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Circulating Serum Interferon and Its Effect on the Activity of Human Natural Killer Cells

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The interferon content in the plasma of 6 healthy donors and 10 patients with multiple sclerosis and the effect of an 1-h treatment of mononuclear cells with autologous plasma on their natural killer activity are studied *in vitro* using ^{3}H-uridine-labeled (3 μCi/ml) human erythromyeloleukosis cells K-562. The serum interferon content in healthy donors is 2.3±0.82 IU/ml, whereas that in patients is higher: 5.2±0.8 IU/ml. Autologous plasma does not affect the activity of natural killer cells *in vitro*, whereas it increases the cytotoxicity of mononuclear cells obtained from patients with multiple sclerosis by 35-64%.

Key Words: *circulating interferon; natural killer activity*

The key role of interferon (IFN) in the mechanisms controlling cell proliferation and differentiation in the human and animal organism, including the maintenance of the dynamic regulatory balance in the natural cytotoxicity system, is well documented [15]. The physiological IFN response has been characterized as a local one, which is confined primarily to the lymphoid system and induced only in certain cells during a certain period [10,11]. Under physiological conditions IFN is not detected in blood plasma [10,11]. However, a certain level of the IFN-dependent enzymes (2'-5')oligo-A-synthetase and protein kinase in the mononuclear cells (MNC) and plasma of healthy donors [10] indicates that there is a permanent induction of IFN that sustains a constant background production in the cellular microenvironment, leading to the activation of IFN-dependent enzymes [10,11,15]. The baseline natural cytotoxicity is a possible manifestation (or consequence) of the physiological IFN response that is provided by a continuous differentiation of natural killer cells to active forms capable of binding and efficient lysis of the target cell [10,15,17].

At the same time, the development of a number of pathological processes is accompanied by an increase in the content of circulating IFN. This increase has been observed in autoimmune diseases [9,20], AIDS [19], myasthenia [18], severe cytomegalovirus infection [21], and diseases of the central nervous system [8]. The antigenic properties of circulating IFN in these diseases vary. While in patients with cytomegalovirus infection and multiple sclerosis the plasma titers of γ-IFN [21] and β-IFN [14], respectively, are increased, in a considerable number of autoimmune pathologies

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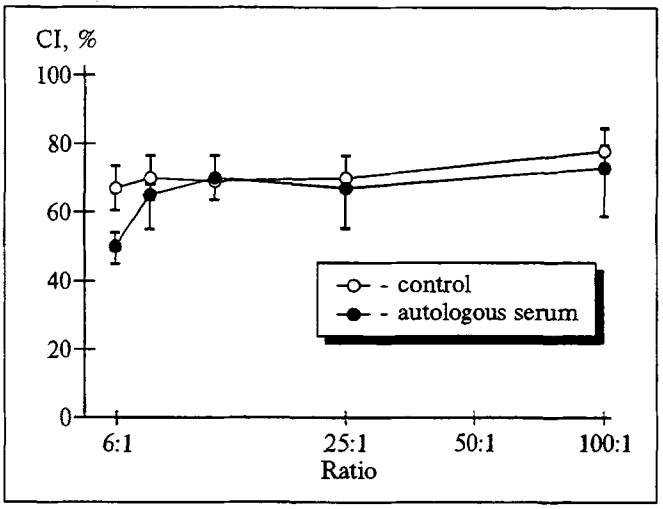

Fig. 1. Activity of healthy donors' NK in the presence of autologous serum *in vitro*.

(rheumatoid arthritis, lupus erythromatosus, scleroderma, Sjögren's syndrome) an unusual acid-labile α-IFN circulates in the plasma; the content of this IFN decreases during the asymptomatic stage of the disease [20]. Acid-labile α-IFN has also been detected in the serum of AIDS patients [19]. The properties of this "pathological" IFN have been intensively studied, but the question as to whether it induces abnormal differentiation of natural killer cells (NK) remains open [8].

Our objective was to determine the serum IFN content and to assess the effect of autologous serum on the cytotoxic activity of NK in healthy donors and patients with multiple sclerosis, which is characterized by the development of an IFN-dependent immunodeficiency of NK [5] and is associated with an increase in the serum IFN titer [14,16].

