Human lymphocyte stimulation with pouchitis flora is greater than with flora from a healthy pouch but is suppressed by metronidazole

A J G Bell, R J Nicholls, A Forbes, H J Ellis, P J Ciclitira

Background/Aims: The gut flora may play an important role in the pathogenesis of inflammatory bowel disease. An ileal reservoir or pouch can be created to replace the excised rectum after proctocolectomy. In patients with ulcerative colitis this is subject to inflammation and termed pouchitis. Using bacteria from patients the authors sought evidence for the presence rather than the identity of a pathogenic species in pouchitis, and for its absence in healthy pouches by the differential effect on lymphocyte proliferation.

Methods: An ex vivo cell culture assay was used in which peripheral blood mononuclear cells or lamina propria mononuclear cells were cultured with sterile sonicates of gut flora from patients with or without pouchitis in the presence of antigen presenting cells.

Results: Sonicated pouchitis flora produced a consistent and intense proliferation of the mononuclear cells but that produced by sonicates from healthy pouches was minimal (p = 0.012 or 0.018, peripheral blood or lamina propria mononuclear cells). Preparation of the sonicates with the antibiotic metronidazole abolished their stimulatory ability (p = 0.005, peripheral blood mononuclear cells). In separate assays neither direct addition of metronidazole nor of its hydroxy metabolite affected the mononuclear cells’ proliferation with alternative stimuli.

Conclusions: These results strongly support a bacterial aetiology for pouchitis.

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INFLAMMATORY BOWEL DISEASE

The construction of a neorectum from the distal ileum and its anastomosis to the anus provides restoration of intestinal continuity in patients who have undergone total proctocolectomy.9 This ileal neorectum, or pouch, is prone to inflammation which is known as pouchitis and which almost exclusively affects those with a diagnosis of ulcerative colitis (UC) rather than familial adenomatous polyposis.6 Evidence suggests a role for bacteria in the pathogenesis of inflammatory bowel disease (IBD).2 One of the hypotheses is that when the faecal stream is diverted away from the diseased area,2 in addition, there is benefit from manipulation of the flora by using antibiotics or probiotics in pouchitis and in animal models11 although in Crohn’s disease the proven efficacy of antibiotics is limited to the specific situations of avoiding postoperative recurrence14 and perianal disease.15 Despite the findings in experimental colitis, there is little evidence for antibiotic use in UC.16 Several candidate species have been investigated as potential aetiological agents. For example, increased numbers of E coli and bacteroides have been found adherent to the mucosa in IBD patients, although moderately raised levels of adherent bacteria in subjects with self-limiting inflammation weaken the case for causation.3 Increased concentrations of the same bacteria together with fusobacteria in the neoterminal ileum have been found to correlate with early postoperative relapse in Crohn’s disease.16 Fusobacterium varium has also been observed within the mucosa of UC patients where it seems to stimulate a species specific antibody. Interestingly, when cultured, the supernatants from the cultures of these human isolates produce ulcers in the colons of mice.19

Investigation of bacteria with distinct metabolic traits has revealed that whereas a majority of UC pouches harbour sulphate reducing species, these are absent from familial adenomatous polyposis pouches.20 However, the intuitive approach of identifying a specific pathogen by comparing the results of culture in IBD and controls is rendered impractical by the number of species present and the lack of universal survival outside the body.21 More indirect methods have been used in attempting to identify a pathogen. Serological studies of excreted and circulating antibacterial antibodies,22,23 and reactivity of bacterial components with commonly found disease related antibodies such as ANCA in UC or ASCA in Crohn’s disease have implicated B vulgatus (53% v 1% seroreactivity in controls) and E coli in active UC. These are found among many commensals also stimulating a serological response. The response to commensals suggests these findings may reflect secondary colonisation of lesions rather than the cause. The same interpretation can be made for the identification of bacterial components from lymphoid follicles or gut lesions.24,25 This argument weakens the otherwise strong case made for Mycobacterium paratuberculosis, a putative pathogen in Crohn’s disease, by the recent demonstration of its DNA in 92% of cases versus 26% of controls using the most sensitive methods.26 DNA fingerprinting using 16S rRNA technology holds more promise than culture but remains a costly method in terms of time and cannot identify all bacteria present. Our aim was to show the presence rather than the identity of a pathogen or pathogens in the gut flora of pouchitis sufferers by an ex vivo lymphocyte stimulation assay.

Abbreviations: IBD, inflammatory bowel disease; LPMC, lamina propria mononuclear cells; MTZ, metronidazole; PBMC, peripheral blood mononuclear cells; PDAI, pouch disease activity index; PHA, phytohaemagglutinin; SI, stimulation index; UC, ulcerative colitis; Aut, a sonicate derived from pouch flora of same patient whose LPMC/PBMC are under stimulation; Het, a sonicate derived from pouch flora of a different patient from the one whose LPMC/PBMC are under stimulation; P, a sonicate derived from pouch flora of a patient with pouchitis; Non, a sonicate derived from pouch flora of a patient without pouchitis; M, a sonicate derived from pouch flora grown in the presence of MTZ
We tested the hypothesis that lamina propria mononuclear cells (LPMC) and peripheral blood mononuclear cells (PBMC) would proliferate more with a sonicate made from the bacterial flora present in pouchitis than they would with one made from the flora in healthy pouches. Secondly, we tested the hypothesis that the antibiotic metronidazole (MTZ) might prevent the growth of the stimulatory bacteria.

