Autoradiographic Cytokinetics of Colonic Mucosal Hyperplasia in Mice

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ABSTRACT

The cytokinetics of a naturally occurring hyperplastic disease of the colon in mice were determined by autoradiography and compared to kinetic changes seen by others in nonneoplastic and neoplastic colonic disease. Cell cycle parameters were determined using the fraction-labeled mitosis method. Labeling index, labeling pattern, and migration rates were also evaluated. During colonic hyperplasia, there was an increase in variation of DNA synthesis times, resulting in prolongation of the S phase. There also was prolongation of the total cell cycle time and G1 phase. In addition, labeling index was increased 2-fold, the proliferative zone was extended to include the entire crypt column and surface mucosa, and the migration rate was accelerated. These findings parallel the "atypical" cytokinetics found in human and murine neoplastic and preneoplastic disorders of the colon and may be a typical proliferative response of mucosa to a variety of stimuli.

INTRODUCTION

Exaggerated mucosal proliferative activity is in some way associated with the development of large-bowel neoplasia. Individuals with mucosal inflammatory proliferative diseases such as Crohn's disease, ulcerative colitis, and familial polyposis suffer an unusually high risk and early onset of colorectal cancer. "Atypical" cytokinetics has been found prior to onset of neoplasia in ulcerative colitis and familial polyposis and prior to polyp formation in familial polyposis. There is widening of the proliferative compartment, sometimes including the surface mucosa, an increased labeling index, and acceleration of the migration rate (8-10, 14, 15, 17, 18, 33). In normal mucosa, DNA synthesis and cell division occur only in the basal crypt. Abnormal proliferative kinetics also occurs in the flat mucosa of humans between existing tumors or polyps and in the "preneoplastic" mucosa of rodents treated with carcinogens (7, 8, 10, 14, 15, 17, 18, 33). These kinetic changes do not necessarily indicate impending neoplasia, but they are present with high frequency in diseases associated with development of colorectal cancer. Their interpretation is of paramount importance in the clinical management of large-bowel disease.

Transmissible murine colonic hyperplasia, a disease of laboratory mice, may provide insight into the relationship of intensified mucosal proliferation and neoplasia. The etiologic agent, a variant of Citrobacter freundii (3), induces a severe but transient mucosal hyperplasia of the distal colon (4). Experimentally, this disease appears to promote 1,2-dimethylhydrazine carcinogenesis by reducing the latent period for appearance of carcinogen-induced neoplastic lesions (5). This may be analogous to the early onset of colonic neoplasia in humans with proliferative bowel disease. Preliminary studies suggested that transmissible murine colonic hyperplasia has abnormal cell kinetics similar to the pattern seen in preneoplastic colonic mucosa. Mitotic activity and DNA synthesis at the peak of the disease have been observed along the full length of the crypt column and within the surface mucosa (4).

In the present study, the autoradiographic cytokinetics of transmissible murine colonic hyperplasia were determined to define changes that occur in colonic mucosa that is markedly hyperplastic but reversible. Results were compared with kinetic changes seen by others in nonneoplastic and neoplastic colonic disorders.

MATERIALS AND METHODS

All animals were obtained from our own cesarean section-derived, barrier-maintained production colony of systematically outbred NIH Swiss [N:(S)J mice. The colony was determined free of disease, including transmissible murine colonic hyperplasia, by periodic microbiological, serological, and pathological monitoring. Experimental mice were housed in a separate room in polystyrene cages (29 x 19 x 13 cm) with hardwood chip bedding. They were fed Purina laboratory chow and hyperchlorinated water (9 mg/liter) ad libitum. Mice were inoculated p.o. with 2 to 3 drops of a thioglycolate broth culture of the hyperplasia-inducing variant of C. freundii or sterile thioglycolate broth (controls). Autoradiography was performed with 5 vials from 2 lots of [3H]dThd8 (specific activity, 50 and 59 Ci/mmol; Schwarz/Mann, Orangeburg, N. Y.). Each mouse was given an i.p. injection of 1 µCi [3H]dThd per g body weight in 0.1 ml sterile water. Mice were killed with ether at various intervals and necropsied. Colons were flushed with Bouin's fixative, opened on filter paper, placed in Bouin's fixative, embedded in paraffin, and sectioned at 5 µm. Three slides with 1 or 2 sections/slide from each mouse were deparaffinized and dipped into 46° Kodak nuclear track emulsion (type NTB 2) diluted 1:1 with sterile water. The emulsion-covered slides were air dried in total darkness and held in sealed, light-tight slide boxes with desiccator capsules at 4° for 14 days. The slides were then developed at room temperature in Kodak D-19 with slight agitation for 3 min, rinsed in distilled water, fixed in Kodak Rapid Fixer for 4 min, and then rinsed for 30 min in running water. Sections were stained with hematoxylin and eosin. All data obtained for this study were derived from samples of the descending colon.

