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Induction of Interferon Production by Conformation-Modified Proteins of Plasma γ-Globulin Fraction

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Plasma γ-globulin fraction proteins, copper and zinc cations, and metal-modified γ-globulins induce the production of IFN by human leukocytes. Binding of zinc cations attenuates the realization of the IFN-inducing effects of human serum γ-globulin, while binding of copper cations potentiated this effect. Activity of IFN and the dynamics of its production correspond to those in response to phytohemagglutinin stimulation. The pool of induced IFN contains acid-labile (up to 60%) and acid-stable (up to 40%) constituents. Anti-IFN-α antibodies do not modify activity of produced IFN. The results indicate the possibility of γ-globulin conformation allowing stimulation or attenuation of the protein capacity to induce the production of IFN pool with predominant content of IFN-γ.

Key Words: *interferon; induction; γ-globulin; conformation*

The capacity of plasma γ-globulins to induce the production of IFN is a well-known fact. Full-sized γ-globulins, Fab fragments of antibody molecules, short oligopeptide fragments of the heavy chain, and supramolecular complexes act as IFN inductors.

Intracellular protein aggregation is mainly regarded as a result of improper folding of protein molecules allowing contact of their hydrophobic surfaces. This can be a result of mutations, post-translation modifications, and local changes in temperature or pH [3]. Extracellular aggregation is also largely determined by hydrophobic protein-protein interactions. It can also be caused by alteration of the vector of transport and exchange of heavy bivalent metal cations forming bridges between protein molecules and virtually not present in the cell cytoplasm in a free state [2], but detected in the plasma or adsorbed by macromolecules loosely binding them [14].

Aggregation is associated with significant conformation changes in protein molecules [3], induction of conformation signal flow [8], and acquisition of a new conformation status modifying the effector characteristics of γ-globulin proteins realized at the expense of Fab or Fc fragments of the molecule [4]. Our previous findings indicate that copper and zinc cations transform γ-globulin molecules under conditions when their aggregation can be with a high probability ruled out [5,6].

The aim of this study was to evaluate the production of IFN in the presence of conformation-modified plasma γ-globulin proteins and characterize acid lability or stability and the type specificity of IFN produced under these conditions.

MATERIALS AND METHODS

Induction of IFN in suspensions of leukocytes from 10 donors (10^6 cells/ml) was carried out in com-

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plete nutrient medium based on double Eagle's medium (M. P. Chumakov Institute of Poliomyelitis and Viral Encephalitis, the Russian Academy of Medical Sciences) supplemented with 2% donor plasma, L-glutamine (from the set attached to a flask of the medium), gentamicin (20 U/ml), and heparin (to the concentration of 5.0 U/ml) for 24 or 48 h at 37°C in a humid atmosphere with 5% CO_2. Plastic round-bottom 24-well plates (Costar) were used. Specimens of γ-globulin (ICN) modified with Cu or Zn cations were used in final concentrations of 5.0, 0.5, and 0.05 μg/ml. In parallel, the effects of control γ-globulin samples and solutions of copper hydrosulfate (Merc) and zinc chloride with cation content corresponding to the volume of protein-bound metal were evaluated. Newcastle disease virus (NDV, 10 CPE/leukocytes) and phytohemagglutinin P (PHA; Difco; 1.0 μg/ml) served as standard IFN inductors.

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

Possible direct antiviral effect of the samples used for IFN induction was evaluated in independent experiments. The results were evaluated after 24-h incubation by the protective effect of the material towards cultured cells infected with MEMV. The samples used for IFN induction were *in vitro* tested for toxicity in special experiments.

Acid lability or stability of the resultant IFN was evaluated by treatment of IFN-containing supernatant with 20% HCl (to pH of 2) and subsequent 24-h incubation at 4°C followed by reduction (pH 7.2-7.4) with 40% NaOH. Titration was then carried out. Control samples were not treated with the acid and incubated under the same conditions as experimental samples.

