Correlations between *in Vitro* Effects of Preparations of Interferon and Its Inducers on Blood Cells in Patients with Multiple Sclerosis

S. B. Cheknev, M. V. Mezentseva, A. N. Narovlyansky, N. A. Kobyakina, and F. I. Ershov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 131, No. 4, pp. 453-456, April, 2001
Original article submitted September 6, 2000

We studied *in vitro* production of interferon-α and interferon-γ by peripheral blood leukocytes from 15 patients with multiple sclerosis. The priming effects of interferon preparations weakly correlated with interferon-α production by leukocytes from patients with multiple sclerosis, but negatively correlated with interferon-γ production. The effects of interferon inducers in most cases positively correlated with its spontaneous production. We found a weak positive correlation between the priming effect of natural interferon-α and the effect of recombinant interferons. There were positive or strong positive correlations between the effects of recombinant interferons on leukocytes from patients with multiple sclerosis. The relationship between the effects of medicinal interferon inducers and interferon preparation varied from negative to strong positive correlations. These data suggest that correlation analysis can be used for dynamic control and elaboration of methods for combined immunotherapy of multiple sclerosis with various interferon preparations or interferon and its inducers.

Key Words: *interferon; inducers; effect; correlations*

The use of interferon (IFN) [2,5,6,12,16] or its inducers [1] for combined therapy of patients with multiple sclerosis (MS) is directed to normalization of the immunoregulatory balance impaired during demyelinating processes [13].

Healthy individuals differ in the sensitivity of peripheral blood leukocytes (PBL) to the priming effect of IFN [9,14]. The reaction of PBL to IFN changes during viral diseases [9], immune deficiency, autoimmune and allergic disorders [14], and MS [3]. Long-term treatment with IFN leads to production of neutralizing anti-IFN antibodies in patients with MS [5,16], which decreases the efficiency of replacement therapy [5]. Therefore, combined therapy with various IFN preparations or IFN and its inducers seems to be promising. These schemes of treatment would allow us to change IFN preparations or its inducers at various stages of patient's management.

In this respect, it is necessary to evaluate the dependence of the effects of IFN preparations and its inducers on functional activity of PBL in patients with MS and to reveal correlations during *in vitro* treatment of PBL with medicinal preparations of IFN and its inducers.

MATERIALS AND METHODS

We studied the sensitivity of PBL from 15 patients (6 men and 9 women aging 25-58 years, mean 42.2 years) with cerebrospinal secondary progressive (5 patients) and remittent MS (10 patients) to the priming effect of IFN preparations. The patients suffered from MS for 6 months-20 years (average 9.3 years). The severity of neurological deficiency was 4.5 points by the EDSS scale.

Departments of Interferons, N. F. Gamaleya Institute of Epidemiology and Microbiology, Russian Academy of Medical Sciences, Moscow

0007-4888/04/1314-0382$25.00

In vitro production of IFN-α and IFN-γ by induced and primed PBL was studied in whole heparinized peripheral venous blood [8]. Titration of IFN was performed in a monolayer culture of human diploid fibroblasts. The content of IFN in conditioned media was estimated by 50% inhibition of cytopathic effects caused by mouse encephalomyocarditis virus (100 ICE_{50}).

In vitro induction of IFN in blood cells was performed as described elsewhere [10]. IFN-α production was stimulated by Newcastle disease virus (ID_{50} per leukocyte). IFN-γ production was induced by staphylococcal enterotoxin A (Immunopreparat) in a dose of 1-10 μg/ml depending on its activity. The cells were treated for 24 h.

Medicinal preparations of human IFN, including human leukocyte IFN for injections (Intekor), Reaferon (recombinant IFN-α_{2b}, Vektor), Betaferon (recombinant IFN-β_{1b}, Schering), and Gammaferon (recombinant IFN-γ, Sanitas), were used for priming of IFN production. The method of high-efficiency induction was used [7]. The preparation (20 IU/ml) was added into blood cell samples 2 (IFN-α and IFN-β) or 4 h (IFN-γ) before treatment with Newcastle disease virus or staphylococcal enterotoxin A, respectively.

To stimulate IFN production with medicinal preparations of IFN inducers, we used Cycloferon (Polisan), Neovir (Issledovatel'skie Laboratorii), Ridostin (Vektor), and Amixin (Lens-Farm) in final doses of 600 μg/ml, 600 μg/ml, 1.0 mg/ml, and 2.5 mg/ml, respectively. The cells were treated for 24 h.

The results were analyzed by Spearman rank correlation test (*r*).

RESULTS

The priming effects of IFN preparations were not related to IFN-α production by PBL (Table 1). The initial functional activity of blood cells from patients with MS estimated by IFN-α production determined the response to priming with recombinant IFN-α and IFN-β (Table 1).

The priming effects of medicinal IFN preparations depended on the ability of PBL from patients with MS to produce IFN-γ. There was a negative correlation between the effect of recombinant IFN-β and production of IFN-γ by blood cells from patients with MS (Table 1).

