Activity of Human Lymphocytes in the Presence of Carbohydrate-Containing Compounds

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Treatment with chelating agent abolished stimulation of human lymphocyte blast transformation observed in the presence of native microbial glycoproteins. Heparin passed through a column packed with aluminum hydroxide inhibited cytotoxic activity in natural killer cells. We discuss the possibility of regulation of cell functions via modulation of the vector of transport and exchange of metal cations in cell microenvironment and the role of macromolecular compounds containing carbohydrate components in this process.

Key Words: *lymphocyte activity; carbohydrate components*

Carbohydrate-rich components associated with the molecule of human γ-globulin, *in vitro* stimulate lymphocytes blast transformation (LBT) and decrease activity of human natural killer cells (NKC) similarly to native γ-globulin [7-9]. Incorporation of these components into the structure of γ-globulin molecule is related via the formation of noncovalent (coordination) bonds between metal cations [1,2].

It can be hypothesized that integration into the γ-globulin molecule is associated with more compact packing of carbohydrates [1,6], while aggregation of γ-globulin is associated with more compact conformation of the protein with the formation of additional bonds between polypeptide chains and domains of the secondary and tertiary structure. These changes are accompanied or result in the exposure of carbohydrate-containing components [7,8].

A correlation was found between mitogenic activity of γ-globulin, protein affinity for transition metals, and protein aggregation capacity [3,4,9]. Metal cations are considered as a factor that determines conformational changes in carbohydrate-containing molecules and realization of effector function under specific conditions. Polyanionic structures formed by free glycans can be stabilized by chelating metal cations from the microenvironment.

Probably, conformational changes in macromolecules resulting from binding or release of cations during their interaction with other molecules modulate the regulation of human lymphocytes.

Here we modified carbohydrate-containing macromolecules (chelation of metal cations associated with aqueous fraction of microbial glycoproteins and treatment of heparin with excess metal) and studied their effector function at the cellular level.

MATERIALS AND METHODS

Human lymphocytes were isolated from the peripheral venous blood of 7 healthy donors (women, 25-46 years) in a Ficoll-Verografin single-step density gradient. Cytotoxic activity of NKC was studied by the standard radiometric method using ^{3}H-uridine-labeled human K-562 erythromyeloblasts. The effector/target cell ratio varied from 100:1 to 12:1. Lymphocytes and target cells (TC) were incubated at 37°C and 5% CO_2 in a humid atmosphere for 14 h. After incubation, the cells were precipitated on fiberglass filters. Radioactivity was counted using a toluene scintillator. The area under the cytotoxicity curve served as the integral parameter of NKC activity (arb. units).

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The reaction of spontaneous LBT was conducted using ^{3}H-thymidine. The suspension of lymphocytes was treated with ^{3}H-thymidine at 37°C and 5% CO_2 in a humid atmosphere for 18 h. The cells were precipitated on fiberglass filters. Radioactivity was counted using a toluene scintillator.

The fraction of glycoproteins (2-5 kDa, 75 μg/ml protein by UV absorption, 20 μg/ml sugars by the Groger glucose equivalent) was obtained by concentration of water samples contaminated with microbial products on a Diaflo UC-05 molecular ultrafilter (Amicon). This fraction was passed through a column packed with DOWEX chelating resin (Serva, volume ratio 1:1). Spectral characteristics of the native and chelating resin-treated fraction were studied in UV light using a PU 8730 UV/VIS scanning spectrophotometer (Philips). The material was equilibrated by salt content and pH with a 10-fold volume of RPMI-1640 nutrient medium (Gibco) and used in dilutions of 1:20, 1:100, 1:500, and 1:1000. Spontaneous LBT was performed at 37°C, 5% CO_2 in a humid atmosphere for 18 h.

Heparin (300 μg/ml, Spofa) was passed through a column with elutriated and washed with deionized water aluminum hydroxide (heparin/hydroxide precipitate volume ratio 1:1). Native and modified heparin samples were equilibrated by salt content and pH and used in doses of 1.0 and 10.0 μg/ml. The lymphocyte suspension was treated with heparin samples (37°C, 1 h) and washed before the cytotoxic test.

