of positive cells were determined by FACS analysis. Cells (2×10^5) were incubated with or without (control samples) the optimal dilution of each appropriate monoclonal antibody in 0.2 ml of complete medium in flat-bottomed 96-well microtiter plates (Costar 3596, Cambridge, Mass., USA) at 4° C for 30 min. Cells were then washed 3 times with cold medium and resuspended in 0.1 ml of 1:30 dilution of fluorescein-conjugated $F(ab')_2$ fragment of goat anti-mouse Ig antibody (Technogenetics, San Mauro Torinese, Italy); after a further incubation at 4° C for 30 min in the dark, the cells were washed 4 times and resuspended in 0.2 ml of 1% paraformaldehyde-RPMI 1640. The following monoclonal antibodies were used: OKT3 (anti-CD3), OKT4 (anti-CD4), OKT8 (anti-CD8) (Ortho Diagnostic, Raritan, NJ, USA) TEC-IL2 R (anti-IL2 receptor or -TAC antigen) (Technogenetics), Leu 19 (Becton Dickinson, Sunnyvale, Calif., USA), B73.1 (anti-CD16) and D1.12 (anti-DR) kindly provided by Dr. B. Perussia (Wistar Institute of Philadelphia, Pa., USA) and Dr. R. Accolla (Ludwig Institute for Cancer Research, Lausanne, Switzerland).

Cytotoxicity assay. The lytic activity of lymphocytes was assessed against autologous F, C, or normal cells (PBL or TIL) and against a panel of allogeneic F or C cells of various histotype. The ^{51}Cr release assay was performed as previously described [4]. Briefly, ^{51}Cr-labeled tumor cells (1×10^3 cells/well) were admixed with different numbers of effector lymphocytes in 0.2 ml of complete medium with 10% human heat-inactivated serum in U-bottomed 96-well microtiter plates; plates were centrifuged and incubated at 37° C for 4 h, then aliquots of supernatants were collected and radioactivity counted. When normal cells were used as target cells, an incubation time of 8 h and an effector/target (E/T) ratio of 100/1 were employed. Data were calculated as percentage of specific release. Cell-mediated cytotoxicity was considered positive when the percentage of cytotoxicity was >15. Spontaneous releases of F or C target cells ranged between 6% and 36%.

Results

Lytic activity of IL2-PBL and IL2-TIL against autologous tumor cells

After 7 or 14 days of culture in 100 units/ml of IL2, PBL, TIL and purified TIL (p-TIL, purified from tumor cells before IL2 stimulation) were assayed for cytotoxicity against autologous cells. As shown in Fig. 1, in four cases (Me 665, Me 1346, Me 9361, and MaCa 5056) IL2-activated effectors were found to efficiently lyse autologous tumor cells, whereas Me 3190, and ReCa 1874 targets were poorly lysed at E/T ratios of 50/1 and 25/1, respectively. In five instances (Me 3190 F, Me 665 C, ReCa 1874 F, MaCa 5056 F, and MaCa 5056 C) the lytic activity of IL2-TIL appeared to be higher than that of IL2-PBL. In three cases illustrated in panel B (Me 1346, Me 9361, and MaCa 5056), autologous cytotoxicity could be assayed against both F and C cells; at least for Me 9361 and MaCa 5056, C cells were more susceptible to lysis by IL2-TIL than the F ones. In three cases (Me 665 and ReCa 1874: all E/T ratios; MaCa 5056: E/T ratio 12/1, F), differences between cytotoxic activities of IL2-TIL and IL2-PBL reached statistical significance ($P < 0.01$ Student's *t*-test).

