STOMACH

Host gastric Lewis expression determines the bacterial density of *Helicobacter pylori* in *babA2* genopositive infection

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Background and aims: We tested if host gastric Lewis antigens and the *babA2* genotype of *Helicobacter pylori* correlated with clinicohistological outcome.

Methods: We enrolled 188 dyspeptic patients (45 with duodenal ulcer, 45 with gastric ulcer, and 98 with chronic gastritis) with *H pylori* infection, proved by culture and gastric histology, reviewed by the updated Sydney system. Gastric expression of Lewis (Le) antigens Le^a, Le^b, Le^x, and Le^y was determined immunochemically to determine intensity (range 0-3). The corresponding 188 *H pylori* isolates were screened for *babA2* genotype by polymerase chain reaction.

Results: All *H pylori* isolates had a positive *babA2* genotype. We identified Le^a in 33.5%, Le^b in 72.9%, Le^x in 86.2%, and Le^y in 97.4% of biopsies from these 188 patients. Patients who expressed Le^b had a higher *H pylori* density than those who did not express Le^b ($p < 0.001$). Among 139 patients who expressed Le^b, *H pylori* density increased with a higher Le^b intensity ($p < 0.05$). Gastric atrophy decreased with Le^b intensity and thus resulted in lower *H pylori* density in the antrum ($p < 0.05$). For the 49 patients without gastric Le^b expression, *H pylori* density was positively related with Le^x and Le^a expression ($p < 0.05$).

Conclusions: Taiwanese *H pylori* isolates are 100% *babA2* genopositive. Gastric Le^b as well as Le^x expression may be major determinants of *H pylori* density. While lacking gastric Le^b expression, Le^x and Le^a were closely related to *H pylori* colonisation.

Helicobacter pylori is a well recognised gastric pathogen in humans.^{1,2} The ability of *H pylori* to achieve persistent colonisation in the human stomach has become the focus of intense research.³ Several studies have proposed that the molecular mimicry of *H pylori* lipopolysaccharide antigens to human Lewis (Le) antigens may help *H pylori* to evade the immune response and enhance bacterial adherence to gastric epithelium.³⁻⁶ As Le antigens are also found on the gastric epithelium in humans,⁷ Le antigen expression may mediate the attachment of *H pylori* to the gastric mucosa.⁸ Strong evidence was provided by Ilver *et al* who purified the blood group antigen binding adhesin (BabA) of *H pylori* and found that BabA selectively adheres to the Le^b antigen of the host.⁹ Their findings suggest that gastric Le^b antigens selectively interact with the products of the *babA2* (blood group associated binding gene) allele of *H pylori* and thus may possibly facilitate a more dense colonisation in the stomach. However, contradictory data focused on the role of the *babA2* genotype in terms of clinicohistological outcome without analysing the host status for Le^b expression in the stomach.¹⁰⁻¹² Therefore, we conducted this study to elucidate if the interaction of the *babA2* genotype of *H pylori* and gastric Le^b antigen expression of the host are correlated with different clinical outcomes.

As gastric Le^b antigens cannot be found in all humans, some other pathways must exist to facilitate adherence of *H pylori*. In contrast with the rare expression of Le^b, Le^x and Le^y antigens are commonly expressed.^{3-5,13} As the adhesion pedestal formation contained Le^x on both *H pylori* and gastric epithelium, these Lewis antigens may be required to establish or maintain infection.^{3,13} Thus we tested if these Lewis antigens have a role in bacterial adherence, when the host has weak or no gastric Le^b expression, interacting with the BabA of *H pylori*.

MATERIALS AND METHODS

Patients and study design

A total of 188 dyspeptic patients (112 men and 76 women; mean age 44.8 years) gave informed consent and were

consecutively enrolled after they were proved to have *H pylori* infection, defined as a positive culture. None had a previous history of anti-*H pylori* therapy. Each patient had undergone panendoscopy to obtain a gastric biopsy for culture and histology of *H pylori* infection. The endoscopic diagnosis of these 188 study patients included uncomplicated chronic active gastritis (n=98), duodenal ulcer (n=45), and gastric ulcer (n=45).

At gastric biopsy, five samples, including two from the antrum, two from the corpus, and one from the cardia, were obtained during endoscopy. Three gastric specimens, each one from antrum, corpus, and cardia, were stained with haematoxylin and eosin as well as with modified Giemsa stains. Apart from analysis of *H pylori* related gastric histology, these three gastric specimens were stained immunochemically for expression of Lewis antigens Le^a, Le^b, Le^x, and Le^y. The remaining two gastric specimens were used for *H pylori* culture.¹⁴ Genomic DNA of these *H pylori* isolates were then extracted by polymerase chain reaction (PCR) to detect the *babA2* genotype. Extraction of DNA was performed using the same method as reported in our previous publication.¹⁵

PCR and primers for *babA2* genotypes

Extracted DNA from each strain was subjected to PCR for amplification of the *babA2* genes, applying one pair of primers (babo-F: CTT AAA TAT CTC CCT ATC CC, corresponding to bp 1 to 20 of AF033654; babo-R: CGA TTT GAT AGC CTA CGC TTA

Abbreviations: Le, Lewis; Lewis-N, total Lewis number; BabA, blood group antigen binding adhesin; *babA2*, blood group associated binding gene; PCR, polymerase chain reaction; TL, total gastric Lewis antigen expression intensity; HPD, *Helicobacter pylori* density; IM, intestinal metaplasia; CIS, chronic inflammatory score; PBS, phosphate buffered saline.

Figure 1 (A, B) Gastric immunohistochemical stains of Lewis antigen Le^b expression. (A) Positive staining over the surface epithelium only. (B) Diffuse staining over the intercryptal epithelium. (C, D) Gastric immunohistochemical stains of Le^x expression. (C) Positive staining over the surface epithelium only. (D) Diffuse staining over the deep glands.

Table 1 Topographic distribution of the intensity of gastric Lewis antigen expression in 188 patients with *Helicobacter pylori* infection

Lewis antigen	Antrum	Body	Cardia	Significance*
Intensity [range 0–3]				
Le^a	0.56 (0.77)	0.41 (0.65)	0.45 (0.81)	A>B; A>C
Le^b	1.37 (1.24)	1.74 (0.95)	1.59 (1.03)	B>A; C>A
Le^x	1.19 (0.72)	0.88 (0.71)	0.75 (0.73)	A>B; A>C
Le^y	1.61 (0.95)	1.76 (1.07)	1.68 (0.88)	B>A; C>A

Values are mean (SD).

*Significant difference by paired *t* test with two tailed analysis ($p<0.05$).

A, antrum; B, body; C, cardia.