The results were analyzed by Student's *t* test.

RESULTS

Adsorbtion of some components on the chelating resin completely blocked the mitogenic effect of microbial glycoproteins. The study with the material in dilutions of 1:100, 1:500, and 1:1000 revealed a tendency to inhibition of human lymphocyte proliferation *in vitro* (Fig. 1). On the whole, the native fraction stimulated LBT in 10 of 11 samples. Microbial glycoproteins treated with chelating resin stimulated blastogenesis in only 1 of 10 samples. For the most active dilution 1:20 adsorption decreased ^{3}H-thymidine incorporation into cells from 2.13×10^3 to 1.32×10^3 cpm (for dilution 1:500 from 1.43×10^3 to 0.77×10^3 cpm, Fig. 1).

The degree of LBT variability differed by 2 times after treatment of cells with native and chelating resin-modified fraction: the error of the mean to the mean ratio was 0.29-0.54 and 0.13-0.25, respectively. These data suggest that rigidity of lymphocyte population develops in the presence of chelated fractions. The mean level of LBT variability (0.18) decreased more than 2-fold compared to that observed in control suspensions (0.39).

Microbial glycoproteins and carbohydrate components associated with γ-globulin exhibit anticoagulant activity, which is similar to that of heparin [1,2]. We found that heparin treated with aluminum hydroxide suppresses natural killer activity of lymphocytes. Native heparin in a dose of 10.0 μg/ml had no effect on cytotoxicity of human lymphocytes *in vitro*. Heparin in a dose of 1.0 μg/ml decreased activity of NKC in 3 of 6 samples (Fig. 2). Activity of the preparation treated with aluminum hydroxide and applied in a dose of 10.0 μg/ml was similar to that of 1.0 μg/ml native heparin. Modified heparin in a dose of 1.0 μg/ml decreased cytotoxicity of cells in 5 of 6 samples by 58% ($p<0.1$).

Modified heparin increased the index of variability from 0.15-0.28 to 0.26-0.46. Variability of NKC activity exceeded that in samples incubated with native heparin and suspension of control cells by 1.5 and 2 times, respectively.

The polyanionic structure of heparin determines the interaction of sulfate groups with metal cations on the column, which results in complex formation, changes in electron density, and increase in liability of the donor-acceptor bonds in amino groups. Amino groups of heparin with specific spatial configuration are involved in the formation of metal-binding ligands in cell microenvironment. Reduced cytotoxicity under these conditions can be explained by deficiency of membrane cations involved in recognition and binding of TC, production of cytotoxic factors by NKC, and generation of reactive oxygen metabolites.

The constant for metal binding to ligands formed by randomly arranged amino groups in the heparin molecule is incomparable with that for true chelates or specialized enzyme systems responsible for the meta-

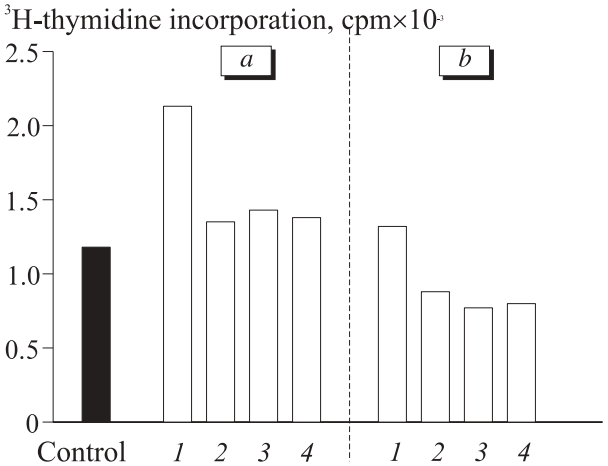

Fig. 1. *In vitro* spontaneous blast transformation of lymphocytes from healthy donors in the presence of native (*a*) and microbial glycoprotein-treated chelating agent (*b*). Dilutions: 1:20 (*1*), 1:100 (*2*), 1:500 (*3*), and 1:1000 (*4*). Here and in Fig. 2: mean values of 3 independent experiments.

