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

B-S Sheu, S-M Sheu, H-B Yang, A-H Huang, J-J Wu

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\(^b\), Le\(^x\), Le\(^y\), and Le\(^z\) was determined immunohistochemically 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\(^b\) in 33.5%, Le\(^x\) in 72.9%, Le\(^y\) in 86.2%, and Le\(^z\) 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\(^x\), *H pylori* density increased with a higher Le\(^x\) intensity (p<0.05). Gastric atrophy decreased with Le\(^x\) intensity and thus resulted in lower *H pylori* density in the antrum (p<0.05). For the 49 patients without gastric Le\(^x\) expression, *H pylori* density was positively related with Le\(^y\) and Le\(^z\) expression (p=0.05).

Conclusions: Taiwanese *H pylori* isolates are 100% *babA2* genopositive. Gastric Le\(^b\) as well as Le\(^x\) intensity may be major determinants of *H pylori* density. While lacking gastric Le\(^x\) expression, Le\(^y\) and Le\(^z\) were closely related to *H pylori* colonisation.
TG, corresponding to bp 369 to 391 of AF033654) designed by Ilver and colleagues 
9 or another self designed primer (bab7-F: CCA AAC GAA ACA AAA AGG GC, 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 µl containing 0.2 µM of each primer, 0.2 mM each of deoxynucleoside triphosphates, reaction buffer with MgCl2, 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

<table>
<thead>
<tr>
<th>Lewis antigen</th>
<th>Antrum</th>
<th>Body</th>
<th>Cardia</th>
<th>Significance*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intensity (range 0–3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lea</td>
<td>0.56 (0.77)</td>
<td>0.41 (0.65)</td>
<td>0.45 (0.81)</td>
<td>A&gt;B, A&gt;C</td>
</tr>
<tr>
<td>Leb</td>
<td>1.37 (1.24)</td>
<td>1.74 (0.95)</td>
<td>1.59 (1.03)</td>
<td>B&gt;A, C&gt;A</td>
</tr>
<tr>
<td>Lex</td>
<td>1.19 (0.72)</td>
<td>0.88 (0.71)</td>
<td>0.75 (0.73)</td>
<td>A&gt;B, A&gt;C</td>
</tr>
<tr>
<td>Ley</td>
<td>1.61 (0.95)</td>
<td>1.76 (1.07)</td>
<td>1.68 (0.88)</td>
<td>B&gt;A, C&gt;A</td>
</tr>
</tbody>
</table>

Values are mean (SD).

