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Mucus-Associated Gastrointestinal Antigens in Transitional Mucosa Adjacent to Human Colonic Adenocarcinomas: their 'Fetal-Type' Association*

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Abstract—*Immunofluorescence studies showed that human gastric M1 antigens and intestinal M3 antigen are associated respectively with the mucus cells of surface gastric epithelium and with the intestinal goblet cells of the normal adult gastrointestinal tract. In the fetus and new born, it was possible to demonstrate by use of double labeling (fluorescein and rhodamine) that some goblet cells of colonic glands contain both the M1 and M3 antigens. This 'fetal-type' association of M antigens, which could not be found in the normal adult colonic mucosa, was observed in some goblet cells of the transitional mucosa adjacent to human colonic adenocarcinomas. Probably it represents the reappearance of a fetal-type pattern associated with the tumors.*

INTRODUCTION

TRANSITIONAL mucosa adjacent to human colonic adenocarcinomas shows histologic [1], histochemical [2] as well as quantitative antigenic [3–5] modifications. Such a mucosa is usually thicker owing to elongation of the crypts which are dilated, often branched, and lined by tall goblet cells [1] showing abnormal histochemical pattern of their mucosecretory activities [2]. This observation prompted us to study the abnormal mucus secretion using a different technique such as immunofluorescence. In a recent work, we have obtained antisera against gastric (M1 antigens) and colonic (M3 antigen) muco-proteins. We have demonstrated that the M1 antigens are associated with mucus cells of gastric surface epithelium and M3 antigen with intestinal mucus cells [6], except in the transitional and tumorous colonic mucosa, where both the M1 and M3 antigens are present. In this study, we wanted to determine whether the M1 and M3 antigens are in the same goblet cells of the transitional mucosa, whether this association could perhaps explain the abnormal pattern of the mucus secretion and if, as already suggested [2], the fetal colonic mucosa show the same modifications.

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MATERIALS AND METHODS

Tissues

Normal colonic tissues. Colonic organs were obtained from 10 human fetuses ranging from 10 to 28 weeks of gestation. One colon was obtained from autopsy of a one-day-old newborn. Three normal colonic mucosae were obtained after surgery from patients with megacolon.

Colonic adenocarcinomas. Several samples of 10 colonic adenocarcinomas having hyperplasic mucosae in the areas adjacent to the tumor (transitional mucosa) were taken no more than 1 hour after surgical ablation. The samples always included a part of transitional mucosa.

Preparation of antigenic material

The source of gastric M1 antigens was the fluid of a mucinous ovarian cyst of pure endocervical type according to Fenoglio's classification [7], because such a cyst contained only the M-antigens located on the surface gastric epithelium [8]. The intestinal M3 antigen was prepared from a pool of three normal colonic mucosae.

Ovarian or intestinal mucoprotein preparations were obtained according to the method previously described [6]. Briefly, ovarian fluid or intestinal crude extracts were homogenized in deionized water. Filtration was done successively on Sepharose 6B and 2B (Pharmacia, Uppsala, Sweden). The excluded material was collected and used for rabbit immunizations.

Immunological reagents

Antisera. Rabbit anti-M1 and M3 sera were prepared as previously described [6]. Briefly, each rabbit received 1 mg of antigenic preparation in complete Freund's adjuvant (Difco, Detroit, Michigan) in the footpads on day 1. During both the fourth and the fifth weeks, they received three booster injections either s.c. or i.v.; each injection contained 1 mg of alum-adsorbed antigenic preparation. Rabbits were bled at the end of the sixth week. Absorption of antiserum was performed as follows: each antiserum was heated to 56°C for 45 min. One volume of a panel of packed human blood red cells (CNTS, Paris, France) was added to one vol of antiserum, and the suspension was kept for 15 min at 37°C and overnight at 4°C. The absorption was repeated until no agglutination occurred. Anti-M1 and M3 sera also had to be absorbed with human plasma polymerized with glutaraldehyde [9] (5 g of polymer was used for absorbing 10 ml of antiserum). Finally, anti-M1 serum was absorbed with crude colonic mucosa extract (50 mg dry powder/ml antiserum) and anti-M3 serum with crude extract of gastric mucosa (50 mg dry powder/ml of antiserum) to remove, from each anti-M serum, the antibodies against those antigens common to gastric and colonic mucosae [10].

