Cytotoxic Activity of Peripheral Blood Platelets and Mononuclears in Cancer Patients and Healthy Donors

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The cytotoxic activity of peripheral blood platelets and mononuclears from 82 patients with cancer, 9 with diseases other than cancer, and 18 healthy donors towards a continuous cell line of pulmonary adenocarcinoma was studied. There were no appreciable differences in the killer activity of platelets in cancer patients (32±3.5%), patients with diseases other than cancer (33±8.1%), and healthy donors (30±6.7%). In contrast to platelets, mononuclears of cancer patients possessed a higher cytotoxicity than mononuclears of healthy donors. The cytotoxicity of platelets of cancer patients was found to reliably drop in cases with disseminated metastases and after polychemo- and radiotherapy.

Key Words: *platelets; mononuclears; cytotoxicity; cancer patients*

Investigations in recent decades have demonstrated the important role of platelets in various biological reactions of the organism not related to blood clotting. It has been shown, for example, that platelets are capable of lyzing, together with complement, antibody-bound red cells and of causing the death of *Schistosoma volvina* and the lysis of tumor cells [2-4, 6-10]. A cytolytic activity of platelets has been demonstrated for a number of tumor cell lines, including K-562, KU 812, and LU 99A [8]. The mechanism of the cytotoxic effect of platelets is still virtually unknown. Some authorities have suggested that they participate in the realization of the cytolytic action of proteases, because joint incubation of platelets with protease inhibitors suppressed the cytotoxic effect in a dose-dependent manner [7,8].

Previously we showed that platelets of cancer patients and healthy donors are characterized by killer activity towards continuous tumor cell lines; a pulmonary adenocarcinoma (PAC) cell line proved to be the most sensitive to the cytotoxic effect of platelets [1].

This study was aimed at comparing the cytotoxic activities of peripheral blood platelets and mono-

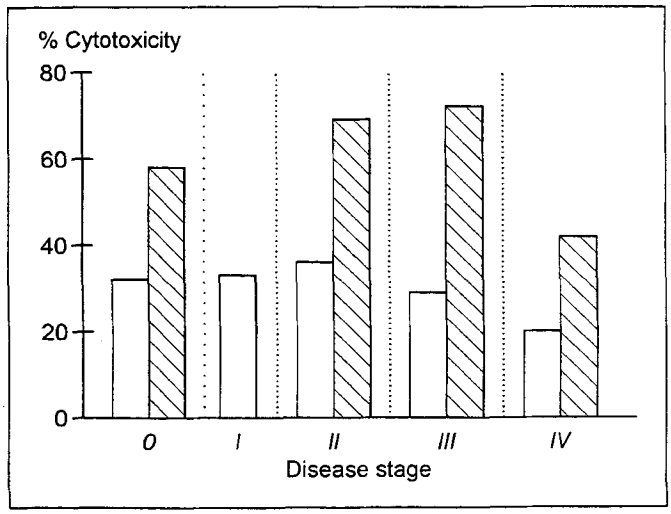

Fig. 1. Relationship between the cytotoxic activity of platelets (light bars) and mononuclear cells (dark bars) and the stage of cancer. 0 - healthy donors.

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nuclear cells (MNC) in cancer and noncancer patients and healthy donors, and at measuring the killer activity of platelets during different stages of the disease, in cases with or without metastases of various degrees of dissemination, and during different modalities of treatment (polychemotherapy, radiation, and surgical intervention).

MATERIALS AND METHODS

Peripheral blood platelet and MNC killer activity was studied in 82 cancer patients with tumors of different localizations: liver cancer (8 patients), gastroesophageal cancer (28), breast cancer (16), cervical cancer (18), and cancer of other localizations (12); in 9 patients with diseases other than cancer (cirrhosis of the liver, peptic ulcer, pancreatitis); and in 18 healthy donors. Continuous PAC cells derived from human PAC served as targets.

Venous blood platelets were isolated in two stages: platelet-rich plasma was obtained by 10-min centrifuging at 200 g, followed by 10-min centrifuging at 1600 g. The resultant platelet pellet was resuspended in RPMI-1640 medium. MNC were isolated from venous blood routinely by centrifuging in a Ficoll density gradient.

