A COMPARATIVE STUDY OF THE ANTIGENIC STRUCTURE OF TUMOR TISSUE AND HOMOLOGOUS NORMAL TISSUE

COMMUNICATION I. AN INVESTIGATION OF THE ANTIGENIC PROPERTIES OF GUEREN'S CARCINOMA AND OF THE NORMAL UTERUS IN RATS BY MEANS OF THE COMPLEMENT FIXATION REACTION

M. S. Lomakin

From the Laboratory of Noninfectious Immunology (Head – Prof. I. N. Maiskii) of the Institute of Experimental Biology (Director – Prof. I. N. Maiskii) of the AMN SSSR, Moscow

(Received July 24, 1958. Presented by Active Member AMN SSSR N. N. Zhukov-Verezhnikov)

The study of the antigenic composition of tumor tissues is of great scientific and practical importance. A number of workers [6, 11, 17] have pointed out that the antigenic composition of carcinoma cells is very complex.

P. N. Kosyakov [6], for example, showed experimentally that human tumor cells contain the same A, B, M, N and rhesus antigens as the normal tissues of the person from whom the tumor was taken. V. K. Kozlov [5] established by means of the anaphylaxis with desensitization test that the ascitic cells of Ehrlich's mouse adenocarcinoma also contain a specific carcinoma antigen and an antigen identical with the organ-specific antigen present in normal mammary gland tissue of mice.

Other investigations have been carried out, however, which have shown that certain malignant tumors of man and animals do not contain all the antigens present in the corresponding normal tissues. Research by Weiler [18-20], for example, showed that cells of a hepatoma of a rat and of a carcinoma of the kidney in a hamster lose their organ-specific antigens during malignant transformation, thereby becoming simpler in structure. It is true that criticism of this work has appeared recently [14, 16], but, nevertheless, this problem remains unsolved.

Grabar, Seligman and Bernard [13] also report the antigenic simplification of tumor cells in relation to the leucocytes and lymphocytes of patients suffering from chronic lymphatic leukemia.

In research by Kay [15], it was observed that certain epithelial tumors of the urogenital tract in man may not contain the group antigens A and B, although they are present in the normal tissues.

Edlinger and Lacour [12] showed, by means of immunological reactions, that the mammary gland tissue of the rat contains antigens in common with epithelioma of the mammary gland, and also antigens which are not found in the tumor tissue.

In view of the contradictory nature of the findings on the problem which we have chosen, we decided to compare the antigenic composition of tumor tissue and of homologous tissue of the organ from which the tumor was derived.

EXPERIMENTAL METHOD

For the comparative study of the antigenic composition, we selected, for our experiments, tissue from a Guérin's rat carcinoma and tissue of the normal rat's uterus. This choice of tissues was made because Guérin's rat carcinoma is known to be derived from spontaneous carcinoma of the uterus of rats, and it is, therefore, homologous with this organ in its origin. The tissues were investigated immunologically by means of the complement fixation test, performed in the classical manner and also after preliminary specific adsorption of the immune sera by the method suggested by P.N. Kosyakov [7].

In our work,we were guided by the research of G.P. Airapet'yan, who succeeded, first, in producing adsorbed tumor antisera, narrow in their specificity, with a reasonably high titer of antibodies (++ at 1:200), and secondly, in showing that specific carcinoma antigens of the same species of animal differed in their antigenic composition [2].

Rabbits were immunized with tissues from metastases of a Guérin's carcinoma in the lymphatic glands and internal organs of experimental animals. The rabbits, of the chinchilla species, were immunized according to the usual scheme. Sera were taken from the immune rabbits on the 8-10th day after the last course of immunization. It should be mentioned that immunization of the rabbits, even with small doses of a saline extract of fresh tissue of normal rat's uterus, was followed by death of a large proportion of the animals. In this case,the fresh tissue of the normal rat's uterus obviously possessed high toxicity to rabbits and was capable of eliciting a fatal anaphylactic shock.