Analysis of specificity of IL2-PBL and IL2-TIL

Each type of IL2-activated effector was examined for cytotoxic activity against autologous F or C cells and against a

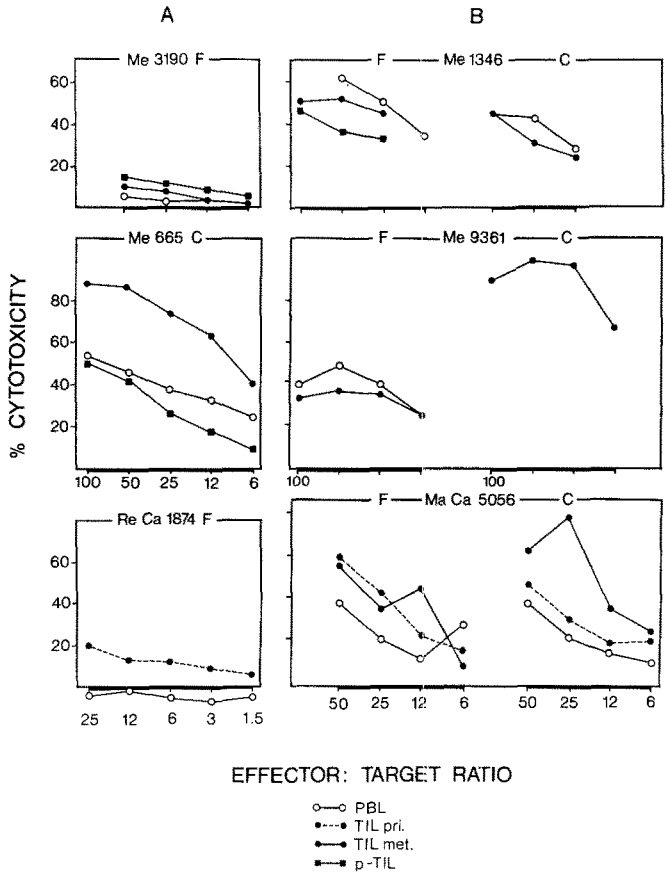

Fig. 1 (A–B). Cytotoxic activity of interleukin 2 (IL2)-activated peripheral blood lymphocytes (PBL) and IL2-activated tumor-infiltrating lymphocytes (TIL) against autologous tumor cells. TIL and PBL were cultured in the presence of IL2 (100 units/ml) for 7 or 14 days before testing. PBL: IL2-activated PBL; TIL pri: IL2-activatd TIL obtained from primary tumors; TIL met: IL2-activated TIL obtained from metastatic tumors; p-TIL: TIL purified from tumor cells before culturing in IL2

panel of allogeneic F or C cells of various histologic origin including K562 and Daudi, melanomas, 6 F (Me 6155, Me 1906, Me 6993, Me 7557, Me 5923, and Me 7653), 7 C (Me 665/1, Me 665/2, Me 665/R, Me 1007, Me 10538, Me 1402, and Me 1402/R); 2 carcinoma (MCF-7, OvCa), and 1 sarcoma (SaOs) cell lines; 1 F carcinoma (MaCa 5557); fibroblasts and a lymphoblastoid cell line from the melanoma patient 665 (665/Fib, 665/LCL).

As shown in Fig. 2, in no case was lysis restricted to the autologous tumor, and K562 and Daudi cell lines were always efficiently lysed. In one case (Me 1346) IL2-TIL, unlike IL2-PBL, appeared to show preferential lysis of autologous F in comparison with allogeneic F of the same histology (at E/T = 25/1: 45% vs 20%–33% for TIL and 50% vs 54% for PBL, Fig. 2A). This preferentiality was not due to particularly high susceptibility of Me 1346 cells to lysis, as demonstrated by cytotoxicity tests performed with allogeneic activated effectors against these and other fresh tumor cells (data not shown). In two other cases (Me 665 C, MaCa 5056 F) similar preferential lysis of autologous tumor cells was observed by both IL2-PBL and IL2-TIL (Me 665 C: 38% vs 16%–18% for PBL, 74% vs 10%–32% for TIL, 26% vs 9%–10% for p-TIL; MaCa 5056 F: 20% vs 15% for PBL, 42% vs 30% for TIL-pri, 34% vs 26% for TIL-met) (Fig. 2B); in the case of Me 665, this phenomenon could be in part related to the greater sensitivity of this cultured tumor to lysis by IL2-activated allogeneic lymphocytes (data not shown). In the cases of ReCa 1874 F (Fig. 2A) and Me 3190 F (Fig. 2B), allogeneic F cells appeared to be slightly more lysed than autologous ones.

