IMMUNOLOGY AND MICROBIOLOGY

Preventive and Therapeutic Effects of α_1-Acid Glycoprotein in Mice Infected with *B. anthracis*

I. G. Shemyakin, A. L. Pukhalsky*, V. N. Stepanshina, G. V. Shmarina*, V. A. Aleshkin, and S. S. Afanas'ev****

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 140, No. 10, pp. 440-445, October, 2005
Original article submitted October 21, 2004

We studied the effects of α_1-acid glycoprotein preparations on the survival rate of BALB/c mice infected with the lethal dose of *B. anthracis* STI-1. Apart from native α_1-acid glycoprotein from donor blood, we studied 3 glycoforms differing in the affinity for concanavalin A and structure of carbohydrate chains. The protective effect of α_1-acid glycoprotein preparations did not depend on its dose and was observed 3 months after treatment (0.3 mg per mouse). The protective effect was revealed in mice receiving α_1-acid glycoprotein preparations 2 h before infection and 24 h after inoculation of the bacterial culture. In the latter case the survival rate of animals was much higher compared to that observed in preventive administration of α_1-acid glycoprotein. The protective effect practically did not depend on the time of treatment with glycoforms. Pretreatment with α_1-acid glycoprotein preparations significantly decreased plasma interferon-γ concentration. Administration of the test preparations 24 h after infection decreased the concentration of tumor necrosis factor-α.

Key Words: *α_1-acid glycoprotein; glycoforms; cytokines; anthrax; endotoxic shock*

α_1-Acid glycoprotein (AGP) is a highly glycosylated protein with a molecular weight of 40-45 kDa. The main function of this protein is transport of lipocaines capable of binding hydrophobic molecules and DNA fragments. Moreover, AGP is one of the major proteins of acute inflammation. The plasma from healthy donors has at least 3 molecular forms of AGP (glycoforms) that differ in the reaction with concanavalin A (Con A). Separation of native AGP on the column packed with Con A-Sepharose allowed obtaining the fractions not bound, weakly bound, and strongly bound to Con A [5]. Expression of the AGP gene is regulated by a variety of neurotransmitters, including glucocorticoid hormones and cytokines (*e.g.*, interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and IL-6) [7].

AGP is a natural antiinflammatory and immunomodulatory compound possessing antioxidant activity and preventing complement activation by the alternative pathway [3]. Moreover, AGP inhibits the proliferative response of lymphocytes stimulated with T cell mitogens and suppresses IL-2 production [2]. Depending on the ratio between glycoforms, AGP can inhibit or stimulate production of TNF-α and IL-1β [1]. *In vivo* studies showed that AGP protects experimental animals from lethal shock produced by administration of TNF-α or lipopolysaccharide (LPS) [12]. AGP increases the resistance of laboratory animals to infection with the culture of gram-negative bacteria *Klebsiella pneumonia* [11].

Here we studied the protective and therapeutic effects of AGP preparations (native AGP and glyco-

State Research Center of Applied Microbiology, Obolensk, Moscow oblast; *Medical and Genetic Research Center, Russian Academy of Medical Sciences, Moscow; **G. N. Gabrichevskii Moscow Institute of Epidemiology and Microbiology. ***Address for correspondence:*** osugariver@mtu-net.ru. A. L. Pukhalsky

0007-4888/05/1404-0439

forms) on mice infected with the lethal dose of gram-positive anthrax bacteria *B. anthracis* STI-1.

MATERIALS AND METHODS

AGP was isolated from the peripheral blood of healthy donors by the method of saline fractionation [5,13]. AGP fractions A, B, and C were obtained by means of affinity chromatography on the column packed with Con A-Sepharose 4B [4]. Fraction A was eluted with a column volume of balanced buffer. Elution of fraction B was performed using a 3-fold volume of the same buffer. Elution of fraction C involved 1 volume of 0.2 M methyl-α-D-glucopyranoside in balanced buffer. The samples were dialyzed and lyophilized. AGP preparations were electrophoretically pure. Endotoxin content was below 500 pg/mg.

