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

COMMUNICATION 3. THE IMMUNOBIOLOGICAL CHARACTERISTICS OF DESOXYRIBONUCLEOPROTEINS ISOLATED FROM GUEREN'S CARCINOMA AND NORMAL RAT'S UTERUS

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In previous research [3, 4] we have shown by means of immunological reactions and the tissue culture method that saline extracts of a transplanted tumor of rats – Gueren's carcinoma – and normal rat's uterus possess different antigenic properties. It was considered of interest to find out whether differences are present in the antigenic properties of nuclear components of the cells of these tissues, and in particular of the desoxyribonucleoproteins (DNP). Several papers have now been published on this subject, but their findings are contradictory. Some writers [1, 2, 8], for instance, point out that the desoxyribonucleoprotein isolated from malignant tissue possesses a definite element of antigenic specificity which is not present in normal tissue. Other writers [12], on the other hand, consider that DNP isolated from malignant tissue has no specificity by comparison with the DNP isolated from the homologous normal tissue. Moreover, the DNP of malignant tissue is simplified in an anitgenic respect by comparison with the DNP of the corresponding normal tissue.

Defendi and Colter [9] isolated a DNP from human cancer cells with which they immunized rabbits intramuscularly. The antisera thus obtained, in the opinion of these workers, acted more intensively on the growth of homologous cells in tissue culture and less intensively on the growth of cultures of some normal human tissues.

The aim of the present research was to study the immunobiological properties of desoxyribonucleoproteins isolated from a malignant tumor and from homologous normal tissue of experimental animals.

EXPERIMENTAL METHOD

DNP was isolated from Gueren's carcinoma and from normal rat's uterus by the method of Mirsky and Pollister [11]. The DNP isolated from cancer tissue was conventionally called CDNP and that from normal tissue NDNP. The preparations isolated were identified by the following characteristics (Table 1); the content of desoxyribonucleic acid (DNA), determined spectrophotometrically [6]; the nitrogen content, determined by Kjeldahl's method (with determination of the nitrogen concentration by Nessler's micromethod); the molecular weight of the DNA forming a component of the DNP of each tissue, determined viscosimetrically [7].

TABLE 1

Chemical Characteristics of the DNP Preparations Isolated

No. of preparation	Preparation	Ratio of nitrogen to phosphorus (N/P)	Concentration (in mg/ml)		Limiting viscosity number (η) 100 ml/g	Asymmetry of molecules (a/b)	Molecular weight of DNP	Molecular weight of DNA forming component of DNP
			DNA	protein				
1 a	NDNP	3.9	0.4	0.575	17.3	179	$9.45 \cdot 10^6$	$4.0 \cdot 10^6$
1 b	CDNP	3.8	0.4	0.545	20.2	193	$10.7 \cdot 10^6$	$4.7 \cdot 10^6$
2 a	NDNP	3.6	0.2	0.236	27.0	223	$13.5 \cdot 10^6$	$6.0 \cdot 10^6$
2 b	CDNP	3.3	0.2	0.200	16.6	175	$7.67 \cdot 10^6$	$3.6 \cdot 10^6$

TABLE 2

Results of Cultivation of Gueren's Carcinoma and Tissue of Normal Rat's Uterus in Sera of Normal Rabbits and of Rabbits Immunized with NDNP and CDNP

Type of serum	Gueren's carcinoma				Normal uterus			
	number of cultures in experiment	results of cultivation			number of cultures in experiment	results of cultivation		
		intensive growth	destruction of cells in zone growth	no growth		intensive growth	destruction of cells in zone growth	no growth
On the 3rd day of explanation								
Serum of normal rabbits	126	94 / 74.6	0	32 / 25.4	125	68 / 54.0	11 / 8.8	46 / 37.2
Serum of rabbits immunized with CDNP	132	2 / 1.5	3 / 2.2	127 / 96.3	126	26 / 20.3	34 / 26.6	66 / 53.2
Serum of rabbits immunized with NDNP	127	37 / 29.1	12 / 9.4	78 / 61.5	133	24 / 18.0	40 / 30.0	68 / 52.0
On the 5th day of explantation								
Serum of normal rabbits	126	119 / 94.4	5 / 3.9	2 / 1.7	125	82 / 65.8	34 / 26.6	9 / 7.6
Serum of rabbits immunized with CDNP	129	0	7 / 5.4	122 / 94.6	126	25 / 19.8	33 / 26.1	68 / 54.1
Serum of rabbits immunized with NDNP	127	49 / 38.5	20 / 15.7	58 / 45.8	133	24 / 18.0	44 / 30.3	65 / 51.7

Numerator – number of cultures showing signs of growth; denominator – percentage of these cultures.

