Mouse Splenocyte Blast Transformation in the Presence of Plasma γ-Globulin Fraction Proteins and Their Complexes with Copper and Zinc

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 150, No. 11, pp. 555-558, November, 2010
Original article submitted May 7, 2009

Plasma γ-globulin fraction proteins, copper and zinc cations, and metal complexes formed by these cations and human serum γ-globulin induce blast transformation of splenocytes from BALB/c mice at a level comparable to that induced by concanavalin A. Zinc bound to γ-globulin reduces by 25% and copper in complex with this protein stimulates by 1.6 times its capacity to induce blast transformation. Combinations with concanavalin A reproduce the effects of γ-globulin–metal complex under conditions of mitogen induction. Incorporation of ^{3}H-thymidine in splenocytes incubated with combinations of γ-globulin–copper metalcomplex, copper cations, and control protein with concanavalin A was by 1.4, 1.3 ($p<0.1$), and 1.25 times higher ($p<0.05$), respectively, than after incubation with concanavalin A alone.

Key Words: *splenocytes; blast transformation; γ-globulins; zinc; copper*

Recent studies have appreciably extended our knowledge of the role of copper and zinc cations in physiological immunoregulation [10,12-14]. Under conditions of chelating by plasma γ-globulin fraction proteins, these cations induce conformation changes in the protein globule, initially involving the spatial configuration of Fc fragments of the antibody molecule [6]. The resultant new protein conformation is sufficiently stable [6] and is realized under conditions of antibody molecule interactions with lymphocyte Fc receptors (FcR) by triggering of intracellular signal pathways not stimulated by Fc fragments in their native conformation [4,5,7].

It was shown that human serum γ-globulin stimulates spontaneous blast transformation (BT) of human lymphocytes *in vitro* [2,3]. The capacities of individual IgG subfractions to stimulate BT correlated with their affinity for transition metals [1]. It was assumed that at certain protein/metal molar proportions in solution, copper or zinc cations formed bridges between individual protein molecules and caused the formation of supramolecular IgG aggregations inducing BT [1]. The release of carbohydrate-rich components directly stimulating lymphocyte proliferation was assumed to be one of the factors providing BT induction [3].

Recent biological testing and EIA studies have shown that chelating of copper and zinc cations by γ-globulins metal-specifically modifies the effector characteristics of proteins towards human blood cells producing IFN-α and IFN-γ [4,5,7].

These effects were recorded in an experimental system ruling out with high probability the formation of supramolecular γ-globulin aggregations [4] and hence, the concomitant exposure of carbohydrate-rich components of the protein molecule [3]. It was therefore hypothesized that induction of lymphocyte BT in the presence of metal-chelating IgG subfractions [1] could also be realized through the direct effect of metal-transformed protein Fc region on cellular FcR.

Here we evaluated mouse splenocyte BT in the presence of γ-globulin fraction proteins, copper and

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0007-4888/11/1505-0624

zinc cations, and metal-bound (and hence metal-modified) γ-globulins.

MATERIALS AND METHODS

Splenocytes were isolated from BALB/c male mice (16-18 g) from Stolbovaya Breeding Center. Fragments of the spleen were grinded and homogenized, washed (65*g*, 10 min, with cooling) with nutrient medium RPMI-1640 (M. P. Chumakov Institute of Poliomyelitis and Viral Encephalitis) containing 10% FCS (PAA Laboratories), 10 mM HEPES buffer (PanEco), and 50 μg/ml gentamicin.

Blast transformation test was carried out with initial splenocyte suspensions (5×10^6 cells/ml in 1 ml complete nutrient medium (CNM) on the basis of RPMI-1640 with above-listed additives and additionally containing 2 mM L-glutamine (PanEco) and 5×10^{-5} M 2-mercaptoethanol (BDH Chemicals Ltd.).

Splenocyte suspension (5×10^5 cells) in CNM was transferred (0.1 ml) to wells of 96-well flat-bottom plastic microplates (Nunc), 0.1 ml test samples in CNM was added, and the mixture was incubated at 37°C for 72 h in a humid atmosphere with CO_2 in a Heraeus CO_2 incubator. The label (^{3}H-thymidine; 10 μCi/ml) was added to the microplate wells 16 h before the end of incubation.

