Gland to Gland Heterogeneity in Histologically Normal Mucosa of Colon Cancer Patients Demonstrated by Monoclonal Antibodies to Tissue-specific Antigens

Norman R. Hughes, Ronald S. Walls, Ronald C. Newland, and John E. Payne

University of Sydney Departments of Surgery [N. R. H., J. E. P.] and Medicine [R. S. W,]. Clinical Sciences Building, and Department of Anatomical Pathology [R. C. N.], Repatriation General Hospital, Concord, New South Wales, Australia 2139

ABSTRACT

Two of the monoclonal antibodies to tissue-specific antigens of isolated colonic glands revealed gland to gland heterogeneity of antigen expression in sections of the histologically normal colonic mucosa from patients with colorectal adenocarcinoma or various nonmalignant conditions. A colon-specific goblet cell antigen (designated 3NM) was absent from rare, solitary glands randomly distributed among the otherwise strongly stained glands of distal colon. These 3NM-negative glands stained normally for the other two antigens studied and appeared morphologically and histochemically normal. They occurred in 11 of 13 cancer patients and in each of the five patients with benign conditions with median incidences of 2.2 and 0.4 per 1000 glands, respectively. Gland heterogeneity was also demonstrated in both patient groups for a cell membrane antigen (designated 6NM). In cecum and ascending colon, glands staining strongly and weakly for 6NM were found intermixed. The weakly stained glands tended to predominate in cecum and first part of ascending colon, but they were completely replaced by strongly stained glands in more distal colon. The heterogeneity shown by both 3NM and 6NM appeared due to phenotypically distinct cell clones. Our observations suggest that normal colonic mucosa contains antigenically diverse gland populations.

INTRODUCTION

In an investigation of the failure of colonic tumors to express some normal tissue components, we prepared a number of monoclonal antibodies to isolated glands of normal colon and used these to demonstrate, by immunohistochemical staining, changes in expression of three tissue-specific antigens in hyperplastic polyps, adenomas, and colorectal carcinoma (1). In the course of this study, we noticed that one of the antigens, which was absent in hyperplastic polyps and colorectal adenocarcinoma, was also absent from rare, solitary glands in the histologically normal mucosa of the cancer patients. To investigate this phenomenon, we prepared sections of Swiss rolls of long lengths of colonic mucosa and used these to determine which of the antigens show such gland heterogeneity, the frequency of unstained glands in individual colons, and their spatial relationship to carcinoma. We now report that glands of the histologically normal colonic mucosa show gland to gland heterogeneity of expression for two antigens at different anatomical sites. In both cases the gland heterogeneity appears to be based upon the presence of distinct cell clones which show absence or markedly decreased expression of antigen. Our observations suggest that normal colonic mucosa contains antigenically diverse gland populations which may be of interest as markers of increased risk or of significance for development of colorectal neoplasia.

MATERIALS AND METHODS

Patients Studied. Tissues were obtained from patients undergoing surgery at the Repatriation General Hospital, Concord, New South Wales. Diseases included 19 colonic adenocarcinomas, 4 diverticular disease of sigmoid colon, and 1 abscess of appendix and cecum. Sigmoid colon was also obtained within 2 h of death from a kidney donor. Anatomical sites of the tumors were cecum (2 tumors), ascending colon (5 tumors), transverse colon (1 tumor), and sigmoid colon (12 tumors), 2 carcinomas being present in one specimen. All tissues were obtained following guidelines set out by the National Health and Medical Research Council of Australia.

Tissues Studied. These were strips of colonic mucosa, 4 to 52 cm in length. A strip of mucosa was taken at a distance of 2 to 16 cm from the carcinoma in 9 of the cancer patients. Excluding a 1-cm length at surgical margins, a single strip running the full length of each colon was taken for study from all other specimens. Ten strips included a wedge of adenocarcinoma. Anatomical sites from which the strips were obtained were cecum and ascending colon (6 samples), transverse colon (2 samples), and sigmoid colon (17 samples).

