Bacterial DNA induces a proinflammatory immune response in patients with decompensated cirrhosis

We read with interest the study of Thalheimer et al (Gut 2005;54:556–63) in which they reviewed actual knowledge regarding the influence of infection on haemodynamics, variceal haemorrhage, hepatic encephalopathy, liver damage, and other effects. We agree with these assumptions and would like to add information not quoted in the paper that may help explain some of the immune abnormalities usually found in patients with advanced decompensated cirrhosis. As the authors detailed in their paper, our group has reported on the detection of bacterial DNA in a significant proportion of patients with cirrhosis and culture negative non-neutrocytic ascites and has also shown that these fragments may last in blood for variable periods of time. In our opinion, the presence of bacterial DNA is not only representative in itself of the presence of bacteria (either viable or non-viable) in our patients, but induces similar immunological changes as endotoxin or viable bacteria. The question of whether bacterial DNA also induces haemodynamic disturbances is currently under investigation.

Bacterial DNA contains a series of CpG motifs that join toll-like receptor 9 and activates a series of intracellular mechanisms leading to the synthesis of proinflammatory cytokines. We therefore observed that peritoneal white cells obtained from ascitic fluid in patients with the presence of bacterial DNA showed a marked activation pattern when the intracellular presence of cytokines involved in a type 1 immune response by means of flow cytometry was analysed, and also an increased ability to secrete this type of cytokines when cultured. Importantly, white cells in culture also displayed a significantly higher ability to secrete nitric oxide than cells obtained from patients without the presence of bacterial DNA, and nitric oxide levels showed a direct and significant relationship with the inducible form of nitric oxide synthase, suggesting that in this setting, asciatic fluid nitric oxide synthesis is, at least in part, induced by this isoform.

Nitric oxide is a key agent in the pathogenesis of haemodynamic disturbances present in patients with advanced cirrhosis, and its levels are further increased in patients with hepaticorenal syndrome. Ascitic fluid nitric oxide levels are independently related to the development of renal impairment in patients with spontaneous bacterial peritonitis.

This relation between the presence of bacterial DNA in blood and the ability to secrete proinflammatory cytokines and nitric oxide by cells of the immune system in patients with decompensated cirrhosis suggests that endotoxin and viable bacteria should not only be taken into account in the design of new research protocols, but also bacterial DNA, or similar molecules, as demonstrated in the presence of bacteria in patients with advanced cirrhosis.

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Conflict of interest: None declared.

References


Author’s reply

We are grateful to Such et al for their comments on our review. As we had outlined, the influence of bacterial infection on the pathophysiology of cirrhosis is indeed an important one and Such et al have contributed significantly to this topic. We were aware of their data, but unfortunately some of it could not be retained in the final version of our paper due to editorial restrictions.

Nevertheless, we agree that the presence of bacterial DNA, in the absence of viable bacteria or endotoxaemia, might be an additional step in the sequence of events outlined in fig 2 of our review, maybe even preliminary to endotoxaemia.

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References


Perinatal passive smoke exposure may be more important than childhood exposure in the risk of developing childhood IBD

The large case control study of patients with inflammatory bowel disease (IBD) in the French paediatric population by Baron et al has clarified the role of well established genetic and environmental risk factors, as well as suggesting novel environmental risk factors (Gut 2005;54:357–63). However, we caution the authors on dismissal of the role of passive smoking in the risk of IBD development in childhood. Our own data would suggest that analysing smoking data during pregnancy and at birth is more important in the development of childhood IBD, rather than assessing smoking during childhood and at disease onset, as performed in this current study.

We have performed a case control study in South East Scotland of children with early onset IBD, matching cases of IBD diagnosed at less than 16 years of age with same sex and age (±1) year controls attending the same general practice. In total, we matched 62 pairs of cases and controls, with a median age of disease onset in cases of 10.6 years. We demonstrated that parental smoking during pregnancy and at birth was also more common in IBD cases than in controls, at 23% versus 6.2% (p = 0.04; OR 4.46 (95% CI 1.05–16.97)). There was no significant effect seen when paternal smoking in pregnancy and at birth was analysed in IBD cases versus controls (p = 0.27). These
data replicate the publication by Lashner and colleagues who studied 72 IBD cases and controls and found a similar relationship to smoking at birth—this was increased in children who later developed IBD in childhood (OR 3.02) and CD in childhood (OR 5.32). The authors of this study also demonstrated that maternal smoking at birth was important in the development of IBD and CD.