Table 2 Lewis antigen expression and clinicohistological features of *Helicobacter pylori* infection

Parameter (mean)	Le^a		Le^b		Le^x		Le^y	
	(+) (n=63)	(-) (n=125)	(+) (n=139)	(-) (n=49)	(+) (n=162)	(-) (n=26)	(+) (n=183)	(-) (n=5)
AIS [0–9]§	2.31	2.61	2.62	2.21	2.81	2.45	2.55	1.18
CIS [0–9]†	6.89	6.97	7.59	6.15	6.95	6.88	6.96	6.40
AT (%)	57.1	57.6	57.6	57.1	54.9	73	56.3	100
IM (%)§	28.6	29.6	28.1	32.7	26.7	57.7	25.1	80
Ulcer rate (%)	49	45.6	47.5	44.9	47.5	42.3	46.9	40
HPD (1–15)†	9.03	8.29	9.29	6.35	8.59	8.03	8.57	7.41
Antrum (1–5)†	2.89	2.69	2.87	2.41	2.73	2.71	2.77	2.14
Body (1–5)*††	3.46	3.08	3.52	2.31	3.31	2.69	3.21	2.85
Cardia (1–5)†	2.67	2.51	2.89	1.63	2.66	2.53	2.57	2.41

AIS, acute inflammatory score; CIS, chronic inflammatory score; AT, antral atrophy; IM, intestinal metaplasia; HPD, total density of *H. pylori*.

Significant difference ($p<0.05$): *between Le^a (+) and Le^a (–) patients; †between Le^b (+) and Le^b (–) patients; §between Le^x (+) and Le^x (–) patients;

§between Le^y (+) and Le^y (–) patients.

TG, corresponding to bp 369 to 391 of AF033654) designed by Ilver and colleagues⁹ or another self designed primer (bab7-F: CCA AAC GAA ACA AAA AGC GT, corresponding to bp 105 to 124 of AF033654; bab7-R: GCT TGT GTA AAA GCC GTC GT, corresponding to bp 357 to 375 of AF033654).

The PCR mixtures were performed in a volume of 50 μ l containing 0.2 μ M of each primer, 0.2 mM each of deoxynucleoside triphosphates, reaction buffer with $MgCl_2$, and 1 unit of

DyNAzyme II DNA polymerase (Finnzymes OY, Espoo, Finland). Amplification was carried out over 30 cycles consisting of 94°C for one minute, 45°C for one minute, and 72°C for one minute in a thermal cycler (Perkins-Elmer Cooperation, Norwalk, Connecticut, USA). The two primers achieved a 391 bp product (by primers designed by Ilver *et al*) and a 271 bp product (using the self designed primers in this study), respectively. The sequences of these two PCR products were

Figure 2 The nucleotide sequence of the 271 bp polymerase chain reaction product gained from the self designed *babA2* primers. The nucleotide sequence of the 10 randomly selected domestic strains (hp250, hp258, hp222, hp116, hp657, hp130, hp238, hp82, hp639, and hp76) was confirmed to be *babA2* in origin with >90% homology to the published sequence of the *babA2* gene (afbaba2) of CCUG 17875. The 10 nucleotides (ATG AAA AAA C), representative of the *babA2* gene of *Helicobacter pylori*, are indicated by “=”.

determined using an ABI PRISM 377 DNA Sequencer (Applied Biosystems, Foster, California, USA).

In addition, we randomly selected 30 *babA2* genopositive strains (proven by the presence of the 271 bp PCR product) to test their BabA producing phenotype by western blotting using BabA specific antiserum. BabA specific antiserum was obtained from Drs Thomas Boren and Stefan Odenbreit.^{8,9,16} Each selected *H pylori* extract was analysed on a sodium dodecyl sulphate-10% polyacrylamide gel. The blot was then subjected to a 1:500 dilution of the anti-BabA antibody and detected with goat antirabbit antibody conjugated to horseradish peroxidase (Chemicon International Inc., Temecula, California, USA).

Analysis of *H pylori* related histology

The same pathologist, unaware of the endoscopic and culture results, analysed the gastric histology. *H pylori* density for each specimen was scored according to Yang and colleagues¹⁷: score 0, no bacteria; score 1, one or two small clusters with less than 10 bacteria; score 2, less than half the superficial crypt area with less than 10 bacteria in each crypt; score 3, less than half

the area but with more than 10 bacteria, or more than half the area with less than 10 bacteria in each crypt; score 4, >10 bacteria in forvelae with some free area; and score 5, >10 bacteria without a free area. Total *H pylori* density (HPD) was defined as the sum of the densities from the three biopsy samples, obtained from the antrum, corpus, and cardia. Thus the HPD score ranged from 1 to 15. The acute inflammatory score (range 0–3), chronic inflammation score (range 0–3), atrophic change (absent, 0; present, 1), and intestinal metaplasia (IM) (absent, 0; presence, 1) were graded using the updated Sydney system.¹⁸ The total acute (AIS) and chronic (CIS) inflammatory scores were also a sum of the three specimens (range 0–9).

Immunochemical staining for gastric Lewis expression

Immunostaining of biopsy specimens for Lewis antigens was performed using the standard avidin-biotin-peroxidase technique. Formalin fixed paraffin embedded tissue sections, including topographical specimens from the antrum, corpus, and cardia from each patient, were deparaffinised through xylene and hydrated with ethanol. Slides were washed with

Figure 3 (A) Total *Helicobacter pylori* density (HPD) was positively correlated with an increase in Le^b intensity (* $p < 0.05$, significant difference analysed using one way ANOVA). (B) Total Lewis number (Lewis-N) of each patient was also positively correlated with higher HPD in the stomach (one way ANOVA, $p < 0.05$).

distilled water and then placed in 1× phosphate buffered saline (PBS) for five minutes. Incubation with 3% hydrogen peroxide for three minutes blocked the endogenous peroxidase activities of these sections. After incubation with 2% bovine serum albumin for two hours and washing with PBS, the primary monoclonal antibodies for detection of gastric Lewis antigens were used (anti-Lewis Le^a, Le^b, Le^x, and Le^y; Signet Laboratories, Inc., Dedham, Massachusetts, USA). The reaction time for the primary monoclonal antibodies (anti-Le^a, Le^b, Le^x, and Le^y) was three hours at 25°C. These slides were again washed with PBS and incubated with the secondary antibody to achieve a 1:2000 dilution of antimouse IgG and IgM conjugated to horseradish peroxidase (Chemicon International Inc., Temecula, California, USA) for two hours at 25°C. These slides were finally washed with PBS, and the AEC kit (Sigma, St Louis, USA) was used as substrate to illustrate the stain. All slides were evaluated blindly by the same pathologist. For each gastric site, the intensity of Le^a, Le^b, Le^x, and Le^y was scored from 0 to 3 (0, no staining; 1, staining of either surface mucous cells or deep gastric glands; 2, staining of surface cells, intercryptal epithelium, and deep glands but expressed in $\leq 50\%$ of the analysed specimens; 3, diffuse staining of $\geq 50\%$ of the analysed specimens on surface cells, intercryptal epithelium, and deep glands). Examples of intensity 1 and intensity 2 gastric Le^b expression are shown in fig 1A and 1B, respectively. Total gastric Lewis antigen intensity (TLI) for Le^a, Le^b, Le^x, and Le^y was the sum of three biopsy samples from the antrum, corpus and cardia (range 0–9).

Statistics

The Student's *t* test and paired *t* test were used as appropriate for parametric differences. One way ANOVA with Bonferroni's method was used for multiple testing of data. Pearson's χ^2 test was used for non-parametric proportions. All significance tests were two tailed and a *p* value < 0.05 was taken as significant.