Conjugated antisera. Anti-rabbit IgG goat antibodies conjugated with tetramethyl rhodamine isothiocynate were obtained from Nordic (The Netherlands). From the anti-M3 serum, γ-globulin fractions were prepared by precipitation with ammonium sulfate at 40% final concentration. One ml of this crude γ-globulin fraction (containing 100 mg/ml of protein after concentration) was added to 2 ml of carbonate buffer (0.5 M, pH 9.0) containing 1.5 mg of fluorescein isothiocyanate (FITC) (Sigma, St Louis, Mo.). The solution was stirred thoroughly at 4°C for 18 hr. FITC–γ-globulin were separated from FITC by filtration on Sephadex G25 (Pharmacia, Uppsala, Sweden) and used in the direct Coons test.

Indirect and direct immunofluorescence methods

Tissues were fixed in ethanol and embedded in paraffin, according to Sainte-Marie's method [11]. Sections of 2 μm thickness were cut from the tissue blocks with an Autocut (R-JUNG, Heidelberg, FRG) and dehydrated in successive baths with xylene and ethanol. The double tracing with FITC and rhodamine-conjugated antibodies was done at room temperature by the following procedure:

(1) The sections were incubated for 1 hr with the anti-M1 serum diluted to 1/10 and were then submitted to repeated washings in phosphate-buffered-saline (PBS).

(2) The second incubation (for 1 hr) was with rabbit anti γ-globulin goat serum conjugated with rhodamine diluted two times and the non-fixed globulins were removed by washings with PBS.

(3) The third incubation (for 30 min) was with a non-immune rabbit serum diluted to 1/5, which is necessary to neutralize active sites of anti-rabbit γ-globulin goat antibody remaining free after step No. 2.

(4) The fourth incubation (for 1 hr) was with the fluoresceinated anti-M3 globulin (20 mg/ml of proteins). Repeated washings in PBS were then performed.

(5) Finally, the sections were restained with 1% hematoxylin for 1 min. This procedure, which stained the cell nuclei, did not abolish the specific staining, and reduced the fluorescent background. Furthermore, it was possible to study the same field alternatively under ordinary or u.v. illumination.

Fluorescence reaction was inhibited by incubation of antisera diluted with crude extract (250 mg/ml) containing either M1 or M3 antigens. Antisera containing the extracts were incubated for 30 min and centrifuges before the immunofluorescence test.

Microscopic observations were made with an Orthoplan Leitz microscope equipped with a Ploem-Type illuminator. Fluorescein staining was observed selectively by placing a 495-nm excitation interference filter and a 525-nm absorption filter in front of the eye piece; this removed from view the staining induced by the rhodamine. Rhodamine staining was examined with a BG 38 and 546-nm excitation interference filter and a 610-nm absorption filter placed in front of the eyepiece. Photographs of fluorescene were taken on color film (Kodak Ektachrome 200) with an Orthomat camera.

