

COMPARIATIVE STUDY OF ANTIGENS OF A TRANSPLANTABLE RAT CARCINOMA AND OF A CONTINUOUSLY CULTIVATED CELL LINE RKC OBTAINED FROM IT

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Like normal cells, malignant cells may undergo biological transformation when cultivated for long periods in vitro and give rise to transplantable lines of cells [5, 9, 12, 13]. So far little attention has been paid to the study of the antigenic properties of such tumor cells grown for long periods in monolayer cultures, and especially the problem of whether they contain specific antigen [6, 11]. As yet no attempt has been made to investigate the antigenic composition of the same transplantable tumor in vivo and in cell culture.

The object of the present investigation was to study the tissue antigens of a transplantable rat carcinoma and of a line of cells obtained from it and grown for long periods in vitro.

EXPERIMENTAL

The test objects were: 1) rat kidney carcinoma strain RA [1, 2], distinguished by its high virulence when transplanted into Wistar rats [3, 4]; 2) a transplantable cell line obtained from the tissues of this carcinoma [10]. The cells of this line, called RKC (rat kidney carcinoma) were grown on a nutrient medium of the following composition: medium No. 199 (50%) 0.5% solution of lactalbumin hydrolyzate (30%), bovine serum (20%). At the time of the investigation the RKC cells had been grown for 9-12 months, and inoculation of cells grown for 9 months in rats, led to the formation of a transplantable tumor.

The investigation was carried out by means of the anaphylaxis with desensitization reaction [7]. Saline extracts of the carcinoma and of normal kidney and spleen tissues, the tissues of two-week rat embryos, and a suspension of line RKC cells in Hanks' solution were used as antigens. All the preparations were tested for sterility and toxicity. The protein content of the extract was determined by Lowry's method. Male guinea pigs weighing 300-350 g were used in the experiments. The animals were sensitized subcutaneously with antigens from the carcinoma tissue in a dose of 2.5 mg protein or with the suspension of line RKC cells (4 million cells per guinea pig). After 25-30 days the guinea pigs were desensitized by the method described previously [8], by intracardiac injections of preparations from the normal organs in doses of between 1.0 and 5.0 mg protein. After testing for completeness of desensitization, the reacting injections were given, using preparations from carcinoma or embryonic tissues in a dose of 4.8 mg protein per guinea pig. Altogether 5 series of experiments were carried out on 128 guinea pigs, including 30 animals used as controls for testing for absence of toxicity of the injected antigens.

EXPERIMENTAL RESULTS

The experimental results are given in the table.

The results of the experiments of series I showed that the tumor tissue contained specific antigens absent from the homologous normal rat kidney tissue. In the experiments of series II the presence of spleen antigens was demonstrated in the tumor tissue. The results of the experiments of series III showed that the rat carcinoma RA tissue contains antigens identical, not only with the spleen antigens, but also with the antigens of the rat embryonic tissues and also specific antigens for the tumor tissues only. The results of the experiments of series IV showed that the cells of line RKC had lost the antigens identical

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Comparative Investigation of Antigens of Rat Carcinoma after Prolonged Transplantation in Vivo and in Vitro

Series of Expts.	Sensitization		Desensitization				Reacting injection	
	antigen	1	2 *		3 *		antigen	reaction of guinea pigs
I	From rat kidney carcinoma tissue	antigen	reaction of guinea pigs†	antigen	reaction of guinea pigs		antigen	reaction of guinea pigs
		From normal rat kidney tissues	+++ (3) ++ (10) + (6) ± (1)				From rat kidney carcinoma tissue	+++ (3) +++ (7) +++ (6) +++ (3) ± (1)
II	The same	The same	+++ (6) +++ (11) ++ (11) ± (1)	From rat spleen tissues	+++ (1) ++ (22) ± (4) - (2)		The same	+++ (3) +++ (11) +++ (13) ± (2)
III	" "	From rat spleen tissues	+++ (2) +++ (8) ++ (15) ± (1)	From rat embryonic tissues	+++ (3) ++ (18) ± (4) - (1)		" "	+++ (1) +++ (5) +++ (19) ± (1)
IV	Cells of RKC culture	From normal rat kidney tissues	+++ (3) +++ (7) ++ (6)	From rat spleen tissues	± (2) - (14)	From rat embryonic tissues	" "	+++ (9) +++ (6) ± (1)
V	The same	From rat spleen tissues	+++ (2) +++ (4) + (1)	From rat kidney carcinoma tissue	+++ (2) +++ (2) + (3)		From rat embryonic tissues	- (7)

*The injections were given after complete desensitization to the preceding antigen.

†The number of guinea pigs responding to injection of the antigen by the corresponding reaction is shown in parentheses.

Legend: - absence of anaphylactic reaction; ± manifestations of anaphylactic reactions ill defined; + transient rubbing of the nose and ears; ++ repeated scratching of the nose, sneezing, coughing; +++ the same symptoms but more sharply defined, urination, defecation, lying on the side; ++++ rapidly developing signs of shock, accompanied by convulsions and ending in death of the guinea pigs.

with those of the spleen. However, by comparison with carcinoma RA the cultivated cells were more closely related to the embryonic tissues and the specific antigens of the tumor cells were less marked.

The considerable resemblance between the antigens of RKC cells and of rat embryonic extract suggested that antigens present in rat embryos, but absent from the transplantable tumor could be present in cells of line RKC. To answer this question the experiments of series V were carried out, but their results did not support this hypothesis.

It may be concluded from the results of these experiments that cells of line RKC during prolonged cultivation lose their antigens identical with spleen antigens, and it may also be postulated that under these circumstances the specific antigenic properties of the rat carcinoma cells are weakened and their antigenic similarity to the tissues of two-week rat embryos is increased.

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