Selective Antitumor Activity of Lymphokine-Activated Killer Cells *In Vitro*

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We compared cytotoxic activity of blood mononuclear leukocytes from healthy donors and lymphokine-activated killer cells generated from them towards tumor and normal cells. Lymphokine-activated killer cells exhibited higher (in comparison with blood mononuclear leukocytes) killer activity towards tumor cells. Lymphokine-activated cells and mononuclear leukocytes had no lytic effect on non-transformed eukaryotic cells. Hence, we demonstrated selective cytotoxic activity of effector cells (lymphokine-activated killers) towards tumor cells of different origin (but not normal cells).

Key Words: *lymphokine-activated killer cells; blood mononuclear leukocytes; cytotoxic activity; tumor cell strains; non-transformed cells*

Lymphokine-activated killer cells (LAKC) similarly to natural killer cells recognize and lyse transformed cells [1-4]. Lysis can be triggered by the absence of the major histocompatibility complex or changes in this complex (associations with a foreign protein or viral transformation of the cell), or absence of the major histocompatibility complex of allogenic non-transformed cells [3-5]. It was reported that LAKC lyse not only malignant cells, but also normal allogeneic or autologous cells which lost the major histocompatibility complex [1,3,6,7], but these data are contradictory.

We compared cytotoxic activity of effector cells towards tumor and normal intact cells.

MATERIALS AND METHODS

Human tumor cell strains (A549 non-small-cell lung carcinoma, SKOV3 ovarian carcinoma, MCF7 breast cancer, and K562 erythroblastic leukemia; American Type Culture Collection), normal cell strains (fetal calf lung cells from specialized collection of somatic cell cultures of agricultural and industrial animals, Ya. R. Kovalenko Institute of Experimental Veterinary, Russian Academy of Agriculture), and human fibroblasts served as objects for the study of cytotoxic effects of effector cells. The cells were cultured in RPMI-1640 with 10% FCS at 37°C and 5% CO_2.

Allogeneic fibroblast cultures were grown from biopsy specimens of donor forearm skin. In order to prepare primary culture, the tissue was washed in saline with gentamicin and treated in a solution containing EDTA, trypsin, and collagenase. Primary culture cells were then centrifuged, washed from enzymes in medium 199, and resuspended in RPMI-1640 with 10% FCS.

Blood mononuclear leukocytes (BML) were isolated from heparin-stabilized (25 U/ml) peripheral blood of 30 donors in a single-step Ficoll gradient (Pharmacia; 1.077 g/cm^3 density) by 30-min centrifugation at 400*g*. Lymphoid cells forming the interphase ring were collected with a pipette and washed 3 times in medium 199. After each washout in 10-fold volume of the medium, the cells were precipitated by centrifugation at 200*g*.

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Peripheral blood mononuclear leukocytes (10^6 cell/ml) were resuspended in RPMI-1640 with 10% FCS, after which IL-2 (Proleukine, Chiron) in a concentration of 1000 U/ml was added. The cells were incubated for 2 days.

Cytotoxic activity of lymphocytes was evaluated on cell strains. The cells were seeded (10^4/well) into flat-bottom 96-well microplates (Costar) and incubated under the above conditions for 24 h. BML and LAKC were then added into wells in target/effector cell ratios of 1:1, 1:2, 1:5, and incubated for 24 h. The cytotoxic effect was evaluated by the MTT test based on reduction of colorless form of the dye to blue formazan crystals soluble in dimethylsulfoxide under the effect of dehydrogenases of living cells.

Optical density of solutions was measured on a Multiscan MS (Labsystems) at λ=540 nm. The death of target cell in the presence of effectors was calculated by the formula:

$$CT=[1-(OD_{E+T}-OD_E)/OD_T]\times 100\%,$$

where CT is cytotoxicity, OD_{E+T} optical density in wells with effectors and target cells, OD_E optical density in wells with effectors, and OD_T optical density in wells with targets.

The error of measurements did not exceed 5%. The cytotoxic effects of LAKC and BML were considered significant, if they caused lysis of at least 50% target cells.

