A SEARCH FOR SPECIFIC CANCER ANTIGENS IN THE ORGANS OF CANCER PATIENTS

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It was shown by several investigators [2, 4, 7, 14, 16, etc.] that in human cancer cells there arise new antigenic substances, not found in normal organs and tissues. It is not clear whether these antigens are present only in tumor tissues or in all the organs of a cancer patient.

Some investigators found specific cancer antigens in cancer tissues and in organs of patients [5] as well as in the blood, some fluids and excretions [1, 3, 13, 15, 17-19], while other found these specific antigens only in tumor tissues [10, 12].

The solution of this problem would be not only of theoretical interest, but would be also important in diagnosis through the demonstration of a specific antigenic substance in tumors.

The purpose of this work was to conduct a comparative serological study of cancer tissues and of organs of the same individuals with a view to isolating an antigen specific for the tumor.

EXPERIMENTAL METHODS

In this work we have studied liver metastases of different primary sources and cancer free tissues of liver, kidney, spleen, heart, lungs, and brain of 6 persons who had died of cancer. The material was kept at −20°C and used as needed.

In these experiments the complement fixation reaction at 37°C in a volume of 2.5 ml was used. The antigens were saline extracts of tissues which were prepared in the proportion of 1 gram of tissue in 20 ml of normal saline. The reactions were done in the presence of excess of antigen whose dose in the initial experiment was determined by titration in the presence of complement.

Antisera were obtained from rabbits following their immunization for 4 weeks, 3 injections per week, with saline extracts of tumors and with 30% suspensions of tumor cells. Antisera were obtained separately against each of the tumors studied. In order to remove nonspecific antibodies from the sera, the latter were absorbed with formalinized normal human tissues (spleen and liver)[9] before being used in experiments.

The anticomplementary properties of sera could be completely removed by means of absorption with formalinized tissues; the use of fresh tissues was not effective for this purpose.

As was shown in the results of our previous studies [6, 8, 11], it is most difficult to differentiate antigens from tumor tissues and from the spleen. Consequently for conducting cross reactions with these antigens we have used organospecific antisera against normal human spleen, which were also subjected to absorption with formalinized liver tissue in order to remove nonspecific antibodies.

RESULTS

Table 1 shows the results of one of the experiments in the comparative study of antigenic properties of tumors and of nonaffected organs of patient P. who died of cancer. It will be seen from Table 1 that antitumor antiserum

TABLE 1. Specific Antigen in Tumor Tissue and in Organs of Patient P

Immune specific antiserum	Serum dilution	Antigen						
		from tumor	from liver	from kidney	from spleen	from heart	from lung	from brain
No. 211 against the tumor of patient P. (metastases into the stomach and liver)	1: 40	+ + + +	–	–	–	–	–	–
	1: 80	+ + + +	–	–	–	–	–	–
	1:160	+ + +	–	–	–	–	–	–
	1:320	+	–	–	–	–	–	–
No. 834 against the spleen of a healthy normal individual	1: 40	–	–	–	+ + + +	–	–	–
	1: 80	–	–	–	+ + +	–	–	–
	1:160	–	–	–	+	–	–	–
	1:320	–	–	–	±	–	–	–

Legend: ±, +, ++, +++, ++++) different degrees of positive complement fixation reaction; –) indicates negative reaction.

TABLE 2. A Comparative Study of Specific Antigens in Tumor Tissues and in Organs of Patients Who Had Died of Cancer

Immune specific antiserum	Serum dilution	Antigen						
		from tumor	from liver	from kidney	from spleen	from heart	from lung	from brain
No. 203 against tumor of patient T. (metastasis into the stomach and liver)	1: 20	+ + + +	–	–	–	0	–	–
	1: 40	+ + + +	–	–	–	0	–	–
	1: 80	+ + + +	–	–	–	0	–	–
	1:160	+ + + +	–	–	–	0	–	–
No. 63 against tumor of patient D. (metastasis into the uterus and liver)	1: 20	+ + + +	0	++	+	+	+	–
	1: 40	+ + +	0	–	–	–	–	–
	1: 80	+ +	0	–	–	–	–	–
	1:160	+	0	–	–	–	–	–
No. 51 against tumor of patient G. (metastasis into the pancreas and liver)	1: 40	+ + + +	–	+	+	–	–	–
	1: 80	+ + + +	–	–	–	–	–	–
	1:160	+ + +	–	–	–	–	–	–
	1:320	+	–	–	–	–	–	–
No. 167 against tumor of patient K. (metastasis into the lung and liver)	1: 40	+ + + +	–	0	–	0	±	0
	1: 80	+ + + +	–	0	–	0	–	0
	1:160	+ +	–	0	–	0	–	0
	1:320	+	–	0	–	0	–	0
No. 73 against tumor of patient V. (metastasis into the lung and liver)	1: 40	+ + + +	–	+++	++	–	0	–
	1: 80	+ + + +	–	+	±	–	0	–
	1:160	+ + + +	–	±	–	–	0	–
	1:320	+ + +	–	+	–	–	0	–

Note: 0) not tested. Other symbols as in Table 1.

No. 211 reacted positively with the tumor extract and did not fix the complement in the presence of antigens of the liver, kidneys, spleen, heart, lungs, and brain of this patient. The positive cross reaction of the spleen antigen with the organospecific antiserum against normal human spleen shows that the spleen antigen dose (the antigen closest to the tumor antigen) had been worked out correctly and that the negative reaction between the spleen antigen and the antitumor serum was not due to the insufficient amount of the antigen in the experiment.

It follows from this experiment that specific tumor antigens were contained only in the tumor tissues and not in other organs of the cancer patient. It is of interest that regions of liver adjacent to the metastasis and macro-

scopically unaffected by cancer, were freed of cancer antigens. This is surprising because the finding of cancer cells in the lymph and blood is common. The results of our experiments however do not exclude the possibility of the penetration of the cancer antigen into tissues surrounding the tumor. But if this takes place, the antigen penetrates in such insignificant amounts as to be outside the limits of sensitivity of the serological method used by us.

Similar results were obtained in the study of malignant tissues and of other organs of five other patients. Table 2 shows the results of complement fixation reactions only with antitumor antisera. Nevertheless these experiments, as the one described above, were also cross-controlled with organospecific antisera against normal human spleen antigens. Each of the antitumor antisera listed in Table 2 was tested correspondingly against the antigens of that patient against whose tumor the serum was made. Thus the antiserum No. 203 was tested against antigens of tumor and organs of patient T., antiserum No. 63 against those of patient D., etc. As seen in Table 2 all the sera prepared by us gave clear positive reactions with antigens from the tumors and did not react with antigens from the nonaffected organs of the same patients. The slight positive reactions of some antitumor antisera (Nos. 63 and 73) with saline extracts of organs may be explained by an incomplete absorption of nonspecific antibodies.

Thus the results of our experiments have shown that the antigen specific for human cancers may be found within the the tissues of the tumor itself and is not present in organs not affected by the cancer process. This indicates that the source of cancer antigens are cancer cells themselves, and that the specific antigenic substances which are the products of metabolism of these cells, are not present in the organs of patients.

SUMMARY

The method of complement fixation test was used to carry out a comparative serological study of the tissues of malignant tumors and organs in one and the same person. The sera were obtained from rabbits individually to each of the six investigated tumors and to a healthy human spleen. Antitumor and organospecific sera were absorbed by normal formalinized human tissues so as to remove nonspecific antibodies.

It was found that the antigen specific of a malignant tumor in man is contained only in the tissue of the tumor itself and is absent in unaffected organs of the same patients.

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