Proliferative and Antigenic Properties of Rectal Cells in Patients with Chronic Ulcerative Colitis

Guido Biasco, Martin Lipkin, Andrea Minari, Paul Higgins, Mario Miglioli, and Luigi Barbara


ABSTRACT

Two markers related to preneoplasia were studied simultaneously in ulcerative colitis (UC). The renewal of the rectal epithelial cells together with expression of second-trimester fetal antigen (STFA) were evaluated in nine patients with UC and four healthy subjects. Endoscopic biopsies were incubated with tritiated thymidine. Cell renewal was studied with microautoradiography, and the antigenic properties of the cells were evaluated by indirect immunofluorescence. At the time of the study, all the UC patients were in a mildly active or in a quiescent stage of the disease; their biopsies did not show dysplastic or neoplastic changes in epithelial cells. STFA was expressed in five UC patients. The analysis of cell renewal in this group revealed a shift of the proliferative compartment towards the luminal surface of the colonic crypts. By contrast, the patient group with STFA-negative reactions showed a pattern of cell proliferation similar to that observed in the controls.

These results suggest that the expression of STFA in colonic mucosa is associated with an expansion of the epithelial stem cell population or with arrested cell differentiation, and it may represent a phenotypic marker of proneness of the mucosa toward neoplastic development.

INTRODUCTION

Several studies suggest that proliferative abnormalities of colorectal cells may be related to the development of neoplastic changes (10, 11, 17, 21, 25). An expansion of the proliferation compartment, normally confined to the deepest part of the crypts (9, 19), has been observed in the colonic mucosa of rodents following the administration of 1,2-dimethylhydrazine (12, 25). An expansion of the proliferation compartment, normally confined to the deepest part of the crypts (9, 19), has been observed in the colonic mucosa of rodents following the administration of 1,2-dimethylhydrazine (12, 25) and in the rectal flat mucosa of population groups at high risk for colon cancer (6, 7, 10, 11, 18, 20).

It could be argued that the cells contributing to this abnormal proliferative pattern fail to repress DNA synthesis during migration and maturation. Changes in RNA and protein synthesis have been reported in these normal-appearing cells (17), but no other biochemical or immunohistochemical characteristics have been investigated.

Recently, a rabbit antiserum prepared to second-trimester human organ extracts (anti-STFA) reacted with cells cultured from benign and malignant tumors of the human large bowel, but not with normal adult colonic tissue (15).

In the present work, human rectal biopsies incubated with [3H]dThd have been used to study the cell kinetics and reactivity with STFA antisera, in order to evaluate whether cells with abnormal growth patterns also have tumor-associated antigens.

The population study groups consisted of normal subjects and patients with UC. The disease is at risk for colon cancer (3, 14, 16, 23), and abnormalities of rectal cell kinetics related to the colon cancer risk have been observed on randomized biopsies of the affected mucosa (4, 5).

MATERIALS AND METHODS

Patients and Controls. Nine patients with chronic UC (4 female, 5 male; age range, 15 to 76 years) were studied. The duration of the disease was less than 2 years in 3 cases, 5 years in 2 cases, 7 years in 2 cases, and 10 and 12 years in the remaining 2 patients. Three patients had extensive ulcerative colitis; in the others, the lesions were limited to the left colon. Six patients were in a mild phase of the disease at the time of the study; in the remaining patients, the disease had been in remission for at least 3 months. The histological activity of the disease was assessed according to the method of Eastwood and Trier (13). All patients ingested salicylazarosulfapyridine (2 g/day). Two patients also were treated with prednisolone (8 and 12 mg/day).

Four healthy subjects (3 male, 1 female; age range, 20 to 42 years) without familial or personal history of colonic neoplasia or inflammatory bowel diseases were studied as controls.

Biopsies of the Rectal Mucosa. During colonoscopy, 8 biopsies were taken from each subject using biopsy miniforceps. Fragments (10 to 15 cm) were removed from flat mucosa from the anal verge. Specimens of mucosa 1 mm thick were incubated for 1 hr in a Dubnoff shaker at 37°. Eagle’s basic salt solution with 10% fetal calf serum and containing [3H]dThd (10 μCi/ml; specific activity, 5 Ci/mmol) was used as culture solution. A mixture of O2 (95%) and CO2 (5%) was bubbled at intervals through the medium. After the incubation, the biopsies were fixed in 100% methanol and embedded in paraffin.

Eight 3-μm sections of the biopsies were placed on each slide. One-half of these slides were used for microautoradiography; the others were used for the immunohistochemical study, except one for each case that was directly processed and stained with hematoxylin-eosin for routine histopathological analysis.

Microautoradiographic and Cell Kinetics Study. Sections were dehydrated and coated with Ilford K9 photographic emulsion. The slides were developed after 21 days of exposure time at 4° and stained with hematoxylin-eosin. The average number of crypts was 15.3 ± 4.7 (S.E.) for UC patients and 12.7 ± 3.5 for controls. The average number of epithelial cells per crypts was 65.4 ± 10.9 for UC patients and 93.5 ± 7.4 for controls.