BALB/c mice received intraperitoneal injections of AGP preparations in a dose of 190-3000 μg. To study the preventive effect, AGP preparations were administered 2 h before infection of spores of the unencapsulated toxigenic vaccinal strain of *B. anthracis* STI-1 to experimental animals. Bacterial spores were injected intraperitoneally in a dose of 5.5×10^8 per mouse (LD_{50}). The therapeutic effect of the test preparations was assayed by the same method. In these experiments AGP was administered 24 h after infection. Cytokine concentration in the peripheral blood was measured 2 h after treatment with the culture of *B. anthracis* STI-1 (evaluation of preventive activity of AGP) or 2 h after administration of AGP (evaluation of therapeutic activity of preparations). The concentrations of IL-1β, TNF-α, and interferon-γ (IFN-γ) were estimated by enzyme immunoassay using commercial diagnostic kits (Cytimmune). The results were analyzed by nonparametric Wilcoxon test. The survival rates were compared by means of log-rank test.

RESULTS

The protective effect of AGP was previously evaluated on 3 experimental models: (1) endotoxic shock, treatment of experimental animals with LPS or TNF-α [12]; (2) ischemia, reperfusion of the intestine [14] or kidneys [8]; (3) and infection with gram-negative bacteria *Klebsiella pneumonia* [11]. The protective effect of AGP depended on its dose. It should be emphasized that this effect was not observed after administration of AGP in doses <1000 μg.

In our experiments 83% animals died over 7 days after infection with *B. anthracis* in the lethal dose. A strong protective effect was observed in animals receiving native AGP and its glycoforms 2 h before infection and 24 h after inoculation of the bacterial culture (Tables 1 and 2). The protective effect was revealed after administration of AGP and its glycoforms in a dose of 187.5 μg. This effect did not depend on the dose, which allowed us to combine the data for each of the test preparations (Fig. 1).

Pretreatment with native AGP was accompanied by a less significant protective effect compared to glycoforms (Fig. 1, *a*). However, native AGP was more potent than fractions A and C in producing the therapeutic effect. The protective effect of native AGP was comparable to that of fraction B (Fig. 1, *b*).

These data show that native AGP was more effective in animals receiving the test preparation as the therapeutic drug. The survival rate of mice receiving AGP

