IMMUNOLOGIC SIMILARITY OF RESIDUAL NUCLEAR PROTEIN ANTIGENS OF DIFFERENT HEPATOMAS

K. A. Perevoshchikova, L. V. Akhabadze, and I. B. Zbarskii*

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The writers showed previously (by immunoprecipitation of nuclei, immunodiffusion in agarose gel, and indirect immunofluorescence) the presence of a residual nuclear protein antigen, characteristic of hepatoma 27 of rats and absent in normal liver [6]. These findings agree with previous results showing the particular features of residual protein of tumor cell nuclei [3, 4] and they are of great importance for the study of the nature of tumor growth. From this point of view it is important to establish whether residual protein antigens of cell nuclei are similar in different tumors or whether they are specific relative to residual proteins in the nuclei of different normal tissues.

The aim of this investigation was to discover whether there are common antigens in the residual protein of the cell nuclei of hepatoma 27, Zajdela's ascites hepatoma, sarcoma M1, Jensen's sarcoma, and Yoshida's sarcoma of rats, and also of hepatoma 22a and Ehrlich's ascites carcinoma of mice.

EXPERIMENTAL METHOD

Hepatoma 27 and the rat sarcomas were tested in the 3rd-4th week after transplantation, Zajdela's ascites hepatoma of rats and ascites hepatoma 22a and Ehrlich's ascites carcinoma of mice on the 5th-6th day after transplantation. Cell nuclei were obtained from normal organs and Zajdela's hepatoma of rats [7] and from hepatoma 22a of mice [8] as described previously. Residual protein was obtained from the isolated nuclei by successive extraction with 0.14 M NaCl, 1.5 M NaCl, and 0.01 M NaOH [5]. Two male Chinchilla rabbits weighing about 1.5 kg were immunized. For the first immunization each rabbit was given an injection of 1 ml of a suspension containing 6 mg residual protein of hepatoma 27 and 0.5 ml of Freund's complete adjuvant (from Calbiochem, La Jolla, USA) in 0.14 M NaCl. Injections of 0.2 ml were given into the footpads of one limb and into the nictating membrane and 0.6 ml subcutaneously into the inguinal region near the lymph nodes. A further 6 mg of residual protein without Freund's adjuvant was injected in the same way 3 weeks after the first injection. Blood was taken from the auricular vein 7 days after the second immunization and, after clotting, serum was obtained for immunological tests. The serum was exhausted from antibodies against normal rat liver and serum (in some cases from antibodies against spleen and kidney antigens). Purification from antibodies against serum antigens was carried out on Sepharose 4B (from Pharmacia, Sweden), activated by cyanogen bromide by the usual method [6]. The immunodiffusion test in agarose gel was carried out with residual protein in capillary tubes as described by Preer [6, 13]. In the agglutination test, 0.1 ml of suspension containing 2×10^6 isolated nuclei in 0.25 M sucrose—3 mM $CaCl_2$ was treated with 0.02 ml of rabbit antiserum against hepatoma 27 residual protein. The appearance of floccules after 1-2 h at room temperature indicated a positive reaction. The indirect immunofluorescence test was carried out on histological sections (3-4 μ thick) of Yoshida's and Jensen's sarcomas and sarcoma M1 of rats. To prepare the sections pieces of tumor were fixed at 4°C in a mixture of acetone, phosphate buffer, and formalin embedded in paraffin wax [2, 11]. The dewaxed sections, and also films of whole cells or isolated nuclei were incubated with rabbit antiserum for 30 min at room temperature, after which unbound antibodies were washed off and the preparation incubated with goat antirabbit serum,

*Corresponding Member, Academy of Medical Sciences of the USSR.

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TABLE 1. Immunological Reactions of Tumors and Normal Tissues of Experimental Animals with Rabbit Antiserum against Residual Protein of Rat Hepatoma 27

Test object	Agglutination of isolated nuclei	Immunodiffusion in gel with residual protein	Indirect immunofluorescence with films and sections
Rat hepatoma 27	+++	+++	+++
Zajdela's rat ascites hepatoma	+++	+++	+++
Mouse ascites hepatoma 22a	+++	+++	+++
Rat sarcoma M1	—	—	—
Jensen's rat sarcoma	—	—	—
Yoshida's rat sarcoma	—	—	—
Ehrlich's mouse ascites carcinoma	—	—	—
Rat liver	—	—	—
Rat spleen	—	+	+
Rat kidney	—	+	+

Legend. +++ strongly positive reaction; + weakly positive reaction; — no reaction

conjugated with fluorescein isothiocyanate (Miles Laboratories, England) and again washed. The preparation was then mounted in a mixture of glycerol and phosphate buffer, pH 7.0 and examined on a Lyumam I-3 luminescence microscope (Lomo, Leningrad). Ascites hepatoma cells were washed three times to remove ascites fluid with 0.25 M sucrose containing 3 mM $CaCl_2$. Films were fixed in methanol at −20°C (30 min). The films were then incubated in 0.05% Nonidet P40 for 15 min at room temperature, washed in Tris-HCl buffer, pH 7.4, and treated with immune serum as described above.

