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Regulation of Human Natural Killer Activity with Autologous Interferon

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A study is performed of the effects of α-interferon and γ-interferon induced in 8 healthy donors and 9 patients with multiple sclerosis on the *in vitro* cytotoxic activity of natural killers in an autologous and allogeneic systems. The general characteristics of regulation are estimated on the basis of the results. There is found to be an inhibitor regulating the effect of interferon on natural killer activity, which is produced in parallel with interferon in response to interferon induction, the efficacy of this inhibitor being dependent on the initial natural killer activity; the inhibitor is absent in commercial interferon preparations.

Key Words: *natural killers; interferon; regulation*

One of the important properties of interferons (IFN) characterizing their immunoregulatory activity is their capacity to activate the natural killer (NK) population, the fundamental component of immunobiological control [3]. By initiating the differentiation of NK precursors, increasing the lytic potential of a single killer, and enhancing recycling of these cells [17], IFN are regarded as factors playing a crucial physiological role in NK functioning [2].

The broad spectrum of immunomodulating effects of IFN, along with their pronounced antiproliferative activity, underlies the therapeutic use of IFN preparations in oncology [13]. However, despite the undoubted clinical efficacy of IFN, in a number of cases their effect falls short of anticipation. IFN fails to repair *in vitro* the reduced NK activity in patients with idiopathic autoimmune hemolytic anemia [9]. In chronic granulocytic leukemia NK insufficiency is partially corrected by IFN *in vitro*, but in the course of IFN therapy NK activity is unchanged in the majority of patients [11]. IFN increases NK activity *in vitro* in myelodysplasia, but no correlation between NK activity and the clinical characteristics of the process is observed in the course of IFN therapy [10].

The above clinical and immunological parallels and the capacity of NK to produce all three types of IFN under conditions of appropriate induction [2] permit us to assume that the interaction between IFN and NK in certain pathological situations changes when the balance characterizing the functioning of the components of the system in health is disrupted. Despite numerous reports about the regulatory interactions between the NK population and IFN [2,3,17], this assumption has not been confirmed on a model of interaction of NK with autologous IFN (auto-IFN).

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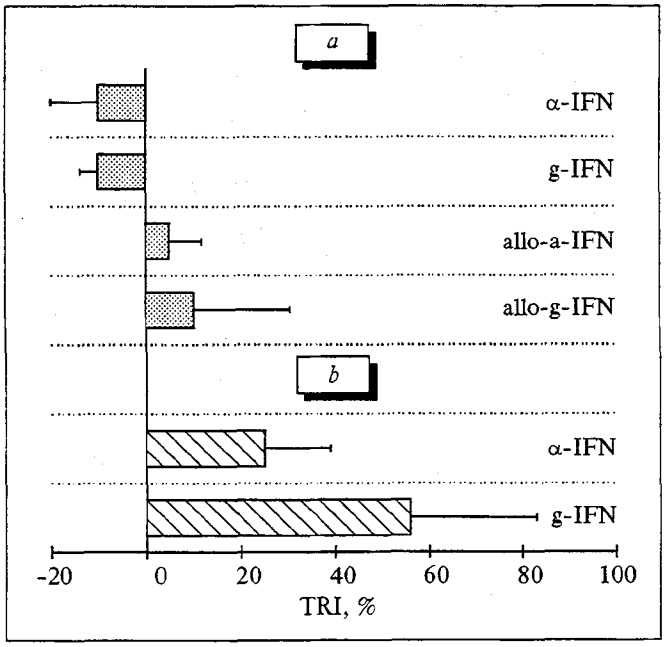

Fig. 1. Effects of auto- and allo-IFN on *in vitro* activity of human NK. *a*) healthy donors; *b*) patients with MS. The data are reliable ($p<0.05$) in comparison with the TRI of healthy donors. Number of cases shown in Table 3.

The aim of this study was to investigate the effects of α- and γ-IFN on the cytotoxic activity of NK *in vitro* in an autologous system in healthy donors and patients with multiple sclerosis (MS). The selection of MS as a model was dictated by the presence of an IFN-related deficit of NK [7] which is associated with a drastic change of cell sensitivity to *in vitro* regulation factors in this disease, as well as by the wide use of IFN preparations in the treatment of MS [14].

Eight healthy donors (2 men and 6 women) aged 18 to 48 and nine patients with MS (4 men and 5 women) aged 19 to 42 with a remitting-relapsing course of the disease with a history of 6 months to 12 years and different degrees of invalidity according to the Kurtzke scale were examined.

MATERIALS AND METHODS

The effects of auto-IFN on NK activity were studied in two-stage experiments. At the first stage blood samples for interferon induction were collected from the peripheral veins of the examinees, and mononuclear cells (MNC) were used to assess NK cytotoxicity. At the second stage after IFN induction (7 days later) MNC were isolated from the blood of the same donors and patients with MS (the latter were administered a placebo course), and the effects of auto-IFN on these MNC were assessed. Parallel experiments with allogeneic IFN (allo-IFN) were set up.

