Cancer Immunol Immunother (1989) 29: 125–132

Cancer Immunology Immunotherapy



# Auto-tumor lysis by blood lymphocytes in vitro

## Strongly activated lymphocytes lack selectivity

**F. Vánky[1, 2] and E. Klein[1]**

[1] Department of Tumor Biology, Karolinska Institute, S-104 01 Stockholm, Sweden
[2] Radiumhemmet, Karolinska Hospital, S-104 01 Stockholm, Sweden

**Summary.** Selectivity of the lysis of the tumor cells by autologous blood lymphocytes and its various subsets was investigated by means of the cold target competition assay. The effectors were autologous lymphocytes passed through a nylon-wool column (unfractionated: U) and their low- and high-density subsets, either without or after activation. The lymphocytes were activated (a) in autologous mixed lymphocyte tumor cell culture in autologous (MLTC), (b) in mixed lymphocyte culture (MLC), without and with interleukin-2, for 6 days, or (c) by phytohaemagglutinin for 3 days. Autologous-lymphocyte-mediated cytotoxicity (auto-tumor lysis: ALC) by the unfractionated, unmanipulated blood lymphocyte (U) population, its high-density fraction and those induced for auto-tumor lysis in the MLTC is regularly weak and affects only the autologous tumor cells. Their ALC function was inhibited only by the target identical unlabelled cells while the effect of separated low-density lymphocytes was inhibited also by allogeneic tumor cells.

The cold-target competition assay indicated that several subsets with different specificities exist simultaneously in the effector populations activated in MLC, because the various targets did not cross-compete or did so only partially. Whenever interleukin-2 was added, at the start of the mixed cultures (MLTC or MLC), the lytic effects were no longer selective. Phytohaemagglutinin-activated effectors lysed several targets. These targets were inhibitory in a criss-cross fashion. Generally, populations showing auto-tumor selectivity had weak lytic effects, while the strongly activated effectors, with strong cytotoxic function, were not selective.

### Introduction

If tumor cells express antigens recognized by the immune system, lymphocytes with the corresponding receptors are expected to be present in the blood of the patients. Such lymphocytes may be deteced in vitro by their reactivity with the tumor cells. In patients with antigenic tumors, immunotherapeutic strategies are expected to provide beneficial effects. The meagre therapeutic results achieved until now could be taken as proof that the tumors that reach the clinically detectable stage do not encounter an immune response; they are either not immunogenic or the patients are unable to mobilize immunological effector mechanisms. However, the results obtained in various types of in vitro experiments indicate the existence of lymphocytes that can recognize autologous tumor cells.

Among the assays used for detection of antitumor reactivity, lysis of the tumor cells has been used extensively. Such experiments, performed mainly with cultured tumor targets, led to the discovery of the natural killer cells. The present concensus is that a direct lymphocyte-mediated attack occurs in vitro on the basis of two essentially different types of recognition. When antigens, presented by the major histocompatibility complex (MHC) molecules, interact with the corresponding receptor-carrying members of the T cell repertoire, the effectors are named for cytotoxic T lymphocytes (CTL). The CTLs are selective, they are generated in the immune response involving the steps of antigen presentation, enlargement of the relevant clones, and establishment of immunological memory. When cytotoxic effectors are induced to function via other mechanisms of interaction, the lysis of the targets occurs in a non-selective manner. Operationally such effectors are designated as natural killer, or as activated killer, also named interferon-activated or lymphokine-activated cells [3, 6–8, 16, 19, 27]. Membrane structures of the interacting cells, other then antigens, which are involved in cytotoxicity, have been recently identified [5]. Antibodies against these cell-surface molecules inhibit contact between the lymphocytes and targets. The CTL and natural killer phenomena are usually distinguished by the MHC restriction, the former but not the latter exhibiting this [12, 20, 24, 37].

The surface moieties recognized by the autologous lymphocytes on the tumor cells are not known. In the majority of cases, the lytic effect of T-cell-enriched blood lymphocytes (autologous lymphocyte-mediated cytotoxicity; ALC) was selective and allogeneic tumors were rarely lysed [31]. We have shown that the MHC class I molecules are required for the lytic interaction [33], therefore it is likely that the ALC represents an antigen-specific CTL effect. On the other hand, with subsets separated on the basis of cell density, in the large majority of experiments the effectors were found in the low-density lymphocyte population [9, 28]. This subset is heterogeneous, containing large granular lymphocytes with non-restricted cytotoxic function [23]. The majority of the cells carry T cell markers. The relatively high spontaneous DNA synthesis and the higher proportion of cells with interleukin-2 (IL-2) recep-

*Offprint requests to:* F. Vánky

tors in this subset, point to the presence of activated T lymphocytes [2, 31].

The specificity of a cytotoxic lymphocyte population can be investigated by the cold target competition assay. The method provides information about whether a particular set of targets, if lysed by the population is recognized by the same or different sets of effectors [11, 21]. With the help of this assay we have investigated the specificity of various auto-tumor-lysing populations including activated cells generated in mixed cultures.

