PRODUCTION OF ANTITUMOR SERA BY IMMUNIZING ANIMALS WITH TUMOR CELLS FREED FROM CONNECTIVE TISSUE STROMA

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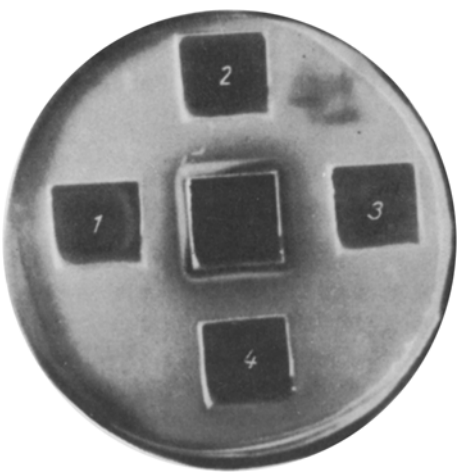
The antitumor sera which has been produced up until now does not possess a sufficient degree of specificity. In serological tests they react to a considerable degree not only with tumor antigens but also with antigens obtained from other tissues. Evidently, this is explained by the fact that the extract prepared from tumor tissue which is used for immunizing animals contains both tumor antigen and antigens from connective tissue stroma which causes the reaction of antitumor sera with antigens from liver and spleen, that is, from organs rich in connective tissue.

One of the ways of producing monospecific antitumor sera might be the use of tumor cells freed from connective tissue stroma as an antigen.

The production of such sera and their serological examination was the subject of the present study.

EXPERIMENTAL METHOD

We used the method of obtaining viable tumor cells devoid of connective tissue stroma described in the literature [3] with certain modifications.



Precipitation in agar of serum No. 41 with tumor cells, tumor tissue, spleen and liver. 1(Tumor cells; 2) tumor tissue; 3) spleen; 4) liver.

A tumor of Geren's metasticizing carcinoma was removed from rats and broken up with scissors. The tumor pulp was treated with an 0.0001% solution of trypsin for 30 min. The tumor tissue suspension was filtered through 2 layers of gauze and the filtrate centrifuged. Physiological solution was added to the sediment and placed for 1 h in a magnetic stirrer, after which this solution again was filtered through 2 layers of gauze and washed several times with physiological solution. The sediment thus obtained contained tumor cells.

We used the tumor cells freed from connective tissue stroma for producing antitumor sera. For this purpose rabbits were intravenously injected 4 times with a 10% suspension of tumor cells in physiological solution every other day with 0.5, 1, 1.5, and 2 ml; in the course of the immunization the rabbits received 15 mg of protein each.

Seven days after the conclusion of the antigen injections the rabbits were bled and the sera obtained tested in the complement fixation reaction and the hemagglutination reaction in the Stavitskii modification [5] with respect to tumor cells separated from connective tissue stroma,* whole tumor tissue, spleen, and liver.

* In the future we shall call them simply tumor cells.

Titer of Antitumor Sera Obtained by Immunizing Rabbits with Tumor Cells and Tumor Tissue

Antigens examined	Titer of antitumor serum obtained by immunizing with tumor cells		Titer of antitumor serum obtained by immunizing with whole tumor tissue	
	in complement fixation reaction	in hemagglutination reaction	in complement fixation reaction	in hemagglutination reaction
Tumor cells	1:1 211 (1:766-1:1 901)	1:3 589 (1:2051-1:6 295)	—	—
Tumor tissue	1:1 114 (1:700-1:1 770)	1:3 589 (1:2051-1:6 295)	1:313 (1:299-1:413)	1:990 (1:690-1:1 419)
Spleen	1:365 (1:251-1:535)	1:766 (1:552-1:1 062)	1:301 (1:292-1:309)	1:735 (1:488-1:1 106)
Reliability of difference of titer with respect to tumor antigen (tumor cells and whole tissue) and spleen	$P < 0.001$	$P < 0.001$	$P > 0.05$	$P > 0.05$
Liver	1:531 (1:334-1:843)	1:1 033 (1:640-1:1 663)	1:96 (1:55-1:166)	1:265 (1:138-1:509)
Reliability of difference in titer with respect to tumor antigen tumor cells and whole tissue) and liver	$P < 0.05$	$P < 0.01$	$P < 0.001$	$P < 0.01$

Note. The average geometric values of the titers and the confidence limits (in parentheses) are given in the table.

The results of the complement fixation reaction are represented by the largest dilution of serum which produces complete inhibition of hemolysis, and the results of the hemagglutination reaction by the largest dilution of serum producing a dense ring of agglutinated erythrocytes.

