SPECIFIC ANTIGEN OF RAT SARCOMA M-1

Z. I. Rovnova

From the Ivanovsky Institute of Virology (Director: Prof. P. N. Kosyakov), Acad. Med. Sci. USSR, Moscow

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L. A. Zilber and his coworkers [1,3,4,5,11] have demonstrated the presence of specific antigenic substances in malignant tumors of man and animals, using the reactions of anaphylaxis and desensitization for this purpose. Evidence of the existence of specific tumor antigens has also been obtained by means of other immunological methods [2,6,7,10, and others].

A number of foreign workers have, however, questioned the existence of specific tumor antigens, and have attempted to explain the specific antigenic properties of tumors from an immunogenetic standpoint. Thus Hauschka [12] considers that tumors, other than those transmittable by ultrafiltrates, do not contain any specific antigens, and that the "antibodies" claimed to have been found in them are in fact isoantibodies, formed as a result of the incompatibility of the tumor tissues with those of the recipient.

It has been shown [8 and others] that normal and tumor tissues contain a variety of antigens. The assortment of antigens, common to both normal and tumor tissues (species, group, type, and others), is identical for tumors and for the various organs of one and the same individual. Additionally, however, tumor tissues contain specific tumor antigens, in the same way that normal tissues contain special organospecific antigenic substances, incidental to their particular functions.

It is also possible that individual antigenic differences may exist, as is shown by the results of experiments on transplantation of skin, for example. Whereas autotransplantation is nearly always successful, homotransplantation is usually unsuccessful.

The question arises, in connection with the study of specific tumor antigens, whether there is any difference between the antigenic properties of tumor tissue and the tissues of other organs of the tumor-bearing animal.

It has been shown in the papers cited above that tumor tissues contain antigens which differ immunologically from those of normal tissues. For their comparative study, these authors took tumor tissue from one animal, and normal tissue from another, as a control.

In view of the absence of incontestable evidence that individual antigenic differences between animals do not exist, we made a comparative immunological study of the antigenic properties of tumors and organs from one and the same animal, since the tumor should contain all the same antigens, species, group, etc., as the normal tissues of the given organism.

For our experiments we obtained serums from rabbits immunized against rat sarcoma M-1, and against liver and spleen from normal rats.*

Serums from 13 rabbits immunized against rat sarcoma M-1 were used. Table 1 presents the results of one of the experiments, in which we made a simultaneous study of absorbed anti-tumor, anti-liver, and anti-spleen serums with rat sarcoma M-1 antigens and with those from the liver and spleen of normal rats.

* The techniques used for obtaining immune serums and for preparing antigens are described in a paper by P. N. Kosyakov, N. I. Kuznetsova, and V. S. Korosteleva [9].

TABLE 1

Reaction of Fixation of the Complement of Specific Serums with Rat Antigens

Serum	No. of rabbit	Dilution of serum	Antigen			Control serum
			sarcoma M-1	normal rat liver	normal rat spleen	
Anti-tumor	169	10	++++	+	++++	—
		20	++++	—	±	—
		40	++	—	—	—
		80	—	—	—	—
Anti-liver	2017	10	—	++++	—	—
		20	—	+++	—	—
		40	—	++	—	—
		80	—	+	—	—
Anti-spleen	1961	10	+	±	+++	—
		20	—	—	+++	—
		40	—	—	+	—
		80	—	—	—	—

TABLE 2

Reaction of Fixation of Complement of Specific Serums with Antigens from Tumor-Bearing Rats

Serum	No. of rabbit	Dilution of serum	Antigen			Dilution of serum	Antigen		
			sarcoma M-1	liver	spleen		sarcoma M-1	liver	spleen
			Rat № 2				Rat № 4		
Anti-tumor	1340	100	++++	—	+	100	++++	±	—
		200	++++	—	—	200	+++	—	—
		400	+	—	—	400	+	—	—
			Rat № 13				Rat № 14		
Anti-tumor	773	40	++++	—	—	40	+++	—	—
		80	++++	—	—	80	++	—	—
		160	++	—	—	160	+	—	—
			Rat № 15				Rat № 16		
Anti-tumor	169	20	++++	±	+	20	++++	+	±
		40	+++	—	—	40	++	—	—
		80	+	—	—	80	±	—	—
			Rat № 15				Rat № 16		
Anti-liver	2017	20	—	++++	—	10	—	++++	—
		40	—	++++	—	20	—	+++	—
		80	—	+(+)	—	40	—	++	—
						80	—	+	—
			Rat № 15				Rat № 16		
Anti-spleen	1961	20	—	—	+++	10	+	±	+++
		40	—	—	++	20	—	—	+++
		80	—	—	—	40	—	—	+
						80	—	—	—

The absorbed anti-tumor serum gave the most intense reaction with sarcoma antigen, and gave only a weak reaction with normal liver and spleen antigens at a dilution of 1:10. In the same dosage, liver and spleen antigens reacted specifically only with their corresponding antisenums.

