# IMMUNOLOGY AND MICROBIOLOGY

# Production of Late IFN-α Induced by Plasma γ-Globulin Fraction Proteins and Their Metal Complexes

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Plasma γ-globulin fraction proteins, copper and zinc cations, and metal complexes they form with human serum γ-globulin induce the production of IFN-α by human blood cells throughout the periods of up to 72 h. Zinc cation-modified protein by 1.6 times ($p<0.05$) more actively induces late IFN-α than the control γ-globulin; γ-globulin-copper metal complex is 2-fold ($p<0.002$) more effective than the control protein. The results indicate that functional relationships between the components inducing the production of late IFN-α differ from the effects realized during the early period of induction.

**Key Words:** *IFN-α; induction; γ-globulins; metal complexes*

According to current notions on the factors and mechanisms of Th1/Th2 polarization of the immune response, IFN-α is not the cytokine determining the immunogenesis direction [7,8]. On the other hand, the production of IFN-α at the beginning of the inductive phase of the immune reaction, its relationship with the production of immunoactive cytokines (including IFN-γ), and involvement in differentiation of cytotoxic effectors suggest this cytokine not only as a factor of antiviral defense, but also as a component of natural physiological immunoregulation. Type I IFN in complex with IL-12 is assumed to play an important role in the formation of functional relationships supporting the development of Th1 response and immunological memory [1,13].

We found that IFN-α is present in parallel with IFN-γ in the IFN pool induced by plasma γ-globulin fraction proteins and their metal complexes with copper and zinc cations obtained and applied under conditions approximating by some parameters the physiological ones [3-5]. It is present in human leukocyte culture fluid during the early (24 h) period of induction with some increment in the content after 48 h [4,5].

Analysis of correlations showed that antiviral activity of the total IFN pool is mainly due to the presence of IFN-α in the pool [5]. The cytokine induction is metal-specifically regulated by copper and zinc cations initially transforming the Fc region of plasma γ-globulin fraction proteins [2] and promoting triggering of intracellular signal routes other than those stimulated by cellular Fc receptors (FcR) during binding of Fc fragments in their native conformation [3-5].

We evaluated the production of late (72-h cell incubation) IFN-α in the presence of γ-globulin fraction proteins, their metal complexes with copper and zinc, and copper and zinc cations alone.

## MATERIALS AND METHODS

Induction of IFN in suspensions of cells from human peripheral venous blood ($10^6$ cell/ml) was carried out

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in complete nutrient medium based on double Eagle's medium (M. P. Chumakov Institute of Poliomyelitis and Viral Encephalitis) with 2% donor plasma, L-glutamine (from the set attached to a flask of medium), gentamicin (20 U/ml), and heparin (to 5 U/ml) for 72 h at 37°C in humid atmosphere with 5% $CO_2$ in flat-bottom 24-well plates (Costar).

Specimens of copper or zinc cation-modified human serum γ-globulin (initial reagent from ICN) were used in a final concentration of 0.5 μg/ml. In parallel, we evaluated the effects of control γ-globulin specimens and copper (hydrosulfate, Merc) and zinc (chloride) solutions, cation content in which corresponded to the quantity of metal bound to protein at the stage of preparations of experimental samples. Newcastle disease virus (10 CPE/cell) and PHA P (Difco, 1 μg/ml) served as the standard inductors of IFN production.

Titration of IFN was carried out on a multilayer culture of diploid human embryo fibroblasts (Medical Genetic Center, the Russian Academy of Medical Sciences) against 1 $CPE_{50}$ mouse encephalomyocarditis virus in plastic flat-bottom 96-well plates (Nunclon or Costar). The initial cell concentration in suspensions was $2\times10^5$/ml.

The content of IFN-α in supernatants of induced cells was evaluated by EIA using ELISA Processor II (Behring). The EIA kits (Vector-Best) were used according to the instruction with extra technological controls.

The results were processed using Student's *t* test for evaluating the significance of differences between the means.

## RESULTS

The data indicate that the pool of late (72 h) IFN produced in the presence of control and metal cation-modified γ-globulin and copper and zinc cations alone contained 5.87±0.66 to 21.55±1.51 pg/ml IFN-α. Spontaneous production of the cytokine by blood cells during this period was 2.35±0.23 pg/ml.

Zinc-transformed protein induced 1.6-fold ($p<0.05$) higher production of IFN-α than control γ-globulin (Fig. 1). Zinc cations used alone exhibited similar activity (Fig. 1). Copper-modified protein 2-fold more effectively induced late IFN-α ($p<0.002$) than control γ-globulin (Fig. 2). Copper cations used alone induced the production of late IFN-α 1.2-fold more effectively ($p<0.1$) than γ-globulin metal complex with copper and 2.4-fold ($p<0.001$) more effectively than control protein (Fig. 2).

Similarly as in evaluation of IFN pool induced in experimental system for 24 and 48 h, γ-globulin complex with copper was more active (in the present study 1.9-fold more active, $p<0.01$) than zinc cation-

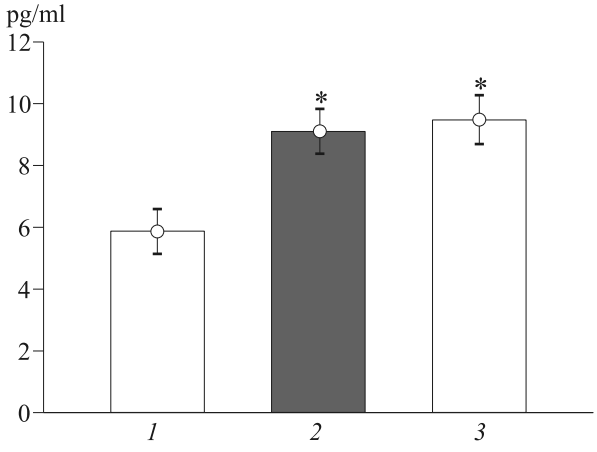


**Fig. 1.** Production of late IFN-α by human peripheral blood cells under conditions of induction with γ-globulin fraction proteins and their metal complexes with zinc (*n*=8). Ordinate: IFN concentrations. *1*) zinc control γ-globulin (0.5 μg/ml); *2*) zinc modified γ-globulin (0.5 μg/ml); *3*) zinc (2.5 ng/ml). *$p<0.05$ compared to control γ-globulin.