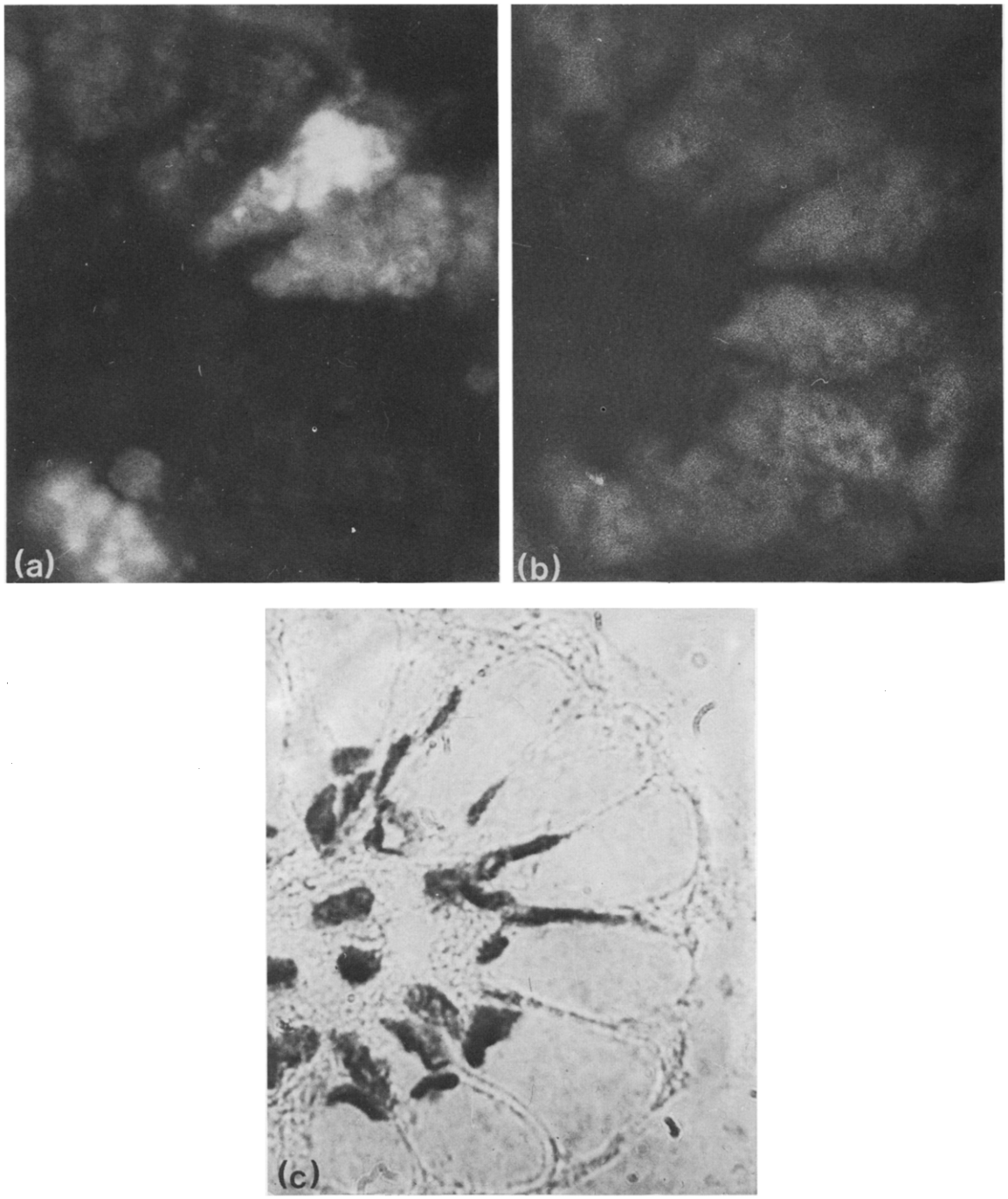


Fig. 1. Paraffin section of a fetal colonic mucosa (28-week *fetus*). *The same section was successively stained by anti-M1 serum and anti-rabbit γ-globulin goat serum labeled with rhodamine* (*a*), *by anti-M3 serum labeled with fluorescein* (*b*), *and by hematoxylin* (*c*). *With the anti-M3 serum, all fetal goblet cells were positive* (*b*); *in contrast, only some of them were stained by anti-M1 serum* (*a*). ×810.

