LYSIS OF TUMOR BIOPSY CELLS BY AUTOLOGOUS T LYMPHOCYTES ACTIVATED IN MIXED CULTURES AND PROPAGATED WITH T CELL GROWTH FACTOR*

By FARKAS VÁNKY, TIKVA GORSKY, YORAM GORSKY, MARIA-GRAZIA MASUCCI, AND EVA KLEIN

From the Department of Tumor Biology, Karolinska Institute and the Radiumhemmet, Karolinska Hospital, S-104 01 Stockholm 60, Sweden

A proportion of patients with carcinomas and sarcomas possess blood lymphocytes that can lyse their freshly harvested tumor cells in vitro (1-4). To enhance such cytotoxicity, we activated the lymphocytes either in mixed cultures with autologous tumor biopsy cells (ATS) or in conventional mixed lymphocyte cultures (MLC). In accordance with the results of Zarling et al. (5), with leukemia cells, MLC-activated lymphocytes were often cytotoxic for autologous and third-party allogeneic solid tumor cells (6). Cocultivation with the patients' tumors generated autotumor cytotoxicity in the majority of experiments (7-9). In contrast to the MLC, these lymphocytes did not lyse allogeneic tumor biopsy cells.

The effects against autologous and third-party tumors by the patients' MLC-lymphocytes might be a consequence of activation of lymphocyte sets that recognize (a) antigens that cross-react with the stimulator lymphocytes (b) tumor-related antigens shared by tumor cells of the lymphocyte donor and the allogeneic tumor, and (c) distinct allo- and/or tumor-related antigens on the different targets. Cold target competition experiments presented in this paper suggest that the third alternative is valid.

Establishment of growing T cell lines with maintained specific functions (cytotoxicity or proliferative capacity) is an important step in the progress of tumor immunology (10-17). Such T cell populations reacting with autologous tumor cells might help in the study of the cell surface antigens of tumor cells, and they may be exploited in therapeutical measures.

Because T cell growth factor (TCGF) (interleukin 2; IL-2) promotes the growth of activated T cells (18), its use imposes a selection for propagation of specific clones when applied to populations preexposed to the relevant antigen. To raise specific autotumor killer T cell cultures from patients with solid tumors, we cultured the blood lymphocytes first with the autologous tumor cells and thereafter added TCGF to...
expand the responding population. We compared these and the MLC-activated, TCGF-propagated lymphocytes from the same patient with regard to cytotoxicity for autologous and allogeneic tumor biopsy cells. The results showed that the reactivity of the growing T cell cultures reflects the characteristics of the population from which they were initiated.

**Materials and Methods**

**Tumor Cells.** These cells were separated from surgical specimens of seven patients with malignancies of various histologic types (tumor 898 is B cell lymphoma; tumors 1,044 and 1,045 are osteosarcomas; tumors 2,207 and 2,285 are oat cell carcinomas of the lung; tumor 2,286 is a squamous cell carcinoma on the lung; and tumor 513 is a hypernephroma) by stepwise application of velocity and density sedimentations of Ficoll-Isopaque (FI) and on human serum albumin gradients, treatment with collagenase and deoxyribonuclease (DNase), as well as adherence to a plastic surface (2, 19). The isolated tumor cells were incubated overnight in culture conditions before their use, to allow resynthesis of cell surface antigens assumed to be affected in vivo and/or during the procedure of cell separation. Experiments were performed only with cell suspensions that had at least 80% cell viability as assessed by trypan blue exclusion and that had <5% obvious contamination with nonmalignant cells.

Samples of tumor cells (2 × 10^6 to 5 × 10^6 vials) were stored in liquid nitrogen as previously described (2) to use as targets for the cultured effectors. The thawed cells were diluted gradually to 10 ml medium plus 20% normal human serum. When necessary, dead cells were removed by centrifugation on FI.

**Cell Lines.** The erythroid line K-562 (20) and the Burkitt’s lymphoma line Daudi (21) were maintained in suspension cultures in RPMI 1640 medium supplemented with 10% fetal calf serum.