1 Supported by National Large Bowel Cancer Project USPHS Research Grant 5 R 28 CA 15405 from the National Cancer Institute.
2 The abbreviation used is: [3H]dThd, [methyl-3H]thymidine.
Fraction-labeled mitosis curves (44) were plotted for control mice and mice with moderate and severe colonic hyperplasia. Each time point in the curve through 24 hr was derived from a minimum of 2 litters of mice, and subsequent time points were derived from 1 litter. Litters of 8 or more mice were weaned at 21 days of age, then prefed for 4 days on Purina laboratory chow. At that time, 2 mice from each litter were set aside as controls, and the remainder were inoculated. At 16 days after inoculation, a point of maximal hyperplasia (4), each litter was pulsed with [3H]dThd and killed at different intervals (0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, and every 2 hr thereafter until 40 hr). All mice in a litter were utilized for the same time point. Because of previously demonstrated differences in the [3H]dThd labeling pattern among colons with different degrees of hyperplasia severity (4), average crypt cell column heights were determined by counting the number of cells along both sides of 5 vertical crypts. Control colons (25 to 30 cells/crypt column) and hyperplastic colons with moderate hyperplasia (50 to 60 cells/crypt column) and severe hyperplasia (70 or more cells/crypt column) were selected for use in this study. Eighty-nine control mice, 90 moderately affected mice, and 103 severely affected mice from a total of 449 mice (62 litters) examined were used for this study. The fraction-labeled mitoses were determined and plotted against time for each mouse by counting the fraction of cells labeled among 100 to 200 cells in mitosis (metaphase and anaphase). The maximal allowable background was 1 grain/cell or less. Mitoses with 2 or more grains were considered labeled (48).

The same mice were utilized for determination of labeling pattern, labeling indices, and migration rate. Colons with moderate and severe hyperplasia and control colons were examined at 2 hr after [3H]dThd injection to obtain the labeling pattern. The pattern of labeling was examined to determine the fraction of the crypt occupied by the proliferative zone. The mean of the highest position of labeling in 10 crypts was determined for each mouse, and the fraction of the total crypt was calculated. Data from colons at 1.5 and 2 hr were combined to obtain labeling indices. Labeling indices, the percentage of crypt cells labeled among a total of 300 cells, were determined for each mouse. Only cells from vertically straight, complete crypts were counted. Migration rates were calculated by determining the shift of position of the highest label in the mean of 10 crypts in each control mouse at 1 and 24 hr and in each hyperplastic mouse at 1 and 12 hr after [3H]dThd pulse.

In addition to examining hyperplastic lesions at 16 days after inoculation, kinetics of regressing hyperplastic lesions was also examined at 28 days after inoculation. Mice were killed 1 and 24 hr after [3H]dThd injection (3 control and 10 to 11 inoculated mice per time point) to obtain migration rates. Fifteen inoculated mice were selected with moderate hyperplasia: 6 at 1 hr and 9 at 24 hr. Labeling indices were calculated from mice killed at 1 hr.

