A COMPARATIVE STUDY OF THE ANTIGENIC PROPERTIES OF CERTAIN MOUSE TUMORS INOCULATED INTO PURE-BRED MICE

G. P. Airapetyan

From the Laboratory of Noninfectious Immunology (Chairman—Prof. I. N. Maisky) and the Laboratory of Antigenic Biology (Chairman – M. M. Kapichnikov, Candidate for Medical Sciences), the Institute of Experimental Biology (Director – Prof. I. N. Maisky), Acad. Med. Sci. USSR, Moscow

(Received May 15, 1957. Presented by N. N. Zhukov-Verezhnikov, Active Member of the Acad. Med. Sci. USSR)

The discovery of specific tumorous antigens [1-7] necessitated a more careful study of them. The problem concerning the specificity of the tumorous antigens (especially with transplanted tumors) must be further developed since many works have appeared in foreign literature which throw doubt on the presence of specific immunity in the case of tumors.

Foreign immunogeneticists [8, 9] explain the existence of immunity in transplanted tumors by a tissue incompatibility between the tissues of the animal bearing the tumor and those of the recipient due to their immunogenetic difference.

Th. Hauschka [9] believes that the antigenic conformity of the tumor donor's tissues with those of the recipient does not cause an immune reaction, and that the tumor develops without the appearance of antibodies. This author believes that the antibodies discovered in non-infiltrating tumors are associated with the phenomena of iso- and autoimmunization. Certain foreign scientists have investigated the problem concerning the existence of specific tumorous antigens using inbred animals [10]. They concluded that a specific cancer antigen was present even when a tumor was passed to pure-bred animals of the same genetic breed, a process effecting maximal exclusion of iso-antigenic differences.

In 1956, V. V. Gorodilova and L. V. Shershulskaya conducted experiments using the anaphylactic reaction with desensitization to study the antigenic properties of normal tissues in mice of various breeds. These authors were unable to discover any antigenic difference in normal tissues of genetically different animals breeds.

We attempted to clear up these differences of opinion. For this purpose, we conducted a series of experiments studying the specific tumorous antigens of 3 transplanted mouse tumors, inoculated simultaneously into the same animal and then passed many times to mice of the A. breed. The inoculation of all 3 tumors into one inbred animal and the subsequent passing of the tumors for a long period of time maximally diminished the influence of the genotypic differences present in non-pure-bred animals, and also reduced the iso-antigenic difference existing between individuals of the same breed.

EXPERIMENTAL METHODS

We made a comparative study of 3 mouse tumors: Ehrlich's adenocarcinoma (subcutaneous form), Kroker's sarcoma and acridine sarcoma. The original inoculation was done by means of a trocar, transplanting uniform pieces subcutaneously to white mice of breed A. Ehrlich's adenocarcinoma was inoculated into the left cervical region, Kroker's sarcoma, into the right flank, and acridine sarcoma into the left flank of the animal.

Therefore, all three tumors developed simultaneously on the same mouse. Fifteen to eighteen days later, the tumors were passed to inbred mice of breed A. in the same locations. A total of 20 passages were done.

We examined the tissues of the passed tumors immunologically, using the complement fixation reaction (CFR) both in its usual form and with preliminary specific absorption of the antitumor immune serums according to the method proposed by P. N. Kosyakov [4].

The immune serums were obtained from chinchilla rabbits intravenously immunized according to one of the 4-week cycle systems. Immunization was produced to each of the 3 tumors inoculated into the same animal at the 1st, 10th and 20th passages. We used the complement fixation reaction in the control experiments with immune serums in relation to the normal mouse liver and spleen.

EXPERIMENTAL RESULTS

In the first series of experiments, we studied the changes in the antigenic properties of tumors passed for a long time to inbred mice. We used immune serums in relation to the Ehrlich's adenocarcinoma (No. 1353 and 1393), to the Kroker's sarcoma (No. 1692 and 3243) and to the acridine sarcoma (No. 1094 and 343) of the 1st and 20th passages. Their examination with the suitable antigens in the complement fixation reaction gave the results presented in Table 1.

As the data in Table 1 shows, the complement fixation reaction did not disclose any change in the antigenic properties of the tumorous tissues to occur during the passing process. Each of the antitumor serums obtained to the tumors of the 1st passage reacted with the corresponding antigen in approximately the same titer as with the antigen from the tissue of the same tumor passed 20 times. The antitumor serums to the tumors of the 20th passage also had the same titer with the antigens from the tissues of the tumors passed 20 times as with the antigens from the tissues of 1st passage tumors. Therefore, prolonged passaging of the tumors to pure bred mice did not affect the antigenic properties of these transplanted tumors. By passing the 3 transplanted tumors simultaneously to inbred mice, we obtained data on the similarity and difference in the antigenic properties of the tumors mentioned.