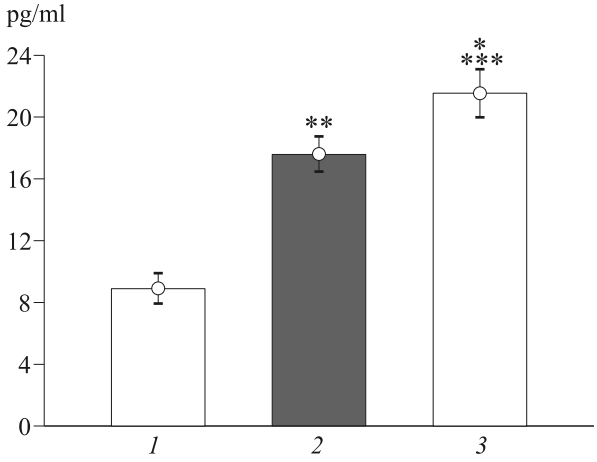


**Fig. 2.** Production of late IFN-α by human peripheral blood cells under conditions of induction with γ-globulin fraction proteins and their metal complexes with copper (*n*=8). Ordinate: IFN concentrations. *1*) copper control γ-globulin (0.5 μg/ml); *2*) copper modified γ-globulin (0.5 μg/ml); *3*) copper (1.0 ng/ml). *$p<0.1$ *vs.* copper modified γ-globulin; **$p<0.002$, ***$p<0.001$ compared to control γ-globulin.

transformed protein (Figs. 1 and 2) [4,5]. Calculations with consideration for differences in activities of copper and zinc control proteins showed that the amount of late IFN-α induced by copper-chelating γ-globulin was by 3.8 pg/ml (or by 42%) greater than that induced by zinc cation-bound protein.

In contrast to 24- and 48-h induction [3-5], cations of both metals and their complexes with γ-globulin exhibited higher IFN-inducing activities than the control proteins (Figs. 1, 2). This changed the ratio of activities of regulatory system components in the pool of late (72 h of induction) IFN and eliminated the relationship between the content of IFN-α and antiviral activity of the samples. No "zinc" drop and "copper" peak described for 24- and 48-h induction were ob-

served, when zinc and copper cation-modified proteins presumably were less or more, respectively, effective inductors of IFN-α production than control γ-globulins and metals alone [4,5].

It was previously found that the relationship between antiviral effects of the samples and the levels of IFN-α and IFN-γ in them was established for the early (24 h) period of induction and was characterized by metal specificity [4,5]. The correlation was retained after 48 h of induction for IFN-α and lost for IFN-γ because of lower activity of copper cation modified protein compared to the control [3,5]. We thought that during this period IFN-γ switched over to realization of biological effects other than its antiviral effect [5].

Under these conditions the contribution of IFN-γ to antiviral activity of samples, which it mediated synergically with IFN-α, was to reduce [6]. The maintenance of a certain level of cellular antiviral status required compensation, presumably at the expense of more intense production of IFN-α. The latter event became possible because zinc cations initiating the production of IFN-α [9] and IFN-γ [10] and potentiating the antiviral effect of IFN-α, but not IFN-γ [9], acted as factors of IFN-α induction in the late IFN pool not only if used alone, but also as components of the protein metal complex (Fig. 1).

It is known in the common immunoregulation context that IFN-α induces the synthesis of metallothioneins (MT) binding zinc cations and reducing zinc content in the plasma by liver cells [11]. MT bind zinc more effectively than copper [12]. Copper cations, less involved in MT binding, can act as IFN-α inductors under conditions of unfolding zinc deficit [12]. Comparison of our data (Figs. 1, 2) showed that in contrast to early induction [4,5], zinc and copper in the late IFN pool acted as components of protein metal complexes similarly, while copper alone induced the production of 2.3 times ($p<0.001$) higher levels of IFN-α than zinc. The direct comparison is justified, because the same cell suspensions served as controls for copper and zinc.

Prolongation of IFN-α induction by γ-globulin fraction proteins to late (72 h) periods and the presence of the cytokine in the late IFN pool were presumably caused by involvement of intracellular signal routes stimulated by FcR. IFN-α and IFN-β were induced in mouse dendritic cell culture by human IgG [14]. The specificity of IgG binding to mouse FcγR corresponded to that of human cells [14]. γ-Globulin metal complexes used in our study had initially transformed molecular Fc regions [2] and, according to previous data, realized the IFN-inducing potential by stimulation of FcR [2,3].

Recent findings [4,5,14] and our results suggest that induction and support of a certain antiviral status in a cell are determined by the antique mechanisms of immunoregulation, involved in metal cation transport and metabolism in cell microenvironment. Copper and zinc induce the production of IFN-α prolonged to the period of unfolding of specific cellular reactions, not only directly, but also as components of metal complexes with γ-globulin acting through cellular FcR. The development of Th1 response and formation of memory T-cells are thus stimulated [1,13].

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