ON THE SPECIFIC ANTIGEN OF HUMAN SARCOMAS

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 52, No. 7,
pp. 84-89, July, 1961
Original article submitted July 15, 1960

Numerous experimental investigations on the antigenic properties of malignant neoplasms, performed by Soviet scientists over the last 10-15 years, have widened our concepts of the antigenic structure of tumor cells considerably. However, fundamental attention has thus far been paid to study of the antigenic properties of carcinomatous tumors, while the antigenic structure of sarcoma cells has remained almost completely uninvestigated.

It is known that sarcomas are differentiated into the grouping factors A, B and O, just as the erythrocytes and tissues of the same individual [5]. Up until now the presence of species-specific antigens and the type factors M, N and Rh has not been established in sarcomatous tumors. There is also very little data relevant to the specific characteristics of the antigenic structure of sarcomas. Former attempts to demonstrate the so-called sarcoma-specific antigens [10,11] can hardly be considered successful. Recently, using the method of anaphylaxis with desensitization, it was shown that antigens are contained in sarcomas which are absent from the muscle and connective tissue of the embryo and adult human [1,9].

The present investigation was carried out with the purpose of detecting a specific antigen in human sarcomas and of studying its resistance to boiling and to treatment with formalin. In addition, our task was a comparative study of carcinomatous and sarcomatous tumors, in order to cast some light on the question of a similarity or a difference in their specific antigenic properties.

METHOD

As material for the investigation we used 2 sarcomas, removed during surgery, 8 carcinomas, taken from patients posthumously, and, as controls, healthy tissue from the liver, kidney, spleen, heart, muscle, stomach and brain. The fresh tissue was preserved in a refrigerator at -20°, and used as needed. A portion of the tumor material from each case was preserved in formalin (1 part 40% formalin plus 7 parts water). The antigens were prepared from untreated tissue, formalinized tissue, and tissue boiled for 30 minutes. The technique by which the antigens were prepared, and also the procedure for setting up the complement fixation and absorption reactions used in this work, have already been described by us in previous reports [3,4,7]. Following the method which we worked out [6], we prepared specific sera against each of the carcinomas being studied. This method was used to obtain sera against the antigens from sarcomatous tissue. Organ-specific sera against the liver and spleen of a healthy human [8] served as the control. Immunization was performed with antigens from the untreated tissues.

RESULTS

Preliminary experiments for the purpose of studying the character of the antibodies in the immune antisarcoma sera with antigens from sarcomas and a number of normal human organs (liver, kidney, spleen, heart muscle, stomach and brain) showed that the most non-specific binding is seen with the antigens from the liver and spleen. We selected these tissues as adsorbents in the acquisition of specific antisarcoma sera, as well as for the control in all further work. By means of absorption of the immune serum with the formalinized tissue of the normal spleen we obtained specific serum to one of the sarcomas in our possession. An attempt to prepare serum specific for the second sarcoma was unsuccessful, due to an insignificant difference in the titer of the specific and non-specific antibodies in the original immune serum.

TABLE 1. Comparative Study of the Antigenic Properties of Carcinomatous and Sarcomatous Tissues, Based on the Complement Fixation Reaction

Serum	Serum dilution	Antigen from formalinized carcinoma No. 48	Antigens from untreated tissues			
			of sarcoma		of a healthy human	
			№ 1	№ 2	Liver	Spleen
No. 991, against sarcoma No. 1	1:20	—	++++	—	—	—
	1:40	—	++++	—	—	—
	1:80	—	++++	—	—	—
	1:160	—	+++	—	—	—
No. 212, against carcinoma No. 48	1:20	++++	—	—	—	—
	1:40	++++	—	—	—	—
	1:80	+++	—	—	—	—
	1:160	++	—	—	—	—
No. 851, against the liver of a healthy human	1:10	—	—	—	++++	—
	1:20	—	—	—	++++	—
	1:40	—	—	—	+++	—
	1:80	—	—	—	++	—
No. 834, against the spleen of a healthy human	1:40	—	±	—	—	++++
	1:80	—	—	—	—	++++
	1:160	—	—	—	—	++++

Arbitrary symbols: ++++, +++, ++, + represent the varying degree of the positive reaction; ± represents a doubtful reaction; – represents a negative reaction.