The cytotoxic activity of platelets and MNC was assessed in a test with Sigma MTT stain as described elsewhere [5]. Target cells were put in the wells of flat-bottom 96-well Costar microplates, and after 24 h a suspension of effectors was added. The effector/target ratio was 100:1 for platelets and 10:1 for MNC, total volume per well 200 μl. RPMI-1640 medium was used as the culture solution, with the addition of 5% fetal calf serum. After 18-h incubation at 37°C in an atmosphere with 5% CO_2, 10 μl of a 0.5% MTT solution in normal saline were added to each well, and the plates were incubated for 4 more hours under the same conditions. Then supernatant was collected from the wells and 150 μl dimethylsulfoxide were added per well to dissolve the forming formazan crystals. After 15 min the optical density (OD) was measured in each well with a Titertek Multiscan MCC 3340 spectrophotometer at wavelength 540 nm. The percentage cytotoxicity was calculated using the formula:

$$1 - \frac{\text{(targets+effectors mixture OD) - effector OD}}{\text{target cell OD}} \times 100.$$

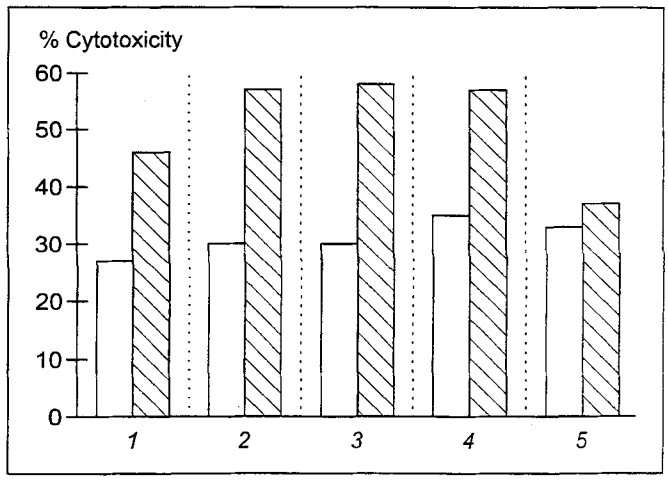

Fig. 2. Killer properties of platelets (light bars) and mononuclear cells (dark bars) in cancer patients with different localizations of the tumor. *1*) liver; *2*) breast; *3*) stomach; *4*) lungs; *5*) diseases other than cancer.

RESULTS

Peripheral blood platelets of cancer and noncancer patients and of healthy donors were characterized by approximately the same levels of cytotoxicity towards PAC cells (32±3.5, 33±8.1, and 30±6.7%, respective-

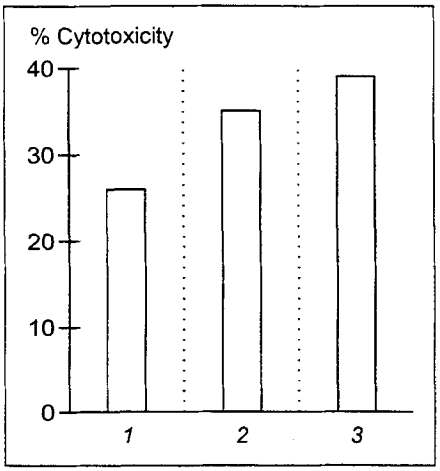

Fig. 3. Platelet cytotoxicity in patients with distant and regional metastases. *1*) disseminated metastases; *2*) regional metastases; *3*) no metastases.

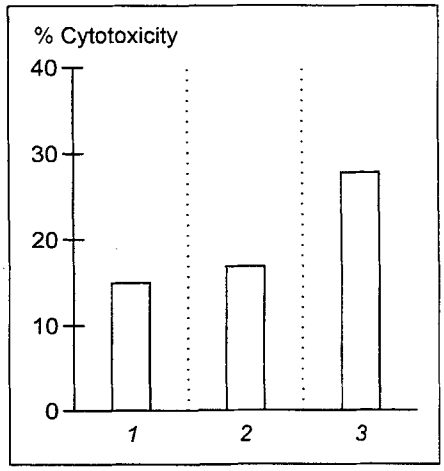

Fig. 4. Effects of polychemotherapy (*1*), radiotherapy (*2*), and surgery (*3*) on the cytolytic activity of platelets.

