Intestinal Mucin Antigens in Ulcerative Colitis and Their Relationship With Malignancy

M. I. FILIPE,* A. SANDEY,* AND J. MA†

The expression of two intestinal mucin-associated antigens large intestine mucin antigen (LIMA) and small intestine mucin antigen (SIMA) were investigated by indirect immunoperoxidase staining of rectal mucosa from patients suffering from ulcerative colitis with (n = 6) and without (n = 31) associated carcinoma and in noncolitic controls (n = 40). The aim was to assess the relationship between antigen patterns and malignant change. SIMA, which is localised predominantly in the small intestine, is virtually undetectable in the normal adult colonic mucosa. However, this antigen is present in the foetal colon and colonic carcinoma. LIMA is expressed in normal colonic mucosa, but absent from the small intestine. LIMA staining patterns were not significantly different among the three groups. In contrast, expression of SIMA was significantly higher in the patients who had developed carcinoma (6/6) than in the noncancer group (7/71) (P < 0.001). The presence of SIMA was also significantly related to areas of dysplasia compared to normal (P = .03) or inflammation (P < .05), but it did not differ from mucosa showing “indefinite” atypia. The finding of 31% SIMA-positive biopsies associated with severe inflammation in colitis with active disease, but no evidence of malignancy, is difficult to explain at the present stage. A followup study would be necessary to determine its significance. Perhaps the most important finding is the increased frequency of SIMA-positive foci in histologically normal mucosa in carcinoma patients compared with the noncancer group (P < .001), suggesting a field change. These observations may be prove useful for the identification of patients who may be at risk of developing carcinoma. HUM PATHOL 19:671–681. © 1988 by W.B. Saunders Company.

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Since the original paper of Morson and Pang1 in 1967, the presence of dysplasia in large bowel biopsies has been the most commonly used indicator of a precancerous state in ulcerative colitis. However, this approach is known to have limitations. First, the patchiness of the lesion may lead to a false negative result, which can be minimized by multiple biopsies and endoscopy expertise. Second, absence of dysplasia in biopsies preceding the development of carcinoma raises problems in the followup of these patients.2-6 Thus, an additional functional criteria needs to be considered.

Studies using carcinoembryonic antigen, IgA, and secretory component,7-10 and more recently lectins,11-12 to distinguish dysplasia from inflammatory changes in ulcerative colitis, have not been successful in defining increased cancer risk in the individual patient. Altered mucin secretion with excess of sialomucins also has been reported in association with dysplasia and in apparently ‘normal’ mucosa in carcinoma fields.13-15 However, its specificity and predictive value as an indicator of malignancy are low when the conventional histochemical methods are employed.16-17

This study was undertaken to establish the patterns of expression of two intestinal mucin-associated antigens SIMA and LIMA,18,19 in the colonic mucosa of patients with ulcerative colitis and its relationship to the development of carcinoma. We were particularly interested in ascertaining whether the nonneoplastic colonic epithelium (normal and diseased) in ulcerative colitis patients who developed carcinoma differed from that of patients who did not, and from normal controls.

Small intestine mucin antigen (SIMA) is a mucin glycoprotein originally isolated from mucus secreting colonic carcinoma with histochemical and biochemical evidence indicating that it is a nonsulphated mucin glycoprotein.18 It is always present in normal

### Table 1. Ulcerative Colitis With Cancer: Summary of Clinical Data and Macroscopic Findings in the Resected Specimens

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex</th>
<th>Duration of disease (years)</th>
<th>Age at operation (years)</th>
<th>Operation</th>
<th>Carcino</th>
<th>Grade</th>
<th>Stage</th>
<th>Site</th>
<th>Site*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>40</td>
<td>67</td>
<td>Proctectomy</td>
<td>Precancer</td>
<td>III</td>
<td>C</td>
<td>Distal</td>
<td>Multifocal</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>22</td>
<td>40</td>
<td>Proctectomy</td>
<td></td>
<td>II</td>
<td>B</td>
<td>Distal</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>31</td>
<td>45</td>
<td>Proctectomy</td>
<td></td>
<td>II</td>
<td>A</td>
<td>Proximal</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>39</td>
<td>68</td>
<td>Proctectomy</td>
<td></td>
<td>III</td>
<td>B</td>
<td>Proximal</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>14</td>
<td>49</td>
<td>Proctectomy</td>
<td></td>
<td>II</td>
<td>B</td>
<td>Proximal</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>24</td>
<td>69</td>
<td>Proctectomy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* I, II, and III: Duke’s grades; well, moderately, and poorly differentiated, respectively.
† A, B, and C: Duke’s stage of invasiveness.
‡ Site in the rectal stump. Proximal, near ileorectal anastomosis; distal, near anal/rectal zone.
adult small intestine and has therefore been named “small intestine mucin antigen”. SIMA has been shown to be an oncofetal antigen of the large intestine, because it is found in 8-12 week old fetal colon and absent thereafter and in the normal adult colonic mucosa, but is present in carcinomas of the colon (and adjacent transitional mucosa), stomach, gallbladder, and ovary. Large intestine mucin antigen (LIMA) is present in the normal large intestine mucosa and, like SIMA, it is also produced to a varying extent in carcinomas of colon and other parts of the digestive system and ovary.\textsuperscript{18-23}

