ONCOLOGY

SIMILARITIES AND DIFFERENCES OF SPECIFIC ANTIGENS OF CANCER IN MAN

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In the course of immunologic studies on malignant tumors sufficient facts have been collected to suggest their practical application for the diagnosis, prophylaxis and therapy of cancer. Of particular significance in this connection is the question of similarity or difference of antigens determining the specificity of cancerous tumors in man; this question has not, however, up to now received a definitive answer.

V. V. Gorodilova and L. V. Shershulskaya [2], using the method of anaphylaxis with desensitization, found that cancerous tumors in man with different localization (stomach, liver, breast, uterus, ovaries) contained both specific antigens common to all the tumors and separate components specific for each particular tumor. Similar results were also obtained by V. A. Korenevskaya [3] who investigated specific antigens from 11 varieties of malignant brain tumors of neuroectodermal and meningo-vascular type. As this author notes, the antigenic similarity of the tumors studied has, as a rule, greater constancy than their differences.

The work of V. V. Gorodilova and V. B. Freiman [1] established that the specific antigen characteristics of tumors are not limited by the species of the host-carrier of the tumor, but extends to tumors in animals of other species. These authors found antigenic similarity between cancerous breast tumors of man and mouse. In addition to the similar components detected in the cancerous tissues of man and mice dissimilar components were also found in these tissues.

Other data were obtained by A. K. Saakov [7] who used absorbed anti-tumor sera and found only one specific antigen common to cancer of the uterus, cancer of the stomach and cancer of the lung in man.

Analogous results were obtained by I. Makari and M. Huch [9], using the Schultz-Dale reaction (anaphylaxis with desensitization on isolated organs of the guinea pig). In their studies on sera from patients the authors found a soluble antigen common to all forms of cancer, independent of its type or site of origin.

In contradistinction to the investigations described above, we [4] found, using anti-tumor sera freed by absorption from antibodies to normal organs, that no single antigen specific for all tumors in man exists. Each of the three cancerous tumors studied by us contained its particular specific antigen which was absent in the other two. This material naturally could not be considered adequate for judging to what extent the observed fact could be applied to other tumors or for answering the question whether each tumor was characterized by specific properties particular to it alone or whether tumors could possess similar antigens. Further work was needed to clarify these problems; the results are presented in the present communication.

The material for the present investigation was formed by 45 cancerous tumors in man, of different localization and structure; moreover, the patients belonged to different blood groups. Comparative studies were made on the antigenic properties of tumors of the stomach, breast, lung, ovary, prostate, liver and metastases in the liver from cancer of the stomach, lung, ovary, esophagus, cecum, colon and others.

EXPERIMENTAL METHODS

The experimental methods for absorption and complement fixation as well as the use of specific anti-tumor sera No. 388 and No. 114 (against tumor No. 1 – liver metastasis of cancer of the colon), No. 528 and No. 990 (against tumor No. 2 – liver metastasis of cancer of the gall bladder), No. 49, No. 1758 and No. 1934 (against tumor No. 3 – primary cancer of the liver) have already been described by us in detail in previous communications [4-5]

Sera organ-specific to liver and spleen in man were used for control simultaneously with anti-tumor sera.

Antigens for absorption and complement fixation were prepared from fresh and formalin-or glycerine-preserved tumor tissues. Formalin-or glycerine-treated tissues were first freed from the preserving fluid by washing in running tap-water or by dialysis in physiologic solution. Absorption of sera by fresh tumor tissue could only be achieved successfully when the material had not been stored for long, so that material obtained at operation gave the best results. The tissue in these cases was macerated and washed immediately before the experiment.

Control antigens were prepared from fresh spleen and liver tissue taken from healthy persons who died accidentally.