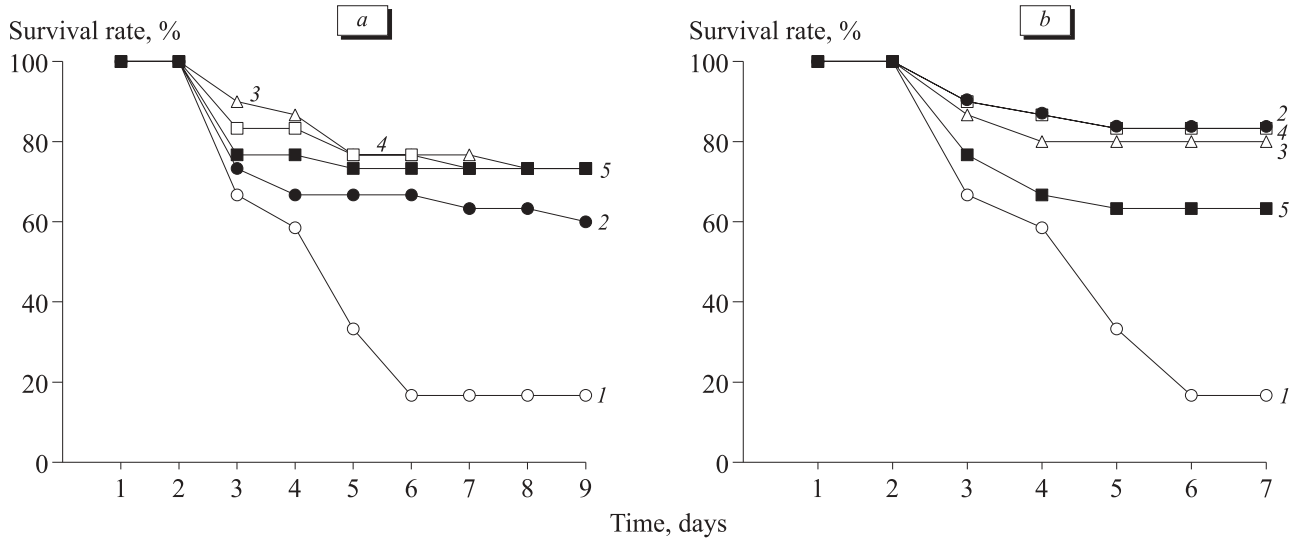

Fig. 1. Survival rate of mice infected with *B. anthracis* in the lethal dose and exposed to preventive (*a*) or therapeutic treatment (*b*) with α_1-acid glycoprotein preparations (AGP). Control (*1*), native AGP (*2*), AGP-A (*3*), AGP-B (*4*), and AGP-C (*5*).

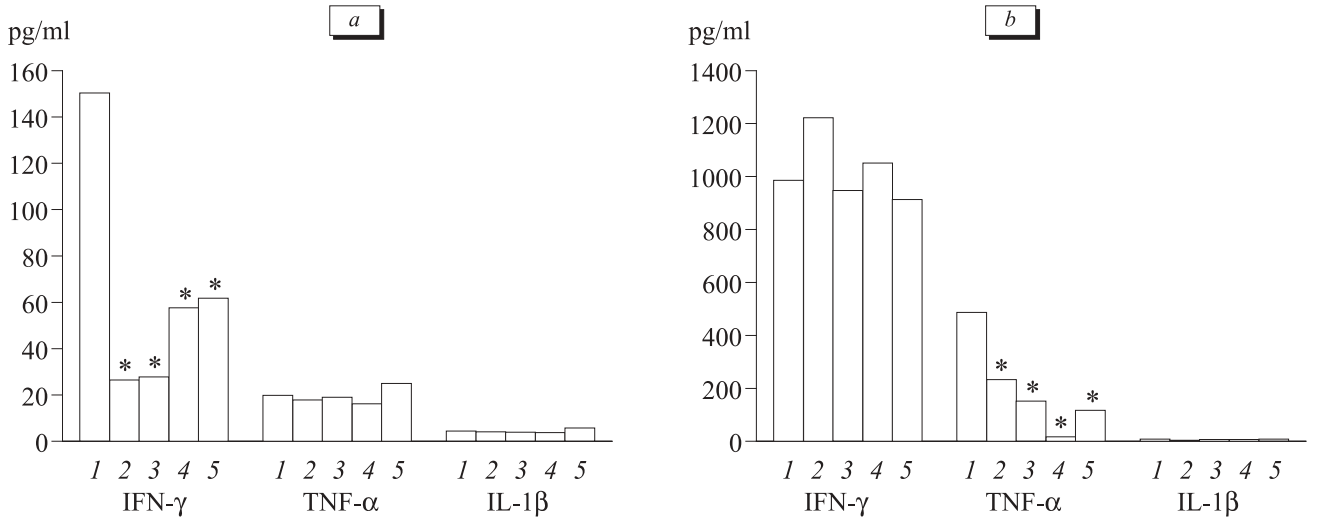

Fig. 2. Blood cytokine concentration in mice infected with *B. anthracis* in the lethal dose and exposed to preventive (*a*) or therapeutic treatment (*b*) with AGP preparations. Control (*1*), native AGP (*2*), AGP-A (*3*), AGP-B (*4*), and AGP-C (*5*). **p*<0.05, Wilcoxon test.

24 h after infection was much higher compared to animals pretreated with this preparation 2 h before administration of the bacterial culture (log-lank test, $p<0.05$). The protective effect of glycoforms practically did not depend on the time of treatment (Tables 1 and 2).

