ONCOLOGY

Effect of Lymphokines on Platelet-Mediated Cytotoxicity

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The effect of several cytokines on the cytolytic activity of platelets from healthy donors was studied. A human lung adenocarcinoma cell line served as the source of targets. IL-1α and IL-1β in a dose range of 10-100 U/ml increased the activity of initially low-toxic platelets, and vice versa. An increase of the dose of any cytokine up to 1000 U/ml caused inhibition of cytolysis, irrespective of the initial cytotoxicity of platelets. Unlike the mentioned cytokines, tumor necrosis factor by itself produced a cytotoxic effect on the targets; however, it had no effect on the cytolytic activity of platelets.

Key Words: *platelet; cytotoxicity; lymphokines*

Earlier it was shown that platelets of both cancer patients and healthy donors cause lysis of autologous and allogeneic tumor cells *in vitro* [1,3,5]. The cells of human lung adenocarcinoma have been found to be highly sensitive targets to the cytolytic action of platelets [1,3]. Electron microscopic studies have revealed that the changes in the ultrastructure of platelets adsorbed on the target cells resemble those characteristic for cells with an intermittent type of secretion, including such features as Golgi apparatus hypertrophy, enlargement of secretory granules, and their orientation to the area of contact [3]. The increase of granulation characteristic for platelets from cancer patients and tumor-bearing animals also provides evidence for the role of the secretory mechanism in the effect described [2]. Evidently, endogenous bioregulators exert a modulatory effect on the cytolytic properties of platelets. In particular, we have shown that the cytolytic effect of platelets is reliably augmented in the presence of platelet-activating factor, whose activating effect is especially pronounced in cases with initially low cytolytic activity (less than 10%) [3]. However, the effect of lymphokines on platelet cytotoxicity has still not been studied, although there are some reports concerning platelet-lymphokine interactions. Thus, it was found that the platelet membrane bears receptors of granulocyte colony-stimulating factor [7]. On the other hand, lymphokines that play a major role in antitumor and antimicrobial immunity (interleukins - IL-1α and IL-1β, tumor necrosis factor - TNF) induce the expression of platelet-derived growth factor receptors on human fibroblasts [9,10]. Moreover, lymphokines can indirectly influence the killer activity of the effectors of the immune system, producing a direct cytotoxic effect on tumor cells or inducing oncogene expression [4,6].

The goal of the present study was an investigation of the modulatory effect of several lymphok-

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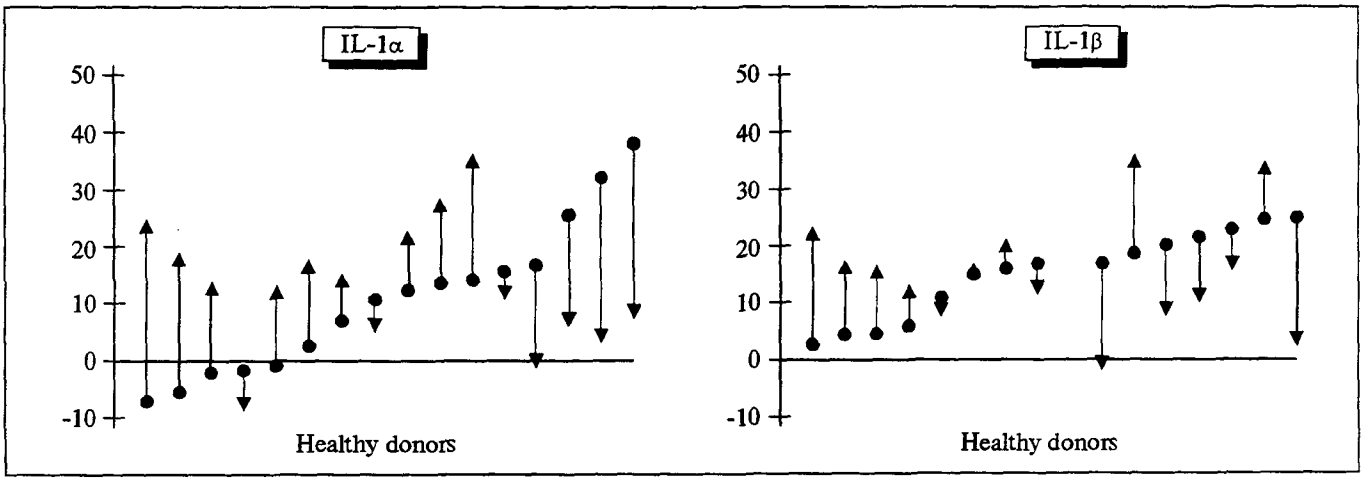

Fig. 1. Spontaneous (circles) and IL–influenced (triangles) platelet–mediated cytotoxicity. Here and in Figs. 2 and 3: the ordinate shows the cytotoxicity index (percentage).

ines (IL-1α, IL-1β, TNF) on the killer activity of platelets and a study of the direct cytotoxic activity of cytokines.