All of the data was submitted to variational-statistical treatment.

In addition, the sera obtained were studied in Ouchterlony agar precipitation reaction [4] against the same antigens.

To clarify the question of in which of the serum protein fractions the antibodies against tumor cells, whole tumor tissue, spleen, and liver are found, we set up a Grabar immunoelectrophoresis reaction. The antitumor serum which we obtained was subjected to electrophoresis in agar with subsequent precipitation with the same antigens which were studied in the other reactions.

RESULTS

A total of 10 sera obtained from experimental rabbits immunized with tumor cells and 16 antitumor sera obtained by immunizing rabbits with whole tumor tissue* were studied.

The control sera were tested for complement fixation and hemagglutination reactions with antigens from tumor tissue, liver, and spleen. The antigens from tumor tissue and tumor cells gave almost the same results.

In analyzing the results of the complement fixation and hemagglutination reactions with sera obtained from immunizing animals with tumor cells, first of all there developed high antibody titers with respect to tumor cells and tumor tissue; moreover, they were far higher than the antibody titer of the antitumor tissue control sera (see table).

The antibody titer with respect to the spleen was approximately the same both in the sera of the experimental animals and the controls. At the same time, the antibody titer with respect to the liver was somewhat higher in the serum of the experimental animals than in the serum of the controls.

* In the future these sera will be designated as the controls.

The antibody titer against tumor antigen in the control sera is almost the same as the antibody titer with respect to antigen from spleen in the complement fixation reaction, but exceeds it a little in the hemagglutination reaction.

The sera of the experimental animals react with tumor antigen $3\frac{1}{2}$-4 times more intensively than with antigen from spleen.

The control sera react less intensively with antigens from liver tissue than sera of the experimental animals.

Thus, the difference between the antibody titer with respect to tumor antigen (tumor cells, tumor tissue) on the one hand, and spleen, on the other, in the control sera is not statistically significant ($R > 0.05$), but in the sera of the experimental animals this difference is significant ($R < 0.001$).

Therefore, the use of tumor cells as an antigen in the immunization of animals makes it possible to increase the antitumor specificity of the sera obtained.

In considering the results of the precipitation reaction in agar, it should be noted that the sera being tested react with a deposit of sharp, clear bands of precipitation against antigen from tumor cells, tumor tissue, and liver, while with antigen from spleen tissue only a weak band of precipitation is deposited (see figure).

The data of the precipitation reaction in agar completely corresponds with the data of the complement fixation and hemagglutination reactions.

From data in the literature it is known that spleen antigen is closely related to metastacized tumor antigen [1, 2]. This is also confirmed by our data. It seems to us that this relationship is connected with the fact that metasticized tumors are rich in connective tissue stroma which in an antigenic respect is very close to spleen. Trypsin dissolves this stroma of the metasticized tumors. The isolated tumor cells obtained are used as an antigen for producing cytotoxic sera which react with tumor antigens in higher dilutions than with spleen antigen.

It is also possible that liver antigen is more closely related to tumor antigen than to spleen antigen since in immunizing rabbits with isolated tumor cells cytotoxic sera is obtained which reacts with spleen and tumor tissue antigens in almost the same way as the control sera.

The data from the immunoelectrophoresis reaction indicates that antibodies against tumor cells, tumor tissue, spleen, and liver are mainly γ-globulins, and, to a small degree, β-globulins. Since the bands of precipitation with respect to the antigens which we examined fall in one or two other zones, this indicates that the antibodies against all the antigens which we examined belong to one or the other protein fractions.

SUMMARY

During treatment of Geren's carcinoma tissue with trypsin it is possible to produce tumor cells freed from the connective tissue stroma.

Rabbits immunized with these cells develop antitumor sera producing a high antibody titer with regard to tumor antigen and 3.5-6 times less titer with regard to the spleen.

LITERATURE CITED

1. V. S. Korosteleva and T. P. Konstantinova, Byull. éksper. biol., 9 (1960), p. 101.
2. P. N. Kosyakov, V. S. Korosteleva, and N. I. Kuznetsova et al., In the book: Malignant Neoplasms, Moscow (1959), p. 177.
3. R. E. Madden and D. Burk, J. nat. Cancer Inst., 27 (1961), p. 841.
4. O. Ouchterlony, Acta path. microbiol. scand., 25 (1948), p. 186.
5. A. B. Stavitsky, J. Immunol., 72 (1954), p. 360.
6. G. C. Velley, C. R. Soc. Biol., 153 (1959), p. 48.
7. C. A. Williams, Jr. and P. Grabar, J. Immunol., 74 (1955), p. 158.