THE STUDY OF ANTIGENIC PROPERTIES OF CERTAIN CORPUSCULAR AND SOLUBLE FRACTIONS ISOLATED FROM TUMORS OF INBRED MICE

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The investigations of L. A. Zil'ber and his co-workers [2] established the presence of specific antigens in the tissues of malignant tumors.

The search for antibodies for a specific antigen, the development of methods of immunization against recurrence of tumors and other problems in tumor immunology required the isolation of the specific tumor antigen in a purer and more concentrated form. One of the ways of solving this problem was the isolation of different fractions from the tumor cell and the study of their antigenic properties.

L. A. Zil'ber, N. V. Nartsissov and G. I. Abelev [2] demonstrated that the specific antigen of the rat tumor-M-1, which possesses serological activity, is associated primarily with the fraction of mitochondria and microsome and with one of the globulin fractions isolated from the hyaloplasm. In these investigations they used tumor induced and transplanted in animals of impure breed which could be distinguished from the tissues of its host by its antigenic composition. In addition, N. V. Nartsissov and N. V. Abelev demonstrated the presence of a specific antigen in experiments with primary rat tumors induced with carcinogens.

This project carried out at L. A. Zil'ber's suggestion was concerned with the distribution of specific antigens in different fractions of tumors transplanted onto animals of pure breed and therefore not having isoantigenic differences with the tissues of these animals – carriers of the tumor.

EXPERIMENTAL METHOD

As material we used two tumor strains transplanted onto mice of pure line C_3HA: strain of breast adenocarcinoma isolated by us from a spontaneous tumor which arose in mouse C_3HA and strain of hepatoma resulting from a tumor induced by ortho-aminoazotoluene (V. I. Gel'shtein) in the laboratory directed by L. M. Shabad. Hepatoma is especially suitable in view of the fact that the liver of healthy mice of the same line can serve as an adequate control for this tumor.

For comparison of antigens of tumor and normal tissues we employed the anaphylactic reaction with desensitization which was also used in conducting basic investigations in the detection of specific antigens in tumors. Tumor tissue was subjected to fractionation by the method of differential centrifugation and separation described in the work of N. V. Abelev and G. S. Bezverkhii [1]. Tumor tissue in a tenfold volume of 8% solution of saccharose was reduced to fragments by a homogenator in 7 minutes. Nuclei and remnants of undestroyed tissue were removed from the homogenate by triple centrifugation at 1,500 revolutions per minute. The supernatant fluid was diluted with an equal volume of saccharose and passed through separator ASG-1 with a pulley giving a maximum speed of 16,000 revolutions per minute. Microscopic control showed that at this speed mitochondria and a considerable portion of microsomes are precipitated out. The precipitate of mitochondria and microsomes is removed from the separator, washed in an 8% solution of saccharose, dissolved in distilled water of pH = 7.6 and used in the investigations.

After separation, the supernatant fluid representing hyaloplasm with an admixture of microsomes was dialyzed to remove the saccharose after which the protein fraction, obtained by salting out the soluble cytoplasmic proteins with ammonium sulfate at 33% saturation in the cold, was removed. Inasmuch as this fraction is obtained by a method analogous to the one for obtaining serum globulins, we assume that with this method we isolate proteins of the globulin type and for this reason name it the globulin fraction.

Anaphylactic reaction with desensitization was used to test the fractions isolated after their solution. Three groups of pigs were sensitized hypodermically with approximately equal amounts of protein (about 4 mg) from each of the fractions of cancerous tissue of mouse breast: the original extract of tumor (supernatant fluid after the removal of nuclei), the mitochondria – microsome fraction and the globulin fraction.

The experiment was performed a month later. The pigs of each group were sensitized with intravenous injections of the corresponding fraction prepared from a mixture of organs of healthy mice of the same line. All pigs responded to this injection with an anaphylactic shock which indicated the presence of normal tissue proteins in the fractions isolated from the tumors. Antigens from normal tissues were injected repeatedly with the purpose of producing desensitization of the pigs to them. After the animals no longer reacted to the injection of a definite dose of antigens from normal tissues (usual dose exceeded the sensitizing dose by $1\frac{1}{2}$ or 2 times), that fraction from tumor tissue to which they were sensitized was administered in a dose not exceeding that for testing the completeness of desensitization.