MATERIALS AND METHODS

MNC were isolated from peripheral venous blood of 6 healthy donors (3 men and 3 women aged 22-47 years and 10 patients with remitting multiple sclerosis and its cerebrospinal form (3 men and 7 women aged 24-31 years) on a one-step Ficoll-Paque (Pharmacia Fine Chemicals) gradient ($d = 1.077$ g/cm^3).

The cytotoxic activity of NK was determined using standard human erythromyeloblastoma cells K-562 labeled with ^{3}H-uridine (3 μCi/ml) [4]. Cells collected from the interphase ring were washed twice with double Eagle's medium (400 *g*, 10 min, 20°C). The initial suspensions contained 10^7 and 10^5 target cells/ml in complete growth medium based on RPMI-1640 (Amimed) supplemented with 12% fetal calf serum (N. F. Gamaleya Institute of Epidemiology and Microbiology, Russian Academy of Medical Sciences), 2 mM glutamine, and 40 μg/ml gentamicin (Pharmachim) on 1 M HEPES buffer (Serva). NK and target cells (0.1 ml of each suspension) were incubated in 96-well round-bottom microplates for 14 h at 37°C in a humidified atmosphere containing 5% CO_2. After the incubation the cells were transferred to Whatman fiberglass filters (2.5 μ pore diameter). The radioactivity of the filters was measured in a Mark-II scintillation β-counter. The cytotoxicity index (CI) for each of 2 or 3 parallel wells with effector:target ratios 100:1, 50:1, 25:1, 12:1, and 6:1 was calculated from the following formula:

$$CI = (1 - \frac{\text{cpm in experimental well}}{\text{cpm in control well}} \times 100\%).$$

For a study of the effect on autologous serum on the activity of NK, before the cytotoxicity test 0.1 ml of native autologous plasma was incubated with the MNC suspension (4×10^6 cells in 0.4 ml of complete growth medium) for 1 h at 37°C in a humidified atmosphere containing 5% CO_2. The cells were then washed and resuspended in 0.4 ml complete growth medium. Positive controls were set up.

The serum IFN content was determined by inhibition of the cytopathic activity of the vesicular stomatitis virus in a monolayer culture of human diploid fibroblasts M-19 infected with 100 CPD_{50} of the virus [2].

The results were analyzed using Student's *t* test.

RESULTS

The IFN content in the serum of healthy donors was 2.3±0.82 IU/ml, varying from 0 to 4 IU/ml. It can be seen from Fig. 1 that autologous serum did not change the cytotoxic activity of the healthy donors' NK *in vitro*. An insignificant 23% decrease in NK activity compared with the control occurred at the effector:target ratio of 6:1. In patients with multiple sclerosis the serum IFN content increased to 5.2±0.8 IU/ml ($p<0.1$), varying from 2 to 8 IU/ml. The activity of patients' NK treated with autologous serum increased *in vitro* at the effector : target ratios 12:1, 25:1, and 100:1 by 64, 35, and 59%, respectively (Fig. 2). The increase in NK activity was observed in 7 out of 10 patients, i.e., treatment with autologous serum had no effect in 3 cases. On the other hand, in healthy donors autologous antiserum activated NK only in 1 case out of 6, inhibition of cytotoxicity was detected in 2 cases, and in 3 cases the effect was not observed.

These data are consistent both with the concept of a low level of physiological production of

IFN and the IFN response, which cannot be detected by conventional biological methods [10,11], and with the results [12,13] indicating that there is a family of IFN inhibitors capable of blocking the effect of IFN in health [13], including the activation of MNC with properties of natural cytotoxicity effectors [12]. Moreover, inhibitors of IFN regulation of NK are probably absent in the blood of patients, as is evidenced by the phenomenon of an autologous increase in cytotoxicity in the presence of α- and γ-IFN [12]. No specific or nonspecific blockers of NK activity [7] were found in the plasma of patients with multiple sclerosis, implying that under the influence of excess IFN [14,16] the differentiation of NK is abnormally accelerated, which leads to the accumulation of functionally inactive NK in the peripheral blood [8]. This may account for the fact that the IFN-containing serum of patients with multiple sclerosis does not alter the cytotoxicity of healthy donors' NK *in vitro* [7]. However, in an autologous system stimulation is observed (Fig. 2), which probably is not realized in the organism, since in patients with multiple sclerosis the cytotoxic activity of effectors *in vitro,* depending on the effector : target ratio, is 14-50% lower than that in healthy donors (Figs. 1 and 2).

