OVERCOMING NATURAL TOLERANCE TO α-FETOPROTEIN IN RATS

A. I. Gusev and A. K. Yazova

UDC 612.6.02.017.1-064

The possibility of overcoming tolerance to α-fetoprotein (α-FP) in rats and mice was studied. Tolerance in rats to their own α-FP is overcome by immunization with cross-reacting mouse α-FP but not with homologous α-FP. Tolerance in mice to α-FP persisted after injection both of homologous mouse α-FP and of heterologous rat α-FP; this result can evidently be explained by the high background level of α-FP in the blood of C3HA mice.

KEY WORDS: natural tolerance, α-fetoprotein.

The organism cannot respond to the injection of most homologous antigens by an immune reaction because of the existence of natural tolerance to them. α-Fetoprotein (α-FP), the principal serum protein during embryogenesis and present in the postnatal period [1, 8, 9], is no exception in this respect. In the adult its concentration in the blood serum is so small that it can be detected only by highly sensitive radioisotope methods [1, 11, 13, 14]. With the onset of hepatomas or teratoblastomas, or during regeneration of the liver and in pregnancy α-FP reappears in the blood in detectable amounts [1], although no antibodies are formed against it.

Artificially induced tolerance can be overcome by the injection of cross-reacting antigens [7, 15, 17] or of antigens previously conjugated with haptens [16], and naturally occuring tolerance can be overcome by injection of the heterologous analogues of the antigen concerned [18]. Recently [10] antibody formation against homologous α-FP has been successfully induced in rabbits, rats, and horses by immunization with human α-FP.

In the investigation described below an attempt was made to overcome natural tolerance to α-FP in rats and mice. Parts of these investigations were published in 1972 [2].

EXPERIMENTAL METHOD

Wistar rats weighing 80-470 g and C3HA mice weighing 18-22 g were used.

Pure preparations of α-FP from mice (α-FPM) and rats (α-FPR) were obtained from pooled sera of newborn mice and rats aged 1-3 days by preparative disc electrophoresis in polyacrylamide gel in tubes and by the method described previously [3, 5].

Antigens were injected subcutaneously, intramuscularly into the hind limb, and into the plantar pads of the hind limbs of the animals twice at an interval of 28-30 days. The first injection was given with Freund's complete adjuvant (Difco, USA), the second without it. Usually 30 μg was given at each injection, but in some cases 50 or 90 μg of the purified preparation of α-FP and 0.1 ml of neonatal rat or mouse serum were used. Blood was taken from the retro-orbital sinus of the mice or from the tip of the tail of the rats before the beginning of immunization, on the 28th-30th day after the first injection, and on the 7th-9th day after the 2nd injection of the antigen.

Laboratory of Immunochemistry and Diagnosis of Cancer, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR G. V. Vygodchikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 78, No. 10, pp. 77-80, October, 1974. Original article submitted December 12, 1973.

TABLE 1. Overcoming Tolerance to α-FP in rats and mice

Species of animals	Sex	Immunization		Immune response			
		first injection of antigen	second injection of antigen	primary antibodies against		secondary antibodies against	
				α-FPM	α-FPR	α-FPM	α-FPR
Rats	Males	α-FPM	α-FPM	31/33	9/30	33/23	31/33
	Females: without offspring	α-FPM	α-FPM	7/10	1/10	22/22	19/22
	with offspring	α-FPM	α-FPM	18/20	3/20	20/20	18/20
		NMS	NMS	3/3	0/3	3/3	3/3
	Males	α-FPM	α-FPR	—	—	10/10	4/10
		α-FPM	α-FPR	0/8	0/8	0/8	0/8
		NRS	NRS	0/3	0/3	0/3	0/3
		α-FPR Heated to 80° for 30 mins	α-FPR	0/9	0/9	0/9	0/9
Mice	Males	α-FPR	α-FPR	—	—	0/16	16/16
		α-FPM	α-FPM	—	—	0/7	0/7

Note: Numerator gives number of animals with antibodies in their serum; denominator gives number of animals tested and NMS and NRS denote neonatal mouse and rat serum respectively.

The antisera obtained from each animal separately were tested by precipitation in agar in a semimicromodification [4] with the aid of a test system for α-FP. The test system for α-FPM consisted of rabbit antiserum against α-FPM and neonatal mouse serum containing 70 μg/ml of α-FPM; the test system for α-FPR consisted of rabbit antiserum against α-FPR and neonatal rat serum containing about 90 μg/ml of α-FPR. The rabbit antisera against α-FPM and α-FPR were obtained as described previously [6].

EXPERIMENTAL RESULTS

Sera taken from the animals before immunization were tested by immunodiffusion in gel with the aid of both test systems. The experiments showed that neither α-FP nor antibodies against it were discovered in any of the 108 rat sera or the 23 mouse sera tested.

Immunization of the rats with heterologous cross-reacting α-FPM led to the formation of antibodies not only against α-FPM, but also against their own homologous α-FPR (Table 1). It should be noted that of the 63 rats only 13 (20.3%) reacted to the first injection of antigen by antibody production against α-FPR. The second injection of the same antigen sharply stimulated the secondary immune response: of 78 rats 71 (91%) now produced antibodies against α-FPR and all 78 rats did so against α-FPM. Not only the comparative content of antibodies against α-FPM and α-FPR in the hyperimmune rat sera is shown (Fig. 1a, b), but also the reaction of partial identity between α-FPM and α-FPR obtained with these antisera (Fig. 1c). The α-FPM completely neutralized antibodies against both α-FPM and α-FPR, but the α-FPR neutralized antibodies only against α-FPR (Fig. 1d, e). These experiments demonstrate that tolerance not to the whole α-FPR molecule, but only to some of its determinants, was overcome.