Results of the specificity study, summarized in Table 1, clearly indicated a lack of killing of autologous tumors only, since all effectors lysed at least some allogeneic tumor cells used as targets.

As targets, normal autologous PBL, IL2-PBL and TIL or allogeneic PBL were not lysed even at a high E/T ratio (100/1) and with an incubation time of 8 h; with the exception of the IL2-PBL and IL2-TIL effectors from ReCa patient (1874) which lysed both lymphoid and fibroblast targets.

Lytic activity of TIL at different days during IL2 culture

To see whether the time of culture in IL2 could modify the lytic activity, TIL from Me 665 and Me 1346 were cultured in 100 units/ml of IL2 for up to 35 and 21 days respectively. Cytotoxicity against autologous melanoma and allogeneic tumors was assayed on different days. As reported in Table 2, IL2-TIL efficiently destroyed autologous tumor cells during all culture periods. At no time was autologous tumor-restricted lysis observed since allogeneic melanomas, K562 and Daudi cells, were always lysed apparently without significant differences among tests performed on different days. No lysis of autologous normal lymphocytes was noted. The p-TIL (uncultured or cultured for 7 days in the absence of IL2) and unstimulated PBL lacked significant cytotoxicity against autologous and allogeneic tumor cells (data not shown). Thus, IL2 activation appeared to be necessary for the development of lytic activity of both types of lymphocytes.

Phenotypic analysis of TIL and PBL

Phenotypic profiles of PBL and TIL from melanoma and carcinoma patients were evaluated at the beginning and on different days during IL2 culture. As shown in Table 3, which reports the mean values (with the range) of the single observations, freshly isolated TIL showed a decreased proportion of cells expressing CD16 (NK cells) (0–3% vs 11%–15%), and a higher proportion of cells expressing DR and TAC antigens (18% vs 4% and 10% vs 0%, respectively) in comparison with PBL. In both PBL and TIL populations, IL2 culture allowed the preferential growth of $CD8^+$ cells compared to $CD4^+$ cells. As expected, the percentage of cells expressing Leu 19, DR, and TAC antigens augmented during the culture period.

Discussion

Freshly isolated TIL had little or no direct cytotoxicity for tumor cells and even for K562, a result reported by other investigators [12, 16, 33]. Immunological functions like proliferation to lectins, lectin-dependent cytotoxicity, and tumor cytotoxicity were shown to be impaired in fresh TIL possibly due to inhibitory factors present in the tumor microenvironment [14]. However, the present report shows that when stimulated by IL2, TIL acquired the capacity to kill autologous tumor cells. In fact TIL directly grown with