MATERIALS AND METHODS

Ten milliliters of peripheral venous blood from 31 healthy donors were taken into heparinized tubes. Platelets were separated as follows. First, heparinized blood was centrifuged at 200 g for 20 min at 4°C. Second, platelets were sedimented by centrifugation of plasma at 1600 g for 20 min at 4°C. The sediment was resuspended in 1 ml of RPMI-1640 medium supplemented with 5% fetal calf serum. The platelets were counted in a Goryaev chamber. The platelet suspension thus obtained was free of nuclear cells.

Human lung adenocarcinoma cells served as targets. The target tumor cells were labeled with $Na_2{}^{51}CrO_4$ and placed in wells of 96-well flat-bottom microplates, 2×10^4 cells per well. Next day the wells were washed, the culture fluid was aspirated, and 200 μl of platelet suspension were added to each well. The effector:target ratio was 100:1. After 18 hours of incubation at 37°C in an atmosphere with 5% CO_2 the radioactivity of supernatants taken from the wells was recorded. The percent of cytolysis was estimated routinely [1,3].

In the assays of the modulatory effect of IL-1α, IL-1β, and TNF (Petro Tech., Inc.) on platelet-mediated cytotoxicity the cytokines were administered in a concentration of 1-1000 U/ml simultaneously with platelets. The reliability of the results was verified using Student's *t* test.

RESULTS

The mean spontaneous cytotoxicity of donor platelets was 12.2±1.8%; however, the level of cytotoxicity varied in different donors. Thus, the maximum level attained 38%, while platelets of 7 out of 31 donors failed to exert any detectable cytotoxic effect (Fig. 1).

IL-1α and IL-1β in the concentration range of 1 to 100 U/ml did not exert any significant cytotoxic effect on the target cells. Unlike interleukins, TNF administered in a concentration of 100 ng/ml induced the death of 15.5±1.9% of cells (Table 1).

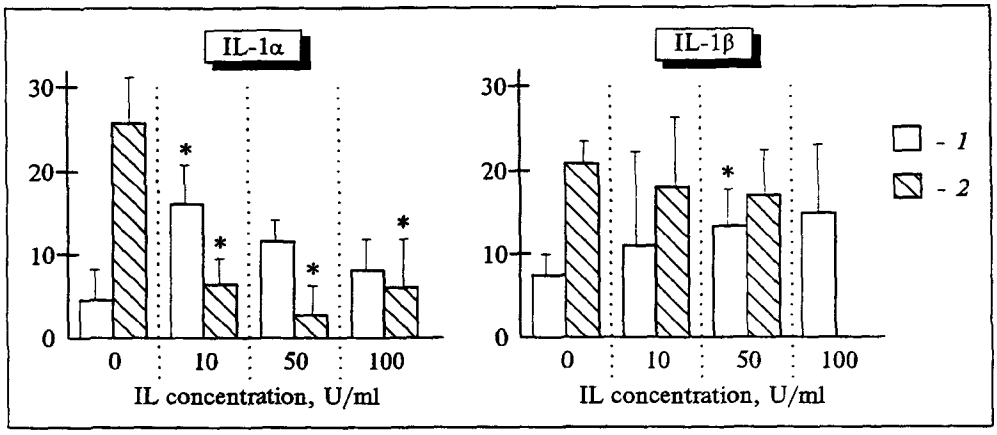

Fig. 2. Modulating effect of IL–1a and IL–1b on the cytotoxicity of platelets with spontaneous low (*1*) and high (*2*) cytotoxic activity. Here and in Fig. 3: *: IL–induced reliable change of cytotoxicity ($p<0.05$).