EXPERIMENTAL RESULTS

In Table 1 are presented the results of such an experiment. The majority of pigs responded to the resolving injection of the antigen studied with an anaphylactic shock. From this the conclusion was drawn that not only the original extract from cancerous mouse breast, but the tested fractions as well – mitochondria – microsomes and globulin – contain a specific antigen distinct from antigens of normal tissues.

In Table 2 is presented data of a similar experiment in which fractions isolated from mouse hepatoma were used for sensitization and for the resolving injection. Pigs sensitized with the original extract and with fractions of mitochondria – microsomes and globulin from the hepatoma and completely desensitized with the same fractions from the liver of healthy mice of the same line C_3HA onto which the hepatoma is transplanted, responded with anaphylactic shock when fractions used for sensitization were injected. Therefore, the original extract of mouse hepatoma as well as fractions of mitochondria – microsomes and globulin contain a specific antigen.

Besides the above-described fractions, those isolated from the same tumors by the method of methanol precipitation were also studied employing anaphylactic reaction with desensitization [5]. As is known, acid albumins of nucleoprotein nature are isolated by this method which is also used to isolate viral proteins.

It became essential to isolate such a fraction from both tumors (one of which was viral) and to determine the antigenic properties of the preparations obtained.

In Table 3 are presented the results of the experiment which shows that specific antigen is present in the methanol precipitate from cancerous breast. In this experiment, pigs were sensitized with the methanol precipitate from spontaneous or transplanted breast adenocarcinoma in mice. Following desensitization with methanol precipitate from a mixture of organs of healthy mice of the same line, and in a number of cases – from the breasts of these mice, the pigs responded with anaphylactic shock when methanol precipitate from cancerous breasts was injected.

In the second part of Table 3 are presented the results of the experiment in which pigs sensitized with methanol precipitate from the liver of healthy mice of the same line responded with anaphylactic shock to the injection of methanol precipitate from hepatoma. Therefore, the methanol precipitate from hepatoma of a mouse in an anaphylactic reaction with desensitization also contains a specific antigen, distinct from antigens in methanol precipitates from the liver of healthy mice of line C_3HA.

Thus, proteins of cellular organoids – mitochondria and microsomes and globulin fraction of hyaloplasm proteins, as well as acid proteins precipitated in methanol precipitate, – contain specific antigen.

Earlier we showed [3] that nucleoprotein fraction isolated from hepatoma contains specific tumor antigen.

TABLE 1

The Study of Fractions from Cancerous Tumor Tissue of the Breast of Mice in a State of Anaphylactic Reaction with Desensitization*

Pig No.	Sensitization		Desensitization			Testing for completeness of desensitization			Resolving injection		
	antigen	protein dose in mg	antigen	protein dose in mg	reaction	antigen	protein dose in mg	reaction	antigen	protein dose in mg	reaction
17	Original extract from	4.5	Original extract	1.2	++	Original extract	5.0	–	Original extract	4.8	+
19	breast cancer of mice	4.5	from a mixture	1.2	+	from a mixture	5.6	–	from breast of	4.5	++
20	of line C_3HA	4.5	of organs of healthy mice of line C_3HA	1.2	++	of organs of healthy mice of line C_3HA	6.2	–	mice of line C_3HA	5.7	+++
123	Mitochondria and	4.4	Mitochondria and	3.7	+	Mitochondria	7.5	–	Mitochondria and	7.2	±
128	microsome fraction	4.4	microsome fraction	2.5	++	and microsome	8.8	–	microsome fraction	8.5	++
132	from breast cancer	4.4	tion from a mixture	5.0	++	fraction from a	10.0	–	tion from breast	9.1	+
133	of mice	4.4	of organs of	2.0	+	mixture of organs	6.5	–	cancer of mice	6.5	+
136	of line	4.4	healthy mice of	2.0	+	of healthy mice	6.5	–	of line	6.5	–
137	C_3HA	4.4	line C_3HA	3.3	+++	of line C_3HA	6.5	–	C_3HA	6.5	++
115	Globulin fraction	3.9	Globulin fraction	1.1	++	Globulin fraction	4.4	–	Globulin fraction	4.3	+
116	from breast cancer	3.9	from a mixture of	1.5	++	from a mixture	5.2	–	from breast can-	5.1	–
118	of mice of line	3.9	organs of healthy	1.6	+	of organs of	4.7	–	cer of mice of	5.1	+++
119	C_3HA	3.9	mice of line C_3HA	2.3	+	healthy mice of line C_3HA	5.5	–	line C_3HA	5.1	++

