# Increased rectal mucosal enteroendocrine cells, T lymphocytes, and increased gut permeability following acute *Campylobacter* enteritis and in post-dysenteric irritable bowel syndrome

R C Spiller, D Jenkins, J P Thornley, J M Hebden, T Wright, M Skinner, K R Neal

**Abstract**

***Background and aims*—Post-dysenteric irritable bowel syndrome (PD-IBS) develops in up to 25% of patients following *Campylobacter* enteritis. Our aim was to define the pathological basis of this subgroup of IBS.**

***Methods*—Twenty one patients (group 1) underwent serial rectal biopsy and gut permeability testing following acute *Campylobacter* enteritis as did 10 PD-IBS patients (group 2) and 12 asymptomatic controls.**

***Results*—In group 1, enteroendocrine cell (EC) numbers were markedly increased initially and at six and 12 weeks ($p<0.001$) compared with controls. Gut permeability, as assessed by the lactulose/mannitol ratio, was significantly elevated, initially and at 12 weeks ($p<0.005$). CD3, CD4, and CD8 lymphocyte counts in the lamina propria and intraepithelial lymphocytes (IEL) were significantly increased initially compared with controls. At visit 1, EC numbers were positively correlated with CD3 counts ($r=0.6$, $p=0.01$). At one year, seven subjects (five with persistent loose stools) had rectal biopsies which showed significantly elevated EC, CD3, and IEL counts. In group 2, EC and IEL counts were significantly increased compared with controls ($p<0.001$), as was gut permeability ($p<0.01$).**

***Conclusion*—Increased EC, T lymphocytes, and gut permeability are acute changes following *Campylobacter* enteritis which can persist for more than a year and may contribute to PD-IBS.**

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Keywords: irritable bowel syndrome; *Campylobacter*; enteroendocrine cell; T lymphocytes

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Irritable bowel syndrome (IBS), characterised by abdominal pain or discomfort associated with altered bowel habit, affects 9–13% of the normal population at any particular point in time[1]; 20–30% of IBS patients describe acute onset to their bowel disturbance following an acute infective enteritis. These patients, who have what Chaudhry and Truelove[2] termed "post-dysenteric IBS (PD-IBS)", appear to have a better prognosis.[3] More recently we have reported that when patients with microbiologically confirmed bacterial enteritis were questioned six months after their initial illness, 25% continued to experience abnormal bowel habits characterised by frequent loose stools, often associated with urgency.[4] Prolonged duration of initial illness, female sex, and age less than 60 years were predictors of persistent disturbance in bowel habit. We found *Campylobacter jejuni* to be the commonest causative organism. Two further studies from Sheffield[5 6] in patients admitted with infective enteritis reported a similar incidence of persistent bowel disturbance with accelerated small and large bowel transit, increased stool weight, and increased rectal sensitivity.[6]

Although conventional histology appears normal, low level inflammation may persist for several months after acute infective colitis.[7 8] We have previously shown a persistent increase in lamina propria mononuclear cells, immunoglobulin A plasma cells, and cytokine mRNAs in apparently normal mucosa, 2–3 months following acute bacterial colitis.[9] It has been suggested that this may underlie persistent "functional" diarrhoea. Enteric infections are known to increase T lymphocyte populations and activate mucosal macrophages but detailed studies after *C jejuni* are lacking. Earlier reports in the 1970s suggested that enteroendocrine cells (ECs) were increased in IBS[10] but there has been little progress since.

The current study aimed to describe quantitatively the subtle morphological changes in the three months following *Campylobacter* enteritis in immune, inflammatory, and EC populations. As *Campylobacter* affects the distal small bowel as well as the colon, we also measured gut permeability as a marker of continuing inflammation in the otherwise relatively inaccessible distal small bowel. Our further aim was to test the hypothesis that persistent bowel dysfunction is related to continuing low grade inflammation and EC hyperplasia and to this aim we studied a further group of patients 12–48 months following acute infective enteritis.

## Subjects and methods

The University Hospital's ethics committee approved all the studies described below and in each case patients gave written informed consent.

**Abbreviations used in this paper**: PD-IBS, post-dysenteric irritable bowel syndrome; EC, enteroendocrine cell; IEL, intraepithelial lymphocyte; DAB, 3'3 diaminobenzidine tetrahydrochloride; 5-HT, 5-hydroxytryptamine; PYY, peptide YY.

