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Variability of Natural Killer Activity during Immunotherapy

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In patients with multiple sclerosis, chronic active hepatitis B, and relapsing genital herpes integral parameters of variability in natural killer activity were higher than in donors. In the dynamics of immunotherapy with IFN preparations (chronic active hepatitis B) and IFN inductor ridostine (relapsing genital herpes) these parameters decreased and approached the normal values. The possibility of using variability of natural killer activity for evaluation of cell function and prediction of the efficiency of immunotherapy is discussed.

Key Words: *natural killers; activity; variability*

High variability of natural killer (NK) activity is a characteristic features of human natural cytotoxicity (NCT) system. This variability is determined at the cellular, sexual, age, and population levels [4,5,7,14]. Reflecting the reaction norm of the system, this variability is a manifestation of mechanisms maintaining high plasticity of NK population related to phylogenetic polymorphism of receptor structures in these cells [2,9,15], differentiation relationships between NCT effectors and T lymphocytes [13], numerous functions of NK, and pleiotropism of NK cytokines [3,11,12].

Our previous findings suggest that as NK population evolutionally developed as a high plasticity system possessing compensatory mechanisms realized through modulation of the reaction norm [5,7]. Increase in variability (compared to the control level) can reflect a transitional state of the population under conditions of variable effector microenvironment. This peculiarity of NK activity was observed during physiological aging [7], in chronic experiments on laboratory animals [4], and in the presence of shifts in the population level of NK cytotoxicity in healthy population of the Earth [7]: NCT effector activity changed through the stage of markedly increased variability of parameters and then (after attaining certain functioning parameters) variability of NK cytotoxicity markedly decreased [4,5,7].

Variability was not evaluated in some our previous experiments and observations, such as repeated measurement of NK activity in donors and patients with multiple sclerosis [10], evaluation of the efficiency of immunotherapy with IFN preparations in patients with chronic active hepatitis B [1] and in patients with relapsing genital herpes treated with IFN inductor ridostine [6].

Here we verified our hypothesis on the significance of NK activity variability for evaluation of the NCT system.

MATERIALS AND METHODS

Donors (3 men and 2 women) aged 18-42 years and patients with multiple sclerosis (remitting course, cerebrospinal form, disease duration 0.6-12 years, EDSS disability degree IV-V; 4 men and 4 women, 19-40 years) were examined twice with one-week interval [10]. Patients with hepatitis B or cirrhosis of the liver (n=6) with proven chronic infection with hepatitis B

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virus (5 men and 1 woman; 17-32 years) with medium or high activity of the process were examined repeatedly during combined immunotherapy with human leukocytic IFN for injections and leukinferone: before treatment and 1 and 4 months after therapeutic course [1]. Patients with severe relapsing genital herpes (8 men and 6 women; 20-42 years, relapses at least once a month lasting for an average of 10 days) were examined during relapses (5 patients) or remissions (9 patients) during the course of immunotherapy with IFN inductor ridostine: before therapy, after the 2nd injection of the drug, immediately and 1 week after a 10-day therapeutic course [6].

The cytotoxic activity of NK in suspension of peripheral blood mononuclear cells was evaluated by the radiometric method against ^{3}H-uridine-labeled standard human erythromyeloblasts K-562 using effector:target (E:T) ratios from 100:1 to 6:1 (from 100:1 to 12:1 in patients with genital herpes). The cytotoxic index was calculated. The area under the cytotoxicity curve was taken as the integral parameter of NCT and expressed in arbitrary units. Variability index (I_{var}) was estimated as the ratio of the maximum deviation to the mean integral NCT value or cytotoxic index obtained under conditions of a certain E:T ratio.

RESULTS

Estimation of the integral NCT parameter and variability of NK activity showed that NCT level in healthy individuals remained virtually unchanged during a week (shifts within 5%), while I_{var} varied from 0.07 to 0.33, *i.e.* 5-fold (mean value 0.2).

