Effect of α_1-Acid Glycoprotein Glycoforms on the Production of Tumor Necrosis Factor and Interleukin-1 by Human Peripheral Blood Mononuclear Leukocytes *In Vitro*

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 124, No. 1, pp. 79-83, January, 1998
Original article submitted December 26, 1996

Effects of various glycoforms of α_1-acid glycoprotein on the production of tumor necrosis factor and interleukin-1 are studied. After fractionation of donor blood or ascitic fluid from cancer patients 4 glycoforms were obtained, differing by affine binding to concanavalin A and by the carbon chain structure. Different glycoforms of α_1-acid glycoprotein inhibit or stimulate the production of tumor necrosis factor-α and interleukin-1β by peripheral blood mononuclears. The glycoform with the highest affinity for ConA produced the strongest stimulating effect.

Key Words: *α_1-acid glycoprotein; tumor necrosis factor; interleukin-1; mononuclear leukocytes*

α_1-Acid glycoprotein (AGP), an acute phase inflammation protein, modulates the function of lymphocytes. It inhibits lymphocyte proliferation stimulated by antiCD3 antibodies [13] and phytohemagglutinin [1]. AGP is microheterogenous due to thedifferences in the structure of carbon chains. At least three different molecular forms (glycoforms) are present in normal serum, which differently react with concanavalin A (ConA). Separation of native AGP in a column with ConA-Sepharose yields the following fractions: not bound to ConA, slightly bound to ConA, and tightly bound to ConA [2,3,6]. The fraction not reacting with ConA most effectively suppresses the proliferative response [7,13] and interleukin-1 (IL-2) production [1]. Experiments with fluorescein-labeled AGP showed that among the peripheral blood cells, monocytes and polymorphonuclear leukocytes, but not lymphocytes, are its targets [4]. These results imply that the effect of AGP on lymphocyte function is indirect, being realized through modulation of IL-1 production. We examined the effect of AGP and its glycoforms on the production of IL-1 and tumor necrosis factor.

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MATERIALS AND METHODS

AGP was isolated from healthy donor peripheral blood (nAGP) or ascitic fluid of patients with gastric cancer (aAGP) by salt fractionation with subsequent chromatography of DEAE-cellulose. Different AGP fractions were obtained by affinity chromatography on a ConA-Sepharose 4B column (100 mg AGP/100 ml ConA-Sepharose). The column was equilibrated with 0.05 M phosphate buffered saline containing 2 mM $CaCl_2$ and 0.15 M NaCl (pH 7.4). Four fractions were obtained. They were designated A, B, C, and D. Fraction A was eluted by one column volume of equilibrating buffer and fraction B with three volumes of the same buffer. Fraction C was eluted with 1 volume of 0.2 M methyl-α-D-glycopyranoside (Sigma) in equilibrating buffer. Fraction D was eluted with 0.1 volume of 0.1 M citrate buffer (pH 3.5) with 2 mM $CaCl_2$ and 0.5 M NaCl. The specimens were dialyzed and freeze-dried.

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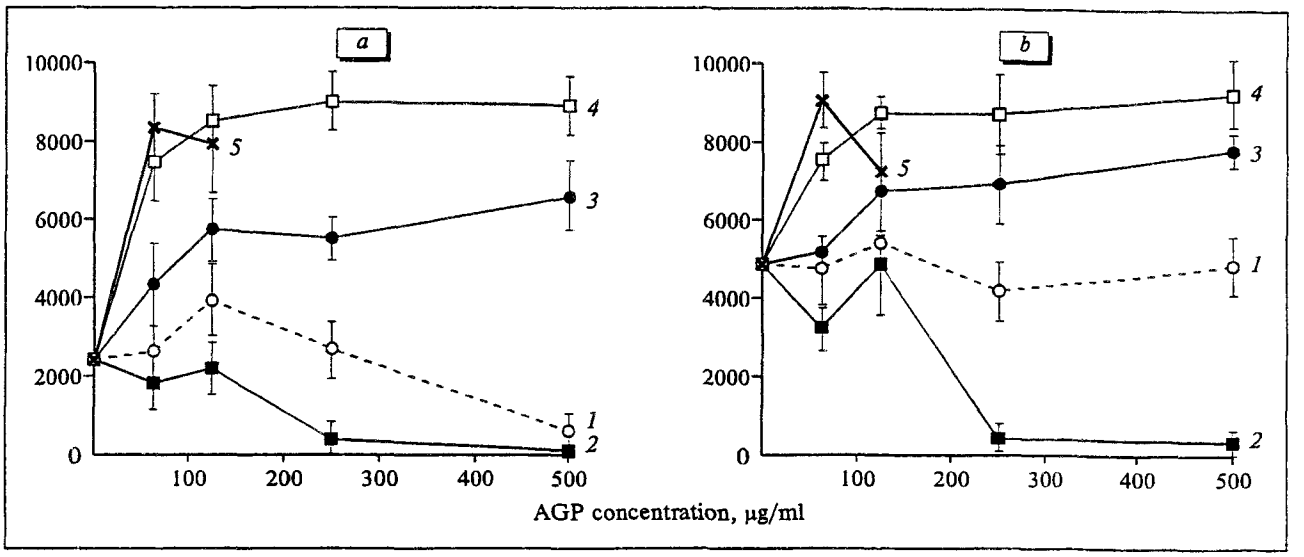

