Interleukin-2 and Staurosporin Cancel Inhibition of Nonspecific Cytotoxicity of Rat Splenocytes by High Doses of Phorbolmyristate Acetate

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Experiments with human erythromyeloleukemic K562 cells as target cells demonstrate that nonspecific cytotoxic activity of rat splenocytes suppressed by 1-day incubation of cells with phorbol-12-myristate-13-acetate is restored by subsequent 3-day incubation with interleukin-2 and staurosporin. Combined effects of both drugs during this incubation time decrease cell permeability for Trypan Blue by 50%.

Key Words: *nonspecific cytotoxicity; interleukin-2; staurosporine; phorbolmyristate acetate*

Treatment of cells with inhibitors of Ca^{2+}, phospholipid-dependent protein kinase C leads to suppression of cytotoxicity toward target tumor cells, unrestricted for the main histocompatibility complex (UCT) [10]. Phorbol-12-myristate-13-acetate (PMA) in a dose of 0.4 μM [6,10] and staurosporine [13] act as such inhibitors.

Previously we showed that 3-day incubation of rat splenocytes with 0.01 μM staurosporine increases UCT activity of these cells toward YAC-1 target cells. The presence of interleukin-2 (IL-2) and staurosporine in the incubation medium exerted additive effect, increasing UCT activity of rat splenocytes and thymocytes in comparison with cultures containing IL-2 alone [1].

In this study human erythromyeloleukemic K562 cells were used as target cells. Our purpose was to assess the effect of combined treatment of cells with staurosporine and IL-2 on UCT activity of rat splenocytes after incubation with high doses of PMA.

MATERIALS AND METHODS

Male Wistar rats weighing 170-220 g were used. Splenocyte suspension was prepared as described previously [1].

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K562 cells were obtained from the Russian Bank of Cell Cultures (Institute of Cytology, Russian Academy of Sciences, St. Petersburg). Cells were cultured and nonspecific cytotoxicity test was carried out in complete RPMI-1640 (Institute of Poliomyelitis and Virus Encephalitis, Russian Academy of Medical Sciences, Moscow) with 10% fetal calf serum (N. F. Gamaleya Institute of Microbiology and Epidemiology, Russian Academy of Medical Sciences, Moscow), 2 mM L-glutamine, 5×10^{-5} M 2-mercaptoethanol (Ferak), and 40 μg/ml gentamycin sulfate.

NBT test (14 h) was performed as described previously [3]. K562 cells labeled with ^{3}H-uridine in a dose of 10 μCi/ml for 2 h were used as target cells. The cytotoxic index was calculated from the following formula: [1-(number of pulses in experimental well)/(number of pulses in control)]×100%. Target cells incubated under the same conditions but without splenocytes were control.

Proliferating cells in 72-h cultures were detected by intensity of ^{3}H-thymidine incorporation in DNA [1].

Cell viability was assessed by the Trypan Blue exclusion test (at least 200 cells per sample), and the viability index was calculated as follows: [(number of live nonstained cells)/(total number of cells)]×100%.

Pretreatment of cells with PMA (400 nM) was carried out in complete medium for 24 h at 37°C in

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a humidified atmosphere with 5% CO_2. An equivalent volume of the solvent (dimethylsulfoxide) was added to control cultures. After incubation, the cells were washed in medium 199 three times.

PMA, staurosporine, Trypan Blue (Sigma), yeast recombinant human IL-2 (roncoleukin, Biotech, St. Petersburg), ^{3}H-uridine and ^{3}H-methylthymidine (Isotop) were used, other reagents were Russian-manufactured, chemically pure.

The significance of differences was assessed using Student's *t* test.

RESULTS

For assessing modulating effect of IL-2, staurosporine, or their combination on UCT activity of rat splenocytes, these cells were incubated with PMA (400 nM, experiment) or dimethylsulfoxide (control) for 24 h and after washing were added to cultures (1 μl) containing IL-2 (200 U/ml), staurosporine (0.01 μM), or both in the above concentrations. After 3-day culturing, cells from each culture were washed once, and their UCT activity toward K562 cells was measured in a 14-h test.