*Table 1 Demographic and illness details of groups 1a and 1b (median (range))*

| | *1a (no biopsy)* | *1b (biopsy)* |
|---|---|---|
| n | 26 | 21 |
| Age | 45 (20–66) | 50 (28–69) |
| Sex (F/M) | 15/11 | 13/8 |
| Duration of fever (days) | 2 (0–14) | 3 (0–8) |
| Duration off normal duties (days) | 7 (0–24) | 4 (0–8) |
| Bowel frequency at 12 weeks | 1 (1–5) | 1 (0.3–3) |
| Urgent bowel movements (days/week) at 12 weeks | 0 (0–7) | 1 (0.3–3) |
| No (%) bowels back to normal at 12 weeks | 15 (58) | 14 (67) |

There were no significant differences between groups.

GROUP 1

Forty seven patients with *Campylobacter* enteritis completed a symptom questionnaire as soon as practicable (2–10 days) after initial isolation of *Campylobacter* from stool cultures, and again at six, 12, and 24 weeks. Twenty one patients (group 1b) agreed to further studies, including measurement of gut permeability at the initial visit and again at 12 weeks. These patients underwent serial rectal biopsy at presentation (visit 1), and six (visit 2) and 12 weeks (visit 3) following onset. In practice, stool sample results were not usually available until 5–7 days after onset and delays in contacting the patient and making an appointment meant that initial biopsies were usually obtained 2–3 weeks after the onset of symptoms. As these were a selected group, it was important to test if they differed significantly from the whole group. The demographic details and symptoms of the 26 patients who had no biopsy (group 1a) and the 21 patients who completed the entire protocol (group 1b) are shown in table 1. There were no significant differences between groups 1a and 1b, suggesting that the results from group 1b can be generalised to the whole group.

Thirty one patients returned bowel symptom questionnaires one year after their original visit but only seven agreed to further rectal biopsy. While the questionnaire showed that only 13 of 31 patients had persistently abnormal bowel habits, five of seven who had a further biopsy had persisting symptoms, suggesting that these represented a more severely affected group.

GROUP 2

A further 10 patients with persistent IBS following an acute bout of diarrhoea and vomiting due to either proven (two *Salmonella*, two *Shigella*, and two *Campylobacter*) or presumed (four) acute infectious gastroenteritis 8–48 months previously were also studied. These patients (six females, four males) had been extensively investigated by haematological, biochemical, and immunological tests to exclude tropical sprue, coeliac disease, and inflammatory bowel disease. Lactose intolerance in those who regularly consumed more than 250 ml of milk was excluded either by lack of response to a 500 ml milk challenge or a negative lactose tolerance test. Barium follow through, duodenal biopsy, oral pancreatic function test, SeHCAT retention, and colonoscopy were performed as clinically indicated and all were normal. They also underwent gut permeability studies and rectal biopsy 8–48 months after their initial episode.

*Table 2 Demographic details and bowel symptoms in those who had rectal biopsies*

| | *Controls* | *Group 1b* | *Group 2* |
|---|---|---|---|
| n | 12 | 21 | 10 |
| Age | 58 (40–76) | 50 (28–69) | 44 (23–64) |
| Sex (F/M) | 9/3 | 13/8 | 6/4 |
| Bowel frequency | 1 (0–2) | 1 (0.3–3) | 3.5 (1–10) |
| Days urgent | 0 | 1 (0.3–3) | 2 (1–7) |
| No (%) bowel habit normal | 100 | 14 (67) | 0 |

CONTROLS

Twelve patients who underwent a negative screening colonoscopy because of a family history of colon cancer, unexplained anaemia, haemorrhoids, or previous colonic polyp and who had no bowel symptoms provided normal rectal biopsies. As shown in table 2, their demographic details were not significantly different from the group under study but their bowel habits were normal.

GUT PERMEABILITY STUDIES

After an overnight fast, subjects ingested 100 ml of a mixed nutrient test meal of Fortisip (200 kcal) followed by 5 g of lactulose together with 2 g of mannitol dissolved in 100 ml of water. Urine was collected for six hours, an aliquot of which was analysed for lactulose and mannitol by high performance liquid chromatography. This was performed on a Hamilton RCX-10 anion exchange column with pulsed amperometric electrochemical detection using cellobiose as an internal standard. Detection limits were 1 μg/ml for mannitol and 10 μg/ml for lactulose. Normal values for gut permeability studies were established in 10 healthy volunteers, aged 25 years (22–45), receiving no non-steroidal anti-inflammatory agents and free of gastrointestinal symptoms, and were in agreement with normal values published in the literature.[11 12]

SYMPTOM QUESTIONNAIRE

All patients completed a detailed bowel symptom questionnaire on the frequency (days per week) that they experienced each of the Rome criteria.[13] These criteria require that in addition to abdominal pain, symptoms of altered stool frequency, altered stool consistency, disordered defecation, bloating, and passage of mucous per rectum be present for more than 25% of the time. Using the new Rome II criteria, which are more stringent, the incidence of IBS would have been slightly less. However, in order that these studies were more readily comparable with previous ones, we retained the standard Rome I criteria in the current study. As previous studies[6] suggested an important role for psychological and life event variables, patients also completed an SF36 (quality of life, mood, impact of symptoms on social, mental, and physical functioning) and adverse life event (bereavement, divorce, job loss, house move) questionnaire.