**MATERIALS AND METHODS**

**Subjects**

Patients aged 18–70 years who had undergone ileal pouch formation and ileostomy reversal for ulcerative colitis (UC) and under regular follow up at a single centre were recruited by letter followed by a telephone call. Pouchitis was defined as a total score of 7 or more on the pouch disease activity index (PDAI) in patients with clinical, macroscopic, and histological changes. Exclusion criteria were significant comorbidity leading to increased hazard with extra biopsies such as ischaemic heart disease, chronic airways disease, insulin dependent diabetes mellitus, and current anti-coagulant use. The subjects gave written informed consent and the Harrow Research Ethics Committee approved the study. Twenty two patients were recruited. One patient failed to attend. Bacterial contamination of the sterile lymphocyte cultures occurred in three. Thus, data from 18 were analysed (10 male, 8 female). The median age range was 38.5 years (range 27–68 years). Median pouch age was 126 months (range 2–266 months) and median PDAI score was 1.75 (range 0–9). One patient had active pouchitis (PDAI score 9) with a history of recurrent episodes and ankylosing spondylitis.

**Cell preparation and harvest**

After at least two weeks patients returned for further biopsies and venesection. LPMC: biopsies were collected into HBSS-CMF (Gibco) and immediately treated with EDTA and EDTA followed by a 14 hour collagenase digestion with DNAase I (Boehringer Ingelheim, Bracknell, UK) in AIM V lymphocyte medium (Gibco). Medium and cells were collected through a cell strainer (Falcon, via Marathon LS, London, UK) and washed before density gradient centrifugation over Ficoll-paque plus (Amersham Pharmacia Biotech, St Alban’s, UK). PBMC: Ficoll density gradient centrifugation was performed. Cells were >95% viable by trypan blue exclusion. FACS analysis revealed that the CD4+ PBMC were 81% CD3+ of which 67% were CD4+ and 14% CD8+. CD4+ LPMC were 89% CD3+ (49% CD4+; 40% CD8+). To avoid degradation of cell surface ligands further purification was not performed. Antigen presenting cells were PBMC that had been irradiated with 8.6 Gy of gamma irradiation.

**Bacterial sonicate preparation**

Four biopsies were taken via a sterile rigid sigmoidoscope from the lower posterior portion of the pouch. Using sterile forceps each biopsy was immediately smeared onto an agar plate preheated to 37°C and kept in an insulated bag. For aerobes, CHB agar (Oxoid, Basingstoke, UK) was used. For anaerobes two plates of fastidious anaerobe agar (E&O Labs, Bonnybridge, UK) peincubated in the “anaerogen compact” (Oxoid) were used and immediately replaced in the “anaerogen” system to optimise conditions for anaerobic growth. One of these was impregnated with MTZ at 200 μg/ml followed by a 14 hour collagenase digestion with DNAase I (Boehringer Ingelheim, Bracknell, UK) in AIM V lymphocyte medium (Gibco). Medium and cells were collected through a cell strainer (Falcon, via Marathon LS, London, UK) and washed before density gradient centrifugation over Ficoll-paque plus (Amersham Pharmacia Biotech, St Alban’s, UK). PBMC: Ficoll density gradient centrifugation was performed. Cells were >95% viable by trypan blue exclusion. FACS analysis revealed that the CD4+ PBMC were 81% CD3+ of which 67% were CD4+ and 14% CD8+. CD4+ LPMC were 89% CD3+ (49% CD4+; 40% CD8+). To avoid degradation of cell surface ligands further purification was not performed. Antigen presenting cells were PBMC that had been irradiated with 8.6 Gy of gamma irradiation.

**Cell culture assays**

Each assay was performed in triplicate in AIM V medium with added Plasmocin antibiotic (Invivogen, Cayla, Toulouse, France) at 1 in 10 000 with 5 × 10^5 LPMC or PBMC and 5 × 10^5 antigen presenting cells per well in 96 well U-bottomed plates (Falcon). The antibiotic was in routine use in the cell culture laboratory, and should not have influenced results as it acts on synthetic pathways of non-eukaryotes. Where cell yields allowed, all six sonicates were tested: AutM, Aut, HetPM, HetP, HetNonM, and HetNon all at 50 μg/ml. As a positive control 0.2 μg of phytohaemagglutinin (PHA) was added, and albumin was added in lieu of sonicate as an unstimulated control.