Processing Tissues. Surgical specimens were collected from the operating theater, transported on ice, and processed immediately. Tissues were fixed in ice-cold 4% paraformaldehyde in 75% ethanol. To obtain mucosa, the opened colon was placed in fixative for 2 h, and a strip of mucosa approximately 1 cm wide was dissected free of muscle coat, placed in fresh fixative, and stored on ice for a period of 24 h. The strip was then trimmed and fashioned into one to 5 rolls (orientation of rolls in relation to the original strip was maintained). Tissue was dehydrated in 4 changes of absolute ethanol at —15°C over 2 days, cleared in 3 changes of chloroform at —15°C, and a further 2 changes at room temperature. The tissue was then vacuum embedded in 4 changes of paraffin wax (Paramat; BDH Chemicals, Ltd.) over a period of 1 h. Serial sections were cut at 3 µm and dried at 58°C for 1 h. From each roll, separate sections were stained for each of the 3 antigens, another with hematoxylin-eosin, and a fifth section with the high iron diamine-Alcian blue procedure to demonstrate sulfomucins and sialomucins (2).

Monoclonal Antibodies. Preparation of monoclonal antibodies 3NM-26-32, 17NM-20-20, and 6NM-56-39 has been described elsewhere (1). In brief, colonic glands were isolated from macroscopically normal mucosa of surgical specimens from patients with colorectal carcinoma, and the freshly prepared glands were used to immunize BALB/c mice. Hybridoma cell lines were prepared from sensitized spleen cells and the P3-NS1-Ag4-1 mouse myeloma cell line and selected for antibody specificity by means of radioimmunoassay and immunohistochemical staining of tissue sections. Hybridoma cell lines were grown i.p. in BALB/c mice, and ascites fluids were harvested, clarified by centrifugation at 20,000 × g, and stored frozen. The same 3 batches of ascites fluids were used throughout the study at dilutions of 1/1000 for antibodies 3NM-26-32 and 17NM-20-20 and at 1/100 for antibody 6NM-56-39.

To obtain a hybridoma secreting antibody to glucose oxidase from Aspergillus niger, a BALB/c mouse was given an injection s.c. of 7 µg of glucose oxidase (type V; Sigma Chemical Co., St. Louis, MO) mixed with Freund’s complete adjuvant and 24 days later of 15 µg in incomplete adjuvant. Thirty days after the second injection, 2 i.p. injections of 4 µg were given 2 days apart, and 2 days later spleen cells were fused with cells of the P3-NS1-Ag4-1 mouse myeloma cell line as described by others (3). Cultures were screened for antibody by radioimmunoassay using glucose oxidase as solid phase and rabbit antibodies to mouse immunoglobulins labeled with iodine-125 as described elsewhere (1). A selected hybridoma was cloned by limiting dilution, and the established cell line was grown i.p. in BALB/c mice to yield ascites fluid containing antibody.

To isolate monoclonal antibody to glucose oxidase, 43 ml of ascites fluid were chromatographed on DEAE-Sephadex (Pharmacia, Uppsala, Sweden) equilibrated with 0.01 M Tris-HCl buffer, pH 7.4. The eluted...
antibodies were then purified by affinity chromatography on a column containing 51 mg of rabbit antibodies to mouse immunoglobulin (1) and by gel chromatography on Sepharose CL-6B (Pharmacia).

Antigens Studied. The antigens recognized by antibodies 3NM-26-32, 17NM-20-20, and 6NM-56-39 were previously (1) designated 3NM, 17NM, and 6NM, respectively, and these names will be used here. The three antigens have yet to be characterized. Each is strongly represented in immunohistochemically stained sections of normal colonic mucosa. 3NM is apparently specific for colon and 17NM for small and large intestine. Both 3NM and 17NM are confined to the mucin vacuole of goblet cells and their secretions. Strong staining for 6NM is seen in the cell membranes separating all colonic gland cells and in the cytoplasmic granules of argentaffin cells (1). The cytoplast of colonic gland cells also stains strongly for 6NM, particularly in the upper part of glands and the surface epithelium, and the antigen is present in the goblet cell secretions. Staining for 6NM is much weaker in small bowel than in colon. 6NM also occurs in certain epithelial cells of some other tissues (1). 3NM tends to low concentrations proximally, but otherwise the three antigens are uniformly distributed throughout the colon (1).

Immunohistochemical Staining of Tissue Sections. A triple-layer immunohistochemical staining procedure with glucose oxidase as a marker enzyme was used. In this procedure, mouse monoclonal antibody which has bound to tissue antigen is then linked via rabbit antibodies to mouse immunoglobulin to an antigen-antibody complex consisting of glucose oxidase and a mouse monoclonal antibody to this enzyme.