Plasma IFN-γ concentration in mice significantly increased 2 h after administration of *B. anthracis* STI-1 in the lethal dose. Pretreatment with AGP and its glycoforms significantly decreased the concentration of this cytokine. However, no significant changes

TABLE 1. Preventive Effect of AGP Preparations in Mice Infected with *B. anthracis* STI-1 Culture in LD_{50}

Preparation	Dose, μg	Number of dead mice/total number of mice	Percent of dead mice	Time-to-death for each mouse, days
Control		5/6	83.3	3, 3, 3, 4, 5
AGP (native preparation)	3000	3/6	50	3, 4, 9
	1500	3/6	50	3, 4, 7
	750	2/6	33.3	3, 3
	375*	1/6	16.7	3
	187.5	3/6	50	3, 3, 3
AGP-A	3000*	1/6	16.7	4
	1500*	1/6	16.7	8
	750	2/6	33.3	3, 5
	375	3/6	50	3, 5, 5
	187.5*	1/6	16.7	3
AGP-B	3000*	0/6	0	—
	1500	2/6	33.3	3, 3
	750	2/6	33.3	3, 5
	375	2/6	33.3	3, 7
	187.5	2/6	33.3	3, 5
AGP-C	3000	2/6	33.3	3, 3
	1500	3/6	50.0	3, 3, 5
	750	2/6	33.3	3, 3
	375*	0/6	0	—
	187.5*	1/6	16.7	3

Note. Here and in Table 2: **p*<0.05, log-rank test.