RECTAL BIOPSY

Patients underwent sigmoidoscopy without bowel preparation. Biopsies were obtained using endoscopic biopsy forceps (FB-13K-1,

Olympus, Japan) and were frozen in liquid nitrogen, or fixed in glutaraldehyde followed by embedding in resin for electron microscopy or 10% normal saline followed by embedding in paraffin wax. Conventional histology was performed on a haematoxylin-eosin stained paraffin embedded section by a single expert pathologist (DJ) using standardised published criteria.[14] All appeared normal in groups 1 and 2.

### IMMUNOCYTOCHEMISTRY ON PARAFFIN EMBEDDED SECTIONS

Following dewaxing, sections were incubated for 15 minutes in absolute methanol containing 10% 20 vol hydrogen peroxide. Sections were rinsed in absolute alcohol followed by running tap water. Normal swine serum (1:5) was added for 20 minutes followed by the primary antibody for 30 minutes (as detailed below). Sections were then washed in Tris/HCl buffered saline pH 7.6 (TBS) and the secondary antibody, a biotinylated goat antimouse and antirabbit antibody (Dako, Cambridge, UK), was added for a further 30 minutes and washed off with TBS. A peroxidase labelled avidin-biotin complex (Dako) was then used followed by 3'3 diaminobenzidine tetrahydrochloride (DAB) solution for 10 minutes. The slides were then washed in running tap water, incubated in 0.5% copper sulphate in 0.9% sodium chloride for 10 minutes, and washed in running tap water. Sections were counterstained with haematoxylin, dehydrated, cleared, and mounted. Individual cell types were stained as follows.

### ENTEROENDOCRINE CELLS

Microwave antigen retrieval in citrate buffer pH 6.0 was performed prior to immunocytochemical staining, as described above using primary antibodies to synaptophysin (Dako) dilution 1:50, peptide YY (PYY; Peninsula Labs, California, USA) dilution 1:4000, and serotonin (monoclonal antibody, Dako) dilution 1:50. EC were easily recognised: the coefficient of variation of repeated measures (n=10) was 2.2% and the 95% limits of agreement between two different observers was ±5%. Electron microscopic images confirmed that these cells had typical morphology and electron dense secretory granules.

### MAST CELLS

Proteinase K antigen retrieval was performed prior to staining using a primary mouse monoclonal antibody to mast cell tryptase (Dako) at a dilution of 1:250.

### MACROPHAGES

The macrophage markers CD68 (Dako), dilution 1:100, and calprotectin (Myeloid Histiocyte Antigen, Dako), dilution 1:200, were assessed using paraffin sections. Sites of antibody binding were visualised using DAB and hydrogen peroxide, as described above.

### LYMPHOCYTES

CD3, CD4, and CD8 were assessed on frozen sections, cut on a sledge microtome (Leitz Weztlar) and stained using an indirect immunoperoxidase technique with antibodies to CD3 (Dako), dilution 1:25, CD4, and CD8 (Nova Castra Lab Ltd, Newcastle upon Tyne, UK), dilution 1:5. Briefly, sections were fixed in acetone at 4°C for 20 minutes and exposed to hydrogen peroxide, swine serum, and primary antibody as above. The secondary antibody was an antimouse monoclonal conjugated with peroxidase (Dako) for CD4 and CD8 and antirabbit for CD3. This was followed by DAB and staining, completed as above.

### METHODS OF CELL COUNTING

The numbers of positively staining ECs and intraepithelial lymphocytes (IEL) per 100 epithelial cells were counted for 5–6 sections and the results averaged. Lamina propria cells were counted in alternate high power fields and the mean of six values calculated. The counts were expressed as counts per square millimetre. Technical problems of sample orientation and small size meant that not all biopsies yielded samples adequate for counting and therefore the numbers at six and 12 weeks were reduced. Only limited paraffin embedded material was available for group 2 and therefore data are limited to EC (synaptophysin stain) and intraepithelial cell counts.

### STATISTICS AND ANALYSIS

Demographic data are shown as median (range). Comparisons were performed using the Mann-Whitney U test. All laboratory results were assessed for normality of distribution using the Shapiro-Wilks test but only the lactose/mannitol relation required log transformation to achieve satisfactory normality. The results are therefore expressed as mean (SEM) except for the lactulose/mannitol ratio which is shown as median (range). Analysis of variance for repeated measures (SPSS for Windows version 9, SPSS Inc Chicago, USA) was used to asses changes from visit 1 through to visit 3, specific differences being assessed using the Student's paired *t* test. Differences from controls were assessed using the unpaired Student's *t* test. Correlations were assessed using the Pearson correlation coefficient.

