Expression of colonic antigens by goblet and columnar epithelial cells in ileal pouch mucosa: Their association with inflammatory change and faecal stasis

A P Campbell, M N Merrett, M Kettlewell, N J Mortensen, D P Jewell

Abstract
Aims—To investigate colonic metaplasia of goblet and columnar epithelial cells in ileal pouch mucosa; to correlate this with the degree of morphological and inflammatory change; and to assess whether such changes are related to the presence of faecal stasis.

Methods—Biopsy specimens of ileal pouch mucosa were taken from 31 patients (30 with ulcerative colitis, one with familial adenomatous polyposis) either before (eight patients) or after (23 patients) ileostomy closure. A simple morphological technique was used to assess changes in villous height. Inflammatory change was estimated using an established scoring system for pouchitis, and acquisition of colonic antigens was determined by immunohistochemistry using three monoclonal antibodies which recognise components of the two major epithelial cell types in the colorectum. The degree of staining with the monoclonal antibodies was graded and the grades correlated with an index of villous atrophy and with the inflammatory scores.

Results—Five of eight (63%) pre-closure and 15 of 23 (65%) post-closure biopsy specimens showed increased staining with an antibody against components of columnar epithelial cells. One of eight (12%) pre-closure and 15 of 23 (65%) post-closure biopsy specimens stained with an antibody for colonic mucin. Although both types of staining showed a positive correlation with the pouchitis score, they also occurred in the absence of inflammation.

Conclusions—Both goblet and columnar cells acquire colonic characteristics which are incomplete, but may represent a true adaptive response as they can develop in the absence of inflammation. As the change in goblet cells occurs after ileostomy closure, faecal stasis is likely to be a major contributory factor. Changes in columnar cells may occur before ileostomy closure in the absence of faecal stasis.

Acquisition of colonic type characteristics by ileal pouch mucosa is well recognised. Morphological and histochemical changes can be demonstrated in over 90% of pouch biopsy specimens. Despite this, complete colonic metaplasia does not occur, because small bowel characteristics, such as disaccharidase activity, are retained by the pouch mucosa. Faecal stasis within the pouch may be an important aetiological factor in the genesis of the colonic phenotype: (1) colonic characteristics are only found following ileostomy closure; (2) they are most pronounced on the posterior wall of the pouch where there is maximal stasis; and (3) similar mucosal changes can be seen in terminal ileum proximal to Crohn's strictures. However, factors other than stasis must also be important as there does not seem to be any correlation between the efficiency of pouch emptying and the severity of any of the mucosal changes.

Previous studies have largely been confined to changes occurring in goblet cells, with little attention being given to the more numerous columnar epithelial cells. The aims of this study were: (1) to investigate the degree of colonic phenotypic change in pouch mucosa using three monoclonal antibodies which recognise components of the two major epithelial cell types in the colorectum—goblet and columnar cells—and to correlate this with the severity of morphological and inflammatory change within the pouch; (2) to confirm previous findings that changes in goblet cells only occur after ileostomy closure; and; (3) to assess whether any changes in the columnar cells occur before or after ileostomy closure—whether they are related to the presence of faecal stasis.

Methods
This study was approved by the Central Oxford Research Ethics Committee. Pouch biopsy specimens were assessed from 31 patients (30 with ulcerative colitis and one with familial adenomatous polyposis). Biopsy specimens from eight patients were taken before and from 23 after (eight weeks to eight years) ileostomy closure. All biopsy specimens were taken from the posterior wall of the pouch 10 cm above the dentate line. They were compared with nine cases of active
ulcerative colitis (six biopsy specimens, three resections) as well as normal large bowel (n = 2) and normal terminal ileum (n = 7) taken from specimens resected for carcinoma. Immunohistochemistry was carried out on 5 μm frozen sections with the primary monoclonal antibodies PR1A3, 3A5, and 5D5 and 5D5 (courtesy of the Imperial Cancer Research Fund, London) (table 1), using a three stage immunoperoxidase technique modified from that described by Mason and Sammons.8 PR3A5 and 5D5 react with constituents of mucus glycoproteins. In the case of PR3A5 this is a colon specific form of O-acetyl sialic acid. PR1A3 reacts strongly with colonic columnar cells but only shows focal weak positivity with normal terminal ileum. The antibodies were supplied as culture supernatant fluid preserved with 0·05% azide and were used neat. Sections were thawed, fixed in acetone for 10 minutes, and dried in air for at least 15 minutes. Endogenous peroxidase activity was blocked by immersing the slides in a solution of 3% hydrogen peroxide in 0·1% sodium azide. After washing in TRIS-buffered saline (TBS) the sections were exposed to either the primary antibody or to TBS alone for one hour. They were then sequentially incubated for 30 minutes each with rabbit anti-mouse immunoglobulin (Dakopatts UK) diluted 1 in 50 with a 1 in 3 solution of normal swine serum in TBS and peroxidase conjugated swine anti-rabbit immunoglobulin (Dakopatts UK) diluted 1 in 100. The end product was visualised with diaminobenzidine (0·5 mg/ml) in the presence of 0·03% hydrogen peroxide. The sections were lightly counterstained with haematoxylin, dehydrated in alcohol, cleared in xylene and mounted in DPX (BDH laboratory supplies). Tissue from the normal large bowel served as a positive control. All sections were assessed by a single observer (AC) who did not know whether the pouch biopsy specimens were taken before or after closure. Strength of staining of goblet and columnar cells was graded from 0 to 3+, where 0 was negative and 3+ was strongly positive involving most of the cells. Loss of villous height in the pouch biopsy specimens was assessed in the following manner. Measurements of total mucosal thickness (TMT) and crypt depth (CD) were taken from routine haematoxylin and cosin stained sections using a calibrated eye piece (Leitz) at a magnification of ×100. Measurements were only taken where the muscularis mucosae could be clearly identified (17 biopsy specimens, four before and 13 after closure). A minimum of three measurements were taken per specimen (mean 3·778, median 3, range 3–9) and the mean values used to calculate the villous height (VH) and an index of villous atrophy (VH/TMT).4 Acute (ulceration 0–3, neutrophil polymorph infiltration 0–3) and chronic (loss of villous height 0–3, chronic inflammatory cell infiltrate 0–3) inflammatory changes were assessed by the same observer, using an established scoring system for pouchitis.10