MNC were isolated from patients' blood in a single-step Ficoll-Paque density gradient (Pharmacia Fine Chemicals, Sweden), $d=1.077$ g/cm^3 according to a previously described method [8].

The cytotoxic activity of NK was assessed by a modified radiometric method [5] against human erythromyeloleukemic K-562 target cells (TC) [12] labeled with ^{3}H-uridine in a dose of 3 μCi/ml. MNC and TC were incubated together in 96-well round-bottom plates for 14 h at 37°C in a humid atmosphere with 5% CO_2. Incubation was carried out in a complete nutrient medium of the following composition: 88 ml RPMI-1640 (Amimed, Switzerland), 12 ml fetal calf serum (N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Russian Academy of Medical Sciences), 10 mM HEPES (Serva), 2 mM glutamine, and 40 μg/ml gentamicin (Pharmachim). NK activity was tested at effector to target ratios (E:T) 100:1, 50:1, 25:1, 12:1, and 6:1. The initial cell suspensions contained 10×10^6 MNC and 10×10^4 TC in 1 ml of medium. After the incubation was over, the MNC and TC

TABLE 1. Time Course of NK activity at Different Stages of the Experiment (CI, %, $M\pm m$)

Group	E:T Ratio				
	100:1	50:1	25:1	12:1	6:1
	Healthy donors, n=4				
Primary examination	82.5±3.6 (75–90)	67.0±8.1 (44–82)	61.8±3.3 (54–70)	62.5±8.8 (50–90)	59.0±11.1 (44–86)
Reexamination (after 1 week)	78.3±8.9 (73–86)	64.5±5.8 (54–75)	61.8±4.6 (53–71)	57.0±7.9 (36–74)	58.7±18.5 (34–94)
	Patients with MS, n=9				
Primary examination	64.0±6.5 (23–83)	72.6±3.0 (63–80)	40.4±9.1 (5–78)	31.2±3.9 (18–57)	31.8±7.9 (4–50)
Reexamination (after 1 week of placebo course)	52.4±8.3 (16–78)	56.7±10.5 (12–98)	60.5±9.6 (20–97)	34.0±5.6 (18–63)	30.6±7.1 (8–48)

Note. The range of CI values is shown in parentheses.

TABLE 2. Distribution of IFN Effects during *In Vitro* Action on Human NK

Number of cases	IFN Type		
	stimulation	suppression	no effect
	Healthy donors		
α–IFN	4(27)	5(33)	6(40)
γ–IFN	3(19)	4(25)	9(56)
allo–α–IFN	6(32)	4(21)	9(47)
allo–γ–IFN	3(23)	4(30)	6(47)
	Patients with MS		
α–IFN	9(28)	10(31)	13(41)
γ–IFN	7(30)	7(30)	9(40)

Note. The percent ratio is shown in parentheses.

were sedimented on fiberglass filters with pore diameter 2.5 μ (Whatman, UK) using a 12-channel harvester of biological fractions (Dynatech, UK). Residual radioactivity on filters was measured with Packard and Mark-II β-scintillators (USA).

The cytotoxic index (CI) was calculated by the formula:

$$CI=(1-\frac{\text{number of pulses in test well}}{\text{number of pulses in control}})\times 100\%.$$

TC incubated under the same conditions as the test cells but without MNC were the control.

α- and γ-IFN in whole blood samples were induced by Newcastle disease virus, strain Kansas, in a dose of 1 CPU/ml and PHA-P (Difco) in a dose of 5 μg/ml for 24 h at 37°C in a 5% CO_2 atmosphere after a previously described [15] modified [1] method. The inductor virus inactivation in cell-free supernatants containing α-IFN was performed by adding 1 N HCl to attain a pH of 2.0 and subsequent 72 h incubation at 4°C and neutralization of acid to pH 7.0 with 1 N NaOH. Interferon was titered in a monolayer culture of human fibroblasts M-19 after a previously described method [6]. The test virus was the vesicular stomatitis virus (VSV), strain Indiana. The reciprocal of the IFN dilution exerting a 50% protective effect on cells infected with 100 CPA_{50} of VSV was taken as the unit of IFN activity.

For a study of the effect of IFN on the activity of NK, IFN-containing supernatants in a dose of 0.1 ml were added to an MNC suspension (4×10^6 MNC in 0.4 ml of medium) for 1 h at 37°C, after which the MNC washed from IFN were pipetted into microplate wells. The results of titration of induced IFN being unavailable by the beginning of the second stage of the study, the IFN dose was regulated by the volume of the native supernatant used, with subsequent conversion to the IFN titer.

