SPECIFIC ANTITUMOR CYTOTOXICITY OF PERIPHERAL BLOOD PLATELETS OF LUNG CANCER PATIENTS

S. N. Bykovskaya, N. G. Blokhina, D. L. Speranskii, and T. A. Kupriyanova

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Because of the rapid development of methods for the immunodiagnosis and immunotherapy of cancer patients, the study of the cellular mechanisms of antitumor immunity is clearly important. It has been shown that monocytes and T and B lymphocytes all participate in tumor destruction [1-3, 5, 6]. However, the role of other cells of the immune system in the mechanism of surveillance of cells undergoing malignant change is still unexplained.

We have studied the ability of platelets of lung cancer patients to induce lysis of autologous and allogeneic tumor cells and examined the question of the specificity of this effect.

EXPERIMENTAL METHODS

Samples (10 ml) of peripheral venous blood were collected from the patients to be investigated and from a control group of blood donors into test tubes containing heparin. Platelet-enriched plasma was obtained by centrifugation of heparinized blood at 200g (20 min, 4°C), after which the plasma was centrifuged at 1600g (20 min, 4°C). The platelet residue was resuspended in 1 ml of medium RMPI 1640 containing 5% fetal calf serum. Platelets were counted in a Goryaev counting chamber. The platelet suspension contained no contaminating peripheral blood mononuclear cells. On the day before the experiment the target tumor cells were labeled with $Na_2{}^{51}CrO_4$, and introduced into the wells of 96-well flat-bottomed panels in a dose of $2 \cdot 10^4$ cells per well. Platelets were added to the target cells in the ratio of 50:1, 25:1, and 12:1. After incubation at 37°C for 18 h in an atmosphere with 5% CO_2 the supernatant was taken from each well for radioactivity determination. The percentage of cytolysis was estimated from the amount of isotope released from the lysed tumor cells. The cytolytic activity of the peripheral blood platelets of 21 lung cancer patients and 24 donors was studied.

EXPERIMENTAL RESULTS

Platelets from 5 of the 13 patients caused lysis of freshly isolated autologous and allogeneic tumor cells (average lysis 15.3 ± 1.7%), and the cytotoxicity of the platelets of 7 patients did not exceed 10%, and the platelets of one patient did not induce lysis of autologous tumor cells (Fig. 1). Platelets of 13 of the 17 patients possessed cytolytic activity against cells of transplantable line AKL of adenocarcinoma of the lung, which averaged 23.2 ± 2.4%, in two cases cytolysis did not exceed 10%, and the platelets of one patient did not cause lysis of HAL cells. The ability of the platelets to induce lysis of autologous tumor cells was exhibited only in 38.5% of cases, the same as the number of positive results found on analysis of the cytotoxicity of lymphocytes against freshly isolated tumor cells [8]. The absence of a cytotoxic effect may be due to the properties of the freshly isolated tumor target cells, for in some cases platelets of the same patient induced lysis of AKL cells but did not exhibit cytotoxicity against autologous or allogeneic tumor cells (Table 1).

To reveal the specificity of action of the patients' platelets on tumor cells, platelets from healthy blood donors and a panel of target cells including cells of transplantable lines HeLa (carcinoma of the cervix uteri), Mel I (melanoma), and K562 (erythromyeloblastosis cells sensitive to natural killer cells) were used as effector cells.

All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow.
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TABLE 1. Cytotoxicity of Peripheral Blood Platelets of Lung Cancer Patients against Freshly Isolated and Transplantable Tumor Cells (percent of lysed target cells)

No. of patient	Freshly isolated lung cancer cells*	Cells of transplantable AKL line	K562	HeLa
1	39,1±4,5	20,2±5,0	5,4±0,7	4,4±0,7
2	0,4±0,1	14,7±0,7	2,6±0,2	—0,9±0,1
3	5,4±0,4	15,0±1,3	3,5±0,3	3,5±0,4
4	—7,8±0,6	47,7±5,4	—1,3±0,1	6,4±0,7
5	1,5±0,1	6,7±0,5	2,9±0,3	2,8±0,1
6	11,4±2,2	11,7±0,1	1,8±0,1	1,6±0,2
7	13,4±0,6	14,4±0,7	5,2±0,9	3,4±0,5
8	0,5±0,1	23,2±2,2	3,6±1,2	—3,8±0,1
9	30,7±3,3	20,1±4,6	2,3±0,5	4,1±0,5
10	1,8±0,1	14,4±0,7	—6,1±1,4	5,3±1,4

Legend. *) Ratio of platelets to target cells 50:1.

