Modification of Humoral Immune Response in C57Bl/6 Mice with a Complex of α-Fetoprotein and Retinoid Acid Derivatives

D. V. Arsenov, M. B. Golubeva, M. A. Kisel', N. A. Konoplya, G. S. Lyubin, B. B. Kuz'mitskii, and O. A. Strel'chenok

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 132, No. 10, pp. 409-412, October, 2001
Original article submitted July 2, 2001

Amides of all trans-retinoic acid and O-phospho-L-threonine, O-phospho-L-tyrosine, and O-phosphoethanolamine injected intravenously in a dose of 6.8 μg/kg 1.5-3.4-fold increased the count of antibody-producing cells in the spleen of C57Bl/6 mice (primary immune response to sheep erythrocytes). Activity of the complex containing α-fetoprotein and L-threonine ligand did not differ from that of free retinoid. This complex holds much promise as a prototype of immunomodulators with a wide range of activity.

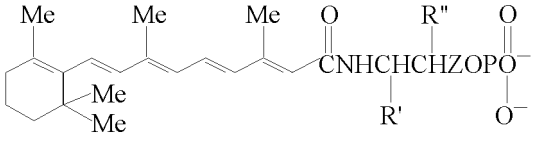
Key Words: *immunomodulators; retinoids; α-fetoprotein; primary immune response*

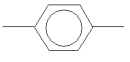
Tumor growth is accompanied by various immunological disorders. Immunomodulators with different activities that modulate the immune response and affect antitumor immunity hold much promise in this respect. Experimental studies showed that α-fetoprotein (AFP) modifies tumor cells, which facilitates their recognition and destruction by immune cells [6]. Retinoic acid and its derivatives activate production of interleukin-2 by mouse splenocytes in mixed lymphocyte cultures and stimulate induction of specific cytotoxic T cells and natural killer cells [4], which are involved in the realization of immune reactions to tumor cells.

Here we studied the effects of complexes containing retinoids and AFP on the primary immune response in mice to evaluate whether these compounds possess immunomodulatory properties (similarly to endogenous analogues).

MATERIALS AND METHODS

We studied amides of all trans-retinoic acid and O-phospho-L-serine (I), O-phospho-L-threonine (II), O-phospho-L-tyrosine (III), and O-phosphoethanolamine (IV):



	R'	R"	Z	Compound
NR-Ser-PA	COOH	H	———	I
NR-Thr-PA	COOH	CH_3	———	II
NR-Tyr-PA	COOH	H		III
NR-EA-PA	H	H	———	IV

We used AFP from retroplacental blood of parturients [2] that can act as the vector molecule [5] and AFP-II and AFP-III complexes containing immunologically active ligands.

Experiments were performed on C57Bl/6 mice displaying weak reactions to sheep erythrocytes (SE). The animals were kept under standard vivarium conditions. Each group consisted of 6 adult mice. Body weight variations were ±3 g.

AFP-ligand complexes were prepared *ex tempore* using purified protein (500 μg in 5 ml distilled water) and retinoids (4.2 mg in 2.1 ml distilled water). Each test complex contained 450 μg AFP and 2.28, 4.55,

Department of Biological Assays, Institute of Bioorganic Chemistry, National Belarussian Academy of Sciences, Minsk. ***Address for correspondence:*** kuzm@ns.iboch.ac.by. Kuz'mitskii B. B.

0007-4888/01/1324-0963$25.00

TABLE 1. Effects of AFP (9 μg/mouse) and Retinoids I-IV (6.8 mg/kg Intravenously) on Humoral Immune Response in C57Bl/6 Mice Immunized with SE (*M*±*m*)

Parameter	Control	AFP	Retinoid			
			I	II	III	IV
Number of APC per 10^6 splenocytes	407.4±10.7	363.0±66.4 (-11)	458.4±57.4 (13)	961.3±56.5* (136)	1354.3±99.9* (232)	639.8±54.6*** (57)
Number of APC in the spleen, 10^3	40.5±2.9	44.8±3.8 (10)	55.0±10.7 (36)	107.0±8.5* (164)	138.9±10.0* (243)	68.3±6.1* (69)
Splenic index	6.2±0.3	5.5±0.4 (-11)	6.3±0.2	6.8±0.2 (10)	6.0±0.5	6.5±0.4
HA titer	6.3±0.3	6.8±0.4 (8)	6.33±0.24 (0)	8.7±0.3*** (37)	7.5±0.2*** (19)	7.5±0.2*** (19)

Note. Here and in Table 2: % of the control is shown in parentheses. *p<0.001, **p<0.01, and ***p<0.05 compared to the control.

TABLE 2. Effects of AFP-II and AFP-III Complexes on Humoral Immune Response in C57Bl/6 Mice Immunized with SE (*M*±*m*)

Parameter	Control	AFP-II, mg/kg			AFP-III, mg/kg		
		2.3	4.6	6.8	2.3	4.6	6.8
Number of APC							
per 10^6 splenocytes	406.4±16.8	716.4±52.4** (76)	827.7±34.6* (104)	889.9±72.3* (119)	405.0±47.9 (0)	692.9±44.8** (71)	811.6±39.9* (100)
per spleen, 10^3	60.4±3.9	89.4±8.7** (48)	90.0±9.5** (49)	145.6±14.6* (141)	55.0±6.7 (9)	87.6±4.3** (45)	99.1±4.6* (64)
Splenic index	6.6±0.3	6.5±0.3	6.6±0.2	6.4±0.4	6.6±0.5	6.3±0.4	6.1±0.3
HA titer	6.2±0.3	7.7±0.2*** (24)	8.0±0.3*** (29)	8.2±0.2** (32)	6.0±0.3 (3)	6.6±0.4 (7)	6.8±0.2 (10)

or 6.83 mg of the corresponding retinoid (per 1 kg body weight). The solutions were injected intravenously during immunization.