After incubation the contents of the wells was precipitated on fiber glass filters with 2.5-μ pores (Whatman) by Titertek 12-channel biological fraction collector (Flow). Residual radioactivity of the filters was evaluated in 5 ml toluene scintillator on an LKB-1215 RackBeta β-counter (1 min counting time).

Specimens of human serum γ-globulin (ICN) modified by metal cation binding were used in appropriate volumes of CNM in final concentrations of 0.5 and 0.05 μg/ml. The effects of control protein specimens in CNM and of CNM with copper and zinc salts in amounts corresponding to the content of metals bound to protein at the stage of experimental samples preparation were evaluated.

Control cell suspensions (spontaneous BT) contained an appropriate volume of CNM without test samples and CNM with 4.0 μg/ml ConA (Sigma). Each sample was tested in 3 parallel wells of the microplate.

The data were statistically processed using common methods of variation statistics. The significance of differences in the means was evaluated using Student's *t* test.

RESULTS

Specimens of γ-globulin transformed by binding of copper and zinc cations and control proteins, as well as by metal cations alone, induced mouse splenocyte BT (Fig. 1). The level of induction was 1.5-2.5 times higher in comparison with ^{3}H-thymidine incorporation under conditions of spontaneous BT and 1.2-2.0 times lower than in the presence of ConA.

Zink-transformed protein realized a 25% lesser inductor potential than control γ-globulin and 1.5-fold lower potential than zinc cations alone (Fig. 1). Copper-modified protein was 1.3-fold more active than copper, 1.6-fold more active ($p<0.05$) than control γ-globulin, and 1.3-fold more active than zinc cation-modified protein (Fig. 1). By the level of BT induction, "copper" γ-globulin was comparable to ConA: the significance of differences *vs*. ConA ($p<0.002$, $p<0.001$), recorded for control proteins, was lost.

Similarly, γ-globulin with bound zinc cations determined a lesser antiviral activity in human leukocyte culture in comparison with protein and cation controls

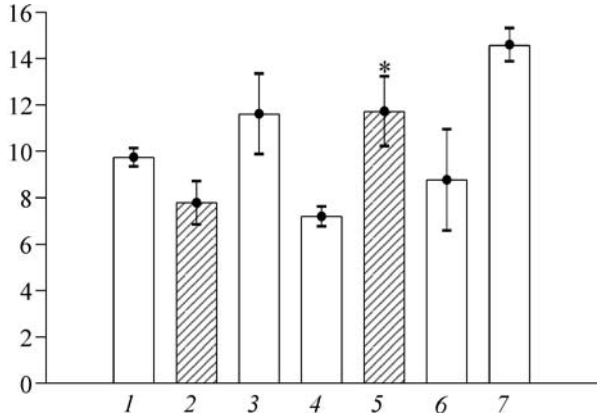

Fig. 1. *In vitro* BT of BALB/c mouse splenocytes in the presence of human serum γ-globulin (*1*, *4*), its metal complexes with zinc (*2*) and copper (*5*), and zinc (*3*) and copper (*6*) cations alone (*n*=6). Abscissa: *1*, *2*: 0.05 μg/ml; *3*: 0.25 ng/ml; *4*, *5*: 0.5 μg/ml; *6*: 0.625 ng/ml; *7* (ConA): 4.0 μg/ml. *$p<0.05$ *vs*. control γ-globulin (*4*). Here and in Fig. 2: ordinate: ^{3}H thymidin incorporation, cpm×10^{-3}.