In patients with multiple sclerosis more pronounced (up to 10%) shifts in NCT coincided in time with less expressed changes in variability. I_{var} changed by only 20%, the mean level of the parameter was 0.43. NCT of lymphocytes from patients decreased by 1 arb. unit, while I_{var} more than 2-fold surpassed the corresponding parameter in donors (Fig. 1).

Hepatitis B and genital herpes were associated with decreased lymphocyte NCT in comparison with normal NK activity (by 2.4 and 3.1 arb. units, respectively, Fig. 1) and increased I_{var} (3- and almost 5-fold, respectively, Fig. 1).

Hence, the studied diseases pathogenetically related to NK insufficiency [1,6,10] are characterized by a tendency to an increase in NK activity variability against the background of decreased cytotoxicity. More pronounced extension of the reaction norm for NCT system was associated with lower NCT in lymphocyte suspensions, *i. e.* more pronounced immunodeficiency (Fig. 1).

We previously detected immunocorrective effect and therapeutic efficiency of IFN preparations in the

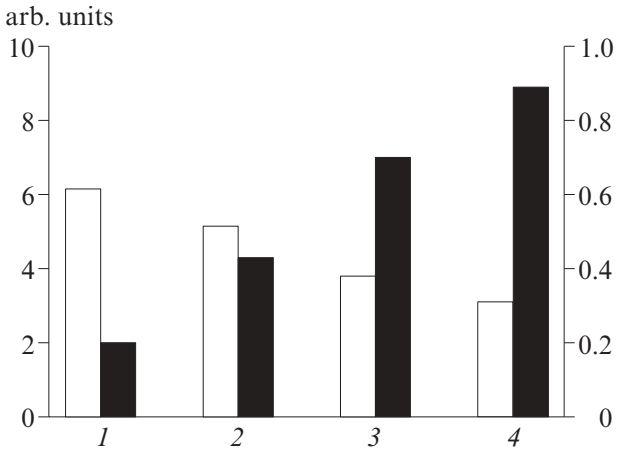

Fig. 1. Cytotoxic activity of natural killers (NK; light bars) and its variability (dark bars) in donors (*1*) and patients with multiple sclerosis (*2*), chronic active hepatitis B (*3*), and relapsing genital herpes (*4*) *in vitro*. Here and in Figs. 2 and 3: left scale: integral parameters of natural cytotoxicity; right scale: variability index.

treatment of chronic active hepatitis B [1]. Treatment with human leukocytic IFN and leukinferone objectively improved patient status and accelerated the dynamics of their clinical laboratory parameters. Seroconversion with disappearance of one or two markers of virus replication was observed in 8 of 14 patients treated with complex preparations (IFN-α and cytokines) [1].

After 4-month therapy, lymphocyte NCT increased by 55% (Fig. 2), which was paralleled by a decrease in NK activity I_{var}. It should be noted that this shift of the reaction norm for this system was observed during therapy with IFN preparations (Fig. 2), while variability level and NCT returned to normal.

Clinical efficiency of ridostine in immunocorrective therapy of relapsing genital herpes manifested in the absence of relapses during the subsequent months

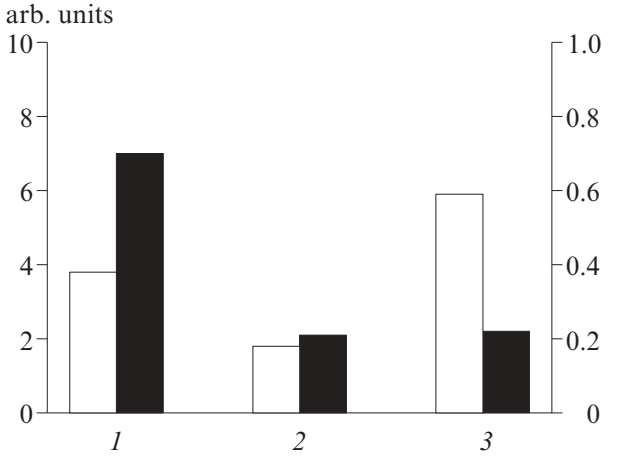

Fig. 2. Cytotoxic activity of NK (light bars) and its variability (dark bars) in patients with chronic active hepatitis B, evaluated *in vitro* before (*1*), during (*2*), and after (*3*) therapy with IFN preparations.

