ONCOLOGY

ON THE POSSIBILITY OF THE EXISTENCE OF A SINGLE ANTIGEN SPECIFIC FOR ALL HUMAN CANCERS

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It has been established that cancerous growths in man, along with antigens common to both malignant and normal tissues as, for example, the specie [8], group [5, 13, 16], type [6, 7], heterophile [14], contain also specific antigens possessed only by them [2, 9, 11]. These specific antigenic substances have been demonstrated in tumors varying as to localization and structure: malignancies in the liver, stomach, gastrointestinal tract, pancreas, long uterus, ovary, breast and others.

However, the question as to whether all these tumors in varying locations, as well as tumors in one location but of a different origin and in different persons, contain a single antigen, uniquely specific for all human cancers, has not been fully settled.

Using as a basis his experiments with absorbed specific sera, A. K. Saakov [12] concluded that tumors in such varying locations as stomach, uterine cervix and the lung – all possess a general, specific antigen which is common to them all. The same conclusion was reached by Makari and Huch [15] who uncovered the cancer antigen in the serum of patients (28 tumors in different locations were studied) by using the method of active and passive anaphylaxis in guinea-pigs sensitized to the extracts prepared from the isolated organs (Schultz–Delya method).

V. V. Gorodilov and L. V. Shershulsky [1] obtained contrary results. Using as a basis their experiments conducted with anaphylaxis from sensitization by the method of L. A. Silver [2], they concluded that tumors from different organs (cancers of stomach, liver, breast, uterus and ovary) contain not only specific antigens common to them all but also various individual antigenic components. Analogous data were obtained by V. A. Korenevsky [3] when he studied human malignancies in the brain.

The goal of the present study was the further investigation of the similarities or differences in specific cancer antigens present in malignancies growing in various locations.

EXPERIMENTAL METHODS

The material for this study consisted of three malignancies all growing in the same human organ. Tumor Number 1 – metastasis in the liver from a primary adenocarcinoma of the cecum, Number 2 – metastasis to the liver from an adenocarcinoma of the gallbladder and Number 3 – primary liver cancer. The patients having tumors 1 and 2 belonged to the same blood group – O, while tumor 3 was taken from an individual in group B. From each tumor immune sera were obtained from rabbits: Number 388 from tumor 1, Number 528 from tumor 2 and Number 1934 from tumor 3. We have described in detail the methods used to immunize the rabbits and also the methods of purifications used to free the sera from other antigens [9].

The specificity of the antitumor extracts of sera were checked simultaneously against saline extracts of normal human organs – liver and spleen. The latter, as shown in previous experiments [4], has the greatest capacity possessed by any normal human organ for having antigenic similarities with human malignancies and therefore extracts from this organ must always be used in deciding the specific nature of any serum used.

As controls we employed, simultaneously with the antitumor sera, sera specific against the normal human organs (liver, spleen), obtained using the method of N. I. Kuznetsov [10].

As malignancies maintain their antigenic properties after fixation in formalin and glycerin [4], we were able to use, alongside with the antigens of the original tumors, also antigens of preserved tumors.

TABLE 1

Comparative Studies on the Specific Antigenic Properties of Cancers Employing the Complement Fixation Method

Sera	Sera dilutions	Antigens					Control sera
		tumor	tumor	tumor	liver	spleen	
Antitumor number 388 (to tumor 1)	1 : 80	++++	—	—	—	—	—
	1 : 160	++++	—	—	—	—	—
	1 : 320	++++	—	—	—	—	—
Antitumor number 528 (to tumor 2)	1 : 40	—	++++	—	—	—	—
	1 : 80	—	++	—	—	—	—
	1 : 160	—	±	—	—	—	—
Antitumor number 1934 (to tumor 3)	1 : 20	—	—	+++	—	—	—
	1 : 40	—	—	++	—	—	—
	1 : 80	—	—	±	—	—	—
Antiliver 930	1 : 40	—	—	—	++++	—	—
	1 : 80	—	—	—	+++	—	—
	1 : 160	—	—	—	±	—	—
Antispleen 57	1 : 40	—	—	—	—	++++	—
	1 : 80	—	—	—	—	++++	—
	1 : 160	—	—	—	—	+++	—
Control antigens		—	—	—	—	—	

Significance of signs used: ++++, +++, ++, +, ± different degrees of positiveness of the reaction; — negative reaction.

EXPERIMENTAL RESULTS

The results of comparative studies on the specific antigenic properties of cancers Numbers 1, 2 and 3 employing the complement binding reaction method is shown on Table 1.

From Table 1 it can be seen that the immune, antitumor serum Number 388 to tumor Number 1, after the removal from it of antibodies to the antigens of normal organs (this goal being achieved by absorbing the serum on spleen extract), loses it ability to react with antigens taken from normal tissues and retains only its capacity to react with water saline extracts of the tumor tissue which had served as the material for immunization. This serum did not react with antigens obtained from tumors Number 2 and 3, this being evidence that any antigen identical with the antigen obtained from tumor Number one must be absent. Similarly, the antitumor serum Number 528, after being treated in the same manner as was serum Number 388, retained only its capacity to react with antigens from tumor Number 2, this being the growth against which this serum was developed. It would not react with antigens obtained from tumors Number 1 and 3. This is taken as proof that these tumors

TABLE 2

Comparative Study of the Specific Antigenic Properties Possessed by Tumors Number 1 and 2 Using the Method of Specific Absorption