EXPERIMENTAL RESULTS

In the first series of experiments, we attempted to study the antigenic composition of Guérin's carcinoma and of normal rat's uterus by means of the complement fixation test, performed in the classical manner. For this purpose,immune sera to Guérin's carcinoma (No. 1153 and 3521) and to normal rat's uterus (No. 3266 and 3521) were used. Only sera with a high titer of immune antibodies – of the order of 1:1280 or 1:2560 – were used in the experiment. Sera with a low titer of immune antibodies were excluded from the experiment since they were completely exhausted by adsorption with the corresponding formalized antigens. In the complement fixation test,both homologous antigens and antigens from the normal organs of the rat (spleen, liver and kidneys) were used. The results of these investigations are shown in Tables 1 and 2.

TABLE 1

Complement Fixation Test on an Immune Serum to Guérin's Carcinoma with Homologous Antigens from Tissue of the Normal Uterus, Kidney Spleen and Liver of the Rat

Antigens	Dilution of serum							
	1:20	1:40	1:80	1:160	1:320	1:640	1:280	1:2560
From Guérin's carcinoma	++++	++++	++++	++++	++++	++++	++++	++++
" normal uterus	++++	++++	++++	++++	++++	++++	++++	++
" kidney	++++	++++	++++	++++	++++	++++	++++	++
" spleen	++++	++++	++++	++++	++++	+++	++(+)	h
" liver	++++	++++	++++	++++	+++	++	h	h
Serum control	++	+	h	h	h	h	h	h

Legend: ++++ or +++ greatest degree of fixation of immune sera with antigens; ++ smaller degree of fixation, + very slight fixation; – no positive reaction, h – total hemolysis

As may be seen from the results in Table 1, the classical complement fixation test revealed no antigenic differences or antigenic similarity between Guérin's carcinoma and normal rat's uterus. The serum of a rabbit, immune to Guérin's carcinoma, reacted at high titer with homologous antigen and at approximately the same titers with antigens from the tissue of the normal uterus, kidney and spleen. A similar picture was observed also in relation to the complement fixation test on the serum of a rabbit, immune to normal rat's uterus, with homologus antigen and with antigens from Guerin's carcinoma and the normal organs of the rat (see Table 2). In both the first and second case, a comparatively slight fixation of the immune sera was observed with antigens from the liver tissue of the rat, and,moreover, there was slightly less fixation of the serum of a rabbit immune to normal rat's uterus with this antigen (+++ at 1:160). From an antigenic point of view, the liver obviously differed more than the other organs from the tumor tissue and the tissue of the normal rat's uterus.

TABLE 2

Complement Fixation Test on an Immune Serum to Normal Rat's Uterus with Homologous Antigen and Antigens from Guérin's Carcinoma, and from Kidney, Spleen and Liver of the Rat

Antigens	Dilution of serum							
	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1 2560
From normal uterus	++++	++++	++++	++++	++++	++++	++++	++
" Guérin's carcinoma	++++	++++	++++	++++	++++	++++	++++	++
" kidney	++++	++++	++++	++++	++++	++++	+++	+ (+)
" spleen	++++	++++	++++	++++	++++	++++	+++	+
" liver	++++	++++	++++	+++	h	h	h	h
Serum control	+++	+++	++	+	h	h	h	h

Legend: ++++ or +++ greatest degree of fixation of immune sera with antigens; ++ smaller degree of fixation, + very slight fixation; – no positive reaction, h – total hemolysis.

The ordinary complement fixation test thus revealed neither antigenic similarity nor antigenic differences between the tumor tissue and the tissue of the normal rat's uterus.

The experiment, performed in this way, failed to show the presence in the tumor tissue of a specific carcinoma antigen, the existence of which has been demonstrated by many workers [1-5, 7-10].

In order to find out whether there was, in fact, any antigenic similarity or antigenic difference between Guérin's carcinoma and normal rat's uterus, we decided to use the method of preliminary specific adsorption of immune sera with the corresponding formalized tissues. Adsorption of the immune sera was carried out, on the one hand, on a mixture of carefully ground up tissues of the tumor, spleen and kidney and, on the other hand, on a mixture of ground up tissues of normal uterus, spleen and kidney. The spleen and kidney of the rat were included in the experiment because they have a great antigenic resemblance to the tissues tested, but at the same time do not contain the organ-specific antigens present in the tissue of the normal rat's uterus.

