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-encephalitic ascitic fluid (Thalheimer et al. Gastroenterology 2004;127:362–9). In these patients, bacterial DNA contained a series of CpG motifs that have been shown to activate immune responses (Wagschall et al. J Immunol 2002;168:903–10). We also demonstrated that these fragments may last in blood for variable periods of time. (Thalheimer et al. Dig Dis Sci 2004;49:2196–205). We are grateful to Such et al. for highlighting the presence of bacterial DNA in patients with advanced cirrhosis.

We are aware of the fact that bacterial DNA is an independent predictor of cirrhosis and ascites. However, we agree with the authors that 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 a representative in itself of the presence of bacterial DNA (Thalheimer et al. Gastroenterology 2004;127:362–9).

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 endotoxinemia, might be an additional step in the sequence of events outlined in fig 2 of our review, maybe even preliminary to endotoxaemia...

U Thalheimer, C K Triantos, D N Samonakis, D Patch, A K Burroughs

Liver Transplant and Hepatology Medicine, Royal Free Hospital, London, UK

Correspondence to: Professor A K Burroughs, Liver Transplant and Hepatology Medicine, Royal Free Hospital, Hampstead, London NW3 2QG, UK; andrew.burroughs@raf.free.nhs.uk

Conflict of interest: None declared.

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;54:357–64). 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 analytical 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 around the time of birth was more common in parents of IBD cases, at 54% compared with control parents at 29% (p = 0.01; odds ratio (OR) 2.87 (95% confidence interval (CI) 1.23–6.66)). Maternal 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 1.16–17.1)), and in mothers of patients with Crohn’s disease, at 27.8% versus control mothers at 8.3% (p = 0.03; OR 4.25 (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/pas sive smoke exposure outside of the perinatal period, including at the time of IBD diagnosis, is not associated with the risk of developing 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 that 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.

R K Russell, R Farhadi, M Wilson, H Drummond, J Satansgi, D C Wilson Gastrointestinal Unit and Department of Child Life and Health, University of Edinburgh, Edinburgh, UK

Correspondence to: Dr R K Russell, Gastrointestinal Unit, University of Edinburgh, Department of Medical Sciences, Edinburgh EH1 2UJ, UK; richardkrussell71@hotmail.com

Conflict of interest: None declared.

Reference
2 Lashner BA, Shaheen NJ, Hanauer SB, et al. Smoking and the risk of IBD in children. The authors of this study also demonstrated that maternal smoking at birth was important in the development of IBD and CD.

We agree that it is important to take into account the role of passive smoking only during childhood and at disease onset but also during the perinatal period. We also looked at this point in our study but came to different conclusions: 9.6% of mothers of IBD patients smoked during pregnancy versus 9.25% of control mothers (odds ratio (OR) 0.95 (95% confidence interval (CI) 0.53–1.72); p = 0.87). Moreover, concerning pas sive smoking during childhood, the findings were 14.2% and 12.8% for IBD patients and controls, respectively (OR 0.87 (95% CI 0.52–1.46); p = 0.60) and 15.3% for Crohn’s disease patients versus 14.4% for controls (OR 0.92 (95% CI 0.53–1.61); p = 0.77).

Due to the high number of questions and findings in our case control study, we only reported positive findings and what we considered as being the most important negative results. In conclusion, we confirm the finding that in our study, smoke exposure during childhood and at disease onset is not associated with the risk of adult onset IBD, investigators should survey smoke exposure in the perinatal period and during childhood.

M Baldé, C Gower-Rousseau Department of Epidemiology and Public Health, CHRU de Lille and Registre Epimad, Lille, France

D Turk Division of Gastroenterology, Hepatology, and Nutrition, Department of Paediatrics, CHRU de Lille and Registre Epimad, Lille, France

J F Colombel Department of Hepato-Gastroenterology and Registre EPIMAD, Hôpital Claude Huriez, CH et U de Lille, 59027 Lille Cedex, France; jfcolombel@chru-lille.fr

Conflict of interest: None declared.

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 way of representing long term survival and that the result of our study was not necessarily specific to HNPCC patients. In HNPCC, QOL after segmental resection may be decreased by the need for colonoscopy (versus rectoscopy after colectomy) and the fear of a second tumour. Secondly, the committee considered our five year survival rates optimistic. The five year survival rates 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 not comparable 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 for an extended resection is based on 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 1, 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 females than males. 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 CRC in surveillance after a second resection, surveillance of the remaining colon will prevent cancer development. It is possible 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.