## Results

### GROUP 1

The demographic features age (46 years (20–69)) and sex (28 females, 19 males) were similar to our previous much larger survey.[4] Median time off normal duties was 4 days (0–24). As expected from our larger study, bowel frequency and urgency had subsided by 12 weeks in most patients but 18 had persistent loose, more frequent bowel habits and variable urgency. Eight patients had developed new recurrent abdominal pain following gastroenteritis which persisted for at least six months. Interestingly, three patients reported lessening in the frequency of previous recurrent abdominal pain.

At one year, 13 of 31 patients reported looser stools than normal, eight more frequent stools, six had abdominal pain at least once a week, and four met Rome I criteria for new IBS.

*Table 3 Enteroendocrine cell (EC) numbers per 100 epithelial cells of rectal biopsy in controls, group 1, and group 2*

| | | Group 1 | | | |
|---|---|---|---|---|---|
| | *Controls* | *Initial* | *6 weeks* | *12 weeks* | *Group 2* |
| n | 12 | 19 | 17 | 16 | 8 |
| EC | 1.8 (0.4) | 10.8 (1.3) | 6.8 (0.6)* | 5.7 (1.0)* | 12.7 (0.4) |
| p | | <0.0001 | <0.001 | <0.001 | <0.001 |

ANOVA showed significant difference between initial, six, and 12 weeks, F=4.8, df 1,13, p<0.05.
*Significant difference from initial value, p<0.05.
p, probability under null hypothesis of no difference from control values, unpaired *t* test.

*Table 4 Peptide staining enteroendocrine cell counts per 100 epithelial cells*

| | *Controls* | *Initial* | *6 weeks* | *12 weeks* |
|---|---|---|---|---|
| n | 8 | 19 | 17 | 15 |
| 5-HT | 0.6 (0.2) | 7.0 (1.0)** | 3.9 (0.5)**† | 3.2 (0.7)**† |
| n | 7 | 12 | NA | 10 |
| PYY | 2.4 (0.3) | 8.4 (1.6)** | NA | 2.0 (0.4) |

ANOVA for repeated measures showed significant differences between initial, six, and 12 weeks for 5 hydroxytryptamine (5-HT) values , F=6.1, df 1,13, p<0.05.
**Significant difference from control values, p<0.01. †Significant difference from initial value, p<0.05.
NB, note reduced numbers in peptide YY (PYY) analysis owing to lack of biopsy material.

*Table 5 Lymphocyte counts*

| *Marker* | *Controls* | *Initial* | *6 weeks* | *12 weeks* |
|---|---|---|---|---|
| n | 12 | 19 | 17 | 16 |
| CD3/mm$^2$ | 5 (0.4) | 26.3 (2.1)*** | 19.0 (1.9)*** | 16.0 (1.5)*** |
| CD4/mm$^2$ | 1.9 (0.25) | 13.3 (1.3)** | 11.4 (1.6) | 9.4 (1.5) |
| CD8/mm$^2$ | 3.0 (0.5) | 12.8 (2.1)*** | 8.6 (1.6)*** | 7.1 (0.9)*** |
| IEL/100 epithelial cells | 0.5 (0.2) | 2.5 (0.4)*** | 1.6 (0.5) | 0.9 (0.2) |
| CD68/mm$^2$ | 57.5 (7.2) | 21.7 (4.1)** | 28.3 (5.3)** | 30.3 (4.4)** |
| Calprotectin/mm$^2$ | 1.4 (0.5) | 20.8 (3.3)** | 8.7 (1.7)** | 7.5 (1.7)** |

Significant difference from controls: ***p<0.001, **p<0.01.

GROUP 2

These patients' symptoms had continued for eight months to four years after the initial infection. As they had been referred because of persistent symptoms it was not surprising that they described more severe diarrhoea with greater frequency and urgency.

RECTAL BIOPSY FINDINGS

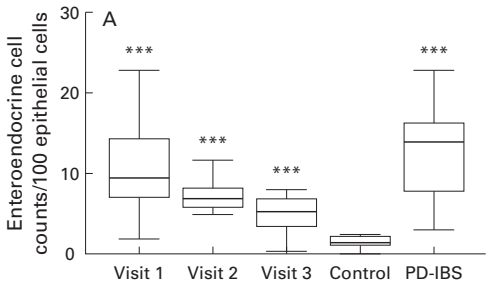
*Enteroendocrine cell counts (tables 3, 4)*