SE (antigen) were injected intraperitoneally in a dose of 2.5×10^8/ml 0.9% NaCl (0.2 ml).

The mice were decapitated 5 days after immunization. The spleens were weighted. The splenic index and the number of antibody-producing cells (APC) per 10^6 splenocytes (relative count) and per spleen (absolute count) were estimated by the method [7] with modifications [3]. Plasma hemagglutinin (HA) was measured [1].

The results were analyzed by Student's *t* test.

RESULTS

Treatment of C57Bl/6 mice with free AFP (9 μg intravenously) did not modify cell and humoral (HA) immune reactions to the T cell-dependent antigen (Table 1). Hence, this oncofetal protein possesses no immunological activity. This is consistent with published data [8].

Free retinoids II, III, and IV in a dose of 6.8 mg/kg potentiated the immune response in immunized mice. Phosphates of NR-Tyr-RA (III), NR-Thr-RA (II), and ethanolamine stimulated the primary immune response by 3.4, 2.4, and 1.5 times, respectively, compared to the control (Table 1). N-retinoyl-O-phosphate-L-serine (I) only slightly potentiated the primary immune response.

Free retinoids elevated HA titers in mouse plasma. It should be emphasized that NR-Thr-RA induced simultaneous changes in test parameters. A close correlation between changes in the relative and absolute APC counts caused by retinoids II and III indicates that stimulation of the immune response is related to a rise in the number of IgM-secreting cells, but not to an increase in the spleen cellularity.

Increasing the dose of retinoid II in the complex with AFP from 2.3 to 6.8 mg/kg was not accompanied by proportional stimulation of the immune response (Table 2). This complex increased the count of APC by 1.8, 2, and 2.2 times compared to the control. Therefore, the threshold for stimulation of the immune response occurred at the lowest content of retinoid II in complexes with AFP. HA titers in mouse plasma underwent similar changes.

The AFP-II complex and free retinoid in a dose of 6.8 μg/kg produced similar effects on the immune response (stimulation by 2.5 and 2.3 times, respectively, Tables 1 and 2). Probably, the immunopositive effect of this complex is determined by the ligand in a high dose. By contrast, in complexes with the lowest content of retinoid II both components contribute to a 2-fold increase in the humoral immune response. A further increase in the content of retinoid II did not enhance immunomodulatory activity of complexes (effect of saturation). Our results indicate that the complex of AFP with retinoid II in a low dose is therapeutically efficient under experimental conditions.

The complex of AFP with retinoid III in a low dose did not modulate immunogenesis. A 2-fold increase in the content of this retinoid in the complex was accompanied by stimulation of the immune response by 1.7 times. The complex containing retinoid III in a dose of 6.8 mg/kg stimulated the immune response only by 2 times (effect of saturation). It should be emphasized that threshold saturation was observed under the effect of complexes containing a 2-fold higher dose of NR-Tyr-RA than NR-Thr-RA. The AFP-III complex increased the count of spleen APC, while plasma HA titers remained unchanged.

Our results indicate that in complexes with retinoids II and III AFP possesses immunopositive and immunonegative properties, respectively. This is probably related to functional characteristics of these ligands. The AFP-II complex 2-fold stimulated the immune response in C57Bl/6 mice immunized with SE. Macromolecules in this complex probably act as functional analogues of products secreted by macrophages or lymphocytes and involved in the regulation of immune reactions. The AFP-II complex induces transformation of B lymphocyte precursors into APC and 2-fold stimulates the immune response in mice with insignificant reactions to SE. This complex holds much promise as a prototype of immunomodulators with a wide range of activity.

REFERENCES

1. E. Zigl', *Immunological Methods*, Ed. Kh. Frimel' [in Russian], Moscow (1979), pp. 108-112.
2. L. I. Survilo, I. I. Vashkevich, M. N. Ermolenko, *et al*., *Izv. Belarus. Akad. Nauk. Ser. Khim. Nauki*, No. 2, 81-85 (2000).
3. A. J. Cunningham, *Science*, **207**, 1106-1107 (1965).
4. G. Dennert, *Ciba Found. Symp*., **113**, 117-131 (1985).
5. C. Esteban, M. Geuskens, and J. Uriel, *Int. J. Cancer*, **49**, No. 3, 425-430 (1991).
6. J. Galea-Lauri, F. Farzaneh, and J. Gaken, *Cancer Gene Ther*., **3**, 202-214 (1996).
7. N. K. Jerne and A. A. Nordin, *Nature*, **140**, 405-407 (1963).
8. S. Sell, H. W. Jr. Sheppard, and M. Poler, *J. Immunol*., **119**, 98-103 (1977).