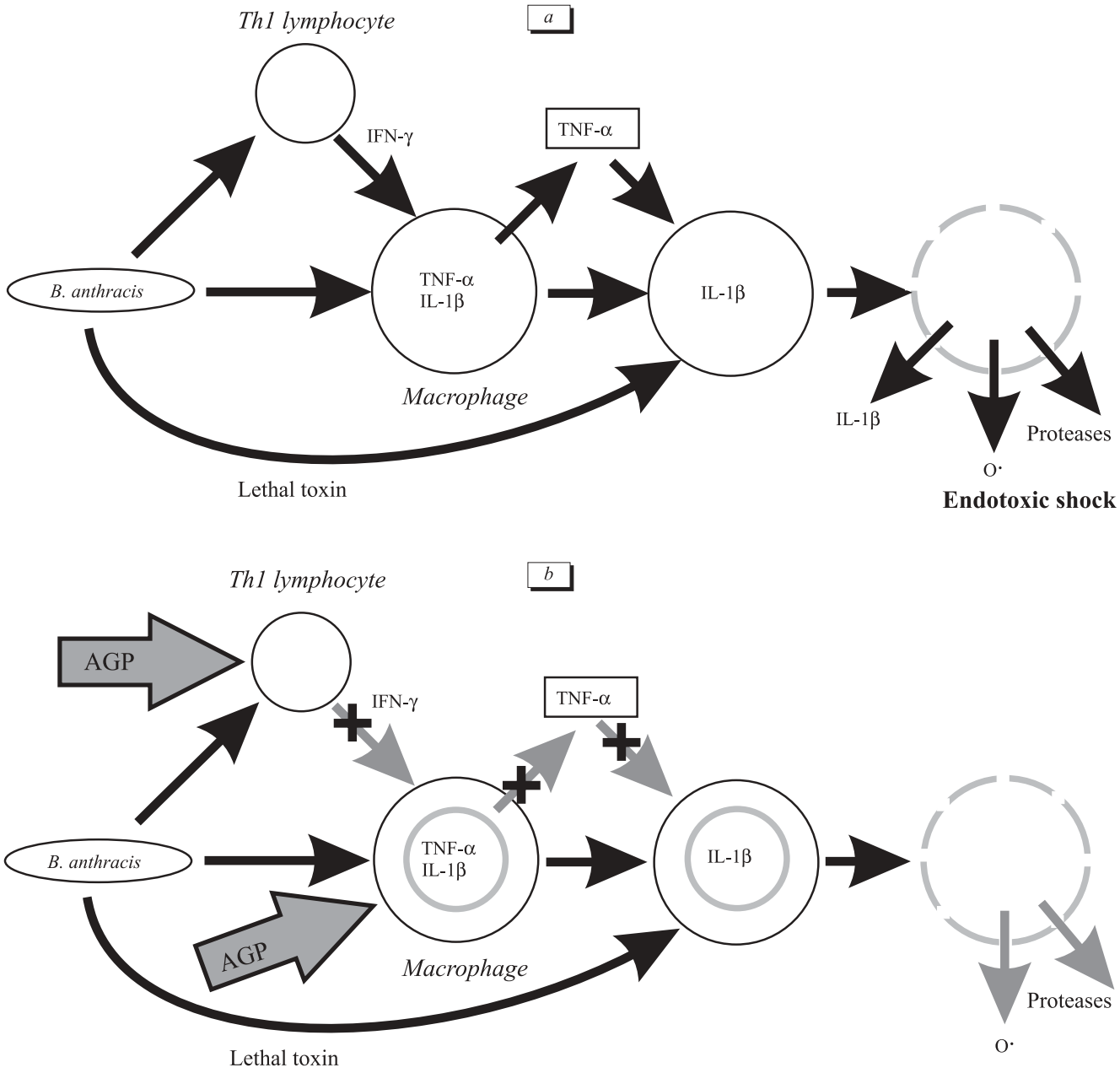

Fig. 3. Hypothetic mechanism for the protective effect of AGP on mice infected with *B. anthracis* in the lethal dose. Endotoxic shock in untreated animals (*a*); prevention of endotoxic shock after administration of AGP (*b*). Crossed arrows: absence of cytokine.

were revealed in the concentrations of other proinflammatory cytokines (TNF-α and IL-1β, Fig. 2, *a*).

The concentrations of IFN-γ and TNF-α in the plasma progressively increased 1 day after administration of the bacterial culture. IL-1β concentration in these mice remained low and did not differ from that observed in intact animals (Fig. 2, *b*). Treatment with AGP preparations had no effect on IFN-γ concentration, but significantly decreased TNF-α content.

The systemic effects resulting in death of animals with anthrax are mediated by so-called lethal toxin (LT). LT produces a selective cytotoxic effect on macrophages that are lysed over 1-2 h after interaction with this compound in high concentration [9]. At the same time, LT in low concentration stimulates production of proinflammatory cytokines IL-1β and TNF-α by macrophages. These changes impair the release of IL-1β, which is accumulated in the cytoplasm of macrophages [10]. It contributes to a decrease in IL-1β concentration in the plasma of infected animals (Fig. 2).

Figure 3 illustrates a possible mechanism for the protective effect of AGP. Administration of *B. anthracis* STI-1 over the first hours after infection was followed by a significant increase in the concentration

TABLE 2. Therapeutic Effect of AGP Preparations in Mice Infected with *B. anthracis* STI-1 Culture in LD_{50}

Preparation	Dose, μg	Number of dead mice/total number of mice	Percent of dead mice	Time-to-death for each mouse, days
Control		5/6	83.3	3, 5, 5, 6, 6
AGP (native preparation)	3000	2/6	33.3	4, 5
	1500	1/6	16.7	3
	750*	0/6	0	—
	375*	0/6	0	—
	187.5	2/6	33.3	3, 3
AGP-A	3000*	0/6	0	—
	1500*	1/6	16.7	4
	750*	1/6	16.7	3
	375	3/6	50.0	3, 3, 3
	187.5*	1/6	16.7	4
AGP-B	3000	2/6	33.3	3, 4
	1500	2/6	33.3	3, 3
	750*	0/6	0	—
	375*	0/6	0	—
	187.5*	1/6	16.7	5
AGP-C	3000	2/6	33.3	3, 3
	1500	3/6	50.0	3, 4, 4
	750	3/6	50.0	3, 3, 4
	375*	0/6	0	—
	187.5	3/6	50.0	3, 3, 5