The IFN neutralization test was carried out using anti-IFN-α IgG (Intecor). Standard anti-IFN-α IgG (L. A. Tarasevich Institute of Standardization and Control of Medical Viral Preparations) were used in some experiments. The antibodies were added to the studied samples with IFN activity brought to 10 U/ml in equal volume proportions in dilutions from 1:100 to 1:800 and subsequent incubation (2 h, 37°C) in plastic 24-well flat-bottom plates (Costar). Activity of antibodies was confirmed in the tests with reference IFN-α (Intecor). All experiments had appropriate controls including MEMV dose control.

The significance of differences between the means was evaluated using Student's *t* test.

RESULTS

The dynamics of IFN production indicated that IFN induced by experimental protein specimens and control samples appeared simultaneously with PHA-induced IFN (24 h) and increase in its titers in the culture medium corresponded to that in the presence of PHA.

The titers of IFN increased 1.2-1.6 times from the 24th to 48th h of the experiment and after 48 h were comparable with the titers of the cytokine induced by experimental, control sample, and PHA (Figs. 1, 2). They were incomparable with the effects of NDV: IFN production in the presence of

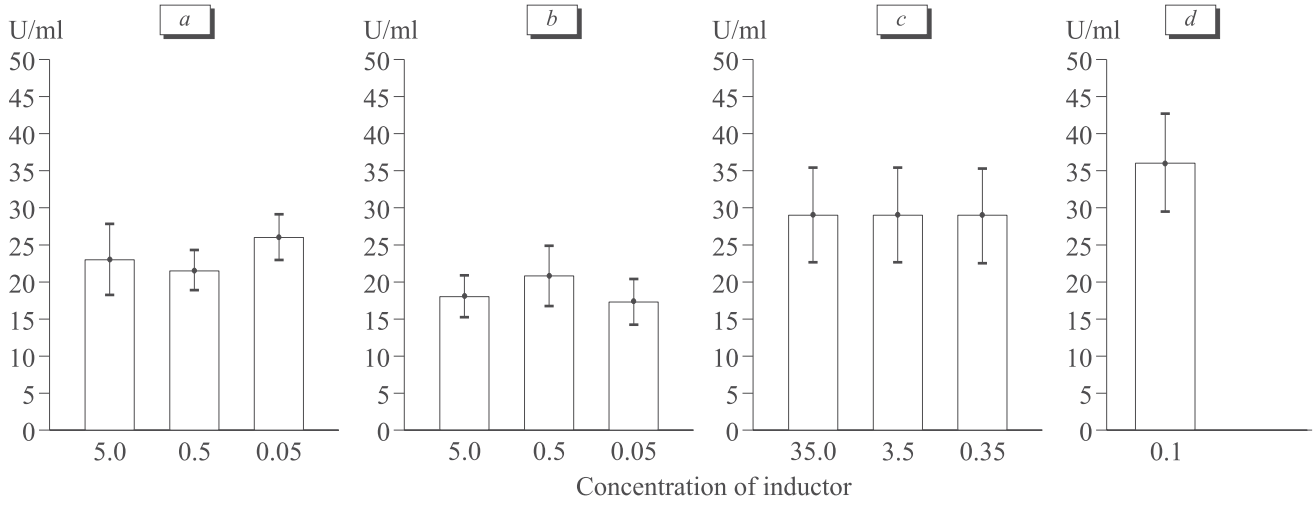

Fig. 1. Production of IFN by human blood leukocytes in the presence of γ-globulin modified by Zn cations *in vitro* (*n*=8). *a*) control γ-globulin; *b*) Zn-modified γ-globulin; *c*) zinc; *d*) PHA. Here and in Fig. 2: induction: 48 h. Abscissa: concentration of inductor (*a*, *b*, *d*: μg/ml; *c*: ng/ml); ordinate: IFN activity. Testing against 1 CPE_{50} MEMV.

