Correlations between Rectal Mucosa Cell Proliferation and the Clinical and Pathological Features of Nonfamilial Neoplasia of the Large Intestine

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ABSTRACT

An in vitro study of proliferative activity as shown by immunohistochemical detection of the uptake of bromodeoxyuridine was run on rectal biopsies from 400 patients with nonfamilial large bowel neoplasia: 200 adenoma; 50 adenocarcinoma; 50 adenoma plus adenocarcinoma. The controls were 400 subjects with negative personal and family histories of colorectal neoplasia. The number and height distribution of bromodeoxyuridine positive cells were determined by dividing the crypt into five longitudinal compartments. The total labeling index and the labeling index of each compartment were higher in all three groups compared with the controls. In subjects with adenoma, total labeling index and labeling index values were correlated with tumor size and decreased in function of the duration of the polyp-free colon state. The major zone of DNA synthesis had shifted to the intermediate and surface crypt compartments in all three groups. This stage II abnormality was more marked in adenoma patients with a high degree of dysplasia and in those with adenoma plus adenocarcinoma. Hyperproliferation and the proliferative compartment shift are cytokinetic abnormalities that coexist in the flat rectal mucosa of patients with colorectal neoplasia. Nonetheless, they are independent, controlled by different factors, and are expressions of different biological aspects of large bowel carcinogenesis.

INTRODUCTION

There is abundant in vitro (1-8) and in vivo (9, 10) autoradiographic evidence of major quantitative and qualitative changes in large intestine mucosal proliferation in colorectal adenoma and adenocarcinoma. These abnormalities have been studied in relatively small case series. Nonetheless, their relation to large bowel carcinogenesis is such that particular cytokinetic profiles are used as markers of enhanced susceptibility to carcinoma (11-13).

We have recently perfected an immunohistochemical assay based on the in vitro uptake of BrdUrd2 (14), which is less time consuming than conventional autoradiography but provides comparable cytokinetic data, and have used this method to investigate rectal mucosal cell proliferation in a large series of patients with nonfamilial colorectal neoplasia. This paper assesses the correlation between such proliferation and the clinical and pathological features of the preneoplastic and neoplastic lesions.

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1 To whom requests for reprints should be addressed.
2 The abbreviations used are: BrdUrd, bromodeoxyuridine; C1-s, mean compartmental distribution of labeled cells; LI-s, percentage ratio between the number of labeled cells and the number of cells in each compartment; MAb, monoclonal antibody; P1-s, percentage of compartments containing at least 1 labeled cell; TLI, percentage ratio between the number of labeled cells and the total number of cells in a column.

MATERIALS AND METHODS

Patients

At least three endoscopic anterior rectum wall mucosa biopsies were taken from each subject at a mean distance of 1.5 cm from the anus. X-prep was used in all cases to minimize changes in histological architecture (crypt height) and cell proliferation modifications (enhanced LI) that might be induced by preparation for endoscopy (15, 16), and the examination itself was always performed between 9 and 11 a.m., since a circadian variation in colorectal mucosa proliferation has been hypothesized (17). No dietary restrictions were imposed and no radiological or continuous drug treatment had been administered in the previous 3 months. Four groups of patients were considered.

Group 1: Controls. Group 1 was composed of 184 men and 216 women ages 15-94 years (median, 58 years) with a negative pancolonic and familial history negative for colorectal neoplasia and inflammatory bowel disease.

Group 2: Adenomas. In this group were 132 men and 68 women ages 25-85 years (median, 60 years) with single (120 patients) or 2-27 (80 patients) nonfamilial adenomas measuring 0.3-4 cm. The sum of the dimensions of synchronous adenomas was used to express the size of the adenomatous tissue. A histological distinction was drawn between adenomas with high-grade and low-grade dysplasia (14). Adenomas were totally removed (Waye’s “clean colon”) (18) at the same times the biopsies were taken in 84 cases. In the remaining 116 cases, endoscopic and/or surgical clearance had been carried out 3-76 months earlier (median, 42 months) and this state (Waye’s “negative colon”) (18) was still present. The duration of Waye’s “polyp-free colon” (18) was assessed in each case with respect to the determination of cell proliferation.

Group 3: Adenocarcinomas. 92 men and 58 women ages 31-90 years (median, 64 years) with nonfamilial colorectal carcinoma diagnosed on inclusion in the study (97 patients) or treated in the preceding 20 months (53 patients). A histological diagnosis of adenocarcinoma and tumor-nodes-metastasis staging and grading (19) were established in all cases. Four patients presented small tumors with the histological features of Foerstott’s de novo adenocarcinoma (20). Neither this nor the previous group included patients with adenomas containing invasive carcinoma.