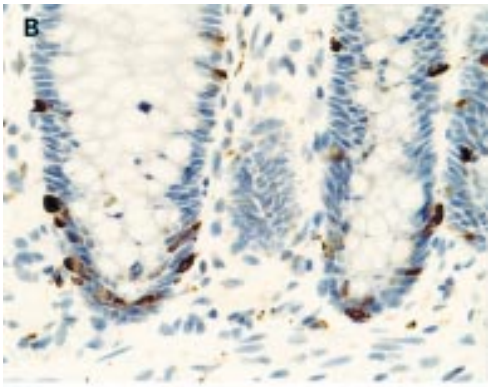
The most striking finding was a marked increase in ECs in both patient groups (table 3; fig 1A, B). This increase declined significantly over the 12 week period (p<0.05). All but two patients in group 2 had levels greater than the highest normal value. As shown in table 4, both 5-hydroxytryptamine (5-HT) and PYY containing cells were increased at the initial visit; PYY values returned more rapidly to normal than 5-HT values which were still significantly elevated at 12 weeks.

The number of CD3 positive T lymphocytes in the lamina propria was markedly increased; at visit 1, numbers were approximately twice those of control values. ANOVA for repeated measures indicated a significant decline from visit 1 to visit 3 (p<0.005) but even at visit 3 values were significantly elevated compared with controls (p<0.001) (fig 2). Analysing these T lymphocytes using the CD4 and CD8 markers showed that both subsets demonstrated a similar increase compared with control values at visit 1 (p<0.01 and p<0.001, respectively). Although numerically both markers declined from visit 1 to 3, ANOVA for repeated measures showed that these changes were not statistically significant. At visits 2 and 3, CD4 counts were no longer significantly different from controls.

*Intraepithelial CD8 lymphocytes*

IEL showed a marked increase, and were five times control values at visit 1 (p<0.001). In



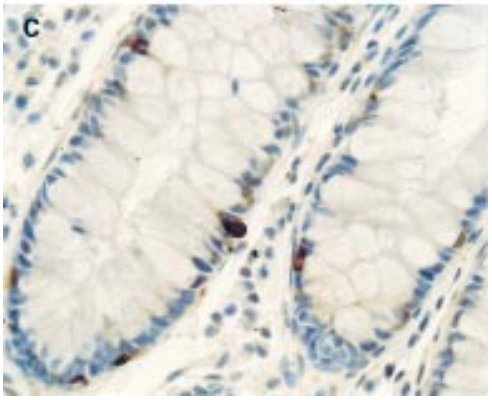




*Figure 1 (A) Enteroendocrine cell counts per 100 epithelial cells in the patient cohort at visits 1, 2, and 3 compared with controls and post-dysenteric irritable bowel syndrome (PD-IBS) patients. ***Significantly increased compared with controls, p<0.001. Values at visits 2 and 3 were significantly lower than those at visit 1, p<0.05. (B) Rectal biopsy stained for synaptophysin showing increased numbers of enteroendocrine cells in the colonic crypt base of a patient three weeks after infection with Campylobacter jejuni. A control biopsy is shown (C) for comparison. Original magnification: ×40. Synaptophysin positive cells are brown, with blue haematoxylin counterstain. CD3, CD4, and CD8 lamina propria lymphocyte counts (table 5)*

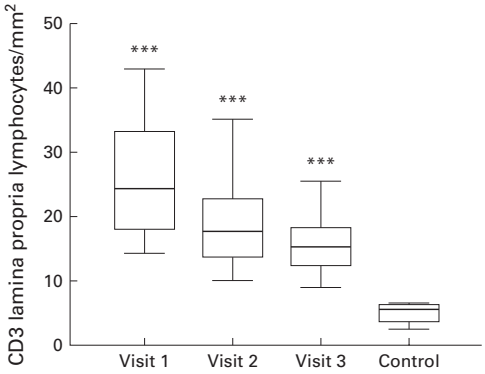



*Figure 2 CD3 staining lamina propria lymphocytes. ***p<0.001 v controls. Values at visit 3 were significantly less than those at visit 1, p<0.005*

common with CD3 lymphocytes, IEL showed a significant decline over the three visits (ANOVAR for repeated measures, p<0.05) and hence the difference between visit 3 and controls was no longer significant (p=0.068) (fig 3). However, PD-IBS patients (group 2) had significantly elevated counts (1.8 (0.3); p<0.001 *v* controls).

*CD68 and calprotectin lamina propria counts*

CD68 positive cells showed a striking decline, with values at visit 1 reaching approximately 50% of control values (p<0.001) (fig 4). These values remained significantly below control values even at 12 weeks (p<0.004). ANOVAR showed no trend between visits 1 and 3. Calprotectin positive cell numbers were markedly elevated at all visits (p<0.01) but ANOVAR did not show any consistent change from visit 1 to visit 3 (see table 5).