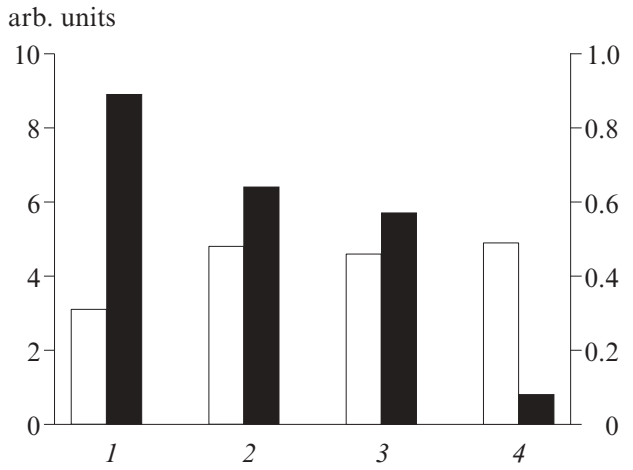

Fig. 3. Cytotoxic activity of NK (light bars) and its variability (dark bars) in patients with relapsing genital herpes, evaluated *in vitro* before (*1*), during (*2*), immediately (*3*) and 1 week after (*4*) therapy with IFN preparations.

of follow-up. The effect of the drug was evaluated as transformation of the process into a long remission and in some patients even convalescence [6]. A rapid (on days 2-5 after the first injection) regression of herpetic eruption was observed in patients in whom the treatment was started during a relapse [6].

During 10-day therapeutic course lymphocyte NCT increased by 58%, reached 4.9 arb. units, and approached the normal values (Fig. 3). These changes manifested as early as after the 2nd injection of ridostine. In parallel, I_{var} of NK activity gradually decreased (Fig. 3). One week after the end of treatment it returned to a level characteristic of normal lymphocyte population.

The detected integral changes in NCT variability were paralleled by shifts depending on the E:T ratio in the experimental system. In donors I_{var} was equal to 0.18-0.61 under conditions of the E:T ratios used in experiments, while in patients with multiple sclerosis I_{var} increased to 0.46-0.93, in hepatitis B to 0.59-1.00, and in genital herpes to 0.85-1.05.

During immunotherapy I_{var} returned to a variability range typical of normal lymphocytes. After the end of therapy I_{var} of cytotoxicity was 0.10-0.45 in patients with hepatitis B and 0.17-0.49 in patients with genital herpes (with cell concentrations used in the study). The profiles of variability curves approximated those characterizing NK function in donors.

It is known that the profile of cytotoxicity curve plotted on the basis of serial cell dilutions can be reflected to a certain measure by the ratio of the highest and lowest cytotoxic indexes. The profile of variability curve can be similarly characterized by the ratio of the highest and lowest I_{var}, calculated for the studied range of cell concentrations.

This I_{var} ratio varied in the dynamics of the pathological process and immunotherapy. However, changes in this parameter are reciprocal to shifts of the integral I_{var}. The parameter in all patients examined before therapy was lower than in donors. The higher I_{var} calculated on the basis of the integral NCT index (Fig. 1), the more pronounced was the decrease of the ratio for the studied range of cell concentrations. NK activity variability values, estimated with consideration for E:T ratios were as follows: in donors the ratio of maximum to minimum I_{var} was 3.39; in patients with multiple sclerosis 2.02; in patients with hepatitis B 1.69 before treatment, 1.51 during, and 4.50 after treatment; in patients with genital herpes 1.24 before treatment, 1.48 during, 2.44 immediately after, and 2.88 one week after the treatment (for donors and patients with multiple sclerosis mean values of measurements carried out at one-week interval are presented).