It will be seen from Table 1 that the DNP preparations isolated were equal in their content of protein and DNA. In preparations 2a and 2b there were differences in the viscosity and molecular weight of the DNA forming a component of the DNA. We considered, however, that it was possible to use them in the experiment, for the available evidence [10] of the connection between the biological activity of DNA and its molecular weight shows that the biological activity is preserved in polymers with a molecular weight greater than 1 million. The results obtained in our work on the study of the immunobiological properties of preparations 1a and 1b on the one hand, and 2a and 2b on the other, confirm this view, for they were found to be identical. In the principal experiment chinchilla rabbits were injected intratesticularly and intramuscularly with 10 ml of the DNP preparations 1a, 1b, 2a and 2b according to the scheme which we described previously [5].

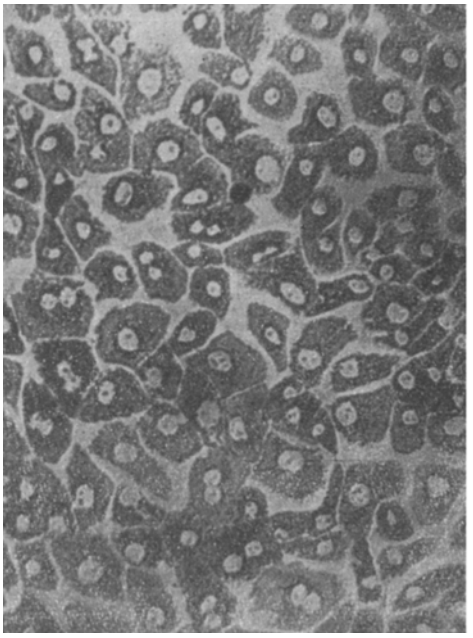

Fig. 1. Living culture of the epithelium of the uterine mucosa of a rat on the fifth day of explantation in normal rabbit serum. Dilution of serum 1:1. Magnification 268×.

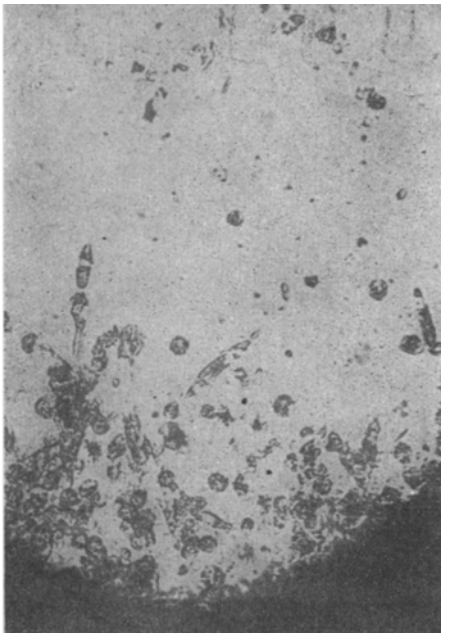

Fig. 2. Zone of growth of a 5-day culture of Gueren's carcinoma explained in the serum of a rabbit immunized with CDNP. Dilution of serum 1:1. Magnification 126×.

TABLE 3

Results of Subcutaneous Inoculation of Normal Rats with the Whole Blood of Animals with a Tumor, after Treatment of the Blood for 45 Minutes in Vitro with the Sera of Rabbits Immunized with CDNP and NDNP

Material used for treatment of blood of animals with carcinoma	Number of animals in experiment	Results of inoculation				
		number of successful inoculations	number of unsuccessful inoculations	time of appearance of tumors (in days)	time of appearance of metastases (in days)	duration of survival after inoculation of tumor (in days)
Serum of rabbits immunized with CDNP	26	4	22	18-22	37-40	47-54
Serum of rabbits immunized with NDNP	26	12	14	10-15	22-28	32-43
Serum of unimmunized rabbits	24	15	9	7-12	30-35	42-48
Saline solution	20	13	7	6-11	28-32	40-43
Blood untreated	22	17	5	13-15	26-30	36-50

The antisera obtained from these rabbits were then tested in two variants of experiments: a) in tissue culture and b) in vitro experiments on inactivation of malignant cells present in the blood of rats with a metastasizing Gueren's carcinoma.

