α_1-Acid Glycoprotein Possesses *in Vitro* Pro- and Antiinflammatory Activities

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We studied the effects of α_1-acid glycoprotein on tumor necrosis factor-α (TNF-α) and interleukin-10 (IL-10) production and lymphocyte response to phytohemagglutinin in cultured peripheral blood mononuclear leukocytes from 6 healthy donors. We observed 2 opposite responses to α_1-acid glycoprotein: first, stimulation of TNF-α and IL-10 production and inhibition of lymphocyte proliferation, and second, suppression of cytokine production and stimulation of lymphocyte proliferation. In cell cultures isolated from 4 of 6 donors, the TNF-α/IL-10 ratio remained unchanged after addition of native α_1-acid glycoprotein, but some fractions isolated by chromatography on concanavalin A-Sepharose changed this parameter. These changes were most pronounced after treatment with fraction C enriched with molecules with incomplete (biantennary) carbohydrate chains. The mechanisms of α_1-acid glycoprotein-induced effects on peripheral blood mononuclear leukocytes are discussed.

Key Words: *α_1-acid glycoprotein; glycoforms; tumor necrosis factor-α; interleukin-10; lymphocyte response to phytohemagglutinin*

α_1-Acid glycoprotein (AGP) is an acute-phase protein possessing various biological activities. AGP can enhance or attenuate the inflammatory response. AGP stimulates production of antiinflammatory cytokines interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-α (TNF-α) [1,10]. At the same time, AGP possesses antiproliferative [2,14] and antioxidant activities, stimulates secretion of IL-1-inhbibiting factor by macrophages [9], and inhibits complement activation by the alternative pathway [3,16]. This diversity of AGP-induced biological effects is related to the fact that some of them are associated with the protein component of AGP molecule (*e.g.*, binding of free radicals and inhibition of complement activation), while others are determined by the carbohydrate component (immunomodulating activity) [3]. Moreover, carbohydrate chains in AGP are characterized by microheterogeneity [3]. Three glycoforms of AGP detected in the plasma from healthy donors differently react with concanavalin A (Con A). Chromatography of native AGP on a column packed with Con A-Sepharose yields 3 fractions: unbound, weakly bound, and strongly bound with Con A (AGP-A, AGP-B, and AGP-C, respectively). These fractions differ in the content of molecular forms with various carbohydrate chain antennarity. AGP-A contains only molecules with triantennary and tetraantennary chains, while AGP-B and AGP-C contain molecules with biantennary chains [1,4,7]. It should be emphasized that AGP-A is most potent in stimulating lymphocyte proliferation [9,14] and production of IL-2 by peripheral blood mononuclear leukocytes (PBML) [2].

MATERIALS AND METHODS

AGP was isolated from the peripheral blood of healthy donors by salt fractionation followed by chromatography on DEAE cellulose. AGP-containing fractions were dialyzed and lyophilized. Endotoxin content in

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chromatographically pure AGP preparations did not exceed 500 pg/mg. AGP fractions were obtained by affinity chromatography on a column packed with Con A-Sepharose 4B (100 mg AGP/100 ml Con A-Sepharose). The column was balanced with 0.05 M phosphate buffered saline containing 2 mM $CaCl_2$ and 0.15 M NaCl (pH 7.4). Fractions A and B were eluted with 1 and 3 volumes of this buffer, respectively. Fraction C was eluted with 1 volume of 0.2 M methyl-α-D-glucopyranoside. The samples were dialyzed and lyophilized.

Human PBML were isolated by gradient centrifugation and cultured (10^6 cells/ml) in RPMI-1640 medium supplemented with 10% horse serum, 2×10^{-6} M 2-mercaptoethanol, 2 mM L-glutamine, and 20 μg/ml gentamicin in the presence of *E. coli* lipopolysaccharide (LPS) gifted by Z. P. Belkin (G. N. Gabrichevskii Moscow Institute of Epidemiology and Microbiology) at 37°C and 5% CO_2 for 16-18 h. The supernatant was collected and stored at -20°C.

The contents of TNF-α and IL-10 in the supernatant were measured using enzyme immunoassay kits (CYTIMMUNE Science Inc.). For evaluation of the inhibitory effect of AGP on lymphocyte proliferation, PBML (5×10^4 cells/well) were cultured in 96-well plates in the presence of 5 μg/ml phytohemagglutinin (Sigma) at 37°C for 72 h. Six concentration of AGP (31.2-1000 μg/ml) were tested. The intensity of cell proliferation was estimated by ^{3}H-thymidine incorporation (Izotop).