RESULTS

Prevalence of *babA2* genotypes of *H. pylori* infection in Taiwan

Fifty per cent (94/188) of *H. pylori* isolates had a positive *babA2* genotype by PCR, applying the primers used by Ilver *et al* to obtain a band of 371 bp. However, the nucleotide sequence of this 391 bp PCR product from the Taiwanese isolates was confirmed as not being *babA2* in origin but had $>90\%$ homology with the published sequence of adenine specific DNA methyltransferase in *H. pylori* 26695. To detect the *babA2* genotype for the domestic strains, we self designed a pair of primers and achieved a 271 bp PCR product whose nucleotide sequence was confirmed with $>95\%$ homology to the *babA2* gene of CCUG 17875 (fig 2). Based on PCR using these primers to obtain a 271

bp band, the prevalence rate of the *babA2* genotype was 100% in all 188 Taiwanese *H. pylori* isolates. Western blotting also confirmed that the 30 randomly selected 271 bp genopositive strains had a uniformly positive phenotype.

Topographic gastric Lewis antigen expression in *H. pylori* infected Taiwanese

Based on the presence of staining of any one of the three gastric specimens, we identified Le^a in 33.5%, Le^b in 72.9%, Le^x in 86.2%, and Le^y in 97.4% of gastric biopsies in these 188 patients. As shown in table 1, the topographic intensity of gastric Le^a expression was higher in the corpus than in the antrum or cardia (1.76 *v* 1.61 and 1.68; paired *t* test, $p < 0.05$). The intensity of Le^b expression was also higher in the corpus and cardia than in the antrum (1.74 *v* 1.37, and 1.59 *v* 1.37; paired *t* test, $p < 0.05$). In contrast, the topographic intensity of Le^a or Le^x was higher in the antrum than in the corpus and cardia (Le^a: 0.56 *v* 0.41 and 0.45, $p < 0.05$; Le^x: 1.19 *v* 0.88 and 0.75, $p < 0.05$).

Lewis antigen expression and clinicohistological features of *H. pylori* infection

There was no difference in ulcer rate between patients with or without Lewis antigen expression in the stomach (table 2). Patients with gastric Le^b expression had significantly higher HPD and CIS than those without Le^b expressions (HPD: 9.29 *v* 6.35, $p < 0.001$; CIS: 7.59 *v* 6.15, $p < 0.05$). We also found that mean HPD of 12 Le^{a+b+} patients was significantly lower than that of either 88 Le^{a-b+} patients or 51 Le^{a-b+} patients (7.42 *v* 9.22 and 9.41; $p < 0.05$ by one way ANOVA). In fig 3A, TLI of Le^b was found to be positively correlated with HPD (one way ANOVA, $p < 0.05$). In table 2, although the statistical significance was limited, HPD was evidently higher in those patients who expressed Le^a, Le^x, and Le^y in the stomach. Furthermore, patients who expressed Le^a and Le^x had a higher bacterial density in biopsies ($p < 0.05$). HPD was even elevated when the total number of gastric Lewis expression (Lewis-N) of each study patient increased (fig 3B). Multivariate logistic regression disclosed that the intensity of Le^b and Le^x expression, rather than Lewis-N, was an independent factor correlated with HPD in *H. pylori* infected patients (table 3).

As also shown in table 2, patients with no expression of Le^x or Le^y had higher rates of IM in the stomach ($p < 0.01$). Among those patients who expressed Le^x, TLI of Le^x was significantly lower in the presence of IM (2.68 *v* 3.83; $p < 0.01$) but higher in the presence of antral atrophy (4.05 *v* 3.01; $p < 0.01$). There was no decrease in TLI of Le^a, Le^b, or Le^y, despite the presence of IM or antral atrophy.

Factors correlating with HPD in non-Le^b patients

Of the 49 *H. pylori* infected patients without Le^b expression, HPD was higher in patients who expressed gastric Le^a and Le^x

Table 3 Multivariate logistic regression for independent factors relevant to total *Helicobacter pylori* density of the stomach

Parameter	Coefficient	Standard error	p Value	95% confidence interval
Total 188 patients				
Total Le ^b intensity	0.491	0.052	0.000*	(0.389–0.593)
Lewis-N	−0.327	0.294	0.267	(−0.906–0.253)
Total Le ^a intensity	0.230	0.075	0.002*	(0.083–0.378)
Total Le ^x intensity	0.092	0.056	0.116	(−0.022–0.199)
Total Le ^a intensity	0.123	0.076	0.221	(−0.057–0.244)
In 139 patients with Le ^b				
Total Le ^b intensity	0.518	0.066	0.000*	(0.386–0.649)
Total Le ^a intensity	0.183	0.074	0.015*	(0.037–0.329)
Total Le ^x intensity	0.061	0.055	0.269	(−0.048–0.169)
Total Le ^a intensity	−0.044	0.073	0.546	(−0.187–0.099)
In 49 patients without Le ^b				
Total Le ^a intensity	0.594	0.136	0.000*	(0.318–0.868)
Total Le ^x intensity	0.242	0.105	0.025*	(0.031–0.456)
Total Le ^a intensity	0.111	0.093	0.241	(−0.077–0.298)

Lewis-N, total number of four Lewis antigen expressions in the stomach of each patient.

*Significant difference.

Table 4 Topographic distribution of *Helicobacter pylori* density and gastric Lewis antigen intensity in 139 Le^b positive patients with and without antral atrophy

Parameter (mean)	Antrum	Body	Cardia	Body+cardia	Total
Le ^a intensity					
AT (n=80)	0.54	0.39	0.54	0.93	1.36
Non-AT (n=59)	0.59	0.51	0.49	1.03	1.64
Le ^b intensity					
AT (n=80)	1.63*	2.43	2.23	4.66	6.05
Non-AT (n=59)	2.15	2.26	2.25	4.51	6.44
Le ^x intensity					
AT (n=80)	1.21	1.31*	0.94*	2.24*	2.49
Non-AT (n=59)	1.28	0.88	0.77	1.66	2.24
<i>H. pylori</i> density					
AT (n=80)	2.52*	3.65*	3.05*	6.71*	9.23
Non-AT (n=59)	3.35	3.25	2.67	5.98	9.38

Body+cardia, the summation of the data from the body and cardia; Total, the sum of antrum, body, and cardia.

*Significant difference between patients with (AT) and without (non-AT) antral atrophy, analysed by the two tailed Student's *t* test (*p*<0.05).

(Le^a: 7.41 *v* 6.05, *p*<0.05; Le^x: 6.81 *v* 4.25, *p*<0.005). In table 3, multivariate logistic regression confirmed that Le^a and Le^x antigens were both independently correlated with HPD in the 49 patients without Le^b expression. In contrast, for the 139 patients who expressed Le^b, only Le^x but not Le^a antigens were independently correlated with HPD (table 3).

Compensatory effect of Le^x to maintain HPD for weak Le^b intensity in antral atrophy

Among the 139 Le^b positive patients, the topographic distribution of *H. pylori* density and the intensity of Le^a, Le^b, and Le^x were compared between patients with and without antral atrophy (table 4). There was no decrease in either Le^a or Le^x intensity over the antrum despite the presence of atrophy. In contrast, patients with antral atrophy had a lower Le^b intensity over the antrum (*p*<0.05) and thus had a significantly lower bacterial density (*p*<0.05).

