Immunomodulating Effects of α_1-Acid Glycoprotein (Orosomucoid) in a Culture of Human Peripheral Blood Lymphocytes

A. L. Pukhal'skii, A. P. Toptygina, E. A. Kalashnikova, S. D. Shiyan, V. V. Nasonov, N. V. Bovin, A. G. Lyutov, and F. T. Bairushin

UDC 615.276.4.015.44:616.155.32].07

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 118, № 7, pp. 71-73, July, 1994
Original article submitted February 2, 1994

The mechanisms of the antiproliferative effect of α_1-acid glycoprotein (AGP) isolated from the blood of healthy donors (nAGP) and from the ascitic fluid of patients with stomach cancer (aAGP) are studied. Three fractions of AGP are divided into 3 groups according to their ability to bind to concanavalin A (ConA): AGP not binding to ConA (AGP-1) and AGP weakly (AGP-2) and strongly (AGP-3) binding to ConA. It is shown that native preparation of aAGP has a more potent inhibitory effect on lymphocyte proliferation than native preparation of nAGP. The most potent inhibitory effect is exerted by AGP-3. Native preparation of aAGP does not affect the secretion of interleukin-2 (IL-2) by lymphocytes, whereas AGP-1 inhibits this process. The weakly bound fraction has a stimulatory effect both on the proliferative response and on IL-2 secretion.

Key Words: *orosomycoid; lymphocyte proliferation; interleukin-2 production*

a_1-Acid glycoprotein (orosomucoid) is an acute phase protein that may play an important role in the adaptation of the immune system to stress. Experimental data suggest that AGP is a naturally occurring immunomodulating agent which is present in blood serum in both health and pathology (neoplasms, rheumatoid arthritis, burn disease), and its blood level in the latter case may be 2- to 5-fold higher than normal [8,9,12]. There is evidence indicating that in some pathological processes an increase in the blood content of AGP is paralleled by structural changes in the hydrocarbon chains of this glycoprotein, which may affect its biological activity [8]. It has also been demonstrated that AGP with different degrees of glycosylation vary in the ability to inhibit the proliferation of cultured lymphocytes [6,11].

Research Center for Medical Genetics, Russian Academy of Medical Sciences; Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow; Immunopreparat Conglomerate, Ufa

In this study we attempted to investigate some mechanisms of the antiproliferative activity of various AGP forms that differ in degree of glycosylation. For this purpose we assessed in a wide dose range the ability of AGP preparations to inhibit the proliferation of lymphocytes and examined the effect of these preparations on the secretion of interleukin-2 (IL-2) by these cells.

MATERIALS AND METHODS

AGP was purified from the blood of healthy donors and from the ascitic fluid of patients with stomach cancer, as described elsewhere [1,5]. Fractionation by the glycosylation level was performed on a ConA-Sepharose column as described previously [4]. Three AGP fractions were obtained: not binding (AGP-1), weakly binding (AGP-2), and strongly binding (AGP-3) to ConA. The immunomodulating effects of these preparations were stud-

0007-4888/94/0007-0755$12.50

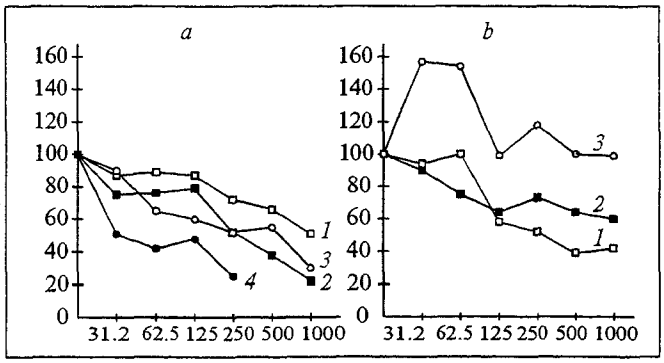

Fig. 1. Effects of nAGP (*a*) and aAGP (*b*) on the proliferative response of PHA-stimulated peripheral blood lymphocytes. *1*) native AGP preparation; *2*) AGP-1; *3*) AGP-2; *4*) AGP-3.