Designations: – absence of reaction; + temperature drop, momentary scratching of the nose, sneezing; + + temperature fall, strong scratching, sneezing, ruffling of fur, cough; +++ same, but more marked; elimination of urine and feces; ++++ convulsive jumping, convulsions terminating in death of the animal.

*In this and the following tables only the results of the first desensitization and the testing for completeness of desensitization are indicated; the intermediate stages of desensitization are omitted.

TABLE 2

A Study of Fractions from Hematoma of Mice in a State of Anaphylactic Reaction with Desensitization

Pig No.	Sensitization		Desensitization			Testing for completeness of desensitization			Resolving injection		
	antigen	protein dose in mg	antigen	protein dose in mg	reaction	antigen	protein dose in mg	reaction	antigen	protein dose in mg	reaction
4	Original	4	Original extract	1.3	+++	Original extract from	7.9	−	Original	7.4	+++
6	extract from	4	from liver of	1.3	+	liver of healthy mice	7.9	−	extract from	5.7	±
7	hepatoma	4	healthy mice of line C_3HA	1.3	++	of line C_3HA	9.2	−	hepatoma	8.2	+++
11	Mitochondria and	4.2	Mitochondria and	1.4	+	Mitochondria and	8.4	−	Mitochondria	7.0	+
12	microsome fraction	4.2	microsome fraction	2.8	+	microsome fraction	8.4	−	and microsome	8.4	++
45	from hepatoma	4.2	from liver	2.8	±	of liver of	8.4	−	fraction	8.4	++
9		4.2	of healthy mice	5.6	±	healthy mice	1.2	−	from hepatoma	9.	−
46		4.2	of line C_3HA	2.8	++	of line C_3HA	9.8	−			±
40	Globulin	4.2	Globulin fraction	1.5	+++	Globulin fraction	7.1	−	Globulin	4.8	±
43	fraction	4.2	from liver of	0.5	+	from liver of	2.7	−	fraction	2.4	+
44	from hepatoma	4.2	healthy mice of line C_3HA	1.5	+++	healthy mice of line C_3HA	3.0	−	from hepatoma	3.0	+

Designations same as in Table 1.

TABLE 3

Anaphylactic Reaction with Desensitization with Methanol Precipitate from Breast Cancer and Hepatoma of Mice