The Wilcoxon test was carried out in order to compare the labeling index data in UC patients and in controls. The height distribution patterns of [3H]dThd-labeled cells were evaluated standardizing the position of these cells within the crypt column (20). Each crypt was divided into 10 compartments of equal size; an additional compartment indicated the luminal surface cells. The compartments were referred to by ordinal numbers from 0 (bottom) to 10 (surface). The labeled cell data for each compartment were compared and analyzed with the Wilcoxon test.
study group were summarized with reference to the crypt height compartments. The total number of labeled cells found in a given compartment defined a frequency of occurrence (f, with i = 0 to 10).

For each case, the number of cells and the labeling index in the upper 40% of the crypts were also evaluated. This zone, corresponding to the crypt height compartments 6 through 10 inclusive, represents the area in which the greatest differences in proliferating cell distribution between population groups at low and high risk for colon cancer have been observed. The average total number of cells in this zone was 50.2 ± 10.7 for UC patients and 37.4 ± 2.2 for controls.

Comparisons of labeled cell frequencies in UC patients versus the controls were carried out by graphically displaying X, intermediate data from the χ² analysis (20). Thus, for any given crypt height compartment i, the frequencies for the patients (f) versus the controls (F) were calculated, and the normalized frequency differences (X) were defined by (X = (f — F)/F). These differences were then plotted as a histogram of X, versus crypt height compartment.

Anti-Second-Trimester Fetal Organ Extracts Antisera (Anti-STFA) and Immunohistochemistry. Since details of experimental procedures have been described previously (15), only the outline is given in this paper. Extracts of lung, liver, and kidney, and large bowel obtained from a fourth-month human abortus were pooled, emulsified, and inoculated s.c. into New Zealand White rabbits using the immunization protocol of Stonehill and Bendich (24).

The IgG fraction of rabbit antiserum was absorbed with A and B blood cells; 0.9% NaCl solution (saline) extracts (100 mg) of normal adult lung, liver, and kidney (tissue obtained at autopsy of cancer-free individuals, 36 to 38 years old); and finally with homogenates of fresh adult colon (surgical specimens). The IgG fraction of the absorbed immunoglobulin preparation was reisolated by DEAE-cellulose chromatography and designated anti-STFA. Anti-STFA was unreactive in agar double-diffusion tests with saline extracts of all adult tissues examined except stomach. Precipitin activity to extracts of several colonic and gastric carcinomas was also evident.

Indirect immunofluorescence tests of paraffin-cleared tissue sections used fluorescein isothiocyanate-conjugated goat anti-rabbit IgG (1:50) as second antibody. Absorption of the IgG isolate with typhoidized extracts of adult spleen and inclusion of 5% goat serum (v/v) in the IgG dilution mixture were essential for removal of nonspecific staining.

RESULTS

No dysplastic or neoplastic changes were observed in the biopsies surveyed during the course of this study. The labeling index in the whole crypt did not differ in the controls and in UC patients (Chart 1). Therefore, it is conceivable that in these patients the turnover time of the mucosa (calculated from the duration of S phase/labeling index × 100) was not altered because the duration of S phase is usually normal in ulcerative disease (4, 6, 8, 22).

In control biopsies, the proliferative area was located below the fifth crypt height compartment; only scattered proliferating cells were observed above this region (Chart 2).

UC patients had cell proliferation maximally located in the lower portions of the glands with a slightly greater fraction of labeled cells in crypt height compartments 5 to 10 compared to controls. The differences are represented by the X, data shown in Chart 3.

The above findings were accompanied by increases in the percentage of [3H]dThd-labeled cells in the upper 40% of the crypts in UC patients compared to controls. In fact, the values of the labeling indices in crypt height compartments 6 to 10 ranged from 0.6 to 8.5% in UC patients and from 0.2 to 1.6% in the controls (Chart 4).
Table 1  
Reactivity with anti-STFA antisera in the rectal biopsies of patients and controls

<table>
<thead>
<tr>
<th>Case</th>
<th>Duration of the disease (yr)</th>
<th>Activity of the disease</th>
<th>Reactivity with anti-STFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>UC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>Mild</td>
<td>Negative</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>Quiescent</td>
<td>Negative</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>Mild</td>
<td>Negative</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>Mild</td>
<td>Negative</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>Mild</td>
<td>Positive</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>Quiescent</td>
<td>Positive</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>Mild</td>
<td>Positive</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>Quiescent</td>
<td>Positive</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>Mild</td>
<td>Positive</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td>13</td>
<td></td>
<td></td>
<td>Negative</td>
</tr>
</tbody>
</table>

The upper 40% of the crypts than the maximum value observed in controls (Chart 5).

