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 Lea, Leb, Le-, and Ley 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 Lea in 33.5%, Leb in 72.9%, Le- in 86.2%, and Ley in 97.4% of biopsies from these 188 patients. Patients who expressed Lea had a higher *H pylori* density than those who did not express Lea (*p*<0.001). Among 139 patients who expressed Leb, *H pylori* density increased with a higher Leb intensity (*p*<0.05). Gastric atrophy decreased with Le- and Ley intensity and thus resulted in lower *H pylori* density in the antrum (*p*<0.05). For the 49 patients without gastric Leb expression, *H pylori* density was positively related with Le- and Ley expression (*p*<0.05).

**Conclusions:** Taiwanese *H pylori* isolates are 100% babA2 genopositive. Gastric Lea as well as Leb intensity may be major determinants of *H pylori* density. While lacking gastric Leb expression, Le- and Ley were closely related to *H pylori* colonisation.

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**STOMACH**

_Helicobacter pylori_ is a well recognised gastric pathogen in humans. The ability of _H pylori_ to achieve persistent colonisation in the human stomach has become the focus of intense research. 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. As Le antigens are also found on the gastric epithelium in humans, Le antigen expression may mediate the attachment of *H pylori* to the gastric mucosa. 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 antigen of the host. Their findings suggest that gastric Le 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 expression in the stomach. Therefore, we conducted this study to elucidate if the interaction of the babA2 genotype of _H pylori_ and gastric Le antigen expression of the host are correlated with different clinical outcomes.

As gastric Le 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-, Leb, and Ley antigens are commonly expressed. As the adhesion pedastal formation contained Le on both _H pylori_ and gastric epithelium, these Lewis antigens may be required to establish or maintain infection. Thus we tested if these Lewis antigens have a role in bacterial adherence, when the host has weak or no gastric Le 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 Lea, Leb, Le-, and Ley. The remaining two gastric specimens were used for _H pylori_ culture.

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.

**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: CCT 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; Le-N, total Lewis number; BabA, blood group antigen binding adhesin; babA2, blood group associated binding gene; PCR, polymerase chain reaction; TI, total gastric Lewis antigen expression intensity; HPD, *Helicobacter pylori* density; IM, intestinal metaplasia; CIS, chronic inflammatory score; PBS, phosphate buffered saline.
TG, corresponding to bp 369 to 391 of AF033654) designed by Ilver and colleagues 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 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

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

<table>
<thead>
<tr>
<th>Lewis antigen</th>
<th>Antrum</th>
<th>Body</th>
<th>Cardia</th>
<th>Significance*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Le⁺ Intensity (range 0–3)</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>Le⁺</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>Le⁻</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>Le⁻</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 2  Lewis antigen expression and clinicohistological features of Helicobacter pylori infection

<table>
<thead>
<tr>
<th>Parameter (mean)</th>
<th>Le⁺ (+) (n=63)</th>
<th>Le⁺ (−) (n=125)</th>
<th>Le⁻ (+) (n=139)</th>
<th>Le⁻ (−) (n=49)</th>
<th>Le⁻ (+) (n=162)</th>
<th>Le⁻ (−) (n=26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIS (0–9)§</td>
<td>2.31</td>
<td>2.61</td>
<td>2.62</td>
<td>2.21</td>
<td>2.81</td>
<td>2.45</td>
</tr>
<tr>
<td>CIS (0–9)†</td>
<td>6.89</td>
<td>6.97</td>
<td>7.59</td>
<td>6.15</td>
<td>6.95</td>
<td>6.88</td>
</tr>
<tr>
<td>AT [%]</td>
<td>57.1</td>
<td>57.6</td>
<td>57.6</td>
<td>57.1</td>
<td>54.9</td>
<td>73</td>
</tr>
<tr>
<td>IM [%]†</td>
<td>28.6</td>
<td>29.6</td>
<td>28.1</td>
<td>32.7</td>
<td>26.7</td>
<td>57.7</td>
</tr>
<tr>
<td>Ulcer rate [%]</td>
<td>49</td>
<td>45.6</td>
<td>47.5</td>
<td>44.9</td>
<td>47.5</td>
<td>42.3</td>
</tr>
<tr>
<td>HPD (1–15)†</td>
<td>9.03</td>
<td>8.29</td>
<td>9.29</td>
<td>6.35</td>
<td>8.59</td>
<td>8.03</td>
</tr>
<tr>
<td>Antrum (1–5)*‡</td>
<td>2.89</td>
<td>2.69</td>
<td>2.87</td>
<td>2.41</td>
<td>2.73</td>
<td>2.71</td>
</tr>
<tr>
<td>Body (1–5)*‡</td>
<td>3.46</td>
<td>3.08</td>
<td>3.52</td>
<td>2.31</td>
<td>3.31</td>
<td>2.69</td>
</tr>
<tr>
<td>Cardia (1–5)*‡</td>
<td>2.67</td>
<td>2.51</td>
<td>2.89</td>
<td>1.63</td>
<td>2.66</td>
<td>2.53</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 Le⁺ (+) and Le⁺ (−) patients; †between Le⁻ (+) and Le⁻ (−) patients; ‡between Le⁺ (+) and Le⁻ (−) patients.
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 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 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