The number of animals giving a secondary immune response to homologous α-FP was independent of their weight and sex and also of the mode of administration of the antigen and its dose, although the strength of the immune response was determined by the character of the antigen injected. The immune response to injection of the pure preparation of α-FPM was clearly stronger than to the injection of serum.

The dynamics of disappearance of antibodies from the rat sera varied: antibodies against α-FPR were eliminated as a rule by 20-25 days, whereas antibodies against α-FPM were found more than 3 months after the second injection of antigen.

Attention is directed to rats (Table 1) immunized 10 days after birth. They gave the same immune response as the other animals even though all the rats of this group retained considerable quantities of α-FPR during the two weeks before the beginning of immunization.

The picture was quite different when mice were immunized with heterologous α-FPR. Antibodies against α-FPR were present in the sera of all 16 animals, but none had antibodies against homologous α-FPM (Table 1).

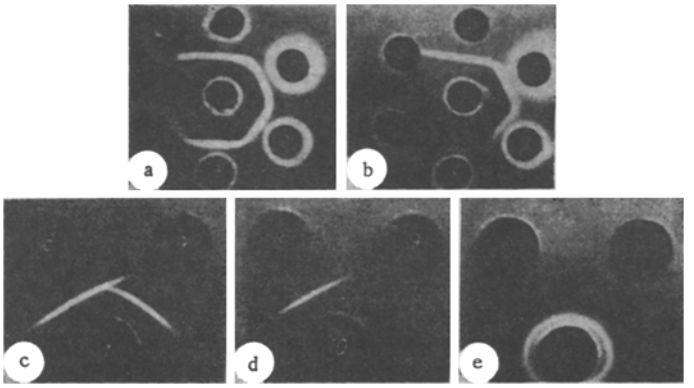

Fig. 1. Immunodiffusion analysis of hyperimmune rat serum: 1-5) serial double dilutions (from 1:1 to 1:16) of rat immune serum; 6, 7) antiserum and antigen of test system to α-FPM (a) and α-FPR (b) respectively; 8, 9) sera of newborn mice and rats in optimal dilutions, respectively – 1:80 and 1:160; 1, 10, and 11) immune rat serum; native, c), absorbed by α-FPR (d) and α-FPM (e) respectively.

The results of the experiments to study immunization of rats and mice with homologous α-FP showed that no immune response was present. None of the 20 rats, including the 9 that received α-FPR previously heated to 80°C for 30 min, showed antibodies against α-FPR. However, if the rats were immunized initially with α-FPM, and later with α-FPR, antibodies against α-FPR were found in 4 of the 10 rats. More convincing data were obtained when the rats received 2 injections of α-FPM, and after elimination of antibodies against their own α-FP, they received an injection of α-FPR. In that case antibody production against α-FPR was resumed, although these antibodies were neutralized not only by α-FPR, but also by heterologous α-FPM. Consequently, in these experiments also tolerance was not overcome to the whole α-FPR molecule.

Unlike in the experiments of Japanese workers [10], according to whom antibodies against homologous α-FP in rats were obtained by immunization with human α-FP, in the present experiments, carried out simultaneously but independently of the Japanese work, tolerance in rats to homologous α-FP was overcome by the injection of cross-reacting α-FPM. The immune response to injection of α-FPM was much stronger than that to injection of human α-FP. Whereas antibodies produced in rats in response to injection of α-FPM were clearly detectable in native and even in diluted antisera, the antisera obtained after injection of human α-FP had to be concentrated fivefold.

The group of female rats immunized 10-12 days after giving birth to their offspring deserves particular attention. α-FP began to appear in these animals on the 13th day of pregnancy, by the end of pregnancy the titer in some of these animals had reached 1:32, but after parturition this protein was eliminated from the serum and by the 10th day its content had evidently reached the background level, for the "half-life" period of α-FPR is 24 h. Presumably the presence of α-FP for a long period in the rats' serum has a tolerogenic action on the organism, and for that reason the female rats gave no immune response to their own α-FP after injection of α-FPM. However, the experiments showed that the females that had given birth to offspring gave the same response as those which had not.

Evidently not all species of animals can overcome tolerance to α-FP. For instance, in the present experiments it was overcome in rats, whereas in mice it could not be overcome by injection of either homologous or heterologous α-FP. These results can probably be explained by the different background levels of α-FP in the blood of the rats and mice. In healthy adult rats the background level of α-FP in the serum is 8-25 ng/ml [11], compared with 30-350 ng/ml in mice [1, 12]. There must evidently be some lower limit of the background level at which an immune response to homologous α-FP is still possible. If, however, the spontaneous level of α-FP is higher than the permitted maximum (as in C3HA mice), antibodies against their own α-FP can evidently be still produced, although they cannot be detected because they are immediately bound by the α-FP present in the blood serum (in vivo exhaustion of the antiserum). In the writers' opinion these hypotheses can be tested by culturing immunocompetent cells taken from the animal after immunization with heterologous α-FP in vitro and following this with an immunochemical study of the

synthesis of antibodies against α-FP and also by determining the α-FP level before and after immunization of the mice.

Tolerance to α-FP (and, probably, to other autologous antigens) can evidently be overcome only in animals whose α-FP level is below a certain limit. The determination of the actual value of this limit is an important task to be undertaken in this field. Another no less important task is the choice of the α-FP-immunogen that would induce the optimal immune response of the recipient animal to its own α-FP, and the study of the effect of such a reaction in vivo on the cells producing α-FP.

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