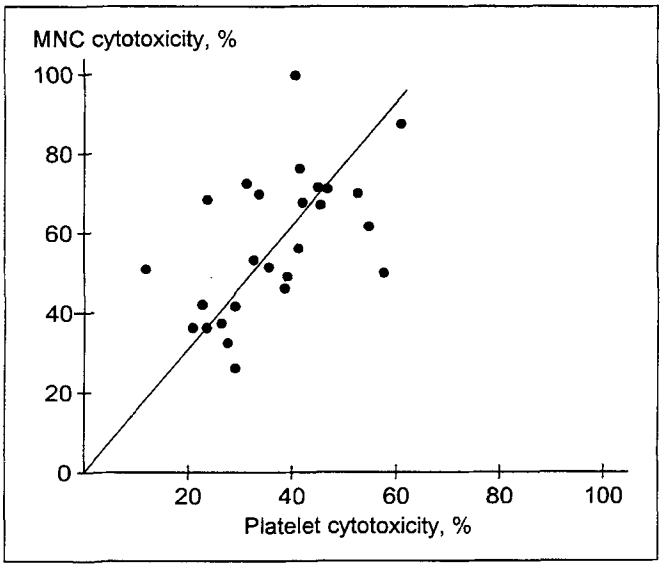

Fig. 5. Correlation between the cytotoxicity of platelets and mononuclears of cancer patients.

ly). On the other hand, the killer activity of platelets in stage I cancer patients did not differ from that in healthy donors and was somewhat increased (to 36%) in stage II patients. At stage III a tendency toward a reduction of platelet cytotoxicity was observed, and at stage IV platelet cytotoxicity dropped to 19.6±3.0%, on average. The cytolytic activity of MNC in cancer patients with disease stages II and III was reliably ($p<0.05$) higher (69±3.3 and 72±4.6%, respectively) than in healthy donors (58±4.7%). In stage IV patients the cytotoxicity of MNC was appreciably lower than the killer activity of mononuclears of healthy donors, amounting to an average of 42±4.3% (Fig. 1). There were no appreciable differences in the cytotoxicity levels of platelets and MNC in tumors of different localizations, but the highest killer activity was observed in patients with lung cancer (35 and 57%, respectively, Fig. 2). It is worth noting that the cytotoxicity of MNC in cancer of any localization was reliably higher than the killer activity of MNC in diseases other than cancer (Fig. 2). The cytotoxicity of platelets in cancer patients depended on the presence of metastases and their dissemination. For example, the lowest lytic activity of platelets was observed in patients with distant metastases (27±2.6%), in cases with regional metastases their killer properties were somewhat higher (34±3.3%), although inferior to the cytotoxic activity of platelets in patients without metastases (39±2.5%, Fig. 3).

Chemo- and radiotherapy reliably ($p<0.05$) affected the cytolytic properties of platelets: their cytotoxicity (15 and 16%) was approximately 50% lower than the mean values for cancer patients (32%); moreover, this treatment affected the killer activity of platelets as well, although to a lesser degree (25%, Fig. 4).

Hence, the results indicate that the use of the MTT stain in the cytotoxic test for assessing the level of viability of target cells after their contact with effectors yielded qualitatively similar data in comparison with those observed with radioactive Cr. For example, we succeeded in validating our previous data [1] on the absence of reliable differences in the cytotoxicity of platelets of cancer patients and healthy donors; an increase of the killer properties of these elements in stage II and a decline in stage IV, as well as the absence of a clear-cut relationship between platelet cytotoxicity and tumor localization. In addition, we discovered that the cytotoxic activity of platelets was virtually the same in cancer and noncancer patients.

In contrast to platelets, MNC of cancer patients were characterized by a higher level of cytolytic activity in comparison with those of donors and noncancer patients. However, the time course of killer properties in patients with different stages of disease was similar for both types of effectors; moreover, there was a correlation between the levels of platelet and MNC cytotoxicity in cancer patients (correlation coefficient 0.67, Fig. 5).

The study revealed a decline in the cytotoxic properties of platelets in cancer patients with different stages and dissemination of the process and a suppressive effect of polychemo- and radiotherapy on the killer properties of platelets.

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