Role of γ-Globulin-Associated Fraction Containing Carbohydrate Component in *in Vitro* Regulation of Human Natural Killer Activity

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Carbohydrate-rich components released from fractions of aggregated and protein aggregate-free γ-globulins decreased the cytotoxicity of natural killer cells in relation ^{3}H-uridine-labeled standard human K562 erythroblasts. In a dose of 0.1 μg/ml, these components decreased activity of natural killer lymphocytes by 57% and 42%, respectively. The data suggest that γ-globulin-associated fractions containing carbohydrate components are involved in the regulation of human natural killer activity.

Key Words: *γ-globulin; carbohydrate component; natural killer cells*

γ-Globulin molecule contains carbohydrate-rich components exhibiting heparin-like activity [1], inducing proliferation of human lymphocytes *in vitro* [4], and responsible for polyclonal activation of immunocompetent cells by aggregated proteins [4,6].

γ-Globulins induce proliferation of human lymphocytes due to their capacity to bind cations of alternating valency [2]. Stimulation of lymphocyte proliferation in the presence of γ-globulin inversely correlates with natural killer (NK) cytotoxicity *in vitro* [6]. The fraction released from γ-globulin after chelation of transition metals and stimulating blast-transformation of human lymphocytes (BTL) [4], probably suppresses function of natural cytotoxicity (NCT) effectors.

MATERIALS AND METHODS

Mononuclear cells were isolated from peripheral venous blood of 5 healthy donors (1 man and 4 women aging 20-28 years) using a Ficoll-Verografin density gradient (d=1.077 g/cm^3). Interphase mononuclear cells were washed twice in medium 199, and initial suspension containing 10^7 cells/ml medium was prepared. The nutrient medium consisted of RPMI-1640 medium (Flow), 12% fetal bovine serum (N. F. Gamaleya Institute of Epidemiology and Microbiology), 2 mM glutamine, and 40 μg/ml gentamicin (Pharmachim). Standard human erythromyeloblasts (K562 cells, 10^5 cells/ml medium) labeled with 3 μCi/ml ^{3}H-uridine [8] were used as the target cells.

Mononuclear cells (0.1 ml) and target cells (0.1 ml) were placed into 96-well round-bottom plates. The effector/target cell ratio varied from 100:1 to 12:1 (serial dilutions of mononuclear cell suspension). Pancreatic RNase (5 μg/ml, Reanal) was added to the suspension of mononuclear cells immediately before the experiment [3]. Mononuclears and target cells were coincubated in complete medium at 37°C, 5% CO_2, and high humidity for 14 h. After incubation, the contents of wells were filtered through fiberglass filters (2.5 μ pore size, Whatman) using a Titertek (Flow) 12-channel harvester. The residual radioactivity of filters was counted in 5 ml toluol scintillator using a Mark-II scintillation β-counter for 1 min.

Cytotoxic indexes for each effector/target cell ratio were calculated by the formula [8]. The area under the cytotoxicity curve [10] served as the integral parameter of NK cell activity (arb. units/1000).

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For fractionation, the preparation of human serum γ-globulin (5 mg/ml, Serva) in 0.15 M NaCl was centrifuged in a Beckman LS-65 centrifuge equipped with an SW-40 rotor at 105,000*g* and 10°C for 2 h. The centrifuged material contained protein aggregate-free and aggregated γ-globulin fractions (the upper and lower thirds, respectively).

For chelation of transition metals, aggregated and aggregate-free γ-globulin fractions in 0.15 M NaCl (pH 6.2) containing 100 μg/ml protein were passed through a column packed with a Dowex chelating resin (Serva) pretreated with 0.1 N HCl and 0.1 N NaOH (1:1 ratio). Eluates obtained by consecutive washings with a 10-fold volume of NaCl were subjected to molecular filtration on a Diaflo XM-50 ultrafilter (Amicon). During separation, spectral characteristics of fractions were recorded using a PU 8730 UV/VIS scanning spectrophotometer (Philips). According to the results of differential spectrophotometry, the aggregate-free portion retained by a Diaflo XM-50 filter and molecular ultrafiltrate of aggregated γ-globulin that have no UV absorption maxima at 280 nm typical of γ-globulin were considered as carbohydrate component-containing fractions. Protein content was measured spectrophotometrically by UV absorption at 280 nm and extinction coefficient of 0.7.

Carbohydrate component-containing fractions obtained from aggregated and aggregate-free γ-globulins after chelation of transition metals [1,4] were used in experiments on human cells (pH was adjusted to 7.4). These fractions were added to the suspension of mononuclear cells immediately before the test for cytotoxicity and remained in the reaction mixture to the end of incubation of mononuclears and target cells [6].

The results were analyzed by standard methods of variational statistics.

RESULTS

The carbohydrate component-containing fraction isolated from serum γ-globulin inhibited the population of NCT effectors (Fig. 1). The samples obtained from preliminary aggregated (Fig. 1, *a*) and aggregate-free (Fig. 1, *b*) γ-globulin contained mainly carbohydrate components [1] and inhibited (in a dose of 0.1 μg/ml) *in vitro* cytotoxic activity of NK of healthy donors from 2.90±0.61 to 1.25±0.35 (by 57%) and to 1.70±0.10 arb. units (by 42%), respectively. Components isolated from aggregated and aggregate-free γ-globulins, suppressed NK cell activity in 8 and 7 of 10 observations, respectively.

