ONCOLOGY

PROLONGED HETEROGENIC TRANSPLANTATION OF MOUSE TUMORS IN RATS

COMMUNICATION II. A STUDY OF THE ANTIGENIC PROPERTIES OF CELLS OF EHRLICH'S ASCITIC MOUSE CARCINOMA DURING HETEROTRANSPLANTATION BY MEANS OF THE COMPLEMENT FIXATION REACTION

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The complement fixation reaction (CFR) is widely used in oncology for the immunological analysis of malignant tumors in man and animals. It has been shown by the CFR that tumors contain both species, organ, group and other antigens in common with normal tissues and also specific tumor antigens [2, 3, 8, 9, 10, 13, 16, 18]. The existence of the latter has also been proved by other immunological methods [5, 6].

The CFR has had only limited application to the study of the constancy of the antigenic structure of tumors during heterogenic transplantation and the investigations which have been done have yielded conflicting results [1, 7, 11, 14, 15, 17, 18]. In addition to its general biological interest, further study of this problem would allow the examination of the subject of vaccination against cancer from a new viewpoint [4].

The purpose of our work was to study, by means of the CFR, the antigenic properties of the cells of a mouse cancer undergoing prolonged heterotransplantation in rats.

EXPERIMENTAL METHOD

We employed a simple method of our own invention for transplanting an Ehrlich's ascitic mouse cancer into rats [12]. This method has definite advantages over those previously employed in the immunological analysis of tumors, since it enabled tumor cells to be obtained separately from ascitic fluid and, more important, it enabled them to be counted.

In the first place we obtained 3 types of immune serum in 15 rabbits (by Kapichnikov's method [6]): against ascitic mouse cancer cells (AMC), against mouse liver (ML) and against rat liver (RL). Only three sera (Nos. 1285, 1579 and 1082) were selected for the experiments whose titers were not less than 1:640 (i.e. they reacted + in this dilution with their own antigen).

The antigens used were saline extracts of fresh tissues (mouse cancer; heterotransplantates; mouse spleen, lung and liver; rat liver) and were prepared by the standard method. The tissue was thoroughly washed with physiological saline, ground in a mortar for 30 minutes; then to it was added physiological saline (0.85% NaCl) in a proportion of 10 ml to 1 g of tissue; the suspension was centrifuged for 15 minutes at 3000 rpm; the supernatant fluid was then aspirated with a Pasteur pipette and was used as antigen. As a preliminary measure the cancer cells were washed five times with physiological saline.

The CFR was performed in the usual manner at 37°C. As seen from Table 1, the serum used were reasonably specific.

TABLE 1

Typical Record of a CFR Experiment

Immune sera	Antigens	Dilutions of serum 1 : 20	1 : 40	1 : 80	1 : 160	1 : 320	1 : 640	1 : 1 280	Antigen Control (AC)
Against Ehrlich's ascitic mouse carcinoma (No. 1285)	Mouse cancer	++++	++++	++++	+++	++	++	+	h
	Heterotransplantate, 25th passage	++++	++++	++++	+++	++	++	+	h
	Mouse spleen	++++	+++	++	+	h	h	h	h
	Mouse lung	+++	++	+	h	h	h	h	h
	Mouse liver	+++	++	+	h	h	h	h	h
	Rat liver	++	+	h	h	h	h	h	h
Against mouse liver (No. 1579)	Mouse cancer	+++	++	+	h	h	h	h	Hemolytic system control (HSC)
	Heterotransplantate, 25th passage	+++	+	+	h	h	h	h	
	Mouse liver	++++	++++	++++	+++	++	+	h	++++
	Rat liver	+++	++	+	h	h	h	h	
Against rat liver (No. 1082)	Mouse cancer	+++	+	h	h	h	h	h	Complement control
	Heterotransplantate, 25th passage	+++	++	++	h	h	h	h	h
	Mouse liver	+++	++	+	h	h	h	h	
	Rat liver	++++	++++	++++	+++	+++	+	h	

Legend: CFR) complement fixation reaction; h) total hemolysis; + + +) complete prevention of hemolysis; + + +, + + and +) partial hemolysis of varying degree.

TABLE 2

Results of a Study of the Antigenic Properties of Cancer Cells from Heterotransplantates by Means of the CFR

Antigens	Immune rabbit sera																	
	Dilutions of sera																	
	Against Ehrlich's ascitic mouse cancer							against mouse liver						against rat liver				
	1 : 50	1 : 100	1 : 200	1 : 300	1 : 400	1 : 600		1 : 50	1 : 100	1 : 200	1:300	1:400	1:600	1 : 50	1 : 100	1 : 200	1:300	1:400	1:600
1st passage MC	++++	++++	++++	++++	+++	++		++	+	h	r	r	r	++	r	r	r	r	r
1st passage HTC	++++	++++	++++	++++	++	++		++	+	h	r	r	r	+++	+	r	r	r	r
	1 : 20	1 : 40	1 : 80	1 : 160	1 : 320	1 : 640	1:1280	1 : 20	1 : 40	1 : 80	1:160	1:320	1:640	1 : 20	1 : 40	1 : 80	1:160	1:320	1:640
25th passage MC	++++	++++	++++	+++	+++	++	+	+++	++	+	h	h	h	+++	+	h	h	h	h
25th passage HTC	++++	++++	++++	+++	++	++	+	+++	+	+	h	h	h	+++	++	h++	h	h	h
29th passage MC	++++	++++	++++	++++	++++	+++	++	+++	++	+	h	h	h	++	+	h	h	h	h
29th passage HTC	++++	++++	++++	+++	+++	++	+	++	+	h	h	h	h	+++	++	+	h	h	h
40th passage MC	++++	++++	++++	+++	+++	++	h	+++	++	h	h	h	h	++	+	h	h	h	h
40th passage HTC	++++	++++	++++	+++	++	++	h	++	+	h	h	h	h	+++	++	+	h	h	h
50th passage MC	++++	++++	++++	++++	+++	++	h	+++	++	h	h	h	h	++	h	h	h	h	h
50th passage HTC	++++	++++	++++	+++	+++	++	h	+	r	h	h	h	h	++	+	h	h	h	h
56th passage MC	++++	++++	++++	++++	+++	++	h	+++	++	h	h	h	h	++	+	h	h	h	h
56th passage HTC	+++	+++	++	++	+	r	h	+	r	h	h	h	h	++	+	h	h	h	h
61st passage MC	++++	++++	+++	+++	++	++	h	+++	++	+	h	h	h	+	h	h	h	h	h
61st passage HTC	+-+	++	+	+	r	r	h	+	r	h	h	h	h	++	+	h	h	h	h
70th passage MC	++++	+++	+++	++	++	+	h	++	+	h	h	h	h	+	r	h	h	h	h
70th passage HTC	+++	++	++	+	r	r	h	r	r	h	h	h	h	++	+	h	h	h	h