EXPERIMENTAL RESULTS

In Table 1 is presented a typical protocol of an experiment on comparative study of water-salt extracts from tumors by means of complement fixation reaction. The table shows that anti-tumor serum No. 1758 gives a positive complement fixation reaction with extract of tumor No. 3, with respect to which this serum was obtained, and also with one of the other tumors under investigation, viz. No. 12. This serum did not, however,

TABLE 1

Comparative Studies on Specific Antigenic Properties of Tumors Using the Complement Fixation Method

Serum	Dilution of Sera	Antigens from Tumors										Antigens from spleen	Serum Control
		№ 1	№ 3	№ 4	№ 5	№ 7	№ 8	№ 9	№ 10	№ 11	№ 12		
Anti-tumor No. 1758 (against tumor No.3)	1:20	–	++++	–	–	–	–	–	–	–	++++	–	–
	1:40	–	+++	–	–	–	–	–	–	–	+++	–	–
	1:80	–	±	–	–	–	–	–	–	–	±	–	–
Anti-spleen No. 57	1:80	–	–	–	–	–	–	–	–	–	–	++++	–
	1:160	–	–	–	–	–	–	–	–	–	–	++++	–
Antigens control		–	–	–	–	–	–	–	–	–	–	–	

Conventional signs: ++++, +++, ++, +, ± different degrees of positive reaction; – negative reaction.

Note. Tumor No. 1 – liver metastasis of cancer of the cecum; No. 3 – primary cancer of the liver; No. 4 – liver metastasis of cancer of the stomach; No. 5 – liver metastasis of cancer of the bile duct; Nos. 7 and 8 – liver metastasis of cancer of the stomach; No. 9 – primary cancer of the liver; No. 10 – liver metastasis of cancer of the ovary; No. 11 – liver metastasis, primary site unknown; No. 12 – liver metastasis of cancer of the stomach.

react with the water-salt extracts of tumors No. 1, 4, 5, 7, 8, 9, 10 and 11; this indicates the absence in these of antigens identical with antigens of tumors No. 3 and No. 12. The anti-tumor serum used in the experiments was specific and did not react with antigen from normal organ (spleen). The working dose of spleen antigen is shown to be correct by the positive reaction obtained with the same dose of spleen antigen and the corresponding organ-specific serum.

TABLE 2

Comparative Studies on Specific Antigenic Properties of Tumors Using the Absorption Method

Serum	Tissue used for Serum Absorption	Dilution of Sera	Antigens from Tumors								Antigen from spleen	Sera control
			№ 1	№ 4	№ 6	№ 7	№ 8	№ 9	№ 10	№ 11		
Antitumor No. 388 (against tumor No. 1)	Spleen	1:80	++++	++++	++++	—	—	—	—	—	—	—
		1:160	++++	++++	++++	—	—	—	—	—	—	—
		1:320	++++	++++	+++	—	—	—	—	—	—	—
	Tumor № 1	1:80	—	—	—	—	—	—	—	—	—	—
		1:160	—	—	—	—	—	—	—	—	—	—
		1:320	—	—	—	—	—	—	—	—	—	—
	Tumor № 4	1:80	—	—	—	—	—	—	—	—	—	—
		1:160	—	—	—	—	—	—	—	—	—	—
		1:320	—	—	—	—	—	—	—	—	—	—
	Tumor № 6	1:80	—	—	—	—	—	—	—	—	—	—
		1:160	—	—	—	—	—	—	—	—	—	—
		1:320	—	—	—	—	—	—	—	—	—	—
	Tumor № 7	1:80	++++	++++	++++	—	—	—	—	—	—	—
		1:160	++++	++++	++++	—	—	—	—	—	—	—
		1:320	++++	++	+	—	—	—	—	—	—	—
	Tumor № 8	1:80	++++	++++	++++	—	—	—	—	—	—	—
		1:160	++++	++++	+++	—	—	—	—	—	—	—
		1:320	++++	++	+	—	—	—	—	—	—	—
	Tumor № 9	1:80	++++	++++	++++	—	—	—	—	—	—	—
		1:160	++++	++++	+++	—	—	—	—	—	—	—
		1:320	++++	+++	+	—	—	—	—	—	—	—
	Tumor № 10	1:80	++++	++++	++++	—	—	—	—	—	—	—
		1:160	++++	++++	+++	—	—	—	—	—	—	—
		1:320	++++	+++	±	—	—	—	—	—	—	—
	Tumor № 11	1:80	++++	++++	++++	—	—	—	—	—	—	—
		1:160	++++	++++	+++	—	—	—	—	—	—	—
		1:320	++++	+++	++	—	—	—	—	—	—	—
Antigen Control			—	—	—	—	—	—	—	—	—	

Conventional signs the same as in Table 1.

Note. Tumor No. 1 – liver metastasis from cancer of the cecum; Nos. 4, 6, 7 and 8 – liver metastasis from cancer of the stomach; No. 9 – primary cancer of the liver; No. 10 – liver metastasis of cancer of the ovary; No. 11 – liver metastasis, primary site unknown.