Such an experimental form excluded the influence of individual antigenic differences in the tissues of the mouse tumor host, since all three tumors developed on one body.

We were also interested in finding additional proof that cancer antigens, specific only to tumors, are present in tumorous tissues as well as the normal antigens. We first tried the ordinary complement fixation reaction for this purpose, without the use of specific absorption. Therefore, in the second series of experiments, we used immune serums to each of the tumors of the 20th passage, with immune serums against normal mouse liver and spleen as the control. Table 2 is a report from a typical experiment with the complement fixation reaction.

Table 2 shows that all the antitumor serums reacted in approximately the same titers with their own antigens and with the antigens from the two other tumors, although the titers were somewhat higher in the reaction with their own antigens. The serum against the normal liver (No. 2991), however, reacted considerably more strongly with its own antigen than with the antigens from the tumors.

The same can be said of the antitumor serums in relation to the normal liver antigen, with which they did not react as strongly as with the tumor antigens. The serum against the mouse spleen reacted with both the tumor and spleen antigens in approximately the same titers, which was also true of the antitumor serums in relation to the antigen from the mouse spleen.

Therefore, the ordinary form of the complement fixation reaction showed that the antigens of these three tumors were very similar, that the antigenic properties of the three tumors differed from those of the normal liver and resembled those of the normal spleen.

In order to establish whether there actually were antigenic differences between the tumors and in order to differentiate the antigenic properties of the tumors from those of the normal spleen, we resorted to the method using preliminary specific absorption of antitumor serums. We used both formalized and native tissues from the tumors to absorb the serums. No material difference was found between absorption by the native tumors and that by the formalized material, although the serums were anticomplementary in some cases, when the serums had been insufficiently centrifuged after absorption by native tissues.

TABLE 1

Comparative Study of the Antigenic Properties of Tissues From the Three Transplanted Tumors (Ehrlich's Adenocarcinoma, Kroker's Sarcoma and Acridine Sarcoma) Passed 1-20 Times

Dilution of serums	Antitumor serums against:											
	Ehrlich's adenocarcinoma				Kroker's sarcoma				Acridine sarcoma			
	1st passage, No. 1353		20th passage, No. 1393		1st. passage, No. 1692		20th passage, No. 3243		1st passage, No. 1094		20th passage, No. 343	
	Antigens obtained from the passed tumors											
	from Ehrlich's adenocarcinoma				from Kroker's sarcoma				from acridine sarcoma			
	1st passage	20th passage	1st passage	20th passage	1st passage	20th passage	1st passage	20th passage	1st passage	20th passage	1st passage	20th passage
1:50	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
1:100	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
1:200	++++	++++	++++	++++	++++	+++	++++	++++	++++	++++	++++	++++
1:400	++++	+++	+++	+++	+++	+++	+++	++++	+++	++	+++	+++
1:800	+++	+++	+++	+++	+++	++	+++	++	+++	++	+++	+++
1:1 600	++	++	++	+	++	+	++	++	++	+	++	++
1:3 200	+	+	+	+−	+−	+−	+	+	+	+−	+	+
1:6 400	−	−	+−	−	−	−	+−	−	−	−	−	+−

Note: All necessary controls were observed in the complement fixation reaction.

TABLE 2

Comparative Study of the Antigenic Properties of Tumorous and Normal Tissues, Using The Complement Fixation Reaction