MAST CELLS

Few mast cells were noted in these biopsies, most of which were in the lamina propria. Counts per high power field were 4.9 (0.9) at visit 1 and 5.2 (1.5) at visit 3, with no significant trend by visit and no difference from normal values (6.7 (1.5)).

GUT PERMEABILITY

The median lactulose/mannitol ratio in group 1 at initial presentation was 0.045 (range 0.025–0.087) and 0.038 (0.023–0.59) at 12 weeks,

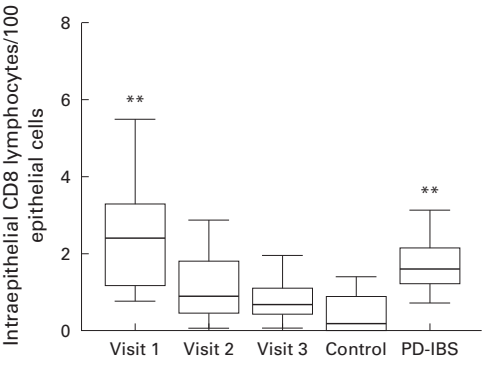



*Figure 3 Intraepithelial lymphocytes stained for the CD8 marker. **Significantly elevated compared with controls. Differences from control were no longer significant at visits 2 and 3. Post-dysenteric irritable bowel syndrome (PD-IBS) patients also showed significant increases.*

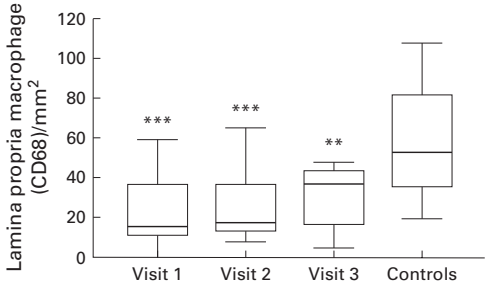



*Figure 4 Macrophages counts (CD68 positive) showed a marked decline at visit 1. ***p<0.001, **p<0.005 v controls.*

both significantly elevated compared with controls (0.0088 (0.0047–0.013); p=0.0001) with 11 and 9, respectively, above the conventional upper limit of normal of 0.03.[15 16] Group 2 ratios were also significantly different from controls (0.060 (0.008–0.22); p=0.005), five having values above the conventional cut off limit of 0.03.

CORRELATION BETWEEN MUCOSAL MARKERS

EC numbers were significantly correlated with CD3 positive lamina propria lymphocytes, (*r*=0.65, p=0.01) at visit 1 but not at visit 3 (p=0.4). There was no correlation between endocrine cell numbers and CD4, CD8, and CD68 counts. Although permeability was significantly increased, there was no relation between permeability and CD3 lymphocyte numbers at visit 1 or 3, or between any other lymphocyte subsets.

CORRELATION BETWEEN MUCOSAL MARKERS AND BOWEL SYMPTOMS

Various markers of disease severity, including duration of initial illness, presence of rectal bleeding, and duration of severe diarrhoea failed to show any significant correlation with CD3 or EC numbers. Only six patients in group 1b showed persistent bowel abnormalities at six months and these small numbers make it difficult to detect any statistically significant relation between histology and persistent symptoms. There was no difference in EC, CD3, or CD8 numbers at visit 1 or 3 in those who did or did not have persistent symptoms at six months. However, at one year follow up, as already noted, five of seven patients attending for repeat biopsy had persistent symptoms compared with only 13 of 31 returning the questionnaire. Not unexpectedly therefore they represent a more severely affected group in which EC numbers were significantly increased compared with controls (9.4 (0.4)/100 epithelial cell; control 1.8 (0.4); p<0.001). CD3 lymphocyte counts in the lamina propria were also increased at 32.7 (5.1) (control 12.5 (0.4)/mm$^2$; p<0.01) as were IEL at 2.1 (0.6) (control 0.5 (0.2); p<0.05).

RELATION BETWEEN LIFE EVENTS, SF36 RESPONSES, AND PERSISTENT SYMPTOMS

There were no significant differences between those who recovered normal bowel function at six months and those who did not with respect to physical, social, and psychological scales of

the SF36. Life events in the preceding six months were slightly more common in those with persistent symptoms (0.7 (0.3) *v* 0.5 (0.1) life events) but this was not statistically significant (p=0.27).

## Discussion

This is the first report of the immunohistological features of resolution of acute *Campylobacter* enterocolitis, and the first to report the striking increase in EC numbers and changes in macrophages and T lymphocytes which, in at least some patients, can persist for more than a year. This description is particularly important because similar changes were found in outpatients with prolonged IBS symptoms following acute bacterial enteritis. The persistence of these changes and their detection in symptomatic outpatients supports our hypothesis that the changes we have described following bacterial enteritis are in part responsible for the persistent symptoms commonly seen in clinical practice. This cohort study required a rather arduous protocol, with patients undergoing three rectal biopsies and two determinations of gut permeability. Although 47 patients agreed to participate, only 21 completed the three visits. However, as shown in table 1, those who did were demographically similar to those who did not and there was no significant difference in illness severity. Therefore, we believe that our biopsy results for visits 1–3 represent the entire cohort; in particular they do not appear to over represent those most severely affected. However, those who attended for the final biopsy at one year appeared to be part of a more severely affected group.