RESULTS

AGP stimulated or inhibited IL-10 production by LPS-stimulated PBML in culture (Fig. 1, *a*). Depending on the effects of AGP on proliferative response and IL-10 production, the donors were divided into 2 groups. In group 1, AGP suppressed IL-10 production and stimulated lymphocyte proliferation. In group 2, this substance stimulated production of IL-10 and decreased proliferative activity of lymphocytes (Fig. 1, *a*, *b*). The effects of AGP on TNF-α production were also different (Fig. 1, *c*). Secretion of TNF-α did not necessarily correlate with IL-10 production and proliferative activity of lymphocytes. In group 1, production of TNF-α was inhibited in 2 donors and stimulated in 1 donor. In group 2, TNF-α secretion was stimulated in 2 donors and suppressed in 1 donor. Previous studies showed that in healthy individuals production of IL-10 positively correlates with secretion of TNF-α [12]. These cytokines play a key role in inflammation. The regulatory effect of IL-10 is related to selective inhibition of NF-κ-B activation, which suppresses transcription of inflammatory cytokine genes in monocytes [15]. At the same time, IL-10 production is reg-

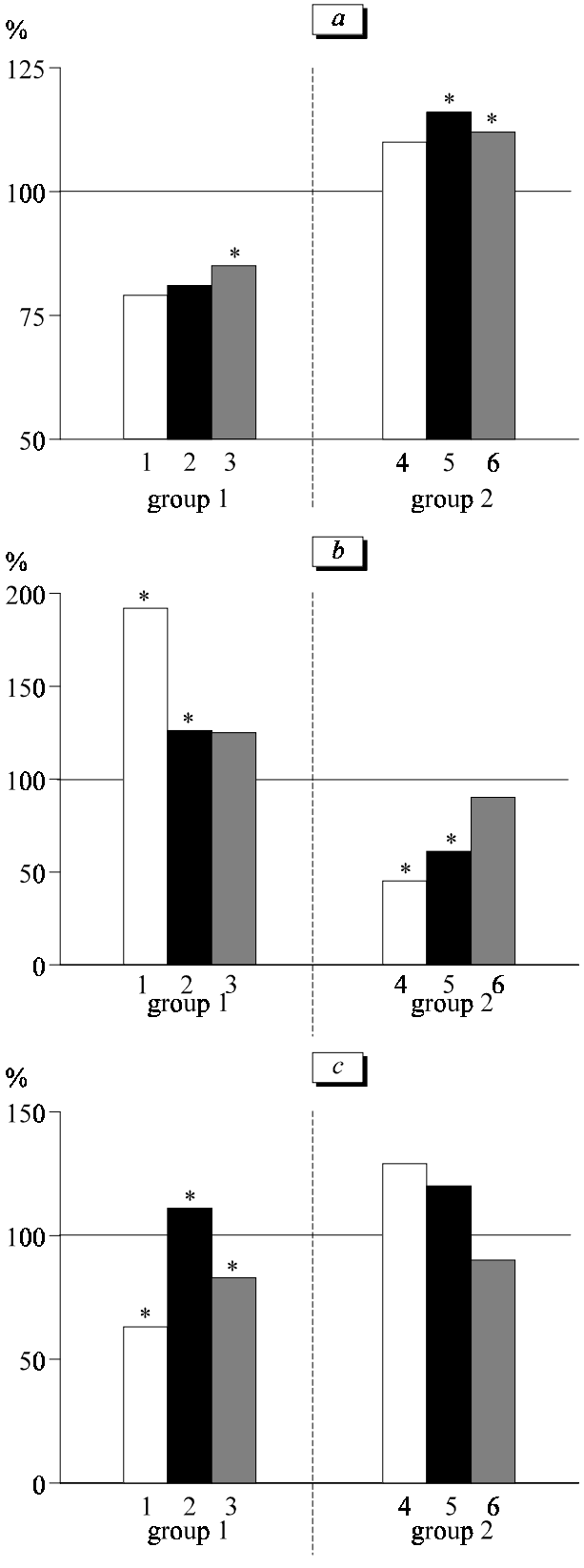

Fig. 1. Effects of α_1-acid glycoprotein on interleukin-10 production (*a*), proliferation of phytohemagglutinin-stimulated lymphocytes (*b*), and tumor necrosis factor-α secretion (*c*). Numerals: numbers of donors. *p<0.05 compared to the initial level (100%).