Fig. 1. Effect of donor blood α_1-acid glycoprotein (AGP) and its glycoforms on production of tumor necrosis factor-α (TNF) by pooled mononuclear leukocytes (*a*) and adherent cells (*b*). *1*) intact AGP; *2*) AGP-A; *3*) AGP-B; *4*) AGP-C; 5) AGP-D. Ordinate: TNF production, pg/ml.

Mononuclear leukocytes were isolated from donor peripheral blood by gradient centrifugation. The cells were washed and resuspended in RPMI-1640 (ICN) with 10% inactivated equine serum, 2×10^{-6} M 2-mercaptoethanol, 2 mM L-glutamine, and 20 μg/ml gentamycin. To obtain the population of adherent cells, mononuclear leukocytes (10^6 cells/ml) were cultured for 2.5 h at 37°C in a humidified atmosphere with 5% CO_2 in 24-well plates (Nunc). Nonadherent cells were removed by double washing with buffered saline. The wells from which nonadherent cells were not removed contained the total pool of mononuclear leukocytes. Then the cells were incubated under the same conditions in the presence of 3 μg/ml *N. meningitidis* lipopolysaccharide and AGP preparations in different concentrations. In control wells the cells were incubated with lipopolysaccharide alone. The incubation medium was collected after 14 h and stored at -20°C.

The activity of tumor necrosis factor was assessed in L-929 cells inoculated in 96-well plates (3×10^4 cells/well) in medium 199 with 10% inactivated bovine serum. Cells were incubated at 37°C in humidified atmosphere with 5% CO_2 until the confluency, after which the culture medium was replaced with fresh medium containing actinomycin D (Serva), and double serial dilutions of the test samples were added to each well. After 18-h incubation under the above-specified conditions, the medium was discarded and cells were stained with 0.2% crystal violet (Sigma). After washing and drying, the intensity of staining corresponding to the number of surviving cells was measured in a Totertek multichannel spectrophotometer at a wavelength of 540 nm. Recombinant tumor necrosis factor-α (Institute of Bioorganic Chemistry, Moscow) was the reference. Experimental and calibration curves were compared by probit analysis. The concentration of tumor necrosis factor in the samples was expressed in pg/ml.

The activity of IL-1 in the samples was assessed using IL-1-sensitive D10.G4.1 cells requiring antigenic stimulation and a feeder layer. Conalbumin (Sigma) was the antigen, and feeder layer consisted of CBA/2 mouse splenocytes ($H\text{-}2^k$). The antigen, feeder cells, and 10% conditioned medium with IL-2 were added to the culture every 7 days. Conditioned medium with IL-2 was prepared by 48-h culturing of DBA/2 mouse splenocytes stimulated with ConA (40 mg/ml). Before the experiment, the cells cultured as described above were preincubated for 18 h in RPMI-1640 with 5% fetal calf serum without IL-2. Then the cells were cultured in 96-well plates for 64 h with serial dilutions of test samples or different concentrations of recombinant IL-1b (code 86/680, National Institute of Biologic Standards and Control). Eighteen hours before the end of culturing, ^{3}H-thymidine (40 kBq/well) was added. Cells were transferred onto filters, and their radioactivity was measured in a liquid scintillation spectrophotometer. The content of IL-1 in the supernatant was expressed in IU/ml.