Although both bacterial density and the intensity of Le^b over the antrum were lower, HPD was not decreased by the presence of antral atrophy (table 4). The paradoxical increase in bacterial density on the body and cardia were found to maintain HPD under the presence of antral atrophy (*p*<0.05). However, there was no significant increase in Le^b intensity elsewhere in the body or cardia (table 4). In contrast, a significant increase in the intensity of Le^x over the gastric body and cardia was found in those patients with antral atrophy compared with those without antral atrophy (*p*<0.05).

DISCUSSION

Identification of specific receptors for *H. pylori* on the gastric mucosa may explain why the organism can only adhere to those cells in humans. Ilver *et al* disclosed that the *babA2* gene of *H. pylori* is a putative determinant allowing it to adhere to Le^b of the gastric epithelium and thus could promote bacterial invasion of the human stomach.⁹ Our prospective study enrolled 188 *H. pylori* infected patients and is the first to analyse both bacterial *babA2* genotype and gastric antigen expression (including Le^b), thus further elucidating the impact of any interactions between BabA and Le^b on the clinico-histological outcome after *H. pylori* infection.

In the present study, after applying the primer of Ilver *et al* to obtain a 391 bp PCR product, we discovered it was non-*babA2* in origin. By applying our self designed primers, a 271 bp PCR product was found and was confirmed to have >95% homology to the published sequence of *babA2*. The nucleotide sequence data confirmed that our self designed pair of primers were suitable for *babA2* genotyping in Taiwan and all 188 isolates in this study were uniformly proven to have a *babA2* positive genotype. The prevalence was higher than in previous reports (38–85%).^{10–12 19} Moreover, such an extremely high prevalence of *babA2* in Taiwan suggests this could be an ideal country in which to study whether *babA2* is a good target for preventive vaccination if BabA interacts strongly with Le^b to impact on *H. pylori* colonisation of patients.

The prevalence rates of the different Lewis antigens in our study were compatible with Kobayashi *et al*, who reported that Le^a had the lowest incidence and that gastric Le^x or Le^y may disappear when *H. pylori* infection is induced by IM.⁷ Such a finding was indirectly supported by our data (table 1) which showed that patients without expression of Le^x or Le^y had higher rates of IM than those with Le^x and Le^y ($p < 0.05$).

Patients with gastric Le^b expression had a higher bacterial density of *H. pylori* than those without Le^b expression ($p < 0.05$) (table 2). TLI of Le^b expression was also positively correlated with HPD (fig 3A). Moreover, HPD was higher in Le^{a+b+} weak and Le^{a+b+} strong secretors than in Le^{a-b-} non-secretors ($p < 0.05$). Accordingly, the intensity of Le^b was proved to be an independent factor in determining HPD (table 3). As all domestic strains were *babA2* positive, our study from Taiwan may be the most rational in elucidating the fact that gastric Le^b really serves as an important receptor for *H. pylori* adherence.

There were 49 patients with *H. pylori* infection but no expression of Le^b in the stomach. Bacterial densities of the body remained higher in patients with positive expression of Le^a and Le^x ($p < 0.05$) (table 2). An increasing trend for HPD was found in patients whose Lewis-N ranked high ($p < 0.05$, by one way ANOVA) (fig 3B). These data imply that there may be some additive effect of expression of other Lewis antigens, apart from Le^b, serving as adherent receptors for *H. pylori*. This is compatible with the finding of Clyne and Drumm who confirmed that blocking with a monoclonal antibody for the Le^b antigen on the gastric epithelium could not totally abolish adherence of *H. pylori*.²⁰ Thus we tested if other gastric Lewis types also enhanced bacterial adherence in the 49 patients without Le^b expression. Our study found that patients who expressed Le^x and Le^a had higher HPD than those who did not express Le^x and Le^a (Le^x: 7.41 ν 6.05, $p < 0.05$; Le^x: 6.81 ν 4.25, $p < 0.005$). By multiple logistic regression, Le^x and Le^a were further confirmed to be independent factors in enhancing colonisation of *H. pylori* (table 3). These clinical data thus support the laboratory findings of Taylor *et al* which found adhesion pedestal formations stained with Le^x on both *H. pylori* and gastric epithelium. Accordingly, our study confirmed that Lewis antigens other than Le^b can be used to establish or maintain *H. pylori* infection in the stomach.^{3 10 16}

Expression of Le^b was stronger in the body, in contrast with the antral dominant distribution of Le^a and Le^x (table 1). Therefore, we tested whether Le^x and Le^a had additive effects when present with Le^b for enhancement of *H. pylori* colonisation in the 139 patients with Le^b expression. Patients with antral atrophy had different topographic distributions of bacterial density but the total density of *H. pylori* did not differ (table 4). The presence of antral atrophy decreased the intensity of Le^b, which was expected, as Le^b usually stained the superficial glands.^{7 9 19} When the intensity of Le^b was lower, bacterial density here decreased. However, overall HPD was maintained by the paradoxical increased density over the body and corpus. As the intensity of Le^x over the body and cardia were higher in the presence of antral atrophy, increased bacterial densities here could be mediated by Le^x expression. These clinical data supported the finding that gastric Le^x antigen can enhance *H. pylori* adherence.¹⁶ Moreover, Le^x may have compensatory or additive effects with Le^b to maintain bacterial loads during ongoing atrophy changes.

Among those patients who expressed Le^x, TLI of Le^x in the presence of IM was 2.68 versus 3.83 ($p < 0.01$) but was higher in the presence of antral atrophy (4.05 ν 3.01; $p < 0.01$). These data confirm that IM and antral atrophy may change Le^x expression and thus alter the *H. pylori* colonisation pattern.

In summary, Taiwanese *H. pylori* isolates are 100% *babA2* positive. Gastric Le^b intensity as well as Le^x intensity appear to be major determinants of bacterial density of *H. pylori*. When lacking gastric Le^b expression, Le^x and Le^a are closely related to *H. pylori* colonisation. To overcome *H. pylori* adherence, genomic

targets such as *babA2* (or others interacting with gastric Lewis antigens) may be promising.

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PostScript

LETTERS

Fatigue in primary biliary cirrhosis

We would like to take the opportunity to clarify some of the points in response to the recent leading article (*Gut* 2004;53:475-7) which accompanied our report¹ of reduced globus pallidus (GP) magnetisation ratios (MTRs) in patients with fatigue and primary biliary cirrhosis (PBC).

As we stated in the paper, fatigue in PBC is a subjective multidimensional symptom with many potential determinants, including sleep disturbance, depression, and personality, in addition to a potential central neurological cause.¹ We therefore wholeheartedly concur with Drs Milkiewicz and Heathcote when they state that brain manganese (Mn) deposition is certainly not the cause of fatigue in all patients with PBC. We certainly do not believe that we drew this conclusion. However, we do believe that our findings of reduced GP MTRs in patients with stage I-II disease, which were associated with hypermanganaemia and measured fatigue, do open up a novel avenue of research into a poorly understood symptom in patients with PBC.

