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Detection of IFN-α Produced in the Presence of Plasma γ-Globulin Fraction Proteins

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Interferon-α was detected in IFN pool produced by human leukocytes in the presence of γ-globulin fraction proteins, copper and zinc cations, and metal-modified γ-globulins. The cytokine appeared in culture medium at early terms (24 h) of incubation, is characterized by acid resistance, and is neutralized by antibodies to IFN-α. The content of IFN-α in supernatants of induced leukocytes reached 60-90 pg/ml and correlated with antiviral activity of the samples. Zinc bound to human serum γ-globulin attenuated and copper stimulated the realization of IFN-inducing characteristics of the protein at early terms of incubation.

Key Words: *interferon-α; production; γ-globulin*

Previous studies showed that the pool of IFN produced in the presence of plasma γ-globulin fraction proteins contains mainly the cytokine corresponding to IFN-γ by its properties [4]. Metal-specific conformation changes of Fc region of the antibody molecule caused by incorporation of copper or zinc cations [5] potentiate or attenuate the effect of IFN induction by the protein which bound the metal and was modified by it [4].

The results are in line with the data on interactions of IFN system with γ-globulins through the Fc receptor (FcR) family. On the one hand, this interaction is realized via stimulation of the expression of FcR γ-subunit and FcR proper under the effect of IFN-γ [7,11] and transduction of signals triggered by FcR [7]. On the other hand, it manifests in induction of gene transcription and production of IFN-γ upon FcR binding to Fc fragment of IgG [9]. Realization of these interactions in an experimental system approximating the physiological system by a number of properties, suggested that conformation changes of Fc fragments of antibody molecule caused by binding of metal cations serve as a natural factor regulating intracellular signaling triggered by activated FcR and normally inducing the production of IFN-γ [4,5].

Up to 40% of late IFN pool is the acid-stable fraction. This indicates that not only IFN-γ, but also IFN-α can be produced by induced human peripheral blood leukocytes.

We evaluated the production of early IFN in the presence of conformation-transformed proteins of plasma γ-globulin fraction and characterized acid resistance and type specificity and measured the concentration of the resultant IFN.

MATERIALS AND METHODS

Interferon induction in suspensions (10^6 cell/ml) of leukocytes from 10 donors was carried out in complete nutrient medium based on double Eagle's me-

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dium (M. P. Chumakov Institute of Poliomyelitis and Viral Encephalitis) supplemented with 2% donor plasma, L-glutamine (supplied with a flask of medium), gentamicin (20 U/ml), and heparin (up to 5.0 U/ml) for 24 h at 37°C in a humid atmosphere with 5% CO_2. Plastic flat-bottom 24-well Costar plates were used. Specimens of γ-globulin modified by copper or zinc cations (ICN) were used in final concentrations of 5.0, 0.5, and 0.05 μg/ml. In parallel, the effects of control γ-globulin preparations and copper (aqueous sulfate, Merck) and zinc (chloride) solutions were evaluated. Cation content in these salines corresponded to the amount of protein-bound metal. Newcastle disease virus (NDV; 10 CPE/leukocyte) and phytohemagglutinin P (PHA; 1.0 μg/ml; Difco) served as standard inductors of IFN production.

Titration of IFN was carried out on a monolayer culture of human embryo diploid fibroblasts (Medical Genetic Center of the Russian Academy of Medical Sciences) against 10 or 1 CPE_{50} of mouse encephalomyocarditis virus (MEMV). Plastic flat-bottom 96-well Nunclon or Costar plates were used. The initial concentration of cells was 2×10^5/ml.

A series of independent experiments was carried out to rule out the direct antiviral effect of the samples used for IFN induction.

In order to evaluate acid sensitivity/resistance of induced IFN, the IFN-containing supernatants were treated with 20% HCl to pH of 2.0, after which they were exposed at 4°C for 24 h and pH was reduced to 7.2-7.4 by adding 40% NaOH. Titration was then carried out. Control samples were not treated with the acid and were exposed under the same conditions as experimental samples.

Interferon neutralization test was carried out with anti-IFN-α IgG (Intercor Company). Standard anti-IFN-α IgG (L. A. Tarasevich Institute of Standardization and Control) was used in some experiments. The antibodies were added to the studied samples with IFN activity of 10 U/ml in an equivalent volume proportion in dilutions 1:400 to 1:3200 and incubated for 2 h at 37°C in plastic 24-well flat-bottom plates (Costar). Antibody activity was confirmed by tests with IFN-α reference preparation (Intecor Firm). All experiments had appropriate controls, including the MEMV dose control.

The content of IFN-α in induced leukocyte supernatants was evaluated by EIA using ELISA Processor II (Behring). The EIA kits (Protein Kontur) were used according to the instruction, extra controls were used.

Specimens of IFN induced by different concentrations of protein or metal cations were pooled in experiment with acid treatment, neutralization test, and EIA in order to obtain a sufficient volume of the experimental material.

The differences between the means were evaluated using Student's *t* test.