In parallel experiments MTZ and its hydroxy metabolite (kindly donated by Aventis Pharma, Vitry-sur-Seine, France) were added to PHA and B. vulgatus cultures at 100 μM and 50 μM, respectively, which equate to reported plasma concentrations. These were to examine the direct effect of MTZ on cell culture, which is debated. They also serve as controls to eliminate possible carry over of MTZ with the bacteria from the culture plates to the cell cultures as the explanation of any effect seen in the sonicate stimulation assays. Precautions were observed to avoid accidental direct contact with PHA. Sonicates were plated on agar to test for sterility. Wells which generated colony forming units overly or when plated were discarded. Incubation in a humidified incubator at 37°C and in 5% CO₂ lasted 96 hours with 1H added at 1 μCi/well for a further 16 hours. 1H incorporation was assessed. Institutional safety guidelines for the handling and disposal of radioactive reagents were adhered to.

Proliferation was recorded as the stimulation index (SI) which is the mean scintillation of the triplicate wells divided by the mean of the three unstimulated wells. An SI >3 was taken to represent increased proliferation beyond experimental error.

The Wilcoxon signed rank test was used for comparison of treatment conditions. Significance for a two tailed test was chosen as p<0.05.
RESULTS
Comparison of proliferation with bacterial sonicates of different origins
In general, mononuclear cells proliferated little when they were stimulated with sonicates derived from either autologous or heterologous pouch bacteria if that pouch was healthy, nor was there a significant difference between the level of proliferation they each induced (p = 0.247, n = 12 PBMC; p = 0.678, n = 12 LPMC) (fig 1A).

However, in the majority of cases strong proliferation was seen with sonicate derived from pouchitis flora whereas weak proliferation close to baseline was observed with non-pouchitis sonicate. This was in eight patients whose mononuclear cells were tested under both conditions in parallel (p = 0.012; PBMC) (fig 1B).

Metronidazole affects proliferation when present at the bacterial culture stage but not when added to the mononuclear cell culture with the sonicate
When pouchitis bacteria were cultured simultaneously in the presence or absence of MTZ the sonicate derived from the MTZ impregnated plate lost the capacity to induce proliferation almost entirely (p = 0.009, n = 10; PBMC) (fig 1C). The proliferation was less for LPMC with no significant difference in that provoked between the two types of sonicate (p = 0.314, n = 10).

Proliferation with non-pouchitis sonicate was close to baseline levels regardless of the presence or absence of MTZ in the original agar.

To examine whether MTZ has a profound effect on lymphocyte proliferation, as has been claimed, in a smaller number of parallel experiments MTZ or its hydroxy metabolite were added to PBMC and LPMC cultures together with medium, B. vulgatus sonicate or PHA.

For each of the subjects, lymphocyte proliferation with progressively stronger stimuli (medium, then B vulgatus sonicate, then PHA) increased as expected. However, for a given subject under any one of those conditions the addition of MTZ or its hydroxy metabolite made little difference to proliferation. No consistent effect was seen in all subjects under any one condition. This was true for PBMC (fig 2A–C) and LPMC (fig 2D–F).

Mononuclear cell proliferation with other stimuli
PHA was used as a positive control to confirm viability of the mononuclear cells. The median SI was 560 for PBMC and 177 for LPMC. Sonicate prepared from E coli reduced proliferation below SI of 1 in nearly all cases—that is, below the spontaneous level of proliferation of the mononuclear cells (fig 3A). The same pattern was seen with sonicate from Bifidobacterium bifidum and from Bacteroides thetaiotaomicron in three patients in whom they were tested. In contrast, the sonicate from B vulgatus proved an exceptionally strong stimulus to mononuclear cell proliferation. (fig 3B)

DISCUSSION
Interaction of the gastrointestinal flora and the gut wall is essential for health. The observation of a specific gut bacterial profile which seems to persist,6 11 like a part of one’s phenotype, supports the theory that flora probably interact with the host genotype.6 21 Certain bacteria may interact with certain genotypes to produce disease states: germ free HLA B27 transgenic rats developed severe colitis only when they were exposed to Bacteroides vulgatus.6 Therefore, it seems reasonable to propose that some bacterial species or their products interact with hosts with a susceptible genotype to produce mucosal disease.

Given the technological constraints on identifying the different species present in the gut flora and on comparing the total flora of any case and control, we sought to establish the presence of pathogenic species rather than their identity.

Using an ex vivo lymphocyte stimulation assay we examined the flora in pouchitis. We found that bacterial sonicate from a heterologous but healthy pouch, HetNon, did not stimulate lymphocytes (PBMC or LPMC) from patients with healthy pouches. Similarly, there was a seemingly anergic response to sonicate of their own bacteria, Aut (fig 1A). The latter result is consistent with the theory that individuals are tolerant of their own gastrointestinal flora. However, our finding of tolerance to the flora of strangers differs from the findings of Duchmann,6 who found a
proliferative response to foreign flora. Our use of feeder cells may reflect the mucosal environment more faithfully, as feeder cells (rather than T cells) have been shown to be the main source of the anti-inflammatory cytokine IL-10 when rodent cells are stimulated with commensal bacterial products. In addition, it is not clear whether their sonicates were derived from inflamed areas. More simply, the explanation may be a dosage effect as their sonicate was 10 times more concentrated than ours.