The results were compared using Student's *t* test.

RESULTS

Table 1 presents the output and characteristics of ConA-bound AGP fractions (glycoforms) B, C, and D and unbound fraction A. The content of two-antenna chains in the glycoforms increased from 0

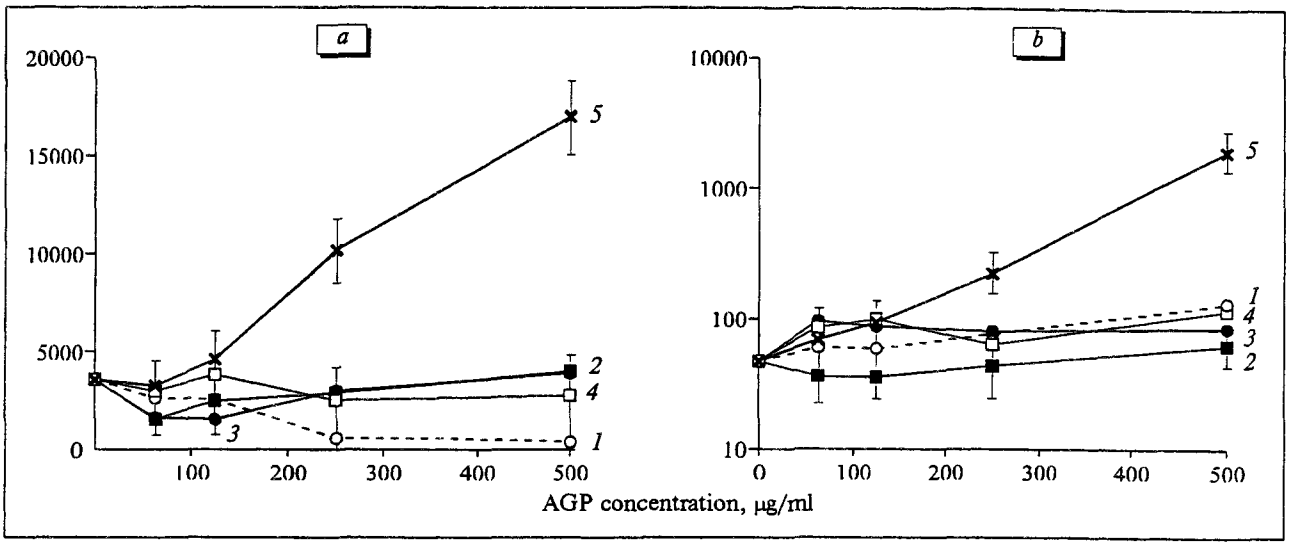

Fig. 2. Effect of α_1-acid glycoprotein (AGP) from ascitic fluid of patients with gastric cancer and its glycoforms on production of tumor necrosis factor-α (TNF) (*a*) and interleukin-1β (*b*) by mononuclear leukocytes. *1*) intact AGP; *2*) AGP-A; *3*) AGP-B; *4*) AGP-C; *5*) AGP-D. Ordinate: Production of TNF, pg/ml (*a*) and interleukin-1β, IU/ml (*b*).

to 3 mole/mole with an increase in the affinity for ConA. Glycoform A contained only three- and four-antenna chains, whereas B, C, and D had one, two, or three two-antenna chains. Homologous forms isolated from nAGP and aAGP differed by the content of fucose residues, but not by the antennas of the carbohydrate part of molecule: aAGP contained more residues than nAGP. The content of ConA-bound forms was 2-2.5 times higher in aAGP than in nAGP. All fractions reacted with anti-AGP antiserum in radial immunodiffusion test, indicating that none of the fractions was a denatured form of AGP.

AGP glycoforms had different effects on the production of tumor necrosis factor. Native AGP in medium doses slightly stimulated the production and suppressed it at a dose of 500 μg/ml, while nAGP-A suppressed it in a concentration of 250 μg/ml. By contrast, nAGP-B, C, and D markedly stimulated the production of tumor necrosis factor (Fig. 1, *a*). Similar results were obtained only with adherent cells, although in this case the intensity of production of tumor necrosis factor in the control was 2 times higher than in experiments with pooled mononuclear leukocytes (Fig. 1, *b*).

