STUDY OF THE ANTIGEN COMMON TO CONTINUOUS EPITHELIAL CELL LINES AND HUMAN GASTRIC MUCOSA

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UDC 612.32.017.1

An antigen common to continuous human epithelial cell lines (CHC) and gastric mucosa, described by the writers previously, was studied. The antigen was found in one other cell line (MDA-MV-231), derived from carcinoma of the human breast, not contaminated by Hela cells. The antigen described was found in exophytically growing adenocarcinomas of the stomach and in the mucosa of a stomach affected by cancer at a distance of 10-12 cm from the site of the lesion. The antigen was not found in endophytically growing carcinomas of the stomach or in areas close to a gastric ulcer. The antigen is not a glycoprotein, for glycoprotein fractions obtained with the aid of 1.2 M perchloric acid from homogenate of normal gastric mucosa and extract E16b were inactive in the immunodiffusion test with a sensitive serum. The antigen described has the electrophoretic mobility of α_2-β_1-globulin. The antigen is interesting because its presence or absence may perhaps help to establish the initial type of cell from which a gastric carcinoma developed and to identify more precisely the histological form of a gastric carcinoma.

KEY WORDS: antigen of continuous cell line, carcinoma of the stomach, gastric mucosa.

The writers previously [3] obtained an antigen common to a continuous human cell line (CHC) of epithelial origin and the human gastric mucosa. The antigen is interesting for it has been found also in certain adenocarcinomas of the stomach and its discovery may prove useful in identifying the histogenesis of certain tumors of the stomach.

Since two of the positively reacting CHC obtained previously, namely HEp-2 and FL, were evidently contaminated by HeLa cells [5,10] and could essentially represent a single CHC, it was decided to test other CHC of epithelial origin for the presence of this antigen, and also to study some of its biochemical characteristics.

EXPERIMENTAL METHOD

Besides the CHC mentioned in the first communication [3], namely HEp-2, E16b, SH3 (epithelial) and Rous ChET and ChET + MRMU (connective-tissue), the MDA-MB-330 CHC isolated by Gailleau [8] from cells of pleural effusions in patients with carcinoma of the breast, and provided in accordance with the program of the Soviet-American agreement, and also CHC 2H-60, derived from a hypernephroma of the human kidney in the Laboratory of Etiology of Tumors, N. F. Gamaleya Institute of Epidemiology and Microbiology, and generously provided by V. I. Chizhevskaya, also were used.

The method of double diffusion in gel followed by treatment of the preparations with iodinated antibodies by the method of indirect autoradiography was used [7].

Extracts of cell membrane antigens obtained by the method described previously [3], homogenates of cell residues after freezing and thawing 5 times, and homogenates from primary tumors and normal human tissues prepared in Hanks's solution and clarified by centrifugation at 600g were used in the immunodiffusion tests. To identify the localization of the antigen, E16b and SH3 cell residues obtained by centrifugation at 200g were broken up in a Potter's homogenizer in RSB buffer, pH 7.2, and the nuclei and cytoplasmic membranes were separated by differential centrifugation, the nuclei were destroyed in an MSE ultrasonic disintegrator,

Laboratory of Sarcoma-Leukemia Viruses and Laboratory of Clinical Biochemistry, Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician G. V. Vygodchikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 86, No. 12, pp. 744-747, December, 1978. Original article submitted March 3, 1978.

 0007-4888/78/8612-1678$07.50

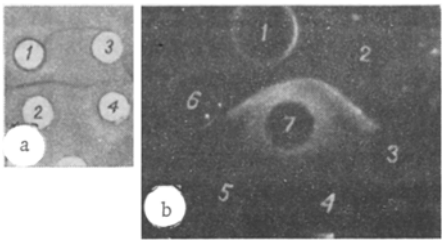

Fig. 1

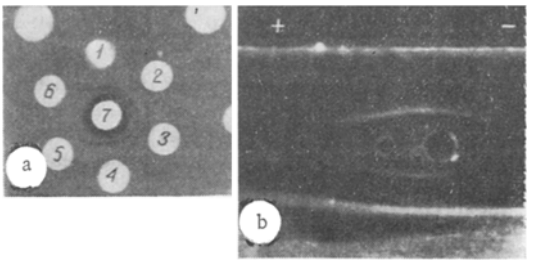

Fig. 2

Fig. 1a. Determination of identity of sera 1 and 2: 1) serum 1; 3) serum 2; 2 and 4) continuous cell line antigen.

Fig. 1b. Precipitation reaction of homogenates of CHC with test system for "continuous cell line antigen." 1) Continuous cell line antigen; 2) homogenate of MDA-MB-231 CHC; 3) homogenate of MDA-MB-330 CHC; 4, 5) physiological saline; 6) homogenate of 2H-60 CHC; 7) serum 2.