The presence in multiple sclerosis of IFN-dependent immunodeficiency associated, on the one hand, with lowered lymphocyte production of induced α- and γ-IFN and functional disorders in the adherent cells providing for α-IFN production by NK, which leads to insufficient production of α-IFN in the natural cytotoxicity reaction [5], and, on the other hand, with the absence of inhibitors of α- and γ-IFN regulation of NK [12] suggests an abnormal interaction between circulating serum IFN and peripheral blood MNC possessing NK activity. This is confirmed not only by the higher IFN titers in patients with multiple sclerosis compared with healthy subjects but also by changes in the spectrum of circulating IFN upon the "switch" to the production of β_2-IFN (interleukin-6) and γ-IFN [14,16]. However, in this case, taking into account the production of other cytokines (primarily interleukin-2) in the experimental system and, consequently, the accompanying cytokine activity of natural IFN preparations that modulate not only the function of MNC which elicit natural cytotoxicity, but also the migration of macrophages and polymorphonuclear lymphocytes, phagocytosis, rosette formation, and the expression of Ia-antigens [1], it is reasonable to assume that the increase in the natural cytotoxicity of MNC treated with IFN-containing autologous serum observed in patients with multiple sclerosis is a consequence of the action of cytokines functionally associated with the IFN system.

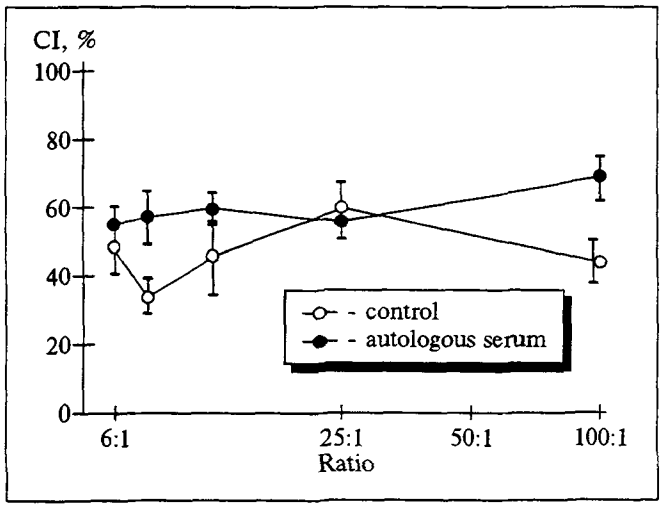

Fig. 2. Changes in the activity of NK of patients with multiple sclerosis under the influence of autologous serum *in vitro.*

An effect similar to that at the effector:target ratio of 100:1 (Fig. 2) was observed by Sorokin *et al.* [3] when the effect of the drug α-IFN (cytoferon) on NK activity was studied in healthy donors. This drug is a complex preparation containing the entire spectrum of cytokines produced in response to an adequate induction of IFN genesis [3]. These experiments showed that cytoferon applied in doses lower compared with all the other Russian-manufactured natural compounds regulates NK activity *in vitro* (either stimulating or inhibiting it) and maximally normalizes the cytotoxicity curve [3]. The effect of this drug was evaluated under the same experimental conditions as in our study. At an effector:target ratio of 12:1 cytoferon, in contrast to the IFN-containing autologous serum of sclerotic patients, reduced NK activity [3].

On the other hand, at low effector:target ratios autologous serum produces on NK of sclerotic patients an effect similar to that produced by human leukocyte IFN injected into MNC of healthy donors [6]. During purification this preparation loses some cytokines accompanying IFN [3]. In both cases an increase in NK activity was observed *in vitro* at the effector:target ratio of 12:1 [6]. At the same time, at the effector:target ratio 100:1 the activity of NK preincubated for 4 h with human leukocytic IFN was lowered [6].