Our findings with sonicate from inflamed tissue agree with theirs, and this stimulatory effect would be consistent with a more aggressive species being present in a greater predominance in inflamed tissue. The degree of inflammation in the bacterial donor bowel has been found to influence profoundly the stimulatory effect of the sonicate derived from it (Sartor RB, personal communication, 2002). This provides evidence for bacterial pathogenesis of the initial inflammation in that local change to a more pathogenic microflora may initiate activation of the local mononuclear cells. This theory is supported by a recent study showing that lymphocytes respond to the presence of pathogens before active inflammation appears. T cells isolated from mesenteric lymph nodes of E. faecalis mono-associated IL-10 knockout mice produced more interferon gamma when pulsed with E. faecalis than with B. vulgatus. T cells from wild type mice without this exposure did not show the increase.

Our results using other specific sonicates are consistent with other published data regarding intense stimulation with B. vulgatus and inhibition of, or low level proliferation of, LPMC and PBMC with E. coli. We have shown that pouchitis derived sonicate (HetP) induces more proliferation in another individual's lymphocytes (PBMC or LPMC) than non-pouchitis derived sonicate (HetNon) (fig 1B). Extraneous factors such as the "cytokine soup", and other host factors in which the bacteria making up the sonicate may have existed before collection, were carefully excluded as colonies were grown from smears of biopsies and were later harvested from the agar. The constituent species of the sonicates was not something we set out to determine. Feasibly, a pathogenic species is present in all pouches but simply in greater number in inflamed ones. However, our examination of the role of the flora in pouches shows that key differences exist and that elements present in pouchitis flora may specifically activate inflammation.

Metronidazole is effective treatment for pouchitis. Because it has been suggested (although not all would agree) that the action of MTZ might be as an immune modulator, we attempted to separate its effect on lymphocyte stimulation from its effect on the bacteria involved in the stimulation. Thus, the MTZ was introduced in the agar rather than into the cell culture. The concentration was similar to that in serum following oral dosing. A fall to baseline of the lymphocyte proliferation when pouchitis flora were grown on MTZ impregnated medium instead of conventional medium was seen (fig 1C).
A smaller number of further assays was conducted to examine the effect of direct addition of MTZ or its main metabolite to cell cultures at a concentration found to exert an inhibitory effect in previous studies. No such reduction in proliferation was seen across assays using, variously, no stimulus, mitogen (PHA), or B. vulgatus sonicate (fig 2 A–F). This makes it unlikely that our results reflect carry over of MTZ into cell cultures via incorporation into growing bacteria where it then directly inhibited lymphocyte proliferation. Rather, the organisms responsible for the proliferation appear to be un inhibited by the MTZ. This suggests that the reason for the in vivo efficacy of MTZ is its antibacterial action.

The pathogenic species whose presence we propose is shown in our experiment and which are important in the aetiology of pouchitis are, therefore, metronidazole sensitive. MTZ has no action against aerobes or facultative anaerobes. It is active against anaerobes especially B. fragilis and other bacteroides and is also bactericidal for fusobacteria, eubacteria, clostridia, and anaerobic cocci. Although it has an antiopozial action it is ineffective against mycobacteria.

CONCLUSION
We have shown that pouchitis derived bacterial sonicate stimulates healthy pouch patients’ mononuclear cells significantly more than non-pouchitis derived sonicate. Taking MTZ into cell cultures via incorporation into growing bacteria where it then directly inhibited lymphocyte proliferation. This makes it unlikely that our results reflect carry over of MTZ into cell cultures at a concentration found to exert antiprotozoal action it is ineffective against mycobacteria.

REFERENCES
Human lymphocyte stimulation with pouchitis flora is greater than with flora from a healthy pouch but is suppressed by metronidazole
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**LETTERS**

**ITPA genotyping test does not improve detection of Crohn’s disease patients at risk of azathioprine/6-mercaptopurine induced myelosuppression**

The thiopurine drugs azathioprine (AZA) and 6-mercaptopurine (6-MP) are effective for the treatment of inflammatory bowel disease (IBD) and their prescription is increasing. Haematotoxicity, which can lead to potentially life threatening bone marrow suppression, represents the most serious side effect of thiopurine therapy. It has been attributed to the accumulation of active cytotoxic metabolites of AZA/6-MP, collectively called 6-thioguanine nucleotides, resulting from a deficiency in thiopurine catabolism specifically catalysed by the thiopurine S-methyltransferase (TPMT) enzyme. Genotyping tests are now available to identify deficient and intermediate metabolizers who are, respectively, homozygous and heterozygous for non-functional alleles of the TPMT gene. As pointed out by Lennard in the leading article (Gut 2002; 51:143–6), it is clear that myelosuppression may be caused by other factors in addition to variable TPMT activity.