We agree with the findings of Baron et al that parental/passive smoke exposure outside of the perinatal period, including at the time of IBD diagnosis, is not associated with the risk of developing IBD in children. This lack of association between passive smoke exposure in childhood and development of childhood IBD has also been replicated by Lashner and colleagues. It is important to note that the other studies quoted by Baron et al in relation to the risk of passive smoking in IBD patients relate to the risk of adult onset IBD after passive smoke exposure during childhood, not the risk of developing IBD as a child. The mechanism by which smoke exposure during pregnancy and at birth leads to an increased risk of childhood IBD can only be a subject for speculation, but it is interesting to note a recent study has demonstrated chromosomal abnormalities in fetal epithelial cells in women who smoke during pregnancy.

In conclusion, our study agrees with previously published data to suggest a role between passive smoke exposure during pregnancy and at birth with the risk of childhood development of IBD. When assessing passive smoking in relation to childhood onset IBD, investigators should survey smoke exposure in the perinatal period and during childhood.

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An alternative to prophylactic colectomy for colon cancer prevention in HNPCC syndrome

The surgical option for treatment of a patient with screen detected colorectal cancer (CRC) from a family with hereditary non-polyposis colorectal cancer (HNPCC) is subtotal colectomy or segmental resection. Using decision analysis, we showed that subtotal colectomy performed at a young age leads to an increased life expectancy (LE) of 1–2.3 years. Based on these results and the high risk of developing a second CRC, we concluded that if CRC is detected in a young patient participating in a surveillance programme, colectomy with ileorectal anastomosis seems to be the treatment of choice. A French Committee on HNPCC commented on our study. Firstly, they stated that using quality adjusted LE would be a more accurate approach. Colectomy for HNPCC patients with Dukes’ C cancer varied in the literature from 70% to 91% and those for patients with Dukes’ C from 19% to 70%.

These survival rates are similar to those used in our analysis. Thirdly, the committee mentioned that the overall five year survival of patients with CRC in HNPCC is approximately 55%. They stated that if the decision is for an extended resection before the pathological staging of the tumour is known, 45% of patients will sustain a substantial decrease in QOL with no counterpart in quantity (that is, LE). The committee referred to the survival (5%) of symptomatic CRC in HNPCC. In our study, we discussed the surgical options for patients with CRC detected during surveillance. In our table, we showed the stage distribution of screen detected CRC based on our study and the Finnish series. As 86% had local cancer, the five year survival will be higher than 55%. Fourthly, the committee indicated that only a very small proportion of patients will be identified with CRC by the age of 27 years and that the increased LE for patients with CRC diagnosed at age 47 years was only one year.

Half of the patients with screen detected CRC will be diagnosed before the age of 50 years and will have a substantial increase of LE of 1–2.3 years. Fifthly, the committee stressed that different indications should be made in men and women because of their different risks for metachronous cancer as well as for the competing risk of endometrial cancer. Although female mutation carriers may have a lower risk of CRC than male carriers, it has not been shown that they also have a lower risk of a second CRC. In fact, among HNPCC patients that developed a second tumour, we found more female than male carriers. Female mutation carriers do indeed have a high risk of developing endometrial cancer but this cancer is only a rare cause of death in HNPCC.

As stated by the committee, it is difficult for a patient diagnosed with CRC to decide between an increase in LE and a potential decrease in their QOL. An increased LE is a somewhat theoretical concept that entails additional years at the end of one’s life. While the negative impact on QOL of subtotal colectomy will start from the first post-operative day. On the other hand, it may be even more difficult for a physician to explain to a patient that has developed a second tumour, that this patient will be happy after removal of the colon as now they are at a substantially lower risk of developing a second CRC. We agree that the patient’s choice is pivotal in decisions on prophylactic surgery, after being fully informed of the pros and cons of the surgical options.

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References


Defective denominators

I was interested in the paper by Langlands et al in which “prebiotic” carbohydrates altered the mucosal flora but apparently had no effect on cell proliferation (Gut 2004;53:1610–16). The matter is of some importance as the products of in vivo fermentation (short chain fatty acids) may increase epithelial cell proliferation, leading to the possibility that such supplements could actually enhance the risk of colorectal cancer.1,2

The authors state that methodology of (gut microflora study) is always an important issue and I argue that this also applies to cell proliferation studies, as the results of the present work may be misleading on two counts. Firstly, I would never recommend the use of Ki67 for cell nuclear antigen as a marker of cell proliferation as: (1) the method is difficult to standardise; (2) the antigen has a long half life; and (3) anomalous expression has been demonstrated in non-cycling near tumours and after administration of growth factors.3 For sections, Ki67 is far better however even using this antibody the results of the present study are unlikely to be conclusive as only 2–4 crypts could be scored; for most studies I would recommend scoring 50 hemi crypts.