RESULTS

During the early period of induction, the culture medium of stimulated leukocytes contained 16-32 U/ml IFN. The production of IFN was as follows: 640-680 U/ml by control cells induced by NDV, 16-48 U/ml by cells induced by PHA, and spontaneous (without inductors) production did not exceed 4 U/ml IFN. Hence, by its biological activity the pool of induced IFN was comparable to that obtained under conditions of PHA induction. On the other hand, experimental samples were weaker than PHA-induced IFN: they could not be tittered against 10 CPE_{50} of the test virus and their activity could be evaluated only by protection of cultured cells infected with 1 CPE_{50} of MEMV.

The complex of γ-globulin with zinc was characterized by 1.4-1.6 lower activity in comparison with zinc cations and 1.3 lower activity in comparison with the control protein (Fig. 1).

By contrast, γ-globulin complex with copper realized a higher potential: the titers of IFN produced in the presence of this complex (neglecting the dose of 0.05 μg/ml) were 1.9-2.0 higher in comparison with copper ($p>0.1$) and 1.3-1.4 times higher *vs.* the control protein (Fig. 2). It is noteworthy that activity of copper-modified γ-globulin was 1.4-1.6 times higher ($p>0.1$) than that of zinc-modified protein (Figs. 1, 2).

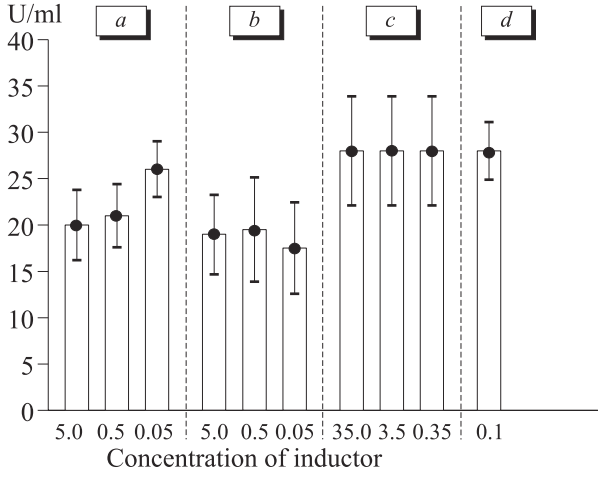

Fig. 1. *In vitro* production of IFN by human blood leukocytes in the presence of γ-globulin modified by zinc cations (n=5): testing against 1 CPE_{50} of MEMV. Ordinate: IFN activity. *a*) control γ-globulin (μg/ml); *b*) zinc-modified γ-globulin (μg/ml); *c*) zinc (ng/ml); *d*) PHA (μg/ml).

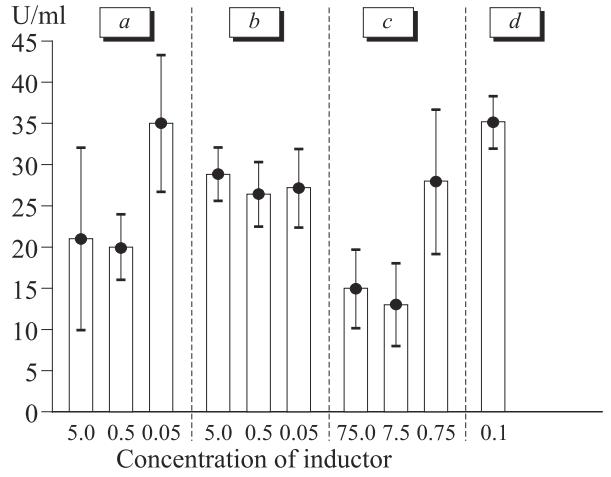

Fig. 2. *In vitro* production of IFN by human blood leukocytes in the presence of γ-globulin modified by copper cations (n=5): testing against 1 CPE_{50} of MEMV. *a*) control γ-globulin (μg/ml); *b*) copper-modified γ-globulin (μg/ml); *c*) copper (ng/ml); *d*) PHA (μg/ml).

The same regularities were observed under conditions of late induction with predominant production of IFN-γ, when zinc reduced and copper stimulated the manifestation of IFN-inducing activity of γ-globulin, binding these cations. In parallel, zinc lost its own IFN inductor potential, while copper (presumably) realizes its potential even as a component of the protein-metal complex [4].

Presumably, the effects of the minimum protein and metal concentrations in experiments with copper (0.05 μg/ml protein and 0.75 ng/ml copper) should be regarded with consideration for high variability of the initial values in the group of donors. Some errors in the mean activities of IFN reached $^1/_3$ to $^1/_2$ of the resultant value (Fig. 2).

The same fact can explain the absence of statistically significant results in the studied sample (Figs. 1, 2). On the other hand, individual analysis showed a reduction of IFN production in the presence of zinc-modified proteins in comparison with the control in about 60% experiments. Increase of titers of IFN induced by copper-bound γ-globulin was observed in 50-70% cases.

Evaluation of IFN pool sensitivity to acid treatment showed that all studied samples contained acid-sensitive and acid-resistant constituents. However, in contrast to delayed induction [4], the pool of early IFN contained up to 60% acid-resistant fraction and up to 40% of sensitive material. Acid-resistant fraction in samples obtained on PHA-stimulated cells constituted 20%.

