ABSTRACT

Apparently normal mucosae adjacent to colon adenocarcinomas were studied by cutting strips of mucosa from the entire length of 120 surgical specimens (94 located on the distal colon and 26 on the proximal colon). These mucosae were coiled into "Swiss rolls." Their mucus alterations were mapped by immunoperoxidase using antibodies against M1 antigens, oncostatic markers associated with precancerous colonic mucosa. We demonstrated mucus modifications in patches of mucosa at a distance from frank tumors. The extent of these alterations was not related to invasion by the adjacent carcinoma according to Dukes' classification. However, these mucus modifications were more frequently observed on the distal than on the proximal side, were more often found adjacent to mucinous hyperplasia or adenoma, and were observed in 8 of 10 mucosae bearing metachronous or synchronous distal colonic adenocarcinomas. Our results suggest that the M1 modifications characterizing an early stage of carcinogenesis could have a putative prognostic value in estimating the risk for metachronous distal colonic adenocarcinomas.

INTRODUCTION

Mucus modifications of a fetal type are observed during 1,2-dimethylhydrazine carcinogenesis in the rat (3, 13, 17). These observations suggest that a putative reversion at a fetal stage may characterize an early stage of carcinogenesis (18). The importance of these mucus modifications lies in the discovery of their presence in such human precancerous lesions as adenomas (5, 9, 10, 22, 30). Moreover, Filipe and Branfoot (18) described mucus alteration in histologically normal mucosa distant from tumors by histological methods. Although these mucosae did not show histological lesions, they were suspected of being potentially precancerous for 2 reasons: (a) the risk of metachronous cancer in these patients was high (1.6 to 4%) in comparison with the risk in the total populations (27); and (b) such mucosae showed the same mucus modification patterns as those obtained after 1,2-dimethylhydrazine treatment in the rat.

Mucus modifications described until now were related to the saccharide part of the mucin-type glycoprotein, a difference in molecular-weight components (4). These M1 antigens were associated with fucomucins (2) common to normal gastric mucosa and ovarian mucinous cyst (7) of a pure endocervical type (15), sometimes present in the small intestine but absent from the adult but not from the fetal colonic mucosa (1). This paper describes modification in the M1 antigen pattern in histologically normal mucosae adjacent to adenocarcinomas just after surgery, and attempts: (a) to estimate the degrees of the disease according to the presence and the intensity of M1 antigen alterations of colonic mucosae; and (b) to determine whether the extent of the M1 modification is more important in mucosae bearing metachronous (interval) or synchronous (simultaneous) cancer, useful in the estimation of risk of metachronous cancer.

MATERIALS AND METHODS

Tissues

Normal Intestinal Tissues. These were obtained from kidney donors. Autopsies were performed on 16 patients (12 men and 4 women) within 5 hr of death. Most patients were in their fourth decade of life (mean age, 35.1), with ages ranging from 17 to 54 years. All had been free of any known neoplastic disease prior to trauma. Tissue samples measuring about 10 x 1 cm were taken from the cecal, ascending, transverse, and sigmoid colon. For 5 patients, samples of duodenal jejunal and ileal mucosa were resected.

Colonic Tumoral Mucosae. Specimens (120) of the large bowel, resected for adenocarcinoma at the Clinique de la Porte de Choisy, Paris, France, between 1981 and 1983, were taken no more than 1 hr after surgery: 94 tumors were located in the distal colon, i.e., from the left flexure to the rectum; 26 were resected from the proximal colon, i.e., the part of the colon between the ileum and the left flexure. Three strips, including the muscularis mucosae, were cut from the entire length of each specimen as shown in Chart 1, one strip to the distal side, a second to the proximal side, and a third perpendicular to the above fragments. Each strip contained a sample (1 cm) of normal areas when possible. The adjacent lesions such as mucinous hyperplasia, hyperplastic polyps, and adenomas were noted and sometimes removed independently with a strip of adjacent mucosa. The degree of dysplasia of adenomas was evaluated (25).

Preparation of Tissues. The mucosa strips (normal or adjacent to adenocarcinomas) were immersed in a 40- x 10- x 5-cm box having a cork bottom and filled with 95% ethanol. They were then pinned on this cork, and mucosae were dissected from the muscularis mucosae immediately. After 2 hr fixation, the mucosae were coiled into "Swiss rolls" (24), fixed in 95% ethanol for 24 hr, processed routinely, and embedded in paraffin wax. Sections 3 μm thick were cut off the bulk using an R-Jung Autocut. Serial sections were obtained and stained using hematoxylin and eosin or the immunoperoxidase method for M1 identification.