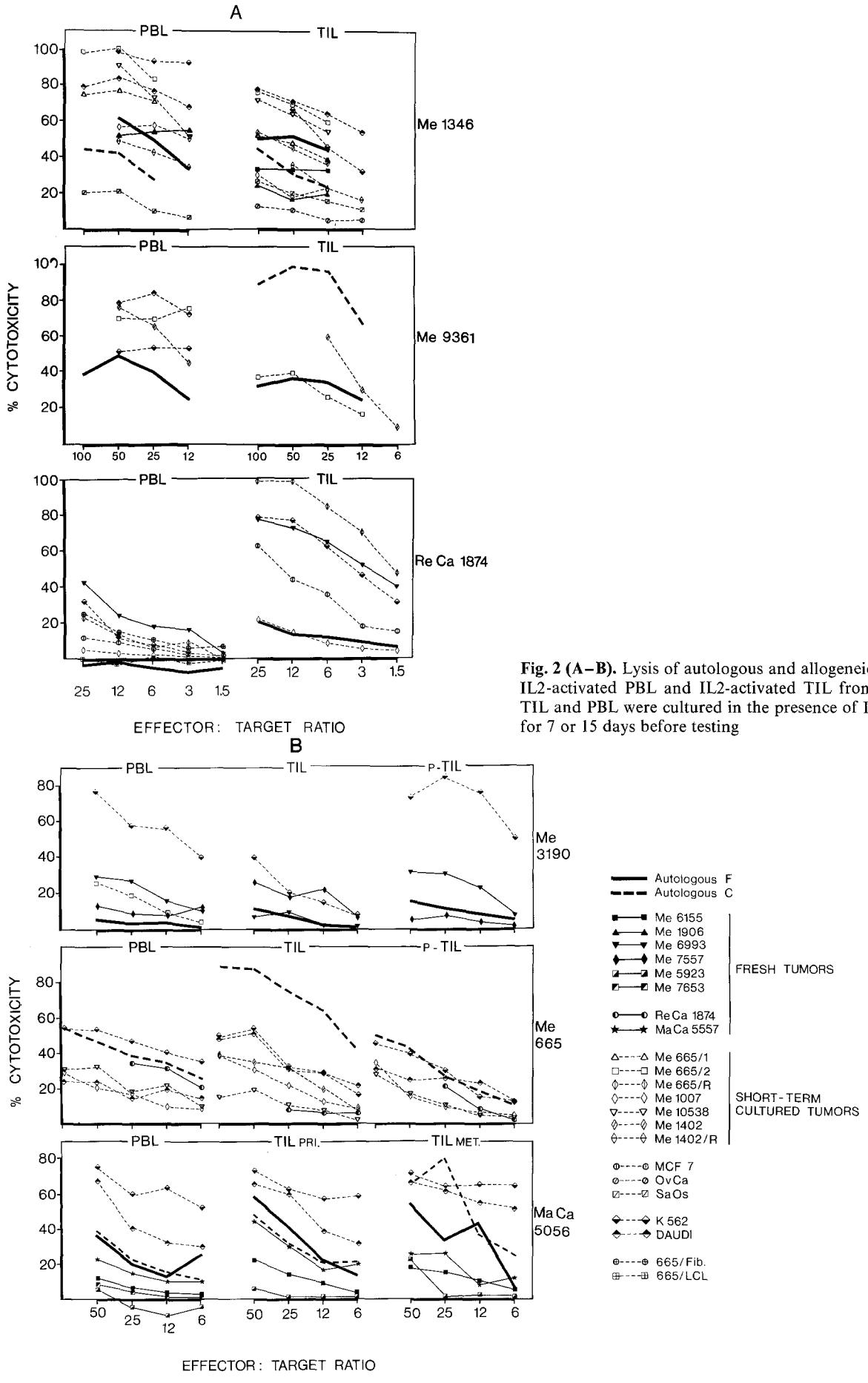

Fig. 2 (A–B). Lysis of autologous and allogeneic tumor targets by IL2-activated PBL and IL2-activated TIL from cancer patients. TIL and PBL were cultured in the presence of IL2 (100 units/ml) for 7 or 15 days before testing

Table 1. Analysis of specificity of killing of IL2-stimulated lymphocytes from cancer patients

Patient	Effectors	Targets											
		Autologous			Allogeneic tumors					Allogeneic			
		Tumor		Lympho-cytes[a]	Melanoma		Carcinoma		Sar-coma	Nor-mal cells[b]	LCL[c]	K562	Daudi
		F	C		F	C	F	C	C				
Melanomas													
1346	PBL	+++[d]	++	–	1/1[e]	5/5		1/2	1/1		0/1	+++	+++
	TIL	+++	++	–	2/2	6/6		1/2	1/1	0/1	0/1	+++	+++
	p-TIL	+++	++	–									
3190	PBL	–		–	2/2	1/1						+++	
	TIL	–		–	1/2							++	
	p-TIL	+		–	1/2							++	
665	PBL		++	–		2/2	1/1					+++	+
	TIL		+++	–		3/3	1/1					+++	
	p-TIL		++	–		2/2	0/1					++	++
9361	PBL	++		–		2/2				0/1		+++	++++
	TIL	++	+++	–		2/2				0/1		++++	++++
Mammary carcinoma													
5056	PBL	++	++	–	1/3		1/1					++++	+++
	TIL pri	+++	++	–	1/2		1/1					+++	+++
	TIL met	+++	+++	–	2/2		1/1					+++	+++
Renal carcinoma													
1874	PBL	–		–	1/1	1/2		1/1		1/1	0/1	++	
	TIL	+		+	1/1	1/2		1/1		1/1	0/1	++++	

