CHANGES IN ACTIVITY OF NATURAL KILLER CELLS FROM NORMAL SUBJECTS AND PATIENTS WITH VIRUS DISEASES UNDER THE INFLUENCE OF INTERFERON *IN VITRO*

R. V. Petrov,* M. Z. Saidov, L. V. Koval'chuk, A. M. Sorokin, and B. S. Kaganov

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Natural killer (NK) cells are nowadays ascribed an important role in antitumor defense of the organism [8], in elimination of virus-infected cells [3]. and control of proliferation and differentiation of somatic cells [7]. NK cells *in vitro* produced spontaneous lysis of tumor cells of various lines without preliminary sensitization or participation by antibodies and complement.

In view of the diversity of functions performed by NK cells *in vivo*, there is no doubt about the importance of their study in clinical practice. Most investigations in this direction have been devoted to the role of NK cells in neoplastic diseases. Depression of their functional activity in cancer is a well-documented fact [9]. Recently NK cells have been found to participate in the pathogenesis of certain diseases of virus etiology [10, 11]. A leading role in the regulation of NK cell activity *in vivo* is ascribed to interferon (IF) of both exogenous and endogenous origin [14].

The aim of this investigation was to study activity of NK cells from normal subjects and patients with disseminated sclerosis and subacute and chronic hepatitis B, i.e., diseases of virus etiology, and the possibility of correcting it by IF.

EXPERIMENTAL METHOD

Activity of NK cells was studied in a suspension of mononuclear cells obtained from peripheral blood by centrifugation on a Ficoll–Verografin gradient [4]. To assess the effect of cytotoxicity, the [^{3}H]uridine method was used [1]. A standard line K-562 of myeloblasts was used as target cells (TC). The cells were cultured in medium RPMI-1640, to which embryonic calf serum (10%), glutamine, and antibiotics were added. TC were labeled with [^{3}H]uridine (3 μCi/ml) for 1 h at 37°C and washed three times with medium 199. The viability of the cells after all manipulations was not less than 95%. Peripheral blood mononuclear cells (TC) were introduced in proportions of 100:1 to 6:1 into wells of round-bottomed microplates and incubated for 14 h at 37°C in the presence of 5% CO_2. Before the beginning of incubation pancreatic RNase was added to the suspension in a dose of 5 μg/ml. At the end of incubation the contents of the wells were deposited on filters with pore diameter 2.5 μm. The level of radioactivity of the control (labeled TC without effector cells — EC) and experimental samples (labeled TC with EC) was determined in toluene scintillator on an Intertechnique counter. The results of the reaction was evaluated as the cytotoxic index (CI):

$$CI = \left(1 - \frac{\text{Number of counts in experimental cells}}{\text{Number of counts in control cells}}\right) \times 100\%.$$

Human leukocytic IF for injection, obtained at the N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, with specific activity of $3 \cdot 10^6$ IU/ml, was used. The dose of IF was worked out beforehand on healthy blood donors [2]. The optimal concentration was 250 IU/ml. The mononuclear cells were incubated

*Academician of the Academy of Medical Sciences of the USSR.

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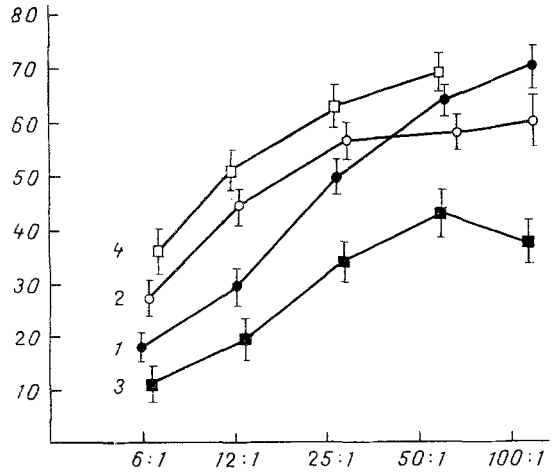

Fig. 1

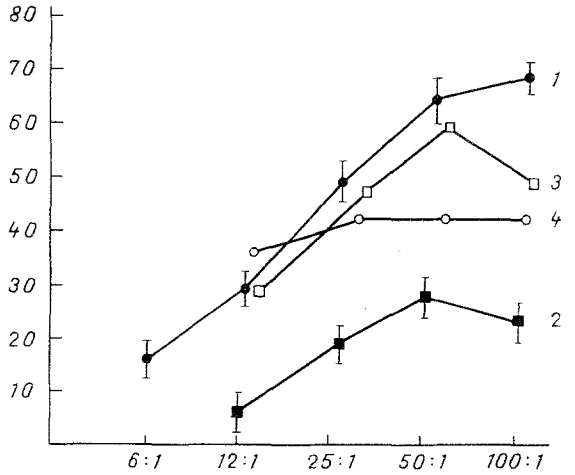

Fig. 2

Fig. 1. Dynamics of NK cell activity in healthy subjects and patients with chronic hepatitis before and after treatment with interferon in a dose of 250 IU/ml. 1) Control group of healthy donors; 2) after treatment of lymphocytes from healthy blood donors with IF in a dose of 250 IU/ml; 3) patients with chronic hepatitis; 4) after treatment of lymphocytes from patients with chornic hepatitis by IF in a dose of 250 IU/ml. Here and in Fig. 2, abscissa, ratio EC:TC; ordinate, CI (in %). Mean values of CI with confidence interval shown.

