CHANGES IN THE SEROLOGICAL PROPERTIES OF TISSUES IN THE PRESENCE OF HETEROGENOUS SERUM

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In the present work the problem was set to study the reasons which cause changes in the antigenic characteristics of cancerous tissue when it is cultured on heterogenous nutritive medium.

We cultivated the tissue of a subcutaneous form of Ehrlich's adenocarcinoma for 3 months in Carrel flasks on nutritive media containing rooster plasma, chicken embryonic extract and rabbit serum.

After the indicated period of time, the explants were removed from the Carrel flasks and washed twice in Ringer's solution, then washed 7 times in physiological solution or a 10% solution of saccharose with a 10 minute centrifuging at 2500 RPM). The wash waters obtained in this way were studied for the presence of proteins in them with the help of a 20% solution of sulfosalicylic acid.

The results are presented in Table 1.

TABLE 1

Presence of Protein (+) in Wash Waters

Wash waters	№ 1	№ 2	№ 3	№ 4	№ 5	№ 6	№ 7
Presence of Proteins	+	+	+	–	–	–	–

On subsequent manyfold immunological investigations of the explants, washed free of nutritive medium, uniform results were obtained which indicated that there were changes in the immunological characteristics of the tissue.

The data from one of these experiments (Experiment No. 4) are presented in Table 2.

As is apparent from Table 2, the tissue partially expends its initial antigenic activity in the complement fixation reaction (CFR) during the comparatively protracted culture in vitro.

Naturally, the question stood before us whether the changes we obtained in the immunological activity of the tissue were the result of changes in its antigenic structure or whether this phenomenon was determined by the physico-chemical interrelations between the explanted tissue and the nutritive medium.

TABLE 2

Result of the Complement Fixation Reaction (CFR) with Serum Immune to Ehrlich's Adenocarcinoma and with Explants of Ehrlich's Neoplasms Cultivated for 3 Months

Dilution serum	Antigens				Serum control
	explant of Ehrlich's adenocarcinoma	spleen of white mouse	liver of white mouse	uncultured Ehrlich's adenocarcinoma	
1 : 10					±
1 : 20					h
1 : 40			h		h
1 : 80			h		h
1 : 160	h		h		h
1 : 320	h		h		h
Antigen control	h	h	h	h	

We set up a number of experiments to answer this question.

In this connection we were guided by the investigation of G. P. Tribulev and P. N. Kosyakov [5], who showed the ability of tissues to bind nonspecific antibodies from heterogenous sera at their surface with subsequent change in the antigenic characteristics of these tissues.

EXPERIMENTAL METHODS

The neoplasms were removed from mice with the subcutaneous form of Ehrlich's adenocarcinoma and were cut in three equal parts. One part of the neoplasm was minced, ground in a mortar, suspended in physiological solution, using 1 : 10, and centrifuged. The supernatant fluid which was obtained was used as the antigen,called "native antigen". The other two parts of the neoplasm were cut with a razor into pieces similar to those made for tissue culture. Half of the pieces were placed in a test tube with physiological solution, the other half of the pieces were also transferred to a test tube and covered in some experiments with only normal rabbit serum, in others with the same serum, but diluted with Tyrode solution (1: 1). The proportions by volume of serum used to the pieces varied in individual experiments between approximately 2: 1 and 4: 1.

Both test tubes with pieces of neoplasm were placed in a thermostat at a temperature of 37° for 18-14 hours in the first experiments, for 1 hour in later ones. Then the pieces of neoplasm were removed from the test tubes, washed with Ringer's solution and physiological solution (or 10% saccharose solution) in the same sequence as was described above with relation to the explants which were taken after culture on a nutritive medium.

The wash waters which were obtained when the pieces were washed free of serum were also studied, testing for the presence of protein.

The results are presented in Table 3.

The pieces which were washed as above were minced and antigens were prepared from them: "antigen, processed with physiological solution" and "antigen, processed with heterogenous serum."

The "native antigen," antigen processed with physiological solution and antigen processed with heterogeneous serum were carefully titrated, then checked by the complement fixation reaction for their ability to react with serum specific against mouse adenocarcinoma.

TABLE 3

Presence of Protein (+) in the Wash Waters Obtained after Processing Pieces of Neoplasm with Heterogenous Serum

No. experiments	Wash waters						
	№ 1	№ 2	№ 3	№ 4	№ 5	№ 6	№ 7
1,2,4,7,10	+	+	+	—	—	—	—
3,5,6,8,9	+	+	+	+	—	—	—

Since almost identical results, with only minor deviations, were obtained in all the experiments we set up, we present only the data obtained in Experiment No. 3 in Table 4.

TABLE 4

Complement Fixation Reaction with Serum against Ehrlich's Adenocarcinoma and with Antigens from Ehrlich's Adenocarcinoma

Serum dilution	Antigens			Serum control
	Native	processed with physiological solution	processed with serum for 14 hours	
1 : 20	++++	++++	+++(+)	±
1 : 40	+++	++++	++	h
1 : 80	++++	++++	+	h
1 : 160	++++	++++	±	h
1 : 240	++++	++++	h	h
1 : 320	++++	++++	h	h
Antigen control	h	h	h	

As is evident from Table 4, preliminary processing of the tumor tissue with physiological solution did not cause changes in its immunological activity, while even comparatively brief (14 hours) contact between the tissue and heterogenous serum led to a sharp decrease in its ability to react with a specific antibody.

We observed similar changs in the immunological characteristics when the duration of the preliminary processing of the tissue with heterogenous serum was cut to 1 hour; the degree of loss of antigenic activity in this case was only slightly less evident than in Experiment No. 3 (1 : 160 ++ or 1 : 160 +(+)).

This let us reach the conclusion that neoplastic tissues, even after brief contact with heterogenous serum, decrease their ability to combine with specific antibodies.

Taking into account the brevity of the presence of the tissue in the heterogenous serum, it is hardly possible in this case to relate the decrease in its immunological activity to changes in antigenic structure. Apparently, the results obtained are explained by the fact that serum proteins, being adsorbed on the surface of the tissue like protective colloids, hinder the interaction of the antigen with the corresponding specific antisera later, when the complement fixation reaction is set up.

This hypothesis is indirectly confirmed also by our investigation of the fall in the titer of antibodies in the nutritive medium due to their adsorption by the cells of the cultured tissue [4].

Our data did not counterindicate the possibility of changes in the antigenic structure of the tissue when it is explanted and heterotransplanted [1, 2, 3], but they did indicate the necessity of taking into account the effect of heterogenous sera with the tissue under investigation in all such work.

We do not touch on the problem of the acquisition of new antigenic properties by the cells and tissues, which is connected, apparently, with changes in the antigenic structure of the tissue during prolonged culture and heterotransplantation.

SUMMARY

Even a short contact (1 hour) of the tissue with heterogenous serum at 37°C decreases its serological activity in the complement fixation reaction.

It is probable that one of the circumstances causing these changes is the fact that the tissue surface absorbs the serum albumins preventing further interaction of antigen and respective antibody.

The data obtained confirm that it does not suffice to use only a serological method of research when solving the problems of changes in antigenic structure of tissue.

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