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The Investigation of Some Antigens of Zajdela Ascitic Rat Hepatoma Cells

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SUMMARY

The study of antigenic properties of Zajdela ascitic rat hepatoma cells shows considerable rearrangement of the antigenic content characteristic of liver cells—normal analogs of tumor cells. In the tumor cells, the synthesis of embryo-specific antigens was renewed and a sharp increase in the concentration of heteroorganic antigens was observed. At the same time no organospecific liver antigens were revealed in these cells, whereas the content of species-specific antigens underwent no significant changes compared with liver cells.

INTRODUCTION

The conversion of cells to malignancy is accompanied by essential changes in the synthesis of some normal cell antigens. According to Day (4), these changes may be classified as follows:

1. The antigenic "simplification" of tumor cells, i.e., the loss or a considerable decrease in the synthesis of some normal cell antigens (1, 19–21).
2. The antigenic reversion, i.e., the ability of tumor cells to restore the synthesis of embryo-specific antigens characteristic of the normal noncancerous tissues in their embryonic period and absent from the adult tissues (2, 11, 12, 18).
3. The antigenic diversion, i.e., an alteration of tumor antigen content which is connected with the appearance in the latter of antigens characteristic of normal tissue nonhomologous to the tumor (3).

All these changes are different aspects of the same process of cell carcinogenesis. However, as is clear from the formula, the antigenic diversion and reversion, contrary to the antigenic simplification, may be considered as a kind of antigenic complication brought about on account of some cell antigens comprising the "repertoire" of a normal genome.

In this connection the data of Pikovski and Witz (14) are worth mentioning. These data reveal the increase of synthesis of some antigens in tumor cells which are characteristic of the initial normal cells. The data obtained in a study of myeloma antigens (5) probably could be similarly interpreted.

The above classification of disturbances of the synthesis of normal cell antigens during cell conversion to malignancy should be taken into account while studying the antigenic rearrangements of tumor cells.

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The present investigation deals with the results obtained in a study of the antigenic structure of Zajdela ascitic hepatoma cells (23). This work is a continuation of our previous studies concerning the antigenic structure of solid hepatomas induced in rats with *N*-nitrozodiethylamine (7–9).

MATERIALS AND METHODS

In the study outbred rats were used. The antigenic content of tumor cells was investigated by means of the agar precipitation reaction and specific inhibition of precipitation according to Björklund. In some cases we used immunoelectrophoresis in micromodification of Hirschfeld (10).

Water-saline extracts of tumor cells and normal tissues of intact rats at different stages of development including the embryonal period were used as antigens for testing. The preparation technic was described earlier (8). The protein concentration in the test extracts was usually 5–10 mg/ml.

In the experiments we used rabbit antitumor serum, sera against liver tissue of newborn rats and embryos, and organospecific serum against liver antigens of adult rats. The immunization was carried out by means of tissue homogenates according to the scheme used in our previous works (7, 8).

We also used immune serum against serum proteins of intact rats. To obtain this immune serum we injected rabbits intravenously with normal rat serum, gradually increasing its volume from 0.5 to 2.0 ml per injection. The whole immunization course comprised 6 injections at three-day intervals. Subsequently after each 1 or 1.5 months the rabbits were reimmunized. Blood was collected from the ear 8 and 14 days after the last injection.

The antitumor serum and antisera against the liver tissue of newborn rats and embryos were exhausted only with respect to normal rat serum. The absorption of all these sera as well as the preparation of organospecific antiliver serum were controlled by the precipitation reaction in agar gel.

To purify the immune sera and make it more concentrated, the γ-globulin fraction was extracted by means of the Cohn alcohol method in the modification of A. J. Gusev (24). Concentrated sera were used undiluted.

RESULTS AND DISCUSSION

Antigenic Simplification

This process mostly involves organospecific antigens that characterize immunologically the differentiation of some organs

and tissues. At the same time, organospecific antigens are considered to play a part of a homeostatic factor providing an interaction of cells in the cell population and balancing the proliferation of differentiated cells (16, 22). This makes clear the significance of the alteration of the synthesis of the mentioned antigens in carcinogenesis.

The content of organospecific antigens in water-saline extracts of Zajdela hepatoma cells was studied by means of anti-liver organospecific serum. In these experiments the precipitation reaction in agar gel and immunoelectrophoresis were used. Thus we revealed no less than six or seven organospecific antigens in the water-saline extract of intact rat liver. This result was obtained by studying a great number of samples of liver extracts. When extracts of Zajdela hepatoma cells were tested under similar conditions, we never observed organospecific antigens (Table 1).