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

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

<table>
<thead>
<tr>
<th>Parameter (mean)</th>
<th>Lea (+) (n=63)</th>
<th>Lea (−) (n=125)</th>
<th>Leb (+) (n=139)</th>
<th>Leb (−) (n=49)</th>
<th>Ley (+) (n=162)</th>
<th>Ley (−) (n=26)</th>
<th>Le (+) (n=183)</th>
<th>Le (−) (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIS (0–9)§</td>
<td>2.31 (2.61)</td>
<td>2.62 (2.21)</td>
<td>2.81 (2.45)</td>
<td>2.55 (1.18)</td>
<td>56.3 (100)</td>
<td>25.1 (80)</td>
<td>49</td>
<td>45.6</td>
</tr>
<tr>
<td>CIS (0–9)†</td>
<td>6.89 (6.97)</td>
<td>7.59 (6.15)</td>
<td>6.95 (6.88)</td>
<td>6.96 (6.40)</td>
<td>54.9 (73)</td>
<td>57.7 (73)</td>
<td>2.89 (2.69)</td>
<td>2.87 (2.41)</td>
</tr>
<tr>
<td>AT [%]</td>
<td>57.1 (57.6)</td>
<td>57.1 (54.9)</td>
<td>54.9 (73)</td>
<td>56.3 (100)</td>
<td>57.1 (54.9)</td>
<td>57.7 (73)</td>
<td>2.89 (2.69)</td>
<td>2.87 (2.41)</td>
</tr>
<tr>
<td>IM [%]†§</td>
<td>28.6 (29.6)</td>
<td>32.7 (26.7)</td>
<td>26.7 (57.7)</td>
<td>25.1 (80)</td>
<td>28.1 (32.7)</td>
<td>26.7 (57.7)</td>
<td>2.89 (2.69)</td>
<td>2.87 (2.41)</td>
</tr>
<tr>
<td>Ulcer rate [%]</td>
<td>49 (45.6)</td>
<td>44.9 (47.5)</td>
<td>47.5 (42.3)</td>
<td>46.9 (40)</td>
<td>47.5 (42.3)</td>
<td>42.3 (46.9)</td>
<td>2.89 (2.69)</td>
<td>2.87 (2.41)</td>
</tr>
<tr>
<td>HPD (1–15)†</td>
<td>9.03 (8.29)</td>
<td>9.29 (6.35)</td>
<td>8.59 (8.03)</td>
<td>8.57 (7.41)</td>
<td>8.03 (8.57)</td>
<td>8.57 (7.41)</td>
<td>2.89 (2.69)</td>
<td>2.87 (2.41)</td>
</tr>
<tr>
<td>Antrum (1–5)†‡</td>
<td>3.46 (3.08)</td>
<td>3.52 (3.21)</td>
<td>3.31 (2.69)</td>
<td>2.31 (2.85)</td>
<td>3.46 (3.08)</td>
<td>3.52 (3.21)</td>
<td>2.89 (2.69)</td>
<td>2.87 (2.41)</td>
</tr>
<tr>
<td>Body (1–5)††</td>
<td>2.57 (2.51)</td>
<td>2.89 (1.63)</td>
<td>2.65 (2.53)</td>
<td>2.57 (2.41)</td>
<td>2.65 (2.53)</td>
<td>2.57 (2.41)</td>
<td>2.89 (2.69)</td>
<td>2.87 (2.41)</td>
</tr>
</tbody>
</table>

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 Lea (+) and Lea (−) patients; †between Leb (+) and Leb (−) patients; ‡between Lex (+) and Lex (−) patients; §between Ley (+) and Ley (−) patients.

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

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

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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.

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 horse-radish 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 colleagues: 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. The total acute (AIS) and chronic (CIS) inflammatory scores were also a sum of the three specimens (range 0–9).

**Immunohistochemical 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
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-Le⁻¹, Le⁺, Le⁺, and Le⁻; Signet Laboratories, Inc., Dedham, Massachusetts, USA). The reaction time for the primary monoclonal antibodies (anti-Le⁻¹, Le⁺, Le⁺, and Le⁻) 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⁻¹, Le⁺, Le⁺, and Le⁻ 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 ≤30% of the analysed specimens; 3, diffuse staining of ≥50% of the analysed specimens on surface cells, intercryptal epithelium, and deep glands). Examples of intensity 1 and intensity 2 gastric Le expression are shown in fig 1A and 1B, respectively. Total gastric Lewis antigen intensity (TLI) for Le⁻¹, Le⁺, Le⁺, and Le⁻ 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 χ² 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 bab2A genotypes of H pylori infection in Taiwan
Fifty per cent (94/188) of H pylori isolates had a positive bab2A genotype by PCR, applying the primers used by Iver 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 bab2A in origin but had >90% homology with the published sequence of adenine specific DNA methyltransferase in H pylori 26695. To detect the bab2A 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 bab2A gene of CCUG 178785 (fig 2). Based on PCR using these primers to obtain a 271 bp band, the prevalence rate of the bab2A 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⁺ in 33.5%, Le⁺ in 72.9%, Le⁻ in 86.2%, and Le⁻ in 97.4% of gastric biopsies in these 188 patients. As shown in table 1, the topographic intensity of gastric Le expression was higher in the corpus than in the antrum or cardia (1.76 ± 1.61 and 1.68; paired t test, p <0.05). The intensity of Le⁻¹ expression was also higher in the corpus and cardia than in the antrum (1.74 ± 1.37 and 1.59 ± 1.37; paired t test, p <0.05).