To prepare the antigen-antibody complex, 70 mg of the purified monoclonal antibody to glucose oxidase were added to 86 mg of glucose oxidase (type V; Sigma Chemical Co.) in 20 ml of Tris buffer, pH 7.2, and concentrated by ultrafiltration to a volume of 4 ml. After standing at room temperature for 7 h and 24 h at 4°C, the solution was chromatographed on Sepharose CL-6B using the same Tris buffer. Electrophoresis and immunoelectrophoresis, using antisera to mouse immunoglobulin and serum from mice immunized with glucose oxidase, showed that the first fraction that eluted from the chromatograph contained a single protein band in the a-2 region of the electrophoretic pattern which corresponded in position to precipitation arcs given by both the antisera to mouse immunoglobulin and that to glucose oxidase. Some free glucose oxidase was present in a fraction that eluted later in the chromatograph. The fraction containing the monoclonal antibody-glucose oxidase complex was made up to 10 ml with Tris buffer and stored at 4°C. For use in the immunohistochemical staining procedure, 20 μl of the solution were diluted with 1 ml of Buffer-S-Alb.3

To carry out the staining procedure, paraffin sections were passed through xylol and rehydrated in a graded series of alcohol solutions, through xylol and rehydrated in a graded series of alcohol solutions, and then in distilled water, stained with 1% Alcian blue in 3% acetic acid for 5 min, rinsed in saline and washed in Buffer-S for 10 min. Buffer-S-Alb containing rabbit antibodies to mouse immunoglobulin (1 mg/ml) (1) was placed on the section for 1 h, the slide was rinsed and washed as before, and the solution of mouse monoclonal antibody-glucose oxidase complex was placed on the section for 1 h. The slide was again rinsed and washed in Buffer-S for 10 min and then in 0.05 M Tris-HCl buffer, pH 8.3, for 10 min. The substrate solution was prepared essentially as described by others (4) 1 h before use by dissolving 400 mg of β-D-glucose (Sigma Chemical Co.) and 40 mg of nitroblue tetrazolium (Sigma Chemical Co.) in 60 ml of the Tris-HCl buffer, pH 8.3, and standing in a water bath at 37°C. Five hundred μl of the Tris-8.3 buffer, pH 8.3, containing 2 mg of phenazine methosulfate per ml (Sigma Chemical Co.) were added to the substrate solution, and the slides were incubated upright in the solution for 1 h in the dark. Sections were then washed in the Tris-HCl buffer for 30 min and in distilled water for 30 min, and they were left in 2% glutaraldehyde in distilled water overnight. Sections were washed in distilled water, stained with 1% Alcian blue in 3% acetic acid for 5 min to stain acid mucins (5), and mounted in PVP medium (6).

Ascites fluid from a hybridoma to colonic glands, but which showed no monoclonal antibody in its electrophoretic pattern and consistently failed to stain tissue sections, was used at a dilution of 1/100 as a negative control throughout the study.

Incidence of Glands Showing Decreased Antigen Expression. For a gland to be scored as unstained for 3NM, at least half its length had to be seen, and with the plane of section sufficiently close to gland lumen to show mucin vacuoles of goblet cells clearly. Unstained glands seen only in oblique or cross-section were recorded separately. 3NM was absent from the body of all glands scored as negative. Some glands scored negative showed one or more cells containing 3NM in the neck of the gland or in surface epithelium immediately surrounding the gland (e.g., as seen in the 3NM-negative gland illustrated in Fig. 1A).

To determine the incidence of glands which did not stain for antigen 3NM, consecutive glands were counted along a strip of mucosa starting from one end, or the mucosa adjacent to carcinoma if present, and continuing in sequence through each roll to the end of the strip. Glands within polyps present in the sections were not included in the count. In recording unstained glands, it was noted in each case whether these were single or a certain number of contiguous glands. In calculating the incidence of unstained glands in a strip of mucosa, any group of contiguous, unstained glands was counted as one gland.

