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.1 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.2 We therefore observed that peripheral 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,3 and also an increased ability to secrete this type of cytokines when cultured.4 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,5 suggesting that in this setting, ascitic 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 hepatoenral syndrome.6 Ascitic fluid nitric oxide levels are independently related to the development of renal impairment in patients with spontaneous bacterial peritonitis.7 This relationship 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 suggest 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 demonstration of the presence of bacteria in patients with advanced cirrhosis.

J Such
Liver Unit, Hospital General Universitario, Pintor Baeza s/n, Alicante, Spain

C Muñoz
Department of Immunology, Hospital General Universitario, Pintor Baeza s/n, Alicante, Spain

P Zapater
Department of Clinical Pharmacology, Hospital General Universitario, Pintor Baeza s/n, Alicante, Spain

M Pérez-Mateo
Liver Unit, Hospital General Universitario, Pintor Baeza s/n, Alicante, Spain

Correspondence to: Dr J Such, Liver Unit, Hospital General Universitario, Pintor Baeza s/n, Alicante, Spain; such_jos@gva.es

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

U Thalheimer, C K Triantos, D N Samonakis, D Patch, A K Burroughs
Liver Transplant and Hepatobiliary Medicine, Royal Free Hospital, London, UK

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

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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 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)). In and mothers of patients with Crohn’s disease, at 27.8% versus control mothers at 8.3% (p = 0.03; OR 4.23 (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 (p = 0.18). 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.

R K Russell, R Farhadi, M Wilson, H Drummond, J Satsangi, 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, Division of Gastroenterology, Hepatology, and Nutrition, Department of Paediatrics, CHRU de Lille and Registre Epimad, Lille, France

M Baldé, C Gowor-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, CHRU de Lille and Registre Epimad, Lille, France

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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 for such a study. Completely but studies on quality of life (QOL) did not specifically consider HNPCC patients. In HNPCC, QOL after segmental resection may be decreased by the need for colostomy (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 HNPC patients with Dukes’ B cancer varied in the literature from 70% to 91% and those for patients with Dukes’ C from 19% to 70%. These survival rates are much lower than 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 made 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 (55%) of symptomatic CRC in HNPCC. In our study, we discuss 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 mutation 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 days 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 who has developed colon cancer surveillance that after segmental 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 Vos tot Nederveen Cappel Department of Gastroenterology, Leiden University Medical Centre, Leiden, the Netherlands

Correspondence to: Dr H F A Vasen, The Netherlands Foundation for the Detection of Hereditary Tumours, Rijnborgerweg 10, 2333 CG, Leiden, the Netherlands; hvasen@wxs.nl doi: 10.1136/gut.2005.075820

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Debate denominators.

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


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 microscopy) 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 cell nuclear antigens 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; 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 merely 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

References


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 Alferz correctly note, the EPIC study showed no 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 study used a fibre supplement, ispaghula, not found in most parts of the world, and at a very small dose of only about 3 g/day.

Conflict of interest: None declared.

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. We therefore elected not to assess individual cell cycle phases in our samples, either by immunostaining or by counting mitotic figures.

Conflict of interest: None declared.

References

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 N1 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 new work-up with computed tomography scan of the thorax and abdomen revealed the early appearance of two metastatic lesions in the liver. The patient underwent liver metastascotomy and in July 2003 was started on post-surgical chemotherapy with the FOL-FIRI (leucovorin, 5-fluorouracil, oxaliplatin) regimen. In February 2004, 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 dexamethasone (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 dexamethasone as a possible cause of hiccup,6 during the following cycles of CT this drug was omitted. This approach allowed the patient to continue CT without recurrence of hiccup.

The strong temporal relation between dexamethasone administration and occurrence of hiccup indicated that this drug was the cause of the patient’s hiccup. Moreover, discontinuing dexamethasone was sufficient to achieve disappearance of hiccup without any further pharmacological intervention.

The mechanism of corticosteroid induced hiccup is unknown, although some hypotheses have been proposed.7 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 suggested that corticosteroids may reduce the synaptic transmission threshold in the midbrain and affect the metabolism of brain neurotransmitters.1,8

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 oxaliplatin, have been implicated as a cause of hiccup.7,9 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).8

Conflict of interest: None declared.

References
1 Veneto (TV), Italy
2 Division of Medical Oncology, Ospedale Civile, Vittorio Veneto (TV), Italy
3 Laboratory of Internal Medicine, Ospedale Civile, Vittorio Veneto (TV), Italy
4 Division of Medical Oncology, Ospedale Civile, Vittorio Veneto (TV), Italy
5 Laboratory of Internal Medicine, Ospedale Civile, Vittorio Veneto (TV), Italy
6 Correspondence to: D Errante, UO Oncologia, Ospedale Civile, Via Forlornia 71, 31029 Udine, Italy; domenico.errante@ussi.it
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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.1 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.2 Despite distinctive biological behaviours of LSTs, only a few genetic alterations have been reported, such as K-ras and p53 mutations2-3 and cyclooxygenase 2 overexpression.4