In Table 1 we present the results of the comparative study on the complement fixation reactions of antigens from the tissues of sarcomas Nos. 1 and 2, carcinoma No. 48, and the liver and spleen of a healthy human with specific sera against the carcinomatous and sarcomatous tumors and organ-specific sera against the liver and spleen.

The clearly manifested positive reaction of the antigen from sarcoma No. 1 with the antisarcoma serum No. 991, in the presence of a negative reaction of that serum with antigens from the spleen and liver, presents a convincing argument that a specific antigen is contained in the human sarcoma which is absent from healthy tissues (in this case, the liver and spleen). The specificity of the reaction is confirmed by the results of the interaction of these antigens with the organ-specific sera Nos. 851 and 834.

Multiple trials of the antigen from the second sarcoma yielded negative results for the complement fixation reaction with the sera obtained by us, as is seen in Table 1. On the basis of this it may be postulated that sarcomas of different localization and structure may contain different specific antigens.

In the comparison of the specific antigenic properties of carcinomatous and sarcomatous tumors we observed that the sarcoma antigen does not react with any of the 8 anticarcinoma sera, just as the antigens from the carcinomas studied by us did not react with the serum against sarcoma. From the data in Table 1 it follows that anticarcinoma serum No. 212 fixes complement only with the antigen from carcinoma and does not react in the presence of extracts from sarcoma No. 1, No. 2 or from the control tissues. At the same time, antisarcoma serum yields a positive reaction with the antigen from sarcoma, but, as has already been noted, does not react with the antigen from carcinomatous tumor. The results of these trials testify to a difference in the specific antigenic properties of carcinomatous and sarcomatous tissues in the human. Carcinomas contain a specific antigen, absent from the sarcoma, and, on the other hand, sarcoma cells contain their own specific sarcoma antigen, which is not encountered in carcinomas.

Besides the complement fixation reaction, a comparison of the specific tumor antigens was carried out in absorption experiments, absorbing the antitumor sera with untreated cells from the carcinoma and sarcoma. Despite inadequate clarity of the obtained results, due to the anticomplementary properties of the sera following absorption,

TABLE 2. The Effect of Formalin and Boiling on the Antigenic Properties of Sarcoma and Carcinoma

Serum	Serum dilution	Antigens from the tissues							
		of carcinoma No. 92			of sarcoma No. 1			normal and untreated	
		untreated	formalinized	boiled	untreated	formalinized	boiled	liver	spleen
No. 991, against sarcoma No. 1	1:20	—	—	—	++++	—	—	—	—
	1:40	—	—	—	++++	—	—	—	—
	1:80	—	—	—	++++	—	—	—	—
	1:160	—	—	—	+++	—	—	—	—
No. 211, against carcinoma No. 92	1:40	++++	++++	++++	±	—	—	—	±
	1:80	++++	++++	++++	—	—	—	—	—
	1:160	++++	++++	+++	—	—	—	—	—
	1:320	++	++	++	—	—	—	—	—
No. 834, against healthy human spleen	1:40	—	—	—	+	±	—	±	++++
	1:80	—	—	—	—	—	—	—	++++
	1:160	—	—	—	—	—	—	—	++++
	1:320	—	—	—	—	—	—	—	++

Arbitrary symbols the same as in Table 1.

an antigenic difference was again indicated between the carcinomatous and sarcomatous tissues.

We have collected data on the impressive stability of the specific antigens from the carcinomas against the action of certain physico-chemical factors, such as freezing, drying, high temperature, and formalin [2,4].

In tests of the antigens from sarcomatous tissue subjected to preliminary boiling for 30 min or treatment with formalin, we discovered, using the complement fixation test, that the antisarcoma serum does not fix complement in the presence of antigens from sarcomatous tissue preserved in formalin or heated for 30 min at 100°, while there is a clearly manifested positive reaction between this serum and the antigen from the untreated sarcoma tissue. This can be seen from the data in Table 2. On the other hand, aqueous-saline extracts of carcinomas, both from the untreated as well as the tissues treated with formalin or heating, yield a positive reaction for fixation with the corresponding anticarcinoma serum No. 211.

Thus, the resistance of the specific antigens from the carcinoma and sarcoma cells to the action of certain physico-chemical factors is not the same.

The experiments showed that the specific sarcoma antigen, in contrast to that of carcinoma, is destroyed by boiling or treatment of the tissue with formalin. These data were confirmed by absorption of the serum on tumor cells subjected to the action of formalin or boiling.

