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Regulation of *in Vitro* Human Lymphocyte Blast Transformation Associated with γ-Globulin Fraction Containing a Carbohydrate Component

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Carbohydrate-enriched components were isolated from human serum γ-globulin by chelation of associated transition metals. Similar to the initial γ-globulin preparation, these components stimulate *in vitro* blast transformation of human lymphocytes from healthy donors and inhibit subsequent phytomitogen-induced lymphocyte blast transformation.

Key words: *γ-globulin; carbohydrate component, lymphocyte blast transformation*

The ability of γ-globulin to maintain a definite level of human lymphocyte blast transformation (LBT) *in vitro* [3] is known to depend on the expression of Fcγ-receptors in cell membrane [8,12]. The effect of LBT stimulation was shown to correlate with the presence of protein aggregates in the γ-globulin preparation [3].

The effector functions of γ-globulin also depend on the presence of carbohydrate-containing fraction bound to polypeptide chains via non-covalent bonds [1]. Carbohydrate component (CC) is associated with γ-globulin molecule via metal ions of alternating valency [1]. These cations interact with specific sites of γ-globulin molecule [13,14] and stimulate aggregation by forming bridges between protein molecules at a certain metal/IgG ratio. A positive correlation between IgG affinity to transition metal and the effect of protein on spontaneous LBT was found [2].

γ-Globulin aggregation is accompanied by significant changes in its conformation stability and enhances the release or exposure of components not bound covalently to polypeptide chains.

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Chelating agent releases CC-containing fraction from γ-globuline molecule, which possesses some properties unrealized by the native molecule [1]. It can be suggested that this fraction stimulates LBT and mediates protein action under condition of its aggregation [3].

MATERIALS AND METHODS

Mononuclear cells (MNC) were isolated from peripheral venous blood of 4 healthy men aged 20-46. The cells were separated in Ficoll-Verografin (d=1.077 g/cm^3) one-step density gradient and MNC were harvested from the interface layer. After repeated washout with medium 199 MNC were resuspended in complete nutrient medium (CNM) consisting of RPMI-1640 (Flow) supplemented with 12% fetal calf serum (Hamaleya Institute of Epidemiology and Microbiology), 2 mM glutamine and 40 μg/ml gentamicin (Pharmachim) to a concentration of 2×10^6 cells/ml. To initiate spontaneous LBT, 0.1 ml MNC suspension was applied to round-bottom 96-well plates, 0.1 ml CNM and 10 μCi/ml ^{3}H-thymidine were added to each well and the plates were incubated for 18 h at 37°C in humidified atmosphere containing 5% CO_2. LBT was induced by 2 μg/ml phytohemagglutinin M (PHA, Serva) or 1 μg/ml con-

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canavalin A (ConA) in 0.1 ml CNM. MNC incubated under the same conditions without inductor served as the control. After incubation the content of the wells was collected with a 12-channel Titertek harvester (Flow) and sedimented on glass fiber filters with pore diameter of 2.5 μ (Whatman). Residual radioactivity was counted in 5 ml toluene scintillator on a MARK-II β-counter during 1 min.

For chelation of transition metals, human serum γ-globulin (Serva) was dissolved in 0.1 M buffered physiological saline (pH 7.4) to a concentration of 100 μg/ml and applied to a Dowex column (Serva) pretreated with 0.1 N HCl (1:1 v/v ratio). Eluate obtained by repeated washing of the column with 10-fold volume of a buffer (in relation to the protein volume) was filtered through Centriflo CF-25 molecular ultrafilters (Amicon) and the filtrate was concentrated on a Diaflo UC-05 molecular filter (Amicon). Spectral characteristics of the fractions were studied with a PU 8730 UV/VIS scanning spectrophotometer (Philips). Protein content was measured by absorption at 280 nm (extinction coefficient 0.7). The content of carbohydrate component was estimated by glucose equivalent according to Groger. CC-containing fraction released from γ-globulin was added to MNC suspension for 1 h at 37°C followed by washing. Simultaneously, the effect of native human serum γ-globulin (Serva) on LBT was examined.

The significance of differences between the mean values was estimated with Student's *t* test.

RESULTS

It was previously shown that chelating agent induces the release of a fraction from human serum γ-globulin characterized by UV absorption spectrum differing from native protein, 3-fold higher CC content, molecular weight below 30 kD, antigen properties, and heparin-like activity. These characteristics distinguish this fraction from the main IgG proteolytic fragments [1].

Our experiments showed that treatment of MNC from healthy donors with CC-fraction of human serum γ-globulin gradually increased the mitogenic effect, while the dose of active component decreased from 1200 to 60 ng/ml (Fig 1, *a*). LBT was stimulated in 8 of 11 cases (72.2%) and reached the level observed under the effect of PHA in 7 cases. γ-Globulin increased the intensity of spontaneous LBT enhancing ^{3}H-thymidine incorporation into MNC in concentrations of 70 and 350 ng/ml (Fig. 1, *b*). In the whole, LBT stimulation by native γ-globulin was noted in 9 of 10 cases (90%). It reached the level corresponding to effects of PHA and ConA in 5 and 2 cases, respectively.