The expression of surface markers on target cells was evaluated by flow cytofluorometry on a FACScan cytometer (Becton Dickinson) using monoclonal antibodies to the corresponding antigens (Catlag Laboratories). The expression of differentiation antigens CD3, CD4, CD8, CD16, activation antigens CD25, CD38, HLA-DR, and adhesion molecules CD57, CD58 was studied. The cell population gate was established on the basis of combination of forward and side light scatter and cell size. A total of 10,000 events per gate were counted. The data were processed using WinMDI 2.8 software.

TABLE 1. Immunophenotypes of BML and LAKC (Expression of Surface Molecules, %)

Marker	BML	LAKC
CD3	59.4±4.1	32.3±8.7*
CD4	43.1±6.6	11.9±6.2*
CD8	30.8±2.3	20.2±4.5
CD16	11.9±1.5	34.6±8.3*
CD25	5.2±2.1	38.3±5.9*
CD38	19.9±4.8	38.5±4.1*
CD56	19.9±2.0	41.9±9.3*
CD57	8.9±2.4	31.2±3.5*
CD58	1.7±0.8	84.2±4.8*
HLA-DR	5.9±1.3	69.2±5.1*

Note. Here and in Table 2: *p<0.05 compared to BML.

RESULTS

Study of immunophenotype of LAKC generated from blood mononuclear leukocytes showed active expression of activation molecules (CD38), class II major histocompatibility complex molecules (HLA-DR), and natural killer cell antigens (CD16, CD56, CD57) on these cells. These cells were also charac-

TABLE 2. Cytotoxic Activities of BML and LAKC towards Cells of Different Tumor Strains (%, $M\pm m$)

Tumor cell strains	Target/effector cell ratio		
	1:5	1:2	1:1
A549 non-small-cell lung carcinoma			
BML (control)	58.0±4.5	53.0±5.2	42.0±3.1
LAKC	77.0±5.8*	60.0±6.4	58.0±6.2
K562 human erythroblastic leukemia			
BML (control)	68.0±9.7	41.0±5.3	33.0±3.4
LAKC	86.0±5.1*	69.0±6.3*	57.0±2.7*
SKOV3 ovarian carcinoma			
BML (control)	28.0±9.2	25.0±8.9	20.0±5.4
LAKC	51.5±10.1*	40.0±9.2*	30.0±7.6
MCF7 human breast cancer			
BML (control)	10.0±5.2	7.5±2.4	6.0±3.1
LAKC	62.5±5.8*	35.5±4.6*	27.0±6.7*

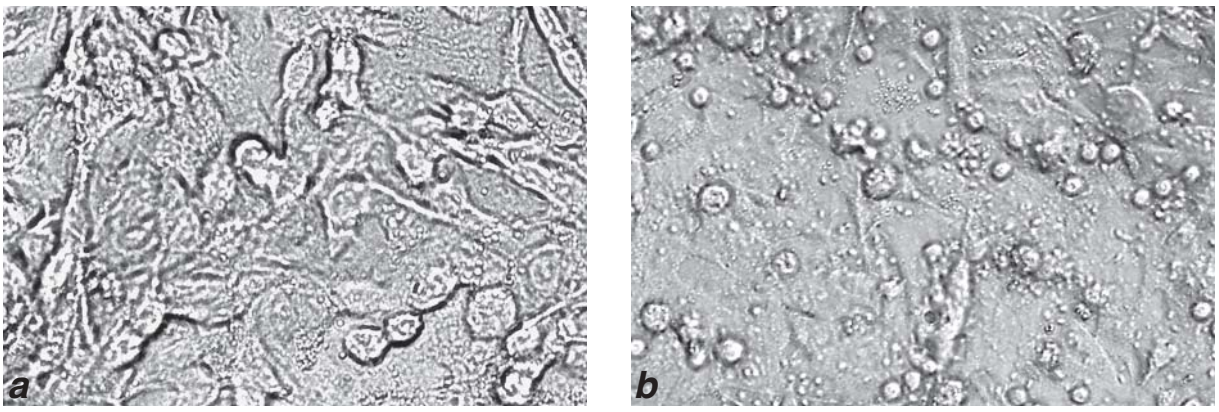

Fig. 1. A549 Non-small-cell pulmonary carcinoma cells in culture before (*a*) and after addition of LAKC (*b*). Here and in Figs. 2-5: target/effector cell ratio 1:5. Microphotographs of culture suspension. Phase-contrast bright-field microscopy, ×200.