Anti tumor serum	Dilutions	Antigens: Ehrlich's adeno-carcinoma	Kroker's sarcome	Acridine sarcoma	Mouse liver	Mouse spleen	Serum controls
No. 198, against Ehrlich's adeno-carcinoma	1:50	++++	++++	++++	+++	++++	—
	1:100	++++	++++	++++	++	++++	—
	1:200	++++	+++	+++	+	+++	—
	1:400	+++	+++	++	—	++	—
	1:800	++	+	+	—	+	—
	1:1 600	+	—	+—	—	—	—
No. 3215, against Kroker's sarcoma	1:50	++++	++++	++++	+++	++++	—
	1:100	++++	++++	++++	++	+++	—
	1:200	++++	++++	++++	+	+++	—
	1:400	+++	+++	+++	+—	++	—
	1:800	+	+++	++	—	+—	—
	1:1 600	+—	+	+—	—	—	—
No. 810, against Acridine sarcome	1:50	++++	++++	++++	+++	++++	—
	1:100	++++	++++	++++	++	+++	—
	1:200	+++	++++	++++	+	+++	—
	1:400	++	+++	+++	—	++	—
	1:800	+	++	+++	—	+	—
	1:1 600	—	+	++	—	—	—
No. 2991, against normal mouse liver	1:50	+++	+++	+++	++++	+++	—
	1:100	++	+	++	++++	++	—
	1:200	+—	+—	+	++++	+	—
	1:400	—	—	+—	+++	+	—
	1:800	—	—	—	+++	+—	—
	1:1 600	—	—	—	+	—	—
No. 12, against normal mouse spleen	1:50	++++	++++	++++	+++	++++	—
	1:100	++++	++++	++++	++	++++	—
	1:200	+++	+++	+++	+	++++	—
	1:400	++	++	+++	+—	+++	—
	1:800	+	+—	++	—	++	—
	1:1 600	+—	—	+	—	++	—
Antigen controls		—	—	—	—	—	—

Table 3 shows the data obtained from the third experimental series using preliminary specific absorption with the complement fixation reaction.

As the data in Table 3 indicate , the preliminary absorption of the antitumor serums by the tumor tissues removed the incidental antibodies from them and left only the antibodies specific for the tumors against which the serums were obtained. For example, before absorption, serum No. 198 against Ehrlich's adenocarcinoma reacted in a titer of 1:400 with all three tumors, showing their antigenic similarity. Serum absorption by the

TABLE 3

Comparative Study of the Antigenic Properties of the Three Transplanted Tumors (20th Passage), Using the Specific Absorption Method in the Complement Fixation Reaction

No. of serum	Absorbed by tissue from:	Dilutions	Antigens obtained from: Ehrlich's adenocarcinoma	Kroker's sarcoma	Acridine sarcoma	Normal spleen	Serum controls
No. 198, against Ehrlich's adenocarcinoma	Before absorption	1 : 50	++++	++++	++++	++++	—
		1 : 100	++++	++++	++++	++++	—
		1 : 200	++++	++++	++++	++++	—
		1 : 400	++++	++++	+++	+++	—
	Ehrlich's adenocarcinoma	1 : 50	—	—	—	—	—
		1 : 100	—	—	—	—	—
		1 : 200	—	—	—	—	—
		1 : 400	—	—	—	—	—
	Kroker's sarcoma	1 : 50	++++	++	++	+	—
		1 : 100	++++	+	+	+—	—
		1 : 200	++	—	—	—	—
		1 : 400	+	—	—	—	—
	Acridine sarcoma	1 : 50	++++	++	++	++	—
		1 : 100	+++	+	+—	+—	—
		1 : 200	++	—	—	—	—
		1 : 400	+	—	—	—	—

Ehrlich's adenocarcinoma tissues removed all the antibodies present, and the serum gave a negative reaction with all the tumor antigens. With serum absorption by the tissues of the Kroker's and acridine sarcomas, the incidental antibodies were absorbed, leaving the antibodies specific to Ehrlich's adenocarcinoma, with the antigen from which, serum No. 198 reacted in considerably higher titers. We obtained analogous data concerning serums No. 810 (against acridine sarcoma) and No. 3215 (against Kroker's sarcoma). Their absorption by the tumor tissues made it possible to distinguish the antigenic substances of the tumors and spleen from each other. The absorbed antitumor serum reacted with the corresponding tumor antigen, but not with the antigen from the tissues of the normal spleen, thereby confirming their antigenic difference.

Therefore, the use of the preliminary specific absorption method in the complement fixation reaction clearly showed that the experimental tumors differed from each other in this relation, in spite of the mentioned antigenic community between them, as well as from the tissues of normal organs.