Since the identification of the molecular basis of inosine triphosphate pyrophosphohydrolase (ITPAse) deficiency, a clinically benign condition characterised by abnormal accumulation of inosine triphosphate in erythrocytes, the possibility of a correlation between thiopurine toxicity and ITPase deficiency has been raised. Complete ITPase deficiency was found to be associated with a homozygous missense 94C>A mutation that encodes a Pro31Thr exchange, whereas an intronic IVS2+21A>C polymorphism was shown to have a less severe effect, homozygotes retaining 60% ITPase activity. It was then postulated that in ITPase deficient patients treated with thiopurine drugs, a 6-thio-ITP metabolite could accumulate resulting in toxicity. A recent study in 62 patients with inflammatory bowel disease reported a significant association between the ITPA 94C>A polymorphism and AZA related adverse effects, specifically flu-like symptoms, rash, and pancreatitis. No correlation was observed with occurrence of neutropenia but only 11 patients were studied. We previously reported TPMT genotype analysis in 41 Crohn’s disease (CD) patients who had experienced leucopenia during AZA/6-MP therapy. Even though this study confirmed the efficiency of TPMT genotyping in identifying patients at risk of developing myelosuppression, it also highlighted its limitations, as only 27% of patients carried mutant alleles of the TPMT gene that were associated with enzyme deficiency. This prompted us to investigate the occurrence of ITPA mutations in this series of patients in order to evaluate whether genotyping of the ITPAse gene could improve the detection rate of patients at risk of thiopurine myelotoxicity. Our population comprising 41 patients with CD has been described in detail previously. Briefly, all patients had either leucopenia (white blood cell count <3000/mm³; n = 24) or thrombocytopenia (platelets <100 000/mm³; n = 30), or both (n = 14), leading either to discontinuation of treatment or reduction of dose by 50% or more during AZA (n = 33) or 6-MP (n = 8) treatment. Patients were genotyped for the ITPA 94C>A and IVS2+21A>C mutations according to a previously described procedure based on endonuclease digestion of polymerase chain reaction products. Distribution of the 41 patients according to their ITPA genotype is presented in table 1 and compared with that of a previously published control population of 100 healthy Caucasians. Allele frequencies in the CD population were 0.085 for the 94C>A mutation and 0.12 for the IVS2+21A>C mutation, similar to frequencies observed in the control population (0.06 and 0.13, respectively). There was no significant difference in the genotypes distribution between the two populations, which confirmed the lack of association between ITPase deficiency and myelosuppression during thiopurine therapy. Due to the retrospective nature of the study, no correlation with other side effects could be investigated.

In conclusion, application of ITPA genotyping tests does not seem to improve the identification of patients at risk of myelosuppression with AZA/6-MP therapy. Although we believe that conventional TPMT genotyping tests should still be applied before the initiation of thiopurine treatment, further work is needed on the role of other candidate genes that may be involved in thiopurine haematotoxicity.

**Acknowledgements**

We thank N Ferrari and A Vincent for their assistance in performing the study and the members of the GETAID for recruiting patients in the study.

**Table 1** Distribution of ITPA genotypes in 41 Crohn’s disease (CD) patients and 100 healthy Caucasians

<table>
<thead>
<tr>
<th>ITPA genotype</th>
<th>CD patients (n = 41)</th>
<th>Control population (n = 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>W/W</td>
<td>26 (0.63)</td>
<td>64 (0.64)</td>
</tr>
<tr>
<td>W/94C&gt;A</td>
<td>6 (0.15)</td>
<td>10 (0.10)</td>
</tr>
<tr>
<td>W/IVS2+21A&gt;C</td>
<td>7 (0.17)</td>
<td>24 (0.24)</td>
</tr>
<tr>
<td>94C&gt;A/94C&gt;A</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>IVS2+21A&gt;C/IVS2+21A&gt;C</td>
<td>1 (0.02)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>94C&gt;A/IVS2+21A&gt;C</td>
<td>1 (0.02)</td>
<td>2 (0.02)</td>
</tr>
</tbody>
</table>

*Values in parentheses represent genotype frequencies.
†The control population comprised 100 healthy Caucasians who were genotyped in a previous study.

**Small bowel malignancy at diagnosis of coeliac disease**

We were very interested in the paper by Rampertab et al (Gut 2003; 52:121–14) and the correspondence by Hawdle et al (Gut 2004; 53:1025–30). Their data are quite similar to ours, from the Italian Registry of Complications of Coeliac Disease.

We collected information on 1968 patients over 18 years of age (mean age at diagnosis: 36.7 years; female/male ratio 3:1), diagnosed with coeliac diseases between January 1982 and December 2002 at 20 Italian clinical centres specialised in gastrointestinal disease. The diagnosis was made according to revised ESPGHAN criteria. We found five (0.25%) patients with a small bowel malignancy at the time of diagnosis of coeliac disease. Age range was 49–69 years (mean 59 years) with a predominance of females (4:1). Survival rate was very poor as three patients died within 36 months of diagnosis.