The second point is that reliance on labelling indices can be misleading as lack of differences does not necessarily mean no proliferative change as both sides of the ratio (labelled cells divided by number of cells) could have altered. This was demonstrated in our studies of epidermal growth factor in parenterally fed rats where no differences in labelling index between orally fed and parenterally fed rats could be seen despite halving tissue weight and crypt cell production. When the data were re-expressed as labelling per crypt, the effects of treatment became apparent; a similar effect was seen in the stomach following misoprostol treatment.4

There is however a far easier and well validated method available for the study of human tissue. This is the so-called microsection technique in which small pieces of stained material are teased apart and mitotic figures scored.5 This literally allows one to score over 100 crypts (if so wished) and as the results are expressed per crypt the effects of changes in denominator are automatically accounted for.

References


Author’s reply

We thank Goodlad for his interest in our article. In our study (Gut 2004;53:1610–16), we assessed expression of Ki67 in three markers most commonly used to indicate cell cycle entry in tissue sections. Importantly, there was no difference in the data obtained for all three. We agree that proliferating cell nuclear antigen is of limited value for the reasons mentioned by Goodlad and also the fact that the protein has a role in DNA repair, which reduces its specificity as a cell cycle marker.

Similarly, Ki67 is not expressed by all cycling cells, may be downregulated by nutritional deprivation, and may also be involved in non-cell cycle related processes, such as ribosomal biosynthesis.

We consider that the most useful markers of cycling cells are the minichromosome maintenance (MCM) proteins, which are abundant at all phases of the cell cycle and are downregulated following exit into quiescence, differentiation or senescence.1 MCMs therefore provide a sensitive and specific indication of cell cycle entry. In our opinion these markers are preferable to counting mitotic figures, which is a subjective and error prone exercise, that by definition provides a limited phase specific indication of cell cycle entry. We further comment that proliferation indices can be misleading and that when assessing large bowel crypts it is important to determine the number of labelled cells per crypt. We confirm that the mucosa in all subjects in our study was microscopically normal, as well as macroscopically normal, as stated. In particular, there was no difference in crypt length and number of cells per crypt between the study groups. The labelling indices determined were therefore valid indicators of cell cycle entry in the samples investigated.

Prebiotic carbohydrates, such as those used in our study, are completely fermented in the large bowel and none is excreted in faeces. The principal products of this fermentation are short chain fatty acids (SCFA). While SCFA have been associated with increased cell proliferation in some animal models, it is hard to believe that what are the major biota in the colon of non-mammalian species should enhance the risk of cancer, particularly as nearly one of these fatty acids, butyrate, is thought to be a differentiating agent. Fermented carbohydrates, such as dietary fibre, when measured properly in the diet, appear to protect against colorectal cancer in observational studies.3 The observed lack of effect of prebiotic carbohydrates on colono-ocyte proliferation in our study suggests that a substantial increase in fermentable carbohydrates intake, as provided by these prebiotics, does not enhance proliferation, as shown in some animal models, and thus might be regarded as adding to the protective role of the fermentable non-starch polysaccharides (fibre).

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References


Author’s reply

Further to Cummings and Coleman’s reply to my letter above, I would like to question the advocacy of minichromosome maintenance (MCM) proteins as proliferative markers, as the number of MCM positive cells can greatly exceed other labels and they are widely distributed on unreplicated chromatin.2 They would appear to be more of an indicator of replication potential and, as such, are likely to be useful markers of dysplasia.3 In addition, scoring immunohistochemical labelled cells is just as, if not more, “subjective and error prone” than scoring mitotic figures (which are far easier to score in “squash” preparations than in sections). My main
concern still stands, as scoring histological sections of human biopsies, unlike squash preparations, leads to the sampling of a very limited number of crypts (2–4 in the present study) which prevents credence of the “observed lack of effect” of prebiotic carbohydrates.

