Carriage of adhesive *Escherichia coli* after restorative proctocolectomy and pouch anal anastomosis: relation with functional outcome and inflammation

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Abstract

Restorative proctocolectomy with pelvic ileal reservoir is a well accepted option for the surgical treatment of ulcerative colitis. Acute pouchitis is a common complication and resembles acute ulcerative colitis. Patients with ulcerative colitis carry *Escherichia coli* that adhere to epithelial cells and thus this study examined whether acute pouchitis is associated with the carriage of adhesive *E coli*. *E coli* isolated from stool samples from 24 patients (median age 34 years, range 16–64; 13 men, 11 women) who had had restorative proctocolectomy with pelvic ileal reservoir were examined by means of the buccal epithelial cell adhesion assay. Patients were studied at a median of 12 months (range 7–21) after operation. Eight of 24 patients had acute pouchitis at the time of study. Adhesive *E coli* were detected in nine of 24 patients with a pelvic ileal reservoir compared with none of 12 controls (p<0.05). The buccal epithelial cell adhesion index was inversely related to the degree of acute pouchitis (r_s=-0.46, p=0.024) and to the functional outcome (r_s=-0.49, p=0.022). Carriage of adhesive *E coli* was not related to the design of the reservoir. By contrast with ulcerative colitis, acute pouchitis is not associated with the carriage of adhesive *E coli*.

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Restorative proctocolectomy with a pelvic ileal reservoir or ‘pouch’ has become a widely accepted operation for patients with ulcerative colitis and familial adenomatous polyposis. The development of acute inflammation in the mucosa of the pouch, pouchitis, is one of the main longterm complications of restorative proctocolectomy. The reported incidence of pouchitis varies from 7 to 45 per cent. The symptoms associated with pouchitis share a number of similarities with those of ulcerative colitis. The condition is characterised by increased frequency of bowel actions with the passage of blood and poorly formed stool and lower abdominal cramp like pains. The condition typically runs a relapsing and remitting course.

The cause of pouchitis remains unknown. Stasis of pouch content with bacterial overgrowth, ischaemia, immunologically mediated inflammation, and deficiencies of short chain fatty acids or glutamine have all been suggested as possible causes but the strong similarities between pouchitis and ulcerative colitis have led many to argue that pouchitis is simply ulcerative colitis occurring in a pouch.

Ulcerative colitis has been shown to be associated with the carriage of *E coli* with the property of mannose resistant adhesion to epithelial cells. This form of adhesion may be a virulence characteristic in a number of other groups of *E coli*. Adherence is more common in *E coli* isolated from the stool from patients with ulcerative colitis than healthy controls or patients with infectious diarrhoea.

The aims of this study were to examine the carriage of adhesive *E coli* in patients who had had restorative proctocolectomy with a pelvic ileal reservoir and to determine its relation to the presence of acute pouchitis.

Patients and methods

PATIENTS

Twenty four patients (13 men; 11 women) were studied. The median age of the patients was 34 years (range 16–64 years). Each patient had had restorative proctocolectomy with either a triplicated (S) (n=11) or a quadruplicated (W) (n=13) pelvic ileal reservoir. The operations had been carried out at a median of 12 months (range 7–21 months) before the study was undertaken. The indication for surgery was ulcerative colitis that had failed to respond to medical treatment in 23 patients and familial adenomatous polyposis in one patient.

Samples of stool were also obtained from 12 healthy controls and 32 patients with ulcerative colitis.

DETAILS OF THE SURGERY

The technique of restorative proctocolectomy with stapled ileoanal anastomosis has been described in detail previously. Briefly, the rectum was mobilised by close perimucosal dissection and it was transected by means of a linear stapling instrument at the anorectal junction (TA30; Autosuture UK Ltd, Ascot, UK). The ileoanal anastomosis was fashioned by means of a circular stapling device (EEA; Autosuture UK Ltd, Ascot, UK).

Ileal reservoirs

A triplicated (S) reservoir was constructed in 11 patients. It consisted of three limbs, each 15 cm long, with a two cm efferent spout. A
quadruplicated reservoir was constructed in 13 patients. This consisted of four limbs, two of which measured 12 cm and two measured 10 cm. Each reservoir was hand sewn with an inner layer of 2/0 chromic catgut and an outer layer of 3/0 Dexon or Vicryl.