These results indicate that there is an increased risk of developing small bowel malignancy in patients with coeliac disease. This correlation was confirmed by the female/male ratio. In fact, while small bowel neoplasms are usually more frequent in males, in our population four of five cases were female. Moreover, mean age at diagnosis of these cases was higher than that of patients overall, emphasising that the risk of a neoplasm increases with longstanding coeliac disease.

**References**


In conclusion, early diagnosis of coeliac disease should be made to prevent small bowel neoplasms from developing, and screening for this cancer should be carried out at diagnosis of coeliac disease, especially in patients diagnosed during adulthood.

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Competing Interests: None declared.

Reference

Hypergastrinaemia in patients infected with Helicobacter pylori treated with proton pump inhibitors

We read with interest the commentary by McColl on Helicobacter pylori infection and long term proton pump inhibitor (PPI) therapy (Gut 2004;53:5–7).

It is remarkable that he did not mention gastrin although hypergastrinaemia is a result of reduced gastric acidity as well as Helicobacter pylori infection, and that patients with H pylori infection treated with PPI have additive hypergastrinaemia. Hypergastrinaemia predisposes to gastric carcinoids in animals and humans as well as to malignant ECL cell derived tumours (gastric carcinomas) in animals and humans.

Interestingly, the carcinogenic effect of H pylori infection may be completely explained by its hypergastrinaemic effect, a work where McColl was one of the authors. Furthermore, the increased gastric cancer frequency in moderate hypergastrinaemic INS-GAS mice concomitantly infected by H pylori infection may also be caused by increased hypergastrinaemia in infected mice.

To conclude, it is odd that gastrin was not taken into consideration when discussing the risk of gastric cancer following treatment with PPI in patients infected with H pylori. Animal as well as human studies linking gastrin to gastric cancer give support for a strategy where H pylori is eradicated in patients on long term PPI treatment.

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Competing Interests: None declared.

References

Terminal ileal biopsies should not be used to document extent of colonicoscopy examination

We commend the British Society of Gastroenterology and the authors for their excellent publication of guidelines for the management of inflammatory bowel disease in adults (Gut 2004;53(suppl V):vi–16).

However, we feel that their recommendation for routine terminal ileal biopsy is inappropriate. Although it is important to biopsy the terminal ileum if there is macroscopic evidence of an abnormality, their statement that “a terminal ileal biopsy performed at colonoscopy documents the extent of examination” is not recommended practice, due to the potential risk of variant Creutzfeld-Jacob disease transmission from prion proteins which are prevalent in the lymphoid tissue of Peyer’s patches in the ileum. Although the use of disposable forceps may reduce the risk of transmission, there could still be contamination of the intubation channel of the colonoscope and prion protein is resistant to the standard endoscopic cleaning process.

If the extent of examination needs to be documented, then a photograph of the ileoccaeval valve or ileal mucosa is preferable.

It is worth emphasising that prion protein may be present in any part of the gastrointestinal tract and random biopsy of gastrointestinal mucosa for reasons other than confirming an endoscopic abnormality or excluding microscopic colitis is not acceptable. Similarly, for surveillance colonoscopy where multiple biopsy is recommended, the risk benefit ratio of this policy must be supported by the clinical indications.

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IgG food antibodies should be studied in similarly treated groups

The recent paper by Atkinson and colleagues (Gut 2004;53:1459–1464) regarding IgG food antibodies and irritable bowel syndrome (IBS) fails to compare like with like.

Regardless of the IgG results, the treatment group excluded significantly different foods to the control group, particularly those foods which appear to exacerbate symptoms of IBS. Of particular concern is the “yeast exclusion” diet. A low yeast diet is not a recognised diet in standard textbooks of dietetics and nutrition. However, alternative practitioners offering such a “yeast exclusion diet” sometimes recommend exclusion of a wide range of foods, such as: bakery products, alcoholic beverages, many other beverages including commercial fruit juices, cereals, condiments, dairy products, fungi, meat products (hamburgers, sausages, and cooked meats made with bread or breadcrumbs), yeast extracts (Bisto, Marmite, Oxo, Bovril, Vegemite, gravy browning, and all similar extracts), all B vitamins preparations, and, most worryingly, “sugar foods” (sugar, sucrose, fructose, maltose, lactose, glycerogen, glucose milk, sweets, chocolate, sweet biscuits, cakes, candies, cookies, puddings, desserts, canned food, packaged food, hamburgers, honey, manniitol, sorbitol, galactose, monosaccharides, polysaccharides, date sugar, turbinoed sugar, molasses, maple syrup, most bottled juices, all soft drinks, tonic water, milk-shakes, raisins, dried apricots, dates, prunes, dried figs, and other dried fruit).