**Lymphocytes.** Lymphocytes from patients and healthy donors were separated from heparinized blood on FI, followed by incubation in plastic culture flasks (type 3023; Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.) for 30 min at 37°C to remove adherent cells. One aliquot of the total nonadherent population was treated with mitomycin-C and used as a stimulator in the MLC. For effectors in the direct cytotoxicity tests and for responders in the various types of mixed cultures, the lymphocytes were passed through a nylon fiber column (22). The nylon column-passed (NCp) lymphocyte population contained mainly T cells; in indirect immunofluorescence, 97-100% reacted with the monoclonal antibody OKT3, specific for circulating T cells (23), and 0-2% reacted with the OK1al antibody, reacting with the Ia antigen (24, 25). The monoclonal antibodies were obtained from G. Goldstein (Ortho Pharmaceutical Co., Raritan, N. J.).

**Tissue Culture Medium.** RPMI 1640 medium (Flow Laboratories Ltd., Irvine, Ayrshire, Scotland) with l-glutamine (200-mM solution, 1% by volume), benzyl-penicillin (100 IU/ml), streptomycin sulphate (100 µg/ml), Hepes buffer (20 mM), and 10% concentration of heat-inactivated serum (from healthy male donors) was used in all experiments. The human sera were not preselected, but cloudy preparations were excluded.

**Stimulation with PHA.** NCp lymphocytes (10^7 cells/10 ml) were cultured with 0.5 µg/ml purified phytohemagglutinin (PHA; Wellcome Reagents Ltd., Beckenham, England) for 3-4 d. These cells, 60-70% T blasts, were used either as effectors or as targets. When used in the cold target competition assays, they were exposed to chilled formaldehyde (0.4% solution in phosphate-buffered saline, pH 7.2) and kept in an ice bath for 30 min. Thereafter, they were washed with phosphate-buffered saline three times, the first wash being at 0°C (26).

**Mixed Lymphocyte Cultures.** Aliquots of the NCp lymphocytes (10^7 to 15 × 10^5 cells/flasks) were mixed either with 0.5 × 10^6 to 1.5 × 10^6 autologous tumor biopsy cells for the ATS (we found the optimum ratios within this range), or with 5 × 10^6 to 7 × 10^6 mitomycin C-treated allogeneic lymphocytes (total population and/or the nylon column-attached [NCa] fraction) for the MLC. The 10- to 15-ml cultures were incubated for 5-6 d in plastic culture flasks (type 1013; Falcon Labware) at 37°C in a 5% CO2-containing humidified atmosphere. On day 5, one-half of the medium was replaced.
The 6-d MLC and ATS cultures contained 95–100% OKT3, 23–30% OKIal, and 0–2% OKM1-reacting cells. The emergence of OKIal reactivity is a sign of T cell activation (25).

Propagation of the T Cell Cultures. MLC and PHA-stimulated aliquots of lymphocytes were exposed to TCGF on day 6, and the ATS cultures were exposed on day 10. They were further propagated in culture medium containing 50% TCGF for 2–4 wk.

The source of TCGF was the supernatant of cultured Fl-separated lymphocytes of healthy blood donors. The lymphocytes were incubated with Wellcome PHA-P (100 μg/10⁸ cells per ml for 30 min at 37°C, followed by six washes and thereafter incubated (10⁶ cells/ml) in serum-free RPMI 1640 tissue culture medium for 48 h. The duplication time of the TCGF cultures was 2.5 d.

All cells from these cultures reacted with OKT3, and 30% also reacted with OKIal antibody. No OKM1-reactive cells were detected.

Cytotoxic Assay. A modification of the method described by Vose et al. (1) has been used (19). The percentage of ⁵¹Cr release was calculated from the following formula: (counts in supernatant per counts in supernatant and pellet × 100). The percent specific ⁵¹Cr release was then determined by the following formula:

\[
\frac{(\text{percent release in test} - \text{percent spontaneous release})}{(\text{percent maximum release} - \text{percent spontaneous release})} \times 100
\]

Tests in which the spontaneous release exceeded 50% were disregarded. Statistical significances were calculated on the cpm values of the triplicate samples by the Student's t test.