**MICROBIOLOGICAL ASSESSMENT**

Fresh samples of effluent were obtained at the time of defecation from each patient. The samples were transported promptly to the laboratory. *E. coli* from the samples of effluent were isolated and 10 colonies were selected at random and stored at a temperature of −70°C. Plasmid profiles of these 10 colonies were obtained by means of the method of Bennett. Plasmid DNA prepared in this way was separated by electrophoresis in a 0.8 per cent agarose gel (2 hours at 80 V), stained by soaking in TEB buffer (40 mM TRIS HCl-Sigma Chemical Company, Poole, UK), 1 mM EDTA, 50 mM boric acid, pH 8.2) containing ethidium bromide 1 mg/l, visualised by transillumination with ultraviolet light and then photographed.

**BUCCAL EPITHELIAL CELL ASSAY FOR ADHESION**

Representative strains selected on the basis of the plasmid profiles were assessed for the property of mannose resistant adhesion to epithelial cells by means of the buccal epithelial cell assay as described previously. Briefly, bacterial strains were grown for 18 hours on nutrient agar slopes at 37°C and washed from the slopes with phosphate buffered saline. Buccal epithelial cells were suspended in phosphate buffered saline, which contained 28 mmol/l D-mannose. Each suspension (0.5 ml) was mixed by means of a rotary roller and cells were harvested over a 5 micron filter (Millipore). An impression smear was made onto a slide, fixed with methanol, and stained by Gram's method. A buccal epithelial cell adhesion index was obtained by counting the number of cells with more than 50 adherent gram negative rods out of 100 non-overlapping epithelial cells minus the number of such cells present in the control in at least 10 high power fields. *E. coli* strains E851/71 and SC13 were included as positive and negative controls respectively. An adhesion index of 25 per cent or greater was used to differentiate between adhesive and non-adhesive strains.

**QUANTITATIVE CULTURE AND CULTURE FOR PATHOGENS**

Approximately 1 g of effluent was placed in a pre-reduced sterile vial that contained glycerol broth (10 per cent glycerol, 1 per cent lab lemo, pH 7.0 with resazurin in a Wheaton vial). This was stored at a temperature of −70°C until analysis.

To culture the stored effluent, the sample was defrosted and serial 10-fold dilutions were made in Trypton broth (phosphate buffered saline, 1 per cent tryptone, 0.1 per cent gelatin at pH 7.0). Aliquots of 50 μl of the dilutions were plated onto selective media and the plates were incubated. Undiluted effluent were used to identify *Campylobacter* species and *Clostridium difficile*. The bacteria isolated were identified to genus level by their appearance on the media and by supplementary standard bacteriological techniques. Colonies of isolates were counted at dilutions where numbers ranged between 10 and 40.

**Dry weight**

Paired samples of effluent were placed in small receptacles of predetermined weight and dried at 60°C in a fan oven to constant weight.

**MORPHOLOGY OF THE ILEAL MUCOSA**

Two biopsy specimens were taken by means of a sigmoidoscope and biopsy forceps from the reservoir of each patient. The specimens were transported in formalin to the laboratory. The tissue was orientated, processed in a standard fashion, and embedded in paraffin wax. Sections 4 μm thick were cut from three different levels. The sections were stained with haematoxylin and eosin. The specimens were assessed by one pathologist (PQ) with a particular interest in gastroenterology and experience with specimens from ileal reservoirs and ileostomies. The pathologist was unaware of any clinical data. Four criteria were assessed on each specimen and subjectively scored on a scale of 0–3 (absent-severe). The criteria assessed were chronic inflammatory infiltrate, acute inflammatory infiltrate, crypt hyperplasia, and lamina propria fibrosis.

**DEFINITION OF ACUTE POUCHITIS**

A patient was deemed to have acute pouchitis only if each of three criteria were satisfied:

- **Clinical** — an increase in the frequency of bowel actions with the passage of blood stained loose motions or lower abdominal pain, or both.
- **Endoscopic** — sigmoidoscopic evidence included an erythematous mucosa with ulceration and contact bleeding.
- **Histological** — an acute inflammatory infiltrate in the mucosa with crypt abscesses and ulceration in areas adjacent to mucosal 'colonisation'.

**EFFICIENCY OF EVACUATION OF THE RESERVOIR**

The efficiency of evacuation of the reservoir was assessed in each of the patients with S reservoirs and in six patients with W reservoirs. This assessment was not carried out successfully in seven patients either because of unsuitability (for example, possibility of pregnancy) or technical reasons (for example, inadequate collections, isotope unavailable).