A 62 year old Japanese woman was referred to our hospital for treatment of a colonoscopic 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.6 Among 350 cancer related genes, bone morphogenetic protein 4 (BMP4) was one of the most differentially expressed genes in tumour tissues and matched normal tissues (fig 1B). BMP4 is a member of the transforming growth factor (TGF)-β superfamily of growth factors. As BMP4 expression is reportedly correlated with oncogenic β-catenin in human colon cancer cells,7 we analysed alterations in β-catenin in tumour tissues. Intense nuclear expression of β-catenin was immunohistochemically seen in the nuclei of tumour cells. (C) Intense nuclear expression of β-catenin protein 4 (BMP4) was one of the most differentially expressed genes in the tumour tissues and matched normal tissues. (D) 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.

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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).1 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).2 However, the functional consequences of this alteration are unclear as yet.

We have now discovered that L-ergothioneine, (ET, 2-mercaptopropionic acid methylthionate), a naturally occurring water soluble thiol compound of dietary origin, is involved in the physiological substrate of OCTN1.3 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 (Km) and a twofold lower maximal transport velocity (Vmax), which resulted in a 50% higher initial transport capacity (Vmax/Km) (503F) 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) 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, K_m, and V_max 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). An equal expression level (relative to the housekeeping gene GAPDH, lowest expression was set to 1) in eight healthy volunteers that were homozygous carriers of the 503L variant (E). MITT assay3 of the proliferation of Caco-2 colon tumour cells with and without OCTN1 mRNA expression after 24 hours of incubation with ET or glutathione. Resulting formazan formation was determined by absorbance at 568 nm (F). Data are means (SEM) of three (A–D) or 8–16 (F) independent experiments. **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 low (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 CD14⁺ 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 tumour necrosis factor α. Moreover, we found transcriptional regulation of SLC22A4 to determine essentially ET uptake: in CD14⁺ monocytes homozygous for the 503L variant, expression levels of SLC22A4 mRNA showed high interindividual heterogeneity and were directly proportional to cellular ET content (fig 1F). Hence rather than antioxidant activities that was independent of OCTN1 expression, we detected no ET (data not shown).

The physiological or pathophysiological functions of ET are as yet unknown. We tested the effect 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 following exposure to ET concentrations above 20 μmol/l for 24 hours: at 200 μmol/l, 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 treatment with 200 μmol/l ET, only differentiation was observed, reaching 6.7 (0.03) nmol/mg protein. When incubated with glutathione, both Caco-2 cell lines exhibited an antioxidant typical inhibition of proliferation that was independent of OCTN1 expression (fig 1E). Accordingly, in CD14⁺ and CD8⁺ lymphocytes lacking OCTN1 expression, we detected no ET (data not shown).


Pharmacology, Medical Hospital of the University of Cologne, Cologne, Germany; dirk.taubert@medizin.uni-koeln.de
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D Taubert and G Grimberg contributed equally to the study.

Conflict of interest: None declared.

References

D Taubert, G Grimberg* Department of Pharmacology, Medical Hospital of the University of Cologne, Cologne, Germany

N Jung, A Rubbert Department of Internal Medicine I, Medical Hospital of the University of Cologne, Cologne, Germany

E Schönig Department of Pharmacology, Medical Hospital of the University of Cologne, Cologne, Germany

Correspondence to: Dr D Taubert, Department of Pharmacology, Medical Hospital of the University of Cologne, Gleueler Str 24, D-50931 Cologne, Germany; dirk.taubert@medizin.uni-koeln.de

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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 infection 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 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 d-, mono-, and non-glycosylated PrP in terminal ileum appeared to be closely similar to the type 4i 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 these 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 might occur through contaminated intestinal endoscopes, 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.

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 ICSM 35). Scale bar, 100 μm. Inset, high power magnification of PrP deposits.

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3 Wadsworth JDF, 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 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/RANKL and BMD are required to validate” his model.

We believe that our study may be a first step towards understand, at least in part, the role 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”.

M T Bardella
Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena, IRCCS, Milano, Italy

M L Bianchi
Bone Metabolism Unit, Istituto Auxologico Italiano, IRCCS, Milano, Italy

A Teti
Department of Experimental Medicine, University of L’Aquila, L’Aquila, Italy

Correspondence to: Dr M T Bardella, University of Milan, Mangiagalli e Regina Elena, IRCCS, Via Francesco Sforza, 35 Milano; manuteeresa.bardella@unimi.it

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References


Acid Related Diseases: Biology and Treatment


This textbook by Irvin Modlin and George Sachs is a welcome addition to the increasing number of textbooks on this subject. It provides useful titbits to amuse the audience during presentations.

A Mahmood

BOOK REVIEW

ACID RELATED DISEASES: BIOLOGY AND TREATMENT


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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 McDonald, 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 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.”
High levels of disease related prion protein in the ileum in variant Creutzfeldt-Jakob disease

S Joiner, J M Linehan, S Brandner, J D F Wadsworth and J Collinge

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