Group 4: Adenomas plus Adenocarcinoma. There were 29 men and 21 women ages 37-78 years (median, 58 years) with colorectal adenocarcinoma and synchronous single or multiple adenomas in group 4. The clinical and pathological parameters examined were those described for groups 2 and 3.

The groups were matched for age, sex, and geographical location in view of the influence of demographic characteristics on large bowel mucosal proliferation (21, 22).

Immunohistochemistry of Cell Kinetics

A slightly modified version of our previously described method (14) was used. Briefly, the specimens were immediately orientated and embedded in paraffin. A slightly modified version of our previously described method (14) was used. Briefly, the specimens were immediately orientated and embedded in paraffin. A slightly modified version of our previously described method (14) was used. Briefly, the specimens were immediately orientated and embedded in paraffin. A slightly modified version of our previously described method (14) was used. Briefly, the specimens were immediately orientated and embedded in paraffin. A slightly modified version of our previously described method (14) was used. Briefly, the specimens were immediately orientated and embedded in paraffin. A slightly modified version of our previously described method (14) was used. Briefly, the specimens were immediately orientated and embedded in paraffin. A slightly modified version of our previously described method (14) was used. Briefly, the specimens were immediately orientated and embedded in paraffin. A slightly modified version of our previously described method (14) was used. Briefly, the specimens were immediately orientated and embedded in paraffin. A slightly modified version of our previously described method (14) was used. Briefly, the specimens were immediately orientated and embedded in paraffin. A slightly modified version of our previously described method (14) was used. Briefly, the specimens were immediately orientated and embedded in paraffin. A slightly modified version of our previously described method (14) was used. Briefly, the specimens were immediately orientated and embedded in paraffin.
BrdUrd taken up by the nucleus with an anti-BrdUrd monoclonal antibody (mouse IgG1) (Becton-Dickinson, Mountain View, CA) diluted 1:50 with phosphate buffered saline (containing 140 mM NaCl, 2.68 mM KCl, 8.1 mM NaH2PO4, and 1.4 mM KH2PO4, pH 7.2) following endogenous peroxidase blockade with 3% H2O2 and DNA denaturation with 2N HCl at 37°C for 30 min. The slices were then weakly contrast-stained with hematoxylin-eosin, dehydrated, clarified, and mounted in Permount (Fisher Scientific, Fair Lawn, NJ) for examination under a standard light microscope.

**Immunohistological Analysis**

Ten sections from each biopsy were examined. Attention was confined to longitudinally oriented crypts visible along their entire length. The histological architecture of the labeled epithelial nuclei, especially its relation to the labeled pericryptal lymphocytes, was used as an indicator of sufficient exposure and uptake of BrdUrd. The restrictions imposed by these criteria resulted in the analysis of a mean number of 30 crypts/subjects. Since the distribution of proliferating cells cannot be effectively and correctly compared in crypts of different height (15), an initial check was made of the absence of significant differences between the mean crypt heights (expressed as the number of cells in each half-crypt): Group 1, 52.4 ± 0.8 (SEM); Group 2, 55.7 ± 0.3; Group 3, 57.2 ± 0.4; Group 4, 55.2 ± 0.8. The number and height distribution of labeled nuclei were determined by dividing each half-crypt or column into five equal longitudinal compartments from the base (No. 1) to the mouth (No. 5) of the crypt. The TLI and the compartment labeling indices (LI1-5) were calculated. Compartment labeling percentages (P1-5) and the mean compartment distribution of BrdUrd positive cells (C1-5) were also determined.

**Statistics**

Student's test for unpaired data was primarily used to determine the significance of differences between the means of the cytokinetic data. Wilcoxon's rank sum test was used when the data were abnormally distributed. The χ² test and Fisher's exact test were used for inference on proportions. Linear regression and Pearson's relation coefficient were used to evaluate relations between the cytokinetic indices and certain clinical and pathological parameters.

**RESULTS**

The mean cytokinetic values for the four groups are shown in Table 1. TLI and LI1-5 were significantly increased in Groups 2-4, together with an increase in P1-5. The compartment distribution of BrdUrd positive cells in general indicated a shift of the zone of maximum DNA synthesis from the base (reduced C1) to the middle and surface (increased C3-5) of the crypt in the three groups. The differences between the means were mostly significant or highly significant. All these differences became more marked in the passages from Group 2 to Group 4 but were only significant for TLI, LI1-5, P5, C3, and C4-5 in Group 4 versus Group 2. From the immunohistological standpoint, the changes in cell kinetics took the form of polymorphic crypt mingling, with different proliferation patterns (Fig. 1). A single microscope field, in fact, displays both crypts in which the BrdUrd positive cells are strictly confined to the deeper two-thirds and others with a uniform distribution of labeled cells along the entire axis. In other crypts, the base is devoid of proliferating cells, whereas these are evident on the middle and mouth segments. Lastly, some crypts present an intermediate proliferation picture, namely positive cells over their entire length, but more heavily concentrated at the mouth.