TABLE 3

Results of Comparative Studies on Specific Antigenic Properties of Tumors in Man

No. of tumor	Localization of tumor	Blood group	Number of anti-tumor serum						
			Against tumor No. 1		Against tumor No. 2			Against tumor No. 3	
			388	114	528	990	49	1758	1934
1	Liver metastasis of cancer of the cecum	0	+	+	−			−	−
2	Liver metastasis of cancer of the bile duct	0	−	−	+	+	−	−	−
3	Primary cancer of the liver	B	−	−	−	−	+	+	+
4	Liver metastasis of cancer of the stomach	B	+		−	−	−	−	−
5	Liver metastasis of cancer of the bile duct	0	+			−		−	−
6	Liver metastasis of cancer of the stomach	0	+		−	−		−	
7	Liver metastasis of cancer of the stomach	0	−		−	−		−	−
8	Liver metastasis of cancer of the stomach	0	−		−	−		−	−
9	Primary cancer of the liver	A	−		−	−		−	−
10	Liver metastasis, of cancer of the ovary	A	−		−	−	−	−	−
11	Liver metastasis, primary site unknown	0	−		−	−		−	−
12	Liver metastasis of cancer of the stomach	A	−		−	−		+	+
13	Liver metastasis of cancer of the uterus		−		−		−	−	−
14	Liver metastasis of cancer of the stomach	A	−		−		−	−	−
15	Liver metastasis of cancer of the stomach	B	−		−		−		−
16	Liver metastasis of cancer of the lung	A	−		−				−
17	Cancer of the stomach		−	−	−				−
	Liver metastasis	B	−	−					
	Lymph gland metastasis		−	−					
18	Cancer of the lung	A	+		−				−
19	Cancer of the lung		−		−				−
20	Liver metastasis of cancer of the lung		−		−				−
21	Cancer of the stomach	A	−	−	−				−
	Liver metastasis of cancer of the stomach		−	−					
22	Cancer of the stomach		−		−				−
23	Cancer of the stomach	0	−		+				−
24	Cancer of the stomach		−						−
25	Cancer of the ovary	A							−
26	Liver metastasis of cancer of the lung	A	−						
27	Cancer of the lung	B	−	−					
28	Cancer of the prostate	0	−	−					
29	Liver metastasis of cancer of the stomach	A			−	−	−	−	−
30	Liver metastasis of cancer of the cervix	0			−		−	−	−
31	Cancer of the breast				−		−		
32	Cancer of the breast	0	−						
33	Cancer of the esophagus growing into bronchus	0		+					
	Liver metastasis			+					
34	Liver metastasis of cancer of the pancreas	0		−					
35	Lung metastasis of cancer of the stomach	A		−					

(Table 3 cont'd)

No. of tumor	Localization of tumor	Blood group	Number of anti-tumor serum						
			Against tumor No. 1		Against tumor No. 2		Against tumor No. 3		
			388	114	528	990	49	1758	1934
36	Cancer of the breast	B		–					
37	Skin (chest) metastasis of cancer of the breast	B		–					
38	Liver metastasis of cancer of the stomach	A					–	–	–
39	Liver metastasis of cancer of the colon	B		–					
40	Liver and spleen from patient with leukemia	A		–					
41	Liver metastasis of cancer of the stomach	B		–					
42	Cancer of the stomach	B		–					
43	Cancer of the lung	AB	–						
44	Cancer of the stomach	0		–					
	Liver metastasis			–					
45	Cancer of the stomach	A		+					
	Lymph gland metastasis			+					

Conventional signs: + positive complement fixation and absorption reaction results; – negative results.

These experiments, on the one hand, confirm the fact established by us earlier [4] concerning the difference of specific tumor antigens and, also, demonstrate that there are tumors which are characterized by the presence of similar specific antigens (tumors No. 3 and No. 12).