## Materials and methods

*Tumor cell suspensions* were prepared from surgical specimens of 16 lung tumors (8 squamous cell, 4 adeno carcinomas, 3 small-cell, and 1 carcinoid), 1 ovarian, 1 breast and 2 renal carcinomas, 2 malignant melanomas and 4 malignant mesenchymal tumors. Macroscopically distinguishable tumor tissue, without necrosis, was excised from the surgical specimens and finely minced with scissors in the presence of collagenase and DNase. The amounts of enzymes depended on the appearance of the tissue. In general, the higher the proportion of dead cells, the larger amount of DNase added, and the more collagen material in the specimen the higher amont of collagenase added. The amount varies between 20 mg and 50 mg for collagenase and between 2 mg and 5 mg for DNase. The tumor material is then gently passed through a stainless-steel mesh into collagenase (3 mg/ml) and DNase (0.2 mg/ml) containing balanced salt solution. By sequential centrifugation, the majority of debris is eliminated and the tumor-cell-enriched (60 *g* sediment) and lymphoid-cell-enriched (500 *g* sediment) suspensions are obtained. Each is further fractionated on Percoll dicontinuous gradients. Three milliliters of 10%, 15% and 20% Percoll solutions are layered into 15-ml Falcon tubes (no. 2057), $(5-10)\times 10^6$ cells are placed on the top of the gradient in 1–2 ml volume and the tubes are centrifuged at 25 g for 3–5 min. The tumor cells usually collect at the lower part and at the bottom of the tube. Lymphocytes, erythrocytes and debris remain at the top and intermediate layers. Contaminating non-viable cells and erythrocytes are eliminated, by further sedimentation on Ficoll/Isopaque (FI) gradients. The gradient is made up of 3 ml 1.055 g/cm$^3$ FI solution (corresponding to 75% stock solution, diluted with balanced salt solution, layered on the stock solution (100% FI, density, 1.077 g/cm$^3$). On this gradient after centrifugation (900 *g* for 10 min at 4° C) the viable cells are recovered at the interface between the 100% and 75% FI. Cells of certain tumor types (glioma, liposarcoma and melanoma) collect on the top of the 75% FI, but generally, the top fraction contains debris, macrophages and damaged cells. Erythrocytes and non-viable cells sediment to the bottom. Following washes, the viable cells are incubated in plastic tissue-culture flasks in RPMI 1640 medium supplemented with 10% human serum, for 30 min at 37° C. Each step can be repeated if necessary. The non-adherent cells, mainly tumor cells, are harvested and incubated overnight in cell-culture conditions, prior to the test. Experiments were performed only with suspensions which contained >80% viable tumor cells and <5% non-malignant cells. Non-malignant tissues were processed similarly.

*Characterization of the tumor cell suspensions.* For morphological characterization, tumor cells were deposited on slides, fixed immediately with the Spray-cyte water-soluble fixative (Clay Adams Div. Becton Dickinson and Co., Parsippany, N. J.) and stained according to Papanicolau. For the evaluation of composition of the suspensions 400–800 cells were scored.

*Treatment of the tumor cells with monoclonal antibody (mAb) W6/32.* One aliquot of the tumor cells was incubated for 30 min at room temperature with W6/32 mAb (1 μg/$10^6$ cells), which react with a monomorphic determinant of the heavy (α) chain of MHC class I molecules. After two washes the cells were used as unlabelled targets.

*Blood lymphocytes.* Nylon-wool-non-adherent (U) lymphocytes were separated from heparinized blood and in some experiments they were further fractionated on Percoll density gradients according to the methods described earlier [23, 31]. The low-density (LD) fraction was collected at the 37.5% and 40% Percoll interface, and the high-density (HD) fraction at the 47% interface. In the LD fraction 58% of the cells (range 38–70) reacted with OKT3, 22% (range 13–25) with NKH1 mAbs and 35% (range 18–42) with Leu19 mAb. The IL-2 receptors (detected with anti-Tac mAb) were expressed on 7%–15% of the cells. Of these cells, 55% (range 38–63) had the morphology of large granular lymphocytes. More than 99% of the cells in the HD fraction reacted with the OKT3 mAb. They did not bind NKH1, Leu19 or anti-Tac mAbs.

In 18 experiments the level of [$^3$H]thymidin uptake was lower in the HD than in the LD ($\Delta\pm$ SD = $365\pm96$ cpm and $986\pm218$ cpm respectively), which indicate the elevated state of activation of the latter.

*Activation of lymphocytes in various types of mixed lymphocyte cultures.* Aliquots of the lymphocytes ($10^7$ cells/flasks) were mixed with $10^5-10^6$ autologous tumor cells for the autologous mixed lymphocyte tumor cell cultures (MLTC) (we found the optimal ratio within this range) and with $5\times10^6-10^7$ mitomycin-C-treated allogeneic non-T cells from one single donor, for the mixed lymphocyte culture (MLC) or a mixture of allogeneic non-T lymphocytes from ten donors for the pool MLC. The cultures (10–15 ml in volume) were incubated in plastic tissue-culture flasks (type 1013, Falcon Labware) in tissue-culture conditions for 6 days. In some instances 2.5 U/ml highly purified IL-2 (Lymphocult-T-HP, Biotest-Serum Institut, 6000 Frankfurt/Main 73, FRG) was also included in the MLTC.

The [$^3$H]thymidin incorporation of the MLTC, MLC and MLTC+IL-2 effectors, increases in this order. $\Delta\pm$ SD was $3914\pm563$ cpm, $17336\pm1339$ cpm and $39150\pm2712$ cpm respectively, indicating the differences in their functional state.

The 6-day MLC and pool MLC contained 90%–98% cells reactive to OKT3, 23%–30% to OKIa and 25%–30% to anti-Tac mAb. The cell yield was between 150% and 200% of the input. The composition of the 6-day MLTC and MLC effectors was similar, but the cell yield was lower and highly variable in the different experiments (30% and 80% of the input). The cell yield in the IL-2 containing cultures was 2–3 times higher for the MLC and between 1–1.5 times the MLTC.

