Effect of Semisynthetic Analog of α_1-Acid Glycoprotein on Immunomodulatory and Antiinflammatory Activity of Natural Glycoprotein

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Pseudo-α_1-acid glycoprotein with carbohydrate chain ratio typical of native form was synthesized by a previously developed original technique of quantitative transfer of α_1-acid glycoprotein carbohydrate chains to other polymeric carrier. Similarly to native glycoprotein, the semisynthetic analog inhibited lymphocyte proliferation and stimulated the production of antiinflammatory cytokines by peripheral blood mononuclear leukocytes. However, it possessed no antioxidant activity and did not inhibit complement activation by the alternative pathway. The role of carbohydrate and protein components of α_1-acid glycoprotein molecule in the realization of its biological effects is discussed.

Key Words: *α_1-acid glycoprotein; neoglycoconjugates; inflammation; immunomodulation; complement; orosomucoid; free radicals; tumor necrosis factor*

Inflammatory reaction is accompanied by the activation of different adaptative mechanisms including accumulation of some acute-phase proteins in the plasma. One of them, α_1-acid glycoprotein (AGP), demonstrates a broad spectrum of biological activities. It suppresses proliferative response in lymphocyte culture induced by anti-CD3 [14] and phytohemagglutinin [2] and in mixed cell cultures [4]. AGP is an important element of the cytokine network: it activates the production of interleukins IL-1β, IL-6, and tumor necrosis factor-α (TNF-α) [1,10], but, on the other hand, it stimulates the release of inhibiting factor IL-1 by monocytes/macrophages [9]. The immunomodulatory properties of AGP depend on the microheterogeneity of its carbohydrate chains. The serum of healthy individuals contains 3 AGP glycoforms, reacting with concanavalin A (Con-A) in different ways. Chromatography of native AGP on Con-A-sepharose usually yields 3 fractions: Con-A-unbound (AGP-A), weakly bound (AGP-B), and strongly bound (AGP-C) fractions differing in the proportion of molecular forms with different patterns of carbohydrate chain branching. Thus, AGP-A consists only of molecules with 3- and 4-antennary chains, while AGP-B and especially AGP-C include also 2-antennary chains [1,3,8]. AGP-A more efficiently suppresses lymphocyte proliferation [9,14] and inhibits IL-2 production by peripheral blood mononuclear leukocytes (PBML) [2]. However, the role of peptide core and carbohydrate chain of AGP in various biological effects of this acute-phase protein remains unclear. Reportedly, glycans are preferentially involved in the inhibition of mitogen-induced lymphocyte proliferation [7,14]. The data on the involvement of the carbohydrate component in cytokine secretion are controversial [10,15]. In this connection, it seemed interesting to study the role of carbohydrate components of AGP in the realization of different biological effects using the method of quantitative transfer of carbohydrate chains from proteins to another polymeric carrier [5,11].

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MATERIALS AND METHODS

AGP was isolated from the peripheral blood of healthy volunteers by salt fractionation followed by DEAE-cellulose chromatography. Electrophoretically pure preparations with endotoxin content below 500 pg/mg were obtained after desalting (by dialysis) and lyophilization of AGP-containing fractions. Pseudo-AGP samples were prepared as described previously [5]. The yield was about 95%.

Human PBML isolated by gradient centrifugation were cultured in RPMI-1640 medium with 10% horse serum, 2×10^{-6} M 2-mercaptoethanol, 2 mM L-glutamine, and 20 μg/ml gentamicin. The cells (10^6/ml) were cultured for 16-18 h at 37°C in the presence of *N. meningitidis* lipopolysaccharide and different AGP concentrations in humid atmosphere with 5% CO_2. Supernatant was collected and stored at -20°C.

TNF-α activity in supernatants was measured by the previously described method [1] based on L-929 cell sensitivity to TNF-α in the presence of actinomycin D.

To assess to inhibition of lymphocyte proliferation by AGP preparations, PBML were cultured in 96-well plates (5×10^4 cells per well) in the presence of 5 μg/ml phytohemagglutinin (Calbiochem). Six AGP concentrations ranging from 31.2 to 1000 μg/ml were tested. The cells were incubated for 72 h at 37°C. Proliferation was evaluated by ^{3}H-thymidine (Isotop) incorporation.

The effect of AGP on the production of superoxide anion-radical ($O_2^{\bullet}$) were studied on leukocytes isolated from the peripheral blood of healthy volunteers. Erythrocytes were removed by sedimentation in gelatin followed by hemolytic shock of the remaining cells in distilled water. Leukocytes (3×10^6 cells/ml) were incubated with different concentrations of test preparations for 25 min at 37°C in the presence of 100 μM cytochrome *c* (Sigma) and 10^{-8} M phorbol myristate acetate. The reaction was stopped by centrifugation at 4°C. Optical density of the supernatants was measured at 550 nm. The intensity of $O_2^{\bullet}$ production was measured by the content of reduced cytochrome *c*.