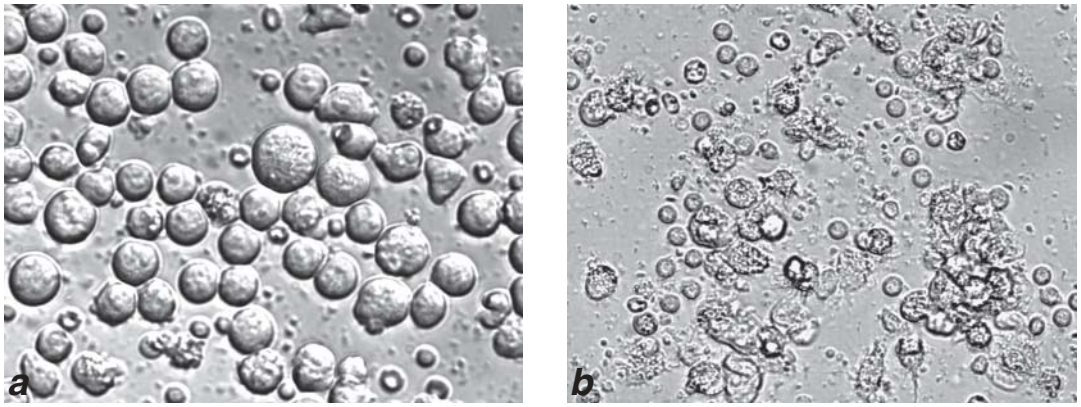

Fig. 2. K562 Erythroblastic leukemia cells in culture before (*a*) and after addition of LAKC (*b*).

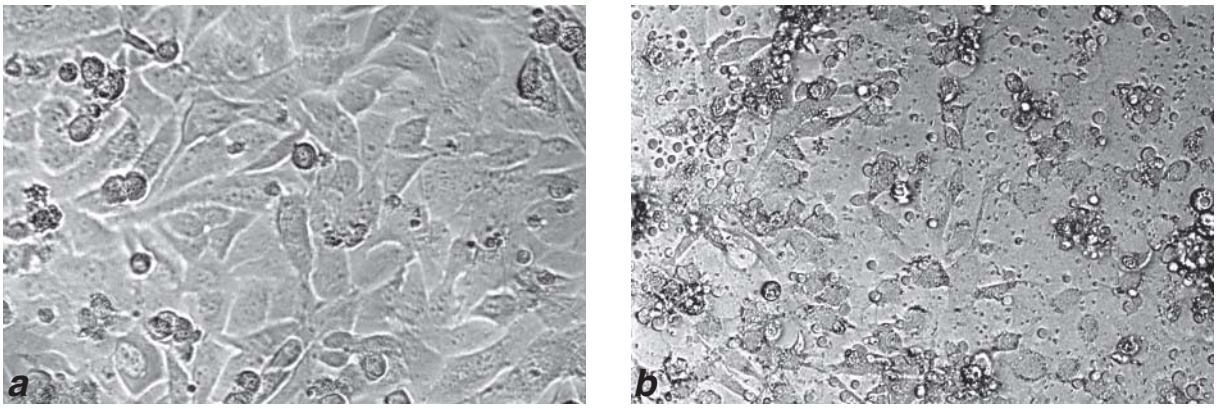

Fig. 3. SKOV3 Ovarian cancer cells in culture before (*a*) and after addition of LAKC (*b*).

TABLE 3. Cytotoxic Activity of BML and LAKC towards Non-Transformed Cells (%, *M*±*m*)

Target/effect or cell ratio	Fetal calf lung cells		Skin fibroblasts	
	BML (control)	LAKC	BML (control)	LAKC
1:5	20.0±3.4	24.0±4.5	18.0±2.7	32.0±4.7
1:2	12.0±5.2	15.0±4.1	22.0±3.6	23.0±5.3
1:1	20.0±3.1	20.0±2.3	15.0±4.2	17.0±2.1