Sera	Absorbing tissue	Serum dilution	Antigens: tumor	tumor	liver	spleen	Control sera
Antitumor No. 388 (to tumor No. 1)	Formalinized spleen	1 : 60	++++	—	—	—	—
		1 : 620	++++	—	—	—	—
		1 : 40	++++	—	—	—	—
	Formalinized tumor No. 1	1 : 80	—	—	—	—	—
		1 : 160	—	—	—	—	—
		1 : 320	—	—	—	—	—
	Formalinized tumor No. 2	1 : 80	++++	—	—	—	—
		1 : 160	++++	—	—	—	—
		1 : 320	++++	—	—	—	—
Antitumor No. 528 (to tumor No. 2)	Formalinized spleen	1 : 40	—	++++	—	—	—
		1 : 80	—	+++	—	—	—
		1 : 160	—	+	—	—	—
	Formalinized tumor No. 2	1 : 40	—	++++	—	—	—
		1 : 80	—	+++	—	—	—
		1 : 160	—	+	—	—	—
		1 : 40	—	—	—	—	—
		1 : 80	—	—	—	—	—
		1 : 160	—	—	—	—	—
Antiliver No. 980	Normal organs	1 : 40	—	—	++++	+	—
		1 : 80	—	—	+++	±	—
		1 : 160	—	—	++	—	—
Antispleen No. 980	Normal organs	1 : 40	±	—	+	++++	—
		1 : 80	—	—	—	++++	—
		1 : 160	—	—	—	+++	—
Antigen controls			—	—	—	—	

Significance of the signs as in the Table 1.

do not possess antigens identical with those obtained from tumor Number 2. The antitumor serum Number 1934, after similar removal of antigens against normal organs, maintained only the ability to react with antigens from tumor Number 3, this being the cancer against which this serum was developed. Again, against tumors Numbers 1 and 2 this serum would not react so that we can assume also in this instance that tumors 1 and 2 did not possess antigens identical to tumor Number 3. As indication of the specific nature of the antitumor antigen, the absence

chosen doses of the antigens from normal organs were sufficiently well gauged in preliminary titrations, can be proven by the fact that these antigens reacted positively with the corresponding organ-specific homologous sera Numbers 986 and 57. The sera against the normal organ extract antigens, in their turn, were quite specific and did not react with the antigens taken from the tumors.

In this fashion, our experiments indicate that the tumors we studied which were obtained from three different individuals differ as to their specific antigenic properties. Each tumor has its own, its absolutely specific antigen.

These conclusions are confirmed by experiments with specific absorptions.

The absorption experiment consisted of two phases. The first phase—absorption of the serum by various fixed tissues (cancers preserved in formalin), the second phase—testing the sera upon complement binding by specific antibodies. Before absorption, the pieces preserved in 5% formalin (1 part 40% formalin plus 7 parts water) were washed in continuously running water for 18-20 hours in order that all the formalin would be removed from the tissues, then the material was ground up in a mortar to which physiological saline was added as the tissues were pulverized. The resulting mixture was then centrifuged, the precipitate being repeatedly washed with physiological saline and recentrifuged until finally there was formed only a clear supernatant liquid. Such precipitates from ground tissues washed in this manner with physiological saline were employed in their capacity as absorbents. Each sample of the serum was absorbed by different volumes of the pulverized tissue extracts. The quantity of tissue used for each absorption was related to the titer of the sera being tested, it being possible for it to be different for each individual sample of serum. As a rule, for 1 volume of precipitate there were 4 volumes of serum, diluted 1:10. The pulverized cancer tissues and the serum were thoroughly mixed, the suspension being permitted to stand at room temperature for 30 minutes. Then it was centrifuged for 20 minutes at 4,000 r.p.m., the supernatant liquid was suctioned off and the second phase of the experiment was performed, the complement fixation by the antibodies being measured.

The results of observations made on tumors Numbers 1 and 2 are given in Table 2.

From Table 2 it can be seen that the antitumor serum Number 388, after absorption by tissues of normal organs (spleen) maintained its capacity to react with saline-water extracts from tumor Number 1. This serum did not lose its specific tumor Number 1 antibodies even after being absorbed by tissues from tumor Number 2. When it was allowed to be absorbed by tissues taken from tumor Number 1, this serum lost completely its antitumor titer. Analogous results were obtained by us when we studied serum Number 528. This serum, after absorption by normal organ tissues (spleen), continued to react with saline-water extracts from tumor Number 2, against which it was prepared originally. Extracts from tumor Number 1 could not remove the specific antibodies against tumor Number 2. In contrast, the tissues from tumor Number 2, against which this serum was prepared, completely removed the specific antibodies so that this serum lost all ability to act with its own specific homologous cancer antigen.

The results of these experiments on specific absorption of the antitumor sera by tissue extracts from various tumors confirm the view that these antigens are qualitatively specifically different from each other at least in the cancers studied.

The general antigenic properties of the tumors we studied could be compared with the normal antigenic properties and could be readily differentiated by our use of absorption of immune antitumor sera by use of spleen extract sera from a normal organ.

In this fashion we believe that we have shown quite conclusively that there is no single antigen specific for all human cancers.

Cancers present in the same metastatic locality (liver) can be quite different from each other having qualitatively distinct specific antigenic properties which do not react in the slightest with sera prepared from another tumor.

There must now be explored the further question as to the reason for qualitative differences between the various human cancers arising in different human patients.

SUMMARY

Specific (absorbed) sera against human cancer and tissues of normal organs were used in a comparative study of antigenic properties of human cancerous tumors.

It was demonstrated by the authors' specific absorption method that tumors of the same localization may differ in the character of their antigenic properties. There is no antigen in existence specific for all human cancerous tumors.

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