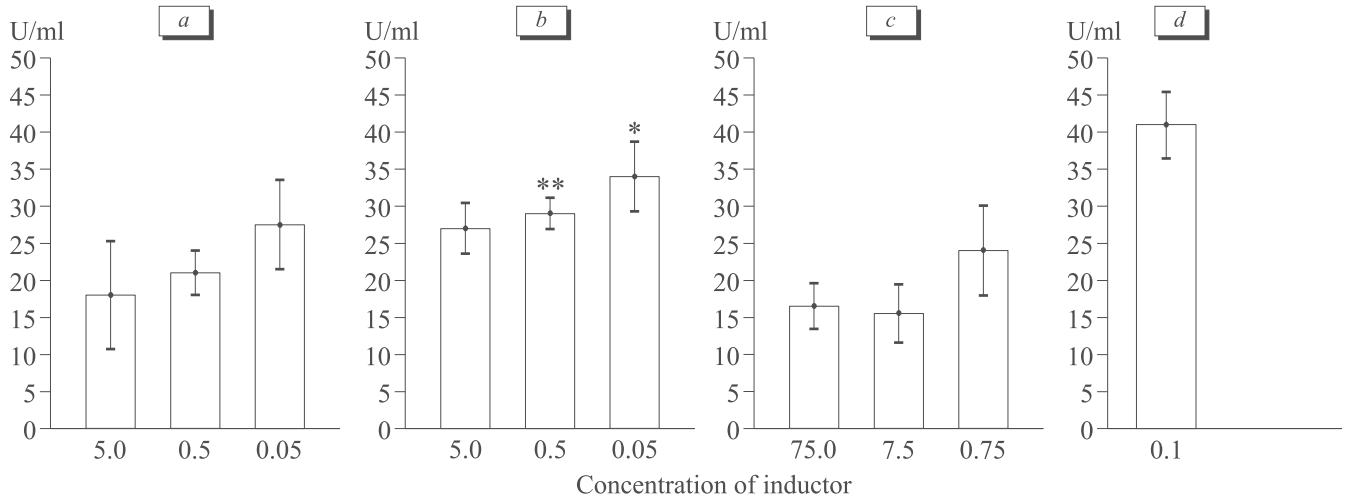

Fig. 2. Production of IFN by human blood leukocytes in the presence of γ-globulin modified by Cu cations *in vitro* (n=8). *a*) control γ-globulin; *b*) Cu-modified γ-globulin; *c*) copper; *d*) PHA. *p<0.1 compared to Zn-modified γ-globulin; **p<0.05 compared to copper cations.

this virus reached 640-680 U/ml after 24 h. The level of IFN induced by PHA 1.2-2.2 times surpassed the corresponding values in experimental samples (p<0.01 for some samples). Spontaneous (without inductors) production of IFN by human leukocytes did not exceed 4 U/ml.

The γ-globulin-Zn complex exhibited a 1.4-1.7 times lower activity in induction of late (48 h) IFN in comparison with Zn cations (p>0.1) and 1.2-1.3 lower in comparison with control protein (p>0.1; Fig. 1).

By contrast, γ-globulin complex with Cu realized higher potential: the titers of IFN produced in the presence of this complex were 1.4-1.9 times higher than production in the presence of copper (p<0.05 for 0.5 μg/ml) and 1.2-1.5 times higher than in the presence of control protein (p>0.1; Fig. 2). Copper-modified γ-globulin was 1.4-2.0 times more active than protein modified with Zn cations (p<0.1 for the dose of 0.05 μg/ml; Fig. 1, 2).

Since the studied factors possess no intrinsic antiviral activity, we conclude that the effector properties of γ-globulin realized by induction of IFN result from conformation changes induced by incorporation of metal cations into the structure of γ-globulin proteins. Zinc attenuated, while copper stimulated the IFN-inducing activity of the protein. Moreover, Zn lost its own potential of IFN inductor, while copper (presumably) realized it being a component of protein-metal complex. Hence, these changes were metal-specific.

No statistical differences were detected in the majority of observations (Figs. 1, 2). This was due to high variability of the initial values in donors.