Cold Target Competition. The effectors (5 × 10⁶ cell/well) were dispersed in 50 μl medium, and various numbers of unlabeled targets were added in 50 μl. The ratios of effector to cold targets were 100:1, 50:1, and 25:1. The plates were then centrifuged at 800 g for 10 s and incubated for 30 min at 37°C. They were then shaken (Titretek plate shaker, Flow Laboratories Ltd, Irvine, Ayrshire, Scotland) and the ⁵¹Cr-labeled targets (10⁴ cells/well) were added in 100 μl medium. (The ratios of labeled:unlabeled cells varied between 5:1 and 1:5). The volume in each well was adjusted to 200 μl. After centrifugation (800 g for 10 s), the plates were incubated and processed as the usual cytotoxic assays. Statistical significances were calculated on the triplicates of cpm values by the Student's t test.

Results

Cytotoxicity of MCL-activated Lymphocytes of Healthy Donors. Lymphocytes cultured in conventional MLC were cytotoxic against blasts of the stimulator. In addition, they lysed the K-562 and Daudi cells in all cases; they lysed third party allogeneic PHA blasts, TCGF blasts, and tumor biopsy cells in 3/4, 3/4, and 5/8 tests, respectively. Autologous TCGF or PHA blasts were not damaged (Table I).

Cytotoxicity of ATS or MLC-activated Lymphocytes of Tumor Patients. Aliquots of the patients’ lymphocytes were activated in ATS and MLC (Tables II and III). Before activation, two patients (513 and 2,285) exhibited cytotoxicity against their own tumor cells (20 and 34% specific release, respectively). In all experiments, the lymphocytes cultured with autologous tumor biopsy cells lysed these cells when tested on day 6. Allogeneic tumor cells and autologous or allogeneic PHA blasts were not lysed. Thus, the cytotoxicity was specific for the stimulator cells.

In contrast, the lymphocytes of the MLC were cytotoxic for the autologous, allogeneic third-party tumor biopsy cells and for allogeneic PHA blasts in 3/5, 5/7, and 4/4 of tests, respectively. Autologous PHA blasts were not lysed (four tests). These results thus confirmed that lymphocytes activated in MLC can lyse autologous tumor cells (5, 6) and we now show that they do not lyse autologous PHA blasts. In accordance with our previous results, the generation of autotumor killer cells occurred more regularly in the autologous mixed cultures than in MLC, and the cytotoxicity was specific for the autologous tumors.
AUTLOGOUS TUMOR KILLER T CELL CULTURES

**Table I**

Cytotoxicity of MLC-activated Lymphocytes from Healthy Donors Tested Directly or after Propagation with TCGF*

| Expe- | Propaga- | PHA blasts | TCGF blasts | Tumor biopsy | Cell lines |
| riment | tion with | Autologous | Allogeneic | Allogeneic | Autologous | Allogeneic | K-562 | Daubi |
| numbers | TCGF | | | | | | |
| 1 | - | 0 | 23 | - | - | 25 | 34 | - | - |
| 2 | - | 0 | 18 | - | - | 30 | 0 | - | - |
| 3 | - | 0 | 27 | - | - | 33 | 0 | - | - |
| 4 | - | 0 | 10 | - | - | 20 | 13 | - | - |
| 5 | - | - | - | 14 | - | - | 37 | 48 | - | - |
| 6 | + | - | - | 64 | - | - | 67 | 59 | - | - |
| 7 | - | - | - | 42 | - | - | 41 | - | - | - |
| 8 | - | - | - | 60 | - | - | 46 | 50 | - | - |
| + | - | - | 0 | 35 | - | - | 35 | 59 | - | - |

* Lymphocytes were cultured with allogeneic lymphocytes for 6 d. Aliquots were removed for cytotoxicity assays and thereafter the cultures were further propagated with TCGF for 2 wk. The values represent the percentage specific ⁵¹Cr release in a 4-h assay at an effector:target ratio of 50:1.

† In experiments 2 and 4, the PHA blasts were derived from third-party donors, otherwise the PHA or TCGF blasts are identical to the stimulators.

§ Each test was performed with a different tumor.