H F A Vasen

The Netherlands Foundation for the Detection of Hereditary Tumours, Leiden, the Netherlands

H De Vaas tot Nederveen Coppel Department of Gastroenterology, Leiden University Medical Centre, Leiden, the Netherlands

W H de Vos tot Nederveen Coppel, Department of Gastroenterology, Leiden University Medical Centre, Leiden, the Netherlands

Correspondence to: Professor H F A Vasen, The Netherlands Foundation for the Detection of Hereditary Tumours, Rijnborgerweg 10, 2333 CA, Leiden, the Netherlands; hfvasen@wxs.nl

doi: 10.1136/gut.2005.075920

Conflict of interest: None declared.
References


Defective denominators

I was interested in the paper by Langlands et al in which they state that the 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 agree 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 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.1 For sections, Ki67 is far better however even using this antibody the results of the present study are unlikely to be as conclusive as only 2-4 crypts could be scored for; for most studies I would recommend scoring 30 hemi crypts.

The second point is that reliance on labelling indices can be misleading as lack of differences may 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.1,3

There is however a far easier and well validated method available for the study of human tissue. This is the so-called microdissection technique in which small pieces of stained material were teased apart and mitotic figures scored.2 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.

R A Goodlad

Correspondence to: Dr R A Goodlad, Cancer Research UK, 44 Lincoln’s Inn Fields, London WC2A 3PX, UK; goodlad@cancer.org.uk

Conflict of interest: None declared.

References

1 Wason HS, Goodlad RA. Fibre-supplemented foods may damage your health. Lancet 1996;348:319-20

2 Goodlad RA. Dietary fibre and the risk of colorectal cancer. Gut 2001;48:587-9

3 Hall PA, Coote PJ, Goodlad RA, et al. Proliferating cell nuclear antigen expression in non-cycling cells may be induced by growth factors in vivo. Br J Cancer 1997;76:244-7


6 Goodlad RA. Defective denominators, or will people never learn? Gastroenterology 1995;108:1623


Author’s reply

We thank Goodlad for his interest in our article. In our study (Gut 2004;53:1610-16), we assessed expression of 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 state in histological sections.

We agree 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.2 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 anaerobic in the colon of all mammalian species should enhance the risk of cancer, particularly since 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.4 The observed lack of effect of prebiotic carbohydrates on colonocyte proliferation in our study suggests that a substantial increase in fermentable carbohydrate 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).

J Cummings

Ninewells Hospital and Medical School, Dundee, UK

N Coleman

Hutchison/MRC Centre, Cambridge, UK

Correspondence to: Dr N Coleman, MRC Cancer Cell Unit, Hutchison/MRC Centre, Hills Rd, Cambridge CB2 2QZ, UK; nc109@cam.ac.uk

Conflict of interest: None declared.

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 chromosome.1 They would appear to be more of an indicator of replication potential and, as such, are likely to be useful markers of dysplasia.2 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
importing observational and intervention studies of fibre has yielded high-quality data in recent years. There are numerous problems with traditional methods, in the context of the current discussion, relate primarily to people treating all sources of fibre as equal, thinking that fibre supplements will yield 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 whole wheat. As Goodlad and Alferz correctly note, the EPIC study showed a beneficial 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 study 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.

**Recurrence of exhausting hiccup in a patient treated with chemotherapy for metastatic colon cancer**

A 61 year old man was surgically treated for a pT3 N2-G2 MO adenocarcinoma of the colon in February 2003. Immediately after surgery, an enteric fistula occurred that caused a delay in administration of adjuvant treatment. At the start of adjuvant chemotherapy (CT) in May 2003, CEA level was 18.2 ng/ml and a scan of the thorax and abdomen revealed the early appearance of two metastatic lesions in the liver. The patient underwent liver metastasectomy and in July 2003 was started on the FOL-FOX (leucovorin, 5-fluorouracil, oxaliplatin) regimen. After day 1 of CT, recurrence of an exhausting hiccup was observed that continued for nine days after therapy. No benefit from the re-use of chlorpromazine was obtained.

