THERMOSTABLE ANTIGENS OF MALIGNANT TUMORS AND NORMAL TISSUES OF EXPERIMENTAL ANIMALS

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Thermostable antigens (TA) were found by the gel precipitation test and by immunoelectrophoresis in malignant tumors of muscle tissue induced by dimethylbenzanthracene, and in the amniotic fluid of 8-12-day embryos and certain normal organs of adult Wistar rats. Relative tissue specificity of these antigens was demonstrated, on the one hand, for tumors, amniotic fluid, and the uterus, and on the other hand for the lung, spleen, and serum. TA detected in tumors and amniotic fluid by immunoelectrophoresis are located in the zones of α- and α_1-globulins.

KEY WORDS: thermostable antigens; induced tumors; α-globulin.

It is known that more active [3, 5, 6] and relatively homogeneous [1, 2, 4] biological preparations can be obtained by the method of temperature fractionation. It has been shown, for instance, that the sera of normal animals can inhibit immunologic reactions in vivo and in vitro [3, 5, 6]. Heating these sera to 100°C for 10 min and more increased their immunosuppressive activity [3, 7], and in the opinion of some workers, this effect is due to the rupture of noncovalent bonds during heating in the protein macromolecule of the serum, with the subsequent liberation of its active source. The present writers have shown [3], that the sera of normal rats have two thermostable antigens (TA), which preserve their immunogenic and antigenic properties, and that one of them, located in the α_1-globulin zone, also possesses an immunosuppressive action in vitro.

The presence of thermostable antigens in malignant tumors and normal organs of animals and also the immunogenic and antigenic properties of some of them were studied in the present investigation. For comparison, the amniotic fluid of animals which, according to data in the literature [9, 10], inhibits immunologic reactions in vitro, was used in the experiments.

EXPERIMENTAL METHOD

Wistar rats of both sexes and aged from 3 to 8 months were used. TA were studied in the gel precipitation tests and by immunoelectrophoresis. The protein concentration in the antigens was determined by Lowry's method on a spectrophotometer with wavelengths of 750 μ. To obtain immune sera chinchilla rabbits were immunized 5 times intravenously, intramuscularly, and intraperitoneally, at intervals of 3-5 days, with the following rat antigens: whole cells of native tumors induced in rats in the thigh muscle by 9,10-dimethyl-1,2-benzanthracene, a saline extract of these tumors, heated to 100°C for 15 min (TTA), a homogenate of normal liver, amniotic fluid from 8-12-day rat embryos, and normal adult rat serum. Saline extracts from tissues of native induced tumors, TTA, thigh muscle, spleen, lung, uterus, liver, kidney, intestine, brain, testes, and erythrocytes, and also amniotic fluid of 8-12-day embryos and normal serum were used as antigens. The above-mentioned tissue extracts and fluids were heated to 100°C for 2, 5, 10, and 15 min, and some of them for 60-180 min. Both native immune sera and sera concentrated by the method described in [8] were used in the experiments. TA of malignant tumors were concentrated by Lifogel (polyacrylamide gel for macromolecular concentration), in accordance with the instructions of the Gelman Company (England), for 5 h in the proportion of 6 ml of extract to 1 g absorbent. Lifogel is known to absorb substances with molecular weights of under 20,000.

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TABLE 1. Distribution of TA in Normal Organs, Induced Tumors, Amniotic Fluid, and Normal Serum of Rats in Gel Precipitation Tests (number of precipitation lines after 48 h)

