REGULATION OF NATURAL KILLER ACTIVITY OF LYMPHOCYTES FROM NORMAL SUBJECTS AND PATIENTS WITH CHRONIC LYMPHATIC LEUKEMIA BY INTERACTION BETWEEN T AND NON-T CELLS

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There is increasing evidence of the essential role of lymphocytes with natural killer activity in the function of immunologic surveillance [6]. For instance, the role of natural killer (NK) cells in the mechanisms of antitumor and antileukemic resistance has been demonstrated [8, 13, 14], including in chronic lymphatic leukemia [11, 15]. On the basis of the results of investigations into the properties of human circulating lymphocytes possessing NK function it has been suggested that this function is linked with certain stages of lymphocyte histogenesis. This hypothesis can be tested by estimating NK activity of human lymphocytes before and after experimental induction of cell differentiation.

Previous investigations showed that short-term incubation of human lymphocytes in culture in vitro is accompanied by changes in the properties of the cells as a result of their maturation. Meanwhile disturbance of the relative numbers of T lymphocytes at different levels of maturity in patients with the B-cell variant of chronic lymphatic leukemia (B-CLL) determines the limited ability of the total T-cell population of these patients to change their own functional properties after preincubation [4].

The aim of this investigation was to study the effect of culture of human cells on functional activity of NK cells and so to investigate the possible mechanisms of regulation of NK activity by acting on cytodifferentiation of lymphocytes in normal subjects and in patients with B-CLL.

EXPERIMENTAL METHOD

NK activity was determined in populations of mononuclear cells (MNC) and of T and non-T lymphocytes isolated from them. MNC were obtained by centrifugation of venous blood obtained from healthy blood donors or from patients with B-CLL in a Ficoll-Verografin density gradient, and T and non-T cells were obtained by sedimentation of the fraction of E-rosette-forming cells (E-RFC) from the MNC suspension. To remove the monocytes, adherent cells were adsorbed in separate experiments on plastic Petri dishes (from the Leningrad Medical Polymers Factory). The cells were preincubated for 24 or 48 h under standard conditions [3] in culture medium containing 80% of medium RPMI-1640 (Flow Laboratories, England), with the addition of L-glutamine (0.3 mg/ml) and 10 mM HEPES buffer (Microbiology Associates, USA), 20% inactivated human group IV (AB) blood serum, and antibiotics. To estimate NK function a membranotoxic test was carried out, using cells of the transplantable line K-562, labeled with ^{3}H-uridine, as the targets [2]. The membranotoxic test was carried out in round-bottomed wells in plates for immunologic investigations (from the Leningrad Medical Polymers Factory), into which 0.15 ml of a cell suspension containing 10^4 target cells (TC) and $4 \cdot 10^5$ lymphocytes (effector cells – EC) in the above-mentioned culture medium was poured. Pancreatic ribonuclease was added to each well in a concentration of 5 μg/ml. After aggitation on a shaker for 1 min to mix the cells the plates were incubated for 18 h at 37°C in a moist atmosphere of air + 5% CO_2. The cytotoxicity index (CTI) was calculated by the equation: $CTI = \frac{C - E}{E} \times 100\%$, where C denotes the level of radioactivity (in cpm) of the nucleoprotein fractions of the

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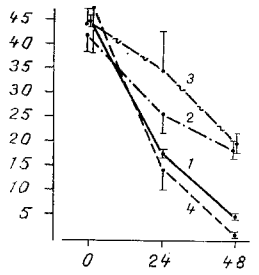

Fig. 1

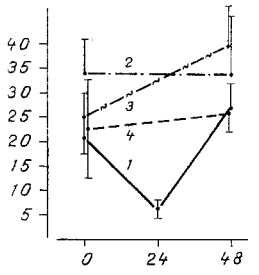

Fig. 2

Fig. 1. Changes in NK activity of healthy human lymphocytes of different types during preincubation. Abscissa, duration of preincubation (in h); ordinate, NK activity, in percent. Unseparated TC (1), T cells (2) and non-T cells (3) isolated from them, or T and non-T cells first separated and then mixed in their original proportions (to correspond to the calculated percentage of E-RFC among the TC) (4), incubated in vitro for 24 or 48 h.

Fig. 2. Changes in NK activity of different types of lymphocytes from patients with B-CLL during preincubation. Legend as in Fig. 1.

