THE USE OF THE METHOD OF ANAPHYLAXIS WITH DESENSITIZATION FOR THE STUDY OF THE ANTIGENIC PROPERTIES OF TUMORS DURING HETEROTRANSPLANTATION

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The method of anaphylaxis with desensitization is widely used in experimental and clinical oncology for studying the antigenic properties of normal and pathological tissues and, in particular, for analysis of the antigens of malignant tumors. The high sensitivity and specificity of the anaphylactic reactions enable the detection by this method of infinitesimal quantities of a protein when mixed with other proteins, and also allow a differential analysis to be carried out of the separate protein components entering into the composition of natural protein complexes.

In view of the special features of this method, we attempted to utilize it in the study of the antigenic properties of a tumor during heterotransplantation. We had in mind here to ascertain to what extent heterologous conditions of growth of the tumor would affect its antigenic properties.

EXPERIMENTAL METHOD

The test object was the mouse adenocarcinoma of Ehrlich. Carcinoma cells were transplanted into the cervical lymphatic nodes of a guinea pig. The lymphatic nodes, according to our previous investigation [1], are the most suitable organ for transplantation of the mouse tumor. As a rule the tumor shows growth in the lymphatic nodes in the course of 6-7 days, after which death of the carcinoma cells begins to take place on the 20th day, and the transplantate is completely absorbed.

In some experiments guinea pigs were sensitized to the original tumor, and in others to a 7-day heterotransplantate of this tumor. From 3-4 weeks from the moment of primary injection of protein, the guinea pigs were desensitized by intracardiac injection of mouse serum, of a saline extract of the heterotransplanted tumor and also of an extract of the original tumor. The order of injection of the antigens was changed according to the character of the experiment.

The use of the guinea pig as a recipient of the tumor and also for the reaction of anaphylaxis with desensitization appeared to have definite advantages for this particular investigation: it made it unnecessary to carry out desensitization of the animals to their own proteins (the proteins of lymphoid tissue), which are inevitably present in extracts prepared from heterotransplantates of the tumor.

EXPERIMENTAL RESULTS

In the first series of experiments (Table 1) guinea pigs were sensitized to mouse adenocarcinoma of Ehrlich. As seen from Table 1, guinea pigs sensitized to this antigen showed anaphylactic reactions to the injection both of mouse serum and of extract of the heterotransplantate of the mouse tumor. However, as the experiments showed, guinea pigs which were completely desensitized to extract of the heterotransplantate of the tumor did not lose the power to react to subsequent injection of the original antigen. The injection of an extract

TABLE 1

Records of the Experiments

Guinea pig no.	Sensitization: mouse adenocarcinoma	Desensitization: mouse serum						Desensitization: heterotransplantate of mouse				Assaulting injection: mouse adenocarcinoma	
	dose, mg	dose ml	reaction	dose ml	reaction	dose ml	reaction	dose mg	reaction	dose mg	reaction	dose mg	reaction
Experiment													
390	15	0,5	Fatal shock	.	.	.	.	.	.	.	.	.	.
3965	15	0,5	» »	.	.	.	.	.	.	.		.	.
1375	15	0,3	++++	0,3	±	.	.	50	+	50	—	50	++++
2437	15	0,3	++++	0,3	±	.	.	50	+	50	—	50	Fatal shock
3128	15	0,3	++++	0,3	—	.	.	50	++	50	—	50	The same
2856	15	0,3	+++	0,3	±	.	.	42	+++	50	—	50	++++
2118	25	0,3	++++	0,5	++	0,5	—	50	++	50	—	50	Fatal shock
1896	25	0,3	++++	0,5	++	0,5	+	50	+++	50	±	50	++++
2539	25	0,3	+++	0,5	+	0,5	—	50	+++	50	—	50	Fatal shock
2508	25	0,3	++++	0,5	+	0,5	—	50	++++	50	±	50	The same
Control													
2905		0,3	—	0,5	±	0,5	—	50	±	50	—	50	—
3674		0,3	—	0,5	—	0,5	—	50	—	50	—	50	—
3131		0,3	—	0,5	—	0,5	—	50	—	50	—	50	—

Conventional signs: — no reaction; +, ++, +++, ++++ reactions of different intensity; . no injection of serum given.

TABLE 2

Guinea pig no.	Sensitization: mouse adenocarcinoma + horse serum (washed 7 times)	Desensitization: horse serum	
	dose, mg	dose, ml	reaction
	Experiment		
5125	15	0.3	+
4214	15	0.3	++
3114	15	0.3	+++
	Control		
2741	.	0.3	–
1102	.	0.3	–

Conventional signs the same as in Table 1.

of the mouse tumor caused in all the guinea pigs a severe anaphylactic shock, terminating fatally in about half the animals included in the experiment. Control, nonsensitized guinea pigs, injected along with the animals of the experimental group with the same doses of the antigens mentioned, in no case developed signs of anaphylactic shock.