The patients were seated on a commode and a viscous paste (420 mMq) of 6 g methyl cellulose in 300 ml normal saline labelled with a non-absorbable radioactive marker, 51Cr (Amersham International, Amersham, England), was infused into the reservoir through a 10 FG Jacques catheter at a rate of 60 ml/min. When the patient felt a constant urge to defecate the infusion was discontinued, the catheter removed, and
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the patient allowed to defecate in private. The activity (cpm) of the original solution, of the evacuated material, and of the solution not infused were measured in a rotating gamma-counter to permit calculation of the percentage of infused solution that was retained after evacuation.

CLINICAL ASSESSMENT OF BOWEL FUNCTION
Bowel function was assessed at the time of the laboratory assessment. Each patient completed a detailed typed questionnaire. Patients were asked about the frequency of bowel action over a 24 hour period and at night, about their ability to defer defecation, and to discriminate flatus from faeces and hence pass wind without visiting the lavatory. Patients were also asked about their use of anti-diarrhoeal drugs, and whether they needed to catheterise the reservoir.

Minor degrees of incontinence were subdivided into 'seepage' – staining of underwear with stains less than 3 cm in diameter – and 'soilage' – the need to wear a perineal pad. No patient experienced major incontinence.

Each patient was given an arbitrary functional score based on the Oresland criteria.13

STATISTICAL ANALYSIS
All grouped data were expressed as medians and interquartile ranges. The groups were compared by means of the Mann-Whitney U test and the χ² test with Yates's correction where appropriate. The significance of association was assessed by means of Spearman's rank correlation.14

Results

ADHESION OF E COLI TO BUCAL EPITHELIAL CELLS

E coli were isolated from each subject studied. Nine of 24 patients carried adhesive E coli

compared with none of 12 controls (χ², p<0·05) (Fig 1). Although more patients carried organisms with this property than controls, the median buccal epithelial cell index, however, was not significantly different between the two groups (median buccal epithelial cell index in patients after restorative proctocolectomy=9·5 per cent (range 0–100 per cent; median buccal epithelial cell index in control group 0·5 per cent range 0–12 per cent, p=0·07).

ADHESION OF E COLI AND INCIDENCE OF ACUTE POUCHITIS

Eight of 24 patients were tested at the time of an acute attack of pouchitis. Seven of eight patients had a triplicated (S) pelvic ileal reservoir while the other patient had a quadruplicated (W) pelvic ileal reservoir. One of eight patients with acute pouchitis showed carriage of adhesive E coli (that is, adhesive index >25 per cent) compared with eight of 16 patients without acute pouchitis (χ², p<0·05). Figure 2 shows the buccal epithelial cell adhesion index according to presence or absence of acute pouchitis. There was an inverse correlation between the degree of acute inflammation and the buccal epithelial cell adhesion index (rₛ=−0·46, p=0·024).

There was no correlation between buccal epithelial cell adhesion index and frequency of bowel action (rₛ=−0·23, p=0·27), efficiency of evacuation of the reservoir (rₛ=0·114, p=0·685), absolute numbers of bacteroides (rₛ=0·509, p=0·162), absolute numbers of bifidobacteria (rₛ=0·104, p=0·76), absolute numbers of coliforms (rₛ=−0·262, p=0·411), or the water content of the stool (rₛ=−0·239, p=0·391).

The overall function of the reservoir showed an inverse correlation with the buccal epithelial cell adhesion index (rₛ=−0·49, p=0·022). In other words, the better the function of the pouch, the higher the buccal epithelial cell adhesion index. The number of clostridia present in the stool correlated with the buccal epithelial cell adhesion index (rₛ=0·583, p=0·046).
Discussion