Table 1 Results of staining with monoclonal antibodies PR5D5, 3A5, and 1A3

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Immunohistochemical specificity in normal colon</th>
<th>Normal colon and active UC</th>
<th>Normal terminal ileum</th>
<th>Pre-closure pouch</th>
<th>Post-closure pouch</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR5D5</td>
<td>Goblet cell mucus 70 kilodaltons protein/glycoprotein</td>
<td>11/11 3+</td>
<td>7/7 3+</td>
<td>8/8 3+</td>
<td>23/23 3+</td>
</tr>
<tr>
<td>3A5</td>
<td>Goblet cell mucus, membranes, brush border, &gt;200–83 kilodaltons. Colon specific O-Acetyl sialic acid</td>
<td>11/11 3+</td>
<td>7/7 3+</td>
<td>1/8 1+</td>
<td>15/23 1+ to 2+</td>
</tr>
<tr>
<td>1A3</td>
<td>Columnar brush border and terminal bar region</td>
<td>11/11 3+</td>
<td>7/7 3+</td>
<td>5/8 3+</td>
<td>15/23 3+</td>
</tr>
</tbody>
</table>

UC = ulcerative colitis.

Results (table 1)
All biopsy specimens and control samples were strongly positive with the antibody 5D5. Normal colon and colon from the nine cases of ulcerative colitis also showed strong positive staining with PR1A3 and 3A5. Normal terminal ileum was weakly positive with PR1A3 but was completely negative with PR3A5. Five out of eight (63%) pre-closure and 15 out of 23 (66%) pouch biopsy specimens showed an increased intensity of staining with PR1A3 (fig 1). Seven of the

Figure 1 Staining of columnar epithelial cells with PR1A3 (a) normal terminal ileum (negative); (b) normal colon (positive); (c) pre-closure pouch biopsy specimen (positive); (d) post-closure pouch biopsy specimen (positive).
eight (88%) pre-closure biopsy specimens were completely negative with PR3A5 but one showed positive staining in occasional goblet cells. Fifteen out of 23 (65%) post-closure biopsy specimens showed variable degrees of positivity with this antibody (fig 2). Total mucosal thickness, crypt depth, and the index of villous atrophy were all significantly increased in the post-closure specimens compared with the pre-closure pouch biopsy specimens \( (p < 0.01) \) (table 2), but did not show any correlation with PRIA3 and 3A5 expression. The index of villous atrophy correlated well with the subjective assessment of villous height used in the pouchitis score \( (rs = -0.632; \ p = 0.007) \). There were no significant differences in the pouchitis scores between biopsy specimens before and after closure, and only one of the post-closure specimens fulfilled the histological criteria for pouchitis (table 3). However, there was a significant correlation between increasing pouchitis score and increased staining with both PRIA3 and 3A5 \( (rs = 0.406, 0.411; \ p = 0.023 \text{ and } 0.021, \text{ respectively}) \) (fig 3). Increased staining with PRIA3 correlated with both increasing score for chronic inflammation \( (rs = 0.410; \ p = 0.022) \) and with the subjective assessment of loss of villous height.