In the above mentioned concentration range γ-globulin fraction reduced the effect of subsequent blastogenesis induction with PHA by 75% (in 6 of 8 cases) and decreased isotope incorporation into human MNC by on average 10% (sometimes by 23%). Similar effect of γ-globulin was observed in 5 of 8 cases (62.5%), but was less pronounced: maximum inhibitory effect of PHA-induced LBT reached 15%.

The inhibition of ConA-stimulated proliferation of human MNC *in vitro* by CC-fraction was less pronounced as compared to the effect of native protein. CC-fraction decreased ^{3}H-thymidine incorporation induced by ConA in 4 of 8 cases (50%) on average by 12% (maximally by 19%). At the same time, γ-globulin decreased the mitogenic effect of ConA in 6 of 8 cases (75%) by 26% on average and by 36% in some experiments. ConA-induced ^{3}H-thymidine incorporation in MNC incubated with 35 and 17.5 ng/ml γ-globulin was 5.26±0.50(×10^3) and 4.70±1.09(×10^3) cpm, respectively, *vs.* 7.33±0.83(×10^3) in control cells. These values did not differ significantly from proliferation level in MNC suspension without mitogen stimulation.

The obtained results point to the presence of CC in γ-globulin molecule, which can realize mitogenic effect of conformed protein and decrease the effect of phytomitogens. Unlike native protein, CC-fraction does not block completely phytomitogen-induced LBT.

The concentration-activity dependence for CC-fraction [1] shows that 60 ng/ml CC-fraction and 70 ng/ml γ-globulin cause similar changes in spontaneous LBT *in vitro* varying depending on the redistribution of peptide components and CC-fraction in the column with chelating resin.

It should be noted that isolation of CC-fraction from γ-globulin was performed under relatively rigid

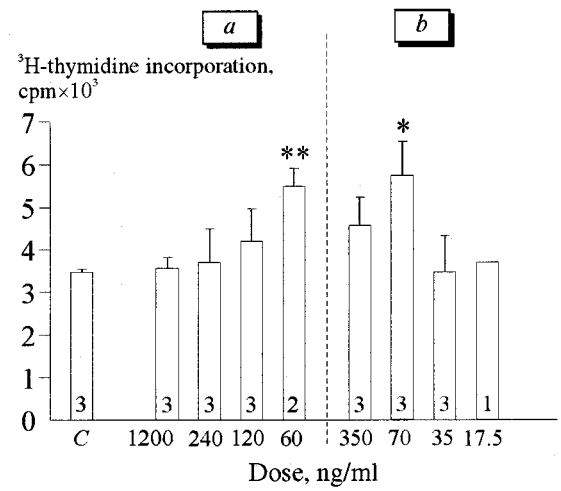

Fig. 1. Effect of carbohydrate-containing fraction isolated from human serum γ-globulin by chelation of associated transition metal ions on spontaneous *in vitro* blast transformation of donor lymphocytes (*a*) in comparison with native γ-globulin preparation (*b*). *p<0.05; **p<0.02 compared to the control (*C*). The number of experiments is indicated in columns.

conditions. Nevertheless, intra- and intermolecule changes accompanying cation-dependent γ-globulin aggregation can be regarded as conformation transformations of the protein molecule inducing CC liberation. This assumption is supported by pronounced spontaneous LBT stimulation *in vitro* by IgG subfraction binding Cu^{2+} from the solution [2]. Theoretically, γ-globulin aggregation can promote the formation of exposed and free CC in concentrations exceeding the carbohydrate content in protein molecule and sufficient for manifestation of their effector functions [3]. Aggregated γ-globulin stimulated human LBT *in vitro* in a concentration of 50 ng/ml, which corresponds to the concentration of active component of CC-fraction [3].

Thus, adsorption of transition metals associated with γ-globulin molecule by chelating resin and closing of intra- and intermolecular cationic bonds during forced protein aggregation induce the release of CC. Spontaneous aggregation typical of γ-globulin under physiological conditions suggests that the pool of this protein includes a number of fractions varying in peptide/carbohydrate ratio.

Thus, apart from its role in specific humoral immune reactions and complement-dependent processes, γ-globulin appears to be a transport protein that associates, conforms, and maintains CC in the circulation, possesses a heparin-like activity [1], and regulates human LBT. Since bonds essential for γ-globulin transport functions are effected via transition metals, any influence on protein molecule either in a model system, or in the circulation (for example, contact with lymphocyte Fc-receptor) changes coordination bonds and induces conformation changes. These changes may result in destruction of the molecule skeleton, liberation of CC fraction, and generation of CC-mediated humoral and cell reactions.

Metals play an important role in a number of cell and humoral immune reactions. Free metals inhibit functional activity of immunocompetent cells. Cytotoxicity of murine lymphocytes decreased in the presence of Zn^{2+} ions [6]. The excess of iron ions suppresses activity of human lymphocytes *in vitro* [5,11], inhibits cell functions in talassemia patients [4], and interferes with leukocyte bactericidal activity [9] and cell-mediated immunity [10]. Iron is also associated with sensitivity to bacterial infections [7]. In light of this, the ability of γ-globulin to bind transition metals forming intra- and intermolecule bonds, thus reducing metal content in the blood, can be regarded as an effective antioxidant protective mechanism underlying metal redistribution and release under certain conditions formed by the contact of microenvironment factors with cell membrane.

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