The main finding of this study was that acute pouchitis is not associated with carriage of adhesive *E. coli*. As the carriage of adhesive *E. coli* is strongly associated with ulcerative colitis, this study casts doubt on the hypothesis that pouchitis is simply a manifestation of ulcerative colitis occurring in a pouch. Indeed, rather than being associated with acute pouchitis, a statistically significant inverse relation was found between adhesive *E. coli* and acute inflammation of mucosa of the pouch. The study also confirmed previous reports of the absence of adhesive *E. coli* in stools from healthy controls. Adherent *E. coli* were isolated, however, in 37.5 per cent of patients, although the median adhesion index did not differ significantly from the controls. This may be a result of the comparatively low numbers studied. Large numbers of *E. coli* of varying genotypic and phenotypic characteristics are carried in the stools of humans. Clearly, it is not feasible to test or identify all of these. In an attempt to reduce sampling error, 10 strains were selected at random and stored. No completely reliable method of distinguishing between *E. coli* strains exists at present. Plasmid profiles act as a useful but not flawless attempt to do this. Strains selected from the faeces in this way carry identical plasmid profiles to those isolated from biopsy specimens of the same patients, apparently representing mucosa associated strains. Adhesion to buccal epithelial cells seems to mirror attachment to colonocytes. The positive association between the buccal epithelial cell adhesion index and overall functional result reflects the influence of acute pouchitis on function – the functional result at the time of assessment was worse if acute pouchitis was present.

The patients in the study did not represent a consecutive series of surgical cases. Rather, these patients were available for study and repeat investigation because they lived near to our centre. It is unlikely that the series was biased by any omissions.

Patients were studied at different times after their initial surgery. The time of study varied from 7 to 21 months after restorative proctocolectomy. The most significant changes occur, however, in the first six months after restorative proctocolectomy with only minor subtle changes taking place between 6 and 24 months.

Responses of patients to direct questioning on the function of their ileal reservoir may have been open to bias as such patients tend to be keen to please their doctors. Initial interviews were conducted, however, by medical practitioners who had not taken part in the surgical treatment of the patients.

The definition of pouchitis is arbitrary. The definition used in this study takes into account clinical, endoscopic, and histopathological criteria. The pathologist’s interpretation is perhaps the most crucial. The pathologist in this study was unaware of the clinical or endoscopic findings.

*E. coli* with the property of adherence to epithelial cells were seen more frequently in patients without acute pouchitis than in those patients with the condition. An explanation might be that patients with acute pouchitis do not express an appropriate receptor for adherence, perhaps as a result of changes in mucus or cell surface glycoproteins. The active moiety of the receptor for the adhesive *E. coli* found in ulcerative colitis is not known but differs from that of enteropathogenic *E. coli* where N-acetyl galactosamine is the active component.

Why should the buccal epithelial cell adhesion index correlate with the absolute numbers of clostridia present in effluent from pouches? We have seen previously that patients with good function from their pouches tend to have greater numbers of clostridia in the effluent. This may be a result of different intestinal transit times with slower transit permitting the microflora of the bowel to adopt a distribution typical more of large bowel than small bowel.

The cause of acute pouchitis remains undefined. Why pouchitis is more likely to occur in patients who had suffered from extensive colitis than those with limited left sided involvement, the presence of backwash ileitis does not seem to be a factor. The suggestion that pouchitis shares a common cause with ulcerative colitis has been questioned by the resemblance of pouchitis to ileal Crohn’s disease rather more than ulcerative colitis, the fact that pouchitis usually responds promptly to treatment with metronidazole, and by occasional well documented cases of pouchitis in patients with familial adenomatous polyposis. Irrespective of the condition for which the operation was originally performed, the mucosa of the pouch secretes colonic rather than ileal type mucus. Indeed, areas of intestinal metaplasia with the development of colonic mucosa occur in 50% of pouches. The prevalence of pouchitis in animal models is high (70–90 per cent) despite the fact that none of the animals suffered from colitis before operation.

Stasis of ileal content would seem to be a possible cause of pouchitis. No correlation has been shown, however, between the efficiency of evacuation of the reservoir and the incidence of pouchitis.

A bacterial cause of pouchitis has been suggested. Stasis of pouch content with inefficient evacuation of pouch effluent is associated with an increase in the proportion of anaerobes to aerobes. There is, however, no correlation between numbers of specific bacteria within pouch effluent and the severity of pouchitis. A reduced value of short chain fatty acids, produced by bacteria, which are known to be present in pouch effluent, does however correlate with mucosal villous atrophy. Short chain fatty acids have a toxic influence on ileal mucosal cells and it is tempting to speculate therefore that pouchitis may be a manifestation of enterocyte fuel deficiency.

Pouchitis is the predominant longterm complication of restorative proctocolectomy. This study has found that patients may carry adhesive *E. coli* after restorative proctocolectomy but that the presence of the organisms is not related to the development of the complication of pouchitis.
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