They appear to be distinct from previously reported antigens of normal and malignant gastrointestinal tissues.\textsuperscript{24-27}

**MATERIAL AND METHODS**

**Patients**

Material was obtained from three groups of patients: (A) Crohn’s disease with cancer was previously studied in six patients who underwent total colectomy and ileorectal anastomosis (IRA) for ulcerative colitis and later developed cancer in the rectal stump (fig 1). Two or three strips of mucosa were taken along the entire length of the resected rectal stump, and each strip divided into several blocks (fig 1).\textsuperscript{2} (B) Crohn’s disease without cancer (control) was studied in rectal biopsies obtained from 31 patients suffering from various large bowel conditions, \textit{ie}, irritable bowel syndrome, diarrhoea which included postinfective Schistosomiasis and postcholecystectomy and postvagotomy etiologies, constipation, amyloid, etc. Ulcerative colitis and malignancy were excluded. There were 29 men and 11 women with a mean age of 40 years (table 2).

**Methods**

All tissues were fixed in 10% formol-saline, routinely embedded in paraffin wax. 5-μm sections were cut and stained with haematoxylin-eosin stain. Eighteen representative tissue blocks (out of 30 total) from six patients in Group A, and all biopsies from control groups B and C, were stained with an indirect immunoperoxidase technique to demonstrate SIMA and LIMA.\textsuperscript{25}

Sections were trypsinized for 15 minutes before staining. Endogenous peroxidase was blocked with 1% H₂O₂ in methanol for 30 minutes, and normal rabbit serum, diluted 1:5 with PBS, was used to block cross-reacting molecules. The monospecific antisera in this study were prepared by Dr Ma and described elsewhere.\textsuperscript{18,19} The appropriate concentrations of the primary antibody (anti-LIMA and anti-SIMA) and the secondary layer of horseradish-conjugated antibody were determined by testing different dilutions on sections of normal colon and small intestine, known to stain for LIMA and SIMA, respectively. The former acts as a positive control for LIMA and negative for SIMA. The converse reaction is obtained in the small intestine.

**TABLE 2.** Controls: Summary of Clinical Data and Morphology of Rectal Biopsies From Noncolitic and Colitic Patients Without Cancer

<table>
<thead>
<tr>
<th>Patients (No.)</th>
<th>Histology*</th>
<th>No. Men</th>
<th>No. Women</th>
<th>Age (years)</th>
<th>Duration of disease (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group B: Crohn’s disease</td>
<td>Active inflammation</td>
<td>6</td>
<td>10</td>
<td>49 (28-72)</td>
<td>2-29</td>
</tr>
<tr>
<td>Group C: Noncolitic (40)</td>
<td>Normal</td>
<td>29</td>
<td>11</td>
<td>40 (18-79)</td>
<td>—</td>
</tr>
</tbody>
</table>

* All biopsies show grade 0 according to Riddell et al, 1983.
FIGURE 2. Hyperplastic rectal mucosa characterized by (top left) taller crypts lined by goblet cells secreting sulphomucins (dark-colored on HID-AB), (bottom) normal pattern of LIMA expression in the goblet cells, and (top right) absence of SIMA. (top left) Approximate magnification ×160; (bottom) indirect immunoperoxidase method; approximate magnification ×160; (top right) approximate magnification ×120.
Grading of Morphologic Features

Morphologic features were defined according to the classification of cellular atypia in inflammatory bowel disease by Riddell et al.\textsuperscript{29} as follows: negative, grade 0, indefinite, grades 1–3 (probably inflammatory, unknown, and probably dysplasia, respectively), and positive, grades 4 and 5 (low and high grades of dysplasia, respectively). In addition, the terms “hyperplasia” and “transitional” were included in grade 0. Hyperplasia is defined by taller crypts, sometimes branched and lined by increased numbers of larger goblet cells secreting predominantly sulphomucins. “Transitional” mucosa shows morphologic features similar to hyperplasia, but goblet cells contain an excess of sialomucins and intermediate cells between them are more conspicuous and produce sulphomucins. Intermediate cells refer to a relatively inconspicuous population of partially differentiated cells that in the normal colonic epithelium occupy the lower crypt.\textsuperscript{30} These show characteristics of both absorptive and goblet cells at the ultrastructural level,\textsuperscript{14,31} and during the course of their differentiation, one or the other function normally predominates.