Gueren's carcinoma and the epithelium of the uterine mucosa were cultivated by the method which we have described previously [4], with the addition to the culture of normal rabbit sera and sera of rabbits immune to NDNP and CDNP in a 1:1 dilution.

In the second variant of the experiments, blood was taken with a glass syringe from the heart of the rats with carcinoma at the moment of appearance of metastases, and placed into a sterile glass receiver of 40-50 ml capacity, and to it was added heparin solution to prevent the blood from clotting. The blood was then mixed to obtain a homogeneous mass, and this was transferred in equal volumes to sterile Wassermann tubes. To each tube containing blood in proportions of 1:1 we added: a) saline solution (1st control), b) normal rabbit serum (2nd control), c) serum of a rabbit immunized with NDNP, and d) serum of a rabbit immunized with CDNP. These substances were not added to certain tubes (3rd control). The tubes were incubated at 37° for 45-50 minutes. At the end of this time 1 ml of blood from the tubes was injected subcutaneously into young male rats (in the case of the blood to which nothing had been added, 0.5 ml was injected).

EXPERIMENTAL RESULTS

The rabbit sera were tested in tissue culture in five experiments. The combined results obtained on the third and fifth days of explantation are given in Table 2.

During the explantation of Gueren's carcinoma in normal rabbit sera not containing antibodies, of the 126 cultures on the third day of the experiment 94 (74.6%) showed growth, and on the fifth day 119 (94.4%) showed growth. In the latter case a few cultures (3.9%) had areas of cell destruction in the zone of growth, but in by far the majority of cases (94.4%) normal rabbit serum had no perceptible inhibitory action on the growth of the tumor tissue explants. A rather lower intensity of growth was observed during cultivation of epithelium of the uterine mucosa in the same sera, but in this case, for example on the fifth day of the experiment, more than half the pieces of tissue (65.8%) showed growth. The cultures of the epithelium of the uterine mucosa of the rat grew exclusively in the form of single-layered epithelial membranes consisting of polymorphic cells with one, two or even three nuclei (Fig. 1). In some cultures the zones of growth consisted of destroyed or lyzed cells (26.6%).

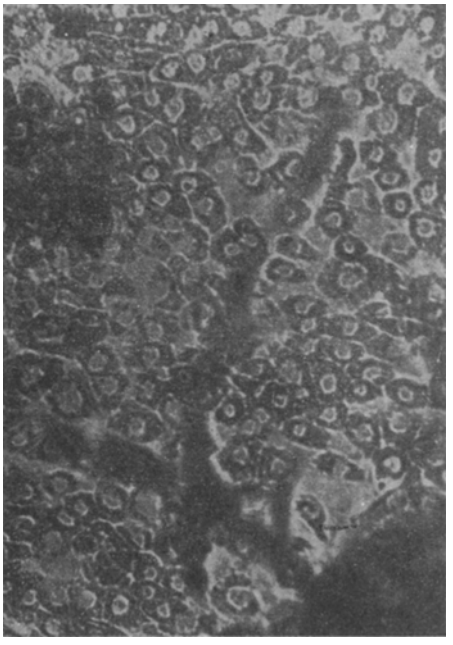

Fig. 3. Living culture of the epithelium of the uterine mucosa of a rat on the fifth day of explantation in the serum of a rabbit immunized with CDNP. Dilution of serum 1:1. Magnification 268×.

A different picture was found when tumor or normal tissues were cultivated in the sera of rabbits immunized with NDNP and CDNP. Of 132 explants of Gueren's carcinoma cultivated in the sera of rabbits immunized with CDNP, only 2 (1.5%) showed growth on the third day of the experiment, and on the fifth day of the experiment there was no culture which had a zone of growth consisting of viable tumor cells (Fig. 2).