During immunotherapy I_{var} values calculated from the integral NCT index decreased to a level characterizing function of NK population in donors (Figs. 2, 3), while in patients with hepatitis B the I_{var} ratio increased and surpassed that in donors; in patients with genital herpes this index was just 15% lower than in donors.

Hence, the variability of NK activity appreciably varied under conditions of pathological processes associated with insufficiency of the NCT system. It decreased at the level of individual cells, which can indicate a deficiency in the mechanisms of autocrine regulation of a solitary killer, and increased at the level of NK population in general, which seems to reflect the involvement of the compensatory mechanisms realized in the complex of cell-cell interactions.

Normalization of the NCT system reaction during treatment permits us explain the initially high integral I_{var} values by the efficiency of subsequent immunotherapy in the patients in whom this correction was pathogenetically justified.

Our findings do not yet allow us to recommend using the index of NK activity variability for the diagnosis and immunocorrection of conditions associated with NCT system insufficiency, as the algorithm of its direct use is not completely formed. We observed no patients in whom immunotherapy was ineffective and its results could be predicted from the initial variability status. Moreover, we studied the group variability. Individualization of diagnostic and prognostic approaches would require prolonged observations of patients, starting before immunocorrective therapy.

However, variability of NK activity reflecting the degree of NCT system plasticity can serve as a highly sensitive characteristic of this lymphocyte population. It depends on mechanisms of autocrine and paracrine regulatory mechanisms, indicates with a sufficient de-

gree of probability the status of the compensatory potential of the system, and can prompt which mechanisms (extra- or intra-system) should (or can) be triggered in order to repair NK functions in a certain disease.

REFERENCES

1. P. E. Krel', V. P. Kuznetsov, D. L. Belyaev, *et al.*, *Ros. Zh. Gastroenterol., Gepatol.*, No. 2, 72-78 (1993).
2. L. N. Fontalin, *Immunologiya*, No. 5, 33-44 (1998).
3. L. N. Fontalin, *Ibid.*, No. 6, 4-11 (1999).
4. S. B. Cheknev, *Vestn. Rossiisk. Akad. Med. Nauk*, No. 2, 10-15 (1999).
5. S. B. Cheknev and A. Ya. Kul'berg, *Immunologiya*, No. 2, 9-12 (1995).
6. S. B. Cheknev, O. I. Mikovskaya, E. N. Meshkova, *et al.*, *Vopr. Virusol.*, **39**, No. 3, 125-128 (1994).
7. S. B. Cheknev, M. Z. Saidov, V. V. Tsvetkov, *et al.*, *Immunologiya*, No. 1, 39-43 (1991).
8. S. B. Cheknev, A. M. Sorokin, and L. V. Koval'chuk, *Ibid.*, No. 3, 48-52 (1989).
9. R. Barten, M. Torkar, A. Haude, *et al.*, *Trends Immunol.*, **22**, No. 1, 52-57 (2001).
10. S. B. Cheknev, Y. G. Ashmanova, A. D. Pritsker, *et al.*, *Mediators of Inflammation*, **3**, No. 5, 341-346 (1994).
11. H. Reyburn, O. Mandelboim, M. Vales-Gomez, *et al.*, *Immunol. Rev.*, **155**, 119-125 (1997).
12. J. C. Ryan and W. E. Seaman, *Ibid.*, 79-89.
13. N. M. Valiante and P. Parham, *Molecular Basis of NK Cell Recognition and Function*, Ed. L. Moretta, *Chemical Immunology*, New York, Basel; Karger (1996), Vol. 64, pp. 146-163.
14. T. L. Whiteside and R. B. Herberman, *Clin. Immunol. Immunopathol.*, **53**, 1-23 (1989).
15. W. M. Yokoyama, A. Reichlin, D. H. Margulies, and H. R. C. Smith, *Molecular Basis of NK Cell Recognition and Function*, Ed. L. Moretta, *Chemical Immunology*, New York, Basel; Karger (1996), Vol. 64, pp. 1-18.