The selection criteria for LIMA and SIMA demonstration of 18 of 30 tissue blocks from rectal stumps were based on the range of morphological atypia.\textsuperscript{29} Furthermore, to avoid possible hybrid patterns of ileo-rectal transition, only blocks of distal rectal mucosa away from the anastomosis were included. Hyperplasia and transitional epithelium were characterised by their distinct patterns of mucin secretion, described in a previous study.\textsuperscript{5}

To map the morphologic features and the varying antigen expression in sections from the rectal stump material, we used a microscope stage micrometer and the areas/foci of change were noted and counted, as illustrated in detail in figure 1.

For statistical analysis, $\chi^2$ and Fisher’s exact tests, as well as two tailed tests, were used.

RESULTS

Clinical Data and Morphology

For group A (ulcerative colitis with cancer), a detailed description of the gross appearances of the whole resected specimens has been reported.\textsuperscript{5} In patient 1, where no invasive carcinoma was present, the rectal stump displayed several polypoid and plaque-like areas. In patient 5, the distal mucosa had a granular velvety texture, and the tumour, a poorly differentiated Duke’s B adenocarcinoma, was located proximally close to the ileo-rectal anastomosis and surrounded by multifocal, plaque-like lesions. Histologically, a range of morphological changes from normal, hyperplasia, and “transitional”, through the various grades of indefinite atypia and dysplasia, was seen in both of these patients. A close correlation was noted between the microscopic and gross appearances. In contrast, the rectal mucosa from the other four cases was pale, flat, and smooth. The carcinoma

\textbf{FIGURE 3.} Normal rectal mucosa from ulcerative colitis with carcinoma showing absence of SIMA (top) and a slightly altered pattern of LIMA staining (bottom) in the luminal border, intermediate cells, and irregular distribution in goblet cells (indirect immunoperoxidase method; approximate magnification $\times$160).
was flat and ulcerated in three of the cases and sited distally in two. Apart from the carcinoma, the mucosa was either normal, hyperplastic, or of "transitional" type. No dysplasia was found (table 1).

Non-cancer Patients

Table 2 summarizes the clinical data and the histological features of the rectal biopsies from 31 cases of colitis without cancer (group B) and 40 cases of noncolitic patients (group C). In group B, 16 cases showed active inflammation, whereas the other 15 cases showed noninflamed mucosa, consistent with ulcerative colitis in remission. In all of the 31 group B patients and the 40 group C cases, the mucosa revealed no epithelial atypia, and was therefore classified as grade 0. Neither hyperplasia nor transitional features were observed in these 71 patients.

Patterns of LIMA and SIMA Expression

In the normal staining pattern observed in the colonic mucosa (normal pattern), LIMA is expressed in the goblet cell mucus all along the crypt epithelium. It may be absent or at the bottom of the crypt and is not conspicuous in the luminal border (fig. 2). Abnormal patterns were characterized by decreased or absent reaction in the goblet cell mucus and the appearance of LIMA in intermediate cells between goblet cells. These intermediate cells showed staining in the luminal border and apical cytoplasm or in the supranuclear cytoplasm (Golgi area) or, alternatively, the loss of antigenic expression. The changes may involve part or the whole crypt (figs. 3 and 4).

SIMA is not expressed in normal large bowel mucosa, though it may occasionally be seen in goblet cells at the bottom of the crypt in patients in the control groups (figs. 2 and 3). The abnormal pattern is defined by the presence of the antigen in goblet cells occupying the lower third of the crypt, scattered along the crypt, or involving the whole crypt epithelium (figs. 5–8). Abnormal expression of both LIMA and SIMA can be focal (1–3 crypts/tissue section), patchy or extensive, and the change from a normal to
an abnormal pattern can be abrupt or gradually increasing in severity.

**LIMA and SIMA in Cancer v. Noncancer Patients**

LIMA is expressed in goblet cells of normal adult colonic mucosa. Abnormal intracellular distribution was observed in the mucosa from both carcinoma patients (group A) and controls (groups B and C). Within group A, atypical staining with LIMA was more marked and more frequent in dysplasia (71%) than in those foci labelled as indefinite (43%) or grade 0 (33%, including normal, hyperplastic, and transitional epithelium), but these differences were not significant. LIMA staining in grade 0 epithelium did not differ significantly between carcinoma patients and controls. (fig. 9 and table 3).