The stability of early IFN is demonstrated by a no more than 2-fold reduction (by no more than one 2-fold dilution of IFN-containing supernatant) of IFN activity after acid treatment or unchanged activity of 40% samples induced by control γ-globulin, of 50% samples induced by zinc-modified γ-globulin, and of 75% samples induced by copper.

Antibodies to IFN-α diluted 1:800 to 1:3200 bind and neutralize IFN produced under conditions of induction by control and metal-modified proteins and by copper and zinc cations alone. The protective effect of IFN-containing supernatants in cell culture was completely canceled after the equivalent concentration of neutralizing antibodies was attained (Table 1).

The need in high dilutions of anti-IFN-α antibodies for the neutralization effect is presumably determined by the above described inertness of the resultant IFN in comparison with IFN-α present in the commercial preparation.

TABLE 1. Identification of IFN Induced by Specimens of Metal-Bound γ-Globulin in Neutralization Test with Anti-IFN-α Antibodies

IFN sample		Anti-IFN-α dilution			
		1:400	1:800	1:1600	1:3200
Reference IFN-α		++++	++++	++++	—
Reference IFN-α (standart antibody)		+++	+++	±	—
After induction by	NDV	++++	++++	—	—
	PHA	—	++++	++++	++++
After induction by	γ-globulin	—	±	++	+++
	γ-globulin with zinc	—	±	++	+++
	zinc	—	±	++	+++
	γ-globulin with copper	—	+	++	+++
	copper	—	+	++	+++

Note. Symbols in the Table show the degree of cell degeneration in MEMV-infected culture. Testing against 1 CPE_{50} of MEMV. Results of two complete independent experiments are united.

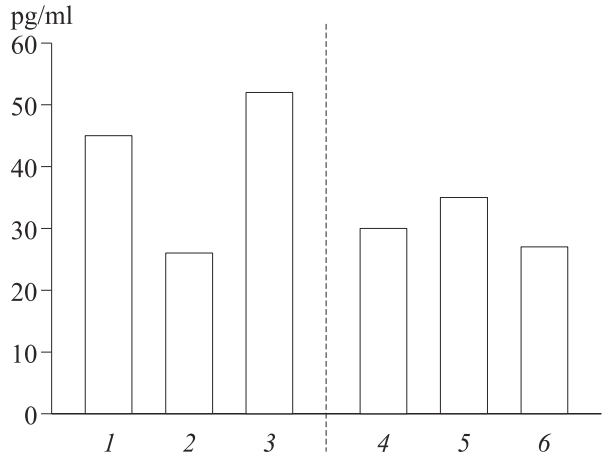

Fig. 3. Content of IFN-α in leukocyte culture fluid after induction by γ-globulin fraction proteins and metal cations. Ordinate: IFN concentration. *1*) γ-globulin (zinc control); *2*) zinc-modified γ-globulin; *3*) zinc; *4*) γ-globulin (copper control); *5*) copper-modified γ-globulin; *6*) copper. Results of 4 complete experiments are summed up.

Our results indicate that the pool of IFN produced in the presence of native and conformation-modified γ-globulin fraction proteins contains not only IFN-γ [4], but IFN-α as well.

The fact of IFN-α production was confirmed by EIA. The concentration of resultant IFN reached 60-90 pg/ml.

The results of EIA correlated with the data on biological activity of the studied material (Fig. 1, 2). γ-Globulin-zinc complex induced 1.7 times less IFN-α than the control protein and 2-fold less than zinc cations (Fig. 3). γ-Globulin-copper complex was 1.2 times more active than the control protein and 1.3 times more active than copper (Fig. 3). Direct correlation was noted in 75% observations.

Presumably, IFN-α present in the early IFN pool produced in cultures containing γ-globulin fraction proteins largely determined the antiviral activity of the samples. The specific features of the model used in the study (similarly as normal conditions of the plasma) admit the possibility of IFN-α stabilization at the expense of binding to antibody Fc fragments, prolonging its half-life period [1].

In contrast to IFN-γ, whose relationships with γ-globulin fraction proteins are focused exclusively on the FcR family and are sufficiently well studied [7,9,11], IFN-α was never regarded in the context of interactions with γ-globulins before [11]. The capacity of mouse FcR expressed on dendritic cells to trigger the production of IFN-α and IFN-β in activation of human IgG [15] is not physiological.

In contrast to IFN-γ, IFN-α was not evaluated as a factor involved in metal metabolism and cation exchange or depending on the presence of certain cations for realization of its biological activity [6,10,14]. It was found however that mouse IFN-α/β induced the production of metallothioneines in animal liver and this production was regulated by exogenous zinc, consumed with food [12].

Since the main signal pathways triggered by activated FcR are considered to be unrelated to the IFN-α induction mechanism [2,3,8,13], this paper presumably for the first time describes functional interrelationships providing the production of IFN-α in the presence of plasma γ-globulin fraction proteins. It will be right to analyze these relationships from the viewpoint of physiological immunoregulation, promoting the development and maintenance of a certain level of antiviral status in the cell, at the expense of conformation of antibody molecule Fc region and regulation of intracellular signaling focused on FcR.

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