Table 1 shows *in vitro* effects of medicinal IFN preparations on PBL from patients with MS. The lower was the ability of cells to produce IFN under conditions of standard induction, the greater was the priming effect of IFN preparations. Thus, the priming effects of type I and II IFN are characterized by functional antagonism [12,15]. There was a negative correlation between production of IFN-γ (type II IFN) and the effect of IFN-α and IFN-β (type I IFN, Table 1). The priming effect of Betaferon inhibiting secretion of antiinflammatory cytokines (*e.g.*, IFN-γ [5]) strongly depended on IFN-γ production by PBL (Table 1).

We found positive correlations between functional activity of blood cells from 5 of 8 patients with MS and IFN production stimulated by medicinal IFN inducers (Table 1). These results indicate that exobiotic IFN inducers produce more pronounced effects on producers characterized by intensive spontaneous secretion of IFN.

It should be emphasized that the correlation coefficients characterizing the relationship between the effects of Ridostin and Amixin and IFN-γ production were different (Table 1). As differentiated from Cycloferon and Neovir stimulating only IFN-α production, these preparations acted as inducers of both IFN-α and IFN-β [4,11].

Correlation analyses allowed us to differentiate IFN inducers with respect to their effects and mechanisms of action: treatment with these agents leads to various IFN reactions in different cell populations. Ridostin stimulates early IFN production by T lymphocytes, which involves macrophages as helper cells. Amixin induces late IFN that is not mediated by helper cells [4,11]. Probably, these inducers would complement each other during combined use. It should be emphasized that the coefficient of correlation between Amixin effects and spontaneous production of IFN-α and IFN-γ surpassed that for other IFN inducers (Table 1).

The analysis of correlations observed during priming of IFN production by medicinal IFN preparations showed that their effects were interrelated. There were insignificant positive correlations between the

TABLE 1. Correlation Coefficients for *in Vitro* Priming Effects of IFN Preparations or Inducers and Production of IFN-α and IFN-γ in Patients with MS

Preparations	IFN-α	IFN-γ
IFN preparations		
human leukocyte IFN	0.14	0.16
Reaferon	-0.2	-0.25
Betaferon	-0.2	-0.49
Gammaferon	-0.06	-0.09
IFN inducers		
Cycloferon	0.42	0.62
Neovir	-0.14	0.1
Ridostin	0.36	0.33
Amixin	0.64	-0.65

TABLE 2. Correlation Dependences (*r*) Revealed during *in Vitro* Effects of Medicinal IFN Preparations and Inducers on PBL from Patients with MS

Preparation	Cycloferon	Neovir	Ridostin	Amixin
Human leukocyte IFN	0.06	-0.02	0.24	0.26
Reaferon	-0.17	0.54	-0.17	0.61
Betaferon	-0.24	0.13	-0.23	0.73
Gammaferon	-0.07	0.04	-0.37	0.77

priming effect of human leukocyte IFN and the influence of Gammaferon, Reaferon, and Betaferon (r=0.07, r=0.3, and r=0.36, respectively). At the same time, we found positive or strong positive correlations between changes in cell activity induced by recombinant IFN preparations. The correlation coefficients for the effects of Betaferon and Gammaferon, Reaferon and Gammaferon, and Reaferon and Betaferon (type I IFN used in combined immunotherapy of MS) were 0.64, 0.7, and 0.86, respectively. This strong positive correlation indicates close relationship between PBL sensitivity to recombinant IFN-α and IFN-β in patients with MS. Therefore, these preparations should be used in combined therapy and substitute for each other, if cell sensitivity decreases [3].

The priming effect of medicinal IFN preparations were interrelated with the influence of medicinal IFN inducers on PBL from patients with MS (Table 2). We found insignificant positive correlations characterizing the effects of human leukocyte IFN. The relationship between the effects of recombinant IFN preparations and IFN inducers varied from negative (Gammaferon-Ridostin) to strong positive correlations (Gammaferon-Amixin, Table 2). The effects of Reaferon and Betaferon negatively correlated with the influence of Ridostin, but positively correlated with the influence of Amixin (Table 2). Therefore, the effects of recombinant IFN preparations are realized during low production of early induced IFN-α and IFN-β, but intensive secretion of late type I IFN under conditions of forced response.

The ratios of mean r to the number of IFN inducers were 0.09, 0.1, 0.135, and 0.2 for Gammaferon, Betaferon, human leukocyte IFN, and Reaferon, respectively (Table 2).

Thus, correlation analysis pathogenically substantiates combined use of natural and recombinant IFN-α preparations for immunotherapy of MS [13]. This approach can be used for the dynamic control and elaboration of methods for combined therapy of patients with various IFN preparations or IFN and its inducers. Moreover, IFN preparations and its inducers can substitute each other during immunetherapy.

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