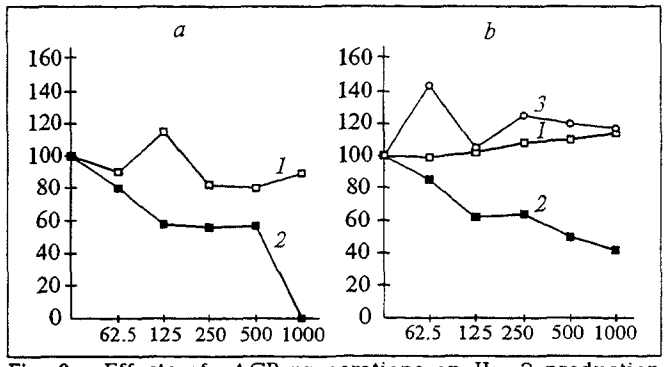

Fig. 2. Effects of aAGP preparations on IL-2 production by lymphocytes isolated from peripheral blood obtained from two donors (*a*, *b*). *1*) native aAGP; *2*) aAGP-1; *3*) aAGP-2.

ied in a culture of lymphocytes isolated from the peripheral blood of healthy donors by centrifugation on a Ficoll-Verografin gradient. Inhibition of lymphocyte proliferation in response to phytohemagglutinin (PHA) was studied as described previously [2,3]. Briefly, the cells were incubated in flat-bottomed 96-well plates with six different concentrations of AGP in the presence of the optimal concentration of PHA (5 μg/ml). Incubation was carried out in RPMI-1640 medium supplemented with additives and 10% horse serum in a humid atmosphere containing 5% CO_2. The intensity of proliferation was evaluated by [^{3}H]thymidine incorporation. The IL-2 secretion was assessed as described [11]. The cells were incubated in the presence of ConA (20 μg/ml) in 24-well plates in 1.5 ml culture medium with various concentrations of AGP or without the agent. After 4 h of incubation the cells were washed, resuspended in fresh medium, and incubated for another 20 h. The culture medium was then aspirated, and the activity of IL-2 was evaluated by its ability to promote growth of the IL-2-dependent cytotoxic cell line CTLL-2. Titration curves were compared using probit analysis.

RESULTS

The characteristics of native AGP preparations and fractions are given in Table 1. It can be seen from the table that individual fractions differ both in the carbohydrate content and in the degree of carbohydrate chain branching. For example, the percentage of biantennary chains in a native AGP preparation isolated from the ascitic fluid is higher than that in AGP isolated from donor blood. The prevalence of such fractions is noticeable in the fraction strongly binding to the sorbent.

The ability of AGP preparations to inhibit the proliferative response of cultured lymphocytes varied, AGP isolated from the blood having a less pronounced inhibitory effect compared with AGP isolated from the ascitic fluid (Fig. 1). The activity of individual fractions differed considerably from the activity of native preparation. For example, all fractions of nAGP with a moderate antiproliferative activity (ED_{50}=1147 μg/ml) had a pronounced inhibitory effect on lymphocyte proliferation. In this case nAGP-3, which strongly binds to ConA due to the presence of three biantennary chains, exhibited the highest activity (ED_{50}=115.1 μg/ml). The nAGP-1 fraction (not binding to ConA) displayed a lower activity (ED_{50}=333.7 μg/ml). The nAGP-2 fraction (moderate binding to ConA) had the lowest antiproliferative activity (ED_{50}= 412.1 μg/ml, Fig. 1, *a*); this AGP fraction contains only two biantennary chains. The results were somewhat different with AGP isolated from the ascitic fluid: nAGP exhibited a high antiproliferating activity (ED_{50}=256.1 μg/ml). The AGP-1 fraction also inhibited lymphocyte proliferation, although less effectively (ED_{50}=2539.1 μg/ml), whereas the AGP-2 fraction had a stimulatory effect (Fig. 1, *b*).

The AGP preparations affected not only lymphocyte proliferation but also IL-2 secretion, which plays a key role in the immune response. We studied the effect of native aAGP and of two fractions, aAGP-1 and aAGP-2, on the *in vitro* secretion of IL-2 by lymphocytes isolated from the peripheral blood of two donors. While in both cases native aAGP had no appreciable effect on the production of this lymphokine, aAGP-1 had a pronounced inhibitory effect (Fig. 2). In contrast, aAGP-2 (tested on lymphocytes of only one donor) exhibited stimulatory activity (Fig. 2, *b*). The fraction strongly binding to ConA-Sepharose was not studied in these series of experiments.