RESULTS

Gland Heterogeneity Shown by Antigen 3NM. Thirteen of the 19 strips of mucosa from cancer patients stained strongly for antigen 3NM (Table 1). Scattered throughout the histologically normal mucosa of 11 of the 13 strips were occasional solitary glands, or groups of 2 to 4 contiguous glands, which failed to stain for 3NM (Fig. 1, A to D). Similar 3NM-negative glands were found in each of the 5 strips of mucosa from sigmoid colon of those patients with benign conditions. In sections counterstained with Alcian blue, these 3NM-negative glands were highlighted by the brilliant blue staining of the mucin vacuole of their goblet cells and secreted mucus. By contrast, in glands heavily stained for antigen the blue mucin stain was generally obscured by the opaque formazan deposit (Fig. 1, A, B, and D).

The PVP medium used to mount immunohistochemically stained sections allowed nuclei, cell, and gland outlines and connective tissue elements to be distinguished by ordinary light microscopy (e.g., Figs. 1C and 4D). Glands which failed to express 3NM did not differ in size or shape from adjoining antigen-positive glands, and they did not show nuclear crowding or stratification. The Alcian blue counterstain showed normal numbers of goblet cells with mature-sized mucin vacuoles to be present in the glands. When recognized in an adjacent serial section stained with hematoxylin-eosin or the high iron diamine-Alcian blue procedure, their unremarkable histological appearance was confirmed, and both the antigen-negative gland and surrounding glands stained strongly for sulfomucins with no difference in intensity of staining seen.

Incidence of 3NM-negative Glands. The anatomical site from which each strip of mucosa was obtained and the incidence of glands which failed to stain for antigen 3NM, found in the plane of section of each strip, are given in Table 1 and Fig. 2. Of a total of 41,950 glands counted in the 11 strips from patients with colorectal carcinoma who had 3NM-negative glands, there were 100 solitary glands and 8 groups of 2 or 3 contiguous glands which failed to stain for 3NM. The median incidence in the 11 strips was 2.2 negative glands in every 1000 glands counted, with a range of 1 to 4.7 per 1000 glands. Nine

3 The abbreviations used are: Buffer-S-Alb, 140 mM NaCl-3 mM KCl-2 mM MgCl2-10 mM Tris-NaHCO3, pH 7.2, containing 1% bovine serum albumin; Buffer-S, 140 mM NaCl-3 mM KCl-2 mM MgCl2-10 mM Tris-0.05% NaN3, pH 7.2; PVP, polyvinylpyrrolidone.
Fig. 1. Localization of antigen 3NM in paraffin sections of rolls of mucosa from sigmoid colons of patients with colorectal carcinoma. A to C, histologically normal mucosa; D, mucosa adjacent to a carcinoma. With the exception of C, all sections were counterstained with Alcian blue stain for acid mucins which, in the absence of antigen, show here as a gray tone. Antigen appears as black. A, a solitary gland completely unstained for antigen (arrow) among normally stained glands, × 55. B, two adjacent unstained glands, × 143. C, a solitary gland showing absence of antigen from gland cells and secreted mucus and sharp demarcation between antigen-negative and -positive cells of surface epithelium. × 221. D, two unstained glands (arrow) present in the nonmalignant mucosa adjacent to a carcinoma. × 55.
**Table 1** Incidence of unstained glands for antigen 3NM and presence of gland heterogeneity for antigen 6NM in rolls of histologically normal colonic mucosa

<table>
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<tr>
<th>Condition and specimen no.</th>
<th>Segment of colon tested</th>
<th>Total no. of glands counted</th>
<th>No. of unstained glands/1000 glands counted</th>
<th>Total no. of glands counted</th>
<th>Gland heterogeneity^b^</th>
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<tr>
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<td>NC</td>
<td></td>
<td>4730</td>
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</table>

^a^ Consecutive glands counted in the plane of section of the strip of mucosa.

^b^ Glands weakly stained for antigen 6NM as described in the text were present (+) in some part of the strip of mucosa or not seen (−).

^c^ NC, glands not counted because antigen concentration was uniformly low throughout mucosa, or antigen 3NM was entirely absent (Specimens 844 and 25).

^d^ Kidney donor (Specimen 147) and abscess of appendix and cecum (Specimen 68).

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**Fig. 2.** Incidence of glands which failed to stain for antigen 3NM in rolls of histologically normal colonic mucosa from patients with colorectal carcinoma and benign conditions. The incidence of unstained glands was calculated as described in "Materials and Methods."