Immunohistochemistry

Antigens. The antigens studied here were associated with fucomucins of the gastric surface epithelium (2, 6, 7). They were isolated from a mucinous ovarian cyst of a pure endocervical type according to Fenoglio's classification (15). We had already shown that these antigens were...
Mucin Antigen Modulation in Mucosa Adjoining Colon Cancer

Chart 1. Diagram of a resected colonic mucosa specimen. Mucosal strips containing small tumoral areas are outlined (black). Tumor areas are located in the center of the tracing (gray).

identical to the antigens of gastric surface epithelium. Preparation of M1 antigens were obtained by chromatography on Sepharose CL 6B (Phar
macia, Uppsala, Sweden).

Antisera. Anti-M1 serum was obtained by immunization of rabbits with the preparation of M1 antigens already described (6). Such anti-
serum was absorbed with normal human plasma and a panel of human RBC. The absence of reactivity of these antisera against plasma antigens and blood group substances was controlled using immunodiffusion and hemagglutination, respectively. Moreover, the anti-M1 serum was ab-
sorbed by normal proximal colon extract (500 mg/ml). Thus, using the

Immunohistological Pattern along the Glands. M1-positive

goblet cells, when present, were observed mainly on the upper
part of the Lieberkühn glands (Fig. 1). Some M1-positive
cells were also present at the bottom of the glands near stem cells
(Fig. 2). Generally, 1 to 5 cells/gland were stained with the anti-
M1 serum. Sometimes, one isolated gland contained 50% or
greater than M1-positive goblet cells (Fig. 3).

Immunohistological Pattern along the Mucosae. Chart 2
shows the pattern of M1 modifications along the mucosae from
the edge of tumors to the limit of the sample section. The mucosa
strips of each patient were classified according to their M1alterations into 3 types as described in "Materials and Methods." Patches of glands scoring 3 were sometimes observed beyond
10 cm from the edge of the tumor. The transitional mucosa
showed patches of glands scoring 3 in 70 of 89 cases (distal
colon) and in 9 of 19 cases (proximal colon). Mucinous hyperpla-
sia was sometimes located in a patch of glands scoring 3.

In 9 cases, adenocarcinomas infiltrated the muscularis along
histologically normal colonic mucosa 0.5 to 2 cm from the tumor.
Four of these 9 mucosae showed M1-negative glands (Fig. 4).
The mucosa adjacent to sigmoidal carcinoma located at the

Statistical Analyses

Student t, F, and $\chi^2$ tests (29) were used to compare the percentage
of associated lesions and mucosal modifications.

RESULTS

M1 Antigens in Normal Intestinal Mucosae

In normal colonic mucosae from the 20 patients without neo-
plastic disease, the anti-M1 serum did not react. In contrast, in
5 small intestinal mucosae, the anti-M1 serum stained faintly
some goblet cells, independently of their intestinal location (du-
denum, jejunum, or ileum).

M1 Antigen Mucus Modifications in Apparently Normal Mu-
cosa Adjacent to Tumors

The mucosa adjacent to sigmoidal carcinoma located at the
tumor site, proximal or distal. They were then ranked into 3 types
according to the presence and the intensity of M1 modifications observed
at a distance from frank tumors, i.e., beyond 1 cm. The length of colonic
mucosa was estimated by counting the glands on the basis of 125 glands
for 1 cm on hematein-stained paraffin sections.

Thus, mucosae were classified into 3 types. At a distance of 1 cm or
more from the tumors, the type I mucosa (like the normal colonic
mucosa) did not contain patches of M1-positive glands; type II contained
patches of M1-positive glands scoring 1 or 2; and type III contained
patches of M1-positive glands scoring 3.

Associated Lesions. Associated lesions adjacent to adenocarcino-
mas were classified into hyperplasia (3), transitional mucosae according
to Filipe's definition (12), or hyperplastic and adenomatous polyps ac-
cording to the WHO classification (26).

M1 Antigen and Associated Lesions

Histological analysis of colonic mucosae from 41 patients
showed that 16 adenocarcinomas were synchronous and 25 metacrine
carcinomas. The distribution of the associated lesions according to the
eroded mucosa showed 26 metachronous cases (17 synchronous and 9 metacrine) and 15 synchronous cases (17 synchronous and 8 metacrine).

Mucosal strips containing small tumoral areas were outlined
in 21 cases (Fig. 1) and 11 cases (Fig. 2) respectively. Tumoral areas were located in the center of the tracing (gray).