Notably, while undergoing the two CT regimens, the patient had received intravenous ondansetron (8 mg) plus intravenous dexmethasone (8 mg), which was used for prophylaxis of delayed emesis. In order to identify the causative drug of hiccup and taking into consideration previous reports indicating dexmethasone as a possible cause of hiccup, during the following cycles of CT this drug was omitted. This approach allowed the patient to continue CT without recurrence of hiccup. This strong temporal relation between dexmethasone administration and occurrence of hiccup indicated that this drug was the cause of the patient’s hiccup.

The mechanism of corticosteroid induced hiccup is unknown, although some hypotheses have been proposed. For example, it has been suggested that there is a hiccup centre in the midbrain that receives input from the thoracic sympathetic nerves and the pharyngeal plexus. It has been proposed that stimulation of the midbrain or these various pathways may be responsible for production of hiccup. Moreover, animal studies suggest that corticosteroids may reduce the synaptic transmission threshold in the midbrain and affect the metabolism of brain neurotransmitters. We reported our case to make oncologists aware that a symptom appearing during CT treatment (hiccup in our case) should not always be ascribed to the use of antineoplastic drugs. It is also true that some cytotoxic drugs, such as irinotecan and oxaplatinum, have been implicated as a cause of hiccup. In particular, the incidence of hiccup after treatment with irinotecan was reported in 49/16518 patients and, as for other cytotoxic drugs, almost exclusively in men (49/9313).
Cyclooxygenase 2 overexpression.

Matched normal tissues (fig 1B). BMP4 is partially expressed genes in tumor tissues and protein 4 (BMP4) was one of the most differentially expressed genes in the tumour tissues and matched normal tissues. Brain 1970;93:851–72.

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, we analysed the gene expression, we analysed the gene expression, we analysed the gene expression, we analysed the gene expression, we analysed the gene expression. 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. 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.

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 cyclloxygenase 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, we analysed the gene expression, we analysed the gene expression, we analysed the gene expression, we analysed the gene expression. 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. 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.

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 cyclloxygenase 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, we analysed the gene expression, we analysed the gene expression, we analysed the gene expression, we analysed the gene expression. 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. 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.

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 cyclloxygenase 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, we analysed the gene expression, we analysed the gene expression, we analysed the gene expression, we analysed the gene expression. 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. 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.
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 etiology 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), 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-fold lower maximal transport velocity (Vmax), which resulted in a 50% higher initial transport capacity (Vmax/Km 503F = 1.5 x 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 x CL 503L) at an ET concentration of 10 µmol/l (fig 1B). ET transport by 503L and 503F was sodium...
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. There has been worldwide avian influenza infections in poultry since 1997. Recently, H5N1 caused severe disease with high mortality in humans in Vietnam and Thailand. Most infected cases usually developed progressive pneumonia with acute respiratory distress syndrome and consequently died. Antipathetic presentations of patients with bird flu were also noted. de Jong et al recently reported a fatal bird flu infected case in Vietnam with a presentation of diarrhoea, without respiratory symptoms.

I performed a mini-study in order to document the magnitude of diarrhoeal presentation among reported Thai patients and the correlation with outcome. A literature review on papers concerning human bird flu in Thailand was performed using databases of published works cited in Index Medicus and the Science Citation Index. I also reviewed published and internet 256 related Thai journals, which are not included in the international citation index, for reports of human bird flu infection in Thailand. Studies that contained incomplete data were excluded from further analysis.

Six reports1–5 of 12 Thai patients with a confirmed diagnosis of bird flu were found. Of 12 infected cases, respiratory symptoms were seen in all cases and diarrhoea was detected at presentation in five cases (41.7%). Considering the five diarrhoeal cases, acute respiratory distress syndrome (ARDS) was detected in four cases and there were three deaths. Concerning the seven non-diarrhoeal cases, ARDS was detected in five cases and there were five fatalities. There was no significant correlation between presentation of diarrhoea and development of ARDS (p = 0.05) or mortality (p = 0.05) but there was a significant correlation between the development of ARDS and mortality (p = 0.001).

There are some reports of diarrhoea in several bird flu infections. Poovoravan recently proposed that diarrhoea was an important presentation of bird flu and could imply a poor prognosis. Here, I attempted to assess the magnitude of diarrhoea among Thai infected cases and its correlation with infection outcome. According to this study, the prevalence of diarrhoeal presentation was high, similar to a recent study in Vietnam (approximately 70%).1,2 I therefore conclude that diarrhoeal presentation had a poor outcome with infection among our subjects.