Finally, I think that the jury is still out on the “protective role” of fermentable non-starch polysaccharides (fibres) as while the EPIC study showed a dramatic effect of intrinsically high fibre diets,7 many others have shown null effects and some of these, especially the intervention ones, demonstrated adverse effects. For example, wheat bran supplementation increased polyp recurrence in women8 and ispaghula had a more general adverse effect on polyps.9

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References

Author’s reply
We sought to identify cells at any point of the cell cycle, regardless of the rate of cycling or the duration of particular cell cycle phases. We therefore selected not to assess individual cell cycle phases in our samples, either by immunostaining or by counting mitotic figures. While additional roles for minichromosome maintenance (MCM) proteins have been suggested, there is strong evidence that they function as essential replication factors.1 MCMs are displaced from chromatin following DNA replication, yet remain abundant in the nucleus throughout the cell cycle.2 Importantly, several groups have shown that MCMs are lost following cell cycle exit (into differentiation, quiescence, or senescence).3 4 MCMs are therefore useful immunohistochemical markers of cell cycle state. It is not surprising that MCMs are more abundant than Ki67 and proliferating cell nuclear antigen (PCNA), as the latter markers are not detectable in all cycling cells.

Interpreting observational and intervention studies of fibre has filled many journal pages in recent years. There are numerous problems which, in the context of the present discussion, relate primarily to people treating all sources of fibre as being equal, thinking that fibre supplements will have the same effect as fibre present in whole foods in the diet and the amounts of fibre considered to be protective. With regard to the study by Alberts and colleagues, the fibre was provided as a supplement and was only of wheat bran. As Goodlad and Alvarez correctly note, the EPIC study showed a protective effect for fibre when intrinsically part of the diet, and from mixed sources. In other words, it is a high fibre diet that protects. The Bonithon-Kopp study1 used a fibre supplement, ispaghula, not found in most diets in the field, and at a very small dose of only about 3 g/day.

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Laterally spreading tumour in which interstitial deletion of β-catenin exon 3 was detected

Laterally spreading tumours (LSTs) of the colon and rectum are defined as lesions greater than 10 mm in diameter with a low vertical axis that extend laterally along the luminal wall. As most LSTs remain as adenomas or early invasive cancers, LSTs have been thought to have relatively little malignant potential. LSTs are divided into two macroscopic subtypes: flat (F)-type, which is composed of superficially spreading lesions with flat and smooth surfaces, and granular (G)-type, which is composed of superficially spreading aggregates of nodules. Despite distinctive biological behaviours of LSTs, only a few genetic alterations have been reported, such as K-ras and p53 mutations and cyclooxygenase 2 overexpression.

A 62 year old Japanese woman was referred to our hospital for treatment of a colonic tumour. Colonoscopy in our hospital showed an F-type LST with a central depression surrounded by a flat elevated area with a smooth surface in the caecum (fig 1A). Microscopically, the tumour consisted of a well differentiated adenocarcinoma with a tubular adenoma and had invaded the submucosal layer.

After obtaining informed consent from the patient, genetic analysis was carried out. No genetic alterations were found in APC, K-ras, or p53 genes. To clarify relevant alterations of gene expression, we analysed the gene expression profiles by a cDNA array. Among 350 cancer related genes, bone morphogenic protein 4 (BMP4) was one of the most differentially expressed genes in the tumour tissues and matched normal tissues (fig 1B). BMP4 expression is reportedly correlated with oncogenic β-catenin involvement in intestine cancer cells.

DNA sequencing showed an interstitial deletion of 394 bp in tumor tissues (fig 2). Three base inverted repeats, AGC and GCT, were found in sequences flanking the interstitial deletion. Short nucleotide sequences at both ends of the deletion were complementary, suggesting that inversely repeated sequences were involved in the somatic rearrangements. These results suggest that β-catenin deletion played an important role in the early stage of tumorigenesis in the present case. Abnormalities of β-catenin may play a crucial role in the morphological features of LSTs, as β-catenin is involved in cell adhesion. It would be interesting to investigate the frequency of β-catenin and APC alterations in a number of LST cases.