All effectors were cultured in IL2

[a] Autologous lymphocytes were normal fresh PBL or PBL or TIL cultured in IL2

[b] Allogeneic normal cells were normal PBL (Me 1346, Me 9361) or cultured normal skin fibroblasts (ReCa 1874)

[c] Lymphoblastoid cell line from patient 665

[d] % specific cytotoxicity: – ≤15%, + 15–25%, ++ 26–50%, +++ 51–75%, ++++ >75%. Effector/target (E/T) ratio = 50/1

[e] Number of positively (>15%) lysed target cells/total tested

Table 2. Autologous and allogeneic tumor cytotoxicity of IL2-TIL from two melanoma patients tested on different days of IL2 culture

Targets	Me 665					Me 1346		
Days of IL2 culture→	7	14	21	28	35	7	14	21
Autologous tumor	74[a]	20	61	57	49	31	52	57
Autologous lymphocytes		–1						–5
Allogeneic melanomas	21[b]–32	10–11	21	16	18	48–71–18	65–46	36–86
K562 – Daudi	27–48	30–42	56	57	47			68–71

[a] Percentage cytotoxicity at E/T ratio 25/1

[b] Each value refers to a different target

autologous tumor cells in the presence of IL2 usually destroyed tumor cells which were undetectable after 6–12 days. In addition these IL2-TIL lysed autologous F and C tumor cells in a short-term ^{51}Cr release assay. Thus, in this respect TIL resemble PBL which, when stimulated by IL2, can kill different types of autologous tumor targets [1, 3, 10, 18, 19].

We have also found that in general IL2-TIL appear to be slightly more cytotoxic for autologous tumors than IL2-PBL, a finding which is in agreement with those reported by others [10, 19]. Differences in tumor cytotoxicity between IL2-TIL and IL2-PBL are usually much less than those described between TIL and LAK cells in the mouse [21, 24] and, therefore, can hardly be taken as an indication for using TIL in a clinical setting.

Next we investigated the degree of tumor specificity of the killing of IL2-TIL compared to that of IL2-PBL. Recently IL2-TIL have been reported [18] to specifically lyse only autologous melanoma cells in three patients while expressing a limited capacity to kill allogeneic F in three other cases. Other studies, however, have shown that the lysis of autologous tumor cells was preferentially rather than absolutely restricted in some but not in other cases [3, 10, 19]. Since the definition of specific reactivity rests on the size of the panel of allogeneic targets used, we tried to include a panel of target cells larger than that usually re-

Table 3. Phenotypic analysis of PBL and TIL at various passages in culture with IL2

Culture passage	Antigenic markers						
	CD3	CD4	CD8	CD16	Leu 19	DR	TAC
0 PBL	62[a] (50–72)	47 (44–51)	31 (29–32)	13 (11–15)	4	4 (0–9)	0
TIL	71 (52–82)	62 (52–73)	20 (13–30)	1 (0–3)	1 (0–2)	18 (7–28)	10 (4–18)
1 PBL	69 (52–78)	37 (21–56)	35 (14–72)	12 (4–21)	14 (0–28)	43 (11–76)	36 (17–59)
TIL	54 (53–55)	34 (16–57)	27 (9–37)	0	15 (7–24)	28 (16–41)	15 (8–19)
2 PBL	63 (52–74)	10 (9–12)	65 (53–77)	30 (19–41)	48 (42–54)	84 (81–88)	24 (16–32)
TIL	56 (39–79)	20 (0–35)	43 (17–84)	2 (0–6)	22 (14–35)	46 (6–73)	20 (2–55)
3 TIL	57 (25–90)	8 (5–10)	49 (21–78)	18 (5–31)	56 (46–67)	49 (47–51)	2 (0–4)

PBL and TIL were cultured in the presence of IL2 (100 units/ml) and passaged every 4–6 days. Percentages of positive cells were determined by FACS analysis after indirect immunofluorescence with the given monoclonal antibodies

[a] Mean value (range) of the individual percentages of positive cells

ported in similar studies. The results of our specificity experiments suggested that IL2-TIL, as IL2-PBL, do not express cytotoxicity restricted to autologous tumor; K562 and Daudi were always lysed and in all cases tested one or more allogeneic tumors were lysed. This reactivity was seen on both fresh and cultured target cells.