The 84 “clean colon” patients in Group 2 were considered separately and were also divided into subgroups according to adenoma diameter (<1 cm and >1 cm) and grade of dysplasia. The mean TLI and LI1-5 of the 40 patients with adenomas <1 cm were used to evaluate relations between the cytokinetic indices and certain clinical and pathological parameters.
cm were not statistically different from the controls, but significantly lower than in the subgroup of 44 patients with adenomas >1 cm (Fig. 2). Distribution of cell proliferation along the crypt was comparable in both subgroups, and the changes in their mean \( P_{1-5} \) and \( C_{1-5} \) values vis-à-vis the controls reflected those of group 2 as a whole and were thus indicative of an upward shift of the proliferation zone. Discrete correlations were noted between adenoma size and TLI and LI\(_{2-5}\) (Pearson’s coefficient: TLI, 0.74; LI\(_1\), 0.39; LI\(_2\), 0.46; LI\(_3\), 0.58; LI\(_4\), 0.41; all \( P < 0.001 \)) but no significant linear relations with \( P_{1-5} \) and \( C_{1-5} \). A comparison between the means for the 65 patients with low-grade dysplasia and the 19 with high-grade dysplasia adenomas showed that the TLI and LI\(_{1-5}\) values were much the same, whereas \( P_{1-5} \) and \( C_{1-5} \) were higher and \( P_{1-2} \) and \( C_{1-2} \) lower in the latter subgroup. Most parameter values were significantly different from the controls in both subgroups, in keeping with the overall proliferation picture for group 2. Lastly, possible proliferation differences in the more superficial segments of the crypt were sought by comparing the compartment 4 and 5 labeling patterns in each subgroup. These were the same for compartment 4, whereas compartment 5 labeling was more frequent in patients with high-grade dysplastic adenomas (Table 2).

The proliferation profile of the 116 polyp-free colon patients in Group 2 mirrored that of the group as a whole. None of the kinetic parameters was linearly correlated with the duration of polyp-free colon, although analysis of the means showed a significant decrease of TLI and LI\(_{1-5}\) when this state lasted longer than 2 years (85 patients) (Fig. 3). Distribution of proliferation along the crypt, on the other hand, was unchanged,

### Table 2 Frequency of crypt compartment 5 labeling in adenomas with low-grade versus high-grade dysplasia

<table>
<thead>
<tr>
<th>Compartments</th>
<th>Labeled( ^a )</th>
<th>Unlabeled</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-grade</td>
<td>8 (12)</td>
<td>57 (88)</td>
<td>65 (100)</td>
</tr>
<tr>
<td>High-grade</td>
<td>8 (42)( ^a )</td>
<td>11 (58)</td>
<td>19 (100)</td>
</tr>
</tbody>
</table>

\( ^a \) Number and percentage (in parentheses) of cases with at least 1 labeled compartment.

\( ^b \) \( P = 0.007 \) versus low-grade dysplasia (Fisher’s exact test).

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Fig. 1. Crypts with expansion and/or shift of the proliferative compartment observed in the same biopsy specimen. Immunohistochemical staining with anti-BrdUrd monoclonal antibody; original magnification × 100.

Fig. 2. Rectal mucosal cytokinetic values. Adenomas, >1 cm versus <1 cm.

Fig. 3. Rectal mucosal cytokinetic values. Polyp-free colon <2 years versus >2 years.

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apart from a slight decrease in the involvement of compartment 5 (Fig. 3).

No significant correlations or differences were observed when groups 3 and 4 were divided into subgroups according to grade and stage and the interval between surgery and admission to this study.

A quantitatively and qualitatively normal cell kinetics was observed in all 4 cases of de novo adenocarcinoma.

DISCUSSION

Hyperproliferation is the most common finding in patients with non-familial colorectal neoplasia and takes the form of an expansion of the proliferative compartment and enhanced DNA synthesis all along the crypt, coupled with an increased TLI (7, 23–26). Recent studies, however, have not revealed significant changes in the mean mucosal TLI (27, 28). Some discrepancies also relate to the different behavior of the TLI in adenoma as opposed to adenocarcinoma. According to the report of Bleiberg et al. (24), higher means are noted in adenocarcinoma, whereas Terpstra et al. (23) found higher values in adenoma. In neither case, however, was the difference significant. Differences in adenoma size may influence the mucosal TLI (23), while in more general terms the wide variability of this parameter from one crypt to another may invalidate calculation of the means (8, 29), especially when a small series is analyzed [the largest (23) comprises only 45 cases of colorectal neoplasia]. In our very much larger series, mucosal proliferation, as shown by the total and compartment LI values, was very much higher in cases of neoplasia than in the controls and rose progressively from adenoma to adenocarcinoma alone and with adenoma. Our results thus point to progressive exacerbation of mucosal epithelial hyperproliferation associated with neoplasia of the large bowel. Adenomas with a diameter of <1 cm were not accompanied by changes in the mucosal TLI and compartment LI values. Terpstra et al. (23), too, observed only slight TLI increases and these were in any event smaller than those found in subjects with larger adenomas. The greater degree of proliferative activity in large adenomas in terms of LI parallels the greater risk of carcinomatous transformation (30, 31) and the onset of metachronous neoplastic lesions (32).

