

ONCOLOGY

INFLUENCE OF HOMOLOGOUS AND IMMUNE ANTIBODIES ON GROWTH OF BROWN-PEARCE TUMOR IN RABBIT TISSUE CULTURES

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The influence of antibodies on the growth of tissue on explantation has long been the subject of attention. Pomerat [2] in his experiments showed that the serum of a rabbit, immunized by the tissue of a rat's spleen, has a most inhibitory effect on the growth of tissue cultures of Walker 319 sarcoma in rats. Interesting findings have been secured recently by Ballowitz [1] in the culturing of cells of the bone marrow of rats, guinea pigs and man in a mixture of immune serum and Louis-Locke solution. Ballowitz found that cultivation of the cells in anti-sera is accompanied by complete absence or contraction of the area of growth. The cells lost their shape, became thicker, furrowed and in them coarse granularity was seen, etc. Heterologous anti-sera caused in the cells the same, but more marked, changes as homologous ones. These differences, in the view of Ballowitz, could not be accounted for by the difference in antibody titer since some homologous sera had the same antibody titer as the heterologous ones.

We conducted our own experiments to study the influence of the homologous and immune antibodies on the growth of tumor tissue culture.

EXPERIMENTAL METHOD

In the experiment we used Brown-Pearce tumor tissue from a rabbit which was cultivated in Carrel flasks on chicken plasma and embryonic chicken extract and on 1) rabbit sera, containing different antibody titers in relation to Brown-Pearce tumor; 2) normal goat's sera; and 3) sera of goats immunized by Brown-Pearce tumor.

Before commencement of the experiment all the sera were checked for presence in them of antibodies by means of the complement-fixation reaction. The effect of the rabbit sera under test in the culture of the tumorous tissue was estimated according to the rate of growth of each sample taken in isolation. The rate of growth of the samples was determined by the ratio of the area of the growth zone to the initial area. This ratio was defined as the coefficient of growth. The area of the samples was determined by drying on millimeter graduated paper with the aid of microscopy and a PA-1 tracing apparatus, and was estimated in square millimeters. The effect of the immune and normal goat sera on the culture of the tumorous tissue was estimated by the morphological picture of the zones of growth of the explants.

EXPERIMENTAL RESULTS

Serological investigations of the tested sera. In the experiment we used sera from four rabbits: 1) the serum of a normal rabbit No. 349; 2) serum of rabbit No. 668, immune to Brown-Pearce tumor; 3) serum of rabbit No. 991 on the 27th day after inoculation with the tumor; and 4) serum of rabbit No. 994 on the 59th day after inoculation with the tumor.

The presence of antibodies in the test sera, as indicated above, was determined by the complement-

fixation reaction performed in the usual way at a temperature of 37°C. Saline extracts from the Brown-Pearce tumorous tissue of the rabbit and from the tissue of the liver of a healthy rabbit served as antigens. Table 1 shows the results of these reactions.

As is clear from Table 1 the largest antibody titer was found in the serum of rabbit No. 668, the lowest in the sera of rabbits No. 349 and No. 991. In the serum of rabbit No. 994 antibodies of the tumorous tissue and liver were in general not found. Sera of goats immune to the rabbit tumor* reacted with an antigen from the tumorous tissue of the rabbit in one case to ++ at an attenuation of the serum 1:640.

TABLE 1

Complement Fixation Reaction with Sera of Experimental Rabbits with Tumorous Tissue and Normal Rabbit Hepatic Tissue Antigens

Serum attenuation	Serum of rabbit							
	№ 349		№ 668		№ 991		№ 994	
	antigen from							
	Tumor	Liver	Tumor	Liver	Tumor	Liver	Tumor	Liver
1:10	++	++	++++	++++	++	++	gg	gg
1:20	+	++	++++	++++	++	gg	gg	gg
1:40	+	+	++++	++++	+	gg	gg	gg
1:80			++++	++++		gg	gg	gg
1:160			++	g		gg	gg	gg
1:320	gg	gg	g	g	g	g	g	g

TABLE 2

Intensity of Growth of 5-day Experimental Rabbit Cultures of the Brown-Pearce Tumor on Sera of Rabbits No. 349, No. 668, No. 991 and No. 994.