The crucial aspect of killing specificity, however, is the lysis of autologous tumor cells but not of autologous normal counterparts. It has been reported that IL2-PBL do not lyse autologous lymphocytes or other autologous normal cells [20]. Unfortunately, the normal autologous cells which could be used in the majority of the studies on tumor killing specificity were lymphocytes. The reported lack of lysability of autologous lymphocytes by IL2-PBL has been questioned by the results of a study specifically addressed to this point [23], and showing that IL2-PBL can destroy autologous and allogeneic lymphocytes in an 8 h ^{51}Cr assay. Therefore we tested the cytotoxic activity of IL2-TIL and IL2-PBL against autologous and allogeneic lymphocytes (including unstimulated PBL, IL2-PBL, or IL2-TIL) in an 8 h ^{51}Cr release assay at an E/T ratio of 100/1. Under these experimental conditions we failed to detect significant killing of autologous lymphocytes (except for the 1874 TIL, Table 1) and of allogeneic PBL or Epstein-Barr virus-transformed cell lines. Cultured fibroblasts from normal skin, however, were lysed in the only case in which they were used (1874).

Therefore we conclude that tumor cytotoxic IL2-PBL and IL2-TIL usually do not kill autologous and allogeneic lymphocytes. Lack of killing of autologous lymphocytes by IL2-TIL has also been found by others [18, 19].

In our study the lymphocyte subsets present in the TIL population appeared similar to that of PBL with a notable exception since TIL contained a negligible number of cells expressing the NK marker CD16 and a low but consistent fraction of cells with activation markers DR and TAC. The majority of lymphocytes were $CD3^+$ with a frequency of $CD4^+$ and $CD8^+$ cells comparable to PBL. This finding is at variance with those reporting a higher percentage of $CD8^+$ than that of $CD4^+$ cells in TIL [6, 19, 34]. It should be noted, however, that we found a great variability in the T cell subsets among the various individual cases examined. During IL2 culture, a shift toward an increase in $CD8^+$ cells with a concomitant decrease in $CD4^+$ and a general augmentation of TAC^+, DR^+, and $Leu19^+$ cells was observed. In contrast others have reported an increase in $CD4^+$ cells in IL2-cultured TIL from melanoma patients [18]. In addition a high number of $CD4^+$ cytotoxic T lymphocyte (CTL) clones were isolated from TIL of different solid tumors [15]. However, a predominance or shift to an increase in $CD8^+$ compared to $CD4^+$ was also noted in IL2-TIL from some cancer patients [6].

Growing evidence indicates that killing of various tumor cells by IL2-PBL (LAK phenomenon) is largely mediated by IL2-stimulated NK cells [6, 7, 27]. Since the results presented here and those from other studies suggest that IL2-TIL display a pattern of lysis similar to that of IL2-PBL, the nature of cells which mediate this type of unrestricted lysis remains unsettled given the fact that few cells with NK markers are found in the TIL population. It may possibly be that both T cells ($CD3^+$, $CD16^-$) or IL2-stimulated NK cells ($CD3^-$, $CD16^+$) are the cytotoxic effector cells although we cannot exclude that $CD3^+$ $CD16^+$ cells are involved in tumor cell lysis, since in two cases we found a higher percentage of $CD16^+$ cells after culturing TIL in IL2 for 3 weeks. A recent report has suggested that the antitumor effector cells in IL2-TIL possess $CD3^+$ Leu 19^+ and $CD3^-$ Leu 19^+ phenotype. In this study, however, the antitumor activity was assessed on allogeneic cells only [6]. Activated $CD3^+$ cells cultured in vitro with IL2 may express nonrestricted killing of tumor cells, a finding frequently noted with CTL clones derived from TIL or PBL of cancer patients [2].