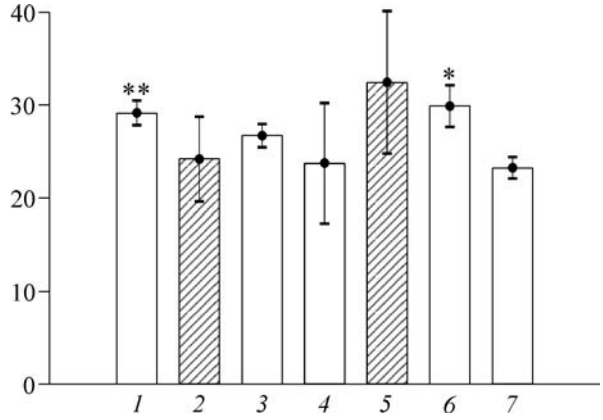

Fig. 2. *In vitro* BT of BALB/c mouse splenocytes in the presence of ConA combinations with human serum γ-globulin (*1*, *4*), γ-globulin metal complexes with zinc (*2*) and copper (*5*), zinc (*3*) and copper cations (*6*) (*n*=6). Abscissa: *1*, *2*, *4*, and *5*: 0.05 μg/ml; *3*: 0.25 ng/ml; *6*: 62.5 pg/ml; *7* (ConA): 4.0 μg/ml. *$p<0.1$, **$p<0.05$ *vs*. ConA (*7*).

[4,5] and induced less intense production of IFN-α and IFN-γ [5,7]. By contrast, antiviral activity of the samples increased in the presence of copper-modified γ-globulin [4,5], similarly as IFN-α and IFN-γ production [5,7].

The same regularities were observed, if the test samples were used in combination with ConA (Fig. 2). Zink cation-modified protein was 10-20% less active than control γ-globulin and zinc cations. The γ-globulin metal complex with copper induced a 10-40% increase of ^{3}H-thymidine incorporation by splenocytes in comparison with the protein and cation controls and induced BT at a level 1.3-fold surpassing that induced by zinc-transformed protein (Fig. 2). The effects of ConA combinations with "copper" γ-globulin was 1.4-fold higher, with copper cations 1.3-fold higher ($p<0.1$), and with zinc control protein 1.25-fold higher ($p<0.05$) in comparison with ConA alone (Fig. 2).

The effects of copper and zinc cations on BT processes in the immune system are attributed to the direct effects of metals on mouse [14] and rat [8] splenocytes and on human lymphocytes [10,11,13]. Copper and zinc act as growth and proliferation factors in T-lymphocyte culture [9,12,14], stimulate spontaneous BT [14] and BT reaction to phytomitogens [8-10], stimulate the expression of IL-2 receptor [14]. Transcription of IL-2 gene and IL-2 production by T-lymphocytes are inhibited [11,12,14] and IFN-γ gene transcription in response to PHA induction is reduced [9] under conditions of cation deficit.

The involvement of copper and zinc in conformation of antibody molecules Fc region has been detected quite recently [6]. Causing these conformations, the metal cations can indirectly modify the time course and intensity of cell response to γ-globulin stimulation of their FcR [4,5,7].

Our data are in good agreement with the results of evaluation of activities of γ-globulin metal complexes with copper and zinc towards cells producing IFN-α and IFN-γ [4,5,7]. Similarly as in previous studies, we used an experimental model for evaluating cell responses under conditions approximating physiological conditions by many parameters [4].

In the above modes, γ-globulin metal complexes with zinc act as splenocyte proliferation inhibitors, while copper complexes act as stimulants of this process at the basal immunoregulation level (Fig. 1, 2). Importantly that these characteristics of metal-transformed proteins are also realized in the forced mode (in combination with ConA). The effects of experimental samples and their controls were cumulative with ConA effects in the majority of observations (Fig. 1, 2). It seems that intracellular signaling routes maintaining splenocyte proliferation in response to γ-globulins (or metal cations) and ConA are largely independent and are realized autonomously.

The fact of obtaining these results in a heterologous experimental system (human γ-globulin and mouse splenocytes) and their agreement with the results of homologous experiments (human γ-globulin and human leukocytes) indicate a universal nature of immunoregulation, mediated by γ-globulin metal complexes, and are in line with the concepts on the evolutional antiquity of the regulatory mechanisms involved exclusively in metal cation transport and metabolism in cell microenvironment.

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