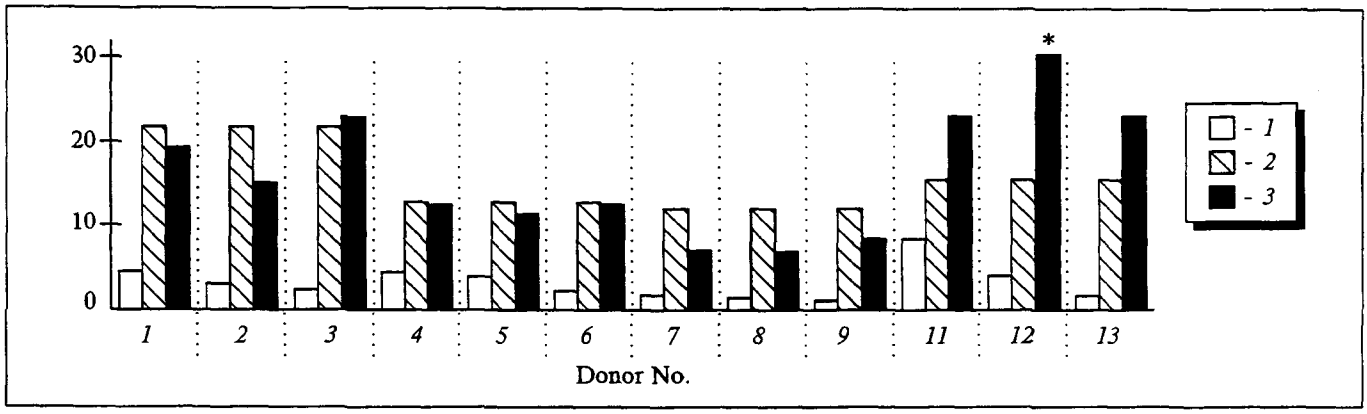

Fig. 3. Effect of TNF on platelet-mediated cytotoxicity of healthy donors in the absence (*1-9*) and presence (*11-13*) of autologous plasma. *1*) spontaneous platelet-mediated cytotoxicity; *2*) TNF cytotoxicity; *3*) platelet-mediated cytotoxocity in the presence of TNF.

In a series of experiments it was shown that both IL-1α and IL-1β administered in a concentration of 10 U/ml could either augment or inhibit the cytotoxic effect of platelets, depending on the initial platelet activity. Thus, platelets with an initial cytotoxic activity of up to 15% produced a 1.5-25-fold increase of cytotoxicity in the presence of IL-1α and IL-1β; however, under the same conditions the cytotoxicity of platelets with an initially high killer activity (15-38%) fell to 10% or even lower (Fig. 1). Therefore, for a more detailed analysis of the effect of interleukins administered in a broad concentration range (1-1000 U/ml) on platelet killer potential, we divided the donors into two groups: those with a spontaneous platelet cytotoxic activity of up to 15% and those with an activity of 15-38%. The cytotoxic activity of platelets from the first group was enhanced by IL-1α and IL-1β administered in a concentration of 10 U/ml; raising the concentration (to 50-100 U/ml) did not cause any further increase in platelet cytotoxicity. Platelets from the second group manifested further inhibition of cytotoxicity ($p<0.01$) in the presence of increased concentrations of IL-α and IL-1β (Fig. 2). Both interleukins induced inhibition of platelet cytotoxicity regardless of the spontaneous platelet activity. Pretreatment of target cells with IL administered in a concentration of 1000 U/ml also mitigated the platelet-mediated cytotoxic effect.

TABLE 1. Cytotoxicity of IL-1α, IL-1β, and TNF, % ($M\pm m$)

Cytokine	Cytokine concentration		
	1	10	100
IL-1α, U/ml	0.4±0.2	1.9±0.9	2.5±2.6
IL-1β, U/ml	−2.4±1.2	−1.5±1.0	−1.8±0.2
TNF, ng/ml	3.1±1.1	1.4±0.7	15.5±1.9*

Note. * reliable difference from the effect of IL-1α administered in a concentration of 100 U/ml ($p<0.05$).

As was noted above, TNF itself had a reliable cytotoxic effect on target cells. However, this cytokine did not potentiate the cytotoxic effect of platelets. Synergism slightly exceeding the summated cytotoxic effect of TNF and platelets could be observed when the platelet suspension was supplemented with autologous plasma in order to prevent aggregation (Fig. 3).

Thus, IL-1α and IL-1β exert a modulatory effect on platelet-mediated cytotoxicity. It is worth noting the paradoxical feature of this effect, i.e., stimulation of platelets with initially low activity and the opposite effect on platelets with initially high activity. The effect of interleukins is of a threshold nature. Maximum efficiency of these lymphokines is observed when they are administered in a concentration range of 10-50 U/ml; further elevation of the concentration (100-500 U/ml) is less efective, and a concentration of 1000 U/ml causes inhibition of platelet-mediated cytotoxicity. Similar concentration-dependent effects were established earlier in our experiments on the effect of platelet-activating factor on the cytotoxicity of platelets. The mechanism of this phenomenon remains unclear, but it can be assumed that platelets possess a certain limited potential of activity and, following its attainment, the action of stimulatory factors leads to their hyperactivation and a subsequent decline or even loss of cytotoxic properties. Apparently, this explains the inhibitory effect of lymphokines and platelet-activating factor administered in high doses as well as the decrease in the killer activity of platelets with spontaneous high cytotoxic potential after incubation with relatively low doses of lymphokines. The variable level of spontaneous platelet-mediated cytotoxicity in different donors may be connected with the pecu-

liarities of the functional status of the individual organism. Indirect evidence of this is provided by our data on the corrrelation between platelet cytotoxicity and the stage of disease in cancer patients [3].

TNF, which, unlike IL-1α and IL-1β, exerts a direct cytotoxic effect, fails, however, to have an appreciable effect on the cytotoxicity of platelets, and synergism of TNF plus platelet-mediated cytotoxicity does not exceed the summation of the separate cytotoxic effects. The addition of autologous plasma in order to prevent aggregation only slightly enhances the combined effect of TNF and platelets.

The phenomenon of resistance to platelet-mediated cytotoxicity following pretreatment of target cells with high doses of IL-1α and IL-1β that has been demonstrated in the present study requires further investigation.

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