Confirmation that our findings in the first three months of follow up can be generalised to the whole population was obtained by a further postal survey during the period of recruitment into the main study. Three hundred and sixty six additional patients with *Campylobacter* infection were sent questionnaires on risk factors, duration of illness, and symptoms, initially and at six months (see Neal and colleagues[4]). Of these, 188 returned both questionnaires (51%). Sixteen of this group had IBS before infection and 16/172 developed new IBS by six months, a rate of 9.3% (95% confidence interval 5–15%). The incidence of new IBS in this larger group and group 1 were comparable, suggesting our cohort is representative in severity and outcome of all *Campylobacter* infections occurring during the period of study. These findings also support the recent report that *Campylobacter* enteritis is the single most important factor in determining the onset of new IBS symptoms in the UK population.[17]

The striking biopsy findings were a fivefold increase in ECs, a doubling in lamina propria T cells (CD3, CD4, and CD8), and a fivefold increase in IEL (CD8), changes which lasted for at least one year in more severely affected individuals. In contrast, there was a halving in resident macrophages (CD68) while incoming activated macrophages (calprotectin positive) were increased. Although *Campylobacter* was no longer cultured at six weeks, the increased T cells and activated macrophages suggest that there was a continuing immune response, although whether this was directed at *Campylobacter* or other commensal bacteria is unknown. The correlation between EC and CD3 lymphocyte numbers raises the possibility of a causative link.

This study provides evidence for a number of mechanisms that might be important in PD-IBS. Although it has been known for many years that ECs are abundant in the lower gastrointestinal tract,[18] the potential clinical significance of an increase in numbers following infection has not previously been recognised. This increase, noticed in the first biopsy, 2–3 weeks after the onset of infection, could be due to either proliferation of stem cells, which then differentiate into ECs, or to decreased apoptosis of existing cells. Our demonstration of increased numbers of 5-HT containing ECs suggests a possible cause for post-dysenteric bowel dysfunction. Release of 5-HT induces vomiting and diarrhoea and can be seen as part of a primitive protective response. This could act to facilitate clearing of the infecting organism by virtue of its physiological actions of stimulating both secretion and propulsive motility.[19 20] Diarrhoea induced by cholera toxin[21 22] and in other conditions such as coeliac disease[23] and carcinoid syndrome[24] appears to be 5-HT driven, at least in part.[25] Some of the features of irritable bowel, including diarrhoea and visceral hypersensitivity, could also be due to excess 5-HT[26] and recently five cases of diarrhoea predominant IBS with exaggerated postprandial release of 5-HT have been published.[27] Most studies of diarrhoea predominant IBS treated with $5\text{-HT}_3$ antagonists have reported an improvement in diarrhoea[28 29] and a reduction in visceral hypersensitivity.[30] Some of the variability in results may reflect the heterogeneity of IBS and failure to separate PD-IBS from IBS with different aetiologies.

PYY is also a major secretory product of rectal ECs[31] and has antidiarrhoeal properties. These include increasing small intestinal absorption,[32] slowing small bowel transit,[32 33] and decreasing ileostomy output induced by vasoactive intestinal peptide.[32 34] The final symptomatology associated with increased EC numbers may therefore depend on the balance between the different peptide products of these cells.

It is important to note that while our controls had a full bowel preparation with either sodium picosulphate (Picolax, Nordic) or polyethylene glycol (Kleenprep, Norgine), patients did not. Since this study we have routinely stained over 150 rectal biopsies from a wide range of unprepared patients and can confirm that most have values lying within the normal range quoted in this paper. It seems unlikely therefore that bowel preparation alters the staining of ECs.

The striking increase in T lymphocytes presumably reflects an immune response to invasion by *C jejuni*. All subsets of T lymphocytes showed a similar trend with a marked increase initially, declining over the three visits, although this decline was significant only for CD3 and intraepithelial CD8 T lymphocytes.