Since the cytoferon dose (2 IU/ml) [3] was the same as the IFN titer in the plasma of patients with multiple sclerosis and the IFN content in the human leukocyte preparation (50 IU/ml) [6], evaluated by the biological activity of the factor, was only two logarithmic units higher than the

IFN titer in the patients' autologous serum, the comparable magnitudes of the effects elicited by the α-IFN preparations [3,6], and the extent of changes in the NK activity of patients with multiple sclerosis after treatment with IFN-containing autologous plasma, suggest that in multiple sclerosis serum factors other than IFN are involved in the stimulation of cytotoxicity at high effector : target ratios, whereas at low effector:target ratios the NK activity is regulated by IFN itself.

The NK population is known to be heterogenous with respect to the increase in cytotoxicity in response to IFN [15]. In light of the alterations in the *in vitro* sensitivity of NK of patients with multiple sclerosis to the serum factors whose contribution to negative regulation is greater than for the cells of healthy donors [7], which may be due to insufficiency of the IFN system [5], the absence of a regulatory effect of IFN on NK activity [12], and the appearance in patients' blood of β_2-IFN and γ-IFN [16], which are not detected in the blood of healthy donors [14], our results indicate that abnormal IFN-associated cytokines are induced and secreted in the plasma of patients with multiple sclerosis.

REFERENCES

1. V. P. Kuznetsov, *Immunologiya*, № 4, 30-34 (1987).
2. V. D. Solov'ev and T. A. Bektimirov, *Vestn. Akad. Med. Nauk SSSR*, № 2, 19-26 (1979).
3. A. M. Sorokin, S. B. Cheknev, and V. P. Kuznetsov, *Immunologiya*, № 1, 17-20 (1991).
4. M. P. Rykova, I. V. Spirande, M. S. Zedgenidze, *et al.*, *Ibid.*, № 3, 88-90 (1981).
5. S. B. Cheknev, *Ibid.*, № 6, 8-12 (1993).
6. S. B. Cheknev, M. Z. Saidov, L. V. Koval'chuk, *et al.*, *Lab. Delo.*, № 3, 231-234 (1987).
7. S. B. Cheknev, O. L. Latysheva, I. S. Frolova, *et al.*, in: *Interferon-89*, Moscow (1989), pp. 42-47.
8. S. B. Cheknev, A. M. Sorokin, and L. V. Koval'chuk, *Immunologiya*, № 3, 48-52 (1989).
9. L. Ya. Babynina, Z. V. Volobueva, and V. M. Ermekova, *Europ. Fed. Immunol. Soc. 10th Meet.*, Edinburgh (1990), p. 65.
10. V. Bocci, *Immunology Today*, **6**, № 1, 7-9 (1985).
11. V. Bocci, *Immunology*, **64**, 1-9 (1988).
12. S. B. Cheknev, in: *Advances in Modern Biotechnology*, Vol. 1, Havana (1992), p. 20.7.
13. W. R. Fleishmann Jr., J. A. Georgiades, L. C. Osborne, *et al.*, *Infect. Immunol.*, **26**, 949-955 (1979).
14. K. Frei, S. Fredrikson, A. Fontana, and H. Link, *J. Neuroimmunol.*, **31**, № 2, 147-153 (1991).
15. R. M. Friedman and S. N. Vogel, in: *Advances in Immunology*, Eds. F. J. Dixo and H. G. Kunkel, Vol. 34, New York (1983), pp. 97-140.
16. K. P. Hammann and H. C. Hopf, *J. Neuroimmunol*, **13**, № 1, 9-18 (1986).
17. R. B. Herberman and J. R. Ortaldo, *Science*, **214**, 24-30 (1981).
18. E. Kott, T. Hahn, M. Huberman, *et al.*, *Quart. J. Med.*, **76**, № 281, 951-960 (1990).
19. S. Krown, F. X. Real, S. Vadhan-Raj, and H. F. Oettgen, *J. Exp. Pathol.*, **3**, № 4, 681-691 (1987).
20. O. T. Preble, R. J. Black, R. M. Friedman, *et al.*, *Science*, **216**, 429-431 (1982).
21. A. Rhodes-Feuillette, M. Canivet, H. Champsaur, *et al.*, *J. Interferon Res.*, **3**, № 1, 45-52 (1983).