In order to control for inter-examination system variability, it is necessary to normalise the raw MTRs against an internal region of interest (ROI). Although it might initially appear easier to analyse the raw MTR data, normalisation to an internal standard allows external sources of variation, unrelated to the patient, to be removed. We followed previously published protocols to calculate GP indices, normalised to the putamen and to the frontal white matter,^{2,3} and these were used to test associations with fatigue and blood Mn levels. The raw MTR data were used for the primary comparison between PBC patients and healthy volunteers. We chose two rather than one internal control ROI because, contrary to the assertion in the editorial, there is evidence for Mn accumulation in brain structures, other than the GP, in patients with cirrhosis.^{4,5} Rose *et al* reported significantly elevated Mn concentrations in the frontal and occipital cortex, pallidum, putamen, and caudate⁴ while Maeda *et al* showed elevated Mn concentrations in the GP, putamen, and frontal white matter.⁵ In both series, the highest Mn concentration was in the GP. Our choice of two standard ROIs was made to maximise the interpretation of the raw data although we accept that the a priori assumption that pathology is absent from these regions in this and all relevant magnetic resonance studies to date, which have used internal controls, may be false. This may explain the unexpected trend towards a positive association between blood Mn and the putamen index normalised to white matter.

Drs Milkiewicz and Heathcote have expressed concern about an apparent autocorrelation in our data that did not equal 1. Table 2 in our paper¹ shows the correlation coefficients between individual MTR indices and blood Mn level. We did not compare the normalised putamen index against the normalised putamen index.

We are grateful to the two commentators for extending our interpretations and naturally agree that bile duct loss, rather than liver fibrosis, governs the severity of cholestasis and that there may be dissociation between these features in PBC. For the purposes of this study, we chose to examine patients with stage I-II disease to remove the possibility of hepatic encephalopathy or cirrhosis as a cause for the MTR findings. We believe that both this patient selection and the demonstration of normal cerebral magnetic resonance spectroscopy (MRS) in these patients, compared with healthy volunteers, does indeed achieve this. We found reduced GP MTRs in patients with stage I-II disease, which were associated with hypermanganaemia and measured fatigue, but we also studied four patients with stage III-IV disease and, as a group, there were no significant differences in GP MTR indices compared with stage I-II patients. Although this may be due to the small number of individuals studied, the lack of clear distinction between stage I-II and stage III-IV disease may also reflect a process that adversely affects the brain long before the development of cirrhosis, owing to early bile duct loss.

The commentators point out that the value of liver biopsy staging of PBC is limited owing to sampling error and that there may not have been a true distinction between the stage I-II and III-IV groups. We accept the possibility of sampling error but, in our view, liver biopsy still remains the gold standard for diagnosing cirrhosis. We disagree with the suggestion that cerebral MRS would have been useful in supporting the histological diagnoses as cerebral MRS abnormalities are only seen in a minority of patients with Child-Pugh A cirrhosis.^{6,7} We did not assume that MRS would be abnormal in stage III-IV patients; in fact, there were no significant differences between these patients and stage I-II patients.

Fatigue in PBC merits further research. We hope that we will be able to take further "steps in the right direction".

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Acute ulcerative colitis during successful interferon/ribavirin treatment for chronic hepatitis

A 54 year old man was treated with pegylated interferon alpha 2a 180 µg weekly and ribavirin 1000 mg daily for chronic hepatitis C genotype 3a ($>5 \times 10^5$ IU/ml). There was no history of gastrointestinal disease or morbidity.

At week 12, hepatitis C virus-polymerase chain reaction (HCV-PCR) was negative and alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels remained elevated at 2-3 times above the upper limit. Continuation of this well tolerated therapy was planned until week 24.

However, at week 14, the patient reported a sudden onset of watery and sometimes bloody diarrhoea. Colonoscopy showed continuous pancolitis, macroscopically suggestive of inflammatory bowel disease (IBD). Histology revealed a severe highly active pancolitis with basal plasmacytosis, crypt abscesses, and crypt distortion, as seen in ulcerative colitis.

The antiviral treatment was stopped and treatment with prednisone and mesalazine (5-ASA) was initiated. Steroids were tapered over four weeks, which had been ongoing with clinical remission. 5-ASA was continued at a dose of 3 g daily for eight weeks followed by 2 g daily.

Three months later (receiving 5-ASA 2 g daily) there was complete clinical and endoscopic remission. Histology showed a mild residual increase in mononuclear inflammatory cells. PCR revealed a virological relapse of HCV (high viraemia $>6 \times 10^5$ IU/ml) and an unchanged twofold elevation in ALT and AST.

We suspect that the ulcerative colitis-like severe pancolitis in this patient with no

history of IBD was probably an adverse effect of the antiviral treatment with interferon/ribavirin rather than a concomitant disease. Similar observations have been made by others.¹⁻³ To our knowledge, the present case is the fourth reported in the literature. Interferon has immune stimulating properties⁴ and may trigger autoimmune diseases and transplant rejections.⁵

Hence, in light of this, the report on interferon treatment in active ulcerative colitis (*Gut* 2003;**52**:1728-33) seems interesting and warrants further research.

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Author's reply

As interferon alpha (IFN alpha) suppresses the synthesis of proinflammatory cytokines and induces various anti-inflammatory cytokines, it may show efficacy in chronic inflammatory disorders of the gut. In Crohn's disease, lamina propria cells manifest increased secretion of IFN- γ whereas in ulcerative colitis lamina propria cells and natural killer T cells demonstrate increased secretion of the Th2 cytokines interleukin 5 (IL-5) and IL-13.^{1,2}

IFN alpha has been demonstrated to potently suppress synthesis of both IL-5 and IL-13 in human leucocytes, making it an attractive agent for the treatment of ulcerative colitis. IFN alpha therapy showed no benefit in patients with Crohn's disease.³ This may be explained by the fact that Crohn's disease is thought to be a Th1 linked disease. IFN alpha therapy seems to be more successful in chronic active ulcerative colitis, a more Th2 linked disorder. Sumer and Palabiyikoglu reported that more than 80% of patients with active ulcerative colitis responded to high dose IFN alpha therapy within two weeks of treatment and were in complete clinical and endoscopic remission after six months of therapy.⁴ Madsen et al recently presented a study comparing systemic IFN alpha therapy and prednisolone enemas in the treatment of left sided ulcerative colitis.⁵ Ulcerative colitis is accompanied by high levels of IL-5 in colonic tissue and IFN alpha effectively suppresses IL-5 synthesis in leucocytes. IFN

beta has been used in a pilot study investigating its role in patients with steroid refractory active UC.⁶ In this study, a high responder rate was observed with a mean time to response of three weeks.

Another IFN beta study in ulcerative colitis has been presented recently.⁷ In this small, placebo controlled, randomised, dose escalating study, clinical improvement was observed in 50% of IFN beta treated patients compared with 14% in the placebo group. We recently presented data on the first placebo controlled use of IFN alpha in the treatment of active UC in patients with or without corticosteroid and/or immunosuppressive treatment.⁸ We observed no significant advantage of any IFN group over placebo but did not observe worsening of disease in any IFN treated patient.