**Table II**

Cytotoxicity of Patients' Lymphocytes Activated in ATS or MLC*

| Lymphocyte donor | Lymphocytes cultured in ATS | Lymphocytes cultured in MLC‡ |
|-----------------|-----------------------------|-----------------------------|
| Autologous targets | Allogeneic targets | Autologous targets | Allogeneic targets |
| Tumor | PHA blasts | A | B | Tumor | PHA blasts | A | B | Tumor | PHA blasts | A | B |
| 2,285 | 21 | 2 | 4 | - | - | 7 | 0 | 20 | 24 | - |
| 1,044 | 33 | 0 | 5 | 0 | 20 | 2 | 25 | 0 | 50 | - |
| 1,045 | 26 | 0 | 0 | 2 | 0 | 29 | 5 | 12 | 38 | - |
| 513 | 22 | - | 5 | 0 | 0 | - | 0 | 65 | - | 45 | 27 |
| 2,286 | 30 | - | 11 | 9 | 2 | 32 | - | 25 | 11 | - |

* Aliquots of lymphocytes were cultured with autologous tumor cells or allogeneic lymphocytes for 6 d. The values represent the percentage specific ⁵¹Cr release in a 4-h assay at an effector:target ratio of 50:1.

‡ Lymphocytes of healthy donors were the stimulators in the MLC.

§ Allogeneic PHA blasts were derived from the stimulators.

‖ Statistical significance, P < 0.01.

Characterization of the Cytotoxicity Generated in ATS and MLC. Cold target competition tests were performed in systems in which ATS (Fig. 1 A) and MLC (Fig. 1 B and C) activated the lymphocytes of a lung carcinoma patient (2,286), and the targets were either autologous (Fig. 1 A and B) or allogeneic (2,285) tumor (Fig. 1 C). The
autotumor killing was inhibited exclusively by the identical cold tumor cells independent of whether this was generated in ATS or MLC. It is important to note that autologous PHA blasts did not compete. The lysis of the third party allogeneic tumor by the MLC-activated effectors was inhibited both by these tumor cells and by PHA blasts from the same individual. In this combination, autologous tumor and PHA blasts did not compete (Fig. 1 C). PHA blasts of the stimulator did not compete either.

These results show (a) the tumor "specificity" of the killer subset, which acts on the autologous tumor cells, independent of whether it was generated in ATS or MLC because autologous PHA blasts did not inhibit; and (b) that neither the autologous nor the third party allogeneic tumor killing of MLC cells is due to cross-reactivity with the stimulator lymphocytes.

Fig. 2 presents a criss-cross cold target competition test with MLC-activated lymphocytes from two patients (hypernephroma 513 and osteosarcoma 1,044). The stimulator lymphocytes were derived from an unrelated healthy donor (Fig. 2 A and B). The cytotoxicities in the allogeneic criss-cross tests were inhibited by the cold tumor cells identical with the labeled ones and also by PHA blasts from the same patient but not by the tumor cells or PHA blasts of the responder (Fig. 2 A and B). The MLC-activated lymphocytes of patient 1,044 were also cytotoxic for autologous tumor cells (Table III), but these did not compete in the assay with the allogeneic tumor 513 (Fig. 2 B).
The cytotoxicity against osteosarcoma cells 1,045, exerted by lymphocytes of a healthy donor activated by lymphocytes of patient 1,045, was inhibited only by the cells, both tumor and PHA blasts, derived from the same patient. PHA blasts from the responder and tumor cells from another individual did not compete (Fig. 2 C).

Because in these experiments the same cells participated in more than one lytic system as competitors and the effect depended on their relation to the effector-target combination, the tests are inherently controlled. Thus, it seems that the lysis of allogeneic tumor cells by MLC-activated lymphocyte populations of tumor patients does not occur on the basis of recognition of cross-reacting tumor-related antigens. The competition by PHA blasts of the tumor cell donor indicate that the cytotoxicity of MLC lymphocytes for third-party allogeneic tumor cells occurs on the basis of alloantigen recognition. In addition to the experiments presented here, results with four further tests involving nine patients led to the same conclusions. The killer cells are probably transactivated as a corollary of the specific events in the culture.

Cytotoxicity of the Activated Lymphocytes after Propagation in TCGF. 10-d-old lymphocyte cultures mixed with autologous tumor biopsy cells were further propagated in TCGF-containing medium. Two cultures (1,045 and 513) were available and could be tested for cytotoxicity 4 wk later. These cells lysed autologous but not allogeneic tumor biopsy cells (Table III). Because the experiment involved a criss-cross test, the specificity was real and did not depend on the sensitivity of the targets.