TABLE 1. Effect of Monocytes (cells adherent to plastic) on NK Activity of TC from Normal Subjects and Patients with B-CLL (M ± m)

Type of cell	CTI of cells from	
	normal subjects	patients with B-CLL
TC	42,4±10,7	28,5±11,7
TC after removal of adherent cells	41,4±13,5	21,0±6,5

TABLE 2. NK Activity of TC and T and non-T Cells from Normal Subjects and Patients with B-CLL (M ± m)

Type of cell	CTI of cells from		p
	normal subjects	patients with B-CLL	
TC	45,8±0,8	21,5±2,4	<0,01
T	37,6±1,1	27,9±2,0	<0,05
Non-T	42,6±1,2	29,1±1,5	<0,05

Legend. P) Significance of differences according to Wilcoxon–Mann–Whitney U test.

control cells (wells containing TC) and E denotes the same in the experiment (wells containing TC and EC).

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that preincubation of healthy human peripheral blood TC is accompanied by reduction of NK activity by more than half after 24 h and by its almost total disappearance by the end of the second day of culture. However, during similar incubation of fractionated T and non-T cells the change in this parameter was much less marked. CTI of fractionated cells after culture of T or non-T cells for 2 days exceeded its value for TC preincubated for 24 h. A mixture of previously separated healthy human T and non-T cells, recombined in their initial proportions (i.e., to correspond to the percentages of the cells among TC calculated previously) during culture lost its NK function by the same degree as intact TC. These results are evidence that incubation of human peripheral blood

lymphocytes in vitro is in fact accompanied by loss of NK activity; this effect is achieved, moreover, through interaction between T and non-T cells.

Changes in the functions of TC after preincubation may take place because of a decrease in the number of monocytes following adhesion of these cells to the glass during preliminary culture in flasks. However, as the data in Table 1 show, elimination of the monocytes was not accompanied by any decrease or increase in the membranotoxic action of TC from normal subjects or patients with B-CLL. It also follows from Table 1 that the NK activity of cells from patients with B-CLL was significantly weaker than the killer effect found in normal human TC. The differences were not due to the well-known gross changes in the ratio between T and B lymphocytes in B-CLL, for isolated T and non-T cells from these patients (Table 2) had CTI which was about equally weaker than the CTI of similar types of healthy human lymphocytes. Incidentally, the NK activity of TC and also of the T and non-T cells of patients with B-CLL was found to be resistant to preincubation (Fig. 2). Culture of autologous T and non-T cells from patients with B-CLL, first separated and then mixed (in the ratio of 7:3, i.e., corresponding to that found among TC in normal subjects) likewise was not accompanied by loss of NK activity. When T and non-T lymphocytes from normal subjects were cultured in similar proportions, as already mentioned, their NK properties were completely lost after 2 days of incubation (Fig. 1).

Analysis of data in the literature and also of previous results suggests that the particular features of the cells from patients with B-CLL thus revealed may be due to ineffective interaction between T and non-T cells during preincubation. It must be stated in this connection that in most experiments described in this paper NK activity was determined in a TC population containing an excess of T lymphocytes. Moreover, removal of monocytes present among the non-T cells was not accompanied by any change in NK activity. It therefore seems most probable that loss of NK activity in recombined mixtures of lymphocytes from normal subjects during preincubation takes place in T cells as a result of interaction with B lymphocytes. This kind of interaction is known to lead to activation of some T cells, namely by young post-thymic lymphocytes, which form rosettes with autoerythrocytes (auto-RFC). Activation is accompanied by proliferation and differentiation of these T cells [1, 5, 7, 10]. It is an interesting fact that a high proportion of T lymphocytes with NK properties is evidently concentrated among the auto-RFC [9]. It can accordingly be postulated that interaction between young T cells, with NK activity, and autologous B lymphocytes leads to activation and differentiation of T lymphocytes, which is accompanied by loss of the killer function. In patients with B-CLL the ability of the T cells, including auto-RFC, to recognize B cells and to interact with them is significantly depressed [1, 3, 12]. As a reuslt the T cells in the cultures are not activated. It is possible that the defective character of the T or B cells of patients with B-CLL, preventing effective self-recognition, may predetermine the distinguishing features of behavior of lymphocytes with NK properties in cultures in vivo.

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