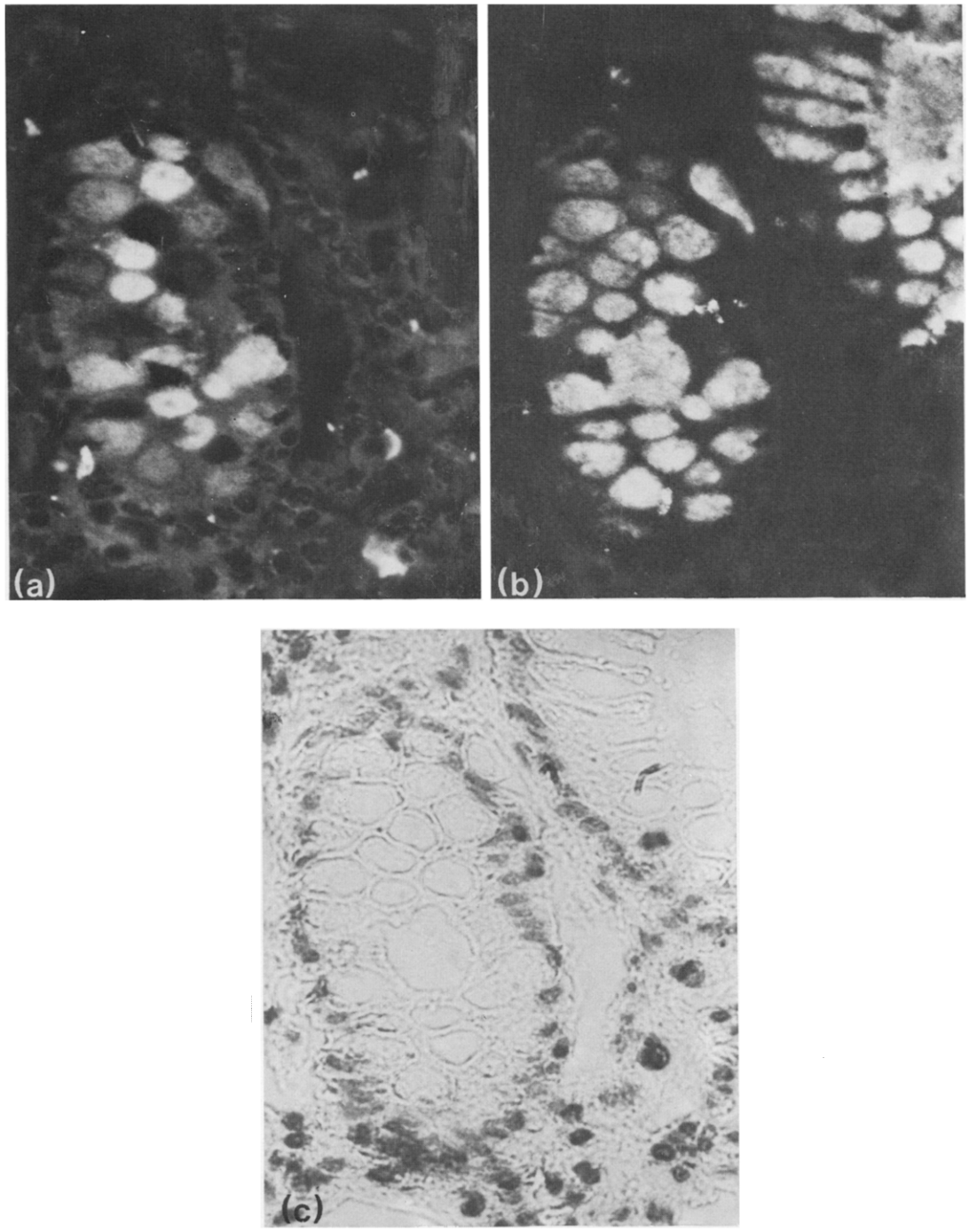


Fig. 2. Paraffin section of a transitional mucosa adjacent to colonic adenocarcinomas. The same section was stained successively by anti-M1 serum and anti-rabbit γ-globulin goat serum labeled with rhodamine (a), by anti-M3 serum labeled with fluorescein (b), and by hematoxylin (c). As in the fetal colonic mucosa, anti-M3 serum stained all goblet cells (b), but anti-M1 serum stained only some of them (a). ×225.