In contrast to the preferential autotumor killing of the ATS-TCGF effectors, the TCGF cultures of the same lymphocytes preactivated in MLC were cytotoxic for both autologous and allogeneic tumor cells. K-562 cells were regularly damaged by these effectors.
**Table III**  
*Cytotoxicity of Lymphocytes Activated in ATS, MLC, or by PHA Tested Directly and after Propagation with TCGF*

| Lymphocyte donor numbers | Type of culture | Propagated with TCGF | Targets |
|--------------------------|-----------------|----------------------|---------|
|                          |                 |                      | Tumor biopsy cells | PHA blasts | K-562 |
|                          |                 |                      | 2285 1044 1045 513 2207 898 |          |
| 2285                     |                 | -                    | 34 - - - - 8 - - - - 34 |
| MLC                      | -               | -                    | 7 - - - - 20 - - 24 32 |
| MLC                      | +               | 47                   | 18 29 - - - - - - 33 |
| ATS                      | -               | 21                   | - - - - 4 - 2 - - |
| 1044                     | -               | -                    | - 0 - - 0 - 0 - - |
| MLC                      | -               | -                    | - 20 - - 25 - - 50 43 |
| MLC                      | +               | 38                   | - - 20 25 - - - - 38 |
| ATS                      | -               | 33                   | - - - - 5 - 0 - - |
| 1045                     | -               | -                    | - 0 6 2 - - - - |
| MLC                      | -               | -                    | - - 29 12 - - - - 38 25 |
| MLC                      | +               | 24                   | - - 24 13 - - - - 45 |
| ATS                      | -               | 2                    | - - 26 0 - - - - 25 |
| ATS                      | +               | 9                    | - - 32 2 - - - - 14 |
| 513                      | -               | -                    | - - 9 - - - - - - 28 |
| MLC                      | -               | -                    | - - 65 - - 0 - - - 45 48 |
| MLC                      | +               | 39                   | - - 30 24 - - - - 41 |
| ATS                      | -               | 5                    | - - - - 25 - 0 - - 20 |
| ATS                      | +               | 0                    | - - 9 29 - - - - 14 |
| Healthy A                | -               | -                    | 11 - - - - - - 0 - 0 |
| MLC                      | +               | 59                   | - - - - - - - - - - 62 |
| PHA                      | +               | 62                   | - - - - - 19 - - 64 |
| Healthy B                | -               | -                    | 7 - - - - - - - - 0 |
| MLC                      | +               | 38                   | - - - - - - - - - - 20 |
| PHA                      | +               | 51                   | - - - - - - - - - - 17 - 47 |

* Aliquots of lymphocytes were cultured in ATS, MLC, and with PHA (0.5 μg/ml). Cytotoxicity against frozen preserved tumor cells or fresh PHA blasts was determined on day 6 in a 4-h 51Cr release assay. The cultures were further propagated with TCGF. Cytotoxicity of the MLC-TCGF cultures was determined after 14 days, and the ATS-TCGF was determined 4 wk later. The values represent the percentage specific 51Cr release.

‡ Tumors 1,044 and 1,045 are osteosarcomas, tumors 2,207 and 2,285 are oat cell carcinomas of the lung, tumor 898 is a B cell lymphoma, tumor 2,286 is a squamous cell carcinoma of the lung, and tumor 513 is hypernephroma.

The tumor biopsy cells were lysed by the healthy donors' lymphocytes propagated in TCGF after activation in MLC or PHA in 3/4 and in 1/4 of the experiments, respectively (Table III).

**Discussion**

The goal of human tumor immunology studies is the demonstration of recognition of autologous tumor cells. Even if its existence does not ensure that the immune mechanisms can control tumor growth, increasing knowledge of the details in the immune events might lead to its therapeutic exploitation. There is considerable interest in the establishment of tumor-reactive T cell cultures. They would provide a tool for characterization of tumor-related antigens and might also contribute to the design of therapeutic strategies.
In previous experiments (6–9) performed in our laboratory, patients’ lymphocytes cultured with their own tumor cells killed these (in 67% of 77 cases) but not allogeneic tumor cells. MLC-activated lymphocytes also killed the autologous tumor biopsy cells, however, less frequently (3/19, 37%) and with lower efficiency (6). These effectors damaged more often the third-party allogeneic tumor cells (11/13, 85%). The experiments presented in this paper are in accordance with our previous results. Similar findings were reported recently by Strausser et al. (27). In 7/10 experiments with MLC-activated lymphocytes (by single donor stimulator cells), lysis of autologous solid tumor cells were obtained. Their effects were also rather weak (the highest 22% at effector:target ratio 25:1), but it could be elevated if “pool stimulation” was performed.