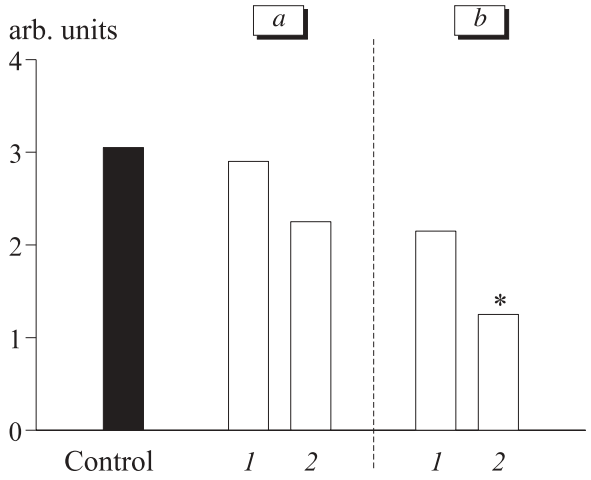

Fig. 2. *In vitro* effect of native (*a*) and aluminum hydroxide-modified heparin (*b*) on cytotoxic activity of natural killer cells from healthy donors. Heparin in concentrations of 10 (*1*) and 1 μg/ml (*2*). *$p<0.1$ compared to the control.

bolism and transport of metals across the cell membrane. Therefore, a paraphysiological dynamic equilibrium exists in the microenvironment of effectors, which is seen from increased variability of NKC activity against the background of decreased cytotoxicity.

Variability is an important characteristic of natural cytotoxicity, which reflects plasticity of NKC. Increased variability observed after treatment of cells with modified heparin reflects adequate compensatory response of lymphocytes to cation deficiency in the microenvironment. Under these conditions the decrease in the strength of cytolytic signals has little effect on the cytotoxic interaction.

Proliferative processes are opposite to the realization of specialized cell functions. We hypothesized that LBT will increase in the presence of compounds that are similar to heparin and human γ-globulin-associated carbohydrate components by general structure and activity. These changes were observed after treatment of lymphocytes with the native microbial material in a dilution of 1:20. However, the effect was blocked and variability of cell activity sharply decreased after delivery of samples through the column with the chelating agent.

Once the metal that stabilizes microbial macromolecules is adsorbed on the column, the polyanionic substance not having intramolecular coordination bonds undergoes elution into the solution. This substance would serve as a more potent chelator than metal-treated heparin. Since LBT is a metal-dependent process, binding of cations mediated by modified glycoproteins plays a critical role in the inhibition of proliferation not compensated by high variability.

Our results confirm the possibility of regulation of cell activity via modulation of the vector of metal transport in the microenvironment of effectors [7,8]. These changes can result from the interaction of biological macromolecules carrying charged carbohydrate groups with coordination metal cations forming intramolecular bonds [1,2,6].

According to the general theory of protein hydrodynamics, binding of metal cations stabilizes biological macromolecules in aqueous solutions, fluids, and tissues of the organism. Published data show that molecules of heparin [5], lectins [3], integrins [4], and γ-globulin contain bound metals [3]. Metal-binding sites are formed in native and aggregated IgG [4,10-12]. Conformational and functional reconstruction of biological macromolecules is determined by binding or donating of cations. Several interactions between biopolymers are associated with metal binding and transition [3,4].

Indirect evidence exists that the cellular microenvironment includes biological macromolecules containing conformational carbohydrate components and differing in the degree of saturation with metals [7,8]. Transport and exchange of cations may be considered as a natural metabolic process in membranes. Effector function of biologically active compounds depends on the occupancy of metal-binding sites and exchange of metal cations during intermolecular interactions.

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