To study the effects of AGP preparations on complement activation by the alternative pathway, cattle erythrocytes (3% suspension) were washed 3 times and incubated with rabbit IgG in a subagglutinating concentration (1:50) for 30 min at 37°C. Sensitized erythrocyte suspension (1.5%) was washed twice from the excess of antibodies and preincubated with different concentrations of test AGP preparations. Then the samples were incubated with rabbit antiserum (1:2) as a source of complement for 1 h at 37°C in the presence of EGTA (selective Ca^{2+} chelator) and $MgCl_2$ (10 nM), the cells were sedimented, and hemolysis was assayed by optical density at 414 nm. The data were expressed in optical density units.

Antioxidant activity of AGP in a cell-free system was measured as described previously [13]. The test preparations were incubated at 25°C in 500 μl K^+-phosphate buffer (pH 7.8) containing 40 μM cytochrome *c*, 0.05 M xanthine, 12×10^{-3} U xanthine oxidase, and 4×10^{-4} M EDTA. Optical density of the reaction mixture was measured at 550 nm every minute for 7 min. The xanthine oxidase/xanthine ratio was so adjusted that the rate of cytochrome *c* reduction remained linear throughout the entire period of measurement. The intensity of $O_2^{\bullet}$ production was measured by the content of reduced cytochrome *c*.

The data were analyzed statistically using Student's *t* test.

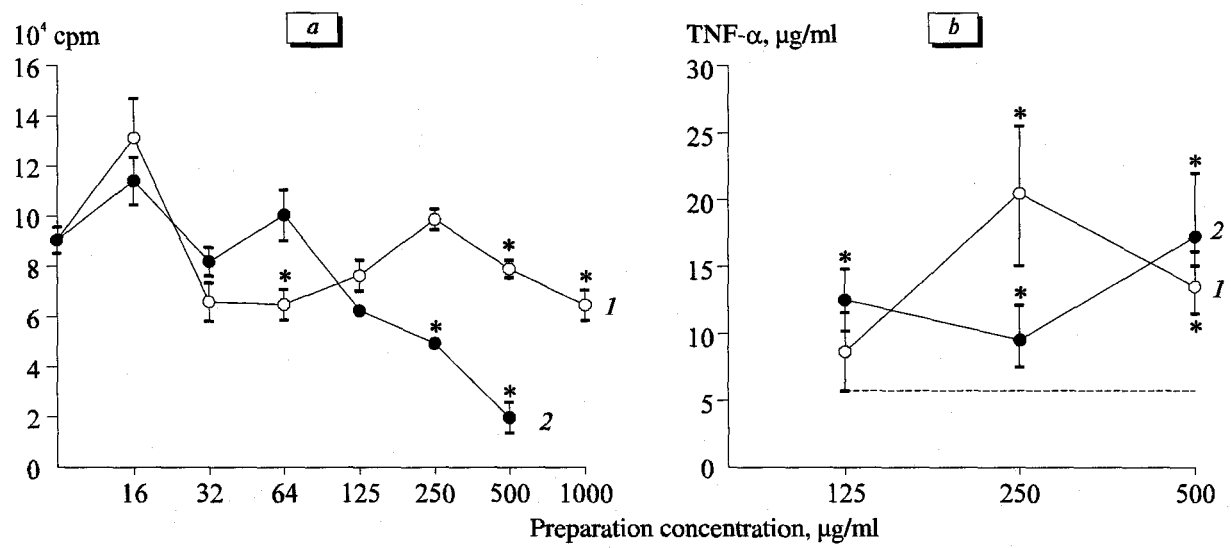

Fig. 1. Effect of α_1-acid glycoprotein preparations on lymphocyte proliferative response (PR) to phytohemagglutinin (*a*) and production of tumor necrosis factor-α (*b*). *$p<0.05$ in comparison with the control (dotted line). Here and in Figs. 2 and 3: *1)* AGP; *2)* pseudo-AGP.

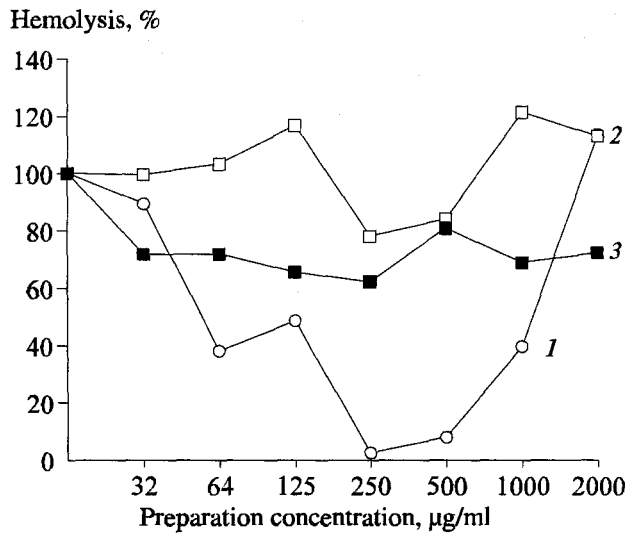

Fig. 2. Effect of α_1-acid glycoprotein preparations on complement activation by the alternative pathway. *3)* control (human serum albumin).