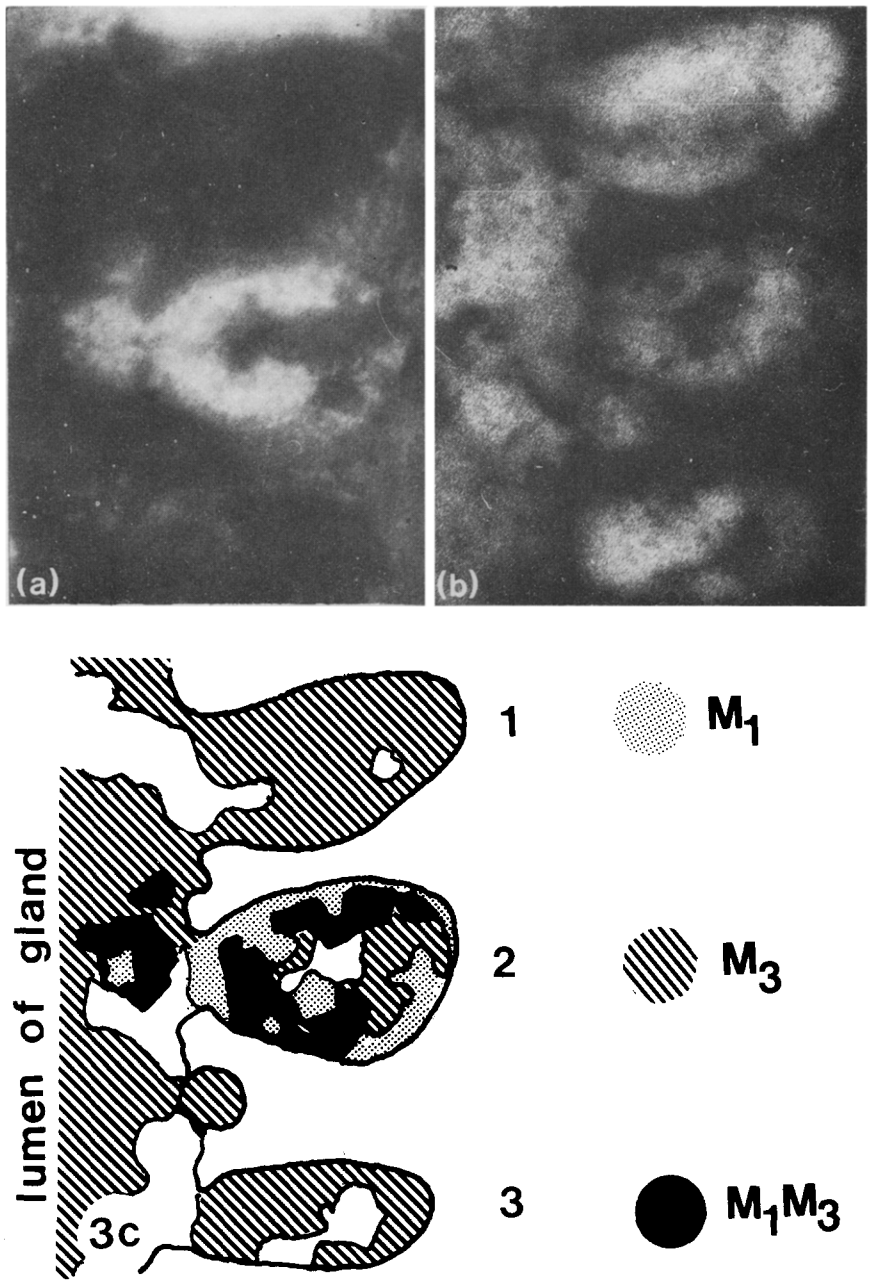


Fig. 3. Detail of three goblet cells in the transitional mucosa. In (a) the section was stained by anti-M1 serum labeled with rhodamine and in (b) the same section was stained by anti-M3 serum labeled with fluorescein. Only one goblet cell was stained by both the anti-M sera, but the pattern of the staining depended on which anti-M serum. (×900). *In (c): schematic representation obtained after superposition of both figures 3 (a) and (b). On the left: lumen of the gland; on the right three numbered goblet cells. Goblet cells No. 1 and 3 contain only the M3 antigen, goblet cell No. 2 contains both the M1 and M3 antigens. In dotted areas M1 antigens predominate, in hatched areas M3 antigens predominate, in black areas, M1 and M3 antigens are present together and in white areas the M antigens are not clearly detected.*

RESULTS

The fluorescence seen when the above antisera were used was considered specific if no staining was found in the colonic mucosae when the specific antisera were replaced by nonimmune serum or absorbed antisera. Both M-antisera showed typical patterns of mucosecretory activity in mucus cells of the three different tissues studied here: fetal, adult and transitional colonic mucosae.