It is also possible that activated cells present in the TIL population, although representing a small fraction, possess a restricted specificity for autologous tumor as suggested by others [18, 30] and that extensive clonal analysis of TIL for autologous-specific killing could shed light on this aspect of tumor immunology.

In conclusion the results of this study indicate that IL2-TIL show a pattern of killing specificity similar to that of IL2-PBL. The high killing of TIL from some patients and the lack of lysis of autologous lymphocytes justify further studies to assess the presence in TIL of tumor-specific T cells, since such effectors have shown a tumor eradicating activity in the mouse much higher than that of LAK cells [21, 24].

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References

1. Allavena P, Zanaboni F, Rossini S, Merendino A, Bonazzi C, Vassena L, Mangioni C, Mantovani A (1986) Lymphokine-activated killer activity of tumor-associated and peripheral blood lymphocytes isolated from patients with ascites ovarian tumors. J Natl Cancer Inst 77: 863
2. Anichini A, Fossati G, Parmiani G (1987) Clonal analysis of cytolytic T cell response to human tumors. Immunol Today 8: 385
3. Burns GF, Good MF, Riglar C, Bartlett PF, Crapper RM, Mackay IR (1984) Activated lymphocyte killer cells derived from melanoma tissue or peripheral blood. Clin Exp Immunol 57: 487
4. Fossati G, Taramelli D, Balsari A, Bogdanovich G, Andreola S, Parmiani G (1984) Primary but not metastatic melanomas expressing DR antigens stimulate autologous lymphocytes. Int J Cancer 33: 591
5. Hayry P, Totterman TH (1978) Cytological and functional analysis of inflammatory infiltrates in human malignant tumours. 1. Composition of inflammatory infiltrates. Eur J Immunol 8: 866
6. Heo DS, Whiteside TL, Johnson JT, Chen K, Barnes EL, Herberman RB (1987) Long-term interleukin 2 growth and cytotoxic activity of tumor-infiltrating lymphocytes from human squamous cell carcinomas of the head and neck. Cancer Res 47: 6353
7. Herberman RB, Hiserodt J, Vujanovic N, Balch C, Lotzova E, Bolhuis R, Golub S, Lanier LL, Phillips JH, Riccardi C, Ritz J, Santoni A, Schmidt RE, Uchida A (1987) Lymphokine-activated killer cell activity. Immunol Today 8: 178
8. Hersey P, Bindon C, Edwards A, Murray E, Phillips G, McCarthy WH (1981) Induction of cytotoxic activity in human lymphocytes against autologous and allogeneic melanoma cells in vitro by culture with interleukin 2. Int J Cancer 28: 695
9. Ioachim HL (1976) The stroma reaction of tumours: an expression of immune surveillance. J Natl Cancer Inst 57: 465
10. Itoh K, Tilden AB, Balch CM (1986) Interleukin 2 activation of cytotoxic T-lymphocytes infiltrating into human metastatic melanomas. Cancer Res 46: 3011
11. Kradin RL, Boyle LA, Preffer FI, Callahan RJ, Barlai-Kovach M, Strauss HW, Dubinett S, Kurnick JT (1987) Tumor-derived interleukin-2-dependent lymphocytes in adoptive immunotherapy of lung cancer. Cancer Immunol Immunother 24: 76
12. Mantovani A, Allavena P, Sessa C, Bolis G, Mangioni C (1980) Natural killer activity of lymphoid cells isolated from human ascitic ovarian tumors. Int J Cancer 25: 573
13. McGovern FJ, Shaw HM, Milton CW, Farago GA (1979) Prognostic significance of the histological features of malignant melanoma. Histopathology 3: 385
14. Miescher S, Whiteside TL, Carrel S, Von Fliedner V (1986) Functional properties of tumor-infiltrating and blood lymphocytes in patients with solid tumors: effects of tumor cells and their supernatants on proliferative responses of lymphocytes. J Immunol 136: 1899