### Table 2 Comparing indices of villous atrophy \( (VH/TMT) \) for pre- and post-closure pouch biopsy specimens

<table>
<thead>
<tr>
<th></th>
<th>Pre-closure</th>
<th>Post-closure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median index</td>
<td>0.302</td>
<td>0.483</td>
</tr>
<tr>
<td>Range</td>
<td>0.202-0.382</td>
<td>0.316-0.727</td>
</tr>
<tr>
<td>( p ) value</td>
<td>&lt;0.01</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3 Comparing total pouchitis score for pre- and post-closure pouch biopsy specimens

<table>
<thead>
<tr>
<th></th>
<th>Pre-closure</th>
<th>Post-closure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median score</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Range</td>
<td>0-6</td>
<td>0-8</td>
</tr>
<tr>
<td>( p ) value</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

### Discussion

Restorative proctocolectomy with ileal pouch-anal anastomosis is now a well established form of treatment for patients with ulcerative colitis, familial adenomatous polyposis, and other colonic disorders such as Hirschprung's disease and idiopathic constipation. Formation of the ileal reservoir results in phenotypic changes in the small bowel mucosa. These include loss of villous height and crypt hyperplasia together with a change from small intestinal type sulfomucin to colonic type sulphomucin. This evidence has been used to support the concept of “colonic metaplasia” in the ileal pouch mucosa. Such a hypothesis is attractive. The syndrome of acute inflammation and ulceration in the

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**Figure 2** Showing staining of goblet cells with PR3A5: (a) normal colon (positive); (b) pre-closure pouch biopsy specimen (negative); (c) post-closure pouch biopsy (positive).
pouch—“pouchitis” is almost entirely confined to patients with ulcerative colitis. Pouches showing colonic metaplasia might be useful as a model in which to study the aetiology of ulcerative colitis because possible pathogenic mechanisms can be studied from the time of pouch formation. However, despite the acquisition of some colonic features, ileal function seems to be quite well maintained in the pouch. Pouch mucosa retains disaccharidase activity (sucrase isomaltase) in the brush border, and nutritional studies on patients with pouchitis have shown normal fat absorption and serum folate concentration with decreased absorption of xylose, lactulose, and iron in only a proportion of cases (up to 30%).

Colonic metaplasia, if present, must therefore be incomplete.

The main functional difference between pouches and conventional ileostomies (in which “colonic metaplasia” is not seen) is the presence of faecal stasis. In general, metaplastic changes only seem to occur following ileostomy closure and they stabilise within three months. A recent study has shown that “colonic metaplasia” is greatest on the posterior wall of the pouch where stasis is maximal. Similar changes can be seen proximal to Crohn’s strictures. However, pouch emptying studies have not shown any relation between incomplete emptying and the degree of “colonic metaplasia” or of “pouchitis”. Therefore, although faecal stasis probably contributes to the changes seen in pouch mucosa, other factors such as bacterial overgrowth may be involved. The focal distribution and incomplete nature of colonic metaplasia seen in biopsy specimens from multiple sites in the ileal pouch prompted Shepherd et al to hypothesise that acquisition of “colonic phenotype” is greatest on the posterior wall of the pouch where stasis is maximal. It is also likely that bacterial flora of the pouch may become altered at an early stage with consequent adaptive change in the columnar epithelial cells. An altered ratio of anaerobes to aerobes may determine the magnitude of pathological change within the established pouch, but the precise role of bacteria in the acquisition of colonic characteristics by the pouch mucosa and in the pathogenesis of pouchitis remains uncertain.

As with PR3A5, staining for PR1A3 showed a positive correlation with the pouchitis score. In contrast to the situation for PR3A5, however, there was a significant correlation between PR1A3 score and pouchitis duration as well as that for loss of villous height. Two of the pre-closure biopsy specimens showing strong staining with PR1A3 had “pouchitis” scores of 5 and 6 (both with acute scores of 1), respectively. Although these were not sufficient for a histological diagnosis of “pouchitis” (this requires a total score of >8 or an acute score of >4), they still indicate the presence of moderate chronic inflammatory change. In one of these patients the inflammation was known to be secondary to a pouch-vaginal fistula; the other the aetiology was unknown. Pouch inflammation before ileostomy closure has...
been reported. It is rare, of obscure aetiology, and seems only to affect patients with previous ulcerative colitis. Whether it is related to the more common post-closure "pouchitis" is unknown. Crohn's disease, chronic ischaemia, mucosal prolapse and diversion pouchitis have to be excluded. Despite the above, it is unlikely that inflammation alone causes acquisition of colonic antigens by columnar cells as three biopsy specimens (one before, two after closure) with a pouchitis score of 0 also showed increased staining with PR1A3.

Both the index of villous atrophy (VH/TMT) and the degree of staining with PR3A5 and 1A3 showed significant correlations with the subjective assessment of loss of villous height included in the pouchitis score. However, VH/TMT did not show a significant correlation with the immunohistochemistry. These disparate findings might be explained by the fact that only 17 biopsy specimens were suitable for morphometric measurements.

In conclusion, staining with antibodies against putative colonic antigens shows acquisition of colonic characteristics by some goblet and columnar epithelial cells in the ileal pouch. Although incomplete, these may represent a true adaptive response as they can develop in the absence of inflammation. Changes in goblet cells occur after ileostomy closure when faecal stasis is likely to be a major contributory factor. On the other hand, adaptive changes in columnar cells may occur before ileostomy closure when factors other than faecal stasis, such as subtle changes in bacterial flora, could be important.

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