of IFN-γ, which potentiates the stimulatory effect of bacterial LPS on macrophages. Activated macrophages synthesize proinflammatory cytokines IL-1β and TNF-α. It should be emphasized that IL-1β undergoes intracellular accumulation, while TNF-α is secreted and accumulated in the peripheral blood. TNF-α is detected in the peripheral blood after 24 h. Blood LT concentration progressively increases during bacterial reproduction. When the concentration of LT reaches a critical level, this compound causes massive death of macrophages and release of proteases, singlet oxygen, and intracellular IL-1β. These changes determine manifestations of endotoxic shock and are accompanied by rapid death of experimental animals.

The protective effect of AGP is probably associated with inhibition of macrophage activity. Pretreatment with the test preparation abolished the increase in IFN-γ concentration over the first hours after infection, which should prevent activation of macrophages stimulated with *B. anthracis*. It cannot be excluded that AGP has a direct inhibitory effect on macrophages, since this compound specifically binds to macrophages and monocytes [6]. Administration of AGP and its glycoforms 24 h after infection is accompanied by a significant decrease in TNF-α concentration, which prevents the progressive activation of macrophages. The intensity of IL-1β production probably decreases under these conditions. Therefore, the amount of toxic products released in AGP-receiving infected animals is insufficient to cause lethal endotoxic shock (even during massive death of macrophages, Fig. 3).

REFERENCES

1. A. L. Pukhalsky, E. A. Kalashnikova, S. D. Shyian, and N. V. Bovin, *Byull. Eksp. Biol. Med.*, **125**, No. 1, 79-83 (1998).
2. A. L. Pukhalsky, A. P. Toptygina, E. A. Kalashnikova, *et al.*, *Ibid.*, **118**, No. 7, 71-83 (1994).
3. A. L. Pukhalsky, G. V. Shmarina, E. A. Kalashnikova, *et al.*, *Ibid.*, **129**, No. 5, 567-570 (2000).
4. A. L. Pukhalsky, G. V. Shmarina, A. G. Lyutov, *et al.*, *Ibid.*, **131**, No. 5, 564-567 (2001).
5. S. D. Shyian, V. V. Nasonov, N. V. Bovin, *et al.*, *Bioorg. Khimiya*, **17**, No. 9, 663-670 (1991).
6. S. D. Shyian, S. V. Khaidukov, A. L. Pukhalsky, and A. P. Toptygina, *Gematol. Transfuziol.*, **41**, No. 2, 24-29 (1996).
7. T. Andus, T. Geiger, T. Hirano, *et al.*, *Eur. J. Immunol.*, **18**, No. 5, 739-746 (1988).
8. M. A. Daemen, V. H. Heemskerk, C. van't Veer, *et al.*, *Circulation*, **102**, No. 12, 1420-1426 (2000).

9. A. M. Friedlander, *J. Biol. Chem*., **261**, No. 16, 7123-7126 (1986).
10. P. C. Hanna, D. Acosta, and R. J. Collier, *Proc. Natl. Acad. Sci. USA*, **90**, No. 21, 10,198-10,201 (1993).
11. T. Hochepied, V. van Molle, F. G. Berger, *et al*., *J. Biol. Chem*., **275**, No. 20, 14,903-14,909 (2000).
12. C. Libert, P. Brouckaert, and W. Fiers, *J. Exp. Med*., **180**, No. 4, 1571-1575 (1994).
13. A. L. Pukhalsky, S. D. Shyian, E. A. Kalshnikova, *et al*., *Mediators Inflamm.*, **7**, No. 2, 115-118 (1998).
14. J. P. Williams, M. R. Weiswer, T. T. Rechet, *et al*., *Am. J. Physiol*., **273**, No. 5, Pt. 1, G1031-G1035 (1997).