*Activation with phytohaemagglutinin.* Blood lymphocytes ($10^7$/flasks) were cultured with 0.5 μg/ml purified phyto-

haemagglutinin (Wellcome Reagents Ltd., Beckenham, England) for 5 days. One-half of the medium was changed with fresh on day 3. The cells (60%–70% blasts) were used as effectors. In three experiments, lymphocytes were exposed to the same concentration of phytohaemagglutinin only during the cytotoxic assay.

*Treatment with concanavalin A.* Lymphocytes used as unlabelled targets were exposed for 3–5 days to 5 mg/ml ConA grade III (Sigma Chemical Co., St. Louis, Miss, 63178 USA). After washing with 0.1 M α-methyl mannose, they were exposed to 0.2% formaldehyde in phosphate-buffered saline for 30 min in an ice bath and washed three times with ice-cold phosphate buffered saline. The advantage of ConA blasts in the cold-target competition tests is the easy removal of the lectin, which is necessary to ascertain that it does not influence the cellular interactions.

*Cytotoxic assay.* The method of the 5-h $^{51}$Cr-release assay for the detection of autologous lymphocyte-mediated cytotoxicity (ALC) was used as described earlier [31]. The percentage of $^{51}$Cr release (cpm) was calculated from the following formula: ($^{51}$Cr in supernatent/$^{51}$Cr in supernatant and cell pellet) 100. The percentage specific $^{51}$CR release was then determined by the formula: (% release in test − % spontaneous release) / (% maximum release − % spontaneous release) × 100. Tests in which spontaneous release exceeded 50% were discarded. In the present series the spontaneous $^{51}$Cr release varied between 11% and 45% (mean 24%). Statistical significances were calculated from the cpm values of quadruplicate samples by Student's *t*-test. The criterion for positivity was that the level of $^{51}$Cr release had to be at least 10% over the spontaneous release and the *P* value $< 0.05$.

*Cold-target competition.* Effectors ($5 \times 10^6$ cells/well) were dispersed in 50 µl medium and increasing numbers of unlabelled targets were added in 50 µl. The ratios of effector to unlabelled targets were between 1:1 and 10:1. 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 $^{51}$Cr-labelled targets ($10^4$ cells/well) were added in 100 µl medium. The ratios of labelled: unlabelled cells varied between 2:1 and 1:10. The volume of each well was adjusted to 200 µl. After centrifugation (800 *g* for 10 s) the plates were incubated and processed in the usual cytotoxic assays. Statistical significances were calculated on the cpm values of quadruplicate samples by Student's *t*-test.

*Strategy of the experiments.* Owing to the limited number of tumor cells and lymphocytes, the experiments representing different combinations between the various types of autologous and allogeneic reactants could not be performed in the same experiments. The effectors were unmanipulated ex-vivo lymphocytes or lymphocytes subjected to various types of activation, such as exposure to autologous tumor cells in MLTC, exposure to allogeneic lymphocytes in the MLC, or exposure to IL-2, which was added to one aliquot of MLTC and MLC cultures, or to phytohaemagglutinin. As targets and unlabelled competitors, autologous and allogeneic tumor or non-malignant cells were used.

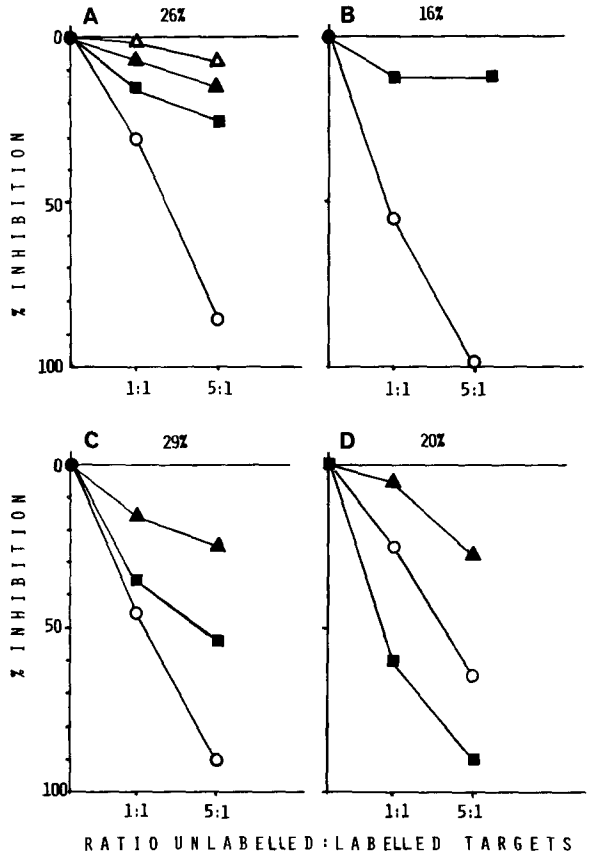


**Fig. 1.** Admixture of unlabelled autologous (○) adenocarcinoma of the lung, lung-tissue-derived non-malignant cells (▲) and concanavalin *A* (ConA) blasts (△) and allogeneic lung adenocarcinoma (■) cells into the auto-tumor lytic test performed with the unfractionated lymphocyte population (**A**), with the high- (**B**) and with the low-density (**C**) subsets. **D** Lysis of the allo-tumor by low-density lymphocytes. The level of cytotoxicity (percentage specific $^{51}$Cr release) without competitor is indicated in these and in the following figures. In all figures inhibitions are expresed as percentage inhibition of specific $^{51}$Cr release

## Results

### *Freshly harvested blood lymphocytes*

Our earlier experiments showed that the unfractionated, nylon-wool-passed blood lymphocyte population of tumor patients rarely lyses allogeneic tumor cells. In accordance with this, an admixture of allogeneic tumor cells did not inhibit the auto-tumor lysis. Autologous ConA blasts and non-malignant lung tissue cells did not inhibit either (Fig. 1 A, Table 1).