Fetal colonic mucosae

In colonic mucosae from fetuses of 12–28 weeks of gestation, M3 antigen was located in all the goblet cells. The anti-M1 serum stained faintly goblet cells located in the deeper part of glands. These latter goblet cells contained both the gastric M1 and intestinal M3 antigens, as seen with filters specific either for fluorescein or rhodamine illumination. In the 28-weeks fetus, goblet cells showing both M-antigens could also be seen on the surface of the colonic glands (Fig. 1), and in the newborn, all goblet cells stained both with rhodamine and fluorescein tracing. It is important to note that the areas positive with the anti-M1 serum do not show the same distribution as those positive with the anti-M3 serum in the cytoplasm. This observation suggests that some secretion vesicules could have a different antigenic content. Colonic mucosa of a 12-yr-old child did not contain such gastric M-antigens. We don't know when these M1 antigens disappear from colonic goblet cells because we have not obtained colonic mucosae of 1–12 years old children.

Normal adult colonic mucosa

As already described [6, 12] the gastric M1 antigens were absent from normal colonic mucosa. In contrast, anti-M3 serum stained each intestinal goblet cell.

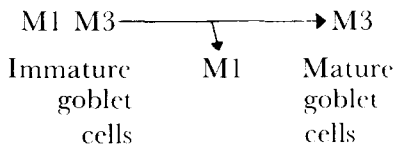
Transitional colonic mucosa

In the areas adjacent to colonic adenocarcinomas, the mucosa was thicker owing to elongation of crypts that were often branched. The M3 antigen was usually present in each goblet cell, as in the normal colonic mucosa (Fig. 2b). Furthermore, some of them were also stained with the anti M1 serum. Thus the presence of both gastric and intestinal antigens was shown in the same mucus cell (Fig. 2a). The intensity of the fluorescence staining (showing M3 antigen) in each goblet cell was relatively uniform. In contrast, the intensity of the rhodamine staining (showing M1 antigens) varied, depending on the mucus cells: some of them were strongly stained; others faintly or not at all. The lumen of the gland was positive with both the anti-M1 and M3 sera but the pattern of localization of this staining was very different according to the anti-M serum used. Many cells were shown to contain only M3 antigen (see Fig. 2b, right). None was positive only with the anti-M1 serum. Furthermore, the pattern of cytoplasmic staining of these double-stained goblet cells differed greatly (Fig. 3). Again, we observed that the areas positive with the anti-M1 serum did not correspond exactly to the areas positive with the anti-M3 serum (Fig. 3c). Some areas contained only M3, or M1 antigens, the others contained both. This indicated that the M1 and M3 antigens are probably not always secreted in the same cytoplasmic inclusions, as previously observed in the fetal goblet cells. Cells containing both M antigens were sometimes observed at more than 1 cm from the tumor.

This phenomenon was noted in transitional areas of the 10 different adenocarcinomas and was independent of the existence of mucus secretory activity in the tumor adjacent to such hyperplasic mucosae.

DISCUSSION

The gastric M1 antigens cannot be regarded as oncofetal antigens because they are found in large amounts in the mucus cells of the surface gastric epithelium [6]. Nevertheless, they are present in fetal and early postnatal colonic mucosae and are absent from adult colonic mucosa. Their association with the intestinal M3 antigen in the same goblet cells was found in fetal and early postnatal colonic mucosae. This antigenic association is typical for fetal colonic mucus cells. The association is also present, according to our results, in transitional mucosae. This is a change of oncofetal type. Our results are in agreement with the histochemical and biochemical data [2] which suggest that the epithelial cells in the transitional mucosa may be of immature type, because they resemble goblet cells of the human fetal colonic mucosa in their mucin content. We can consider that the association of the M1 and M3 antigens in the same mucus cell is characteristic for the immature colonic goblet cells and that their maturation necessitates the loss of the M1 antigens according with the following scheme:



The reappearance of this 'fetal-type association' of M-antigens remains to be explained. One possible hypothesis is that the modification in biosynthesis of M-antigens in these intestinal mucus cells corresponds to an abnormal expression of normally 'silent' genes. Such a genetic modification could be explained by a retrodifferentiation phenomenon as described by Uriel [13] or due to the presence of immature cells [2].

We are now studing the association of these M-antigens in the colonic tumors and hope to determine the role of the cell differentiation phenomenon ir the malignancy process.

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