Intact aAGP did not stimulate the production of tumor necrosis factor. Moreover, in high concentrations (250 and 500 μg/ml) aAGP exerted marked inhibitory effect. Like nAGP-D, aAGP-D stimulated the release of this factor (Fig. 2, *b*).

Intact nAGP suppressed the production of IL-1 both in the presence of pooled mononuclears and fractions of adherent cells. Forms A and B stimulated it only after treatment of a pool of mononuclear leukocytes with high doses of the preparation. Form C

TABLE 1. Characteristics of Glycoforms of AGP of Different Origin

Glycoforms	Output, %	Molecular weight, Kd	Carbohydrates, %	Carbon chain antennas, %		
				Tetra-	Tri-	Bi-
nAGP	100	43.5	40	49	39	12
nAGP-A	48	44.0	40	53	47	0
nAGP-B	39	42.0	39	43	38	19
nAGP-C	10	41.2	37	32	29	39
nAGP-D	1	39.5	34	22	18	60
aAGP	100	44.0	40	42	37	21
aAGP-A	42	44.5	41	54	46	0
aAGP-B	35	42.5	40	42	36	22
aAGP-C	20	41.5	38	31	29	40
aAGP-D	3	39.0	35	23	19	58

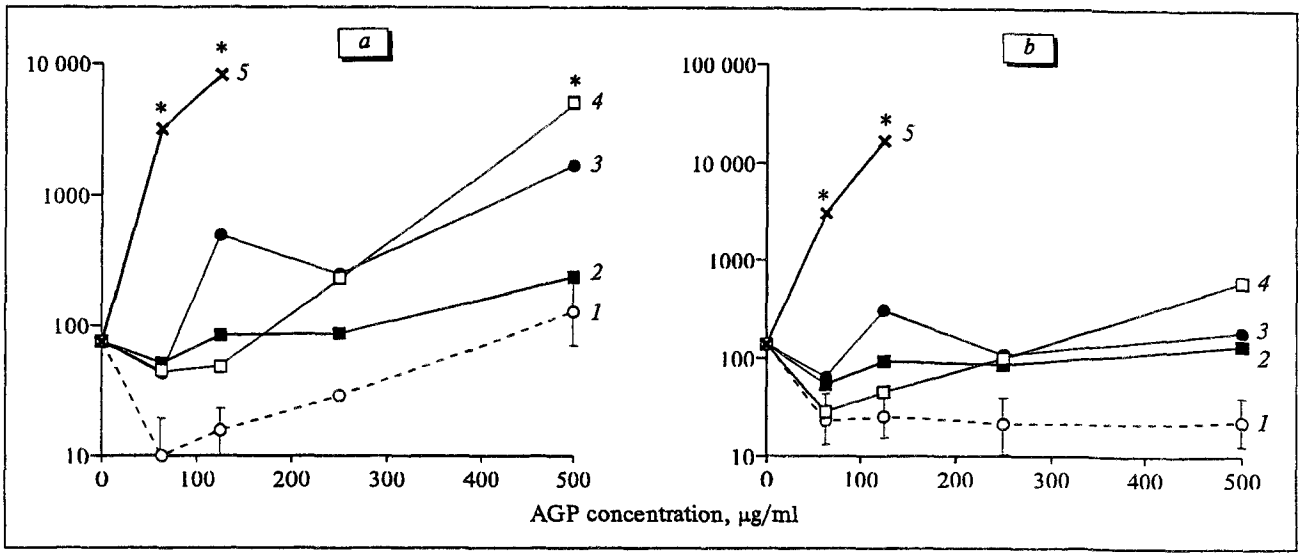

Fig. 3. Effect of donor blood α_1-acid glycoprotein (AGP) and its glycoforms on production of interleukin-1β by a pool of mononuclear leukocytes (*a*) and adherent cells (*b*). *1*) intact AGP; *2*) AGP-A; *3*) AGP-B; *4*) AGP-C; *5*) AGP-D. Ordinate: Production of interleukin-1β, IU/ml. *$p<0.01$ vs. the control.

showed a higher stimulating activity, and the activity of form D was very high (Fig. 3). Similar results were obtained with fractions isolated on the basis of aAGP. Intact preparation and forms A, B, and C were characterized by low stimulating activity, whereas fraction D elicited a pronounced stimulating effect (Fig. 2, *b*).