In contrast, all six patients with ulcerative colitis and carcinoma (fig. 9 and table 4) had intense and extensive SIMA staining at some point in the rectal mucosa compared with 2/40 (5%) noncolitic and 5/31 (16%) colitic with no evidence of malignancy ($P < .001$) (table 3).

**SIMA and Epithelial Atypia in Ulcerative Colitis With Cancer**

The expression of SIMA was related to increased severity of epithelial atypia, since it was observed in 12/30 (40%) grade 0 epithelium as opposed to 5/7 indefinite (71%) and 6/7 dysplastic areas (86%), but the numbers are too small for valid statistical significance. However, in this group of cancer patients, grade 0 includes three phenotypes. It is of interest to analyze separately the antigenic expression of normal, hyperplastic and transitional epithelium. A positive SIMA staining was significantly more common in transitional epithelium (100%) than in normal (23%)
FIGURE 6. Normal rectal mucosa (left) from ulcerative colitis associated with carcinoma showing (right) SIMA-positive goblet cells scattered along the crypt. (Left) Hematoxylin-eosin stain, approximate magnification ×160; (Right) Indirect immunoperoxidase method; approximate magnification ×120.

FIGURE 7. Rectal mucosa showing (left) indefinite grade epithelial atypia with slightly enlarged and crowded nuclei, fewer goblet cells, and conspicuous intermediate cells. (Right) Expression of SIMA is seen in the cytoplasm and luminal border in two of the crypts. (Left) Haematoxylin-eosin stain, approximate magnification ×160; (Right) Indirect immunoperoxidase method; approximate magnification ×160.
FIGURE 8. Rectal mucosa showing marked SIMA expression in (left) low grade and (right) high grade dysplasia, in ulcerative colitis associated with carcinoma. Indirect immunoperoxidase method; approximate magnification x160.

or hyperplastic foci (20%) (P = .004). In contrast, there was no significant difference in SIMA expression in transitional, indefinite, and dysplastic epithelium, probably reflecting a higher percentage of immature cells in these three categories. Thus, it seems valid to classify transitional epithelium as a separate grade. If so, positive SIMA phenotypes are significantly related with dysplasia (P < .01) and indefinite atypia (P = .05), compared with normal or hyperplastic epithelium.

SIMA in Grade 0 Epithelium in Carcinoma v Non-carcinoma Patients (Table 3)

SIMA staining was more frequent (40%) in grade 0 foci (normal, hyperplastic, and transitional) in the six patients with ulcerative colitis associated with cancer than in patients without cancer, both colitics (5/31, 16%) and noncolitics (2/40, 5%) (P < 0.001). The incidence of SIMA staining was similar in both control groups as a whole (table 3). However, the presence of inflammation seems to influence SIMA expression, as reported below.

Inflammation

In colitis with active inflammation, SIMA was observed in 5/16 biopsies (31%). This result was different from the incidence of SIMA in the noninflamed mucosa (colitis in remission 0/15, noncolitis 2/40, P = .03) and significantly lower to the incidence in dysplastic mucosa (6/7, P < .05) (table 3). Differences in SIMA staining between severely inflamed mucosa in colitis (5/16) and indefinite atypia in the cancer group (5/7) failed to reach statistical significance due to the relatively small number of subjects studied.

DISCUSSION

In assessing cancer risk in the individual patient with ulcerative colitis, importance has been attached to the presence of dysplasia. However, the knowledge that severe dysplasia is sometimes absent in endoscopically biopsies from colitic patients who later developed carcinoma should prompt us to pay attention to both morphologic and functional mucosal abnormalities that may antedate the development of malignancy.

We demonstrated a gradation of aberrant distribution or loss of LIMA and the resurgence of an oncofetal antigen (SIMA) in all patients with ulcerative colitis and carcinoma.

Changes in LIMA staining pattern tended to be more common in patients with carcinoma than in controls and more marked in dysplasia than in both
The presence of SIMA in 31% of cases of colitis without evidence of malignancy may reflect an early stage in the process that may lead to carcinoma or, alternatively, the expression of SIMA in the colon may be related to cell proliferation and nonspecific.

These patients in the colitis group showing SIMA-positive biopsies had disease duration of >9 years. It is known that the risk of developing carcinoma in ulcerative colitis increases after the first decade of disease. Therefore, these five cases of SIMA-positive colitis would be in a high risk group for the development of carcinoma. Further studies with a larger number of patients and longterm followup are required to correlate SIMA expression both with activity and duration of diseases and with development of malignancy.