In six experiments, density-fractionated lymphocytes were the effectors (Table 1). In one of four tests the HD cells lysed the autologous but not the allogeneic adenocarcinoma cells, and the auto-tumor lysis was not influenced by the admixed unlabelled allogeneic tumor (Fig. 1 B). In three of five tests the LD cells lysed both autologous and allogeneic tumor cells and these effectors could be inhibited in a criss-cross fashion (Fig. 1 C–D). However, the inhibitory effects were not complete, indicating the existence of effector sets each acting on only one of the targets. Cross-reactive and selective effectors were detected with LD lymphocytes of two out of three additional patients (Table 1).

**Table 1.** Summary of the cold target competition tests with blood lymphocyte populations

| Effector populations | Target tumor | No. of positive test[a] | Inhibition of the lysis by admixed | | | |
|---|---|---|---|---|---|---|
| | | | Tumor | | Non-malignant | |
| | | | Auto. | Allo. | Auto. | Allo. |
| U[b] | Auto. | 9/21 | 5/5[c] | 0/5 | 0/5 | 0/5 |
| U | Allo. | 0/10 | | | | |
| LD | Auto. | 5/6 | 3/3 | 2/3 | 0/1 | |
| LD | Allo. | 3/5 | 2/2 | 2/2 | 0/1 | |
| HD | Auto. | 1/4 | 1/1 | 0/1 | 0/1 | |
| HD | Allo. | 0/3 | | | | |

[a] The results obtained in the series of experiments during the period when the competition tests were performed
[b] U, unfractionated, blood lymphocytes passed through nylon wool; LD and HD are the high- and low-density fractions of U
[c] At least 50% inhibition/number of tests

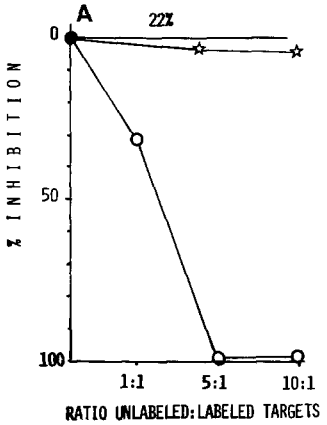


**Fig. 2.** Auto-tumor lysis (squamous cell carcinoma) in the presence of unlabelled identical untreated (○) and W6/32-mAb-treated cells (☆). The W6/32-mAb-treated tumor cells were not lysed by the lymphocytes when used as targets

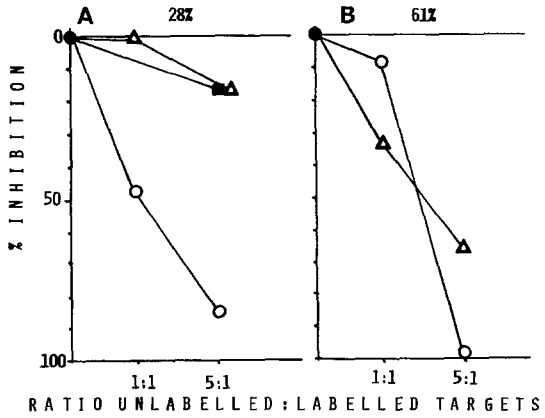


**Fig. 3.** Auto-tumor lysis by lymphocytes activated in 6-day mixed lymphocyte tumor cell culture (MLTC) (squamous cell carcinoma). **A** Admixture of unlabelled target (○), ConA blasts (△) and allogeneic adenocarcinoma (■) (not lysed by these effectors). **B** Auto-tumor lysis by effectors generated in MLTC in the presence of interleukin-2. Unlabelled targets: autologous adenocarcinoma (○) and ConA blasts (△) (lysed by these effectors)

In one experiment, the unlabelled tumor cells were added both without and with pretreatment with W6/32 mAb (Fig. 2). The antibody-treated cells were not lysed and they did not inhibit the lysis of untreated cells. Thus, the targets were not recognized by the lymphocytes when their class I antigens were blocked by antibodies.

*Mixed lymphocyte tumor cell cultures, MLTC*

Auto-tumor cytotoxicity is often generated in autologous MLTC. The effect is usually selective, in that allogeneic tumors are rarely lysed [26, 36]. The cold-target competition confirmed the selectivity, i.e. the lysis of autologous tumor was not inhibited by allogeneic tumor cells (eight experiments) or by autologous and allogeneic ConA blasts (seven and six experiments respectively) (Table 2). The results of one experiment are shown in Fig. 3A.

When IL-2 (5 U/ml) was added to the mixed culture, considerably stronger auto-tumor cytotoxicity was generated. This cytotoxicity was not selective, allogeneic tumor cells and autologous ConA blasts were also lysed. These blasts competed for the tumor target (Fig. 3B). Two additional experiments gave similar results (Table 2).

