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INTERACTION OF INTERFERON WITH OTHER IMMUNOMODULATORS REGULATING HUMAN NATURAL KILLER CELL ACTIVITY

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In our research into regulation of activity of natural killer cells (NKC), which constitute the main cell population in the natural cytotoxicity (NCT) system, we found a definite similarity in the properties of T-activin (TA), a stimulator of antibody producers and interferon (IFN), manifested in particular as stimulation of NKC activity in the presence of low effector to target (B:T) ratios was discovered [7]. Considering that the question of the origin of NKC has not yet been answered (compare [1] and [2]) this result has been regarded as evidence that all three factors are involved at a certain stage in regulation of maturation of NKC precursors. The key place of IFN in activation of NKC and induction of their maturation [14, 15] suggested that the study of regulation of NKC by IFN in combination with peptides controlling individual stages of immunity, and possessing a definite action on NKC, would lead to an understanding of the principles governing differentiation of these cells in man.

The aim of the investigation was to assess the action of reaferon (RF) (human recombinant IFN-α_2) in conjunction with regulatory peptides of varied origin on NKC activity in vitro in healthy individuals and patients with multiple sclerosis (MS). MS was chosen as the model because in this disease there is an IFN-dependent NKC deficiency [5], IFN preparations are widely used in the treatment of MS [12, 13], and definite positive results have been obtained in the clinic for nervous diseases of the N. I. Pirogov Second Moscow Medical Institute by treatment of patients with MS by T-activin, myelopide (MP), and dalargin (DL). Altogether 20 healthy blood donors (four men and 16 women) aged from 18 to 46 years and 34 patients with MS (12 men and 22 women) aged from 16 to 55 years, with a remittent course of the disease, the duration of which varied from 6 months to 12 years, and with different degrees of disability on the Kurtske scale, were investigated.

EXPERIMENTAL METHOD

Mononuclear cells (MNC) were isolated from peripheral venous blood of the healthy subjects and patients in a one-step Ficoll—Paque density gradient (Pharmacia Fine Chemicals, Sweden), d = 1.077 g/cm^3 [9].

The cytotoxic activity of NKC was determined by a radiometric method, against target cells (TC) of human erythromyeloleukemia K-562 [10], labeled with ^{3}H-uridine in a dose of 3 μCi/ml, in the modification in [3]. Combined incubation of MNC and TC was carried out for 14 h at 37°C in a humid atmosphere containing 5% CO_2. Complete nutrient medium based on RPMI-1640, used for incubation, had the following composition. RPMI-1640 (Amimed, Switzerland) 88 ml; bovine embryonic serum (N. F. Gamaleya Research Institute of Bpidemiology and Microbiology, Academy of Medical Sciences of the USSR) 12 ml, HEPES (Serva, Germany) 10 mM, glutamine 2 mM, gentamicin (Pharmachim, Bulgaria) 40 μg/ml. The E:T ratio ranged from 100:1 to 6:1.

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The cytotoxic index (CTI) was calculated by the equation:

$$CTI = \left(1 - \frac{\text{number of counts in experimental well}}{\text{number of counts in control}}\right) \times 100\,\%.$$

As a control for the cytotoxic test we used TC incubated under the same conditions as the experimental, but without MNC.

As regulators of NKC activity the following immunoactive peptides were used: T-activin (Research Institute of Physicochemical Medicine, Ministry of Health of the RSFSR, batch 126, activity 100 μg/ml), myelopide (Institute of Immunology, Ministry of Health of the USSR, batch 10, activity 3 mg per ampul), dalargin (All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, activity 1 mg per ampul), and reaferon ("Ferment" Research-Production Combine, Ministry of the Medical and Biological Industry of the USSR, activity $1 \cdot 10^6$ IU per ampul). TA, MP, and DL were added to the suspension of MNC in a dose of 1.0 and 10 μg/ml, whereas RF was added in a dose of 100 IU/ml. The dose of the peptides given in combination with RF was 10 μg/ml. The duration of incubation of MNC with the preparations was 1 h at 37°C ($4 \cdot 10^6$ MNC in 0.5 ml of medium), after which the preparations were washed and the MNC and TC were introduced into wells of 96-well round-bottomed microplates, the experiments being accompanied by a positive control.

The efficacy of the immunomodulators was assessed with the aid of regulation indices (RI), consisting of the absolute change in CTI divided by the original NKC activity, together with the appropriate sign ("+") or ("−") and calculated as the arithmetic mean of all E:T ratios [8]. Groups of subjects were characterized by a general regulation index (GRI), calculated as the arithmetic mean of RI of the persons composing the group.

The significance of differences between the mean values was determined by Student's t test.

EXPERIMENTAL RESULTS

The study of the action of RF on NKC function showed that in healthy individuals NKC activity was increased under the influence of RF by a much lesser degree than when the natural preparation of human leukocytic IFN-α was used (HLI, 125 IU/ml); GRI of RF for healthy donors was 20%,whereas GRI of HLI was 143% [8]. The action of RF in patients with MS corresponded to the effect of HLI, the corresponding GRI values being: RF 30% HLI 24% [8].

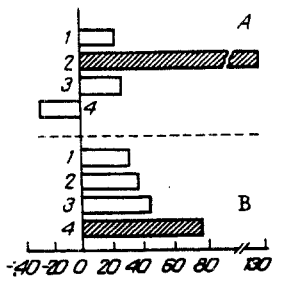
TA in healthy donors in a dose of 1.0 μg/ml caused marked stimulation of NKC activity (GRI 127%; Fig. 1a). A combination of RF and TA led not only to reduced efficacy of action of the latter, but even caused NKC activity to fall below its initial values (GRI −27%). Under the influence of TA, GRI for patients with MS in doses of 1.0 and 10 μg/ml, GRI was 36 and 42% respectively (Fig. 1B). A combination of TA with RF increased NKC activity with GRI of 75%.