![Figure 2](http://www.gutjnl.com)
confirmed with >95% homology to the domestic strains, we self designed a pair of primers and with the published sequence of adenine specific DNA methylase 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-Lea, Leb, Lec, and Led; Signet Laboratories, Inc., Dedham, Massachusetts, USA). The reaction time for the primary monoclonal antibodies (anti-Lea, Leb, Led, and Lea) 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 reaction time for the primary monoclonal antibodies (anti-Lea, Leb, Led, and Lea) was three hours at 25°C. These slides were 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 Lea, Leb, Led, and Lea 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 Lea, Leb, Led, and Lea 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 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 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 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 Lea in 33.5%, Leb in 72.9%, Led in 86.2%, and Lea 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 v 1.61 and 1.68; paired t test, p<0.05). The intensity of Lea 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 Leb or Lea was higher in the antrum than in the corpus and cardia (Lea: 0.56 v 0.41 and 0.45, p<0.05; Lea: 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 Lea expression had significantly higher HPD and CIS than those without Lea 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 Lea+ patients was significantly lower than that of either 88 Lea− patients or 51 Lea+ patients (7.42 v 9.22 and 9.41; p<0.05 by one way ANOVA). In fig 3A, TLI of Lea 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 Leb or Lea was higher in the antrum than in the corpus and cardia (Lea: 0.56 v 0.41 and 0.45, p<0.05; Lea: 1.19 v 0.88 and 0.75, p<0.05).

**Factors correlating with HPD in non-Lea+ patients**

Of the 49 *H pylori* infected patients without Lea expression, HPD was higher in patients who expressed gastric Leb and Lea.
Compensatory effect of Le\textsuperscript{x} to maintain HPD for weak Le\textsuperscript{a} intensity in antral atrophy

Among the 139 Le\textsuperscript{x} positive patients, the topographic distribution of \textit{H pylori} density and the intensity of Le\textsuperscript{a}, Le\textsuperscript{x}, and Le\textsuperscript{b} were compared between patients with and without antral atrophy (table 4). There was no decrease in either Le\textsuperscript{a} or Le\textsuperscript{x} intensity over the antrum despite the presence of atrophy. In contrast, patients with antral atrophy had a lower Le\textsuperscript{a} 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\textsuperscript{a} 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 was found to maintain HPD under the presence of antral atrophy (p<0.05). However, there was no significant increase in Le\textsuperscript{x} intensity elsewhere in the body or cardia (table 4). In contrast, a significant increase in the intensity of Le\textsuperscript{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 \textit{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 \textit{H pylori} is a putative determinant allowing it to adhere to Le\textsuperscript{x} of the gastric epithelium and thus could promote bacterial invasion of the human stomach.\textsuperscript{9} Our prospective study enrolled 188 \textit{H pylori} infected patients and is the first to analyse both bacterial babA2 genotype and gastric antigen expression (including Le\textsuperscript{x}), thus further elucidating the impact of any interactions between BabA and Le\textsuperscript{x} on the clinicohistological outcome after \textit{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%).\textsuperscript{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\textsuperscript{x} to impact on \textit{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' had the lowest incidence and that gastric Le' or Le' 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' or Le' had higher rates of IM than those with Le' and Le' (p<0.05).

Patients with gastric Le' expression had a higher bacterial density of H pylori than those without Le' expression (p<0.05) (table 2). TLI of Le' expression was also positively correlated with HPD (fig 3A). Moreover, HPD was higher in Le' weak and Le' strong secretors than in Le' non-secretors (p<0.05). Accordingly, the intensity of Le' was proved to be an independent factor in enhancing gastric epithelial cell infected in patients. Gastroenterology, 1998;115:1113-22.


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

Acknowledgements

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References


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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 report 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. 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 hypermanganeseemia and measured fatigue, do open up a novel avenue of research into a poorly understood symptom associated with hypermanganesaemia and believe that our findings of reduced GP MTRs that we drew this conclusion. However, we do indeed achieve this. We found reduced GP MTRs in patients with stage I–II disease, which were associated with hypermanganeseaemia 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. 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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Conflict of interest: None declared.

References
6 Taylor-Robinson SD, Sargentoni J, Marcus CD, et al. Regional variations in cerebral proton spectroscopy in patients with chronic hepatitis C genotype 3a (>5x10^5 IU/ml). There was no history of gastrointestinal disease or morbidly.

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 (>5x10^5 IU/ml). There was no history of gastrointestinal disease or morbidly.