V Wiwanitkit
Correspondence to: Dr V Wiwanitkit, Department of Laboratory Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand 10330;
vwriju@yahoo.com

doi: 10.1136/gut.2005.072488
Conflict of interest: None declared.

References

High levels of disease related prion protein in the ileum in variant Creutzfeldt-Jakob disease

Disease related prion protein (PrPSc) is readily detectable in lymphoreticular tissues in variant Creutzfeldt-Jakob disease (vCJD) but not in other forms of human prion disease. This distinctive pathogenesis together with the unknown population prevalence of asymptomatic vCJD infection1,2 has led to significant...
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 PrP<sub>Sc</sub> levels and prion titres within intestinal tissues in vCJD patients. Because of its high content of lymphoid folicles, 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 PrP<sub>Sc</sub> 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 PrP<sup>Sc</sup> in brain). Terminal ileum was analysed for PrP<sub>Sc</sub> 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 PrP<sub>Sc</sub> (fig 1A). In three vCJD cases, 2/2 homogenates prepared from each ileum specimen were positive for PrP<sub>Sc</sub> 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<sub>Sc</sub> in terminal ileum appeared to be closely similar to the type 4 PrP<sub>Sc</sub> pattern seen in vCJD tonsil. Although there was variation in PrP<sub>Sc</sub> concentration between different homogenates of vCJD terminal ileum, PrP<sub>Sc</sub> 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 PrP<sub>Sc</sub> concentration, terminal ileum appears to be closely similar to lymph nodes in vCJD.

These findings, together with our previous studies, show that PrP<sub>Sc</sub> deposition within the intestine is not uniform in vCJD. From the four cases of vCJD with PrP<sub>Sc</sub> positive terminal ileum studied here, 0/2 cases with available tissue had detectable PrP<sub>Sc</sub> in the appendix and only 1/3 cases had detectable PrP<sub>Sc</sub> in the rectum. In contrast with findings with vCJD terminal ileum, no detectable PrP<sub>Sc</sub> was found in homogenates of terminal ileum prepared from sporadic CJD patients (fig 1A). The lack of detection of PrP<sub>Sc</sub> in sporadic CJD terminal ileum extends our previous findings for one of these cases in which we have previously reported a lack of detectable PrP<sub>Sc</sub> in tonsil, rectum, and appendix.

In agreement with findings from immunoblotting, immunohistochemistry showed abnormal PrP<sub>Sc</sub> deposition in the terminal ileum in vCJD (fig 1C) but not in sporadic CJD (data not shown). The irregular distribution of abnormal PrP<sub>Sc</sub> positive lymphoid folicles seen in vCJD terminal ileum is consistent with variation in PrP<sub>Sc</sub> concentration detected in different terminal ileum samples by immunoblotting.

Albeit from necessarily limited numbers investigated, the uniform presence of PrP<sub>Sc</sub> in vCJD terminal ileum, at concentrations of up to 1% of those found in vCJD brain, reinforces concerns that iatrogenic transmission of vCJD prions might occur through contaminated intestinal endoscopies, 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.

Acknowledgements
This study was funded by the UK Medical Research Council and was performed under the approval of the Institute of Neurology/National Hospital for Neurology and Neurosurgery Local Research Ethics Committee.

S Joiner, J M Linehan, S Brandner, J D F Wadsworth, J Collinge MRC Prion Unit and Department of Neurodegenerative Disease, Institute of Neurology, University College London, National Hospital for Neurology and Neurosurgery, London, UK

Correspondence to: Professor J Collinge, MRC Prion Unit and Department of Neurodegenerative Disease, Institute of Neurology, University College London, National Hospital for Neurology and Neurosurgery, London, UK

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


**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 osteoclastogenesis, 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 found 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 referred to the observation that studies of OPG/RANKL and BMD are required to validate their model.

We believe that our study may be a first step towards understand, 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”.

**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 broad historical perspective, although in the case of the latter there is a touch of overdue 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. Getting many individuals who have contributed to this field over the last hundred or so years. I thought the cartoons in the chapter on *Heliococcus pylori* were particularly pleasing and informative.

I particularly liked the background to 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 oesopha gus 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 tibbits to amuse the audience during presentations.

A Mahmood

**CORRECTIONS**

doi: 10.1136/gut.2004.055699corr1

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 McKiernan, and D Kelleher. The CCR5-A32 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 compar ing percutaneous radiofrequency thermal ablation, punctuate 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”.