In Table 3 we present the protocol of the absorption experiment, involving antisarcoma (No. 991) and anticarcinoma (No. 204) sera absorbed on the cells of formalinized sarcoma and carcinoma. In the first horizontal column we show the reaction of each of the sera with the test antigens before absorption. In the remaining four are reflected the results following the interaction of the sera with the tumor cells. From the data in Table 3 it is apparent that absorption of the antisarcoma serum by the cells of the formalinized carcinoma or sarcoma did not deprive it of its ability to enter into reaction with the antigen from untreated sarcoma tissue. In other words, the sarcoma or carcinoma cells treated with formalin did not extract the specific antibodies from the antisarcoma serum. This may be explained by the fact that these tissues did not contain the antigen specific for sarcoma: in the carcinoma it is absent, and in the sarcoma it is inactivated by formalin. As a result of absorption of the anticarcinoma serum with homologous tissue, i. e. carcinoma, a complete extraction of the specific antibodies occurred. When the second portion of this serum was treated with sarcoma tissue preserved in formalin, the reaction with the carcinoma antigen was not lost.

TABLE 3. Absorption Studies of the Antigenic Properties of Sarcoma Treated with Formalin

Serum	Formalinized tissue, used for the absorption of the serum	Serum dilution	Antigens from the tissue			
			of formalinized carcinoma No. 48	of untreated sarcoma No. 1	of formalinized sarcoma No. 1	of untreated healthy human spleen
No. 991, against sarcoma No. 1	—	1:20	—	++++	—	—
		1:40	—	++++	—	—
		1:80	—	++++	—	—
	Carcinoma No. 48	1:20	—	++++	—	—
		1:40	—	++++	—	—
		1:80	—	++++	—	—
	Sarcoma No. 1	1:20	—	++++	—	—
		1:40	—	++++	—	—
		1:80	—	++++	—	—
No. 204, against carcinoma No. 48	—	1:20	++++	—	—	—
		1:40	+++	—	—	—
		1:80	+	—	—	—
	Carcinoma No. 48	1:20	—	—	—	—
		1:40	—	—	—	—
		1:80	—	—	—	—
	Sarcoma No. 1	1:20	++++	±	—	—
		1:40	+++	—	—	—
		1:80	+	—	—	—

Arbitrary symbols the same as in Table 1.

TABLE 4. Absorption Study of the Antigenic Properties of Sarcoma Following Boiling

Serum	Tissue used for the absorption of serum	Serum dilution	Antigens from the tissues		
			of untreated sarcoma No. 1	of sarcoma No. 1 following boiling	of untreated healthy human spleen
No. 991, against sarcoma No. 1	—	1:20	++++	—	±
		1:40	++++	—	—
		1:80	+++	—	—
	Sarcoma No. 1 after boiling	1:20	++++	—	—
		1:40	++++	—	—
		1:80	+++	—	—
No. 834, against healthy human spleen	—	1:20	—	—	+++
		1:40	—	—	++
		1:80	—	—	±

Arbitrary symbols the same as in Table 1.

The absorption experiments showed that not only extracts but even the sarcoma cells thenselves lose their specific antigenic properties when treated with formalin.

The data obtained in the absorption experiments with antisarcoma and anticarcinoma sera absorbed upon tumor cells subjected to boiling for 30 min correspond to the results of the experiments with the formalinized cells.

As an example, in Table 4 we present the protocol of an absorption experiment involving antisarcoma serum absorbed upon previously boiled sarcoma cells.

From the data in Table 4 it follows that serum does not lose its specific antibodies after treatment with cells from a sarcoma exposed to heat, which can be discerned from the positive reaction of the serum with untreated antigen from the sarcoma.

Our investigations indicate that a specific antigen is contained in the sarcoma cells which is absent from carcinoma and normal human tissues. The specific sarcoma antigen, in contrast to that of carcinoma, is destroyed by boiling or treatment of the tissue with formalin.

SUMMARY

The presence of specific antigen was established in the crude tissue of human sarcoma by immunological methods of investigation. This antigen differed from those of the normal tissues and of the human cancer tumors. A comparative study of some physico-chemical properties of specific antigens of cancer and sarcomatous tumors has demonstrated that the sarcoma specific antigen, as distinct from the cancer one, disintegrates during boiling or formalin treatment of the tissue.

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