In contrast, the topographic intensity of Le⁻¹ or Le⁺ was higher in the antrum than in the corpus and cardia (Le⁻¹: 0.56 v 0.41 and 0.45, p <0.05; Le⁺: 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⁻¹ expression had significantly higher HPD and CIS than those without Le⁻¹ expression (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⁻¹⁺ patients was significantly lower than that of either 88 Le⁺⁻ patients or 51 Le⁺⁺ patients (7.42 ± 9.22 and 9.41; p <0.05 by one way ANOVA). In fig 3A, TLI of Le⁺ 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⁻¹, Le⁺, and Le⁺ in the stomach. Furthermore, patients who expressed Le⁻¹ and Le⁺ 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⁻¹ and Le⁺ 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⁻¹ or Le⁺ had higher rates of IM in the stomach (p <0.01). Among those patients who expressed Le⁺, TLI of Le⁺ 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⁻¹ or Le⁻, despite the presence of IM or antral atrophy.

Factors correlating with HPD in non-Le⁻¹ patients
Of the 49 H pylori infected patients without Le⁻¹ expression, HPD was higher in patients who expressed gastric Le⁺ and Le⁺.
Compensatory effect of Le\(^b\) 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\(^b\) 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\(^a\) intensity elsewhere in the body or cardia (table 4). In contrast, a significant increase in the intensity of Le\(^a\) 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. 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 clinicohistological 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 BabA is a good target for preventive vaccination if BabA interacts strongly with Le\(^b\) to impact on \(H\) \(pylori\) colonisation of patients.

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Coefficient</th>
<th>Standard error</th>
<th>p Value</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total 188 patients</td>
<td>0.491</td>
<td>0.052</td>
<td>0.000*</td>
<td>(0.389–0.593)</td>
</tr>
<tr>
<td>Total Le(^a) intensity</td>
<td>-0.327</td>
<td>0.294</td>
<td>0.267</td>
<td>(0.096–0.533)</td>
</tr>
<tr>
<td>Total Le(^b) intensity</td>
<td>0.230</td>
<td>0.075</td>
<td>0.002*</td>
<td>(0.083–0.378)</td>
</tr>
<tr>
<td>Total Le(^x) intensity</td>
<td>0.092</td>
<td>0.056</td>
<td>0.116</td>
<td>(0.022–0.199)</td>
</tr>
<tr>
<td>Total Le(^x) intensity</td>
<td>0.123</td>
<td>0.076</td>
<td>0.221</td>
<td>(0.057–0.344)</td>
</tr>
<tr>
<td>In 139 patients with Le(^a)</td>
<td>0.110</td>
<td>0.093</td>
<td>0.413</td>
<td>(0.030–0.293)</td>
</tr>
<tr>
<td>In 139 patients with Le(^b)</td>
<td>0.068</td>
<td>0.071</td>
<td>0.339</td>
<td>(0.011–0.214)</td>
</tr>
<tr>
<td>In 49 patients without Le(^a)</td>
<td>0.092</td>
<td>0.093</td>
<td>0.413</td>
<td>(0.030–0.293)</td>
</tr>
<tr>
<td>In 49 patients without Le(^b)</td>
<td>0.068</td>
<td>0.071</td>
<td>0.339</td>
<td>(0.011–0.214)</td>
</tr>
</tbody>
</table>

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

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

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).
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^b^ or Le^y^ may disappear when *H. pylori* infection is induced by IM. Such a finding was indirectly supported by our data (table 1) which showed that patients without expression of Le^a^ or Le^b^ had higher rates of IM than those with Le^a^ and Le^b^ (p<0.05).

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

There were 49 patients with *H. pylori* infection but no expression of Le^a^ in the stomach. Bacterial densities of the body remained higher in patients with positive expression of Le^a^ and Le^b^ (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^a^, serving as adherent receptors for *H. pylori*. This is compatible with the finding of Clyne and Drummon who confirmed that blocking with a monoclonal antibody for the Le^a^ antigen on the gastric epithelium could not totally abolish adherence of *H. pylori*. Thus we tested if other gastric Lewis types also enhanced bacterial adherence in the 49 patients without Le^a^ expression. Our study found that patients who expressed Le^b^ and Le^b+^ had higher HPD than those who did not express Le^a^ and Le^b^: 7.41 v 6.05, p<0.05; Le^a^: 6.81 v 4.25, p<0.005. By multiple logistic regression, Le^b^ and Le^b+^ were further confirmed to be independent factors in enhancing *H. pylori* infection (table 3). These data support the laboratory findings of Taylor et al which found adhesion pedestal formations stained with Le^a^ on both *H. pylori* and gastric epithelium. Accordingly, our study confirmed that Lewis antigens other than Le^a^ can be used to establish or maintain *H. pylori* infection in the stomach.1 11 15 16 18