RESULTS

AGP inhibited the proliferative response of lymphocytes induced by T-cell mitogens. Protein-free neoglycoconjugates (pseudo-AGP) exhibited similar activity (Fig. 1, *a*). Similarly to AGP, pseudo-AGP in all test concentrations stimulated *in vitro* production of TNF-α (Fig. 1, *b*), although the efficiency of the same dose varied in different donors.

Similarly to native AGP, our AGP preparations inhibited complement activation by the alternative pathway. However, pseudo-AGP did not inhibited erythrocyte hemolysis in this experimental model. It should be noted that human albumin exhibited minor inhibitory activity, but this effect seemed to be nonspecific and did not depend on the dose (Fig. 2).

Our experiments confirmed that AGP inhibits $O_2^{\bullet}$ production by activated leukocytes. However, the new protein-free glycoconjugate exhibits no antioxidant activity (Fig. 3, *a*). The study of the mechanisms of this antioxidant activity showed that this glycoprotein inhibited free radical production in the xanthine-xanthine oxidase system (Fig. 3, *b*). This suggests that antioxidant activity of AGP is due to binding of free radicals, rather than inhibition of neutrophil activation.

The effects of AGP can be conventionally divided into two groups: immunomodulatory (inhibition of lymphocyte proliferation and stimulation of cytokine production) and antiinflammatory (inhibition of complement activation by the alternative pathway). It can be assumed that the immunomodulatory effects are associated with AGP–cell membrane interactions mediated by carbohydrate chains [6]. These effects can be realized irrespective of the molecular carrier for these chains, therefore, pseudo-AGP preparations retained immunomodulatory activity of native glycoprotein. The antinflammatory effect of AGP does not depend on AGP–cell membrane interactions, but is largely determined by chemical properties of its molecule, in particular, by the presence or absence of the protein core.

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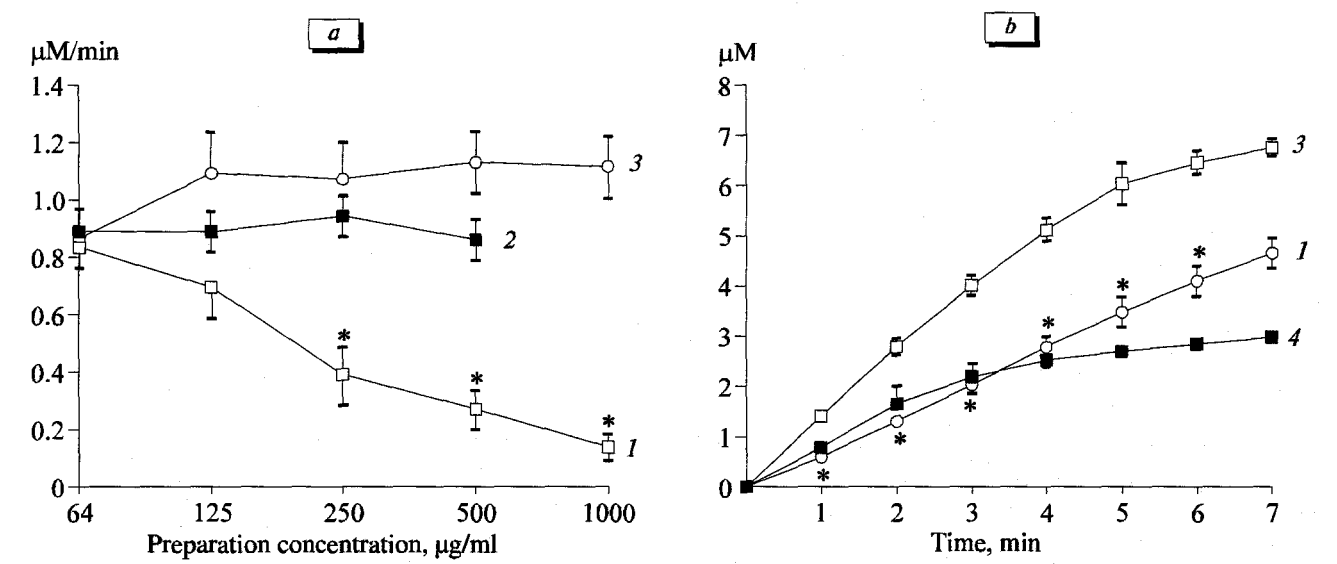

Fig. 3. Effect of α_1-acid glycoprotein preparations on $O_2^{\bullet}$ production by peripheral blood leukocytes activated by phytohemagglutinin (*a*) and in the xanthine-xanthine oxidase system (*b*). Ordinate: reduction rate (*a*) and the level of reduced cytochrome *c*. *3)* BSA (control, *a, b*); *4)* Mn-SOD, 40 μg/ml (control, *b*). *$p<0.05$ in comparison with BSA.

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