The sera of rabbits immunized with CDNP also had an inhibiting action on the growth of explants of the normal uterus, but this action was weaker than in the first case. For instance, of 126 explants of normal uterus, on the third day of the experiment 26 pieces of tissue in culture (20.3%) showed growth, and on the fifth day 25 pieces (19.8%). One such culture of normal uterus explanted in the serum of a rabbit immunized with CDNP is shown in Fig. 3. These results show that the sera of rabbits immunized with CDNP differ in the intensity of their action on the growth of tumor tissue and of homologous normal tissue.

The action of the sera of rabbits immunized with NDNP on the experimental cultures was somewhat weaker. For instance, of 127 explants of Gueren's carcinoma cultivated in such sera, on the fifth day of the experiment 49 (38.5%) showed growth. These sera had a more intensive inhibiting action on the growth of cultures of normal uterus: of 133 explants, on the fifth day of the experiment 24, or 18%, showed growth.

It is quite probable that sera of this type should be less active serologically than the sera of rabbits immunized with CDNP. In view of the results of our experiments, however, it may be postulated that the sera of rabbits immunized with CDNP have a greater immunological affinity for the antigens contained in the DNP of tumor tissue

than those in the DNP of normal tissue. If this were not so, then the sera of rabbits immunized with CDNP would have an equal inhibiting action on the growth of both tumor tissue and normal tissue. We did not, however, observe this.

In the second variant of the experiments on the inactivation of malignant cells in the blood stream of rats with a progressively growing Gueren's carcinoma by means of the sera of rabbits immunized with CDNP and NDNP, the following picture was found (Table 3).

Altogether four such experiments were carried out, the results of which are summarized in Table 3. It will be clear from the results given that the sera of rabbits immunized with CDNP had the strongest inactivating action on the malignant cells present in the blood of the animals with carcinoma. For instance, of the 26 rats inoculated subcutaneously with the blood of animals with carcinoma, treated with these particular sera, tumors of the Gueren's carcinoma type developed in only four experimental animals, or in 15.3% of cases. The sera of rabbits immunized with NDNP were less effective in this experiment: of the 26 rats which were inoculated with the blood of rats with carcinoma, treated with these sera, tumors developed in 12 (46.1%), i.e., the incidence of development was three times greater than in the first case.

In the control animals tumors developed in 62.5-77.2% of cases. An interesting picture was observed in those rats which were inoculated with whole blood from animals with carcinoma, treated with the sera of rabbits immunized with CDNP. These rats developed at the site of inoculation a severe inflammatory reaction with the formation of large, firm nodules, resembling tumors in their external appearance. On the 8th-15th day these nodules began to decrease in size, and eventually they disappeared completely. Some nodules ulcerated and became necrotic, finally undergoing regression.

The results obtained thus show that the sera of rabbits immunized with CDNP possess comparatively more marked carcinolytic properties than the sera of rabbits immunized with NDNP. Whereas the sera of the first type completely inhibit growth of tumor tissue during explantation, the growth of the homologous normal tissue in the same conditions is inhibited far more weakly. From this fact two hypotheses may be put forward: 1) either CDNP contains antigens which are not present in NDNP, or 2) antigens present in NDNP are not found in CDNP.

The results of the second variant of experiments, namely the inactivation of malignant cells in the blood stream of animals with carcinoma, which was shown by our experiments to be possible, may be utilized as a method of testing the antitumor action of various biological antitumor preparations both in vivo and in vitro.

SUMMARY

Sera of rabbits immunized with DNP isolated from Gueren carcinoma tissue in conditions of explantation exert a more pronounced cytotoxic effect on the growth of homologous tumor tissue cultures than on that of the homologous normal tissue cultures (rat uterus). Sera of rabbits immunized with DNP isolated from the tissue of normal rat uterus excercise an identical effect on the growth of the tumor and homologous normal tissue explants. At the same time sera of rabbits of the first type were more effective with respect to inactivation of malignant cells in the blood of animals suffering from cancer than the sera of the second type, or the sera of rabbits immunized with DNP, isolated from the tissue of normal uterus. Evidently DNP, isolated from the cancer tissue, possesses some degree of specificity, but on the other hand – contains the same antigens, as DNP, isolated from normal tissue.

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