Thus, orosomucoid preparations isolated from the peripheral blood of healthy donors and from the ascitic fluid of patients with stomach cancer inhibit proliferation of PHA-stimulated lympho-

TABLE 1. Characterization of Different Forms of AGP

Preparation	M_r (kD)	Content				
		carbohydrates, %	Neu5Ac, mol/mol	antennary chains, %		
				4-	3-	2-
nAGP	43.5	39.5	15.0	48	39	12
nAGP-1	43.5	40.0	16.0	47	41	11
nAGP-2	41.2	37.0	13.0	32	30	37
nAGP-3	39.5	34.0	11.0	21	16	62
aAGP	44.0	40.0	16.0	43	38	18
aAGP-1	44.0	41.0	17.0	47	42	10
aAGP-2	41.5	37.5	13.5	32	31	36
A-AGP-3	40.0	35.0	12.0	25	16	57

cytes, the inhibitory activity of the preparation isolated from the ascitic fluid being considerably higher. All nAGP fractions had a pronounced antiproliferating activity, the highest activity being displayed by the fraction strongly binding to the sorbent. These results agree with the published data [10]. Although aAGP had a higher inhibitory activity compared with nAGP, one of its fractions (aAGP-2) stimulated lymphocyte proliferation. Evidence confirming the ambivalent action of orosomucoids has been obtained in other experimental systems [7,10,12]. Our data on the effect of AGP on IL-2 production contribute to the concepts regarding the mechanisms of the immunomodulating effect of this orosomucoid. Despite the fact that native aAGP had a pronounced inhibitory effect on lymphocyte proliferation, we did not find it to have any appreciable effect on the production of IL-2, whereas the aAGP-1 fraction strongly inhibited this (Fig. 2). On the other hand, while this fraction inhibited the proliferation of lymphocytes; it did so to a lesser degree than the native AGP preparation.

Thus, several mechanisms seem to be responsible for the immunomodulating effect of AGP. The inhibition of IL-2 secretion is one of these mechanisms. At the same time, it was demonstrated that aAGP-2 stimulates both lymphocyte proliferation and IL-2 production. It can be assumed that the direction of the immunomodulating effects is determined by the structural peculiarities of the oligosaccharide chains in the AGP molecule.

This study was financially supported by the Russian Foundation for Basic Research, Grant № 93-04-6256.

REFERENCES

1. L. I. Novikova, in: *Development of Techniques for Isolation of BOF, Preparation of Antisera to Them, and Some Aspects of their Application* [in Russian], Abstr. of PhD. Dissertation, Moscow (1990).
2. A. L. Pukhal'skii, A. P. Mezhneva, and L. A. Pevnitskii, *Byull. Eksp. Biol. Med.*, **105**, № 2, 196-198 (1988).
3. A. 1. Pukhal'skii, A. P. Toptygina, O. E. Dolomanova, *et al., Pediatriya*, № 4, 39-43 (1991).
4. S. D. Shiyan, V. V. Nasonov, N. V. Bovin, *et al., Bioorgan. Khimiya*, **17**, № 5, 663-670 (1991).
5. S. D. Shiyan, V. V. Nasonov, and N. V. Bovin, *Eksp. Onkol.*, **15**, № 5, 53-61 (1993).
6. S. D. Shiyan, N. N. Nasonov, N. V. Bovin, and A. E. Medvedev, *Ibid.*, № 2, 46-50 (1993).
7. K. M. Chiu, R. F. Mortensen, A. P. Osmand, *et al., Immunology*, **32**, 997-1005 (1977).
8. M. Fujii, N. Takahashi, H. Hayashi, *et al., Anticancer Res.*, **8**, 303-306 (1988).
9. A. Koji, in: *Structure and Function of Plasma Proteins*, Ed. A. C. Allison, Vol. 1, New York., (1974) pp. 94-95.
10. O. Pos, R. A. J. Oostendorp, M. E. Van der Stelt, *et al., Inflammation*, **14**, 133-141 (1990).
11. A. L. Pukhalsky, E. A. Kalashnikova, V. N. Lyashko, and L. A. Pevnitsky, *Int. J. Immunopharmacol.*, **12**, 657-663 (1990).
12. K. Schmid, in: *The Plasma Proteins*, Ed. F. W. Putnam, Vol. 1, New York., (1975), pp. 183-228.
13. K. V. Singh and H. H. Fudenberg, *Immunol. Lett.*, **14**, 9-13 (1986).