Microsatellite instability (MSI) due to defective DNA mismatch repair occurs in the majority of hereditary non-polyposis colorectal cancers (HNPCC) and in 10–15% of sporadic colorectal cancers. It has been reported that β-catenin mutations occur more often in MSI positive colorectal cancers. However, tumour tissues in the present case were MSI negative. Samowitz and colleagues reported that β-catenin exon 3 mutations can be an early event in colorectal tumorigenesis. However, Johnson and colleagues recently reported that β-catenin exon 3 mutations were rare in small (<1 cm) sporadic adenomas (1/83, 1.2%), HNPCC adenomas (1/37, 2.7%), and in both MSI positive (3/34) and MSI negative (9/78) sporadic colorectal cancers. In contrast, a significantly increased frequency (8/44, 18.2%) was found in HNPCC cancers. The

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**Figure 1** (A) Endoscopic picture with indigo carmine dye spraying showing an F-type laterally spreading tumour with a central depression surrounded by a flat elevated area in the caecum. (B) cDNA array hybridisation image of the tumour and non-tumour tissues. Bone morphogenic protein 4 (BMP4) was one of the most differentially expressed genes in the tumour tissues and matched normal tissues. (C) Intense nuclear expression of β-catenin immunohistochemically seen within the nuclei of tumour cells. (D) Intestinal deletion examined by polymerase chain reaction spanning the genomic region flanking exon 3 and the surrounding introns. A shorter band was detected in both carcinoma and adenoma tissues compared with the normal size of 931 bp. CA, carcinoma tissue; TA, tubular adenoma tissue; N, normal tissue.

**Figure 2** DNA sequencing showing interstitial deletion of the 394 bp region in tumour tissue. Three base inverted repeats, AGC and GCT, were found in sequences flanking the interstitial deletion. The deletion included the part of exon 3 containing critical serine and threonine codons for GSK-3β phosphorylation.
present patient had no past history or family history of cancer. It would be interesting to investigate whether β-catenin mutation positive HNPCC cancers have any specific morphological features.

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References

Functional role of the 503F variant of the organic cation transporter OCTN1 in Crohn’s disease

Several susceptible gene loci were identified as being involved in the aetiology of Crohn’s disease (CD). Recently, a non-synonymous single nucleotide polymorphism in the SLC22A4 gene encoding the organic cation transporter OCTN1 has been linked with CD in Caucasian populations (a 1672CT transversion, resulting in the amino acid substitution L503F). However, the functional consequences of this alteration are unclear as yet. We have now discovered that L-ergothioneine (ET, 2-mercaptoproline trimethylbenzine), a naturally occurring water soluble thiol compound of dietary origin, is a physiological substrate of OCTN1. Analysis of the concentration dependence of ET transport in OCTN1 transfected HEK293 fibroblasts by liquid chromatography tandem mass spectrometry revealed that the 503F variant was associated with a threefold higher substrate affinity (1/Km) and a two- to three-fold lower maximal transport velocity (Vmax), which resulted in a 50% higher initial transport capacity (Vmax/Km (503F) = 1.5 × Vmax/Km (503L)) at low ET levels (< 10 µmol/l) (fig 1A). Analysis of the time course of ET transport showed a higher clearance for the 503F variant (CL (503F) ≈ 1.65 × CL (503L)) at an ET concentration of 10 µmol/l (fig 1B). ET transport by 503L and 503F was sodium

Figure 1 Ergothioneine and OCTN1. Concentration dependence, Km, and Vmax of specific ergothioneine (ET) uptake in HEK293 cells constitutively expressing the 503L variant or the 503F variant after one minute of loading (A); specific uptake and clearance (CL) over a time course after incubation with 10 µmol/l ET (B); effects of sodium (C) and pH (D) on specific uptake after one minute of loading with 10 µmol/l ET. In sodium reduced transport buffer, NaCl was isotonically replaced with choline chloride (which did not interfere with ET transport). Resulting formazan formation was determined by absorbance at 568 nm. Data are means (SEM) of three (A–D) or 8–16 (F) independent experiments. *p<0.05, **p<0.01, ***p<0.001: significant differences between OCTN1 variants (A–D); significant differences compared with buffer controls (F), as determined by one way ANOVA with Holm-Sidak correction (α=0.05).
and pH dependent; only at unphysiologically low Na+ and pH values were the differences in transport activity between both variants lost (Fig 1C). Considering that maximal levels of ET found in tissues and in common foods are in the nanomolar to low micromolar range, our data suggest that carriers of the 503F allele accumulate higher ET concentrations in OCTN1 expressing cells compared with carriers of the wild-type 503L allele. Therefore, high tissue levels of ET may constitute a possible risk factor for CD.