Another confirmation of this fact came from investigation of tumor antigens by means of specific absorption. Table 2 presents a protocol of experiment on absorption of anti-tumor serum No. 388 (against tumor No. 1) by different samples of tumor tissues. The experiment included tumor tissues No. 4 and No. 6, whose water-salt extracts, like that of tumor No. 1, gave a positive complement fixation reaction with anti-tumor serum No. 388 and tumor tissues Nos. 7, 8, 9, 10 and 11 whose water-salt extracts gave negative complement fixation reactions (see Table 2, absorption of serum by spleen tissue). Absorption of serum No. 388 by tumor tissues Nos. 1, 4 and 6 led, as can be seen from Table 2, to complete loss of its ability to react with water-salt extracts from tumors Nos. 1, 4 and 6. Conversely, tumor tissues Nos. 7, 8, 9, 10 and 11, whose water-salt extracts gave a negative complement fixation reaction, could not extract from the serum anti-tumor antibodies in the course of absorption. In the course of absorption the serum retained its ability to react with water-salt extracts of tumors Nos. 1, 4 and 6.

Thus, the experiments performed by us on specific absorption provided new proofs in favor of the existence of tumors both differing in their specific antigenic properties and similar with respect to specific antigen.

As has been mentioned above, comparative studies were carried out on 45 cancerous tumors of man. The results of all these investigations are summarized in Table 3 which shows that of 32 tumor antigens tested with sera No. 388 and No. 114 (against tumor No. 1) only 6 antigens (Nos. 4, 5, 6, 18, 33, 45), as well as antigen No. 1, gave a positive reaction, i. e. proved to be similar to antigen No. 1.

The majority of the antigens, however, reacted negatively thus demonstrating qualitative differences between the antigenic properties of these 27 tumors from those of tumor No. 1 and tumors Nos. 4, 5, 6, 18, 33 and 45 similar to No. 1.

Of 26 tumor antigens tested with sera Nos. 528 and 990(against tumor No. 2) only one antigen, No. 23, like antigen No. 2 which was used for the serum, reacted positively, the remaining 24 antigens gave a negative result.

Twenty-nine tumor antigens were studied with anti-tumor sera Nos. 49, 1758 and 1934 (against tumor No. 3). Apart from antigen from tumor No. 3, a positive reaction was found only with antigen from tumor No. 12.

It must be noted that sera of one series which were obtained from different rabbits but were immunized by the same tumor antigen (e. g. sera Nos. 49, 1758, 1934) behaved consistently in the experiments, in other words they all gave either a positive or a negative reaction when treated with the antigen being tested.

As can be seen from Table 3, attempts to establish some relationship between the ability of a tumor antigen to enter into immunologic reaction with one or other anti-tumor serum and localization of this tumor proved unsuccessful. Thus, for example, tumors Nos. 18, 33 and 45 gave a similar positive reaction with sera Nos. 388 and 114 despite the fact that they differed in localization. On the other hand, tumors with similar localization behaved differently in their reactions, e. g. tumors Nos. 3 and 9 reacting with sera Nos. 1758 and 1934.

This ability of tumors to enter into reaction did not depend on the host's blood group either, and hence did not depend on the group antigens present in the tumors.

It should be noted particularly that primary tumors and their metastases obtained from one and the same patient possessed similar specific antigenic properties. If the original tumor entered into immunologic reaction with one or other serum, its metastases also reacted positively, and vice versa (e.g. tumor No. 17 or No. 45, see Table 3).

We did not observe a single case of divergence in the behavior of primary tumor and its metastases.

Unlike the data of L. A. Silber and his collaborators, A.K. Saakov, Makari and Huch and others, these investigations have shown that no single antigen, specific for all cancerous tumors of man, exists. Some tumors are qualitatively different as regards antigens and have no common specific "cancer" antigens; other tumors, on the contrary, are characterized by the presence in them of similar, and possibly identical, specific antigens.

This has also found support in the work of P. N. Kosyakov and N. I. Kuznetsova [6].

In connection with the results obtained by us it is of interest to note the data reported by J. Graham and R. Graham [8], according to which patients with cancer show antibodies which react with antigen from tumors of other patients.

The same authors, however, report that in some, more rare, cases tumors did react similarly with one and the same serum, i. e. showed similar antigenic properties.

The question concerning the causes of similarity of specific antigens in some cancerous tumors in man and, conversely, of differences in others remains unanswered and requires further study.

SUMMARY

The authors employed reactions of complement fixation and specific absorption for comparative study of the antigenic properties of tumors in man. As a result of these experiments it was established that there is no single antigen which is specific for all tumors of man. Certain tumors are qualitatively different and do not have common specific "cancer" antigens. On the contrary, other tumors are characterized by the presence of similar specific antigens.

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