The mechanisms of action of IFN alpha are probably multiple but the possible interactions of IFN alpha with the cytokine cascade and immune system are usually not considered. Favouring Th1 responses and suppressing Th2 type immune responses could imply that type I IFNs may be therapeutic in diseases such as ulcerative colitis or allergic disorders. We agree with the authors that IFN alpha might have the potential to enhance inflammatory reactions and alloreactivity in certain situations but are also convinced that it has strong immunomodulatory and anti-inflammatory properties. Larger controlled trials with IFN alpha in ulcerative colitis are eagerly awaited.

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The toll-like receptor 4 (TLR4) Asp299Gly polymorphism is associated with colonic localisation of Crohn's disease without a major role for the *Saccharomyces cerevisiae* mannan-LBP-CD14-TLR4 pathway

It is with great interest that we read the paper by Frachimont and colleagues (*Gut* 2004;**53**:987-92) in which they describe a novel association of the toll-like receptor 4 (TLR4) +896 A>G polymorphism with both Crohn's disease (CD) and ulcerative colitis (UC), supporting the genetic influence of pattern recognition receptors (PRRs) in triggering inflammatory bowel disease (IBD). PRRs are sensors of pattern associated molecular patterns of microorganisms in the intestinal flora. Independently, we performed a similar study. However, special attention to the presence of anti-*Saccharomyces cerevisiae* antibody (ASCA) was taken, as Tada and colleagues¹ have recently reported that the *S. cerevisiae* mannan-LBP complex is recognised by CD14 on monocytes and signalling through TLR4 leads to the production of proinflammatory cytokines in a manner similar to that induced by lipopolysaccharide (LPS).

Patients and controls were recruited from the Outpatient Department of Gastroenterology, VU University Medical Centre, Amsterdam, the Netherlands. The group consisted of 112 CD patients and 170 unrelated Dutch Caucasian controls. Diagnosis of disease was based on clinical, histopathological, and endoscopic findings. CD patients were categorised using the Vienna classification (general patient characteristics are described elsewhere²). ASCA IgA and IgG ELISAs were performed as described previously.³ Genotyping for the *CD14*-260 C>T and *TLR4*+896 A>G single nucleotide polymorphisms (SNPs) was performed as described previously by our group.⁴ The *CD14*-260 and *TLR4*+896 genotypes, allele, and carrier frequencies were compared between the different clinical patient groups and controls. In addition, synergism between *CD14* and *TLR4* genotypes and alleles (carrier trait analyses) was studied. Vienna classification and ASCA status were included in the statistical modelling.

The results are shown in table 1. The frequency of the G allele of *TLR4*+896 was significantly increased in CD patients compared with controls (19% v 10%; p=0.049; odds ratio (OR) 2.1 (95% confidence interval (CI) 1.0-4.1)). Disease phenotype was assessed in patients using the Vienna classification. Carriage of *TLR4* +896*G significantly increased the risk of colonic localisation of CD compared with non-colonic localisation (43% v 12%; p=0.0017; OR 5.5 (95% CI 1.9-15.4)). There was a clear trend (test for trend: χ^2 : 16, p<0.0001) when we compared the increasing frequency of the G allele of *TLR4* +896 in controls (10%) to CD patients (19%) and to CD patients with colonic localisation (43%).

We also assessed if ASCA status was correlated with carriage of the *TLR4* G allele. However, there was no difference between

Table 1 CD14-260 and TLR4+896 genotype distribution in Crohn's disease (CD) patients and healthy controls (HC)

Group	Vienna classification	n _{total}	CD14-260 C>T			TLR4+896 A>G		
			1.1 (CC)	1.2 (CT)	2.2 (TT)	1.1 (AA)	1.2 (AG)	2.2 (GG)
HC		170	48 (28)	82 (48)	40 (24)	153 (90)	16 (9)	1 (1)
CD		112	35 (31)	54 (48)	23 (21)	91 (81)	19 (17)	2 (2)*
	A1	97	29 (30)	49 (51)	19 (20)	77 (79)	19 (20)	1 (0)
	A2	15	6 (40)	5 (33)	4 (27)	14 (93)	0 (0)	1 (6)
	B1	43	14 (33)	20 (47)	9 (21)	36 (84)	6 (14)	1 (2)
	B2	45	15 (33)	21 (47)	9 (20)	37 (82)	7 (16)	1 (2)
	B3	24	6 (25)	13 (54)	5 (21)	18 (75)	6 (25)	0 (0)
	L1	41	14 (34)	17 (41)	10 (24)	36 (88)	4 (10)	1 (2)
	L2	23	6 (26)	13 (57)	4 (17)	13 (57)	9 (39)	1 (4)†
	L3	47	15 (32)	23 (49)	9 (19)	41 (87)	6 (13)	0 (0)
	L4	1	0 (0)	1 (100)	0 (0)	1 (100)	0 (0)	0 (0)

*TLR4*G was more frequent in CD patients compared with HC (19% v 10%; p=0.0489; odds ratio (OR) 2.076 (95% confidence interval (CI) 1.041–4.142)).

†TLR4*G was significantly associated with colonic localisation compared with non-colonic localisation (43% v 12%, p=0.0017; OR 5.455 (95% CI 1.931–15.410)).

Vienna Classification: (age) A1: age <40 years; A2: age >40 years; (behaviour) B1: non-stricturing, non-penetrating; B2: stricturing; B3: penetrating; (localisation) L1: ileal; L2: colonic; L3: ileocolonic; L4: upper gastrointestinal tract.

TLR4 G allele carriage in ASCA positive and ASCA negative patients (23% v 14%; p=0.33) (data not shown) and there was no difference between TLR4 G allele carriage in ASCA positive and negative CD patients with colonic localisation (40% v 46%; p=1.00) while the frequency of G allele carriage was identical to that of CD patients with colonic localisation (43%) without correcting for ASCA status.

Several studies have described both TLR4+896 A>G and CD14-260 C>T in CD. Klein *et al* have described a German population and found an increased incidence of CD14-260 heterozygous and homozygous mutants in CD patients compared with healthy controls.⁵ This association could not be confirmed in our population. Preliminary data by Braat *et al* demonstrated an increased risk of suffering from CD in a Dutch population carrying the TLR4+896 SNP,⁶ confirming our results. Franchimont and colleagues (*Gut* 2004;**53**:987–92) corroborated the results of Braat *et al*. In contrast with Franchimont *et al*, we found a clear association between the G allele of TLR4+896 and disease phenotype (colonic localisation). In contrast with the aforementioned studies and results, Arnott *et al* were unable to demonstrate an association between susceptibility to CD and the TLR4 and CD14 SNPs in a Scottish and Irish population.⁷

The association between TLR4 and CD underscores the role of impaired innate immunity in CD. TLR4 signalling is based on both exogenous (for example, LPS) and endogenous (for example, human HSPs) agonists, and as heterozygous carriership of the TLR4+896 A>G does not seem to impair LPS signalling,^{8,9} further agonist identification to elucidate the microorganisms involved in CD and especially in colonic localisation is essential to obtain insight into both the pathophysiological and immunogenetic aspects of CD. This insight may be helpful in developing strategies for the prevention and treatment of CD.