RESULTS

The fraction-labeled mitosis curve for cells of the hyperplastic colonic mucosa was altered compared to that for controls (Charts 1 and 2). Little difference was apparent between the data from moderate and severe lesions except between 8 to 12 hr. At that time, the shape of the curve of moderate lesions was similar to that of controls. Only the curve for the severe lesion was plotted for illustration since it differed most from the control curve. The interval from the midpoint of each peak (cell cycle time) was approximately 22 hr in controls and between 27 and 28 hr in severe hyperplasia. The duration of the maximum G2 and M phases was estimated by measuring the interval between [3H]dThd injection and the point at which 100% of the mitoses were labeled. Both control and hyperplastic colons were equally and near maximally labeled at 2 hr. The M phase did not differ appreciably during hyperplasia, when measured from the point of labeling onset to the point of maximal labeling. It was approximately 1.5 hr. Marked alterations of the S phase occurred during hyperplasia. The mean duration of DNA synthesis, approximated by the time between the point of 50% labeled mitosis on the ascending and descending portions of the curve, was 8.75 hr in controls. In comparison to the curve derived from control data, the angle of the slope of transition from the peak to the trough of the curve was more obtuse, the trough was shallower, and the interval between peaks was longer in hyperplasia. This indicated S phases of prolonged and unequal length in hyperplasia. Since the trough did not dip below the 50% label point, S phase could not be estimated as in controls. However, if
the midpoint of the descending portion of the curve is used instead of the 50% label point, the S phase in hyperplasia was approximately 12 hr long. This would actually be an underestimate. The total of each of these intervals was subtracted from the cell cycle time to obtain G₁ phase lengths of approximately 10 hr in controls and 13 hr in hyperplasia.

The position of labeling during hyperplasia differed markedly from controls (Table 1). The relative position of cells in the proliferative zones of control and hyperplastic colons was calculated from mice sampled 2 hr after [³H]dThd injection. There was an expansion of the proliferative zone from the basal one-third of the crypt in controls (Fig. 1) to the basal one-half in moderate hyperplasia (Fig. 2). Severe hyperplasia had DNA synthesis and mitotic activity throughout the crypt column and surface mucosa (Figs. 3 to 5). Labeling indices of moderate and severe hyperplasia were significantly elevated above that of controls (Table 2).

Table 1

<table>
<thead>
<tr>
<th>No. of mice</th>
<th>Crypt column height (cells)</th>
<th>Proliferative zone height (cells)</th>
<th>Fraction of crypt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10 27.0 ± 2.4</td>
<td>9.0 ± 0.9</td>
<td>0.33 ± 0.02</td>
</tr>
<tr>
<td>Hyperplastic</td>
<td>5  52.8 ± 3.4</td>
<td>30.5 ± 4.0</td>
<td>0.58 ± 0.05</td>
</tr>
<tr>
<td>Moderate</td>
<td>6  72.7 ± 1.0</td>
<td>67.7 ± 2.9</td>
<td>0.93 ± 0.03</td>
</tr>
<tr>
<td>Severe</td>
<td>5  672.7 ± 1.0</td>
<td>67.7 ± 2.9</td>
<td>0.93 ± 0.03</td>
</tr>
</tbody>
</table>

Mean ± S.E.

Table 2

<table>
<thead>
<tr>
<th>No. of mice</th>
<th>Crypt column height (cells)</th>
<th>Labeling index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13 27.6 ± 2.4</td>
<td>10.7 ± 1.9</td>
</tr>
<tr>
<td>Hyperplastic</td>
<td>12 54.4 ± 3.3</td>
<td>16.8 ± 2.8</td>
</tr>
<tr>
<td>Moderate</td>
<td>13 90.5 ± 12.1</td>
<td>23.3 ± 6.1</td>
</tr>
<tr>
<td>Severe</td>
<td>15 27.7 ± 2.0</td>
<td>9.5 ± 1.1</td>
</tr>
</tbody>
</table>

Mean ± S.E.

The indices from 1.5 and 2 hr after [³H]dThd injection were combined for control colons and for moderately and severely hyperplastic colons for comparison. Statistically significant differences occurred between each of the groups (p < 0.001 between controls and moderate or severe hyperplasia and p < 0.01 between moderate and severe hyperplasia).