These results show that AGP glycoforms produce different effects on the production of inflammation cytokines. The high stimulating activity of form D is noteworthy. It does not depend on the source of the initial AGP preparation or on the target cells. Inflammation involves an increase of AGP production and alteration of the ratio of its glycoforms [9,12]. In addition, AGP enhances the secretion of tumor necrosis factor-α and interleukin-1β by cells stimulated with lipopolysaccharide [8]. On the other hand, these cytokines regulate the production of acute phase proteins, including AGP, by hepatocytes [11] or hepatoma cells [5]. Besides stimulation of inflammation cytokine production, AGP induces the production of IL-1 antagonist and soluble receptor for tumor necrosis factor [14,15]. Therefore, the differences in the effects of AGP glycoforms can be due to different combinations of the above factors.

Our results do not agree with the data on a similar stimulating effect of ConA-bound fraction, ConA-nonbound fraction, and native AGP on lipopolysaccharide-induced production of IL-1β and tumor necrosis factor-a [8]. This contradiction may be explained by different conditions of experiment. We used four, but not two AGP fractions, and tested a wider spectrum of doses.

Thus, we can assert that glycoform AGP-D bound by ConA and containing 3 two-antenna carbohydrate chains produces the highest stimulating effect on the production of IL-1β and tumor necrosis factor-α.

The study was supported by the Russian Foundation for Basic Research (grants 93-04-06256 and 96-04-49203). The authors thank Dr. A. G. Lyutov for initial AGP preparations and Dr. V. G. Korobko for recombinant tumor necrosis factor-α.

REFERENCES

1. A. L. Pukhal'skii, A. P. Toptygina, E. A. Kalashnikova, *et al., Byull. Eksp. Biol. Med.*, **118**, No. 7, 71-73 (1994).
2. S. D. Shiyan, V. V. Nasonov, N. V. Bovin, *et al., Bioorgan. Khimiya*, **17**, No. 9, 663-670 (1991).
3. S. D. Shiyan, V. V. Nasonov, N. V. Bovin, *et al., Ibid.*, **20**, No. 10, 11-25 (1994).
4. S. D. Shiyan, S. V. Khaidukov, A. L. Pukhal'skii, and A. P. Toptygina, *Gematol. Transfuziol.*, **41**, No. 2, 24-29 (1996).
5. H. Baumann, K. K. Morella, and G. H. Wong, *J. Immunol.*, **151**, 4248-4257 (1993).
6. M. Bierhuizen, M. De Witt, C. Govers, *et al., Eur. J. Biochem.*, **175**, 387-394 (1988).
7. P. N. Bories, J. Feger, N. Benbernou, *et al., Inflammation*, **14**, 315-322 (1990).
8. A. Boutten, M. Dehoux, M. Deschenes, *et al., Eur. J. Immunol.*, **22**, 2687-2695 (1992).
9. A. Drechou, N. Perez-Gonzalez, K. Agneray, *et al., Eur. J. Cell Biol.*, **50**, 111-116 (1989).
10. T. Fournier, N. Mejdoubi, D. Monnet, *et al., Hepatology*, **20**, 1584-1588 (1994).
11. V. Pimeneau, P. Midoux, G. Durand, *et al., Glycoconj. J.*, **6**, 561-574 (1989).
12. O. Pos, A. Drechou, G. Durand, *et al., Clin. Chim. Acta*, **184**, 121-131 (1989).
13. O. Pos, R. A. F. Oostendorp, M. E. Van der Stelt, *et al., Inflammation*, **14**, 133-141 (1990).
14. J. Rydberg, H. Miorner, A. Chandramuki, and M. Lantz, *J. Infect. Dis.*, **172**, 301-304 (1995).
15. H. Tilg, E. Vannier, G. Vachino, *et al., J. Exp. Med.*, **178**, 1629-1636 (1993).