Summary

A membrane-associated antigen (or antigens) of the normal colonic mucosa was found to be lacking in carcinomas and diminished in polyps of the colon. There seems to be a correlation between the loss of this antigen and the reappearance of the carcinoembryonic antigen of gastrointestinal cancers.

Introduction

Several authors (1, 2, 5–8, 10–13, 17) have studied the fate of normal tissue antigens in human and animal cancerous cells. Generally these were cytoplasmic antigens. Little is known of membrane antigens of human cells and the changes they may undergo in cancer. We report here the loss of a cell membrane-associated antigen of the normal colonic mucosa. The loss is generally total in the colonic carcinomas; it may be partial in the histologically normal pericancerous areas and in benign polyps of the colon. The localization of this deletion was compared to the distribution pattern of the CEA

Material and Methods

Antiserum. We used several antisera against normal colonic mucosa prepared with phosphate-buffered 0.85% NaCl solution or butanolic extracts of normal colonic mucosa. Antiserum were obtained by foot pad injections of extracts emulsified with complete Freund’s adjuvant and followed by successive s.c. injections of alumina-adsorbed extract. Antiserum against human fetal intestine was obtained according to the same scheme. Antiserum against CEA was prepared with extracts of cultured tumors (3). All antisera were absorbed with normal human plasma and O and AB red cells stromata.

Organ. We studied 10 colonic adenocarcinomas and 10 colonic polyps, most of them having a partially or totally adenomatous structure; samples of normal colonic mucosa obtained from the pericancerous area or from noncancerous specimens removed because of sigmoiditis or ulcerous colitis; 3 fetal intestines, taken from fetuses expelled after at least 3 months of gestation; and colonic tumors maintained in organ culture, obtained from Professor Wolff’s laboratory (18).

Methods. Small pieces of the organs were snap frozen and cut in a cryostat. Sections were fixed in cold ethanol for 20 min and then air dried and rehydrated. Some pieces were fixed in ethanol at 4° for 24 to 48 hr and then embedded in paraffin, as described by Sainte-Marie (14). Indirect immunofluorescence was performed with a fluorescent goat anti-rabbit γ-globulin serum (Institut Pasteur, Paris).

Extracts of normal colonic mucosa and of surgical and cultured tumors were prepared by homogenization in phosphate-buffered 0.85% NaCl solution, centrifugation at 20,000 × g for 30 min, and lyophilization. The concentration of proteins was measured by the biuret method after trichloroacetic acid precipitation. For absorption studies, the lyophilized powder was added to the antiserum. The mixture was put under gentle agitation for 1 hr at 37° and then stored overnight at 4°. It was then centrifuged at 20,000 × g for 30 min.

Results

Anti-NCM applied on sections of normal colonic mucosa gives 2 generally associated images (Fig. 1): (a) an intense fluorescence of the cytoplasm and of the intraglandular deposits, the latter often having the same localization as the mucus; and (b) a fluorescence apparently located on the plasmic membranes of glandular mucosa cells. The membrane fluorescence is bright on frozen sections but weaker on sections prepared according to Sainte-Marie’s technique.

In colonic adenocarcinomas, the membrane fluorescence is absent from all, or almost all, of the glands (Fig. 2). The cytoplasmic fluorescence is diminished but in a variable manner.

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1 The abbreviations used are: CEA, carcinoembryonic antigen of gastrointestinal tumors; anti-NCM, immune serum against normal colonic mucosa; CMA, colonic membrane antigen.
Our study shows that in man it is possible to demonstrate immunologically an alteration of the membrane of cancerous cells.

The CMA that we describe here is different from the specific gastrointestinal antigen found by Nairn et al. (11) to be present in normal digestive mucosa and diminished or absent in gastrointestinal cancers. CMA is present on the cell membranes and only in the colonic mucosa. On the contrary, Nairn's antigen is cytoplasmic and is found in the mucosa of stomach, small intestine, and colon. The reason for having found 2 different antigens lies perhaps in the preparation of the antiserum. Our antiserum were prepared with the whole extract of normal colonic mucosa. Nairn used a semipurified microsomal fraction to immunize rabbits.

There seems to be an alternate synthesis of CMA and CEA in adults. In the fetal colon, however, one finds CEA and some CMA. After birth, CEA synthesis is stopped, and the amount of CMA increases. In adult colons, one of the antigens is always present, CMA in normal and CEA in cancerous and adenomatous glands. As the loss of the CMA (at least as judged by immunofluorescence) is sometimes more diffuse than the reappearance of CEA, it is likely that the cessation of CMA precedes the derepression of CEA synthesis. It is remarkable that the localization of both antigens in tissue sections is not the same. CEA is found mainly on the membrane bordering the glandular lumen, and CMA is found on the intercellular membranes, possibly due to a change of the cell polarity during its differentiation.

The nature of the CMA is still not determined. It was found to be sensitive to Pronase in one experiment; thus at least part of the antigen is protein. Our findings may be compared with recent results that showed the loss of a protein at the surface of cultured tumor cells, transformed cells being strongly agglutinable by concanavalin A while normal cells were not (9). After trypsin treatment, however, normal cells have the same titer in agglutination tests as the tumor cells.

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Loss of a Membrane Antigen in Human Cancer
Loss of a Normal Colonic Membrane Antigen in Human Cancers of the Colon

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