Source of antigens	Sera of rabbits immunized with: native tumor No. 2					TTA No. 11					amniotic fluid No. 7					liver homogenate No. 6					Serum No. 44				
	Duration of heating of antigens at 100°C, min																								
	н	2	5	10	15	н	2	5	10	15	н	2	5	10	15	н	2	5	10	15	н	2	5	10	15
Tumor*	7	4	3	3	3	3	3	3	3	3	5	3	3	2	2	4	0	0	0	0	5	0	0	0	0
Thigh muscle	3	0	0	0	0	1	0	0	0	0	3	0	0	0	0	3	0	0	0	0	3	0	0	0	0
TTA*	—	—	—	—	3	—	—	—	—	3	—	—	—	—	2	—	—	—	—	0	—	—	—	—	0
AF*	5	2	2	2	1	3	3	3	2	2	7	2	2	2	0	4	0	0	0	0	3	0	0	0	0
Serum	5	1	1	1	1	1	0	0	0	0	4	1	1	1	1	3	0	0	0	0	6	2	2	2	1
Spleen	4	2	2	1	1	1	0	0	0	0	5	0	0	0	0	3	1	1	1	1	4	1	1	1	1
Lungs	4	2	2	1	1	2	0	0	0	0	5	0	0	0	0	3	0	0	0	0	3	1	0	0	0
Uterus*	4	1	1	1	1	2	2	2	2	2	4	1	1	1	1	3	0	0	0	0	3	0	0	0	0
Liver	3	0	0	0	0	2	0	0	0	0	4	0	0	0	0	6	0	0	0	0	3	0	0	0	0
Kidneys	3	0	0	0	0	2	0	0	0	0	4	0	0	0	0	3	0	0	0	0	3	0	3	0	0
Brain	2	0	0	0	0	2	0	0	0	0	3	0	0	0	0	2	0	0	0	0	3	0	0	0	0
Testes	2	0	0	0	0	1	0	0	0	0	3	0	0	0	0	2	0	0	0	0	2	0	0	0	0
Intestine	2	0	0	0	0	1	0	0	0	0	4	0	0	0	0	2	0	0	0	0	3	0	0	0	0
Erythrocytes	2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2	0	0	0	0	2	0	0	0	0

* Extracts did not form visible coagulated precipitates after heating to 100°C for 60 min or more.

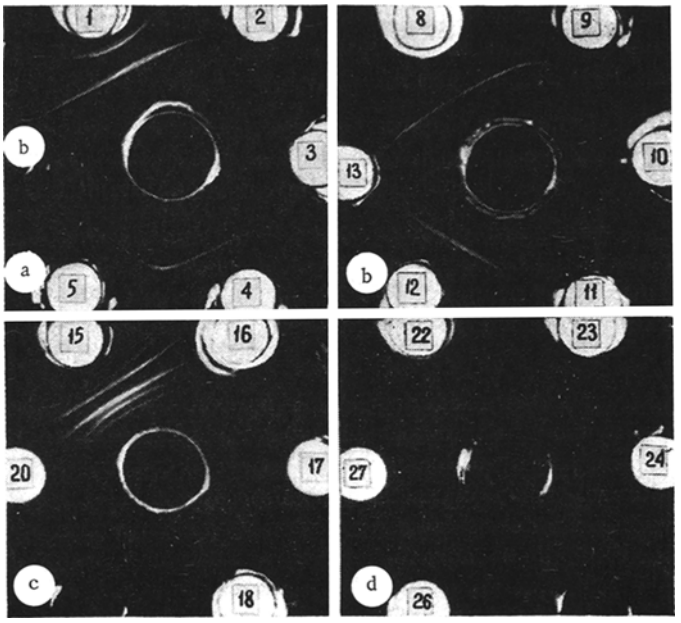

Fig. 1. Gel precipitation reaction between sera of rabbits immunized with native induced tumor (a, b) or with amniotic fluid (c, d) and rat antigens heated to 100°C. a: 1) TTA, 2) liver (2 min), 3) kidney (2 min), 4) spleen (15 min), 5) lungs (15 min), 6) testes (2 min); b: 8) TTA (180 min), 9) uterus (180 min), 10) thigh muscle (2 min), 11) intestine (2 min), 12) pregnant rat serum (180 min), 13) normal rat serum (180 min); c: 15) native amniotic fluid, 16) kidneys (2 min), 17) liver (2 min), 18) lungs (2 min), 19) spleen (2 min), 20) thigh muscle (2 min); d: 22) TTA, 23) testes (2 min), 24) intestine (2 min), 25) brain (2 min), 26) erythrocytes (2 min), 27) empty.