The cold target competition experiments were performed with the aim of characterizing the cytotoxicities directed against autologous or allogeneic tumor biopsy targets. Lysis of different targets by the same activated population can be performed by the same, by different, and by partially overlapping subsets. The experiments were designed to elucidate whether (a) the autotumor killer lymphocytes generated in MLC are members of the same subset that recognize the stimulator cells and (b) whether the allotumor killer subset of the patients act via recognition of shared antigen(s) with the autologous tumor.

The inhibition of the autologous tumor killing by the identical and not by other unlabeled cells suggests that the effector lymphocytes recognize tumor- or organ-specific antigens. The fact that the antitumor cytotoxicity of the MLC population was not inhibited by the stimulator lymphocytes points also to the specificity of the lysis. This rules out the possibility that the tumor biopsy cells are attacked indiscriminately by the activated lymphocytes as is the case with the K-562 or Daudi cells (6, 28).

On the basis of our previous studies, we consider the lysis of K-562 cells to be a sign of the activated state of the lymphocytes. The mechanisms of this activity are different than the lysis of freshly harvested tumor cells. It seems that the effect against the cell lines is independent of antigen recognition, and it is due to some as yet undetermined properties of their plasma membrane (28, 29). This view is substantiated by the general competing potential of the K-562 cells in cytotoxic assays (30). Consequently, in the judgment of the specificity of a certain reactivity exerted by lymphocyte populations, we do not take into account the anti-K-562 or anti-Daudi effects but only the results against targets of similar type.

Competition with the PHA blasts of the tumor cell donor in the system with allogeneic tumor target suggests that the cytotoxicity occurs on the basis of alloantigen recognition even when the MLC-activated effectors are derived from tumor patients. These arguments are valid provided the cold target competition assays can be interpreted to decide between distinct and overlapping populations. The method is widely used, and up to the present there is no indication against this assumption.

Similar selective cytotoxicities were revealed in our previous experiments with interferon (IFN)-activated fresh lymphocytes. Cold target competition tests showed that different allogeneic tumor targets are lysed by distinct subsets of the same effector population. Effects against an allogeneic tumor were inhibited only by identical cells.
or by PHA blasts from the same individual but not by other tumors or PHA blasts, although they were also lysed by the same effector population.\(^2\) We interpreted the results as a polyclonal activation induced by short-term IFN treatment. In addition to the generation of specific CML, similar events occurred in the MLC cultures.

In view of the high frequency of alloreactive cells in the lymphocyte population, clonal expansion might not always be necessary, but activation suffices to generate allospecific cytotoxicity. It is likely that in the MLC, production of IL-1 and IL-2 as a corollary of antigen recognition even leads to activation and proliferation of cells that do not bear receptors for the stimulating antigen (31, 32).

Characterization of cytotoxic clones in primary murine MLC populations also showed distinct effector subsets against different targets. This approach revealed (33) that in addition to the enlargement of the specific clone (representing 81% of the clones), cytotoxic cells reacting with third-party targets proliferated simultaneously (11% of the clones).

The lack of alloactivation in the ATS is probably due to the relatively low proportion of autoreactive cells. In a mouse system, comparison between the interaction of cytotoxic lymphocytes with syngeneic and allogeneic tumors showed that in the syngeneic combination the frequency of the conjugate-forming lymphocytes and their binding avidity was lower compared with the allogeneic one (34). Such a difference is reflected in the lower blastogenic response in cultures of autologous lymphocytes and tumor cells (ATS) compared with MLC (35). The proportion of antigen-reactive cells may determine the quantity of amplifying factors produced. The comparison of the generation of autotumor killing effectors by single or “pool” MLC stimulation indicates that this is the case (27).

In mice, the production of soluble mediators differed when K/D and I-region stimulation were compared with regard to the requirement of the Ly phenotype of the helper cells (31). Therefore, the different nature of the antigens recognized in ATS and MLC may also be held responsible for the differences in the cytotoxic target spectrum generated.