Pig No.	Sensitization		Desensitization			Testing for completness of desensitization			Resolving injection		
	antigen	protein dose in mg	antigen	protein dose in mg	reaction	antigen	protein dose in mg	reaction	antigen	protein dose in mg	reaction
120	Methanol precipi-	4.3	Methanol precipitate	2.9	+	Methanol precipitate	5.8	−	Methanol pre-	4.5	++
121	tate from trans-	4.3	from a mixture of	2.2	++	from a mixture of	5.8	−	cipitate from	5.4	±
125	planted	4.3	organs of mice of	1.4	−	organs of healthy	5.4	−	transplanted	5.4	+
126	breast	4.3	line C_3HA	2.2	++	mice of line C_3HA	5.8	−	breast cancer	5.4	−
131	cancer	4.3		2.2	−		5.8	−		5.4	++
168	From spontaneous	4	From breast of mice	0.8	+		4.6	−	From spon-	4.3	+++
169	breast cancer	4	of line C_3HA	1.6	++		4.6	−	taneous breast cancer	4.3	±
173	The same	4	From breast of mice	2.2	++	From breast of mice	4.5	−		4.3	−
165	"	4	of line C_3HA	1.3	+++	of line C_3HA	3.1	−	From trans- planted breast cancer	3.1	++
167	"	4	From a mix-	1.3	−	From a mixture of	6.3	−	The same	6.2	+
170	"	4	ture of organs	1.9	++++	organs of healthy	8.8	−	"	7.4	+
172	"	4	of healthy	1.9	−	mice of line	6.3	−	"	6.2	+
174	"	4	mice of line	3.2	++	C_3HA	6.3	−	"	6.2	+
169	"	4	C_3HA	0.6	++		6.3	−	"	6.2	++
173	"	4		0.6	++				"		
135	Methanol	4.2	Methanol precipitate	1.4	+	Methanol precipitate	4.2	−	Methanol pre-	4.4	+
140	precipitate from	4.2	from liver of	1.8	+	from liver of	5.4	−	cipitate from	5.5	+
142	transplanted	4.2	healthy mice of	3.6	+	healthy mice of	6.0	−	hepatoma	6.0	++
143	breast cancer	4.2	line C_3HA		·	line C_3HA	·	·		6.5	++
214		2.9		0.9	+		6.2	−		6.0	+
215		2.9		0.9	+		6.2	−		6.0	+
219		2.9		0.9	+		6.2	−		6.0	+
222		2.9		0.9	+		6.2	−		6.0	+

Designations same as in Table 1. Dot indicates that no injection was given.

Naturally, the question arises: how to correlate the data of the previous experiment dealing with antigenic activity of the nucleoprotein fraction with the data dealing with antigenic activity of individual fractions established in this investigation.

Inasmuch as the nucleoprotein fraction is isolated from cellular cytoplasm, undoubtedly, it contains also nucleoproteins of cellular organoids, and therefore the observation of antigenic activity in the fraction of mitochondria – microsome may be regarded as antigenic activity of one part of the antigenic complex of the common nucleoprotein fraction.

The question of whether or not globulin is present in the nucleoprotein fraction and what its role is in the antigenic reaction brought about by nucleoprotein is awaiting further study and is the subject of the next communication.

The presence of specific tumor antigen in cellular organoids and in hyaloplasm proteins indicates the wide distribution of this antigen through various parts of the tumor cell.

On further investigation it is necessary to study the presence of specific tumor antigen in fractions from other cellular organoids (nuclei) as well as other fractions of hylaoplasm proteins (globulins isolated at 66% saturation with ammonium sulfate, albumins) and compare the antigenic properties of various fractions.

The experiments described indicate that mitochondria – microsomes and globulin fractions, isolated from hepatoma tissues and from breast concer of inbred mice, contain a specific tumor antigen which manifests itself in anaphylactic reaction with desensitization in guinea pigs. This specific antigen is also detectable in the fraction isolated from both tumors by the method of methanol precipitation. The detection of the specific tumor antigen in cellular organoids and in hyaloplasm proteins indicates the wide distribution of this antigen in various parts of the tumor cell.

SUMMARY

The presence of specific antigens was studied in certain fractions of mice tumors, which were induced and transplanted on purebred animals and, thus, had no isogenic variations.

It was established by anaphylactic reaction with desensitization in guinea pigs that there is a specific antigen present in the fractions of mitochondria, microsome, globulin and methanol precipitate of the hepatoma tissue and breast cancer in mice C_3HA. This antigen differed from the antigens which were present in the same fractions obtained from the normal liver or a mixture of organs of healthy mice.

The presence of specific tumor antigens in the cellular organoids, as well as in the proteins of the hyaloplasm shows the widespread distribution of this antigen in various parts of the tumor cell.

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