Fig. 2. Correction of activity of NK cells from patients G. and Sh., with disseminated sclerosis in the remission stage, by IF in a dose of 250 IU/ml. 1) Control group of healthy donors; 2) patients with disseminated sclerosis, remission stage; 3) activity of NK cells from patient Sh. after treatment with IF in a dose of 250 IU/ml; 4) activity of NK cells from patient G. after treatment of lymphocytes with IF in a dose of 250 IU/ml.

with IF for 2 h at 37°C, and then washed three times with buffered physiological saline. In parallel experiments lymphocytes from the same patient were incubated in complete nutrient medium without IF. Incubation with IF did not affect the viability of the lymphocytes.

EXPERIMENTAL RESULTS

To estimate activity of the NK cells the principle of serial dilutions was used as being more informative than the optimal ratio EC:TC. Activity of NK cells was determined in 35 healthy subjects aged from 20 to 40 years (control group), in 8 patients with subacute and chronic virus hepatitis B, and in 10 patients with disseminated sclerosis in the remission stage.

Dependence of CI on the ratio EC:TC in the control group was found to be directly proportional (Fig. 1). The CI curve flattens out on a plateau at ratios of 50:1 and 100:1. IF stimulated activity of NK cells in proportions of 25:1, 12:1, and 6:1 ($P < 0.05$). In proportions of 100:1 and 50:1 there was a tendency for activity to be inhibited. The action of IF on NK cells *in vitro* is thus definitely immunomodulating in character. Stimulation of NK cell activity through the action of various types of IF *in vitro* has been described in the literature [14]. The mechanism of stimulation involves interaction of IF with a receptor on the surface of the NK cells followed by activation of the cyclic nucleotide system and of killer cell function [13].

The results of investigation of activity of NK cells from patients with subacute and chronic hepatitis B are given in Fig. 1. They show that this activity was lower than in the control at all values of the EC:TC ratio. Treatment of lymphocytes of five of these patients with IF in a dose of 250 IU/ml significantly increased the value of CI, and approximated the curve of cytotoxic activity to normal.

Investigations of activity of NK cells from patients with chronic hepatitis B in different laboratories have yielded contradictory results [5, 6, 15]. In the modern view, protraction of the infectious process or its conversion to the chronic type in virus hepatitis B is associated with weakening of the cellular immune response [10]. It can be tentatively suggested that the reduced activity of NK cells recorded in the present investigation, and also

elsewhere [12, 15] is the cause of the insufficiently complete elimination of infected hepatocytes and persistance of hepatitis B virus.

The virus theory of the pathogenesis of disseminated sclerosis has now assumed great prominance and special attention is attached to persistent virus infection. Information in the literature obtained by the study of NK cells in disseminated sclerosis is highly contradictory [11]. The present writers investigated 10 patients with disseminated sclerosis in the remission stage. The results showed that these patients have low levels of NK cell activity. The results of investigation of the patients of this group are given in Fig. 2. CI was sharply reduced at all values of the EC:TC ratio compared with the control ($P < 0.01$). Activity of NK cells was corrected by IF in a dose of 250 IU/ml in two patients. Treatment with IF considerably stimulated the NK cells and CI values became close to control level.

The results indicate a corrective action of IF *in vitro* on NK cells from patients with persistent virus infection, suggesting that controlled administration of IF may have a place in the combined treatment of this pathology.

The effect of IF *in vivo* on NK cell activity has been investigated in several diseases and, in particular, in patients with chronic active hepatitis [12]. Elevation of the initially depressed activity of NK cells against the background of IF treatment correlated with stimulation of these cells by IF *in vitro*. After a 4-week course of treatment, the level of cytotoxicity returned to its initial value. The authors cited explain this fact by exhaustion of the pool of pre-NK cells, which are functionally inactive before exposure to IF and which acquire the properties of mature cells after exposure.

The results described above are evidence of an immunomodulating action of IF on activity of NK cells, but further research is needed with comparison of results obtained *in vitro* and *in vivo* in patients with virus diseases.

The method of evaluating activity of NK cells used in the present investigation can be used for preliminary determination of sensitivity of NK cells to IF in clinical practice.

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