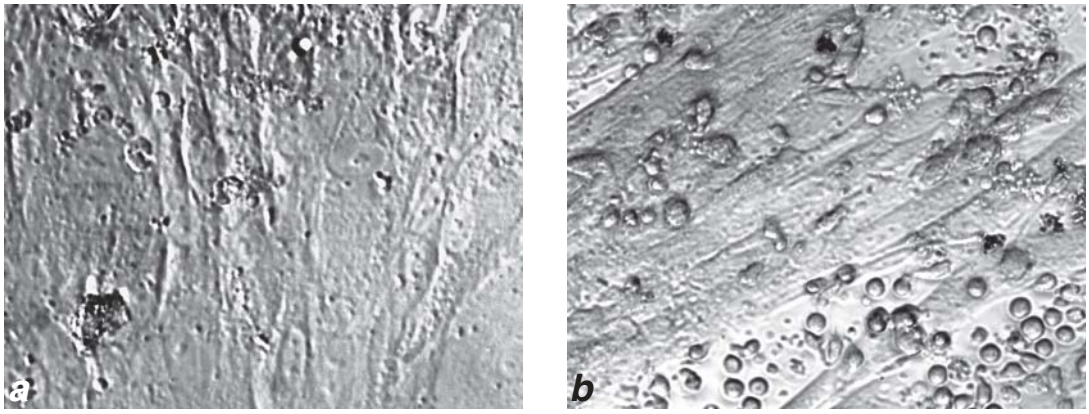

Fig. 4. Donor skin fibroblasts in culture before (*a*) and after addition of LAKC (*b*).

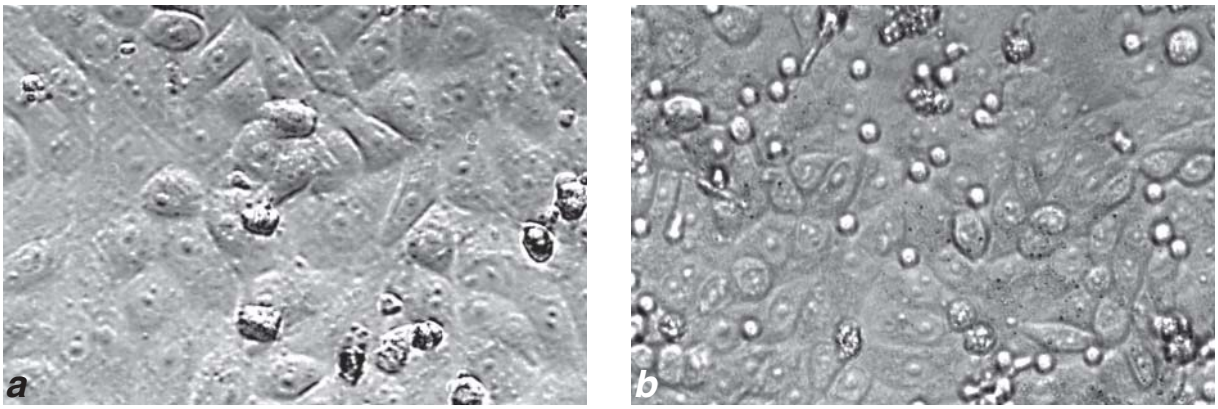

Fig. 5. VER normal fetal calf lung cells in culture before (*a*) and after addition of LAKC (*b*).

terized by intensive expression of adhesion molecules (CD58) (Table 1). The expression of surface markers on LAKC cells was significantly higher than on BML.

Intact BML and LAKC generated from them exhibited cytotoxic activity towards all tested tumor cell strains (Table 2). Mononuclear leukocytes activated with IL-2 caused lysis of tumor cells of different strains (Figs. 1-3). The maximum cytotoxic effect was observed at tumor/effector cell ratio of 1:5 for all tested tumor strains.

Killer activity of LAKC was significantly higher than cytotoxic activity of BML in all studied target/effector cells ratios. The highest cytotoxicity of BML and LAKC was observed towards K562 and A549 cells. SKOV3 and MCF7 strains were less sensitive to the lytic effects of BML and LAKC.

BML and LAKC in these ratios had virtually no cytotoxic effect on normal human fibroblasts and fetal calf lung cells (Table 3; Figs. 4, 5).

Hence, selective lytic activity of effector cells towards tumor cells of different histogenesis was demonstrated. LAKC exhibited higher killer activity towards all tumor strains in comparison with BML activity. The absence of LAKC cytotoxicity towards non-transformed cells confirms specificity of adoptive IL-2/LAKC immunotherapy. These data necessitate further study of the mechanism of selective antitumor effect of effector cells.

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