The involvement of OCTN1 in the inflammatory process is further supported by observations that OCTN1 is strongly expressed in intestinal epithelial and immunological cells, particularly in CD4+ monocytes/macrophages playing a key role in the immunopathogenesis of CD, as well as by the finding that levels of SLC22A4 mRNA were upregulated by proinflammatory cytokines such as tumor necrosis factor α. Moreover, we found transcriptional regulation of SLC22A4 in CD4+ monocytes homozygous for the 503L variant, expression levels of SLC22A4 mRNA showed high interindividual heterogeneity and were directly proportional to cellular ET content (Fig 1E). Accordingly, in CD4+ and CD8+ lymphocytes lacking OCTN1 expression, we detected no ET (data not shown). The physiological or pathophysiological functions of ET as yet are not known. We tested the efficacy of ET on proliferation of the colon cancer epithelial cell line Caco-2 that was shown to be homozygous for the susceptible 503F allele and to express high levels of OCTN1 mRNA. Cell proliferation was enhanced in a dose dependent manner after exposure to ET concentrations above 20 nM for 24 hours: at 200 nM, proliferation was increased to 120 (3%) of the buffer control and intracellular ET concentration reached 6.7 (0.3) nmol/mg protein. In contrast, no stimulation of proliferation was seen when a Caco-2 variant without OCTN1 transporter was tested. Moreover, we found transcriptional regulation of SLC22A4 expression was employed; consequently, after exposure to ET concentrations above 150 nM, only diffusion was evident. Moreover, we found transcriptional regulation of SLC22A4 expression was employed; consequently, after exposure to ET concentrations above 150 nM, only diffusion was evident.

**Diarrhoea as a presentation of bird flu infection: a summary on its correlation to outcome in Thai cases**

Bird flu or avian flu, caused by H5N1 virus, is a new emerging infectious disease. The physiological or pathophysiological functions of ET as yet are not known. We tested the efficacy of ET on proliferation of the colon cancer epithelial cell line Caco-2 that was shown to be homozygous for the susceptible 503F allele and to express high levels of OCTN1 mRNA. Cell proliferation was enhanced in a dose dependent manner after exposure to ET concentrations above 20 nM for 24 hours: at 200 nM, proliferation was increased to 120 (3%) of the buffer control and intracellular ET concentration reached 6.7 (0.3) nmol/mg protein. In contrast, no stimulation of proliferation was seen when a Caco-2 variant without OCTN1 expression was employed; consequently, after exposure to ET concentrations above 150 nM, only diffusion was evident.

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concerns that secondary transmission of vCJD prions will occur through a wide range of surgical procedures. Risk assessment for intestinal endoscopy, biopsy, and surgery is currently limited by a lack of knowledge about relative PrPSc levels and prion titres within intestinal tissues in vCJD patients. Because of its high content of lymphoid follicles, terminal ileum is regarded as the intestinal tissue having the highest potential for iatrogenic transmission of vCJD prions. Here we provide the first report of relative PrPSc concentrations in vCJD terminal ileum.

Tissues were obtained at autopsy with consent from relatives from four patients with neuropathologically confirmed vCJD and two patients with neuropathologically confirmed sporadic CJD (both PRNP codon 129MM with type 2 PrPSc in brain). Terminal ileum was analysed for PrPSc by high sensitivity immunoblotting and for abnormal PrP immunoreactivity by immunohistochemistry. Using these methods, terminal ileum from all four vCJD cases showed high levels of detectable PrPSc (fig 1A). In three vCJD cases, 2/2 homogenates prepared from each ileum specimen were positive for PrPSc whereas 2/4 ileum homogenates were positive in the other vCJD case. The glycoform ratio of protease resistant fragments of di-, mono-, and non-glycosylated PrP in terminal ileum appeared to be closely similar to the type 41 PrPSc pattern seen in vCJD tonsil. Although there was variation in PrPSc concentration between different homogenates of vCJD terminal ileum, PrPSc levels in positive samples were typically in the range 0.1–1% of that present in vCJD brain (fig 1B). With respect to both sampling variation and PrPSc concentration, terminal ileum appears to be closely similar to lymph nodes in vCJD. These findings, together with our previous studies, show that PrPSc deposition within the intestine is not uniform in vCJD. From the four cases of vCJD with PrPSc positive terminal ileum studied here, 0/2 cases with available tissue had detectable PrPSc in the appendix, and only 1/3 cases had detectable PrPSc in the rectum. In contrast with findings with vCJD terminal ileum, no detectable PrPSc was found in homogenates of terminal ileum prepared from sporadic CJD patients (fig 1A). The lack of detection of PrPSc in sporadic CJD terminal ileum extends our previous findings for one of these cases in which we have previously reported a lack of detectable PrPSc in tonsil, rectum, and appendix.