*Mixed lymphocyte cultures, MLC*

Lymphocytes stimulated in conventional MLC can often lyse autologous tumor cells [1, 14, 17, 18, 22, 28, 35]. When lymphocytes from one ovarian carcinoma patient were stimulated in MLC, the admixture of tumor cells (rhabdomyosarcoma) from the "stimulator" patient altered the lysis of autologous tumor only marginally and the lysis of these rhabdomyosarcoma cells was not inhibited by admixture of the autologous tumor cells (Fig. 4A, B). Thus there was no cross-reactivity between the autologous and allogeneic targets, the sets of effectors against them did not overlap. MLC-stimulated effectors from one healthy individual lysed the cells from allogeneic small-cell lung carcinoma and squamous cell carcinoma of the lung (Fig. 4C). These allogeneic cells did not cross-inhibit. Admixture of ConA blasts, derived from the same individual, inhibited the lysis of tumor cells. Similar results were obtained with MLC effectors in 7 autologous and 15 allogeneic combinations (Table 2).

In three experiments MLC effectors, stimulated by lymphocytes from one individual, were compared with the aliquots exposed to pooled lymphocytes from ten donors (Table 2). The auto-tumor inhibition obtained with allogeneic tumors on the MLC and pool MLC effects was comparable. However, in one out of three experiments a partial cross-competition was obtained between two squamous cell carcinomas of the lung when pool-MLC-activated cells were the effectors (Fig. 5A, B). The same effectors did not affect the ovarian carcinoma cells, and these did not cross-compete with the two squamous cell carcinomas. It is important to note that autologous ConA blasts did not inhibit in either tests, while ConA blasts from the another individual, whose tumor was the target, inhibited the effect. Since lymphocytes from the patient who provided the allogeneic tumor target were included in the stimulator pool this reactivity is expected and represents a regular allo response. The experiments are therefore well-controlled and suggest the specificity of the auto-tumor lysis on the effector level.

**Table 2.** Summary of the cold target competition tests with activated lymphocyte populations

| Lymphocytes activated[a] | Tumor targets | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Autologous | | | | Allogeneic | | | |
| | Tumor | | Non-malignant[b] | | Tumor | | Non-malignant | |
| | Auto. | Allo. | Auto. | Allo. | Auto. | Allo. | Auto. | Allo. |
| MLTC | 9/9[c] | 0/8 | 0/7 | 0/6 | | | | |
| MLC | 7/7 | 0/7 | 0/3 | 0/3 | 1/15 | 17/17[d] | 0/13 | 12/12 |
| Pool-MLC | 3/3 | 1/3 | | | 0/1 | 1/1 | | |
| MLTC + IL-2 | 3/3 | 2/2 | 1/1 | | 2/2 | 1/1 | 1/1 | |
| MLC + IL-2 | 4/4 | 4/4 | 2/2 | 2/2 | 5/5 | 5/5 | 0/1 | 2/2 |
| PHA | 3/3 | 2/2 | 1/1 | | 7/7 | 7/7 | 1/1 | 3/3 |

[a] Blood lymphocytes were exposed to autologous tumor cells (MLTC), to allogeneic lymphocytes from one (MLC) or ten donors (pool-MLC), with or without admixture of interleukin-2 (IL-2) and phytohaemagglutinin (PHA) (0.1 μg/ml)
[b] ConA blasts or ex-vivo non-malignant tissue cells
[c] Al least 50% inhibition/total number of tests
[d] Tumors from the individuals whose lymphocytes were the stimulators. The competition pattern with lymphocyte targets was similar to that obtained with the tumor cells

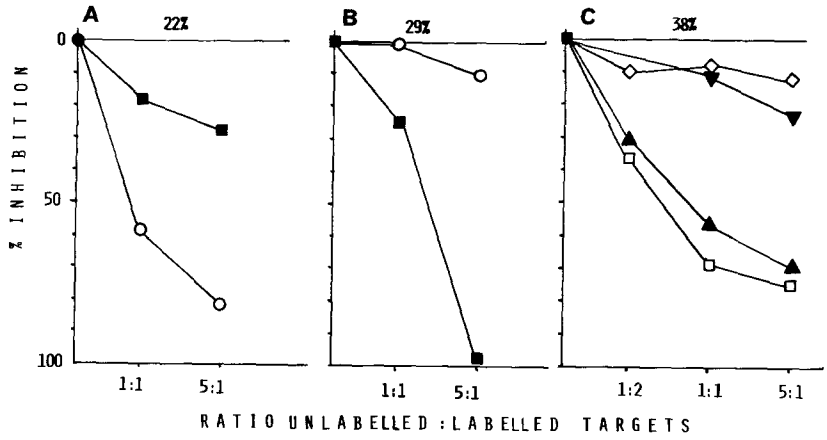


**Fig. 4.** Cytotoxicity by allogeneic lymphocyte-activated (MLC) effectors on the autologous ovarian carcinoma (**A**) and allogeneic rhabdomyosarcoma (from the stimulator) (**B**). Admixture of the unlabeled autologous (○) and allogeneic (■) tumor cells (criss-cross assay). **C** Allo-tumor lysis. The MLC-activated effectors are from a healthy individual. The target is a squamous cell carcinoma. Unlabelled targets: the target identical (□), another tumor (small-cell lung carcinoma, lysed by the effectors) (◇) and ConA blasts from both patients (▲, ▼ respectively)