The association we demonstrated between TLR4 and CD is most likely not strongly based on the *S cerevisiae* mannan-LBP-CD14-TLR4 pathway but, as we have demonstrated, on

the ASCA data in our group. It would be interesting to know whether Franchimont *et al* tested for ASCA in their CD patients and whether or not an association between ASCA and TLR4 was observed.

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Conflict of interest: None declared.

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Reoperative chemoradiotherapy for oesophageal cancer: a systematic review and meta-analysis

Just as the weakest link in a chain determines how much weight the chain will hold, the weakest link in the data used by Fiorica *et al* will determine how much weight we as readers should give to their findings and conclusions regarding neoadjuvant chemoradiotherapy for oesophageal adenocarcinoma (*Gut* 2004;**53**:925–30). Clearly, the weakest link in their data is the material by Walsh *et al*, and prior to placing any confidence in the conclusions by Fiorica *et al*, a careful assessment of the reliability of the Walsh data is imperative.¹ Well known criticisms of the Walsh trial include the lack of routine staging with computed tomography scanning that led to five patients undergoing surgery alone for stage 4 disease, the exclusion of a number of patients in the neoadjuvant arm for "protocol violations" when in fact several had evidence of progressive disease and should have been considered treatment failures, and the lack of a uniform surgical technique that led to five different types of operations being performed and what are arguably the worst surgical results for oesophageal adenocarcinoma reported in the literature. However, these concerns are overshadowed by an even greater problem in the Walsh trial related to internal inconsistencies in the survival data. Careful review of the Walsh manuscript reveals that the survival data in the text of the report does not match the data in the Kaplan-Meier survival curves, but interestingly the discrepancy is only for the neoadjuvant arm.¹ In all cases the survival data for the surgery alone arm matches up precisely. For example, in the text of the manuscript, survival by intention to treat at three years in the neoadjuvant arm is reported as 32%, yet on the Kaplan-Meier graph survival by intention to treat in the neoadjuvant arm is approximately 48%.¹ Similar discrepancies occur at essentially every data point for both the intention to treat and the treatment actually received graphs, but only for the neoadjuvant arm, with survival on the Kaplan-Meier graphs much improved over the data in the text. Importantly, the statistics for survival are calculated from the Kaplan-Meier curves, raising concern that the difference in survival between groups is in fact not significant. This alarming discrepancy has never been adequately addressed despite a letter to the *New England Journal of Medicine* and a subsequent reply by Dr Walsh.²

The response by Walsh was that the graphs were mislabelled, but even with a different label the data points continue to be incongruent.

In light of this, I would like to know how Fiorica *et al* handled the data from the Walsh trial. Did they use data from the Kaplan-Meier survival curves or from the text and tables in the manuscript? Were they aware of the discrepancy and if so why did they not comment on it in their manuscript and specify how they dealt with it in their meta-analysis? In light of these concerns, as well as other issues regarding this trial, is it appropriate to even include it in a meta-analysis unless the raw data are independently reviewed and the statistics validated? This is an especially important issue as the Walsh study is the only trial that included just patients with adenocarcinoma, and as stated in the manuscript by Fiorica *et al*, robust analysis showed that exclusion of the Walsh trial would lead to loss of statistical significance for overall mortality (*Gut* 2004;**53**:925–30). This would leave us where we started, lacking any significant evidence that neoadjuvant therapy improves survival for patients with oesophageal adenocarcinoma.

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Systemic lidocaine and mexiletine for the treatment of a patient with total ulcerative colitis

In basic research, neural modulation in ulcerative colitis has been shown.¹ In clinical settings, local anaesthetics such as lidocaine and ropivacaine were used, administered per rectum, for the treatment of distal ulcerative colitis with a response rate of 83% after long treatment periods (6–34 weeks) for proctosigmoiditis (n = 49).² We report a patient with total ulcerative colitis that was ameliorated by continuous intravenous administration of lidocaine followed by oral administration of mexiletine (a congener of lidocaine).

A 24 year old man suffering from exacerbation of ulcerative colitis was admitted to our hospital. Total ulcerative colitis had been initially diagnosed one year previously. Disease extent was re-examined by barium study to reveal a total type of colitis. Conventional medical therapies, including steroids (60 mg prednisolone and steroid pulse therapy), 5-aminosalicylate, and leucocytapheresis were not effective. Abdominal pain with bleeding per rectum was very severe at night. We administered continuous systemic lidocaine (1 mg/min on the first day and 1.3 mg/min on subsequent days) only at night, resulting in complete disappearance of abdominal pain and bloody diarrhoea on the

first day of treatment. This therapy was given for one week followed by oral mexiletine (300 mg/day on the first two days and 200 mg/day thereafter) administration. Prednisolone was tapered without exacerbation of colitis during this treatment, and the patient left our hospital.

Clinical reports by Kemler and colleagues,³ who reported on a patient with ulcerative colitis exacerbated by spinal cord stimulation, and by Peck and Wood,⁴ who obtained complete remission of a patient with ulcerative colitis after spinal cord injury, support the involvement of neural control in ulcerative colitis. Systemic lidocaine, which has been shown to suppress only spontaneous ectopic discharges without blocking nerve conduction,⁵ and mexiletine may modulate central and/or peripheral nerve function. Thus, in this case, the effectiveness of these drugs could be attributed to modulation of nerve function. Björck *et al* found that when using a 2% gel (400 mg lidocaine), maximum plasma levels were 0.5–1.9 mg/l in patients with proctitis two hours after application of the gel.² In experimental models, plasma concentrations of 1.2–2.1 mg/l of lidocaine has been shown to be effective for neuropathic pain.^{6,7} Therefore, it is possible that in ulcerative colitis, lidocaine administered per rectum could exert its pharmacological effects after being absorbed into blood and has an effect on central and/or peripheral nerves. Another possibility is direct anti-inflammatory effects of these drugs on immune cells.⁸ However, it is not known whether systemic administration of lidocaine can achieve adequate concentrations in colonic tissue to have a direct anti-inflammatory effect on immunocytes.

A prominent feature of this case was the close association between pain and other symptoms such as bloody diarrhoea. Systemic lidocaine caused prompt symptomatic relief followed by amelioration of ulcerative colitis which was assessed by sigmoidoscopy and blood inflammatory parameters (data not shown), suggesting that pain or pain inducing substances could be a cause of exacerbation of ulcerative colitis as well as a result of the disease.

Lidocaine and mexiletine therapy could be useful for the treatment of the subgroup of patients with ulcerative colitis that are refractory to conventional medical treatments. While we do not know how to select responders to this treatment, pain could be one of the indicators.

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New treatment for bile salt malabsorption

Currently available binding resins used for symptomatic bile salt malabsorption are generally poorly tolerated because of unpalatability and associated gastrointestinal side effects. We suggest that there is now a viable alternative, colessevelam hydrochloride (WelChol, Sankyo Pharmaceuticals Inc., Japan).

A 30 year old man presented with steatorrhoea, progressive weight loss, marked abdominal bloating, and lethargy after a right hemicolectomy following a road traffic accident in 1966.

