THE PROLONGED HETEROGENEOUS IMPLANTATION OF MOUSE TUMORS IN RATS

COMMUNICATION III. THE USE OF THE AGGLUTINATION REACTION TO STUDY THE ANTIGENIC PROPERTIES OF THE CELLS OF EHRLICH'S ASCITIC CARCINOMA OF MICE AFTER PROLONGED IMPLANTATION IN RATS

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It is only recently that the agglutination reaction has been introduced into oncology as a method of studying the immunology of malignant cells, and then only in respect to the ascitic form of tumor [1, 2, 8]. By this means it was shown that antigenic structures of a species-specific and cancer-specific nature are present on the surface of cancer cells.

The antigenic composition of heterotransplanted tumors, as has already been pointed out [7], has not been adequately studied, although research in this direction has revealed the character of the relationship between the tumor and the host animal, as well as the variation and signs of adaptation of the tumor cells of the heterotransplantates [3-5].

Our investigations were made on the prolonged transplantation of a mouse carcinoma in rats, in the course of which the tumor grew intensively and retained its ascitic character [6]. This feature enabled the agglutination reaction to be used to analyze the antigenic composition of the surface of the cancer cells of the heterotransplantates.

When studying the different generations of the tumor, we directed special attention to the possibility of modifying the species-specific antigenic structures.

EXPERIMENTAL METHOD

In performing the agglutination reaction we employed a new modification of the method, as suggested by M. M. Kapichnikov for the immunological study of the cancer cells of homotransplantates [2].

As antigens we used suspensions of cancer cells in physiological saline (0.85% NaCl in concentrations corresponding to a standard bacterial suspension of $1.5\text{-}2.0 \times 10^9$ organisms. Freshly obtained cancer cells were washed five times in physiological saline (with gradual sedimentation for $1\frac{1}{2}$-2 hours at +4° three times, and rapid sedimentation by centrifugation for 30 seconds to 1 minute at 3000 rpm twice). The material used in each experiment was heterotransplantates — cancer cells obtained from the ascitic exudate of rats — and the permanent control investigations were made on cancer cells of the original Ehrlich's mouse carcinoma (homotransplantates).

TABLE 1

Results of the Study of the Antigenic Properties of Cancer Cells by Means of the Agglutination Reaction

Suspensions of ascitic carcinoma cells			Dilutions of rabbit antisera to Ehrlich's ascitic carcinoma of mice							mouse liver				rat liver				Physiological saline
			1 : 20	1 : 40	1 : 80	1 : 160	1 : 320	1 : 640	1 : 1 280	1 : 20	1 : 40	1 : 80	1 : 160	1 : 20	1 : 40	1 : 80	1 : 160	
Expts.	Heterogenous passages in rats	1	++++	++++	++++	+++	++	+	±	++	+	±	−	++	+	±	−	−
		20	++++	+++	+++	++	+	±	−	++	+	−	−	+	+	±	−	−
		29	++++	+++	+++	+++	++	+	±	++	+	±	−	++	+	±	−	−
		50	++++	+++	++	++	+	+	±	+	±	−	−	++	+	±	±	−
		57	++	++	+	+	±	−	−	+	±	−	−	++	+	±	−	−
		62	++	++	++	+	±	−	−	±	−	−	−	++	+	±	−	−
		65	++	++	++	+	±	−	−	±	−	−	−	++	+	±	±	−
		70	++	++	+	+	±	−	−	±	−	−	−	+	+	−	−	−
Controls	Ordinary Ehrlich mouse strain		++++	++++	++++	+++	++	+	±	++	+	±	−	+	±	−	−	−
			++++	+++	+++	++	+	±	−	++	+	±	±	++	±	±	−	−
			++++	+++	+++	++	++	+	±	++	+	±	−	++	±	−	−	−
			++++	+++	+++	++	++	+	±	++	+	±	−	+	−	−	−	−
			++++	+++	+++	++	++	+	±	++	+	±	−	+	±	−	−	−
			++++	++++	+++	+++	++	+	−	++	+	±	−	+	±	−	−	−
			+++	+++	+++	++	++	+	±	++	+	−	−	+	±	±	−	−
			++++	+++	+++	++	+	±	−	+	+	−	−	+	±	−	−	−

Legend: ++++, +++, ++, + various degrees of agglutination of cells; ± agglutination feebly marked; − agglutination absent.