15. Miescher S, Whiteside TL, Moretta L, Von Fliedner V (1987) Clonal and frequency analyses of tumor-infiltrating T lymphocytes from human solid tumors. J Immunol 138: 4004
16. Moy PM, Holmes EC, Golub SH (1985) Depression of natural killer cytotoxic activity in lymphocytes infiltrating human pulmonary tumors. Cancer Res 45: 57
17. Mukherji B, Wilhelm SA, Guha A, Ergin MT (1986) Regulation of cellular immune response against autologous human melanoma. I. Evidence for cell-mediated suppression of in vitro cytotoxic immune response. J Immunol 136: 1888
18. Muul LM, Spiess PJ, Director EP, Rosenberg SA (1987) Identification of specific cytolytic immune responses against autologous tumor in humans bearing malignant melanoma. J Immunol 138: 989
19. Rabinowich H, Cohen R, Bruderman I, Steiner Z, Klajman A (1987) Functional analysis of mononuclear cells infiltrating into tumors: lysis of autologous human tumor cells by cultured infiltrating lymphocytes. Cancer Res 47: 173
20. Rosenberg SA (1985) Lymphokine-activated killer cells: a new approach to immunotherapy of cancer. J Natl Cancer Inst 75: 595
21. Rosenberg SA, Spiess P, Lafreniere R (1986) A new approach to adoptive immunotherapy of cancer with tumor-infiltrating lymphocytes. Science 233: 1318
22. Santoli D, Tweardy DJ, Ferrero D, Kreider BL, Rovera G (1986) A suppressor lymphokine produced by human T leukemia cell lines. J Exp Med 163: 18
23. Sondel PM, Hank JA, Kohler PC, Chen BP, Minkoff DZ, Molenda JA (1986) Destruction of autologous human lymphocytes by interleukin 2-activated cytotoxic cells. J Immunol 137: 502
24. Spiess PJ, Yang JC, Rosenberg SA (1987) In vivo antitumor activity of tumor-infiltrating lymphocytes expanded in recombinant interleukin 2. J Natl Cancer Inst 79: 1067
25. Taramelli D, Fossati G, Balsari A, Marolda R, Parmiani G (1984) The inhibition of lymphocyte stimulation by autologous human metastatic melanoma cells correlates with the expression of HLA-DR antigens on the tumor cells. Int J Cancer 34: 797
26. Totterman TH, Hayry P, Saksela E, Timonen T, Eklund B (1978) Cytological and functional analysis of inflammatory infiltrates in human malignant tumours. II. Functional investigations of the infiltrating cells. Eur J Immunol 8: 872
27. Trinchieri G, Matsumoto-Kobayashi M, Clark SC, Seehra J, London L, Perussia B (1984) Response of resting human peripheral blood natural killer cells to interleukin 2. J Exp Med 160: 1147
28. Underwood JCE (1974) Lymphoreticular infiltration in human tumours: prognostic and biological implication: a review. Br J Cancer 30: 538
29. Vose BM (1982) Quantitation of proliferative and cytotoxic precursor cells directed against human tumours: limiting dilution analysis in peripheral blood and at the tumour site. Int J Cancer 30: 135
30. Vose BM (1987) Activation of lymphocyte anti-tumour responses in man: effector heterogeneity and the search for immunomodulators. Cancer Metastasis Rev 5: 299
31. Vose BM, Moore M (1979) Suppressor cell activity of lymphocytes infiltrating human lung and breast tumours. Int J Cancer 24: 579
32. Vose BM, Vanky F, Klein E (1977) Human tumour-lymphocyte interaction in vitro. V. Comparison of the reactivity of tumour-infiltrating, blood and lymph node lymphocytes with autologous tumour cells. Int J Cancer 20: 895
33. Vose BM, Vanky F, Argov S, Klein E (1977) Natural cytotoxicity in man: activity of lymph node and tumour-infiltrating lymphocytes. Eur J Immunol 7: 753
34. Whiteside TL, Miescher S, Hurlimann J, Moretta L, Von Fliedner V (1986) Separation, phenotyping and limiting dilution analysis of T-lymphocytes infiltrating human solid tumors. Int J Cancer 37: 803

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