Physical examination, relevant blood tests, barium follow through, colonoscopy, and microscopic examination of colonic biopsies were normal. A trial of cholestyramine in preference to a SeCHAT scan caused cessation of diarrhoea on one sachet per day. However, his abdominal bloating continued unabated and he found the treatment unpalatable. Cholestyramine was therefore changed to colessevelam 2.5 g/3.75 g on alternate days. This was well tolerated, with complete cessation of his steatorrhoea and lethargy, and no side effects. In addition, he rapidly gained weight.

A further four patients with markedly symptomatic bile salt malabsorption resistant to antidiarrhoeal agents and intolerant of cholestyramine were subsequently commenced on colessevelam (table 1). In all of these cases colessevelam was well tolerated with no side effects.

Colessevelam is a non-absorbed water insoluble polymer which sequesters bile.¹ It has been approved for usage by the US FDA, and has been received as a valuable alternative for lowering cholesterol.² Colessevelam has high affinity for dihydroxy and trihydroxy bile acids in the intestine which causes increased faecal bile acid secretion, reducing the enterohepatic circulation of bile acids.² This allows 7-hydroxylase, the rate limiting enzyme in bile acid synthesis, to increase the conversion of hepatic cholesterol to bile acids.³ It has not yet been approved for use in the UK. One abstract suggests that colessevelam may be beneficial for patients with diarrhoea who have undergone small bowel resection for Crohn's disease.³ There are no other published data to support its role in bile salt induced diarrhoea. Colessevelam is reported to be 4–6 times as potent as traditional bile salt sequestrants, possibly due to its greater binding affinity for glycocholic acid.⁴ It is administered in tablet form, and in one study the rate of compliance with colessevelam was 93%.⁴ The unique hydrogel polymeric

Table 1 Characteristics of four patients with markedly symptomatic bile salt malabsorption resistant to antidiarrhoeal agents and intolerant of cholestyramine given colessevelam

Age (y)	Sex	Reason for bile salt malabsorption	Outcome with cholestyramine	Outcome with colessevelam	Duration of colessevelam treatment (months)	Current dose of colessevelam
37	M	Idiopathic	Diarrhoea improved but not tolerated because it induced severe dyspepsia	Diarrhoea resolved, no side effects	7	3.75 g/day
59	F	Right hemicolectomy	Diarrhoea improved but not tolerated due to unpalatability	Diarrhoea resolved, no side effects	3	3.75 g/day
68	F	Radiation enteritis and right hemicolectomy	Diarrhoea improved although suffered intractable vomiting	Diarrhoea resolved, no side effects	2	2.5 g/day
40	F	Radiation enteritis	Diarrhoea improved although suffered intractable nausea	Diarrhoea resolved, no side effects	2	1.25 g/day

structure enables greater tolerability with less potential drug interactions than with resins.¹

Reported adverse events from the largest clinical trial to date include flatulence, dyspepsia, and diarrhoea although the incidence of adverse events does differ significantly from that observed with placebo, and is lower than with cholestyramine.² It is rarely associated with constipation, unlike cholestyramine.⁴ Colessevelam is non-absorbed and is excreted entirely via the gastrointestinal tract, preventing systemic side effects.⁵ Furthermore, there is little evidence for clinically significant interactions involving colessevelam.⁴ Pharmacokinetic studies with colessevelam have not shown clinically significant effects of absorption of six other coadministered drugs.⁶

There is a theoretical risk of fat soluble vitamin deficiency following such efficient bile acid sequestration. None of our patients developed any significant change in fasting triglycerides or fat soluble vitamin levels to date.

Each film coated tablet contains colessevelam 625 mg (active ingredient).² The recommended starting dose for monotherapy for hypercholesterolaemia is 3.75 g once a day or 1.875 g twice per day, although the optimal dose is 4.375 g in adults.² The optimal dose for bile salt malabsorption is not clear but an effective dose has varied between two and six tablets/day in our series. Colessevelam was obtained from IDIS Ltd.

Thus colessevelam is a novel bile acid binding resin in tablet form that maintains the benefits of cholestyramine, yet is palatable, associated with decreased adverse effects, and has greater potency. It provides a very attractive alternative therapy for patients with bile salt malabsorption and further study is warranted.

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Any researchers interested in applying for access to information held within the national register should contact Dr Helen Harris (Register Co-ordinator) or Ms Shirley Cole (Research Assistant), Immunisation Department, CDSC, Centre for Infections, Health Protection Agency, 61 Colindale Avenue, London NW9 6EQ, UK (tel: +44 (0)20 8200 6868 ext. 7676 (Wednesday to Friday) or ext. 7906 (Monday to Friday); fax: +44 (0)20 8200 7868; email: helen.harris@hpa.org.uk or shirley.cole@hpa.org.uk).

No data will be released that could identify individual patients directly or via linkage to other data. Any study proposals should then be submitted to the register co-ordinator for consideration by the steering group by Thursday 31 March 2005 (deadline).

6th International Symposium on Functional Gastrointestinal Disorders

This symposium is co-sponsored by the Office of Continuing Medical Education, University of Wisconsin Medical School, and the International Foundation for Functional Gastrointestinal Disorders (IFFGD). It will take place on 7–10 April 2005 in Milwaukee, Wisconsin, USA, at The Pfister Hotel, 424 E. Wisconsin Avenue, Milwaukee, Wisconsin 53202 (tel: +1 414 273 8222; toll free tel: +1 800 558 8222; fax: +1 414 273 5025; email: info@thepfisterhotel.com; web: http://www.iffgd.org/symposium2005.html).

NOTICES

The national register of hepatitis C infections with a known date of acquisition

A new call for study proposals.

In 1998, a national register of hepatitis C virus (HCV) infections with a known date of acquisition was established. The register was set up to help inform the natural history of HCV related disease in the UK and now contains anonymous data for one of the largest cohorts of individuals with known date HCV infections, with over 1120 registered patients. The majority of infections in the register are those that were acquired following transfusion of HCV infected blood that was issued before the introduction of routine screening of the blood supply for HCV, but other routes of acquisition are represented.

In order to get maximum benefit from this national resource, the register steering group would like to invite clinical and epidemiological researchers to submit proposals to access data held in the register. It is envisaged that a variety of studies might benefit from linkage with or access to the register, and proposals from all specialties and institutions are welcomed. Such studies are urgently needed to help determine the current and future burden of HCV related disease on healthcare services, and to assess the impact of currently available treatments as well as those that may become available in the future.

CORRECTIONS

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In reference 38 of the paper by C Gasche and P Grundtner, published in the January issue (Genotypes and phenotypes in Crohn's disease: do they help in clinical management? *Gut* 2005;**54**:162–7), the page span is incorrect, it should read 1658–64.

In the paper by Sheu *et al* in the July 2003 issue of *Gut* (B-S Sheu, S-M Sheu, H-B Yang, A-H Huang, and J-J Wu. Host gastric Lewis expression determines the bacterial density of *Helicobacter pylori* in *babA2* genopositive infection. *Gut* 2003;**52**:927–932), the B and C slides of figure 1 have been transposed and the arrow on D should be labelled Le^x not Le^b.