It is possible that the immunological differences between tumor antigens and those of organs of healthy animals derive from the immunological peculiarities of two different animals – the tumor-bearing animal, and the healthy prospective recipient.

We applied the complement fixation test to specific absorbed serums with antigens from rat sarcoma M-1, liver, and spleen, all taken from one individual (Table 2).

As appears from the data of Table 2, anti-tumor serum reacts most strongly with sarcoma antigen, and very weakly, if at all, with other antigens from the same animal. Anti-liver and anti-spleen serums absorb complement only in the presence of their respective organospecific antigens.

TABLE 3

Reaction of Fixation of Complement of Specific Serums with Antigens from Preserved Tissues

Serum	No. of rabbit	Dilution of serum	Antigen					
			sarcoma M-1 (native)	liver (native)	Spleen (native)	sarcoma M-1(glycerol)	sarcoma M-1(5% formalin)	sarcoma M-1(1% formalin)
Anti-tumor	773	40	++++	+	+++	++++	++++	++++
		80	++++	–	–	++++	++	++
		160	++++	–	–	+++	–	±
Anti-tumor	169	20	++++	±	+	++++	++++	Not tested
		40	+++	–	–	++++	++	
		80	+	–	–	++	±	
Anti-liver	2017	20	–	++++	–	–	–	Not tested
		40	–	++++	–	–	–	
		80	–	++	–	–	–	
Anti-spleen	1961	20	–	–	+++	–	–	Not tested
		40	–	–	++	–	–	
		80	–	–	–	–	–	

TABLE 4

Reaction of Fixation of the Complement of Serums Taken from Rabbits Immunized Against Antigens from Preserved Sarcoma M-1 Tissue, with Rat Antigens

No. of rabbit	Dilution of serum	Antigens				
		sarcoma M-1 (native)	liver (native)	spleen (native)	sarcoma M-1 (glycerol)	sarcoma M-1(5% formalin)
1288, immunized against tumor tissue preserved in glycerol	100	++++	–	++	++++	++++
	200	+++	–	–	++	++
	400	±	–	–	±	–
1211, immunized against tumor tissue preserved in formalin	100	++++	–	+++	++++	++++
	200	+++	–	–	+++	+++
	400	+	–	–	±	±

It has thus been possible, using specific anti-tumor and anti-organ serums, to provide a clear-cut immunological differentiation between sarcoma M-1 tissue and those of the liver and spleen of the same animals; this is incontrovertible evidence of the antigenic specificity of rat sarcoma M-1, and cannot be ascribed to individual antigenic differences between the tumor tissues and the organs of a different, healthy animal, as has been maintained by some authors.

We also studied some of the specific antigenic substances of the tumor.

V. S. Korosteleva [7] found that the specific antigenic substances of human cancerous tissue are resistant to the action of glycerol and formalin solutions, and that they retain their antigenic and immunogenic properties after being treated with such solutions.

We used 1 and 5% formalin and 80% glycerol for the preservation of rat sarcoma M-1 tissues. The results of testing the reaction of fixation of the complement by specific serums with native and preserved sarcoma M-1 antigens are presented in Table 3.

Aqueous saline antigens of sarcoma tissues preserved in glycerol or formalin specifically absorbed complement with anti-tumor serums, but did not react with organospecific serums (Nos. 2017, 1961).

Antigens from tissues preserved in glycerol reacted in the same way as native sarcoma antigen, while those from formalin-preserved tissue gave a somewhat weaker reaction.

Immunization of rabbits with preserved sarcoma tissues shows that these tissues retain their specific antigenic and immunological properties after storage in formalin or glycerol.

Table 4 gives the results of tests of the reaction of fixation of complement of serums taken from rabbits immunized against preserved tissues, with rat antigens.

It is evident from the data of Table 4 that serum No. 1288 (immunized against tissues preserved in glycerol) contains specific anti-tumor antibodies, which react with both the antigens of native and preserved sarcoma tissue. Antigens from tissues preserved in formalin (serum No. 1211) also give rise to production of antibodies to native tumor tissue.

It follows that preservation of rat sarcoma M-1 tissues in 80% glycerol or 1 and 5% formalin does not affect the specificity of the tumor antigen.

Our experiments thus show that rat sarcoma M-1 contains specific tumor antigens, which are different from those of other tissues of the same animal from which the tumor tissue was taken.

The specific antigen of sarcoma M-1 is resistant to the action of 1 and 5% formalin and of glycerol, and the tumor tissue retains its specific antigenic properties after storage in such solutions.

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