EXPERIMENTAL RESULTS

All the tumors tested were divided into two groups: 1) nonhepatic tumors (Jensen's, Yoshida's, and M1 rat sarcomas and Ehrlich's mouse ascites carcinoma), 2) Zajdela's rat hepatoma and mouse hepatoma 22a.

In group 1 the residual protein gave no precipitation bands during immunodiffusion with antihepatoma serum in gel by Preer's method [13]. The precipitation test also was negative with isolated nuclei of the rat sarcomas and Ehrlich's mouse carcinoma. In the indirect immunofluorescence test [9, 15] antiserum against residual protein of hepatoma 27 likewise did not react with films of nuclei from sarcoma M1, Jensen's and Yoshida's sarcomas, and Ehrlich's ascites carcinoma. The indirect immunofluorescence test with sections of rat sarcomas also was negative.

Meanwhile, during the study of the tumors of group 2 (Zajdela's rat hepatoma and mouse hepatoma 22a) all immunological tests were positive. The residual protein of both hepatomas gave a clear precipitation band during Preer's immunodiffusion test; the band was absent; moreover, when the serum was replaced by nonimmune serum or when residual liver protein was used. Common antigens in all three hepatomas also were discovered by the agglutination test with isolated nuclei (Table 1). Rabbit serum against residual protein of hepatoma 27 gave strong immunofluorescence with goat antirabbit serum conjugated with fluorescein isothiocyanate, both with isolated nuclei (Fig. 1a, b) and with cells of both ascites hepatomas. On examination of cell films in the luminescence microscope fluorescence was discovered in both nuclei and cytoplasm. However, in the case of Zajdela's hepatoma (Fig. 1c), just as in the case of hepatoma 22a (Fig. 1d), fluorescence was brighter in the nuclei. In the control, when nonimmune serum was used, fluorescence was absent or very weak.

When immune serum against residual protein of hepatoma 27, exhausted with normal rat liver, was used the reaction was weakly positive with residual protein of the kidneys and spleen in the immunodiffusion test in gel and with sections of these organs in the indirect immunofluorescence test. The weakly positive reaction disappeared after exhaustion of the immune serum with an acetone powder of kidney or spleen, respectively.

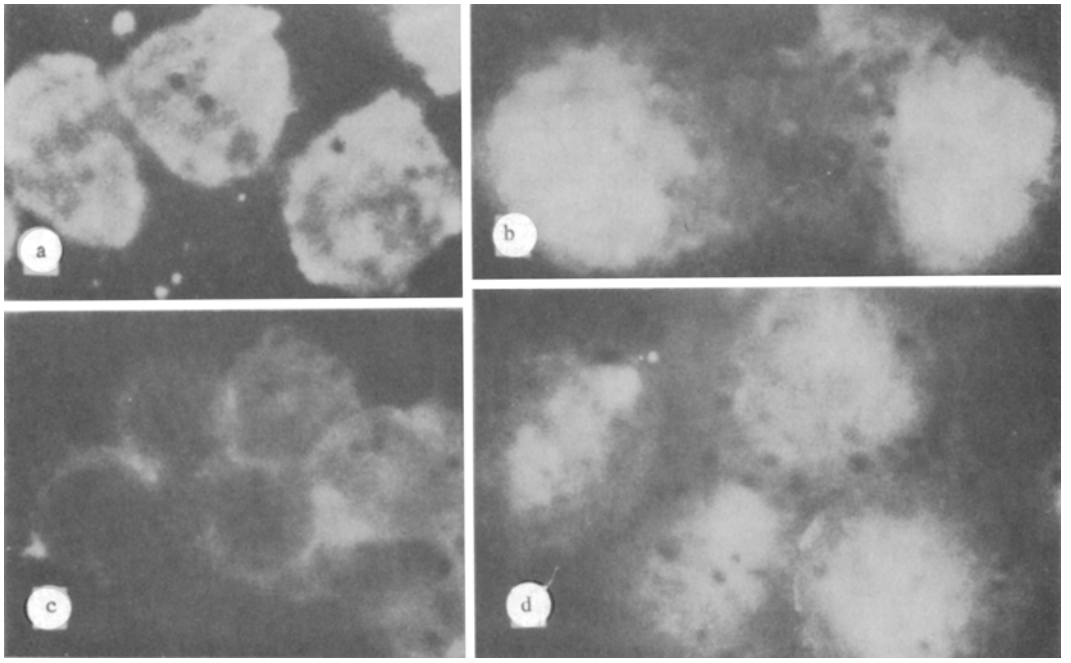

Fig. 1. Indirect immunofluorescence with immune rabbit antiserum against residual protein of rat hepatoma 27 and with goat antirabbit serum conjugated with fluorescein isothiocyanate. a) Isolated cell nuclei of Zajdela's rat hepatoma; b) isolated cell nuclei of mouse hepatoma 22a; c) whole cells of Zajdela's hepatoma; d) whole cells of hepatoma 22a. Objective 99, ocular 7.