Histologically normal mucosa adjacent to adenocarcinomas
were divided into 2 areas according to the tumor site, proximal or distal. They were then ranked into 3 types
according to the presence and the intensity of M1 modifications observed
at a distance from frank tumors, i.e., beyond 1 cm. The length of colonic
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Statistical Analyses

Student t, F, and $\chi^2$ tests (29) were used to compare the percentage
of associated lesions and mucosal modifications.
Chart 2. Pattern of the M1 antigen mucus modifications in 34 of 94 distal colonic mucosae adjacent to adenocarcinoma studied here. Each band corresponds to a mucosa strip of one specimen resected for carcinoma. Patients were classified according to the presence and intensity of M1 modification at 1 cm from the tumors. Type 1, no M1 modification; type 2, patches of glands scoring 1 or 2; type 3, patches of glands scoring 3. Right, M1 patterns of mucosa bearing metachronous or synchronous adenocarcinomas. ■, patch scoring 1; □, patch scoring 2; △, patch scoring 3; ◀, patch of M1-negative glands. Each patch corresponds to approximately 25 glands and about 0.20 cm of mucosa.

Table 1

<table>
<thead>
<tr>
<th>M1 modification and tumor location</th>
<th>Colonic mucosae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proximal (26 tumors)</td>
</tr>
<tr>
<td>Type 1 (-)</td>
<td>12 (46%)</td>
</tr>
<tr>
<td>Type 2 (+)</td>
<td>8 (31%)</td>
</tr>
<tr>
<td>Type 3 (++)</td>
<td>6 (23%)</td>
</tr>
</tbody>
</table>

Table 2

Relationship between M1 modification of the distal colonic mucosa and the Dukes' stage for the tumors

<table>
<thead>
<tr>
<th>M1 modification</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1 (-)</td>
<td>1</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Type 2 (+)</td>
<td>2</td>
<td>8</td>
<td>18</td>
</tr>
<tr>
<td>Type 3 (++)</td>
<td>6</td>
<td>18</td>
<td>21</td>
</tr>
</tbody>
</table>

Table 3

M1 modification of proximal or distal colonic mucosae and associated lesions

<table>
<thead>
<tr>
<th>Associated lesions</th>
<th>Colonic location</th>
<th>M1 modification</th>
<th>Mucinous hyperplasia</th>
<th>Hyperplastic polyp</th>
<th>Adenoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distal</td>
<td>Type 1 (-)</td>
<td>1/18 (5%)</td>
<td>4/18 (22%)</td>
<td>3/18 (16%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Type 2 (+)</td>
<td>2/29 (6%)</td>
<td>5/29 (17%)</td>
<td>6/29 (20%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Type 3 (++)</td>
<td>16/48 (33%)</td>
<td>10/48 (20%)</td>
<td>21/48 (43%)</td>
<td></td>
</tr>
<tr>
<td>Proximal</td>
<td>Type 1 (-)</td>
<td>1/12</td>
<td>0/12</td>
<td>5/12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Type 2 (+)</td>
<td>2/8</td>
<td>0/8</td>
<td>2/8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Type 3 (++)</td>
<td>1/6</td>
<td>0/6</td>
<td>5/6</td>
<td></td>
</tr>
</tbody>
</table>

Lesions and Age of Patients

Dukes' Grade. Our data showed that there was no relationship between the extent or intensity of M1 modifications of mucosae and parietal invasiveness of carcinomas (Table 2). The percentages of type 1, 2, or 3 mucosae were found to be independent of Dukes' grading. The number of proximal tumors was too small to permit statistical analysis and to draw any conclusions.

Associated Lesions. Table 3 reports the degree of M1 modifications according to the associated lesions found on the adjacent histologically normal mucosa. On the distal side, we could establish a correlation between the increase in M1 modifications and the presence of mucinous hyperplasia and adenomas; mucinous hyperplasia was present on 1 of 18 type 1 mucosae versus 16 of 48 type 3 (p < 0.05, χ²; Yates), and adenomas were observed to be associated with 3 of 18 type 1 mucosae versus 21 of 48 type 3 mucosae (p < 0.05). In contrast, hyperplastic polyps were found in the same percentage independently of the M1 modification.
Age and Sex of Patients and Histological Type of Tumors.
We were unable to relate M1 modifications in mucosae with the histological type of the adjacent adenocarcinomas, the extent of the staining of tumors with the anti-M1 serum or the age and sex of patients.

DISCUSSION

Using immunohistological methods, we demonstrate that mucus modifications associated with goblet cells can be observed in apparently histologically normal colonic mucosa distant from a frank adenocarcinoma, as already described using a histochemical approach (18).

The patterns of M1 mucus modifications in a colonic Lieberkühn gland are similar to those described for resurgence of sialomucins (18). The proportion of M1 goblet cells varies along the crypt and, like sialomucin, predominates in their upper part. We demonstrate that the change in the expression of these M1 antigens showing a fetal program of differentiation of these mucus cells (1) occurs most often in the proliferation compartment, as defined by Lipkin (23). However, such a change in differentiation can also be observed in the deeper part of the crypt, suggesting that these M1 alterations can occur during the differentiation of stem cells. The mucus changes observed with M1 antigens and sialomucins are not synchronous; Decaens et al. (13) demonstrated the presence of sialomucins in the same histological lesion as were M1 antigens, although they were not always associated in the same goblet cells. Such a difference could be explained as: the histochemical method characterizes surface properties of the saccharide part of mucin-type glycoprotein (carboxylated or sulfated groups); in contrast, the anti-M1 antibodies characterize antigenic conformational determinants which could be related to the peptidic part of these molecules (4).