**Fig. 3.** Distribution of 3NM-negative glands in relation to 4 colorectal carcinomas. Consecutive glands were counted along the whole length of strips of mucosa extending proximally 15 to 52 cm from carcinomas of rectosigmoid or sigmoid colon (carcinoma is present to the left of each distribution shown). Each vertical bar represents a solitary unstained gland (60 glands in all) or a group of 2 adjacent unstained glands (3 examples in all) encountered at the points shown.

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of these strips of mucosa also showed glands seen only in cross-section which did not stain for 3NM. The incidence of such glands ranged from 0.2 to 1.3 per 1000 glands counted, with a median incidence of 0.6. On two occasions a group of 4 contiguous unstained glands were seen in cross-section.

A total of 16,720 glands was counted in the strips of sigmoid colon from the 5 patients with benign conditions (Table 1). Twenty-one solitary, 4 groups of 2, and 1 group of 5 3NM-negative glands were found. The incidence of 3NM-negative glands in the individual colons ranged from 0.2 to 2.9 per 1000
COLONIC GLAND HETEROGENEITY
glands counted, with a median incidence of 0.4. This incidence did not differ significantly from that found in the 13 patients with colorectal carcinoma (\( t = 1.41, \text{df } 16 \)).

Spatial Distribution of 3NM-negative Glands. There was no obvious relationship between spatial distribution of the unstained glands and carcinoma site. The distribution of 3NM-negative glands along strips of mucosa extending proximally from carcinoma of sigmoid colon for distances of 15 to 25 cm is shown in Fig. 3 for 4 colons with a high incidence of the negative glands. Although there appeared to be some degree of clustering in each of the strips (in the sense of most gap lengths being short), calculation of an autocorrelation coefficient (7) to measure dependence between successive gap lengths between the unstained glands found in the longest strip shown in Fig. 3 indicated that the gap lengths were randomly distributed (\( r_1 = -0.101, \) with 95% confidence limits, \(-0.37\) to \(0.31\)).

Staining of Glands for Antigen 17NM. Uniform staining of glands for antigen 17NM was seen in the histologically normal mucosa of all 25 colons studied.

Gland Heterogeneity Shown by Antigen 6NM. Heterogeneity of staining of glands for antigen 6NM was seen in 4 of the 5 strips of mucosa from cecum and ascending colon of the cancer patients (Table 1). It consisted of segments of mucosa showing weakly stained glands intermingled with normally stained glands (Fig. 4, A to D). There was either no obvious staining of plasma membranes, or weak staining of basolateral plasma membranes between some gland cells could be seen. In some weakly stained glands antigen was mostly associated with the mucin vacuole of goblet cells. Others glands showed no staining for 6NM except in some cells of the neck of the gland and its surface epithelium (e.g., Fig. 4C). The microvillous layer of the surface epithelium of the weakly stained glands was usually positive for 6NM in continuity with that of adjacent normally stained gland cells (Fig. 4, C and D). Cells derived from normally stained and weakly stained glands were sharply demarcated in the surface epithelium (as illustrated in Fig. 4, C and D). Segments of mucosa containing such heterogeneity were found adjacent to and extending distally from the 4 carcinomas for a distance up to 9 cm. Staining of glands at the distal ends of the 4 strips was normal. Only 2 of the strips of mucosa from transverse or sigmoid colon showed gland heterogeneity for 6NM. In each case only one unstained gland was found, and these were an estimated 7- and 20-cm distance from the carcinomas present.

The patient with an abscess of appendix and cecum showed a similar heterogeneity of staining of glands for 6NM extending from an unaffected part of the cecum along the ascending colon for a distance of 17 cm. There were relatively few normally stained glands in the cecum, but these became more frequent in passing along the strip, and all glands at the distal end of the strip stained normally.

DISCUSSION

The distinguishing feature of the gland heterogeneity revealed by staining for antigen 3NM was the absence of antigen in small numbers of otherwise unremarkable solitary glands randomly scattered throughout histologically normal mucosa. This discrete appearance of 3NM-negative glands suggested that the heterogeneity was unlikely to be due to transient physiological fluctuations in synthesis by individual glands. If synthesis of 3NM was in fact permanently repressed in the 3NM-negative glands, the question would arise whether they represent a minor population of functionally distinct glands normally present in colonic mucosa, or if they are aberrant.5998