Figure 1, *a*, shows that UCT activity of PMA-treated rat splenocytes in cultures with staurosporine and IL-2 increased during incubation. In control splenocytes incubated with dimethylsulfoxide for 3 days, none modulator stimulated cell lysis.

Staurosporine in a concentration of 0.01 μM (dose optimal for inhibition of mainly protein kinase C [5]) proved to be toxic for cells; therefore, in a parallel experiment we examined the effects of staurosporine and IL-2 on viability of splenocytes incubated under the same conditions as for determination of UCT activity (Fig. 1, *b*). Obviously, 3-day incubation of cells with staurosporine decreased cell permeability for Trypan Blue irrespective of preincubation with PMA. Combination of staurosporine and IL-2 produced the maximum toxic effect on rat

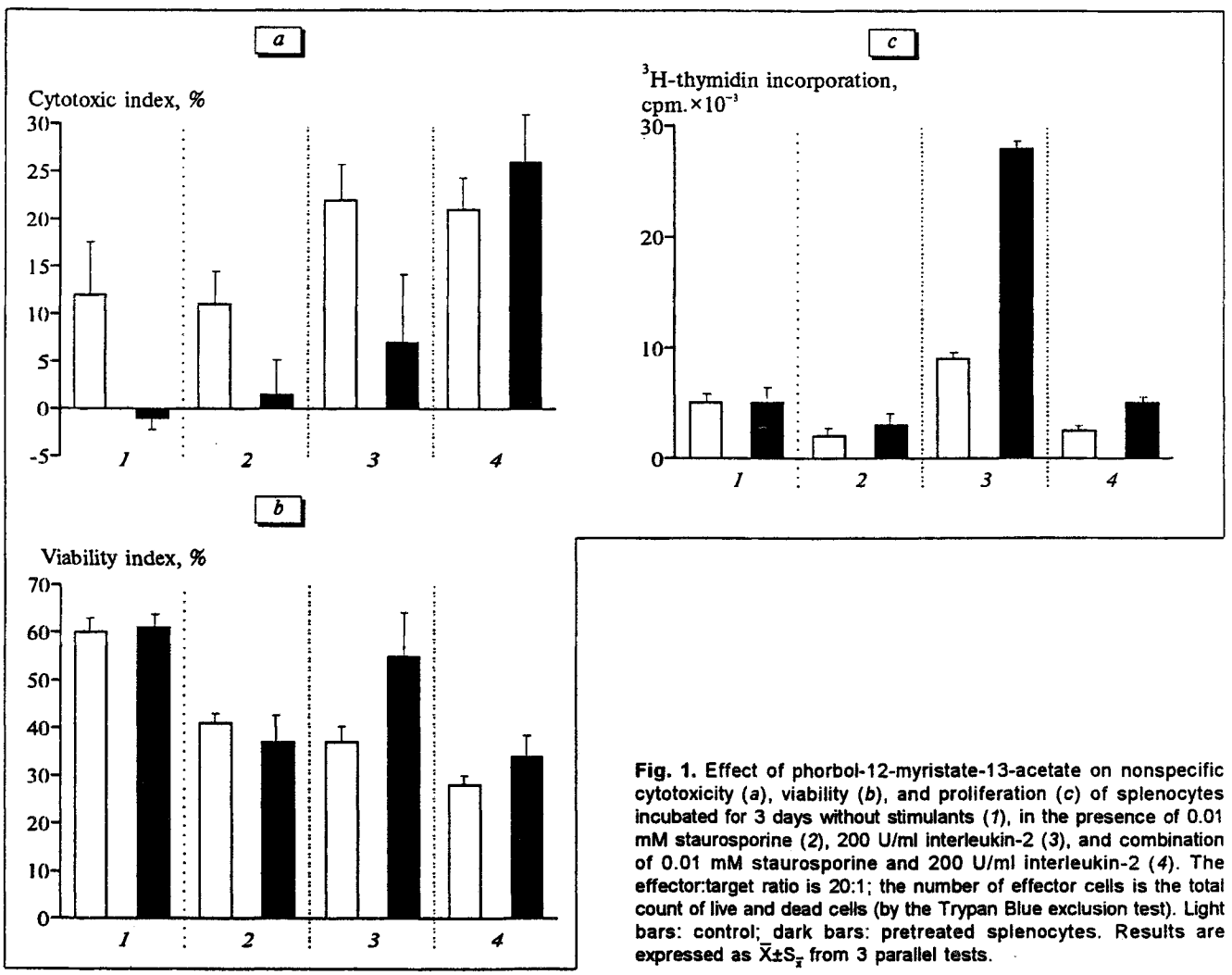

Fig. 1. Effect of phorbol-12-myristate-13-acetate on nonspecific cytotoxicity (*a*), viability (*b*), and proliferation (*c*) of splenocytes incubated for 3 days without stimulants (*1*), in the presence of 0.01 mM staurosporine (*2*), 200 U/ml interleukin-2 (*3*), and combination of 0.01 mM staurosporine and 200 U/ml interleukin-2 (*4*). The effector:target ratio is 20:1; the number of effector cells is the total count of live and dead cells (by the Trypan Blue exclusion test). Light bars: control; dark bars: pretreated splenocytes. Results are expressed as $\bar{X}\pm S_{\bar{x}}$ from 3 parallel tests.

splenocytes under the chosen conditions (28.4±0.6 and 34.9±5.8% viability for control and PMA-treated cells, respectively) and is the best stimulator of UCT activity in PMA-treated rat splenocytes (Fig. 1, *a*). The number of effector cells in the effector:target ratio in Fig. 1, *a*, reflects the total cell count but not their viability. Fig. 1, *b*, shows that the high total (for the culture) cytotoxicity of cells from PMA-treated splenocyte culture containing staurosporine and IL-2 is mediated by 2-fold less live cells than in a culture of the same cells without these agents.

Since staurosporine and IL-2 exert additive effect on UCT activity of PMA-treated rat splenocytes toward K562 cells, it was interesting to analyze functional interactions between these agents during induction of cell proliferation in response to IL-2. This growth factor is a classical inductor of lymphokine-activated killer cells [8], whose appearance is associated with cell proliferation. Therefore, the enzyme (protein kinase) suppressing UCT-inducing (and probably growth) activity of IL-2 may be the intracellular target of staurosporine in PMA-treated splenocytes.