Note. MC) ordinary Ehrlich's ascitic mouse cancer; HTC) heterotransplantate; passage of mouse cancer through rats; remaining symbols as in Table 1.

Not less than 2-3 reactions were set up with each experimental antigen (heterotransplantate, passage) and in each case with 3 sera (AMC, ML, RL). Altogether 15 experiments were carried out.

Included in the investigation was material from 70 repeatedly transplanted heterogenic tumors corresponding to about one year in time. The heterotransplantates were studied selectively (after an average of 5-10 passages), and each time material from the original mouse tumor and also from mouse and rat liver was used as a control. The AMC serum was used to investigate the degree to which the original antigenic composition had been preserved in the cells of the cancer heterotransplantates; the ML serum to ascertain the degree of preservation of mouse antigens common to both mouse and liver and mouse cancer and the RL serum — the degree of acquisition of common antigens with rat liver by the cells of the cancer heterotransplantates.

All the CFR experiments included controls of antigens, complement, hemolytic system and sera.

EXPERIMENTAL RESULTS

The results of the investigation of the antigenic composition of the cells of the heterotransplanted tumor are summarized in Table 2 in which mouse cancer and its successive transplantates in rats are shown selectively.

As seen in Table 2, the mouse ascitic cancer antiserum (AMC) reacted with antigen of ordinary Ehrlich's ascitic mouse cancer in dilutions of 1:20 and 1:40 to + + + +, in dilutions of 1:80 and 1:160 — to + + + + and + + +, in dilutions of 1:320 and 1:640 — to + + + and + +, and in a dilution of 1:1280 — to + (and this only in the first CFR experiments when no fall in the titer of the antisera had yet been observed).

The AMC antiserum reacted equally intensively in the same dilution with the antigens of the 1st, 25th, 29th, 40th and 50th serial heterotransplantates. In the case of tumor antigens from the 56th, 61st and 70th passages in rats, however, the same antiserum reacted noticeably more feebly, i.e. in dilutions of 1:20 and 1:40 — to + + + and + +, in dilutions of 1:80 and 1:160 — to + + and +, and in dilution of 1:320 — to + (and this only with the 56th passage of the tumor).

It may also be seen in Table 2 that mouse liver antiserum (ML) reacted with antigen of the ordinary ascitic cancer in dilution of 1:20 to + + +, in dilution of 1:40 to + + and in dilution of 1:80 to +. ML antiserum reacted almost equally with antigens from the tumor after the 1st and 25th passages, rather less with antigens after the 29th and 40th passages and perceptibly more feebly with antigens after the 50th, 56th, 61st and 70th passages of the tumor.

The results given in Table 2 show that a positive reaction was obtained with antigens of the original tumor and RL antiserum in dilution of 1: 20 mainly to + + and +, in dilution of 1:40 to +, whereas with antigens from the tumor after the 1st, 25th, 29th, 40th, 50th, 56th, 61st and 70th passages the RL antiserum reacted in much higher titer: in dilution of 1:20 — to + + + and + +, in dilution of 1:40 — to + + and +, and sometimes (in the earlier experiments, i.e. up to the 50th generation) in dilution of 1:80 — to +.

It can be concluded from the results given in Table 2 that the antigenic composition of the cells of Ehrlich's ascitic mouse carcinoma is modified to some degree during transplantation in rats.

Of greatest interest is the "weakening" of the antigenic properties of the mouse cancer after the 40th-55th passage through rats, which corresponds in time to marked changes in the biological properties of the transplanted tumor [12].

There are, therefore, grounds for the belief that at this period the cancer cells lose some of their antigens which are common to the tumor and to mouse liver, i.e. some of the species antigens of the mouse.

With regard to the acquisition of antigens, characteristic of rat liver, by the transplanted tumor, we consider that in the first few passages this fact may be due to the simple adsorption of rat proteins on the cancer cells, although our results do not exclude the other possibility (inclusion of these proteins in the composition of the cancer cells). Further experiments are necessary to test these hypotheses.

The results obtained call for a much more profound immunological study, for they introduce fundamentally important questions of the mechanism of adaptation and variation of cancerous tissue.

SUMMARY

The antigenic properties of the cells of mice ascitic Ehrlich's carcinoma transplanted to rat's offsprings for a year was studied with the aid of the reaction of complement fixation. From the first transplantations the cells acquire low quantities of the rat's species-specific antigens, while in 40-55 reinoculations they lose a number of mice species-specific antigens. The loss of the mice antigens is associated with the decreased growth of the heterogenic strain of the tumour.

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