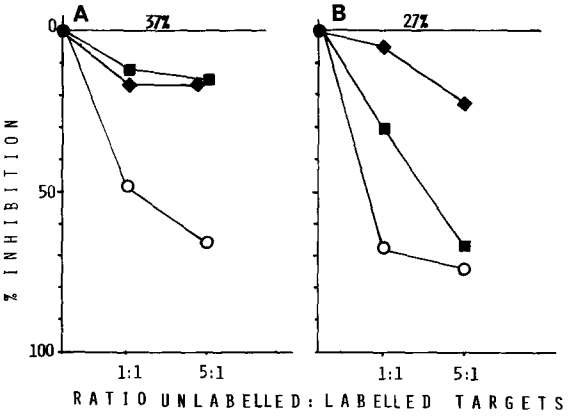


**Fig. 5.** Auto-tumor (squamous cell carcinoma) lysis by lymphocytes activated by MLC (**A**) and pool-MLC (**B**). Unlabelled targets: autologous (○), allogeneic tumor (squamous cell carcinoma) (■) and ovarian carcinoma (◆). The ovarian carcinoma was not lysed by any of these effectors and the allogeneic tumor was not tested as labelled target

When IL-2 was added at the initiation of the MLC, the cytotoxicity was no longer selective, i.e. allogeneic tumors and ConA blasts were also lysed and these competed for the tumor targets in all cases (Table 2).

*Phytohaemagglutinin-activated lymphocytes*

The lectin-induced cytotoxicities were shown to be non-selective (Fig. 6). This was the case also when phytohaemagglutinin was added to the cytotoxic assay (Fig. 7A, B). Auto-tumor lysis by phytohaemagglutinin-pretreated lymphocytes was inhibited by the autologous ConA blasts, (contrasting the lack of inhibition for the auto-tumor lysis by cells generated in MLTC, shown in Fig. 3A). Allo-tumor lysis was also competed for by ConA blasts from the tumor patient. Lymphocytes derived from healthy donors, treated in similar way, were cytotoxic for the tumor cells and the competition pattern was similar (Fig. 7C, D). Similar results were obtained in three autologous and seven allogeneic combinations (Table 2).

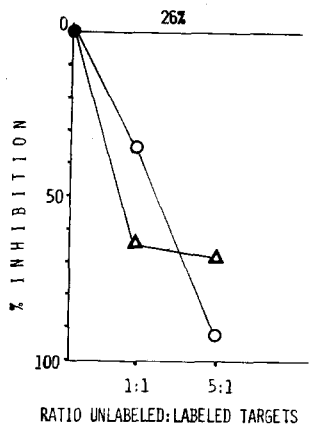


**Fig. 6.** Auto-tumor (squamous cell carcinoma) lysis by lymphocytes cultured in 0.1 μg/ml phytohaemagglutinin. Unlabelled targets: autologous tumor (○) and autologous ConA blasts (△)

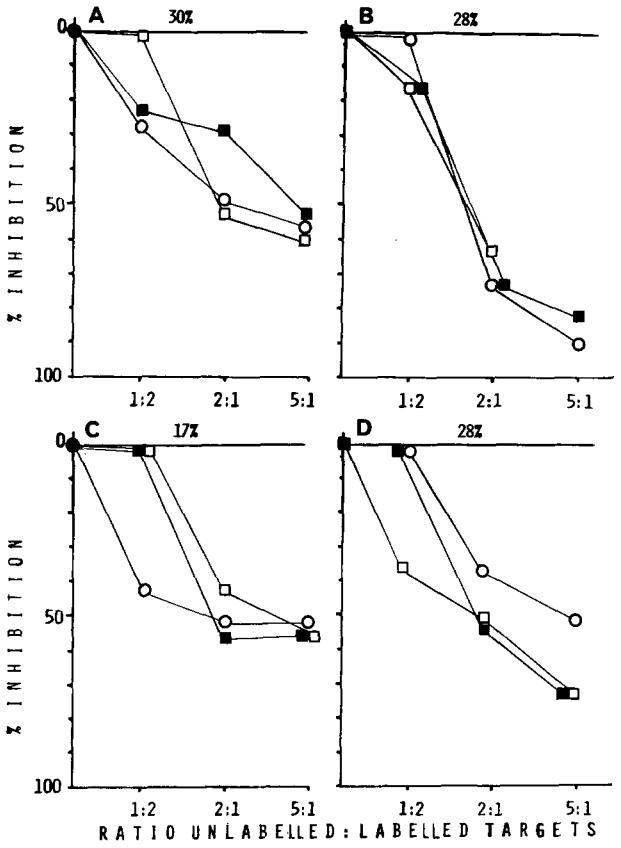


**Fig. 7.** Lysis of melanoma cells by lymphocytes in the presence of phytohaemagglutinin (0.1 μg/ml). **A**, **B**, Autologous effectors. **C**, **D**, Effectors from a healthy donor. Unlabelled targets: autologous (○) and allogeneic (■) melanomas and breast carcinoma cells (□)

## Discussion

The auto-tumor reactivities of blood lymphocytes measured in vitro point to the existence of tumor-recognizing T lymphocytes with the characteristics of antigen specificity. One important feature in which the auto-tumor-lysing lymphocytes resemble CTLs is the requirement of the MHC class I antigens on the target [10, 32–34].

In a group of patients with lung carcinomas and with malignant mesenchymal tumors we have obtained a correlation between the auto-tumor lysis, measured at the time of surgery, and the post-surgical clinical course. This suggests that immunological recognition may control tumor growth [30]. Selectivity of the lysis for the autologous tumor and correlation of the results with the clinical course were observed only with the T-cell-enriched, unfractionated population of blood lymphocytes. When tested separately, the low-density subsets (LD) damaged the autologous tumor cells in a higher proportion of cases and they had a broader target panel, including allogeneic tumors [29, 33]. The LD cells show elevated DNA synthesis, their size is large (compared to the HD) and a high proportion express IL-2 receptors. Thus, the subset contains activated T cells, which is reflected in the broader target panel in their lytic potential. The results of the cold-target inhibition assay suggest that while a proportion of LD cells can lyse more than one target, selective effectors also exist among them.