TABLE 3 (continued)

No. of serum	Absorbed by tissue from:	Dilutions	Antigens obtained from: Ehrlich's adeno-carci-noma	Kroker's sarcoma	Acri-dine sarcoma	Normal spleen	Serum controls
	Before absorption	1 : 50	++++	++++	++++	++++	—
		1 : 100	++++	++++	++++	++++	—
		1 : 200	++++	++++	++++	+++	—
		1 : 400	+++	++++	++++	++	—
No. 810, against acridine sarcoma	Acridine sarcoma	1 : 50	+	+	++	+—	—
		1 : 100	+—	—	+	—	—
		1 : 200	—	—	—	—	—
		1 : 400	—	—	—	—	—
	Kroker's sarcoma	1 : 50	+++	+	++++	+	—
		1 : 100	+	—	+++	—	—
		1 : 200	—	—	++	—	—
		1 : 400	—	—	+	—	—
	Ehrlich's adeno-carcinoma	1 : 50	++	++++	++++	++	—
		1 : 100	+	++	+++	+	—
		1 : 200	—	+	++	—	—
		1 : 400	—	+—	++	—	—
	Before absorption	1 : 50	++++	++++	++++	++++	—
		1 : 100	++++	++++	++++	+++	—
		1 : 200	++++	++++	++++	+++	—
		1 : 400	++++	++++	++++	++	—
	Kroker's sarcoma	1 : 50	++	+++	++	+	—
		1 : 100	+—	+	+	+—	—
		1 : 200	—	—	—	—	—
		1 : 400	—	—	—	—	—
No. 3215, against Kroker's sarcoma	Acridine sarcoma	1 : 50	++	++++	++	++	—
		1 : 100	+	+++	+—	+	—
		1 : 200	+—	++	—	—	—
		1 : 400	—	+	—	—	—
	Ehrlich's adenocar-cinoma	1 : 50	+++	++++	+++	+++	—
		1 : 100	+	++++	++	—	—
		1 : 200	—	++	—	—	—
		1 : 400	—	+	—	—	—
Antigen controls			—	—	—	—	—

Since the three tumors were passed to the same animal, we can consider that the differences disclosed in the antigenic properties of the tumor tissues are not antigenic differences in the tissues of individual animal tumor-bearers, as all three tumors developed on the same mouse and had been passed to inbred animals for periods of over a year. Moreover, the investigation of the tumors passed 20 times established the presence of a specific antigen, differing from the antigenic substances of normal tissues, as a rule, since, in the first experimental series, we found that the antigenic properties of the tumors of the 1st and 20th passages were the same.

SUMMARY

A comparative study of the antigenic properties of the 3 transplanted mice tumors was conducted. These tumors were passed for a long period of time in inbred animals, with simultaneous inoculation of all of the 3 tumors to the same mouse. The method of specific absorption of antitumor serums was used in the reaction of complement fixation.

A clear antigenic difference of the tumor tissues from the tissues of the normal organs of the mouse, the carrier of the tumor was revealed as a result of this investigation. Besides, it was demonstrated that the antigenic substances of the cancer cells are common in different tumors. An actual difference between the antigenic properties of the 3 mice transplanted tumors was, likewise, revealed.

The use of these methods allows us to show the fallacy of the opinion of certain foreign immunogeneticists on the absence of the specific immunity in tumors.

The above data not only make the presence of the specific antigenic substances of the pathologically changed cancer cell quite certain, but, likewise, the presence of the variations between the antigenic properties of the 3 different transplanted mice tumors.

LITERATURE CITED

[1] G. L Avdeev, On Certain Features of Immunity to Transplanted Tumors (in Russian), Author's Abstract of Dissertation, Moscow, 1953.

[2] V. V. Gorodilova, and L. V. Shershulskaya, in the book: Problems in the Pathogenesis and Immunology of Tumors (in Russian), Moscow, 1956, pp. 115-125.

[3] M. M. Kapichnikov, Byull. Eksptl. Biol. i Med., 1956, No. 3, pp. 63-66.*

[4] P. N. Kosyakov, V. S. Korosteleva, and N. L Kuznetsova, Byull. Eksptl. Biol. i Med., 1955, No. 9, pp. 63-65.

[5] D. M. Levina, in the book: Problems in the Pathogenesis and Immunology of Tumors (in Russian), Moscow, 1956, pp. 127-150.

[6] A. K. Saakov, in the book: Questions on the Immunology of Normal and Malignant Tissues (in Russian), Moscow, 1956, pp. 54-70.

[7] V. B. Freiman, Zhur. Mikrobiol., Epidemiol. i Immunobiol., 1950, No. 10, pp. 36-41.

[8] P. A. Gorer, Cancer res., 1947, Vol. 7, pp. 634-641.

[9] Th. S. Hauschka, Cancer res, 1952, Vol. 12, pp. 615-633.

[10] M. A. Fink, G. D. Snell, and D. Kelton, Cancer res., 1953, Vol. 13, pp. 666-671.

* Original Russian pagination. See C.B. Translation.