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DISCOVERY OF A STABLE CELL LINE ANTIGEN IN HUMAN INTERNAL ORGANS

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A cell membrane antigen was found in stable cell lines of epithelial origin, identical to an antigen in homogenates of the human gastric mucosa. The antigen was detected by means of antiserum against extracted membrane antigens of HeP-2 cells, absorbed by a mixture of homogenates of human lung, liver, and papillomas of the human larynx and breast. The antigen described was found in some of the adenocarcinomas of the stomach that were tested. It differs from Gold's carcinoembryonic antigen and from the secretory component of IgA, it is not the structural antigen of the D-type primate oncornavirus produced by HeP-2 cells and, evidently, it is not coded by the genome of that virus.

KEY WORDS: stable human cell lines; cell membrane antigen; carcinoma of the stomach; gastric mucosa

For a number of years the writers have been engaged on research into the virion structural antigens of D-type primate oncornaviruses and the cellular antigens induced by these viruses in human tumors. In this connection the need arose for extraction of the membrane antigens of HeP-2 cells producing D-type oncornavirus (HeP-2b) [2, 6] and for the preparation of an antiserum against it.

In the present investigation, by means of the test system thus obtained, antigens of a number of stable human cell lines and of the gastric mucosa were investigated.

EXPERIMENTAL METHOD

Antigens. Solubilization and extraction of the cell membrane antigens by treatment with 3M KCl solution were described previously [8]. By this method antigens were extracted from stable human cell lines (HCL) HeP-2 (carcinoma of the larynx) and E16-b (carcinoma of the breast) [9], and also from primary human tumors: carcinomas of the kidney, tongue, stomach, three types of carcinoma of the breast, nodular mastopathy, and myoma of the uterus. The protein concentration in the preparations was determined by Lowry's method and they were concentrated with Lifogel to bring the proteins up to 1.55-1.7 mg/ml.

Homogenates also were prepared in Hanks' solution from stable HCL Fl (embryonic skin), ChET (human fibroblasts transformed by OB40 virus), ChET Rous (fibroblasts transformed by Rous virus), a suspension culture of Burkitt's lymphoma (the last three were obtained from G. I. Deichman), MB-157 and SH3 (carcinoma of the breast obtained from Texas Univeristy and Cancer Institute, Houston, USA), ChET + MPMV (human fibroblasts infected with Meson-Pfizer virus in the writers' laboratory), and also from numerous human primary tumors and normal tissues, and clarified by centrifugation at 9000 rpm.

Immune sera were obtained in rabbits by immunization into a lymph node by the method described previously [1]. Serum against the extracted membrane antigen of HeP-2 cells was obtained by the writers and designated AS against HeP-2 cell. Antiserum against twice purified and destroyed HeP-2b was provided by K. V. Il'in and designated AS against HeP-2b. It reveals the basic polypeptide of the nucleoid membrane, p27, of D-type oncornaviruses [6].

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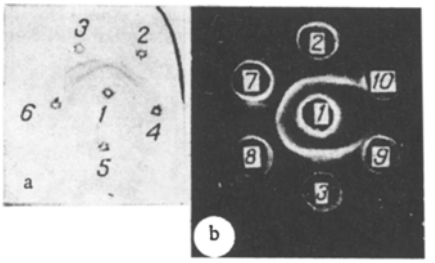

Fig. 1. Double diffusion in gel reaction of antiserum against membrane antigens of HeP-2 cells with extracts of membrane antigens and homogenates of human tumors and normal tissues: a) reaction with membrane antigens of tumor cells; b) reaction of identity of homogenates of gastric mucosa and small intestine and of extracts of membrane antigens from E16-b cells (autoradiograph). 1) AS against HeP-2 cell; 2) extract of HeP-2 cells; 3) extract of E16-b cells; 4) extract of primary carcinoma of the tongue; 5) extract of primary carcinoma of the kidney; 6) extract of primary carcinoma of the breast; 7) homogenate of embryonic small intestine; 8) homogenate of adult gastric mucosa; 9) homogenate of embryonic spleen; 10) homogenate of embryonic large intestine.

Antiserum against human IgA was obtained from the Laboratory of Labeled Sera, N. F. Gamaleya Institute of Epidemiology and Microbiology.

The method of double diffusion in gel followed by treatment of the preparations with iodinated antibodies by the method of indirect autoradiography was used [3]. Donkey globulin against rabbit globulins, labeled with ^{125}I (from the "Medradiopreparat" Factory, Ministry of Health of the USSR), was used.

EXPERIMENTAL RESULTS

AS against HeP-2 cell before absorption revealed two identical antigens for HeP-2 and E16-b by indirect autoradiography but gave an additional precipitation band with certain tumor homogenates. After exhaustion with homogenates of human lung, liver, breast, and papillomas of the larynx in children (used as the source of epithelial tissue of the same localization as HeP-2) it completely preserved its activity toward extracts of HeP-2 and E16-b cells, detecting the same two antigens in them (Fig. 1) and also a third antigen in the extract of HeP-2 cells. Identical antigens were found in homogenate of SH3 cells, but in no cell line of connective-tissue origin.

To find out whether both antisera detect the same or different antigens, AS against HeP-2 cell and HeP-2b were compared in the reaction with homogenate of HeP-2 cells. Precipitation lines formed by the different sera with the same preparation were found to intersect; consequently, the antisera revealed different antigens in the HeP-2 cells. Since HeP-2 cells produce virus whereas E16-b cells do not produce virus, do not contain virus particles, but contain an antigen partly identical to the nucleoid antigen of HeP-2b, the antigens found in the E16-b cells by AS against HeP-2 cell evidently did not belong to the group of structural virion antigens, but were cellular antigens of another kind. The first precipitation band formed by AS against HeP-2 cell with extract of membrane antigens of HeP-2 cells but not E16-b cells (Fig. 1), evidently belonged to the virion structural antigens.