Figure 1, *c*, shows that staurosporine suppresses both spontaneous and IL-2-induced proliferation of rat splenocytes. Higher level of ^{3}H-thymidine incorporation by PMA-treated splenocytes in response to IL-2 in comparison with control samples may reflect a possible suppressive effect of protein kinase C on proliferating cells [6].

These results can be interpreted as follows. Treatment of cells with high doses of PMA (0.4-1.5 μM) for 8-48 h [6,10] exhausts the activity of protein kinase C in the cytoplasm by at least 80-85%. PMA under such conditions can suppress UCT activity of purified natural killer cells [10], and therefore the inhibitory effect of its UCT on rat splenocytes is directed toward cytotoxicity effector cells. Increased UCT activity of cells in response to IL-2 can be related to activation of protein kinase C [2], specifically, its **inactive** pool bound to the plasma membrane [7]. Increased UCT activity of PMA-treated splenocytes can be observed only if the above-mentioned part of molecules of protein kinase C family escapes complete exhaustion under the effect of PMA.

Since the increase in UCT of PMA-treated splenocytes in our experiments was observed only in response to the staurosporine-IL-2 combination, we supposed that the inhibitor is responsible for suppression of some negative signal in rat splenocytes. Natural killer cells incubated for several days with high doses of IL-2 express gp42 molecule on their surface. This molecule is homologous to human type I Fc-receptor (CD64) [11]. K562 cells are of human origin, and it is probable that gp42 as a receptor can react with a ligand on their surface. Rat natural killers possess C-type lectin receptors (Ly49 and NKR-P1) capable of reacting with class I main histocompatibility complex [4]. These receptors are expressed in K562 cells [9]. If such a reaction between rat splenocytes and K562 cells takes place, it can lead to an increase in UCT activity of splenocytes [4], negligibly expressed in control cells. Thus, staurosporine suppressing the activity of intracellular protein kinases of different types, including thyrosine protein kinases [12], can suppress signal activity of gp42 or type C lectin receptor at the expense of suppressing a negative signal in UCT effector cells or a probable increase of gp42 expression.

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