Expression of Le^a^ was stronger in the body, in contrast with the antrum, the dominant distribution of Le^a^ and Le^b^ (table 1). Therefore, we tested whether Le^a^ and Le^b^ had additive effects when present with Le^a^ for enhancement of *H. pylori* colonisation in the 139 patients with Le^a^ 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^a^, which was expected, as Le^a^ usually stained the superficial glands.13 14 When the intensity of Le^a^ 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^b^ over the body and cardia were higher in the presence of antral atrophy, increased bacterial densities here could be mediated by Le^a^ expression. These clinical data supported the finding that gastric Le^a^ antigen can enhance *H. pylori* adherence.17 Moreover, Le^a^ may have compensatory or additive effects with Le^b^ to maintain bacterial loads during ongoing atrophy changes.

Among those patients who expressed Le^b^, TLI of Le^b^ 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 v 3.01; p< 0.01). These data confirm that IM and antral atrophy may change Le^b^ expression and thus alter the *H. pylori* colonisation pattern.

In summary, Taiwanese *H. pylori* isolates are 100% babA2 positive. Gastric Le^a^ intensity as well as Le^b^ intensity appear to be major determinants of bacterial density of *H. pylori*. When lacking gastric Le^a^ expression, Le^a^ and Le^b^ are closely related to *H. pylori* colonisation. To overcome *H. pylori* adherence, genomic targets such as babA2 or (other interactions with gastric Lewis antigens) may be promising.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


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 hypermanganesaemia 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,1 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.2,3 Rose et al reported significantly elevated Mn concentrations in the putamen and to the frontal white matter,1 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.2,3 Rose et al reported significantly elevated Mn concentrations in the putamen and to the frontal white matter,1 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.2,3 Rose et al reported significantly elevated Mn concentrations in the putamen and to the frontal white matter,1 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.2,3 Rose et al reported significantly elevated Mn concentrations in the putamen and to the frontal white matter,1 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.

In the current 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 hypermanganesaemia 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 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.2,3 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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References

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

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 (>5×10^6 IU/ml). There was no history of gastrointestinal disease or morbiditity.

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 of normal. While we had anticipated therapy would be stopped, treatment 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 treatment with prednisone and mesalazine (5-ASA) was initiated. Steroids were tapered over four weeks, which had been ongoing treatment with prednisone and mesalazine (5-ASA) was initiated. Steroids were tapered over four weeks, which had been ongoing. Three months later (receiving 5-ASA 2 g weekly and misoprostol 100 µg daily), the patient was well and the colitis had resolved.

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. 1,2 To our knowledge, the present case is the fourth reported in the literature. Interferon has immune stimulating properties 3 and may trigger autoimmune diseases and transplant rejections.

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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References

Author’s reply
As interferon alpha (IFN alpha) suppresses the 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 IFN-g production in ulcerative colitis lamina propria cells and natural killer T cells demonstrate increased secretion of the Th2 cytokines interleukin 5 and IL-13.1,2

IFN alpha has been demonstrated to potentiate synthesis of both IL-5 and IL-13 in human leukocytes, making it an attractive agent for the treatment of ulcerative colitis. IFN alpha therapy showed no benefit in patients with Crohn’s disease. 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 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.3 Madsen et al recently presented a study comparing systemic IFN alpha therapy and prednisolone enemas in the treatment of left-sided ulcerative colitis.1 Ulcerative colitis is accompanied by high levels of IL-5 in colonic tissue and IFN alpha effectively suppresses IL-5 synthesis in leukocytes. IFN beta has been used in a pilot study investigating its role in patients with steroid refractory active UC.4 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.5 In this small, placebo controlled, randomised, dose escalating study, clinical remission 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.6 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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Conflict of interest: None declared.