The absence of SIMA in 14% of dysplastic foci is consistent with the heterogeneity of SIMA expression observed in gastric and colonic carcinomas and may reflect cell lineage differentiation.

We believe that the availability of new markers for colonic cell differentiation represents a novel approach to the definition of cancer risk, looking at functional atypia related to cell proliferation and impaired or aberrant cell differentiation rather than reliance solely on the morphologic features of dysplasia.

In fact, the present study clearly demonstrates that morphologically normal epithelium (grade 0) from carcinoma patients reveals a significantly higher proportion of altered cell populations expressing SIMA than in the noncarcinoma group (table 3) (P < .001). This is not surprising, as neoplastic transformation occurs in a background of disorderly cell proliferation and differentiation.

The presence of SIMA in cases 2, 3, 4, and 6, shown in table 4, is of interest in these four patients; atypia or dysplasia were absent in the rectal stumps but SIMA is expressed in the nondysplastic epithelium. Whether or not the resurgence of SIMA means that the tissue is invariably committed to malignant change is open to question. At the moment, we can only speculate that the probability of hitting SIMA-positive foci in the absence of dysplasia in colitis associated carcinoma seems higher than in controls.
In nondysplastic mucosa, SIMA is more frequently found in transitional than in normal or hyperplastic epithelium ($P = .004$). Although hyperplastic and transitional epithelially are morphologically similar and labelled grade 0 in Riddell et al classification, the latter has distinct histochemical features in common with those of immature colonic cells of the adult and foetal gut and neoplasia and we believe that the presence of transitional epithelium should not be included in the grade 0 classification.

The resurgence of SIMA in adult colonic epithelium is highly atypical since SIMA is a glycoprotein normally produced by fetal gut. In adult life, SIMA persists in the goblet cells of the small intestine, but is absent in gastric and colonic epithelium. It is re-expressed in intestinal metaplasia of gastric mucosa and in both gastric and colonic carcinoma. Recent studies with lectins and monoclonal antibodies to carbohydrate structures of glycoproteins during embryogenesis and oncogenesis support the concept that aberrant oligosaccharides in the glycoproteins of malignant cells are related to incomplete or inappropriate synthesis. This may be a result of incomplete glycosylation, availability of the substrates, or differences in glycosidase activities, or it may be related to the reexpression of a native glycoprotein of the fetal gut. An increased population of immature and intermediate cells has been noted in foci of morphologically normal mucosa at various distances from colorectal carcinoma, and it is possible that the altered phenotypes described above in ulcerative colitis-harboring carcinoma reflect this shift in cell differentiation.

We conclude that markers for colonic cell differentiation, such as SIMA, may enable detection of altered cell populations in an unstable epithelium prone to malignant transformation. The findings of persistent abnormal expression of SIMA in multiple followup biopsies in ulcerative colitis may be useful for the identification of patients who may be at risk for developing cancer. This is being evaluated in our prospective studies.

Acknowledgment. The authors thank Mrs Rosemary Brown for statistical analysis.

### Table 4. SIMA Distribution in the Six Patients With Ulcerative Colitis and Carcinoma

<table>
<thead>
<tr>
<th>Histology</th>
<th>Normal, n = 13</th>
<th>Hyperplasia, n = 10</th>
<th>Transitional, n = 7</th>
<th>Indefinite, n = 7</th>
<th>Dysplasia, n = 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 0/2</td>
<td>1/7</td>
<td>1/1</td>
<td>1/1</td>
<td>1/7</td>
<td>5/6</td>
</tr>
<tr>
<td>2 0/1</td>
<td>0</td>
<td>1/1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3 1/3</td>
<td>0/1</td>
<td>3/3</td>
<td>2/2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4 1/3</td>
<td>0</td>
<td>1/1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5 1/2</td>
<td>0/1</td>
<td>1/1</td>
<td>0/1</td>
<td>1/1</td>
<td>1/1</td>
</tr>
<tr>
<td>6 0/2</td>
<td>1/1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total 6</td>
<td>3/13</td>
<td>2/10</td>
<td>7/7</td>
<td>5/7</td>
<td>6/7</td>
</tr>
</tbody>
</table>

Note: Patients 2, 3, 4, and 6 had no dysplasia in the resected rectal stump and patients 2, 4, and 6 had no indefinite atypia. Abbreviation: n, number of foci.

### References

37. Feizi T: Demonstration by monoclonal antibodies that carbohydrate structures of glycoproteins and glycolipids are oncodevelopmental antigens. Nature 314:55, 1985