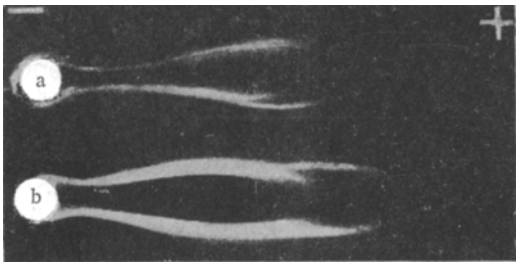

Fig. 2. Immunoelectrophoresis of fraction IV of concentrated serum from rabbit immunized with TTA and antigens from TTA and amniotic fluid of rats. a) TTA in well; b) amniotic fluid heated to 100°C for 5 min in well. Concentrated rabbit immune serum in gutters.

EXPERIMENTAL RESULTS

The investigations by the gel precipitation tests showed that the sera of immune rabbits gave from 1 to 7 precipitation bands with antigens from the sources mentioned above. Fewest bands were observed with extracts with erythrocytes, most with antigens of tumors, amniotic fluid, and serum. Different results were obtained with heated antigens. The data on distribution of TA in rat organs and tissues are summarized in Table 1. They show that as regards thermostability the organs and tissues of rats can be divided into three groups: 1) tumors, amniotic fluid, and uterus; 2) spleen, lung, and serum; 3) organs not containing TA (liver, kidney, muscle, brain, testes, intestine, and erythrocytes). It is interesting to note that even after boiling for a comparatively long time (60-120 min) no precipitation of visible coagulated proteins was observed in extracts of tumors and uterus and of amniotic fluid, whereas extracts of the other organs and tissues, including serum, when heated for 1-2 min, formed large coagulated masses with a small volume of supernatant fluid. With respect to this index, the extracts of tumors, uterus, and amniotic fluid differed from extracts of the other organs in their high thermostability.

Depending on the character of the reaction of the various immune rabbit sera with heated antigens, relative tissue specificity was observed. For instance, the serum of rabbit No. 6, immunized with liver homogenate, gave one precipitation band with heated antigen from splenic tissue but did not react with the other heated antigens, including isologous antigens. On the other hand, serum of rabbit No. 44, immunized with rat serum, reacted with heated antigens of serum, spleen, and lungs but gave no precipitation bands with other heated antigens. It is also interesting to note that the serum of rabbit No. 2, immunized with native tumor, reacted with heated antigens from spleen and lung tissues, amniotic fluid, uterine tissue, and serum, but the serum of rabbit No. 11, immunized with TTA, reacted only with heated isologous antigens of amniotic fluid and uterus and did not react with the other heated antigens. Specimens of the reactions of the immune sera of rabbits with native and heated antigens are illustrated in Fig. 1.

The unusual character of the reaction, for example between the serum of rabbit No. 2 and heated tumor and serum antigens, will be noted (Fig. 1b). For instance, with heated (for 180 min) antigen from the tumor the rabbit's serum gave an unusual and very clear precipitation line, whereas with rat serum heated for the same time the bands were somewhat indistinct and were not identical with the band of the tumor antigen. TA of the tumor and amniotic fluid formed identical precipitation bands in the test. Since TA were detected with the greatest constancy and similarity by the gel precipitation test in the tumors and amniotic fluid, these were subjected to immunoelectrophoresis. Figure 2 shows that the TA of the tumor and amniotic fluid are located in the zones of α- and α_1-globulins.

The results of concentration of the TTA with Lifogel showed that after 24 h three distinct bands appeared in the gel precipitation test with isologous antigen heated to 100°C for 15 min, whereas if unconcentrated TTA were used the third band did not begin to appear until after 48 h. These results also indicate that the tumor TA have a molecular weight of over 20,000.

TA were thus found in the gel precipitation test in malignant tumors and in certain normal organs of rats. The greatest identity and the largest quantity of TA were found in tumors, amniotic fluid, and uterus. The relative tissue specificity of TA for tumors, amniotic fluid, and the uterus, on the one hand, and for the spleen, lung, and normal serum of rats, on the other hand, was demonstrated.

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