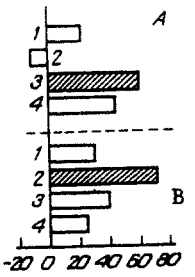
GRI of MP in a dose of 10 μg/ml was 58% in healthy donors, but 12% in a dose of 1.0 μg/ml; a combination of MP and RF stimulated NKC activity with GRI of 42% (Fig. 2A). In patients with MS, MP in a dose of 1.0 μg/ml had a significant stimulating action on NKC activity with GRI of 70% (Fig. 2B). In a dose of 10 μg/ml GRI of MP was 37%. A combination of MP with RF led to a reduction in the efficacy of action of MP to a GRI of 25%.

In healthy donors DL and also a combination of DL with RF were ineffective as regulators of NKC activity (Fig. 3A). In patients with MS, GRI for DL was about 70% with the preparation acting in both doses, and a combination of DL with RF led to a further strengthening of NKC activity by 30% of GRI (Fig. 3b).

From the standpoint of stage-specific regulation of differentiation of NKC from their bone-marrow precursors to mature forms, are evidence that the maturation of these cells, which constitute the evolutionarily oldest system of immunologic surveillance [11], is under the control not only of IFN, but also of factors of thymic and bone marrow origin. NKC of healthy individuals respond by marked stimulation to the action of T-lymphocyte differentiation factor (TA) and, to a lesser degree, but also by stimulation, to the action of MP, which is primarily a regulator of the function of the B-component of immunity. Under these circumstances RF has no marked action on the cytotoxicity of NKC, confirming the conclusion that preservation of the whole complex of cytokines in commercial preparations of IFN is necessary for the preservation of their biological efficacy as factors of immunoregulation [4].

In patients with MS, against the background of changes described previously in the NCT system, developing in the manner of IFN-dependent deficiency [5], the functional insufficiency of NKC linked with a sharp fall in IFN production by virtually all the cells involved in this process, is observed. As the results of the present investigation show, in this situation NKC lose their sensitivity in vitro to TA, the degree of activation of the cells under the influence of MP is increased, and





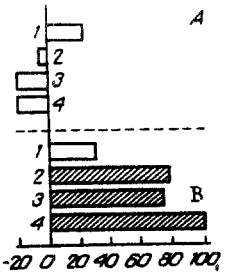


Fig. 1 Fig. 2 Fig. 3

Fig. 1. Action of reaferon, T-activin, and their combination on NKC activity in vitro. Abscissa, GRI (%). A) Healthy blood donors: 1) RF (n = 6); 2) TA (1.0 μg/ml, n = 6); 3) TA (10 μg/ml, n = 6); 4) RF + TA (n = 4). B) Patients with MS: 1) RF (n = 9); 2) TA (1.0 μg/ml, n = 9); 3) TA (10 μg/ml, n = 8); 4) RF + TA (n = 8). Here and in Figs. 2 and 3, oblique shading indicates $p < 0.05$ compared with NKC activity without preparations.

Fig. 2. Action of reaferon, myelopide, and their combination on NKC activity in vitro. Abscissa, GRI (%). A) Healthy donors: 1) RF (n = 6); 2) MP (1.0 μg/ml, n = 5); 3) MP (10 μg/ml, n = 6); 4) RF + MP (n = 6). B) Patients with MS: 1) RF (n = 9); 2) MP (1.0 μg/ml, n = 9); 3) MP (10 μg/ml, n = 9); 4) RF + MP (n = 7).

Fig. 3. Action of reaferon, dalargin, and their combination on MKC activity in vitro. Abscissa, GRI (%) A) Healthy donors: 1) RF (n = 6); 2) DL (1.0 μg/ml, n = 4); 3) DL (10 μg/ml, n = 4), 4) RF + DL (n = 4). B) Patients with MS: 1) RF (n = 9); 2) DL (1.0 μg/ml, n = 9); 3) DL (10 μg/ml, n = 9); 4) RF + DL (n = 6).

a response to DL, a preparation of the opiate series, absent in healthy donors, arises. In these patients the use of RF restores the action of TA in vitro by about 50% (Fig. 1) and abolishes excessive stimulation of NKC by MP (Fig. 2). When combinations of RF with DL and RF with TA were used, a cumulative effect was observed, which could not be detected with a combination of RF and MP.

Thus the immunodeficiency for NKC in patients with MS is manifested not only as weakening of the cytotoxic activity of the cells and of the intensity of their differentiation, as we showed previously [5]. It is also associated with a marked change in the sensitivity of NKC to the action of regulatory factors in vitro, which we have observed in other diseases of the CNS also [6]. Disturbance of the fine mechanisms of NKC maturation and a change in distributive processes during the formation of the circulating lymphocyte pool were discovered. These processes were characterized by a distinctive shift toward activation of the B component of immunity, which becomes possible as a result of a deficiency of IFN, which under conditions of a normal balance between the factors regulating NKC activity, can abolish the excessive stimulation of the cells by myelopeptides.

It can be concluded from these results that on the attempt to restore MKC activity when sharp changes have occurred to the sensitivity of the cells to the action of their physiological regulating factors, if there is a marked deficiency of one of these mediators (in the present case – IFN), introduction of the recombinant analog of the deficient factor to the system is not sufficient to normalize the function of the NKC population. Its use in combination with regulatory peptides of thymic or bone marrow origin, evidently through abolishing the imbalance between initiation of the early stages of maturation, leads to restoration of the initial sensitivity of the cells to the action of regulatory factors in vitro and normalization of the cytotoxic and, perhaps, regulatory activity of NKC.

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