The efficacy of IFN was assessed using the regulation index (RI), representing the absolute change of the cytotoxic index with respect to the initial NK activity and calculated as the arithmetic mean for all E:T ratios and taken with the relevant sign. The total regulation index (TRI) in a group of experiments was calculated as the arithmetic mean of the respective RI. The reliability of differences in the mean values was assessed using the Student *t*-test.

RESULTS

Since the effect of IFN is related to the initial NK activity, the cytotoxicity was monitored over the course of the experiment. The results demonstrate that NK activity during one week of the experiment remained virtually unchanged in healthy donors and patients with MS as regards both the mean CI values and the differences in the values (Table 1), this indicating the stability of the system of evaluation used and permitting us to exclude the NK activity variable from the analysis of the regulatory effect of IFN on the cellular cytotoxic function.

Study of the effect of auto-IFN on *in vitro* NK functioning confirmed that it showed immunomodulating activity vis-a-vis this lymphocyte population (Table 2). It is noteworthy, however, that

TABLE 3. IFN Doses Actually Used for Regulation of NK Activity ($M\pm m$)

IFN Type	IFN dose, IU/ml	Range of values
	Healthy donors	
α–IFN, $n=4$	130.4±22.52	51.2–209.6
γ–IFN, $n=4$	28.8±4.18	12.8–51.2
allo–α–IFN, $n=4$	128±15.51	51.2–204.8
allo–γ–IFN, $n=3$	51.2±12.68	25.6–102.4
	Patients with MS	
α–IFN, $n=9$	174.1±27.02	51.2–409.6
γ–IFN, $n=8$	35.4±5.74	1.6–103.4

Note. The IFN content in the final suspension of MNC is shown.

among healthy donors the share of cases where NK activity failed to be stimulated with α-IFN was 73%, and with γ-IFN 81%. A similar situation was recorded during exposure of NK of healthy donors to allo-IFN. The respective values were 68% for α-IFN and 77% for γ-IFN.

The absence in the majority of cases of NK stimulation under the effect of auto- and allo-IFN tells on the RI and TRI values (Fig. 1, *a*). In healthy donors TRI of auto-α-IFN was -11±20% and of γ-IFN -12±4%. Allo-IFN changed NK activity with TRI 3±8% for α-IFN and 11±19% for γ-IFN. Comparison of the efficacies of experimental and commercial IFN (evaluated in our previous research) revealed an appreciable difference between the agents. The TRI of commercial human leukocytic IFN in doses of 50 and 125 U/ml were 270 and 143%, and of commercial γ-IFN in the same doses 46 and 31%, respectively, while the TRI of reaferon (human recombinant α_2-IFN) in a dose of 100 IU/ml was equal to 20%. The doses of commercial and experimental IFN were comparable, as is seen from Table 3, the logarithmic difference not exceeding 2.

In patients with MS auto-IFN had a reliable stimulating effect on *in vitro* NK activity (Fig. 1, *b*). The TRI of α-IFN was 26±10% ($p<0.05$), while the TRI of γ-IFN was 52±33% ($p<0.05$). The distribution of IFN effects corresponded to that in the cells of normal donors: the proportion of cases where auto-IFN did not stimulate NK was 72% for α-IFN and 70% for γ-IFN (Table 2). The ratio of cases with RI higher than TRI in healthy donors to the total number of RI observations in MS patients 7:9 (78%) for α-IFN and 6:7 (86%) for γ-IFN. Such an increase of the activity of NK with initially normal cytotoxicity under the effect of auto-IFN in MS patients was not due to the use of a different (in comparison with donors) dose of the factor, because the titers of IFN induced in patients' cells did not reliably differ from the IFN doses used in healthy donors (Table 3).

When comparing the pronounced stimulating effect of IFN preparations on the activity of NK, which is related to the initiation of differentiation of these cells and enhancement of the cytotoxic properties [17], and the absence of a general stimulating effect of auto- and allo-IFN on the activity of NK with the production of an inhibitor of the IFN effect (IEI) induced by IFN genes, manifested by attenuation of its antiviral activity [4], it is logical to deduce that the present investigation yielded data on the existence of an inhibitor of the regulating effects of α- and, moreover, γ-IFN (IRE-IFN) on NK activity, induced by IFN genes and produced simultaneously by α- and γ-IFN.

The nature of IRE-IFN is unclear. Its production associated with IFN induction, the obvious relation of the efficacy of the blocking action to the initial activity of NK, and the absence of IRE-IFN in commercial IFN preparations give grounds for speculating that this factor may be identical to IEI, whose activity manifests itself vis-a-vis the antiviral effect of IFN.

From this viewpoint, the increase of NK activity under the effect of auto-IFN in patients with MS suggests that one of the principal components in the pathogenesis of this disease is a defect of IRE-IFN production which distorts the regulatory and cytotoxic properties of NK in the presence of an altered sensitivity of this lymphocyte population to regulating factors. This interpretation helps explain some aspects of the activation of the autoimmune mechanism involved in the process of demyelinization in patients with MS [16].

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