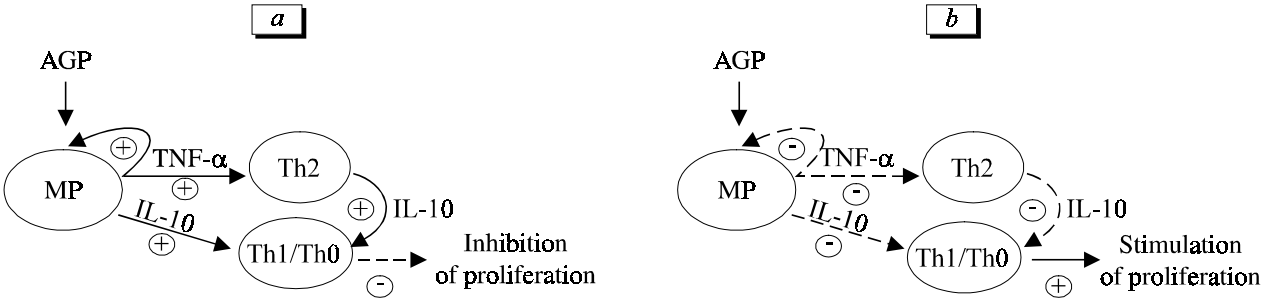

Fig. 2. Hypothetical mechanisms of *in vitro* effects of α_1-acid glycoprotein (AGP) on peripheral blood mononuclear leukocytes in case of stimulation (*a*) or inhibition (*b*) of tumor necrosis factor-α (TNF-α) production. "+" and "-": potentiation or attenuation, respectively. MP: macrophage. IL-10: interleukin-10.

ulated by TNF-α that activates protein-1 recognized by human IL-10 promoter [11].

Since AGP does not bind to lymphocyte membranes, its effects are mediated via macrophages and monocytes [5]. Hence, it can be hypothesized that stimulation of TNF-α production in donors 4 and 5 was accompanied by activation of IL-10 synthesis in macrophages and Th2 lymphocytes, blockade of NF-κ-B activation in T cells, and inhibition of proliferative response (Fig. 2, *a*). In donors 1 and 3, production of TNF-α and IL-10 was suppressed. Under these conditions IL-2 production by Th1 lymphocytes and proliferative response to T-cell mitogens increased due to the absence of the inhibitory effects of IL-10 (Fig. 2, *b*).

It should be emphasized that AGP had no effect on the TNF-α/IL-10 ratio in 4 donors, but changed this parameter in 2 donors (it increased in donor 2 and decreased in donor 6). Changes in the TNF-α/IL-10 ratio indicate severe disturbances in the immune system, *e.g.*, activation of inflammatory reactions [6,8,12,13]. Such disturbances can be suspected in donors 2 and 6.

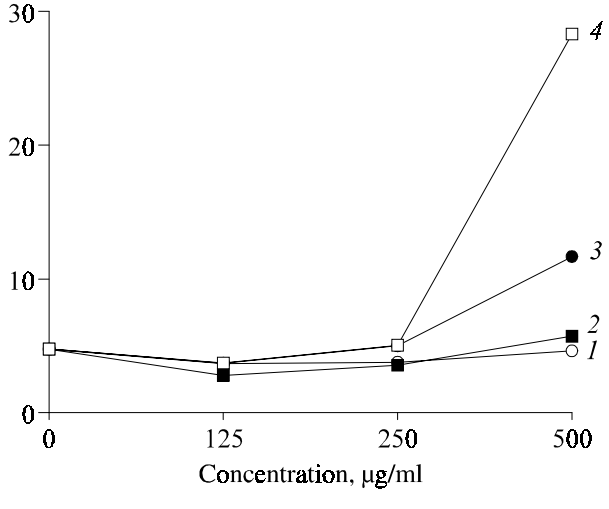

Fig. 3. Changes in the tumor necrosis factor-α/interleukin-10 ratio in the supernatant obtained after culturing of peripheral blood mononuclear leukocytes in the presence of native α_1-acid glycoprotein (*1*) and fractions A (*2*), B (*3*), and C (*4*).

We studied the effects of various AGP fractions on the TNF-α/IL-10 ratio. Native AGP in various concentrations had no effect on the TNF-α/IL-10 ratio in cell culture (Fig. 3). Similar results were obtained after culturing of cells with fraction A. However, fraction B and, especially, fraction C increased the TNF-α/IL-10 ratio. These data suggest that unlike native AGP containing tri- and tetraantennary chains, AGP glycoforms with incomplete (biantennary) chains enhance the inflammatory reaction.

Thus, the effects of AGP on inflammation depend on its composition (content of different glycoforms) and individual peculiarities of donors.

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