Negative results were obtained even in the experiments in which the protein concentration of the tumor test extracts was increased several times (20–25 mg/ml).

Hence, the synthesis of liver antigens in the cells of Zajdela ascitic hepatoma was found to be considerably reduced (at least below the sensitivity of the methods used).

It is worth mentioning that similar results were obtained earlier in a study of solid forms of hepatocellular tumors induced in rats with nitrosamines (6). The overwhelming majority of the primary tumors were able to perform synthesis of at least two or three organospecific antigens, while no such antigens could be detected in the extracts of hepatomas of transplantable strains derived from primary hepatomas just mentioned after 2–15 passages. In this case, as well as in the case of long-term transplanted Zajdela ascitic hepatoma, the fact that we failed to reveal any organospecific antigen might be attributable to more profound disturbances in their synthesis.

Besides the organospecific antigens, some other groups of normal cell antigens (species-specific antigens and isoantigens) may be involved in the process of antigenic simplification (15, 17). For example, T. A. Pokrovskaya (15), in experiments with heterotransplantation of Ehrlich ascitic carcinoma cells on the chick embryo chorionallantois, observed a decrease in ascitic cell content of species-specific antigens which are in common with mice sera antigens.

Making use of immune serum against serum proteins of normal rats and of the methods of the precipitation reaction in agar gel and immunoelectrophoresis, we were able to compare the content of corresponding antigens in Zajdela hepatoma cells and initial normal tissue. We failed to observe any difference (Table 1). In this case the carcinogenesis of cells followed by passages under conditions of homotransplantation probably did not induce any significant changes in the synthesis of species-specific antigens.

Table 1

Test extracts (10 mg/ml)	Organospecific anti-liver immune serum	Immune serum against rat serum
Liver	+++++	++++++
Cells of hepatoma Zajdela	−	++++++

Investigation of antigenic simplification in the cells of Zajdela ascitic hepatoma. The volume of all the ingredients of the precipitation reaction and specific inhibition of precipitation are 0.04 ml. +, one precipitation band revealed in the test extract with the use of corresponding immune serum; ±, doubtful result; −, no precipitation band.

Antigenic Reversion

The conversion of cells to malignancy is often accompanied by the appearance of embryo-specific antigens characteristic of normal tissues during their embryonal development. To study these antigens, immune serum against the rat embryo liver and antitumor serum were used.

We shall first analyze the results obtained with the use of antitumor serum. In this series of experiments, by means of the specific inhibition of precipitation in the agar, an attempt was made to compare the antigenic content of the tumor and normal liver tissue in different stages of development: in embryos (18 days), newborn rats (6–12 hr), and then in rats 4 days, and 1, 2, and 3 weeks old, as well as in adult intact animals (2 months old and older).

When absorbed with liver of adult rats at concentrations required for complete neutralization of antibodies against test antigens of the same tissue, the antitumor serum is still able to react not only with antigens of tumor tissue, but also with liver antigens of embryos and newborn rats forming two and one precipitation bands respectively (Table 2). On the other hand, after the removal of antibodies against antigens of livers of 18-day-old embryos, the antitumor serum still yielded a positive reaction not only with tumor, but also with liver extracts of adult intact rats (two precipitation bands).

However, the most peculiar feature of these experiments is that an increase in the concentration of the embryo liver extract completely neutralizes the antibodies against antigens of adult animals, whereas in the tumor extracts at least one antigen is still detectable. In principal, similar results were obtained also in the absorption of antitumor serum with liver extracts of newborn rats.

The comparison of the experimental data (Table 2) shows that the antigenic content of the liver tissues of embryos and newborn rats in the early postnatal period has a closer resemblance with the tumor antigenic content than that of liver tissues obtained from the animals at later stages of development. One can draw such a conclusion from a comparison with the number of antigens revealed in the cell extracts of Zajdela hepatoma, making use of antitumor serum previously absorbed by the liver extracts of newborn and adult rats in the same concentrations (e.g., 20 mg/ml). It must be noted that the synthesis of embryo-specific antigens decreases along with the increase of the definitive cell antigen synthesis (evidently on account of organospecific antigens), and during the third week of development, the antigenic content of liver tissue becomes definitive.