References


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. 7 In this recent and well-devised study the authors 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 colleagues 8 have recently reported that the S cerevisiae mannan-LBP complex is recognised by CD14 on monocytes and can signal through TLR4 leading 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 elsewhere).1 ASCA IgA and IgG ELISAs were performed as described previously.2 Genotyping for the CD14-260 C>T and TLR4+896 A>G single nucleotide polymorphisms (SNPs) was performed as described previously by our group.3 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 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 +896G 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 no clear trend (test for trend; y2: 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
TLR4 G allele carriage in ASCA positive and ASCA negative patients (23% vs 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% vs 46%; p = 1.00) while the frequency of G allele carriage was positively and negatively CD patients with non-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 CD +260 heterozygous and homozygous mutants in CD patients compared with healthy controls. 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, 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, Arnot et al were unable to demonstrate an association between susceptibility to CD and the TLR4 and CD14 SNPs in a Scottish and Irish population.

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 carrierrship of the TLR4 +896 A→G does not seem to impair LPS signalling, 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 mannann-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.

References
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. 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 proctitis two hours after application of the gel.1 In experimental models, plasma concentrations of 1.2–2.1 mg/l of lidocaine has been shown to be effective for neuro-pathic pain. 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. 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 immune cells. 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.

References


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, colesevelam hydrochloride (WelChol, Sankyo Pharmaceuticals Inc., Japan). A 30 year old man presented with steatorrhea, 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 unacceptable. Cholestyramine was therefore changed to colesevelam 2.5 g 3 times daily 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 anti diarrhoecal agents and intolerant of cholestyramine were subsequently commenced on colesevelam (table 1). In all of these cases colesevelam was well tolerated with no side effects.

Colesevelam is a non-absorbed water insoluble polymer which sequesters bile. It has been approved for usage by the US FDA, and has been received as a valuable alternative for lowering cholesterol.2 Colesevelam has high affinity for dihydroxy and trihydroxy bile acids in the intestine which causes increased faecal bile acid secretion, reducing the entero-hepatic circulation of bile acids.3 This allows 7-hydroxylase, the rate limiting enzyme in bile acid synthesis, to increase the conversion of hepatic cholesterol to bile acids. It has not yet been approved for use in the UK. One abstract suggests that colesevelam may be beneficial for patients with diarrhoea who have undergone small bowel resection for Crohn’s disease.2 There are no published data to support its role in bile salt induced diarrhoea. Colesevelam 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 colesevelam was 93%.5 The unique hydrogel polymeric
structure enables greater tolerability with less potential drug interactions than with resins.\textsuperscript{3}

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.\textsuperscript{4} It is rarely associated with constipation, unlike cholestyramine.\textsuperscript{4} Colesevelam is non-absorbed and is excreted entirely via the gastrointestinal tract, preventing systemic side effects.\textsuperscript{5} Furthermore, there is little evidence for clinically significant interactions involving colesevelam.\textsuperscript{6} Pharmacokinetic studies with colesevelam have not shown clinically significant effects of absorption of six other coadministered drugs.\textsuperscript{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 colesevelam 625 mg (active ingredient).\textsuperscript{1} 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.\textsuperscript{1} 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. Colesevelam was obtained from IDIS Ltd.

This colesevelam 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.

\begin{table}
\centering
\caption{Characteristics of four patients with markedly symptomatic bile salt malabsorption resistant to anti diarrhoeal agents and intolerant of cholestyramine given colesevelam.}
\begin{tabular}{|c|c|c|c|c|c|}
\hline
Age & Sex & Reason for bile salt malabsorption & Outcome with cholestyramine & Outcome with colesevelam & Duration of colesevelam treatment (months) & Current dose of colesevelam \\
(y) & & & & & & \\
\hline
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 \\
\hline
\end{tabular}
\end{table}


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. 7767 (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).

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.

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 Helicobacter pylori in hba2 genepositive 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$^c$ not Le$^b$.

doi: 10.1136/gut.2003.035600corr1

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