The results given demonstrate that the antigens of the original tumor are preserved in the heterotransplantate. The reaction of the guinea pigs to injection of an extract of the heterotransplanted tumor was shown to be present after they had been completely desensitized to the species antigen (mouse serum); for this reason it may be asserted that the antigenic similarity between the tumor before and after heterotransplantation is determined by a complex of antigens, which differs in its properties from the species antigen of the tumor.

Meanwhile the experiments also show that in addition to common antigenic properties, certain antigenic differences are also present between the tumor before and after heterotransplantation. This is demonstrated by the fact that the extract of heterotransplanted tumor did not entirely remove the sensitivity of the guinea pigs to the subsequent injection of an extract of the original tumor. For an explanation of this phenomenon we postulated that the difference in the antigenic properties of the tumors being compared is determined not by the loss of certain antigens of the tumor at the time of heterotransplantation, as might be thought, but because this difference is connected with a change in the antigenic properties of the tumor under the influence of heterogenic protein.

In fact the extract of the heterotransplanted tissue used in the experiments as an antigen for desensitization of the guinea pigs, besides the proteins of the carcinoma cell, naturally contained a vast quantity of the proteins of lymphoid tissue. It is very probable that under such conditions the heterogenic proteins, firmly adsorbed onto the surface of the tumor cells, could to some extent block the active groups of tumor antigens and thereby restrict the antigenic powers of these antigens in relation to the appropriate receptors of the tissues of the sensitized animal. We attempted to check the grounds for this hypothesis experimentally. For this purpose we carried out a special experiment in which guinea pigs were sensitized to cells of ascitic carcinoma which had, as a preliminary measure, been placed in contact for 16 hours with horse serum at a temperature of 37°C, and then washed free from serum seven times with physiological saline.

The experiments showed (Table 2) that contact of the carcinoma cell with heterogenic serum leads to the firm adsorption of the heterogenic protein on the surface of the carcinoma cells, which can only be removed with difficulty by extensive washing of the carcinoma cells in physiological saline.

The effect of heterogenic protein on the antigenic activity of the tumor extract was examined by G. V. Suvorova [2], who succeeded in demonstrating by means of the complement fixation reaction that the intensity of the reaction between the antigens of the tumor and the corresponding immune sera is reduced if the tumor from which the antigen was prepared was placed as a preliminary measure in contact with heterogenic serum for a few hours. The weakening of the reactivity of the carcinoma antigen, in the author's opinion, is connected with the adsorption of foreign protein onto the surface of the tumor protein.

In another series of experiments to study the antigenic properties of a heterotransplanted tumor we attempted to shed light on the fate of the species antigen of the tumor after heterotransplantation. In contrast to the previous experiments, the guinea pigs were sensitized not to the original tumor but to a heterotransplantate of this tumor in the lymphatic nodes of the guinea pig.

The results of these experiments are shown in Table 3.

As seen from the findings in Table 3, guinea pigs sensitized to a heterotransplantate of the tumor were insensitive to the mouse serum. On subsequent injection of an extract of mouse tumor the guinea pigs reacted by anaphylactic shock. It was further discovered that desensitization to the mouse tumor caused total loss of

TABLE 3

Guinea pig no.	Sensitization	Desensitization												Assaulting injection	
	heterotransplantate of mouse adenocarcinoma	mouse serum				mouse adenocarcinoma								heterotransplantate of mouse adenocarcinoma	
	dose (mg)	dose (ml)	reaction	dose (ml)	reaction	dose (mg)	reaction	dose (mg)	reaction	dose (mg)	reaction	dose (mg)	reaction	dose (mg)	reaction
						Experiment									
958	25	0,4	—	0,3	—	60	++++	60	++++	60	++++	60	+	50	—
386	25	0,5	—	0,3	—	60	++	60	+++	60	+(+)	60	—	50	—
1604	25	0,5	±	0,3	—	60	+++	60	++++	60	++(+)	60	—	50	—
						Control (horse serum)									
1396	25	0,4	—	0,3	—	60	++++	50	+++	60	++	60	+	50	—
1658	25	0,4	+	0,3	—	60	++++	50	+	60	+++	60	—	50	—
555	25	0,3	+	0,3	—	60	++	50	++	60	+++	60	—	50	—