Using mixed liver extracts of adult and newborn rats for the absorption of antitumor serum, we could completely neutralize the antibodies against tumor antigens. Such an effect is evidently due to the mixture's greater content or

Table 2

Antitumor immune serum exhaustion according to Björklund		Test extract (5 mg/ml)			
		Livers of rats			Zajdela hepatoma
Rat liver extracts	Dose (mg/ml)	Embryos	Newborns	Adults	
Embryos	10	−	+	+±	++
	20	−	±	±	++
	30	−	−	−	+
Newborn rats	10	−	−	+	+
	20	−	−	±	+
	30	−	−	−	±
Four-day-old rats	10	−	+	+	+++
	20	−	±	−	++±
	30	−	−	−	+±
One-week-old rats	10	+	+	++	+++
	20	−	+	±	+++
	30	−	−	−	++
Two-week-old rats	10	+	+	+	+++
	20	−	±	±	++±
	30	−	−	−	++
Three-week-old rats	10	+±	++	++	+++
	20	+	+	−	+++
	30	+	+	−	++
Adult rats	10	+±	+±	±	+++
	20	+	+±	−	+++
	30	+	+	−	++
Mixture of extracts from newborn and adult rats	10	−	−	−	+
	20	−	−	−	±
	30	−	−	−	−

Investigation of embryo-specific antigens in the cells of Zajdela ascitic hepatoma. For explanation of symbols see Table 1.

larger amounts of antigens typical of adult or newborn rats than there are in the extract prepared from the tissues of animals of only one developmental stage. Consequently, the antigenic content of the investigated Zajdela ascitic hepatoma cells was represented only by a combination of the mentioned normal cell antigens. It must be pointed out that the above data are in good agreement with the results yielded by the testing of solid forms of hepatocellular tumors (6). In the experiments with antitumor serum, results were obtained indirectly demonstrating the synthesis of embryo-specific antigens in tumor cells.

Direct evidence of this phenomenon could be obtained only by means of immune serum against the embryo liver antigens. For such kinds of experiments, a specific inhibition of precipitation reaction in the agar gel was used (Fig. 1). It is seen that in the course of neutralization of antibodies against adult rat liver antigens, the serum still reacts with test extracts from the livers of newborn rats (two precipitation bands), embryos, and Zajdela hepatoma (one band for each). Here a phenomenon of identity of antigens revealed in tumor and embryonal tissues was observed. On the other hand, the embryo liver extract totally absorbed antibodies against all the test extracts studied, including Zajdela hepatoma extract.

We have not enough information to discuss what happens in the tissues of adult animals, either cessation of embryo-specific antigen synthesis or considerable quantitative shifts in their synthesis; but in any case, during carcinogenesis the embryospecific antigen production rises considerably, sometimes reaching in tumor cells the levels of corresponding embryonal tissues.

Antigenic Diversion

In tumor cells one may observe an increase in the content of some antigens characteristic of definitive tissues nonhomologous to the tumor studied. Such antigens may be conditionally denoted as "heteroorganic." The term "heteroantigens," which we used in our previous publications, is less suitable as it does not differentiate clearly between heteroorganic antigens and heterogenous ones of the Forssman types common for different animal species.

As a detailed description of heteroorganic antigens was given earlier (13), we shall confine ourselves to a discussion of the experimental evidence concerning Zajdela ascitic carcinoma.

In these experiments we used the reaction of specific inhibition of the precipitation in the agar gel: antitumor serum after absorption with extracts of normal initial tissues was tested

with extract of nonhomologous tissues. To absorb immune serum, as in the case of the study of antigenic reversion, we used liver extracts from rats at different stages of development.

The results are presented in Table 3. It can be seen that the antitumor serum exhausted with liver extracts with the protein concentration 20 mg/ml still reacted with extracts of normal tissues nonhomologous to the tumor; the control testing of liver extracts used to exhaust the immune serum revealed no precipitation bands (see Table 2). This result shows that the content of heteroorganic antigens in the extracts of normal homologous tissue is not sufficient to neutralize corresponding specific antibodies in the antitumor serum. However, increase of the concentration of the extract used for serum absorption to 30 mg/ml markedly changed the result: the serum, exhausted with the liver extracts of newborn rats and embryos, yielded negative results with all normal organ test antigens studied, whereas the extract of definitive liver tissue failed to neutralize serum antibodies against the majority of heterologous tissues. It should be mentioned that the antigenic content of liver extracts from rats at later stages of development (in our case from two- and three-week-old rats) sharply differed from the antigenic content of embryo tissue, being nearer to the definitive tissue, while the liver antigen sets of rats of the first week of development were still nearer to the antigenic profile of embryo tissues.

The concentration of heteroorganic antigens is higher in liver extracts of newborn animals and embryos than in extracts of the same tissue in adult rats. Hence, with the growth of the animal, the content of heteroorganic antigens in the liver considerably decreases.

