A COMPARATIVE IMMUNOCHEMICAL STUDY OF ANTISERA TO A TISSUE HOMOGENATE AND A MIXTURE OF ITS NONPROTEIN FRACTIONS

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In a preliminary communication [1] it was shown that immunization of animals (16 rabbits, 2 donkeys) with a mixture of human tissue fractions (a malignant growth of the stomach) and Freund's adjuvant leads to the formation of antibodies which are specific to these fractions (polysaccharide, lipid, DNP). It was found that the titer of antibodies against the fractions is higher and more stable following immunization with a mixture of fractions than following immunization with tissue homogenate. In the present investigation this phenomenon was examined in more detail.

EXPERIMENTAL METHOD

Experiments were conducted on 6 horses and 4 donkeys. The animals of the 1st group (5 altogether) were immunized with tissue homogenate. Tissue from a malignant tumor of the human stomach, removed at operation, was obtained directly from the surgeon and homogenized in the cold in 10 volumes of physiological saline in the presence of a 0.01 M solution of sodium citrate (as an inhibitor of depolymerases).

The cycle of immunization consisted of 6-7 subcutaneous injections at intervals of 2-3 days. Antigen was injected in increasing doses, to which was added 100,000-200,000 units of penicillin. Each donkey was injected with from 50 to 300 mg protein nitrogen per injection, and this dose was doubled for the horses.

The antigen used to immunize the animals of the second group was a mixture of fractions of a carcinoma of the human stomach with Freund's adjuvant [4, 5]. The polysaccharides were extracted from 100 g carcinoma tissue by Sevag's method [7], the nucleoproteins (DNP) by the method of Mirsky and Pollister [6], and the lipids by ether extraction. These particular fractions were practically free from soluble proteins. For immunization of each animal, a definite proportion of the tissue polysaccharide and also of the lipid were emulsified in 8 g lanolin. To the resulting mixture was added 22 ml of mineral oil, preliminarily mixed with 50 mg BCG (killed culture). Immediately before immunization, 20 ml of a solution of DNP in 1 M NaCl solution was added to the above mixture.

The animals received 4 injections of antigen (with added penicillin) at intervals of 10 days, subcutaneously in the dorsal region at two points. The animals were immunized in the immunization clinics of the N. F. Gamaleya Institute of Epidemiology and Microbiology (A. V. Ushakova). In the course of immunization, blood samples were taken from the animals of both groups for the preparation of sera, which were tested in the complement fixation reaction (CFR) with the test antigens (saline extracts of malignant and normal gastric tissue and of human spleen). At the moment of appearance of antibodies to malignant tissue in higher titer than to normal tissue, massive bleeding of the animals was performed. The blood was allowed to stand in the cold, after which the plasma was aspirated from it under sterile conditions, and γ-globulin was then obtained from the plasma by Cohn's method.

Titer of γ-Globulins of Antisera from Horses and Donkeys Immunized with Homogenate and Fractions of Tumor Tissue of the Human Stomach in the Quantitative CFR at 50% Titer

Test antigen	Tissue from which test antigens obtained	Antigen used for immunization					
		tumor homogenate		mixture of fractions of antigen with Freund's adjuvant			
		batch of γ-globulins					
		No. 9 (from donkeys)	No. 3 (from horses)	No. 12 (from horses)	No. 13 (from donkeys)	No. 14 (from horses)	No. 15 (from donkeys)
DNP	Malignant	1:400	1:40	1:25	1:400	1:1800	1:1800
	Normal	1:400	1:40	1:25	1:400	1:1800	1:1800
Polysaccharide	Malignant	1:80	1:40	1:10	1:640	1:80	1:640
	Normal	1:80	1:40	1:10	1:640	1:80	1:320
Lipid	Malignant	1:40	1:40	1:20	1:160	1:160	1:160
	Normal	1:40	1:40	1:10	1:80	1:160	1:80
Saline extract	Malignant	1:80	1:40	1:80	1:40	1:160	1:320
	Normal	1:40	1:20	1:20	1:40	1:160	1:160
Cytotoxic action on monolayer culture of carcinoma cells	HeLa	–	1:128	1:256	1:128	–	–
	HEp_2	–	–	1:128	1:128	1:128	1:128

Each batch of γ-globulin was tested for sterility and for nontoxicity towards the animals. The preparation was stored in ampules at 4°.

