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ACTIVITY OF ENZYMES OF ADENOSINE METABOLISM IN HUMAN NATURAL KILLER (NK) CELLS DURING ACTIVATION AND INHIBITION OF THEIR CYTOTOXIC ACTIVITY

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Experiments in vivo and in vitro have shown that the principal modulators of the cytotoxic activity (CTA) of natural killer (NK) cells are interferon (IF) and prostaglandins of various types, mainly of type E [4, 7]. IF and certain interferonogens induce differentiation of NK cells and increase the rate of killing, so that the CTA of these cells is quickly and essentially increased [7, 12]. Prostaglandins of type E (PGE), which are locally active short-living hormones, exhibit different biological activity depending on their concentration [4, 6]. However, the concrete biochemical mechanisms determining CTA of NK cells have hardly been studied at all. It has been shown that an important role in the functioning and differentiation of immunocompetent cells is played by enzymes, namely adenosine deaminase (ADA; EC 3.5.4.4) and 5'-nucleotidase (5-N; EC 3.1.3.5), which regulate the intracellular adenosine concentration [11].

The aim of this investigation was to study activity of ADA and 5-N in human NK cells when treated in vitro with the IF inducer – Newcastle disease virus (NDV) or a preparation of PGE_2.

EXPERIMENTAL METHOD

Human NK cells were isolated from packed lymphocytes obtained on an "Amicon" blood separator (USA) at the bone marrow bank of the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, by the method described previously [2] in a Ficoll–Verografin density gradient, followed by purification and concentration of NK cells in a Percoll stepwise density gradient [3]. CTA of the NK cells was studied in the cytotoxic test (CTT) with target cells consisting of human lymphoma MOLT-4 cells labeled with ^{51}Cr [3]. The percentage lysis of MOLT-4 target cells was determined with ratios of effector to target cells of 50:1 and 25:1. The isolated NK cells were treated for 15 min with NDV at 20°C. After the end of treatment the NK cells were washed 3 times with medium RPMI-1640, containing 10% of bovine serum. A commercial preparation of PGE_2 (Sigma, USA) was diluted in medium RPMI-1640 and added (in a concentration of 10^{-5} to 10^{-12} M) to the test NK cells. After incubation for 30 min at 37°C the NK cells were sedimented by centrifugation and their CTA was investigated in the CTT. In parallel tests, some cells obtained after treatment with MDV and PGE_2 were lyzed in a solution containing 20 mM Tris-HCl, 5 mM $MgSO_4$, pH 7.4, and activity of ADA and 5-N was determined in the lysate with the aid of (8-^{14}C)-adenosine and (8-^{14}C)-adenosine monophosphate by ascending paper chromatography [1] in a micromodification. ADA activity was expressed in nanomoles of inosine and hypoxanthine per minute per 10^8 cells, and

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TABLE 1. Effect of NDV on CTA and Activity of ADA and 5-N in Human NK Cells (M ± m; n = 5)

Experimental conditions	CTA, %	Activation index	ADA	5-N
Control	33,6±4,6	—	137,4±9,2	38,8±3,7
NDV	68,3±5,6*	2,3	199,9±12,3*	17,2±1,4*

Legend. Here and in Table 2: *$p < 0.05$ compared with control; ratio of effector to target cells 25:1.

TABLE 2. Effect of PGE_2 on CTA and Activity of ADA and 5-N in Human NK Cells (M ± m; n = 5)

PGE_2 concentration, M	CTA, %	Inactivation index	ADA	5-N
Control	63,0±3,8	—	149,2±10,1	45,9±4,6
10^{-5}	24,5±4,6*	2,6	74,6±5,2*	134,4±10,6*
10^{-6}	22,8±3,5*	2,8	60,2±6,3*	171,6±11,9*
10^{-7}	29,6±4,0*	2,1	81,2±7,0*	127,5±9,9*
10^{-8}	36,9±3,4*	1,7	96,6±7,8*	102,0±8,4*
10^{-9}	52,9±4,7*	1,2	127,8±10,9*	67,1±6,3*
10^{-10}	60,9±3,5	1,0	147,0±12,5	44,2±3,9
10^{-11}	61,4±2,4	1,0	153,2±13,4	46,8±4,1
10^{-12}	66,8±3,6	1,0	157,1±11,2	42,1±3,7

Legend. Ratio of effector to target cells 50:1.

5-N activity in nanomoles of adenosine, inosine, and hypoxanthine per minute per 10^8 cells.

EXPERIMENTAL RESULTS

After treatment of human NK cells with NDV, a significant increase in CTA was observed compared with the initial level (Table 1). This was accompanied by an increase in ADA activity (by 1.5 times) and a decrease in 5-N activity (by 2.3 times) in these cells compared with the control, leading to a fall of the intracellular adenosine concentration.

Combined incubation of NK cells with the PGE_2 preparation in various concentrations gave dissimilar results (Table 2). Under the influence of high doses of PGE_2 (10^{-5}-10^{-7} M) a marked decrease in CTA of the NK cells was observed compared with cells not treated with the preparation. Meanwhile there was a sharp decrease in ADA activity and an increase in 5-N activity in the NK cells, creating the conditions for the intracellular adenosine concentration to rise (unlike in experiments with treatment with NDV). A further reduction of the PGE_2 concentration in the incubation medium led to a gradual weakening of the inhibitory effect of the preparation on CTA of the NK cells. Normalization of this parameter of functional activity of NK cells was observed when PGE_2 was used in a concentration of 10^{-10}-10^{-12} M. Parallel with the changes in CTA, normal activity of the enzymes of adenosine metabolism was restored in the human NK cells.

The results are evidence that under the influence of modifiers of the CTA of natural killer cells (NDV and PGE_2) changes in activity of enzymes of adenosine metabolism take place in these cells. An increase in the CTA of human NK cells under these circumstances was accompanied by changes in ADA and 5-N activity which led to a decrease in the intracellular adenosine concentration, and vice versa. We know that NDV and other inducers of IF facilitate differentiation of inactive precursors into mature cytotoxic NK cells and thereby increase the number of effector cells forming conjugates with target cells [7, 12]. On the other hand, the addition of ADA inhibitors or adenosine to the medium led to inhibition of

differentiation of NK cells in vitro [9, 13]. The increase in ADA activity which we found in NK cells under the influence of NDV is in agreement with data in the literature. The mechanism of the inhibitory action of PGE_2 on CTA of NK cells is considered to be connected with activation of adenylate kinase and a subsequent increase in intracellular cyclic adenosine monophosphate synthesis [6, 10]. It has also been shown that adenylate kinase is closely bound in the lymphocyte membrane with 5-N, which is involved in adenosine transport inside the cell [5, 8]. It can therefore be postulated that PGE_2 has an activating effect on adenylate cyclase through 5-N and inhibits the CTA of NK cells by raising the intracellular adenosine concentration.

One of the mechanisms of the change in functional activity of NK cells under the influence of various exogenous and endogenous factors may therefore be modification of the activity of enzymes of adenosine metabolism in these cells.

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