A comparison of the fractions of labeled cells in each of the crypt height compartments 0 to 10, in anti-STFA-negative and -positive biopsies versus the controls, demonstrated that the anti-STFA-positive biopsies exhibited an abnormal distribution of proliferating cells (Chart 6). The differences in comparison to the controls are significant ($p < 0.005$) in compartments 1 and 2 and 6 to 10 (except 9 in which $p < 0.05$).

The $X^2$ values show the direction and the significance of these differences (Chart 7). The results are very similar to that observed in population groups at high risk for colon cancer (20). By contrast, the anti-STFA-negative patients showed a proliferating cell distribution pattern similar to the controls ($p$ is not significant).

DISCUSSION

Several attempts have been made to identify phenotypic markers of colon cancer risk (19). Studies in rodents and in humans demonstrated that in the colorectal mucosa a shift of the proliferative compartment toward the luminal surface can be considered, under certain conditions, an early expression of proneness toward cancer (5, 7, 10, 11, 18, 20). In particular, the study of frequency histograms of the proliferating cell distribution in 11 crypt compartments of equal size appears to be a very reliable...
tool for the identification of population groups with hereditary predisposition to colon cancer and shows some prospect for the detection of persons at risk in the general population (20). In our study, this analysis has been applied to the colonic mucosa of patients affected by UC. This disease represents a satisfactory model for studies of the relationship between colonic cell renewal and cancer risk in humans. Several observations support this view: (a) UC is characterized by periods of active inflammation and altered cell turnover (4, 6, 8, 13, 22); (b) the disease represents a condition at risk for colon cancer (14, 16, 23); in our experience, this risk appears after 7 years of clinical history and increases along with the duration of the disease (3); (c) the inflammatory lesions are uniformly distributed in the mucosa, thus representing an advantage for in vitro studies on randomly removed endoscopic biopsies.

In our UC patients, the major proliferative zone was located in the lower third of the crypts as occurred in the controls. However, in 7 of 9 patients, the frequency of [3H]TdR-labeled cells in the upper crypt region was greater that in the controls, thus indicating a shift of the proliferative area towards the surface.

Under certain conditions, the rectal mucosa of patients with UC can show an expansion of the proliferative compartment that may be considered a physiological phenomenon. Previous studies hypothesized that in active UC the abnormal distribution of proliferating cells could represent reaction to an enhanced cell extrusion from the lumen that requires a high number of newly formed cells. This result could be obtained by a shortening of the cell turnover time and by an enlargement of the proliferating cell pool (4, 6, 8, 13, 22).

Our UC patients did not show clinical or morphological signs of severe active disease, and the labeling index values in the whole rectal crypts confirmed that the cell renewal was not enhanced. Therefore, the high labeling index in the upper 40% of the crypts observed in some of our cases may indicate a failure in the control of DNA synthesis and in cell differentiation and maturation. The preneoplastic significance of this alteration is suggested by the similarity with results obtained in population groups in which the high colon cancer risk is well defined (10, 11, 18, 20).

Five UC patients exhibited a positive immunohistochemical reaction indicating the presence of STFA antigen in the epithelial cells of the rectum. A relationship between this antigenic property and an abnormal proliferative pattern is suggested by the cumulative results in STFA-positive and -negative patients. Biopsies unreactive with this antiseraum had a cell growth pattern very similar to that of the controls; on the contrary, antigen-positive biopsies exhibited a proliferating cell distribution in the crypts significantly different from that in the normals.

Anti-STFA was previously utilized to identify subpopulations of colonic neoplastic cells (15). High percentages of antigen-positive cells were observed in cultures from villous and tubulo-villous human colonic adenomas. Moreover, cell lines of human colonic carcinomas or explants of colonic carcinomas showed a STFA positivity. By contrast, the normal colonic mucosa does not exhibit these antigenic characteristics. The specificity of these STFA antigens in colonic neoplasms has been established by their absence in human tumors of other sites (15). The significance of fetal antigens in neoplastic cells is not yet clarified, but it is possible that it reflects a disturbed differentiation of a specific cell population (2, 26).

In our study, the presence of STFA was demonstrated in rectal epithelial cells without morphological abnormalities. However, these cells exhibited an abnormal growth pattern related to a predisposition to neoplastic development. These results suggest that the presence of certain fetal antigens may indicate an expansion of the colonic stem cell population or arrested differentiation, thus representing a phenotypic marker of cancer risk in the colonic mucosa. We have also observed antigenic and cell renewal abnormalities in patients affected by UC for 1 and 2 years. It is generally accepted that colon cancer risk in UC patients occurs many years after the beginning of UC (3, 14, 16, 23). A relationship between duration of UC and cell-proliferative abnormalities has been also demonstrated (1, 4, 5). However, the presence of phenotypic abnormalities related to colon cancer risk in patients affected by the disease for a few years suggests that the cancer proneness in UC may be evident after the first stages of the disease. Work is currently in progress to verify this hypothesis.

REFERENCES


Proliferative and Antigenic Properties of Rectal Cells in Patients with Chronic Ulcerative Colitis

Guido Biasco, Martin Lipkin, Andrea Minarini, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/44/11/5450

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.