Serum of rabbit	Total No. samples	No. samples exhibiting growth	Growth coefficient	
			Mean	Maximum
№ 349	50	31	1.04	4.36
№ 668	50	8	0.077	1.27
№ 991	50	28	0.96	3.30
№ 994	50	43	2.16	7.62

In the second case, with attenuation of the serum 1:800, normal goat sera served as control in the given case. On the sera indicated we started to grow a culture of rabbit tissue of Brown-Pearce tumor. In the experiment, perfectly fresh tumorous tissue without the slightest traces of necrosis, chiefly from the metastases in the liver, was used. The tissue was prepared and inoculated in Carrel flasks with stringent aseptic technic. The rate of growth of the experimental cultures on the tested sera varied, as shown in Table 2.

As can be seen in Table 2 the most intensive growth of experimental cultures occurred on the serum of rabbit No. 994, in which no antibodies were revealed by the complement-fixation reaction. The mean growth coefficient in the experimental cultures cultured on the serum of this rabbit was 2.16; the maximum coefficient of growth, 7.62. Somewhat poorer growth occurred on the serum of rabbit No. 349.

* Provided by P. P. Filakov, coworker in Laboratory of Noninfective Immunology.

Serological reaction of the serum of this rabbit revealed the presence of antibodies associated both with the tumorous tissue antigen and with the hepatic tissue antigen. In this case the mean growth coefficient in the experimental cultures equalled 1.04; the maximum, 4.36. On the serum of rabbit No. 668 the experimental cultures showed poor growth. In the serum of this rabbit, a comparatively large quantity of antibodies was found. The mean growth coefficient in these experimental cultures equalled 0.077; maximum, 1.27. In the following experiment we studied the effect of normal and immune goat-sera on growth in the culture of Brown-Pearce tumorous tissues from rabbits. Table 3 shows the summarized findings of such cultivation. The most intensive growth of experimental cultures took place on normal goat sera. On the 15th day of cultivation growth was observed in the majority of samples. In one case 42 out of 54 samples showed growth, in another 38 out of 52. The growth of the samples was essentially of a purely epithelial nature in the form of a single membranous layer in which the cells were closely contiguous, forming a cellular complex.

TABLE 3

Intensity and Character of Growth of the Experimental Cultures from Brown-Pearce Tumor in Rabbit, Cultivated on Normal and Immune Goat Sera

Type of serum	Total No. samples	No. samples exhibiting growth on 15th day cultivation	Basic character of growth
Normal serum of one goat	54	42	Epithelial
Normal serum another goat	52	38	
Immune serum of one goat with antibody titer 1:640 at ++	62	11	Destructive
Immune serum of another goat with antibody titer 1:800 at ++	50	8	

In addition a mixed type of growth was observed where, alongside the epithelial membranes, there were clearly visible connective tissue cells of the fibroblast type. Photograph No. 1 shows the zone of growth of the culture of the tumorous tissue, growing on normal goat serum (on the 12th day of cultivation). It is true that the growth of the experimental culture in the case illustrated is weak, but this illustration was chosen, since in this culture each separate cell is comparatively clear. As is evident from Photograph No. 1, the cells in the growth zone are of an oblong shape with large bright nuclei, and are intact, without visible signs of destruction.

Photograph No. 2 shows the zone of growth of a 12-day culture of the tumorous tissue on immune goat serum with an immune antibody titer 1:800 at ++. Destruction of all the cells in the growth zone is clearly visible. The cells are of smaller dimension, round in shape, with deep granular protoplasm. In some places cell fragments, agglutination of them in small deposits, and lysis are visible. In the bulk of samples cultivated on the immune goat sera, growth was generally not demonstrated. Thus, in the first case, when the titer of the immune serum was 1:640 at ++, of 62 samples 51 showed no sign of growth whatsoever; in the second case, with a serum titer of 1:800 at ++, of 50 samples 42 explants did not grow. These experiments revealed that immune goat sera exert, on the one hand, a suppressive effect on the culture of the corresponding tumorous tissue, and on the other, where growth does occur in the cultures, it is of a destructive character. Such a strong influence of the immune goat sera on the tumorous tissue culture is apparently caused by the value of the titer and specificity of the antibodies. However, in order to confirm this hypothesis, it is necessary to perform special experiments in which the effect of the given immune goat sera on culture growth, both of the tumorous and normal rabbit tissue, is observed.