TABLE 4

Guinea pig no.	Sensitization: heterotransplantate of mouse adenocarcinoma, dose (mg)	Desensitization: mouse serum, dose (ml)	reaction	dose (ml)	reaction	dose (ml)	reaction	dose (ml)	reaction	mouse adenocarcinoma: dose (mg)	reaction
					Experiment						
2054	15	0,3	++	0,4	++	0,5	+	0,5	—	50	Fatal shock
3194	15	0,4	+++	0,4	++	0,4	+	0,5	—	30	The same
3043	15	0,3	++	0,4	—	0,4	+(+)	0,5	±	20	» »
2604	15	0,3	++	0,4	±	0,4	+(+)	0,5	—	20	» »
					Control						
2813	.	0,3	—	0,4	—	0,5	±	0,5	—	50	—
2402	.	0,3	—	0,4	—	0,5	—	0,5	—	50	—
3930	.	0,3	—	0,4	—	0,5	—	0,5	—	50	—

the sensitivity of these animals to the original antigen, i. e. to an extract of the heterotransplanted tumor.

In Table 4 are shown the results of a similar experiment. In contrast to the previous experiment guinea pigs were sensitized to a heterotransplanted tumor showing intensive growth in the lymphatic nodes of a guinea pig. This experiment showed that in this particular case sensitization of the guinea pigs was developed both to mouse serum and to the tumor. In these circumstances there was observed a more or less moderate reaction to the species protein and a violent reaction to the protein of the malignant tumor, terminating in the death of all the experimental animals. In some guinea pigs death ensued after the injection of a comparatively small dose of the carcinoma antigen. Unsensitized animals did not react to the injection of these antigens mentioned.

The results in Tables 3 and 4 demonstrate that a heterotransplantate of a tumor has in its composition antigens which are identical with the antigens of the mouse tumor. In addition, by means of mouse serum, carrying protein with species-specific properties, it is possible to show that a species antigen forms an indispensable component of the antigenic complex of the carcinoma cell. Analysis of the results of these experiments shows that the species antigen in the tumor heterotransplantate stands in a definite relationship with the remaining antigenic components of the carcinoma cell. If, for instance, in the first case the reaction of the guinea pigs to mouse serum is absent and the reaction to the tumor is moderate, then in the second case a clear reaction will be observed to the species antigen and a violent reaction of shock, terminating in the death of all the animals taking part in the experiment will occur in response to the injection of the remainder of the antigenic complex of the carcinoma cell.

Furthermore guinea pigs sensitized to a heterotransplanted tumor, in contrast to guinea pigs sensitized to unchanged tumor, are desensitized to the corresponding antigen with great difficulty. As a rule frequent injections of antigen are required in order to obtain complete desensitization of the animal.

As the experiments showed, the sensitivity to protein of the carcinoma cell (see Table 3) was removed only after the guinea pigs had received 4 injections of comparatively large doses of antigen. Complete desensitization to the species antigen (see Table 4) also took place after 4 injections of mouse serum. Meanwhile complete loss of sensitivity of the guinea pigs sensitized to the nontransplanted tumor is attained as a rule after two injections of a suitable dose of serum.

It can be concluded on the basis of the results obtained that the method of anaphylaxis with desensitization in guinea pigs enables a highly accurate analysis to be made of the antigenic properties of heterotransplanted tumors. By means of this method it was possible to show that the temporary growth of a mouse tumor in an animal of another species does not lead to a sharp change in its antigenic properties. Under these conditions the heterotransplantate of the tumor largely preserves the antigens of the original tumor and, in particular, those antigens which characterize the species to which the proteins of the tumor cell belong.

Certain antigenic differences found in the experiments between the original and the heterotransplanted tumors are connected, in our opinion, with physicochemical changes in the reactive properties of the tumor proteins as a result of the adsorption of foreign protein. At the same time, during investigation of the antigenic properties of tumors after long-standing heterotransplantation, it must be considered that the changes of this sort in the tumor are associated not only with adsorption of proteins but also with the loss of some of the tumor antigens in the process of heterotransplantation.

For a final solution of these problems additional investigations will be required in order to minimize the opportunity of a dual interpretation of this phenomenon.

SUMMARY

The method of anaphylaxis with desensitization makes it possible to analyze the antigenic properties of the heterotransplanted tumors in guinea pigs with considerable precision. It was demonstrated that the temporary growth of the mouse tumor in an animal of another species does not alter considerably its antigenic properties. The antigens of the original tumor, particularly the antigen characterizing the species specificity of the protein, are preserved in the heterotransplant of the tumor in these conditions.

LITERATURE CITED

[1] V. K. Kozlov, Biull. Eksptl. Biol. i Med. 37, 4, 56-59 (1954).

[2] G. V. Suvorova, Biull. Eksptl. Biol. i Med. No. 1, 82-85 (1957).*

* See English translation.