Auto-tumor lysis can be generated in mixed cultures. Cocultivation with autologous tumor cells represents the specific stimulus and in the MLTC selective cytotoxicity was induced. Lysis of allogeneic tumors of the same histological types, was rare [26, 28, 35, 36]. In accordance with these earlier observations, the auto-tumor lysis induced in MLTC was not inhibited by admixture of allogeneic tumor or autologous ConA blasts.

In a recent publication, reporting the results in a mouse tumor system, the IL-2-activated population contained effector subsets with narrow ranges of specificity, i.e. separate subsets lysed the autologous or allogeneic tumor cells and the allogeneic blasts. Lysis of each allogeneic blast was competed for only by cells of the same haplotype. The LAK cells acting on trinitrophenol-modified blasts were shown to stem from Thy-1$^+$, while those acting on tumor cells came from Thy-1$^-$ precursors [13]. In human ovarian carcinomas, effectors maintained in the presence of IL-2 (1000 U/ml) lysed a wide range of autologous and allogeneic tumor cells and the different tumor targets cross-competed with each other [7]. Our results are consistent with the latter observation, i.e. the MLTC effectors did not have selectivity when IL-2 (5 U/ml) was added at the initiation of the culture.

Activation of lymphocytes for auto-tumor lysis can occur in MLC [1, 14, 17, 18, 22, 28, 35]. We showed that in these cultures distinct alloreactive populations coexist. The different tumor targets did not cross-compete but each was competed for by the ConA blasts from the same individual. In addition to the specific alloreactive subsets, a proportion of effectors lysed more than one target, as indicated by the cross-inhibitions. This was more evident with the pool-MLC-stimulated effectors. The beneficial effect of in vivo allosensitisatiton in murine malignancies [14, 15] and the graft-versus-leukemia phenomenon [25] are consistent with the selectivity of MLC effectors acting on autologous tumor but not on non-malignant cells. In this context, lysis of allogeneic tumors is not a relevant function.

Auto-tumor-selective cytotoxic lymphocytes could not be detected in strongly activated populations, i.e. when IL-2 or phytohaemagglutinin was added.

Il-2-activated cells are now used for therapeutic purposes. The mechanism of the observed beneficial effects in vivo is difficult to asses. It is, however, likely that they do

not act through direct damage of the tumor cells. If their direct effect were the relevant function, their widened tumor target panel would not necessarily create problems, because this is irrelevant in the autologous host. However, when the lymphocytes can also attack non-malignant cells in vitro, such as ConA blasts, they may also harm normal cells in vivo.

*Acknowledgements.*
This work was supported by the Swedish Cancer Society, and by a grant from the Cancer Society of Stockholm. We thank Mrs Birgitta Wester and Mrs Eva Oldmark for skillful technical assistance.