If 3NM-negative glands are abnormal they must contain a phenotypically distinct clone of cells, since each colonic gland is an isolated proliferative unit (8). That a distinct cell clone could be limited to solitary glands scattered throughout mucosa is rendered likely by the demonstration by other workers that intestinal glands are each derived during histogenesis from a single progenitor cell (9). A programming error arising in a stem cell whose progeny become dispersed throughout epithelium of developing hind gut prior to gland formation could thus give rise to the form of gland heterogeneity seen. Alternatively, 3NM-negative glands could arise later in life due to some acquired error in gene expression affecting the stem cells of individual glands. To distinguish between these 2 possibilities, we need to determine if 3NM-negative glands are present in the neonate and if their incidence in the general population is constant at all ages. This would be the case if the glands arise during histogenesis rather than being acquired with age.

We have previously reported that antigen 3NM is also absent from colorectal carcinomas and hyperplastic polyps (1). Concentrations of 3NM are decreased in adenomas in keeping with the decreased mucus production usually shown by adenomatous glands, but there is not the complete loss seen in the other 2 lesions. Although hyperplastic polyps and adenomas are due to different etiological factors, both of which are required in the development of a carcinoma (10). Although we found no obvious spatial relation between 3NM-negative glands and carcinoma or hyperplastic polyps, the common antigen deletion suggests that the 3NM-negative glands in normal mucosa may be a marker of one set of factors relevant to development of colonic neoplasia. Further patients and controls are being studied to determine if a significant association can be confirmed between the incidence of 3NM-negative glands and hyperplastic polyps or colonic neoplasms.

Gland heterogeneity for antigen 6NM differed from that shown by 3NM in both form and anatomical site affected. In contrast to the solitary 3NM-negative glands, those showing decreased expression of 6NM were relatively numerous and, intermingled with normally stained glands, occupied mucosa extending over centimeters of cecum and ascending colon. The sharp demarcation that could be seen in surface epithelium between individual cells of normally stained glands and those staining weakly for 6NM (e.g., as shown in Fig. 4, C and D) indicated that the difference between such glands was based upon the presence of phenotypically distinct cell clones rather than due to physiological fluctuations in activity of individual glands. The uniform staining of glands for 6NM seen in more distal colon also suggested that physiological variations in synthesis between individual glands were an unlikely explanation of the heterogeneity. The appearance of this gland heterogeneity suggests that it is based upon cell clones exhibiting a form of discontinuous phenotypic variation (12).
be a transition zone between small and large bowel for full expression of this antigen. The presence of carcinoma in the specimens could therefore have been coincidental to the heterogeneity for 6NM. That mucosa adjacent to carcinomas of sigmoid colon did not show this heterogeneity for 6NM also suggests that there is no relationship with development of carcinoma. In this context it should be noted that we did find decreased expression of 6NM in carcinomas of proximal colon, although some carcinomas of distal colon did show absence or marked depletion of 6NM (1). It will be necessary to define more closely the anatomical extent of this heterogeneity for 6NM and its incidence in subjects with other bowel disorders in order to determine if there is any association with neoplasia.

Although the significance of our observations has yet to be determined, the presence of gland to gland heterogeneity of expression of normal tissue components within histologically normal mucosa raises certain questions relevant to development of colorectal neoplasia. Differences in expression of normal cell components by individual glands or groups of glands could result in differences in susceptibility to carcinogenic or other substances present in the gut (13, 14). Increased susceptibility to transformation due to decreased antigen expression would perhaps be most pronounced at anatomical regions of the colon normally showing high concentrations of the antigen, e.g., as seen for antigen 3NM in distal colon. Differences in the incidence of such gland heterogeneity at different anatomical sites and in different subjects should then correlate with risk of developing polyps or carcinoma at the site, at least for populations exposed to the appropriate agent(s) (14). Under such circumstances gland heterogeneity for an antigen might prove a marker of risk.

The two examples of gland heterogeneity described in this paper appear to be due to the presence of cell clones which are distinguished from those in the majority of glands by the absence or decreased expression of a normal tissue component. If, as we have proposed elsewhere (1), a progressive antigen deletion is characteristic of neoplastic change, absence of tissue components in some apparently normal glands may represent one of the first consequences of tissue instability in subjects at risk of colonic carcinoma (15). It seems likely that other examples of antigenically distinct gland populations are present in histologically normal colonic mucosa and that some of these may prove relevant to neoplastic change.

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REFERENCES

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