Similarity of residual protein antigens of hepatoma 27 and Zajdela's hepatoma of rats and of mouse ascites hepatoma 22a was thus demonstrated immunologically by three methods. Meanwhile, the antigen characteristic of hepatomas is evidently absent in tumors of nonhepatic origin. At the same time, the antiserum gave a weak reaction with heterologous kidney and spleen antigens.

Comparison of the results with those in the literature on tumor immunology reveals that as a rule tumor antigens discovered were specific purely for that particular type of neoplasm and were not detected in tumors of a different type or in normal organs [12]. The complex of nonhistone proteins with DNA behaved rather differently as an antigen. Such a complex (but not nonhistone proteins or DNA separately) from Novikoff's hepatoma, according to complement fixation tests, contains a common antigen with hepatoma 30D and Walker's carcinosarcoma [14]. Rabbit antihepatoma serum, exhausted with liver chromatin, gave an immunoprecipitation reaction in agarose with hepatocellular tumors and with liver on the 4th-7th day after injection of a carcinogen (dimethylaminoazobenzene) into rats. Despite a negative reaction with the nonhistone proteins—DNA complex from liver, a weak reaction was observed with the preparation from kidney [1]. Finally, an antigen of isolated nucleoli of Novikoff's hepatoma and of human tumors has been described, which is evidently specific for different tumors but is absent in normal organs [10].

Since the residual protein antigen of hepatoma 27 exhibits common properties with other hepatocellular tumors and gives a weak reaction with the preparation from kidney, it is evidently nearest in its properties to the nonhistone proteins—DNA complex, but it is by no means identical with it.

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MOLECULAR MECHANISM OF THE COMBINED ACTION OF AMPHOTERICIN B AND CYCLOPHOSPHAMIDE ON VIRUS-INDUCED NEOPLASIA

M. A. Shneider and A. Ya. Oksman

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The effect of enhancement of the selective action of antitumor compounds by antibiotics of the polyene group [5, 7, 11] is generally regarded as the result of increased penetration of the chemotherapeutic agent into the neoplastic cell [15]. The writers showed previously that administration of the polyene antibiotic levorin to rats with Pliss lymphosarcoma in a dose not inhibiting neoplastic growth causes some degree of proteolytic degradation of the tumor chromatin proteins. Combined administration of the polyene and cyclophosphamide potentiates the antitumor action of the cytostatic. Proteolytic degradation of chromatin proteins induced by levorin is potentiated under these circumstances (administration of cyclophosphamide alone does not change the fractional composition of the tumor chromatin proteins) [4]. These observations indicate that when administered in conjunction with cytostatics, the polyene antibiotic can not only increase the permeability of the cell membranes for cytostatics but can also essentially damage the chromatin proteins.

Since experience in clinical oncology [12, 14] and experiments on animals with tumors [6, 10] have clearly demonstrated the prospects for the use of amphotericin B for potentiating the specific antitumor action of various alkylating preparations, the investigation described below was carried out to study the molecular mechanism of this phenomenon on models of virus-induced neoplasia.

EXPERIMENTAL METHOD

Experiments were carried out on 9-11-day chick embryos and 12-day-old chicks of the Russian White breed with C/O phenotype belonging to the VNIIRGZh "leukemia-free" cross line. Contamination of the birds with viruses of the avian leukemia-sarcoma group was monitored by the complement fixation test, indirect hemagglutination test, and pathomorphologically. The tumor was induced by Rous sarcoma viruses (RSV) belonging to different serological groups, using strains RSV (RAV-1), RSV (RAV-2), RSV (RAV-49), and RSV (RAV-50). The titer of viruses was $10^{-4.5}$-$10^{-6.5}OD_{50}$/0.1 ml.* The chicks were divided into four groups. In the special experimental group, 3 h after inoculation of the virus amphotericin B was given by mouth in a dose of 2 mg, dissolved in 1 ml water, and 1 h later 10 mg/kg cyclophosphamide in physiological saline was injected intraperitoneally. Chicks of the three control groups received one of the preparations, respectively: amphotericin B — group C_p, cyclophosphamide — group C_c, and

*OD: oncogenic dose.

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