Although no comparison between M1 and sialomucin-positive goblet cells was performed in our study, the pattern of distribution of M1-positive glands along the histological normal mucosa adjacent to adenocarcinoma is similar to those described by Filipe (18). Moreover, M1 modifications in the distal colon are more frequently observed distant from the tumor than in the proximal side, as already described (18). Such a difference in the production of M1 antigens between proximal and distal colon has already been reported in adenocarcinomas (8). However, no correlation can be established between M1 mucus alterations of mucosae and the degree of parietal invasion by the adjacent carcinoma according to Dukes’ classification, in contradiction with histochemical data (18, 20). We feel that a more extensive histochemical study including a larger number of cases would permit a statistical analysis leading to the same conclusion as our approach.

In contrast, M1 modifications are well correlated with the presence of mucinous hyperplasia and adenomas. These latter lesions are regarded as precancerous. This observation agrees with the conclusion of Hancock (21) who demonstrated that patients with synchronous tumors or with polypse and single carcinoma have an increased risk of developing metachronous cancer. This idea is reinforced by the fact that 8 of 10 distal mucosae containing metachronous or synchronous adenocarcinomas belong to type 3; i.e., they show mucosae containing patches of M1-positive glands scoring 3 distant from frank tumors. Moreover, the resurgence of sialomucins and M1 antigens corresponding to a fetal program of differentiation of the intestinal mucus cells (1) confirms that a putative reversion at a fetal state may characterize an early stage of carcinogenesis (18).

Moreover, these mucus modifications were described as a response to unknown stimuli rather than a local secondary effect of tumor growth (18). We agree with such a conclusion because: (a) patches of M1-positive glands are found at 20 cm from the edge of the tumor; (b) we observed 4 mucosae adjacent to colonic carcinomas infiltrating the muscularis mucosae which did not show M1-positive glands (see Fig 4); and (c) 2 metastases of 2 carcinoid tumors located in the muscularis mucosae showed M1-negative glands in the adjacent areas.

If such conclusions are exact, our results suggest that the intensity of M1 mucus alterations of mucosae could characterize a fairly strong response to unknown stimuli and, as described during rat carcinogenesis, could be proportional to the intensity of a stimulus [for example, dose of carcinogen (28) received by the patient].

Thus, apparently histologically normal mucosae adjacent to tumors and which show severe mucus modifications appear to be more likely to form a carcinoma. Moreover, the risk is perhaps not the same for each patient. Severe M1 modifications observed in the mucosae of patient having undergone ureterosigmoidoscopy confirm this view. Indeed, it is well known that the colonic carcinogenesis process occurs when urine is surgically diverted into the fecal stream (11).

Thus, our immunohistological approach using anti-M1 serum could be useful in estimating the risk of metachronous colonic cancer just after the first surgical resection. Patients who had had a colorectal cancer resected in the past are at high risk for development of other neoplasms of the large intestine. The incidence of such a metachronous cancer varied from 1.6 to 4.6% (27). This incidence is probably underestimated, since some patients fail to return for a follow-up visit and colonoscopic examination. Other patients die from the original cancer or from another disease before the second cancer develops.

We classified these 120 patients from the Clinique de la Porte de Choisy into 3 groups according to the presence and the extent of M1 mucus modifications and, consequently, according to the risk of developing another colonic cancer. The follow-up of these patients will determine whether such a classification could be useful in the prognosis.

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Unpublished data.
J. Bara et al.


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Figs. 1 to 4. Histologically normal colonic mucosa adjacent to adenocarcinoma.

Fig. 1. Lieberkühn gland showing 3 goblet cells strongly stained using anti-M1 serum (arrows). V, surface epithelium. × 250.

Fig. 2. Bottom of Lieberkühn glands showing 3 M1-positive goblet cells (arrows) near stem cell area. M, muscularis mucosa. × 250.

Fig. 3. Lieberkühn gland containing more than 50% of goblet cells stained using anti-M1 serum (right); in contrast, a negative gland can be observed on the left. V, surface epithelium. × 250.

Fig. 4. Undifferentiated M1-positive colon adenocarcinoma infiltrating the muscularis mucosa on the bottom (>). The adjacent histologically normal mucosa is not stained with the anti-M1 serum. V, surface epithelium. × 100.
Abnormal Pattern of Mucus-associated M1 Antigens in Histologically Normal Mucosa Adjacent to Colonic Adenocarcinomas

J. Bara, J. André, R. Gautier, et al.

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