One of the original objects of the investigation was to seek antigens common to the producer cells of D-type oncornavirus and carcinoma of the breast, in view of the discovery of homology between the nucleotide sequences in the breast cancer cells and, in some cases, in antigens of the D-type viruses [4, 7, 10]. However, no antigens identical with the membrane antigens of HeP-2 cells could be found in any of the preparations from carcinoma of the breast (or indeed, in tumors in other situations, except carcinoma of the stomach) (Table 1). Carcinoma of the stomach was the exception: Homogenates of two of the four verified tumors and all four homogenates of the mucous membranes taken from areas remote from the tumor gave lines completely identical with the lines of the test system to membrane antigens of HeP-2 cells (Fig. 1). Identical antigens were found in the mucous membranes of embryonic and adult stomachs, in the mucous membrane of the embryonic small intestine but not of the adult small (but not large) intestine, and in trace amounts in the embryonic spleen (Table 1, Fig. 1).

TABLE 1. Autoradiographic Investigation of Extracts of Membrane Antigens and Homogenates of Human Tumor and Normal Tissues with AS against HeP-2 Cell

Preparation studied	Number of specimens tested	Number of specimens giving positive reaction
Tumors:		
Carcinoma of the breast, kidney, tongue, and ovary	9	0
Melanoma	1	0
Myoma of uterus	1	0
Fibroadenoma of breast, fibrocystic mastopathy	5	0
Papilloma of larynx	3	0
Carcinoma of stomach	4	2
Mucosa of stomach affected with cancer	4	4
Serous membrane of stomach	2	0
Normal tissues:		
Saliva	3	0
Mucous membrane of stomach	3	3
Mucous membrane of small intestine	3	0
Mucous membrane of large intestine	3	0
Embryonic tissues:		
Lung	3	0
Skin	3	0
Thymus	2	0
Spleen	1	1
Heart	2	0
Liver	3	0
Pancreas	2	0
Small intestine	2	2
Large intestine	2	0
Mucous membrane of stomach	3	3
Purified preparations of carcinoembryonic antigen:		
From sera of patients with carcinoma of stomach	4	0
From small intestine	2	0
From large intestine	2	0
From pancreas	2	0
Stable cell lines:		
of epithelial origin (HeP-2, FL, E16b, SH3, MB-157)	5	4
Of connective-tissue origin (ChET, ChET Rous, ChET + MPMV, Burkitt's lymphoma)	4	0

The antigens discovered differed from Gold's carcinoembryonic antigens: Comparison of test systems for purified carcinoembryonic antigen provided by É. R. Karamova [3] with the test system for membrane antigens of HeP-2 and other HCL showed absence of identity between them; none of the preparations of purified carcinoembryonic antigen likewise was active with AS against HeP-2 cell.

The antigens described are not a secretory component of IgA: Antibodies against IgA did not react with preparations of membrane antigens of HCL, but reacted clearly with saliva, whereas AS against HeP-2 cell did not react with saliva (Table 1).

The antigens discovered are interesting because they may be antigenic markers for malignant transformation of certain types of cells: Their absence or presence in human malignant tumors must evidently correlate with whether the tumor arises from the cell producing them or from a cell not producing them.

The discovery of these antigens in stable human cell lines deserves attention: All HCL synthesizing them have for a long time been maintained as an infinitely transplantable line, and as a result of prolonged culture they must have lost their differentiated cells. Since the stomach is a derivative of the entoderm, and the breast and larynx (HeP-2) are derivatives of the ectoderm, it is difficult to postulate the participation of a common stem cell in the synthesis of the common antigens.

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ANTIBODY FORMATION AGAINST ANTIGEN-RECOGNIZING RECEPTORS OF T LYMPHOCYTES IN A SYNGENEIC SYSTEM

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Antibody formation against antigen-recognizing receptors of T lymphocytes were shown to be capable of being formed in a syngeneic system. Antiserum of CBA mice receiving intravenous injections of CBA lymphocytes immune against C57BL cells specifically inhibited blast transformation of CBA T lymphocytes against C57BL cells only in a mixed culture. The same antiserum had no effect on proliferative activity of CBA T lymphocytes reacting to "foreign antigen" – i.e., DBA/2 cells. No antibodies against C57BL cells likewise were found in the antireceptor antiserum. A regulatory influence of autoantireceptor antibodies on the immune response is postulated.

KEY WORDS: antigen-recognizing receptor; antireceptor serum; blast transformation

In recent investigations [2, 3, 7, 8, 13, 14, 16] so-called antireceptor sera, which specifically inhibit the response of lymphocytes to one antigen only without affecting immunoreactivity to other antigens, have been obtained in a xenogeneic or semiallogeneic system. It has been suggested [9, 12, 15, 17, 18] that the production of autoantireceptor antibodies, which may play an essential role in the regulation of the immune response, can take place in the body in situ.

The object of the present investigation was to study the possibility of formation of antireceptor antibodies in a syngeneic system and to examine their effect on T-lymphocyte function. The aim was to obtain antibodies specifically inhibiting proliferation of T lymphocytes of mice of one strain only against cells of mice of another strain in a syngeneic system in a unidirectional mixed lymphocyte culture.

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