In agreement with findings from immunoblotting, immunohistochemistry showed abnormal PrP deposition in the terminal ileum in vCJD (fig 1C) but not in sporadic CJD (data not shown). The irregular distribution of abnormal PrP positive lymphoid follicles seen in vCJD terminal ileum is consistent with variation in PrPSc concentration detected in different terminal ileum samples by immunoblotting. Albeit from necessarily limited numbers investigated, the uniform presence of PrPSc in vCJD terminal ileum, at concentrations of up to 1% of those found in vCJD brain, reinforces concerns that iatrogenic transmission of vCJD prions may occur through contaminated intestinal endoscopy, biopsy forceps, or surgical instruments. These findings should assist policy makers in the UK and elsewhere in risk assessments about the use of disposable forceps for intestinal biopsy. Alternative approaches to risk reduction may now be possible as practical means of prion decontamination for endoscopes and surgical instruments are now feasible using enzymatic methods.

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3 Wadsworth JBF, Joiner S, Hill AF, et al. Tissue distribution of protease resistant prion protein in variant CJD using a highly sensitive

Figure 1 (A, B) High sensitivity immunoblots using anti-prion protein (PrP) monoclonal antibody 3F4. (A) Proteinase K digested sodium phosphotungstic acid pellets from 0.5 ml of 10% terminal ileum homogenates from variant Creutzfeldt-Jakob disease (vCJD) patients 1–4 or sporadic CJD (sCJD) patients 1 and 2. (B) Proteinase K digested sodium phosphotungstic acid pellets from 0.5 ml of 10% normal human tonsil homogenate (normal tonsil) or 0.5 ml of 10% normal human tonsil homogenate spiked with 2.5 μl of 10% brain homogenate from vCJD patient No 4 (spiked tonsil) were compared with a proteinase K digested sodium phosphotungstic acid pellet from 0.5 ml of 10% terminal ileum homogenate from the same vCJD patient. (C) Photomicrograph showing abnormal PrP immunoreactivity in a lymphoid follicle in vCJD terminal ileum (anti-PrP monoclonal antibody 3F4). Scale bar, 100 μm. Insert, high power magnification of PrP deposits.


10 Joiner S, Linehan J, Branden H, et al. Irregular presence of prion in gastrointestinal diseases was mainly considered a direct consequence of malabsorption. The article of Moschen et al. and a previous one of our group on bone loss in coeliac disease, a disorder similarly characterised by intestinal inflammation, offer a new perspective on the pathogenesis of bone loss and reveal a more complex picture. Moschen et al demonstrated overproduction of OPG in the cells of colonic mucosa in IBD whereas Taranta and colleagues showed the direct role of the soluble cytokines in the serum of coeliac patients on bone cells. In fact, they found an increased RANKL/OPG ratio in untreated coeliac patients and different effects of the sera of untreated coeliac patients with respect to those on a gluten free diet, on cultured bone cells. These effects included increased in vitro osteolastogenesis, and lower interleukin 18 and OPG expression in osteoblasts. In both studies, these biochemical observations were translated in a reduction of bone mass. Moschen et al observed a negative correlation between OPG plasma levels and spine and femoral neck bone mineral density (BMD). Taranta and colleagues observed a significant negative correlation between BMD z score and interleukin 6 levels and RANKL/OPG ratio. In the discussion, Moschen et al observed that “studies of OPG/RANK and BMD are required to validate” his model. We believe that our study may be a first step towards understanding, at least in part, the relevance of inflammation to bone loss in intestinal diseases. These results are also in accordance with recent studies on primary osteoporosis, which are beginning to show a relevant role of local and systemic factors on cell activity.”