Therefore, regardless of IgG antibody status, the dietary restrictions in one group are not controlled for by the other group, and hence the conclusion may not be valid.

It would also be helpful to know if any of the patients with IgG antibodies to a particular antigen also had IgE antibodies to the same antigen.

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Competing Interests: None declared.
IgG antibodies to foods in IBS
We read with interest the article by Atkinson et al (Gut 2004;53:1459–64). The authors describe an important advance in our understanding of the putative role of inflammation in irritable bowel syndrome (IBS). However, we wonder whether their conclusion that assay of IgG antibodies may have a role in identifying candidate foods for elimination to treat patients with IBS may be a step too far. The four foods to which the patients most commonly formed antibodies and hence the four foods most commonly eliminated from the “true diet” were yeast (86.7%), milk (84.3%), whole egg (58.3%), and wheat (49.3%). The “sham diet” involved eliminating foods to which the patients had not formed antibodies and, therefore, in the sham group the exclusion rates for yeast, milk, whole egg, and wheat were very low (0%, 1.3%, 26.7%, and 8% respectively). It is therefore difficult to assess whether a diet excluding these foods would have led to symptomatic improvement in all patients, regardless of their antibody status.

Furthermore, the foods to which the study group commonly formed antibodies were similar to those already identified as leading to symptomatic benefit in patients with IBS when excluded from their diet. In a review cited by Atkinson and colleagues,1 it was noted that in eight trials of exclusion diets in IBS, seven identified dairy products and five identified wheat as worsening symptoms. It is not clear whether the difference in improvement in symptoms seen in the current study between true and sham groups can be explained simply by the omission of these foods. This could in practice eliminate the need for antibody testing.

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Reference

Influence of dietary factors on the clinical course of inflammatory bowel disease
Jowett et al reported in their elegant study on the role of diet in maintaining remission in patients with ulcerative colitis (Gut 2004;53:1479–84). Surely the effect of diet has an essential, but often forgotten, role in altering the course of disease in all types of inflammatory bowel diseases. This role does not necessarily act by maintaining patients in remission clinically, but perhaps more importantly by modifying the activities of the disease and rendering it quiescent.

We have recently reported a case of active strictureing Crohn’s disease in an adult female patient with high stoma output.1 We were able to reduce the stoma output significantly after adopting a casein based formula (Modulen IBD-Nestle, Vevey, Switzerland) for three weeks. Her stoma output was reduced from 2800 ml to 400 ml per day by day 10. Serum albumin and serum protein significantly increased also. She subjectively felt better and pain free and stopped her opiate and non-opiate formula. The casein based formula is a nutritionally complete formulation containing a natural anti-inflammatory growth factor, transforming growth factor α2. The mechanism for inducing remission in our patient was possibly inhibition of expression of MHC class II protein in downregulating the inflammatory response.2

Previous studies have shown that there is a decrease in plasma antioxidant defences in all types of inflammatory bowel disease.3 This is mirrored by an increase in free radical peripheral leucocyte DNA damage. It is therefore possible that the casein based formula acts as an antioxidant to minimise the oxidative stress that occurs in patients with active Crohn’s disease. Another possible mechanism is that this formula may have a role as a prebiotic by stimulating the activity of bacteria which are already present in the gut.

Remission induced in our case study highlights the part played by a casein based formula in the management of adult Crohn’s disease. The encouraging result demonstrates the need to treat similar cases with dietary measures first. This opportunity should not be missed as it may well obviate the need for surgical intervention or administration of potent pharmacotherapeutic agents which carries the risk of several comorbidities.

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References

Identification of ferroportin disease in the Indian subcontinent
Haemochromatosis is a common inherited disorder of iron metabolism, characterised by excessive iron absorption and deposition in tissues. The majority of cases are associated with mutations in the HFE gene and inherited in an autosomal recessive manner.1 Autosomal dominant forms of haemochromatosis have been reported, mainly associated with mutations in the ferropontin 1 gene.2 This syndrome, termed type 4 haemochromatosis or more recently ferroportin disease, is usually characterised by an early increase in serum ferritin with normal transferrin saturation. Iron accumulation is most prominent in Kupffer cells and other macrophages, in addition to hepatocytes. Some patients do not tolerate venesection therapy well and can develop anaemia. Hereditary iron overload disorders appear to be uncommon in Asia. Secondary iron overload due to beta thalassaemia trait is relatively common in the Indian subcontinent. However, primary iron overload disorders and HFE mutations appear to be rare and cases have not been well characterised in this region.3 We identified a patient from the Indian subcontinent with features typical of ferroportin disease.

A 36 year old female of Sri Lankan origin presented for a routine medical examination in December 2003. She was found to have an elevated serum ferritin of 17160 ng/ml and transferrin saturation (29%) were normal. Liver function tests, blood glucose, and thyroid studies were all normal. Physical examination was normal and she had no significant past medical history or risk factors for iron overload.