TABLE 2

Agglutination Experiments with Cancer Cells of the Ehrlich Mouse Strain with Controls to Evaluate Adsorption of Serum Protein

Expt. No.	Suspensions of ascitic mouse carcinoma cells	Dilutions of rabbit antisera to															
		Ehrlich's ascitic carcinoma of mice							mouse liver				rat liver				Physiological saline
		1 : 20	1 : 40	1 : 80	1 : 160	1 : 320	1 : 640	1 : 1 280	1 : 20	1 : 40	1 : 80	1 : 160	1 : 20	1 : 40	1 : 80	1 : 160	
45	Washed five times	++++	++++	+++	+++	++	±	−	++	+	±	−	+	±	−	−	−
	Kept in rat serum for 1 hour at 37°	++++	+++	+++	++	+	±	−	+	+	±	−	++	+	±	−	−
	The same	++++	++++	+++	++	+	±	−	++	+	±	−	++	+	±	−	−
	61st passage in young rats	++	++	++	+	±	−	−	+	−	−	−	++	+	±	±	−
46	Washed five times	++++	++++	+++	+++	++	+	−	++	+	±	−	+	±	−	−	−
	Kept in rat serum for 1 hour at 37°	++++	+++	+++	++	+	−	−	++	+	±	−	++	++	±	−	−
	62nd passage in young rats	++	++	++	+	±	−	−	±	−	−	−	++	++	±	−	−
47	Washed five times	++++	+++	+++	++	++	+	±	++	+	±	−	+	±	−	−	−
	Kept in rat serum for 1 hour at 37°	++++	+++	++	++	+	+	−	++	+	−	−	++	+	±	−	−
	64th passage in young rats	++	++	++	+	±	−	−	±	−	−	−	++	+	±	±	−

Legend as in Table 1.

As in the performance of the complement fixation reaction, so also in these experiments we used three types of immune sera: against cells of Erlich's mouse carcinoma, against mouse liver and against rat liver.

By means of the cancer antiserum we investigated to what degree the heterotransplantates retained the original antigenic composition of the mouse tumor, and by means of the mouse liver antiserum, the extent to which they retained the species antigens of the mouse, common to both tumor and liver. Using the rat liver antiserum, we studied the extent to which the heterotransplantates had acquired the species antigens of the rat.

The results of the agglutination reaction were read on the 2nd day, i.e., after the mixed cells and sera had been kept at +4° for 48 hours. Altogether 70 tumor passages were studied, corresponding to a heterotransplantation time of 1 year.

EXPERIMENTAL RESULTS

Some comparative results of this investigation are shown in Table 1.

As may be seen from Table 1, the antisera to the carcinoma and to mouse liver reacted with the cells of the homo- and heterotransplantates in the same titer (+ in dilutions of 1:320 and 1:64) almost to the 50th passage, but in subsequent generations they reacted appreciably weaker (+ in dilutions of 1:80 and 1:160). The heterotransplantate antigens constantly reacted more weakly (+ in dilution of 1:40) with the rat liver antiserum than did the homotransplantate antigens (+ in dilution of 1:20).

It might have been thought from these findings that, after their first transplantation in rats, the cells of the heterotransplanted Ehrlich's tumor acquired a small quantity of rat antigens, and that later on, after the 50th passage, a marked loss of certain mouse antigens was combined with this process. The idea could not be ruled out, however, that these findings could have been due to adsorption of rat protein on the surface of the cancer cells of the heterotransplantates.

In order to test this hypothesis we carried out control experiments. Cells of the original mouse tumor were washed in the usual way and kept in rat serum for 1 hour at 37°, and were then used as a third antigen in the reactions.

As may be seen from Table 2, the serological activity of these cells was essentially unchanged. It was observed simply that they reacted with rat antiserum just as did the cancer cells of the heterotransplantates (+ in a dilution of 1:40). These findings suggested that the "acquisition" of rat antigens could be accounted for by adsorption of rat protein on the surface of the cancer cells, but that the degree of adsorption was very slight, since no "weakening" of the mouse antigenic properties was observed.

Thus a true change in the antigenic composition of the surface of the mouse carcinoma cells appeared only after a long period of heterotransplantation (after 50-56 passages, i.e., in the 8th-9th month of heterotransplantation). It took the form of loss or modification of certain antigens common to both tumor and mouse liver, i.e., specific mouse antigens. It is interesting that an analogous weakening of the original species properties was also found during a study of the heterotransplantates by the complement fixation reaction method [7], and also that at the same time as the antigenic structure of the Ehrlich's tumor was changed after 40-55 generations in rats, so also were its biological properties, as shown by a weakening of its growth in mice [6].

SUMMARY

The antigenic content of Ehrlich's mouse carcinoma transplanted for more than a year on young rats was studied with the aid of cancer cell agglutination reaction (in M. M. Kapichnikov's modification). Rat species-specific antigens appear on the surface of cancer cells begining from the first generation of rats, evidently, at the expense of the rat protein adsorption. In addition to this, after 50 generations, certain mouse species-specific antigens disappear from the surface of cancer cells. Identical results are obtained in complement fixation reaction. The loss of mouse antigens is accompanied by a simultaneous decline in the growth of the tumor heterotransplants on mice.

LITERATURE CITED

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