## References

1. Balsari A, Fossati G, Taramelli D, Tona G, Delia D, Giardini R, Parmiani G (1985) Allostimulation of patients lymphocytes generates both T and NK-like cells cytotoxic for autologous melanoma. Br J Cancer 52: 73
2. Galili U, Galili N, Vánky F, Klein E (1978) Natural species restricted attachment of human and murine T lymphocytes to various cells. Proc Natl Acad Sci USA 75: 2396
3. Grimm EA, Mazumder A, Zhang HZ, Rosenberg SA (1982) Lymphokine activated killer cell phenomenon. Lysis of natural killer cell resistant fresh solid tumor cells by interleukin-2 activated autologous human periferal blood lymphocytes. J Exp Med 155: 1823
4. Heo DS, Whiteside TL, Kanbour A, Herberman RB (1988) Lymphocytes infiltrating human tumors. I. Role of Leu-19 (NKH1)-positive recombinant IL-2-activated cultures of lymphocytes infiltrating human ovarian tumors. J Immunol 140: 4042
5. Hynes RO (1987) Integrins. A family of cell surface receptors. Cell 48: 159
6. Itoh K, Tilden AB, Balch CM (1986) Lysis of human solid tumor cells by lymphokine-activated natural killer cells. J Immunol 136: 1910
7. Klein E (1982) Lymphocyte mediated lysis of tumor cells in vitro, antigen restricted colnal and unrestricted polyclonal effects. Springer Semin Immunopathol 5: 147
8. Klein E, Vánky F (1981) Natural and activated cytotoxic lymphocytes which act on autologous and allogeneic tumor cells. Cancer Immunol Immunother 11: 183
9. Klein E, Vánky F, Masucci MG, Bejarano MT (1984) Experimental and clinical data for the role of NK cells in immunosurveillance. Behring Inst Mitt 74: 140
10. Klein E, Vánky F, Stuber G (1988) Lysis of autologous tumor cells by blood lymphocytes in vitro requires MHC class I antigen expression. Springer Semin Immunopath (in press)
11. Landazuri OM, Herberman RB (1972) Specificity of cellular immune reactivity to virus induced tumors. Nature 238: 18
12. Lanier LL, Cwirla S, Ferderspiel N, Philips JH (1986) Human natural killer cells isolated from periferal blood do not rearrange T-cell receptor beta chain genes. J Exp Med 163: 209
13. LeFor AT, Eisenthal A, Rosenberg SA (1988) Heterogenicity of lymphokine activated killer cells induced by IL-2. Separate lymphoid subpopulations lyse tumor, allogeneic blasts, and modified syngeneic blasts. J Immunol 140: 4062
14. Leshem B, Gotsman B, Kedar E (1984) In vitro elicitation of cytotoxic response against a nonimmunogenic murine tumor by allosensitisation. Cancer Immunol Immunother 17: 117
15. Longley RE, Good RA (1986) Leukemia prevention and long-term survival of AKR mice transplanted with MHC-matched or MHC-mismatched bone marrow. Cell Immunol 101: 476
16. Masucci MG, Klein E, Argov S (1980) Disappearance of NK effect after explantation of lymphocytes and generation of similar non-specific cytotoxicity correlated to the level of blastogenesis in activated cultures. J Immunol 124: 2458
17. Mazumder A, Grimm EA, Rosenberg SA (1983) Lysis of fresh human solid tumor cells by autologous lymphocytes activated in vitro by allosensitization. Cancer Immunol Immunother 15: 1
18. Paciucci PA, Macphail S, Zarling JM, Bach FH (1980) Lysis of syngeneic solid tumor cells by alloantigen stimulated mouse T and non-T cells. J Immunol 124: 370
19. Santoli D, Koprowski H (1979) Mechanism of activation of human natural killer cells against tumor and virus-infected cells. Immunol Rev 44: 126
20. Schlossman SF (1972) Antigen recognition: the specificity of T-cells involved in the cellular immune response. Transplant Rev 10: 97
21. Seely JK, Golub SH (1978) 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
22. Strausser JL, Mazumder A, Grimm EA, Lotze MT, Rosenberg SA (1981) Lysis of human solid tumors by autologous cells sensitized in vitro to alloantigens. J Immunol 127: 266
23. Timonen T, Saksela E, Ranki A, Häyry P (1979) Fractionation, morphological and functional characterization of effector cells responsible for human natural killer activity againast cell-line targets. Cell Immunol 48: 133
24. Thorsby E, Berle E, Nousianen H (1982) HLA-D region molecules restrict proliferative T-cell responses to antigen. Immunol Rev 66: 39
25. Truitt RL, Shih CC-Y, Lefever AV (1986) Manipulation of graft versus host disease for a graft versus leukemia effect after allogeneic bone marrow transplantation in AKR mice with spontaneous leukemia/lymphoma. Transplantation 41: 301
26. Vánky F, Vose BM, Fopp M, Klein E (1979) Human tumor-lymphocyte interaction *in vitro*. VI. Specificity of primary and secondary autologous lymphocyte mediated cytotoxicity. J Natl Cancer Inst 62: 1407
27. Vánky F, Argov S, Einhorn S, Klein E (1980) Role of alloantigens in natural killing. Allogeneic but not autologous tumor biopsy cells are sensitive for interferon-induced cytotoxicity of human blood lymphocytes. J Exp Med 151: 1151
28. Vánky F, Argov S, Klein E (1981) Tumor biopsy cells participating in systems in which cytotoxicity of lymphocytes is generated. Autologous and allogeneic studies. Int J Cancer 27: 273
29. Vánky F, Masucci MG, Bajarano MT, Klein E (1984) Lysis of tumor biopsy cells by blood lymphocytes of various densities. Autologous and allogeneic studies. Int J Cancer 33: 185
30. Vánky F, Klein E, Willems J, Böök K, Ivert T, Péterffy A, Nilsonne U, Kreicbergs A, Aparisi T (1986) Lysis of autologous tumor cells tested at the time of surgery. Correlation with the postsurgical clinical course. Cancer Immunol Immunother 21: 69
31. Vánky F, Klein E, Willems J, Böök K, Ivert T, Péterffy A (1987) Recognition of autologous tumor cells by blood lymphocytes in patients with lung cancer. In: Byers VS, Baldwin RW (eds) Immunology of malignant diseases. MTP Press Ltd, Lancaster, pp 105–128
32. Vánky F, Roberts T, Klein E, Willems J (1987) Auto-tumor immunity in patients with solid tumors. Participation of CD3 complex and MHC class I antigens in the lytic interaction. Immunol Lett 16: 21
33. Vánky F, Stuber G, Willems J, Sjöwall K, Larsson B, Böök K, Ivert T, Péterffy A, Klein E (1988) Importance of MHC antigen expression on solid tumors in the in vitro interaction with autologous blood lymphocytes. Cancer Immunol Immunother 27: 213
34. Vánky F, Stuber G, Rotstein S, Klein E (1989) Auto-tumor recognition following in vitro induction of MHC antigen expression on solid human tumors: stimulation of lymphocytes and generation of cytotoxicity against the original MHC antigen negative tumor cells. Cancer Immunol Immunother 28: 17

35. Vose BM, White W (1983) Tumor reactive lymphocytes stimulated in mixed lymphocyte and tumor culture. Clonal analysis of effector cells in cytotoxic and proliferative assays. Cancer Immunol Immunother 15: 227
36. Vose BM, Vánky F, Fopp M, Klein E (1978) Restricted autologous lymphocytotoxicity in lung neoplasia. Br J Cancer 38: 372
37. Zinkernagel R, Doherty P (1979) MHC-restricted cytotoxic T cells: studies on the biological role of polymorphic major transplantation antigens determining T-cell restriction-specificities, function and responsiveness. Adv Immunol 27: 51

Received 19 October 1988/Accepted 31 January 1989