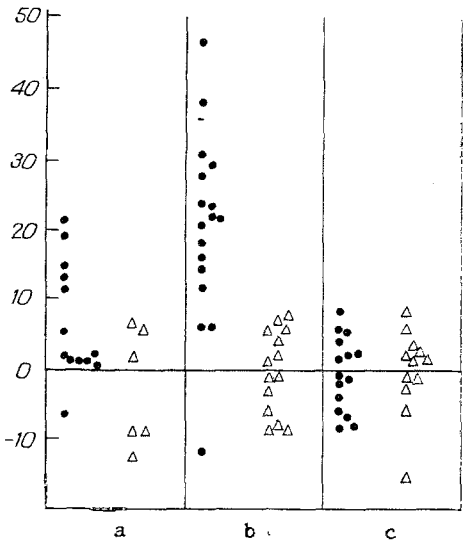

Fig. 1. Cytotoxicity of platelets of lung cancer patients (•) and healthy blood donors (△) against freshly isolated tumor cells (a), AKL cells (b), and K562 cells (c). Ordinate, cytolysis of target cells (in percent). Ratio of platelets to target cells 50:1.

The cytolytic activity of the platelets of subjects of the control group against freshly isolated tumor cells and AKL cells did not exceed 8.0% and was significantly lower than the cytolytic activity of platelets of lung cancer patients ($p < 0.001$; Fig. 1a, b). Platelets from patients and blood donors of the control group had no marked natural killer activity: lysis of K562 cells did not exceed 10% (Fig. 1c). The cytolytic activity of platelets from patients and blood donors against HeLa cells was the same and did not exceed 6.4% (Table 1). The patients' platelets also caused only slight lysis of Mel I cells, on average 2.9 ± 0.7%. Thus the cytolytic activity of the patients' platelets against K562, HeLa, and Mel I cells was negligible and was significantly lower than cytotoxicity against AKL cells ($p < 0.001$; Table 1).

However, platelets from healthy blood donors were able to induce lysis of Mel I cells, on average by 18.7 ± 3.1%. Similar data on cytotoxicity of platelets from healthy blood donors against transplantable melanoma cells were obtained by Grethen et al. [4], who suggested that the mechanism of the lytic action of the platelets is connected with secretion of a cytotoxic mediator [4, 7].

The results show that besides known populations of cytotoxic effector cells, such as lymphocytes and monocytes, platelets also can carry out destruction of tumor cells. It was thus shown for the first time that platelets of lung cancer patients possess specific cytolytic activity and cause lysis of freshly isolated and transplantable lung cancer cells. Elucidation of the mechanisms of the cytolytic action of platelets, including the study of receptors and structures participating in interaction with tumor cells, isolation of the cytotoxic factor from platelets, investigation of the specificity of their action in other groups of cancer patients also, are tasks of fundamental importance for characterization of the cellular systems involved in interaction with malignantly transformed cells.

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HUMAN TUMOR CELL LINES OBTAINED BY TISSUE CULTURE PASSAGE FROM STRAINS TRANSPLANTED INTO NUDE MICE

T. V. Yudicheva, T. I. Milovanova, Yu. N. Solov'ev, and E. S. Revazova

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It has rarely proved possible to obtain a continuous cell line of a human tumor by passage in tissue culture from biopsy or operative material. A more promising method is to obtain cell lines from strains of human tumors maintained by long-term passage through nude mice. As a result of repeated serial transplantations, cells with shortened cell cycles accumulate in tumors [1, 2]. Cells of transplantable strains are capable of unlimited multiplication, and for that reason the possibility of obtaining immortalized cell lines from them is much greater.

Two human cell lines are described in this paper: carcinoma of the body of the uterus and Wilms' tumor, obtained from strains transplanted into nude mice.

EXPERIMENTAL METHODS

The strain of carcinoma of the body of the human uterus was obtained from operative material by serial transplantations in nude mice. This strain was transplanted subcutaneously and has undergone 60 passages at intervals of 7-8 days. A uterine tumor with dimorphic structure of adenocarcinoma and undifferentiated carcinoma was used as original material. Throughout the period of passage the tumor basically preserved its stereotyped structure and cell composition and corresponded to the pattern of an undifferentiated carcinoma. No sign of a glandular structure (adenocarcinoma), present in the original material, could be detected in the transplants [3]. The strain of Wilms' tumor has undergone subcutaneous transplantation into nude mice at intervals of 22-24 days. For 57 generations it has maintained the structure of a nephroblastoma, with some predominance of the epithelial over the mesenchymal component. In all passages the strain has corresponded in principle to the original tumor

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All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow.

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