Batches Nos. 3 and 12 from horses and batch No. 9 from donkeys were obtained by immunization with homogenate of tumor tissue from a human stomach, and batches Nos. 14 and 15 from horses and batch No. 13 from donkeys by immunization with a mixture of fractions of tumor tissue practically free from water-soluble proteins. Altogether 65 liters of antiserum was obtained. Preparation of the γ-globulins was undertaken in the laboratory of the Moscow I. I. Mechnikov Institute of Vaccines and Sera, and 6.5 liters of the preparation was obtained.

The serological investigation of the sera of the immunized animals was undertaken by the classical and quantitative CFR [3] with the following test antigens: saline extracts, polysaccharides, lipids, and preparations of DNP. Saline extracts of malignant and normal gastric tissue and also of human spleen were obtained by homogenization of the tissue in 10 volumes of physiological saline. The centrifugate was poured into ampules, frozen and thawed three times, and then stored in the frozen state for 3 months. Polysaccharides were isolated from the tissue of the gastric carcinoma removed at operation and from normal human stomach tissue obtained at necropsy, by Sevag's method. After being purified from protein admixtures, the polysaccharides gave very feeble biuret reaction. The Molisch reaction was positive. The reducing properties of the polysaccharides from malignant and normal stomach tissues were identical. As test antigens in the CFR solutions of polysaccharides were used containing 1 mg dry substance/ml. Lipid was extracted with ether from the tumor tissue removed at operation and from the normal human stomach tissue removed at necropsy. Weighed samples (each of 15 mg) of chromatographic paper were immersed for 1 h in an ethereal solution of lipid with a concentration of 10 mg/ml, and after being soaked with the ether-soluble fraction, they were used in the CFR at 50% titer as the lipid test antigen [2].

The DNP preparations were isolated by the method of Mirsky and Pollister from freshly frozen human gastric carcinoma tissue taken at operation (malignant antigen) and also from tissues obtained after curettage of the uterus (nonmalignant antigen).

The test antigen consisted of malignant and nonmalignant tissues, adsorbed on paper, for which purpose weighed samples of Whatman's No. 1 paper (9 mg) were immersed for 20 min in a cold solution of DNP in 1 M NaCl, and then washed three times (for 5 min each time) with physiological saline.

EXPERIMENTAL RESULTS

It is clear from the results given in the table that the serological activity of the γ-globulins obtained from animals immunized with tissue homogenate was several degrees lower than from animals immunized with a mixture of fractions. This result was obtained in relation to all fractions – lipid, polysaccharide, and DNP. The differences between the serological activities of the malignant and normal human tissues were very slight. In other words, the antisera obtained were polyspecific.

The cytotoxic action of anticarcinoma γ-globulins was tested in a monolayer culture of human carcinoma cells (strains HeLa and HEp_2). The γ-globulins obtained from horses and donkeys immunized with homogenate or mixture of tissue fractions exhibited a cytotoxic action, in approximately the same titer (1:128) on the human carcinoma cells.

Preliminary clinical observations on a series of patients has shown that anticarcinoma γ-globulins of batches Nos. 13 and 15 donkeys and horses immunized with a mixture of tissue fractions free from soluble protein, when injected subcutaneously, cause a less violent cytotoxic reaction than the globulins of batches Nos. 9 and 12 obtained by immunization with tissue homogenate. Consequently, simplification of the composition of the immunizing antigen apparently leads to weakening of the reaction to injection of the γ-globulins, as shown by a less intensive general pyrexial reaction, leukocytosis, or inflammatory changes at the site of subcutaneous injection, and so on. These observations will be described in more detail in a special article.

Hence, immunization with an antigen of simplified composition yields an antiserum with a higher titer of antibodies against the individual fractions of the tissue. The cytotoxic action of the γ-globulins of the antisera in a culture of carcinoma cells was equal in intensity on both immune antigens. Pilot observations showed that injection of γ-globulins obtained by immunization with a mixture of fractions into patients is accompanied by a less marked cytotoxic reaction than injection of antibodies against a homogenate of malignant tissue.

SUMMARY

Homogenate and a number of fractions free from soluble protein (lipid, polysaccharide, DNP) were obtained from the resected tissue of a human stomach cancer. Donkeys and horses were immunized with the homogenate or a mixture of the fractions mentioned with Freund's adjuvant. Gamma-globulin preparations were prepared from the antisera; their serological activity was studied in the complement fixation reaction with the test antigens – lipid, polysaccharide, DNP, saline extract.

Immunization with the antigen of a simplified composition (fractions without soluble protein) led to the formation of antibodies to these fractions in a higher titer and with a less marked cytotoxicity than immunization with a more complicated tissue antigenic mixture.

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