Chronic inflammatory intestinal diseases and bone loss

We were very interested in the recent article by Moschen et al on activation of the RANKL/OPG system in inflammatory bowel disease (IBD) (Gut 2005;54:479–87). Until recently, osteoporosis secondary to gastrointestinal diseases was mainly considered a direct consequence of malabsorption. The article of Moschen et al and a previous one of our group on bone loss in coeliac disease, a disorder similarly characterised by intestinal inflammation, offer a new perspective on the pathogenesis of bone loss and reveal a more complex picture. Moschen et al demonstrated overproduction of OPG in the cells of colonic mucosa in IBD whereas Taranta and colleagues showed the direct role of the soluble cytokines in the serum of coeliac patients on bone cells. In fact, they found an increased RANKL/OPG ratio in untreated coeliac patients and different effects of the sera of untreated coeliac patients with respect to those on a gluten free diet, on cultured bone cells. These effects included increased in vitro osteolastogenesis, and lower interleukin 18 and OPG expression in osteoblasts. In both studies, these biochemical observations were translated in a reduction of bone mass. Moschen et al observed a negative correlation between OPG plasma levels and spine and femoral neck bone mineral density (BMD). Taranta and colleagues observed a significant negative correlation between BMD z score and interleukin 6 levels and RANKL/OPG ratio. In the discussion, Moschen et al observed that “studies of OPG/RANK and BMD are required to validate” his model. We believe that our study may be a first step towards understanding, at least in part, the relevance of inflammation to bone loss in intestinal diseases. These results are also in accordance with recent studies on primary osteoporosis, which are beginning to show a relevant role of local and systemic factors on cell activity.”

Finally, these studies may also open the way to different therapeutic approaches—namely, drugs specifically acting on cytokines release and/or activity—for bone loss secondary to “inflammatory intestinal diseases”.

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References


BOOK REVIEW

Acid Related Diseases: Biology and Treatment


This textbook by Irvin Modlin and George Sachs is a welcome addition to the increasingly important and dynamic field of gastric acid and related disorders. It is very well laid out and provides quite a comprehensive understanding of this field. Compared with the first edition, this second edition has a few additional sections, such as reports of studies on knockout and transgenic animals, which help keep the reader up to date.

It concentrates on cellular events with great focus, and at the same time provides a very enlightening and broadly historical prospective, although in the case of the latter there is a touch of overdose at times. I found the chapters on biology and pharmacology particularly interesting. This acted as a useful exercise in revision and brought back memories (mostly pleasant) from my medical student days.

Each chapter is not separately referenced although at the end of each chapter the authors do provide a list of suggested reading for further introduction to the scientific literature.

The information is generally presented in a refreshing and amicable style. I think the book is friendly enough to be of benefit to an average student, but at the same time it caters adequately for the more seasoned learner too. It features some beautiful pictures and drawings. There are many individuals who have contributed to this field over the last hundred or so years. I thought the cartoons in the chapter on Helicobacter pylori were particularly pleasing and informative.

I particularly liked the background on the development of the first proton pump inhibitor (PPI). This I thought was thoroughly stimulating and will no doubt enable me to create a greater impression in front of the next PPI rep that I meet. The chapter on peptic ulcer disease is by and large par for the course, but the section on Barrett’s oesophagus presents a very logical and sensible approach towards tackling an area which remains controversial.

As a matter of personal taste, I would like to have seen a few key messages or take home points at the end of each chapter. These can also act as a quick source of reference for those who find that spare time is generally an elusive commodity, which, I suspect, is nearly all of us.

All in all, it is a timely and a creditable addition covering a very important and rapidly evolving field of gastroenterology, and the authors ought to be congratulated for their efforts. Would I buy it? Probably yes, but only if I did not have a copy of the first edition. I would certainly recommend it as a departmental book as, among its many virtues, it provides useful tibits to amuse the audience during presentations.

A Mahmood

CORRECTIONS

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In the August issue of Gut one of the authors was omitted from the paper by Goulding et al (C Goulding, A Murphy, G MacDonald, S Barrett, J Crowe, J Hegarty, S McKierman, and D Kelleher. The CCR5-32 mutation: impact on disease outcome in individuals with hepatitis C infection from a single source. Gut 2005;54:1157–61). R McManus (Department of Clinical Medicine and the Dublin Molecular Medicine Centre, Trinity Centre for Health Sciences, St James Hospital, Dublin 8, Ireland) should have been listed as the second author on the paper.

doi: 10.1136/gut.2004.045203corr1

In the August issue of Gut the following paper, Randomised controlled trial comparing percutaneous radiofrequency thermal ablation, percutaneous ethanol injection, and percutaneous acetic acid injection to treat hepatocellular carcinoma of 3 cm or less (S-M Lin, C-J Lin, C-C Lin, C-W Hsu, and Y-C Chen. Gut 2005;54:1151–1156), was published without one of the author corrections being made. On page 1154 under the heading “Local and new HCC recurrence”, the first line reads “...a median of 35 months” and should have been revised to “...a median of 24.3 months.”

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