The transit time of cells migrating through the nondividing segment of the crypt column was markedly decreased in hyperplasia (Table 3). Only moderately hyperplastic lesions could be evaluated since label was already present in the surface mucosa in severely hyperplastic lesions at 1 to 2 hr. Since the crypt cell column heights of moderately hyperplastic colons averaged only about twice that of controls, although the migration rate was approximately 9 times faster, cell loss via surface extrusion or death was apparently accelerated in hyperplasia.

In regressing hyperplasia (28 days), crypt heights were comparable to moderate lesions at 16 days except that the labeling index was slightly decreased, the migration rate was greatly prolonged, and the proliferative zone was diminished (Table 4). Although the labeling index and the position of label were evaluated at 1 hr in this study and 2 hr in the previous study, no differences existed between control groups. The cell column height of the proliferative zone in regressing hyperplasia was twice that of controls, but it occupied approximately the same relative position. Migration rates were identical for controls in both studies. The rate in regressing hyperplasia was slower than that found in peak hyperplasia, but it was still accelerated compared to that in controls.

DISCUSSION

Transmissible murine colonic hyperplasia offers an opportunity to examine the colonic mucosa at the limits of benign but marked proliferative response. Data obtained from this disease can be extrapolated to provide insight into diseases with a lesser degree of proliferation. During hyperplasia, an expansion of the proliferative zone, an increased labeling index, a prolongation of the cell cycle time, an increase in variation of DNA synthesis times result-
Cytokinetics of Colonic Hyperplasia

It is tempting to consider these cytokinetic changes, particularly atypical labeling pattern, as characteristic of neoplasia or impending neoplasia. The present study indicates that they occur in severe hyperplasia and probably more minor proliferative states. Clearly, intestinal cell renewal is modified by numerous factors, including establishment of microflora, bile acids, hormones such as pentagastrin and growth hormone, partial surgical resection, starvation, lactation, age, and general state of health (1, 11, 12, 19, 30-32, 37, 46, 50). Mucosal injury results in a more zealous compensatory proliferative response. The labeling index increases, the proliferative zone expands, and crypts become hypercellular following irradiation, acute 1,2-di-methylhydrazine toxicity, induction of enteritis in mice by Salmonella typhimurium, and nonspecific injury to the cecal mucosa by foreign body implantation (2, 13, 25, 36, 41). Therefore, the intestinal mucosa appears to respond similarly to various stimuli that induce proliferation. When the need demands, the pool of dividing cells enlarges by expansion of the proliferative zone from the crypt base to higher levels along the crypt column. The number of cells synthesizing DNA (and dividing) increase with acceleration of the migration rate. Extra cells are retained and crypts elongate. When stimulation is more intense, the proliferative zone is capable of expanding to include the entire length of the crypt and surface mucosa. Under these circumstances, a prolonged S phase and cell cycle time occur and resemble the kinetics seen in neoplasia. Despite prolongation of the cell cycle time, the expanding population of dividing cells results in an increase in migration rate and retention of immature cells. If human colonic mucosa responds in the same way, interpretation of atypical cytokinetic changes must be performed with caution. The high correlation of hyperplasia with eventual neoplasia and the known promoting effect of hyperplasia on experimental colon carcinogenesis (5, 20, 39) imply that the finding of atypical cytokinetics is highly significant.

ACKNOWLEDGMENTS

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REFERENCES


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24. Fig. 3. Autoradiograph of the descending colon from a mouse with severe hyperplasia. Labeling is present in the crypt base and crypt cell columns are greater than 70 cells high. H & E, x 125.

25. Fig. 4. Autoradiograph of the descending colon from a mouse with severe hyperplasia. Labeling is present in the crypt base and crypt cell columns are greater than 70 cells high. H & E, x 125.

26. Fig. 5. Descending colon from a mouse with severe hyperplasia. A mitotic figure is present in the surface epithelium (arrow). H & E, x 340.
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