Fig. 2a. Precipitation reaction of serum 2 with homogenates from normal stomach and from tissues of human gastric carcinoma. 1, 4) "Continuous cell line antigen; 2) homogenate of gastric carcinoma (exophytic); 3) homogenate of gastric mucosa of patients with gastric ulcer, taken at a distance of 10-12 cm from site of lesion; 5) homogenate of gastric mucosa of patients with gastric ulcer, taken 1-2 cm from site of lesion; 6) homogenate of gastric carcinoma (endophytic), 7) serum 2.

Fig. 2b. Determination of electrophoretic mobility of "continuous cell line antigen." Well contains continuous cell line antigen; top gutter – serum 2 (exhausted), bottom gutter – serum 2 (not exhausted).

and the two fractions – nuclear and cytoplasmic – were concentrated in a current of air and used in the microprecipitation test in gel, with subsequent treatment with iodinated antibodies [7].

Both antiserum against extracted membrane antigens of HEP-2 cells (AS against HEP-2, or antiserum 1) [3] and rabbit antiserum against the washed off precipitate [1] were used in the immunodiffusion test. The precipitate was obtained in immunodiffusion between homogenate of normal human gastric mucosa and antiserum 1.

To study the biochemical characteristics of this antigen the glycoprotein fraction was isolated with the aid of 1.2 M perchloric acid [2] from homogenates of the gastric mucosa and E16b extracts.

AS against HEP-2 was tested in the immunodiffusion test with seven strains of mycoplasmas that are the most frequent contaminants of cell lines, and which were obtained from the Laboratory of Mycoplasmas, N. F. Gamaleya Institute of Epidemiology and Microbiology.

The electrophoretic mobility of the antigen discovered was determined by immunoelectrophoresis in 1% agar on veronal-medinal buffer, pH 8.6, with an ionic strength of 0.05 [6]. Electrophoresis was carried out for 2 h with a current of 10 mA to the frame and with a voltage of 140 V.

EXPERIMENTAL RESULTS

The newly obtained serum 2, if applied to the precipitation band after removal of antibodies against species-specific and isoantigens by appropriate absorption, revealed the same antigen as serum 1. This meant that in future both these antisera could be used in the work (Fig. 1a).

The antigen described above could not be detected in the newly tested CHC (MDA-MB-330 and 2H-60), whereas it was found in MDA-MB-231 CHC. It formed a line of complete identity with the test system for "continuous cell line antigen" (Fig. 1b).

The nuclear fraction from E16b and SH3 cells was completely inactive, whereas the fraction containing cytoplasmic proteins reacted clearly with a visible precipitation line identical with the line of the test system.

Homogenates of three of the six gastric tumors and of five of the samples of gastric mucosa taken from the same patients with carcinoma of the stomach at a distance of about 10-12 cm from the site of the lesion gave a line of complete identity with the test system for "continuous cell line antigen." It was noted that the antigen was not found in endophytically growing tumors, by contrast with exophytically growing carcinomas of the stomach.

In two homogenates of gastric mucosa taken 1-2 cm from the site of a gastric ulcer the antigen was not found, but in homogenates of gastric mucosa from the same patients taken at a distance from the site of the lesion (10-12 cm away) the antigen was detected (Fig. 2a).

The antigen described is not a glycoprotein, for glycoprotein fractions from homogenates of the mucosa of the normal stomach and from E16b extracts were inactive with the test serum.

Testing antiserum 1 for the possible presence of antibodies against mycoplasmas gave a negative result.

The electrophoretic mobility of the antigen which was found in the mucosa of the normal stomach and of certain carcinomas of the stomach and CHC corresponded to that of α_2-β_1-globulin (Fig. 2b).

The antigen described previously by the writers was thus found in another cell line of epithelial origin, namely MDA-MB-231, which was not contaminated with HeLa cells [9], by contrast with the epithelial lines used in the previous investigations, which were perhaps so contaminated.

Cell lines MDA-MB-330 and 2H-60 evidently do not contain this antigen.

The study of CHC antigen is interesting because its presence or absence could perhaps help to establish the initial process causing the development of carcinoma of the stomach – atrophic-hyperplastic gastritis or a proliferating polyp. Proliferation processes are known to take place in both cases, the only difference being in the character of growth of the foci of proliferation: In hyperplastic gastritis it is endophytic and in proliferating polyps it is exophytic [4]. The CHC antigen can also perhaps enable the histological forms of carcinoma of the stomach to be identified more precisely.

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