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. Chronic hepatitis C treatment was continued as the patient wished, 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 treatment with prednisone and mesalazine (5-ASA) was initiated. Steroids were tapered treatment with prednisone and mesalazine (5-ASA) was initiated. Steroids were tapered...
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.1,4 To our knowledge, the present case is the fourth reported in the literature. Interferon has immune stimulating properties1,2 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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Conflict of interest: None declared

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 secretion of the Th2 cytokines interleukin 5 (IL-5) and IL-13.1,2 IFN alpha has been demonstrated to potent 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 patient 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 active ulcerative colitis, a more Th2 linked disorder. Sumer and Palabiylkoglo reported that more than 80% of patients with active ulcerative colitis responded to high dose IFN alpha therapy within two weeks of treatment and we 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 1 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

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6 Musch E, Anind T, Malek M. Induction and maintenance of clinical remission by interferon beta in patients with steroid-refractory active ulcerative colitis: an open-label pilot trial.

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 mannann-LBP-CD14-TLR4 pathway

It is with great interest that we read the paper by Frachimont and colleagues (Gut 2003;52:987–92) in which they described 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 have recently reported that the S cerevisiae mannann-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 elsewhere). ASCA IgA and IgG ELISAs were performed as described previously.1 Genotyping for the CD14-260 C>T and TLR4+896 A>G single nucleotide polymorphisms (SNPs) was performed as described previously by our group.2 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 a clear trend (test for trend: χ²: 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

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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 while the frequency of G allele carriage was identical to that of CD patients with colonic localisation (43%) without correcting for TLR4 was observed.

Several studies have described both TLR4+896 A/G and CD4−260 C/T in CD. Klein et al have described a German population and found an increased incidence of CD4−260 heterozygous and homoygous mutants in CD patients compared with healthy controls. This association could not be confirmed in our population. Preliminary data by Braat et al demonstrated that, despite an increased risk of suffering from CD in a Dutch population carrying the TLR4+896 SNP, confirming our results. Franchimont and colleagues (citetext) 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 CD4 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 carriehers 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 mannain-LBP-C1D4TLR4 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.

### Table 1

<table>
<thead>
<tr>
<th>CD4−260 C−T</th>
<th>TLR4+896 A−G</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD</td>
<td>CD</td>
</tr>
<tr>
<td>HC</td>
<td>170</td>
</tr>
<tr>
<td>CD</td>
<td>112</td>
</tr>
<tr>
<td>A1</td>
<td>97</td>
</tr>
<tr>
<td>A2</td>
<td>15</td>
</tr>
<tr>
<td>B1</td>
<td>43</td>
</tr>
<tr>
<td>B2</td>
<td>45</td>
</tr>
<tr>
<td>B3</td>
<td>24</td>
</tr>
<tr>
<td>L1</td>
<td>41</td>
</tr>
<tr>
<td>L2</td>
<td>23</td>
</tr>
<tr>
<td>L3</td>
<td>47</td>
</tr>
<tr>
<td>L4</td>
<td>1</td>
</tr>
</tbody>
</table>

**TLR4+896 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+896 was significantly associated with colonic localisation compared with non-colonic localisation (43% v 46%; p = 0.10) while the frequency of G allele carriage was identical to that of CD patients with colonic localisation (40%) v 14%; p = 0.33).

### References


### 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 (2004;53:925−30). Clearly, the strongest 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. 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 carried out and what are arguably the worst surgical results for oesophageal adenocarcinoma reported in the literature. However, these criticisms are overshadowed by the 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 surprisingly the discrepancy is only for the neoadjuvant arm. In all cases the survival data for the surgery alone arm matches up precisely. For example, in the text of the manuscript, survival at 1 year for 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%. 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 matching 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.
Systemic lidocaine and mexiteline for the treatment of a patient with total ulcerative colitis

In basic research, neural modulation in patient with total ulcerative colitis mexiletine for the treatment of a systemic lidocaine and 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 (564;3925–30).

This would leave us where we started, lacking any significant evidence that neoadjuvant therapy improves survival for patients with oesophageal adenocarcinoma.

S R DeMeester

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Conflict of interest: None declared.

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, colesvelam hydrochloride (WelChol, Sankyo Pharmaceuticals Inc., Japan).

A 30 year old man presented with steatorrhoea, progressive weight loss, marked abdominal bloating, and lethargy. He underwent small bowel resection for ileal stricture. Clinical reports by Kemler and colleagues, changes in bile acid synthesis, to increase the conversion of ileal bile acids in the intestine which causes increased faecal bile acid secretion, reducing the enterohepatic circulation of bile acids. 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 colesvelam may be beneficial for patients with diarrhea who have undergone small bowel resection for chronic disease.

Colesvelam 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. 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 colesvelam may be beneficial for patients with diarrhea who have undergone small bowel resection for chronic disease.

Colesvelam 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.

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structure enables greater tolerability with less potential drug interactions than with resins.7

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.8 It is rarely associated with constipation, unlike cholestyramine.1 Colesevelam is non-absorbed and is excreted entirely via the gastrointestinal tract, preventing systemic side effects.9 Furthermore, there is little evidence for clinically significant interactions involving colesevelam.7 Pharmacokinetic studies with colesevelam have not shown clinically significant effects of absorption of six other coadministered drugs.8

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).7 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.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 IBIS 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.

Table 1

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


8th 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: +1 800 538 8222; fax: +1 414 273 5025; email: info@thepfisterhotel.com; web: http://www.iffgd.org/symposium2005.html).

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.

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).

REFERENCES


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