Thus, aggregated and aggregate-free human serum γ-globulins contained carbohydrate-rich fractions inhibiting cells of the NCT system. In the molecule of γ-globulin, carbohydrate components did not inhibit NK cells because of their high conformational stability. Native γ-globulin in a dose of 0.5 μg/ml *in vitro* did not change the cytotoxicity of NK cells from healthy donors [5], while its suppressive effect in extremely low doses of 5.0×10^{-5} and 5.0×10^{-6} μg/ml can be attributed to activation of spontaneous protein aggregation and induction of LBT by aggregates [6] bearing in mind the reciprocal relationship between differential functions of cells and their response to nonspecific mitogenic stimuli.

Our findings and previously reported data [4] indicate that LBT and NCT observed *in vitro* in the presence of human γ-globulin [5,6] are due to the release of carbohydrate-rich components inhibiting NK cells and stimulating lymphocyte proliferation. This process results from intra- and intermolecular cross-linking in γ-globulin molecule, which improves protein stability in diluted water solutions. Comparative analysis using

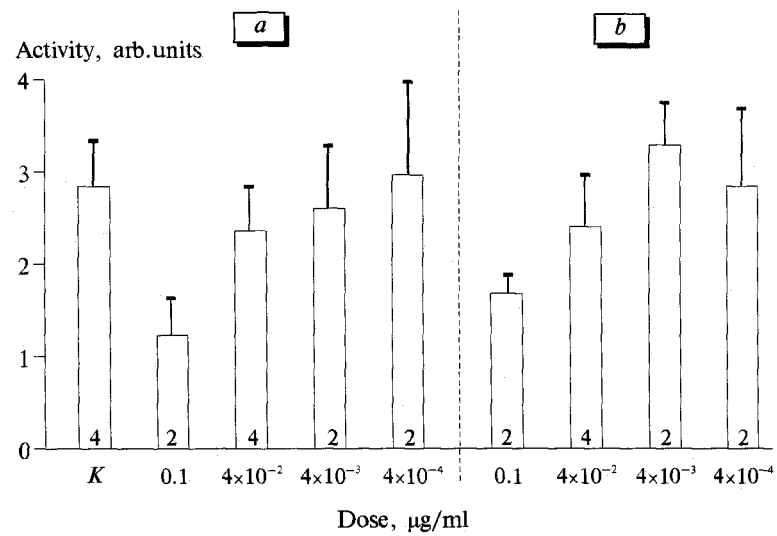

Fig. 1. *In vitro* effects of carbohydrate-rich components isolated from fractions of aggregated (*a*) and aggregate-free (*b*) γ-globulins after chelation of associated transition ions on cytotoxic activity of natural killer cells obtained from healthy donors. *C*: the control; figures in bars show the number of observations.

a special algorithm showed that the effective dose of carbohydrate-rich components (0.1 μg/ml) inhibiting *in vitro* cytotoxic functions of human mononuclear cells (Fig. 1) corresponded to concentrations of 60 ng/ml carbohydrate component and 70 ng/ml γ-globulin stimulating lymphocyte proliferation [4]. Therefore, the revealed effects are mediated by γ-globulin-associated fractions released during the action of chelating agents not only under rigorous [1,4], but also under neutral conditions.

Intra- and intermolecular rearrangements accompanying γ-globulin aggregation were observed during protein fractionation in buffered isotonic NaCl (acid medium). This was obviously due the effects of negatively charged molecular groups of carbohydrate-rich components exposed on the molecular surface after rearrangement of intramolecular cationic bonds, which stabilized protein conformation. That is why the ability of γ-globulin to stimulate LBT correlated with its Cu^{2+}-binding capacity [2].

It has been proposed that the serum contains γ-globulin fractions with varying protein/carbohydrate ratio [4]. In this case, the binding of γ-globulin to Fcγ-RII and Fcγ-RIII receptors of NK cells [7,9] can for regulation of the functional activity of NCT effectors by carbohydrate-enriched components, whose conformation in the protein molecule depends on microenvironment and the presence of cations of transition metals. These components have an amphipathic structure and, therefore, form a layer of a varying potential on the membrane of cytotoxic lymphocytes. This layer can be hydrated or dehydrated and, therefore, the transport of metals can be realized in various directions (due to chelating properties of γ-globulin molecules) [4]. These mechanisms regulate cytotoxic activity of NK cells.

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REFERENCES

1. A. Ya. Kul'berg, E. E. Babaeva, and A. A. Rodnikova, *Immunologiya*, No. 4, 12-14 (1994).
2. A. Ya. Kul'berg, Yu. B. Berkun, and S. B. Cheknev, *Ibid.*, No. 6, 7-9 (1997).
3. M. P. Rykova, I. V. Spirande, M. S. Zedgenidze, *et al.*, *Ibid.*, No. 3, 88-90 (1981).
4. S. B. Cheknev, *Byull. Eksp. Biol. Med.*, **127**, No. 6, 668-671 (1999).
5. S. B. Cheknev, D. V. Kuyavskaya, I. A. Tarkhanova, *et al.*, *Ibid.*, **118**, No. 7, 54-59 (1994).
6. S. B. Cheknev, Ya. G. Ashmanova, E. E. Babaeva, *et al.*, *Ibid.*, **118**, No. 12, 625-630 (1994).
7. W. H. Friedman, J.-L. Teillaud, C. Bouchard, *et al.*, *J. Leukocyte Biol.*, **54**, No. 5, 504-512 (1993).
8. Y. Hashimoto and H. Sudo, *Gann.*, **62**, No. 2, 139-143 (1971).
9. B. Perussia and G. Trinchieri, *J. Immunol.*, **132**, No. 3, 1410-1415 (1984).
10. T. P. Sheeran, F. R. Jackson, P. T. Dawes, *et al.*, *J. Immunol. Methods*, **115**, No. 1, 95-98 (1988).