In Table 3 are shown the results of one experiment of this second series.

The serum of a rabbit, immune to Guérin's carcinoma, after adsorption on a mixture of formalized tissues of the tumor, spleen and kidney, now contained no antibodies to either the homologous tumor or the tissue of the normal rat's uterus. In this case, therefore, these particular adsorbents had completely extracted the antibodies from the immune serum. The serum of a rabbit, immune to Guérin's carcinoma, after adsorption on a mixture of formalized tissues of the normal uterus, spleen and kidney of the rat continued to react at a comparatively high titer (++ at 1:160) with only the homologous, i.e.,the tumor antigen. By its antigenic properties, Guérin's carcinoma thus differed from both the tissue of the normal uterus and the tissues of the spleen and kidney. If this difference had not been present, the normal tissue of the homologous organ and the tissues of the other normal organs would have adsorbed all the immune antibodies, but this did not occur during the experiment.

The serum of a rabbit, immune to normal rat's uterus, after adsorption on a mixture of formalized tissues of the normal uterus, kidney and spleen of the rat, did not contain, in a comparatively high titer, antibodies to any one of the four antigens (tumor, uterus, kidney and spleen). The serum of a rabbit, immune to normal rat's uterus, after adsorption on a mixture of formalized tissues of the tumor, kidney and spleen, continued to react, in the complement fixation test, only with antigens from the tissue of the normal uterus (+++ at 1:80) and no fixation took place with antigens from the other tissues. The results of these tests showed that the normal uterine tissue of the rat differed in its antigenic properties from the carcinoma of the uterus and contained antigens which were not present in the tumor. If this difference had not been present, the tissue of the Guérin's carcinoma would have adsorbed all the immune antibodies contained in the serum of the rabbit, immune to normal rat's uterus.

TABLE 3

Comparative Study of the Antigenic Properties of Guérin's Carcinoma and of the Normal Uterus of the Rat by the Complement Fixation Test and the Method of Specific Adsorption

Antigens	Serum (1153), immune to Guérin's carcinoma										Serum (3266), immune to normal rat's uterus									
	Adsorbent tissues																			
	Guérin's carcinoma + spleen + kidney of the rat					Normal uterus + spleen + kidney of the rat					Guérin's carcinoma + spleen + kidney of the rat					Normal uterus + spleen + kidney of the rat				
	Dilution of sera																			
	1:20	1:40	1:80	1:160	1:320	1:20	1:40	1:80	1:160	1:320	1:20	1:40	1:80	1:160	1:320	1:20	1:40	1:80	1:160	1:320
From Guérin's carcinoma	+(+)	h	h	h	h	++++	++++	+++	++	h	++	h	h	h	h	++	h	h	h	h
From normal uterus	++	h	h	h	h	++	h	h	h	h	++++	+++	+++	±	h	++	h	h	h	h
" spleen	++	h	h	h	h	++	h	h	h	h	++	h	h	h	h	++	h	h	h	h
" kidney	++	h	h	h	h	++(+)	h	h	h	h	++	h	h	h	h	++	h	h	h	h
Serum control	+	h	h	h	h	+	h	h	h	h	+(+)	h	h	h	h	+(+)	h	h	h	h

Legend: ++++ or +++ greatest degree of fixation of immune sera with antigens; ++ smaller degree of fixation; ± very slight fixation and h – absence of positive eaction or total hemolysis.

The results of our experiments showed that the tissue of a carcinoma of the uterus (Guérin's strain) contains an antigen which is not present in the tissues of the normal organs of the rat (uterus, kidney and spleen), but at the same time an antigen or antigens present in the normal uterine tissue of the rat are not found in the tumor. In this connection, it is postulated that carcinoma of the uterus and normal uterus of the rat differ in their antigenic properties.

SUMMARY

Antigenic differences between the uterine carcinoma (Guérin's strain) and the normal rat's uterus may be revealed by specific adsorption of immune serums. An antigen exists in the tumor tissue which is absent in the normal rat's uterus, spleen and kidney. At the same time, certain antigens which are absent in the tumor of this organ are present in the tissue of a normal uterus. In other words, these tissues differ from each other by their antigenic properties.

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