In further study an attempt was made to characterize quantitatively the content of heteroorganic antigens in the cells of Zajdela hepatoma and in the livers of adult and newborn rats. For that purpose we determined the minimal concentration of the mentioned extracts and extracts of heterologous organs of adult rats (lungs, spleen, kidneys, and skeletal muscle) sufficient to neutralize antibodies against some or other heteroorganic antigens. Being used for the exhaustion of immune serum in these minimal concentrations, each extract caused negative results in testing by the precipitation reaction with heterologous test extracts. As the minimal concentration of the heterologous extract was taken for 100 percent, we were able to calculate the percent concentrations of heteroorganic antigens in tumor tissue and homologous normal tissue (Table 4).

It turned out that, as a rule, the content of heteroorganic antigens in tumor cells exceeded their concentration in the definitive liver tissue 1.5–2 times. The only exceptions were muscle antigens the level of which was the same in both normal and tumor tissues. As for the livers of newborn rats, only in two cases (lung and kidney antigens) did we observe an elevation of the level of the heteroorganic antigens in comparison with the definitive tissue. The content of spleen antigens in the liver of adult and newborn rats was the same, while the content of muscle antigens in the liver of adult rats was even higher. The comparison of the experimental data dealing with liver extract of newborn rats and the tumor extracts revealed a higher level of heteroorganic antigens in tumor cells, although in the case of lung antigens the result was inverse.

It should be mentioned that similar conclusions were drawn earlier from the investigation of solid forms of hepatocellular tumors (13).

Thus, the above experimental material shows that the synthesis of some antigens typical of liver cells in the embryonal period of development is renewed in the cells of Zajdela hepatoma.

All these considerations allow a suggestion that tumor cells show some likeness to the normal initial cells at certain stages

Table 3

Antitumor immune serum exhaustion according to Björklund		Test extracts (5 ml/mg)				
Rat liver extracts	Dose (mg/ml)	Muscles	Spleen	Lungs	Kidneys	Zajdela hepatoma
Embryos	20	+	+	+	+	++
	30	−	−	−	−	+
Newborn rats	20	−	±	−	±	+
	30	−	−	−	−	±
Four-day-old rats	20	−	+	−	+	++±
	30	−	−	−	−	+±
One-week-old rats	20	−	±	−	+	++±
	30	−	−	−	−	++
Two-week-old rats	20	±	+	−	+	++±
	30	−	±	−	±	++
Three-week-old rats	20	+	+	+	+	++±
	30	−	+	−	+	++
Adult rats	20	+	+	+	+	+++
	30	−	+	±	+	++

Investigation of heteroorganic antigens in the cells of Zajdela ascitic hepatoma. For explanation of symbols see Table 1.

Table 4

Test antigens in the organs of adult rats (5 mg/ml)	Extract for exhaustion of antitumor serum	Minimal extract concentrations sufficient to neutralise the antibodies against test antigens (mg/ml)	The heteroorganic antigen content in the tissue (%)
Lungs	Zajdela hepatoma	7.5	33
	Liver of newborn rats	5	50
	Liver of adult rats	15	16.6
	Lungs	2.5	100
Muscles	Zajdela hepatoma	10	25
	Liver of newborn rats	15	16.6
	Liver of adult rats	10	25
	Muscles	2.5	100
Kidney	Zajdela hepatoma	15	75
	Liver of newborn rats	20	50
	Liver of adult rats	25	40
	Kidney	10	100
Spleen	Zajdela hepatoma	10	10
	Liver of newborn rats	15	7
	Liver of adult rats	15	7
	Spleen	1.0	100

The heteroorganic antigen content in the Zajdela hepatoma cells in comparison with normal homologous tissue.

of their embryonal development. However, one should not overestimate significance of this likeness, since not only the data of general oncology, but also our own evidence concerning heteroorganic antigens, show that no reversion to the state of embryonal omnipotency occurs in the process of malignant transformation.

We suppose that in tumor cells both the synthesis of embryospecific and heteroorganic antigens and the antigenic simplification, opposite in their consequences, are in fact different aspects of the same alteration or perversion process in the regulation of normal cell antigen synthesis. This disturbance of the regulation is based on changes in the activity of genes, i.e., the derepression of some genes (as in the case of antigenic complication) and the repression of others (antigenic simplification); however, in the case of antigenic simplification, a supposition of its mutational nature cannot be completely ruled out. In normal adult animals, the repressed state of the one kind of gene and the derepressed state of the other characterize to a considerable extent the high level of definitive tissue differentiation.

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Fig. 1. The specific inhibition of precipitation reaction in agar gel. *IS*, immune serum against embryo rat liver; *E*, embryo rat liver; *N*, newborn rat liver; *A*, adult rat liver; *H*, Zajdela hepatoma cells.

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