Only IEL numbers returned to normal by visit 3. The pathophysiological role of T cells in the control and resolution of inflammation in the gut after an invasive bacterial infection remains unclear. The T cell response following *Campylobacter* infection may be part of a continuing cell mediated or humoral defensive response to the infecting organism, may be involved in terminating the acute immune and inflammatory responses, or may be a response to other host intestinal flora. Transgenic and other mouse models have shown the importance of CD4 T cells in initiating and controlling immune mediated inflammation in the gut.[35 36] Studies of the mouse parasite *Trichuris muris* have shown that persistence of infection and the nature and duration of the inflammation depend on polarisation of the Th1 or Th2 cytokine response.[37] It has also been shown that specific regulatory T cell subsets can suppress antigen specific responses and downregulate intestinal inflammation in vivo.[38 39] Both CD4 and CD8 subsets have been involved and interleukin 10 and/or transforming growth factor β identified as key mediating mechanisms. Intraepithelial CD8 lymphocytes in rodents and humans may have suppressor functions and be activated by intestinal epithelial cells acting as antigen presenting cells through non-classical pathways.[40] Additionally, human CD4 T cells may downregulate T cell responses to commensal bacterial antigens through interleukin 10 release in vitro.[41] Whatever the nature of the T cell response it may play an important part in determining the pattern of continuing mucosal response. Demonstration of a correlation between EC numbers and CD3 lymphocyte counts suggests the possibility that both are part of a continuing coordinated immune and motor response.

In addition to these functions T lymphocytes have been shown experimentally to play a key role in mediating changes in neuromuscular function following gastrointestinal infection. The cholinergic hypersensitivity of jejunal longitudinal muscle and the increased substance P content of the myenteric plexus of *Trichinella* infected rats is absent in athymic animals but restored by reconstituting them with splenic cells.[42 43] It is therefore possible that such T cell mediated effects may also contribute to the continuing bowel symptoms in some of our patients.

The marked fall in CD68 cells, a marker of resident macrophages, is interesting as *C jejuni* is known to be taken up by macrophages, which it has the ability to destroy,[44] in common with other enterotoxic pathogens such as *C difficile*.[45] The apparent increase in calprotectin positive cells, thought to represent incoming macrophages derived from circulating monocytes,[46] suggests recruitment to replace damaged macrophages. These activated macrophages would be expected to produce substantial amounts of the proinflammatory cytokine interleukin 1β. Interleukin 1β is known to stimulate ECs in the gastric antrum[47] as well as T cells and may possibly account for some of the observed changes.

Mast cell counts appeared to vary considerably within individuals, some increasing and others decreasing with time. As mast cells were few, sampling errors may have accounted for some of this variability. Others have reported increased mast cell numbers in the terminal ileum and right colon of some patients with IBS.[48] These may be a distinct group from those with EC hyperplasia described here.

In group 2 subjects only paraffin embedded sections were available for study and hence they cannot be compared fully. However, clinically, they were similar and have the advantage of representing cases with a much longer follow up. Evidence from these patients suggests that some of the lymphocyte and EC changes described here can be sustained for many years. This is supported by our data on the seven subjects from our initial 47 who have been studied for more than a year after their initial illness. Their increased EC, CD3, and IEL counts clearly indicate that such changes can be sustained and appear to be associated with a poorer outcome.

Our own recent audit of gastroenterological outpatients indicates that about 25% of patients (13 of 64) with unexplained diarrhoea report an acute onset compatible with acute gastroenteritis.[49] Accurately defining this distinct subgroup from other aetiologies will be important in facilitating our understanding of IBS, which at present is a large heterogenous ill defined population. Symptoms are highly subjective and hard to interpret, correlating poorly with currently available objective assessments. We would suggest that increased EC and IEL numbers in a rectal biopsy, which are both relatively easy to assess in routine pathological practice, may allow objective definition of this subgroup. This is particularly important as these patients may well respond specifically to anti-inflammatory agents and/or 5-$HT_3$ receptor antagonists.

These patients have normal mucosa using conventional histological criteria and can be clearly distinguished histologically from microscopic colitis, in which unequivocal inflammatory changes are apparent. We have not found increased EC numbers in lymphocytic colitis; we found that IEL counts averaged 7.4 (1.8)/$mm^2$, three times the peak average value after *Campylobacter* enteritis (unpublished data). Similar increases in IEL counts have been reported by others.[50]

The implications of persistent immune, inflammatory, and EC changes in colorectal mucosa following *Campylobacter* infection and in acute onset IBS are numerous. If inflammatory cytokines and/or an immune response drive EC cell hyperplasia, it may be possible to suppress cytokine production pharmacologically and prevent the development of PD-IBS. Furthermore, 5-HT antagonists may have specific efficacy in such patients. Increased EC and IEL counts in a rectal biopsy may be a histological marker of a previous infectious insult in patients with an uncertain history or otherwise unexplained diarrhoea and may also predict a therapeutic response. However, this study was not designed to assess the diagnostic

value of this finding which will require a separate prospective study of patients attending with acute onset diarrhoea. Providing a definitive histological diagnosis would be valuable if it could reduce the burden of unnecessary further tests which these patient often undergo.

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