C282Y, H63D, and S65C HFE gene mutations were all negative and she had no family history of iron overload. Her mother and three siblings all had normal serum ferritin levels. Her father died of ischaemic heart disease aged 48 years.

A magnetic resonance imaging scan showed hepatic iron overload. Liver biopsy showed grade 3–4 iron deposits in the hepatocytes and Kupffer cells; no fibrosis or cirrhosis was evident (fig 1). The hepatic iron concentration was 17 700 μg/g dry weight which was hepatic iron index was 9.1.

Venesection therapy was initially poorly tolerated with the development of anaemia following the first two 500 ml venesections. Her haemoglobin is now stable on a programme of 300–500 ml venesections every three weeks.

The features of ferroportin disease in this patient led us to sequence the ferroportin 1 gene, as previously described.4 Analysis of the DNA sequence revealed a heterozygous three base pair deletion (TTG) in exon 5. This is the same deletion, V162del, described by us and others in haemochromatosis patients from Australia, the UK, Italy, and Greece.5–6 This is the first report to identify V162del or indeed any ferroportin 1 mutation in an individual from the Indian subcontinent. Identification of V162del in an Asian patient confirms that this mutation is likely to be the most common mutation of ferroportin 1 and the most common cause of non-HFE associated haemochromatosis. The wide geographical distribution of this mutation suggests that it is a recurrent mutation that has repeatedly arisen in distinct populations, probably by slippage mispairing.

Iron overload in this patient was typical of ferroportin disease. At the time of diagnosis she was asymptomatic and had no fibrosis on liver biopsy. Whether fibrosis or clinical complications will develop with age if iron stores are not depleted is unknown.

In conclusion, we have identified the V162del mutation of ferroportin 1 in a fifth geographical location, emphasising that this mutation is the most common and widely distributed mutation which causes non-HFE haemochromatosis. We have identified V162del in a region where iron overload disorders have not been well characterised. Analysis of this and other ferroportin 1 mutations may be useful in iron overload disorders in this region and may be the basis of hitherto unexplained cases of iron overload.

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Figure 1 Liver biopsy sections from our patient stained with (A) haematoxylin and eosin and (B) Perls’ Prussian blue (magnification 100×). Grade 3–4 iron is prominent in hepatocytes and Kupffer cells.

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References

BOOK REVIEW

Morson and Dawson’s Gastrointestinal Pathology, 4th edn

Why do people buy a book such as this, which involves a not inconsiderable financial outlay (even if you box clever and make it tax deductible)? I think for two main reasons—firstly, for use as a bench book, and secondly, for information on the pathological basis of gastrointestinal disease for interest, teaching, or indeed research purposes.

On the first criterion, this book succeeds, usually quite brilliantly. As a vade mecum on gastrointestinal pathology it should be on the shelf of every pathologist who engages in the reporting of such material. In my view, the book is more user friendly than the competition—Fenoglio-Preiser and Goldman to name but two—and is certainly more readable. I would therefore extol its virtues unreservedly in this respect.

On the second criterion, as a source book, I suppose the correct word is patchy. Some sections, for example that on colorectal tumours, is admirable in this respect, whereas other sections are more limited in scope and even cursory in their treatment of the pathobiology. There is also the problem of the unavoidable intrinsic delay in producing such a book, resulting in reference lists which are some years away from the publication date. I am aware however that my personal outlook is not that of most individuals who will purchase this volume so I am probably being over critical. It is, after all, quintessentially a bench book, and excellent at that.

However, I do have one real beef. In any multi-author work there is bound to be variation, but here we are not told which one of the stellar cast were responsible for which section or chapter. Of course we can make informed guesses about the Barrett’s or colorectal carcinoma sections, but who did the GIST bit? Because of some (minor) errors in the criteria for the diagnosis of malignancy, I have tried to berate a number of authors who have all denied responsibility, and blamed someone else—usually the author(s) absent at the time. Not good enough.

I have to concede however that the authors have succeeded in producing perhaps the test in gastrointestinal pathology, which is a credit to both themselves and the discipline in the UK. I congratulate them.

N A Wright

CORRECTIONS

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In the January 2005 issue of Gut, one of the author’s names of the paper entitled Human peripheral and gastric lymphocyte responses to Helicobacter pylori NapA and APhC differ in infected and uninfected individuals (H J Windle, Y S Ang, V A Morales, R McManus, and D Kelleher. Gut 2005;54:25–32) was cited incorrectly. V A Morales should read V Athie-Morales. The journal apologises for this mistake.

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In the December issue of Gut fig 1 in the paper by AJG Bell et al (Human lymphocyte stimulation with pouchitis flora is greater than with flora from a healthy pouch but is suppressed by metronidazole. Gut 2004;53:1801–1805) is incorrect. The labels for fig 1C are inverted; the squares should have been labelled HetNon and the triangles HetPM. The legend is also incorrect because the label for flora grown on agar without metronidazole is HetNon, not HetP as stated.