The experiments with MLC lymphocytes show that cells that react with the autologous tumor can be activated by nonspecific means. The results with solid tumors were thus similar to those obtained with leukemias. Cytotoxicity against autologous leukemia cells were generated in mixed cultures by confrontation with a pool of allogeneic lymphocytes or leukemia blasts and BCG extract (5, 36). Our experiments indicate that the ATS cultures provide the best system for generation of autotumor cytotoxicity.

In our previous experiments, fresh lymphocytes were activated by IFN to kill allogeneic but not autologous tumor cells. The observation with the ATS system concerning the high proportion of cases (67%) with autotumor-recognizing lymphocytes conflicts with the lack of IFN-activated killing against autologous tumors. The obvious cause may be the absence or low number of tumor-recognizing lymphocytes in a state of differentiation that can be enhanced for lytic functions by IFN. We have shown (37) that IFN does not act on separated Fcy receptor-negative T cells. This subset represents a high proportion of the blood lymphocyte population and contains the lymphocytes that are triggered for multiplication by confrontation with antigens (38).

The selective cytotoxicity for the autologous tumor cells of the ATS cultures and
the wider reactivity of the MLC was maintained in the growing cultures. Because of the exclusive reactivity of activated T cells with the growth-promoting factor IL-2, the initial events in the cultures determine thus the characteristics of the TCGF cultures (18).

In experiments with auto- and allogeneic lymphoblastoid lines (LCL), Sumiya et al. (39) have showed that in primary mixed cultures cytotoxicity was generated in autologous cultures with specificity for the auto-LCL. After reciprocal restimulation, the secondary cytotoxicity reflected the characteristics of the primary cultures, i.e., auto-LCL specificity was maintained after the secondary exposure to allo-LCL. In this system, cocultivation with allo-LCL also generated some auto-LCL lysis, although this was weak.

The early commitment determining specific reactivity has been directly demonstrated with T cell colonies raised after brief exposure to allogeneic lymphocytes and grown with TCGF (16).

It remains to be seen whether the distinctness of the cytotoxic lymphocyte subsets for different targets, as it was seen in MLC, is maintained when these cultures are further propagated.

In vivo events that parallel the autotumor reactivity of the MLC population have been described in the experimental system. Allosensitivation was shown to confer protection of mice and rats against the outgrowth of syngeneic tumor grafts (41).

Nonspecific immunostimulation has been attempted in the therapy of human tumors. On the basis of the results in experimental models and in vitro assays, more substantial success would have been expected. However, the possible reasons for the failure of such measures are numerous.

The generation of autotumor-reactive T lymphocyte cultures by a single exposure of the lymphocytes to the autologous tumor cells is important in the view of the limitation in the amount of tumor tissue that can be collected from the patients. Because "transactivation" in the MLC may also induce the autotumor-reactive cells to exert cytotoxicity, "nonspecific" measures can be exploited in the absence of or in extreme scarcity of available tumor tissue.

Summary

Blood lymphocytes from tumor patients were cocultivated with allogeneic lymphocytes (MLC) or autologous tumor cells (ATS), and their cytotoxicity was characterized. The main objective of the study was the lysis of autologous tumor biopsy cells by such effectors. Lymphocytes of patients activated in MLC lysed allogeneic third-party cells and in some cases also lysed autologous tumor cells. Allogeneic but not autologous PHA blasts were also damaged by these effectors. The cytotoxic potential of MLC-activated lymphocytes from healthy donors was similar; allogeneic tumors and phytohemagglutinin (PHA) blasts but not autologous PHA blasts were lysed.

The cytotoxicity of lymphocytes activated in ATS were specific for the stimulator because they acted only on the autologous tumor cells. Allogeneic tumors and autologous and allogeneic PHA blasts were not lysed. The pattern of cytotoxicity with regard to this target panel was maintained when the MLC or ATS cultures were further propagated with TCGF.

Results obtained in cold target competition assays suggested (a) activated lymphocyte lyse the third party tumor targets because of alloantigen recognition; (b) in MLC
several different sets of alloreactive cytotoxic lymphocytes are present simultaneously; and (c) the alloreactive cells are different than those that act on the autologous tumor cells. Thus, the lysis of allogeneic tumor cells by lymphocytes of the patient is not due to recognition of cross-reacting tumor-related antigens, and the autotumor cytotoxicity of the patients' MLC-activated lymphocytes is performed by specifically reacting cells.

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