A different alteration (stage II abnormality) (29) has been described in the mucosa of patients with colorectal neoplasia. It consists of a shift of the major zone of DNA synthesis from the base to the intermediate and superficial portions of the crypt and has been observed as a focal cytokinetic situation in individual crypts or columns of patients with sporadic adenomas (29) or adenocarcinomas (7), familial polyposis (4), familial colorectal carcinoma without polyposis, and their collateral relatives (11). The literature suggests that its low frequency should not influence mean cytokinetic values (8, 27). In our study, however, it was readily observable immunomorphologically, and its frequency was such as to alter certain within-individual and within-group means. The concentration of BrdUrd positive cells, in fact, was lower than the controls in the deep compartments and higher in the surface compartments in groups 2, 3, and 4.

The most interesting finding to emerge from this study is that hyperproliferation and the stage II shift coexist in the flat mucosa of patients with neoplasia but are independent, dissimilarly related to the pathological and clinical parameters, and probably under the control of different factors and mechanisms. In brief, our data indicate that proliferation is related to the size and chronology of preneoplastic lesions, whereas these factors do not alter the location of the proliferative compartment, the shift of which to the surface is linked to histological parameters indicating the degree of differentiation of preneoplastic tissue (dysplasia) or the adenoma plus carcinoma association. This independence of hyperproliferation and the stage II abnormality, in effect, has been very recently established in other preneoplastic colorectal conditions. In ulcerative colitis, for example, inflammation stimulates compensatory epithelial cell proliferation (33). The stage II shift, on the other hand, is not triggered in this way and persists when the TLI returns to normal during the quiescent stages (28). Moreover, we have previously observed this shift as a late kinetic expression of the mutagenic effect of radiation (34) occurring when the hyperproliferation induced by acute damage has subsided (35). Under these conditions, however, the onset of hyperproliferation may be related to an inflammation induced change in the mucosal microenvironment (33), but this explanation does not apply to colorectal adenoma or carcinoma. Since external factors, particularly eating habits, have been shown to be important in the genesis of colorectal carcinoma (36) and maintenance of hyperproliferation (25, 37, 38), it may be that radical changes in such habits or significant alteration of the lumen chemistry (39) following endoscopic and/or surgical establishment of a negative colon normalize proliferation. In any event, persistent hyperproliferation is of substantial significance in intestinal carcinogenesis, since neoplastic transformation is facilitated in tissues with a high proliferative index (33, 40). Another reason is that expansion of the proliferation zone results in the persistence of S-phase cells on the surface of the mucosa in direct contact with mutagens present in the feces (40).

The stage II shift has a different nature. It is a reliable premorphological marker of an intrinsic risk of neoplastic transformation (29). In addition, it is a kinetic profile frequently noted in the adenomatous crypt (10, 14, 41) and an early step in the histogenesis of adenoma (1). It was not observed in our four cases of de novo adenocarcinoma. This is in line with the data on carcinogenesis in animal models. Colorectal carcinoma induced with 1,2-dimethylhydrazine in CF-1 mice is accompanied by kinetic abnormalities similar to those observed in humans, including an initially reversible reaction in the form of expansion of the proliferative compartment (42), followed by an upward shift of the major zone of DNA synthesis (29). Mice develop multiple adenomas, some of which may progress to adenocarcinoma (43). By contrast, BD IX rats treated with this carcinogen develop microinvasive de novo adenocarcinomas (29), accompanied by a downward shift from the middle third to the base of the crypt. Support can thus be given to Deschner and Maskens’ (29) suggestion that the stage II shift is correlated to the histogenesis of colorectal carcinoma through the adenoma-carcinoma sequence, but not to the genesis of de novo adenocarcinoma.

In conclusion, two cytokinetic abnormalities (hyperproliferation and shift of the major zone of DNA synthesis to the crypt surface) coexist in the flat mucosa of patients with colorectal neoplasia and appear to have different roles and biological implications in intestinal carcinogenesis. It may thus be proposed that they should be regarded as separate markers of the risk of colorectal carcinoma.

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