Our experiments showed also that the rate of growth of the experimental cultures depends directly on the presence of antibodies in the rabbit sera and mainly on their titer. The greater the amount of antibodies in the

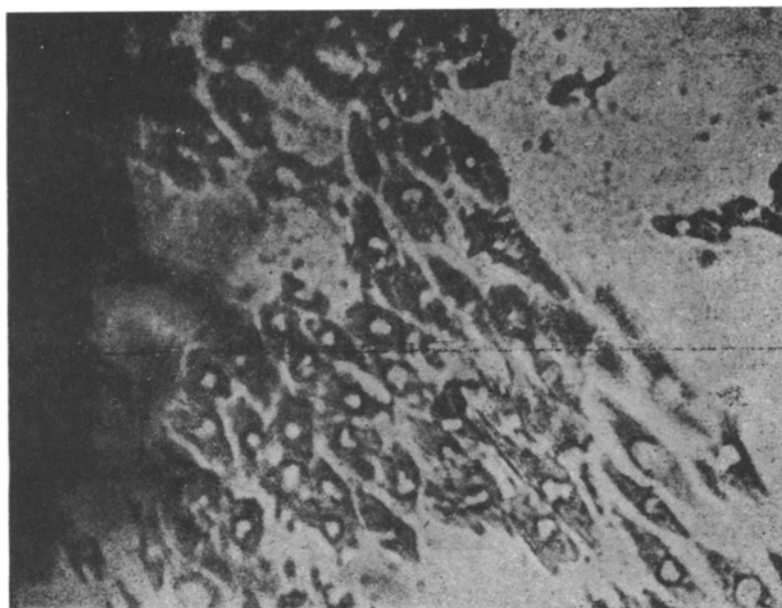


Fig. 1. Zone of growth of a 12 day culture of Brown-Pearce tumor in rabbit, cultured on normal goat serum. X 268.

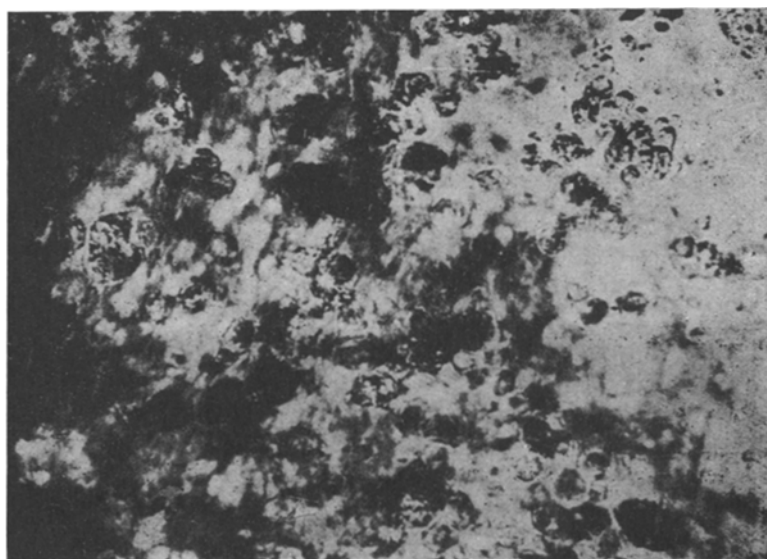


Fig. 2. Zone of growth of a 12-day culture of Brown-Pearce tumor in rabbit, cultivated on goat serum immune to this tumor. X 268

test serum, the weaker the growth of the experimental culture.

In connection with the experimental investigations carried out, it would be useful to determine which antibodies (homologous or heterologous) exert a more marked effect on the culture of normal and tumorous tissues. In addition, it would be interesting to clarify the question of the specificity of the effect of normal rabbit antibodies on the culture of an homologous tumorous tissue. In order to decide this question, it is necessary to establish a suitable form of control in which the effect of the same sera on the growth of the culture

of the normal rabbit tissue would be observed, and to conduct special investigations.

LITERATURE CITED

- [1] Ballowitz, Z. Immunitätsforsch. III, No. 2, pp. 90-109 (1954).
- [2] Pomerat, Cancer Research Vol. 5, No. 12 (1945).