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

IFN sample		Dilution of anti-IFN-α			
		1:100	1:200	1:400	1:800
Reference IFN-α		++++	++++	++++	++++
Reference IFN-α (reference antibodies)		++++	++++	+++	+++
After induction by	NDV	++++	++++	++++	++++
	PHA	—	—	—	+
After induction by	γ-globulin	—	—	—	±
	Zn-modified γ-globulin	—	—	—	±
	Zn	—	—	—	±
	Cu-modified γ-globulin	—	—	—	+
	Cu	—	—	—	+

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

IFN production in the presence of control γ-globulin varied from 8 to 64 U/ml. On the other hand, individual analysis showed reduced IFN production in the presence of Zn-modified proteins in comparison with the corresponding controls in 58-67% observations. The titers of IFN induced by Cu-bound γ-globulin increased in 50-67% cases.

Evaluation of IFN sensitivity to acid treatment showed the presence of acid-labile (about 60%) and acid-stable (about 40%) constituents in all the studied samples, including PHA-induced. Reduced IFN activity as a result of acid treatment was noted under conditions of induction with Zn-modified γ-globulin in 75%, with Cu-modified one in 63%, and under conditions of PHA induction in 89% cases.

Anti-IFN-α-antibodies in dilutions from 1:100 to 1:400 did not neutralize IFN induced by control and metal-modified proteins and by Zn and Cu cations alone. The protective effect of IFN-containing supernatants was fully retained in cell culture (Table 1).

The effect reproduced the absence of antibody reaction with PHA-induced IFN. The studied samples differed from those containing IFN-α (induced by NDV or a component of IFN preparations), neutralized by anti-IFN-α, which abolished its protective effect in cell culture: the cells degenerated in the presence of MEMV (Table 1).

Hence, the pool of immunoactive compounds produced in the presence of γ-globulin fraction proteins and metal cations (Zn and Cu) contains mainly the cytokine corresponding (by the sum of its properties) to IFN-γ.

Interaction of the IFN system with γ-globulins is realized through the Fc receptor (FcR) family. IFN-γ stimulates the expression of FcgRI by the cells [15] and the transduction of the triggered FcgRI signals [11,15]. The effect is caused by accumulation of FcgRI mRNA [9] and expression of the receptor γ-subunit [11], which is also a component of the macrophage FcgRIIA and lymphocyte FcgRIII structure [11]. It manifests under conditions of no more than 10 IFN molecules per cell [15].

In turn, FcgRIII reaction with IgG Fc fragment induces the transcription of genes and production of IFN-γ [12,13]. This is paralleled by more intense transcription of IL-2 genes, synergically with FcgRIII causing accumulation of IFN-γ mRNA [10].

Our previous data indicate that Fc fragments of antibody molecules undergo primary conformation under conditions of metal cation binding to γ-globulin fraction proteins. This is also seen from the results of EIA, showing significant differences in the dynamics of solid phase saturation (direct EIA) by control and Cu- and Zn-modified proteins [7]. Recognition of the samples by human specific anti-IgG in the sandwich variant reproduces the results of direct EIA [7].

The formation of a monolayer on the solid phase is determined for γ-globulin fraction proteins primarily by the status of their Fc region, and the detected differences agree with specific features of realization of IFN-inducing activities of protein-metal complexes.

In general, the involvement of antibodies in IFN-γ induction can be regarded in the context of maintenance of the basal level of immunoregulation in the absence of antigenic challenge. The physiological nature of this model is confirmed by the conditions of experimental samples preparation, their activity realized at low concentrations and detectable in an infected cell culture only against a low virus dose (the produced IFN is not titered against 10 CPE_{50} MEMV), and absence of the resultant IFN toxicity towards cell culture.

For the studied interactions, we can rule out with high probability the adsorption of metal by molecules of protein binding it and inducing cation effects directly on the membrane structures of effector cell or metal-dependent formation of IFN dimers, characterized by a different (in comparison with monomers) antigenic specificity and reducing the clinical efficiency of IFN dosage forms containing them [1]. This conclusion is based on a drastic reduction of the capacity of metal binding γ-globulin to aggregation in solution, shown by EIA; hence, there are no free or easily exchanged metal cations in the perimembrane space of IFN-producing cells [6].

Hence, conformation changes of antibody molecule Fc fragments by metal cation binding can serve as the natural factors regulating the flow of intracellular signals triggered by activated FcR and normally providing IFN-γ induction.

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