STOMACH

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

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Gut 2003;**52**:927–932

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Accepted for publication 12 December 2002

Background and aims: We tested if host gastric Lewis antigens and the *babA2* genotype of *Helicobacter pylori* correlated with clinicohistological outcome.
Methods: We enrolled 188 dyspeptic patients (45 with duodenal ulcer, 45 with gastric ulcer, and 98 with chronic gastritis) with *H pylori* infection, proved by culture and gastric histology, reviewed by the updated Sydney system. Gastric expression of Lewis (Le) antigens Le^a, Le^b, Le^x, and Le^y was determined immunochemically to determine intensity (range 0–3). The corresponding 188 *H pylori* isolates were screened for *babA2* genotype by polymerase chain reaction.
Results: All *H pylori* isolates had a positive *babA2* genotype. We identified Le^a in 33.5%, Le^b in 72.9%, Le^x in 86.2%, and Le^y in 97.4% of biopsies from these 188 patients. Patients who expressed Le^b had a higher *H pylori* density than those who did not express Le^b ($p<0.001$). Among 139 patients who expressed Le^b, *H pylori* density increased with a higher Le^b intensity ($p<0.05$). Gastric atrophy decreased with Le^b intensity and thus resulted in lower *H pylori* density in the antrum ($p<0.05$). For the 49 patients without gastric Le^b expression, *H pylori* density was positively related with Le^x and Le^a expression ($p<0.05$).
Conclusions: Taiwanese *H pylori* isolates are 100% *babA2* genopositive. Gastric Le^b as well as Le^x intensity may be major determinants of *H pylori* density. While lacking gastric Le^b expression, Le^x and Le^a were closely related to *H pylori* colonisation.

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

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

MATERIALS AND METHODS

Patients and study design

A total of 188 dyspeptic patients (112 men and 76 women; mean age 44.8 years) gave informed consent and were consecutively enrolled after they were proved to have *H pylori* infection, defined as a positive culture. None had a previous history of anti-*H pylori* therapy. Each patient had undergone panendoscopy to obtain a gastric biopsy for culture and histology of *H pylori* infection. The endoscopic diagnosis of these 188 study patients included uncomplicated chronic active gastritis (n=98), duodenal ulcer (n=45), and gastric ulcer (n=45).

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

PCR and primers for *babA2* genotypes

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

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

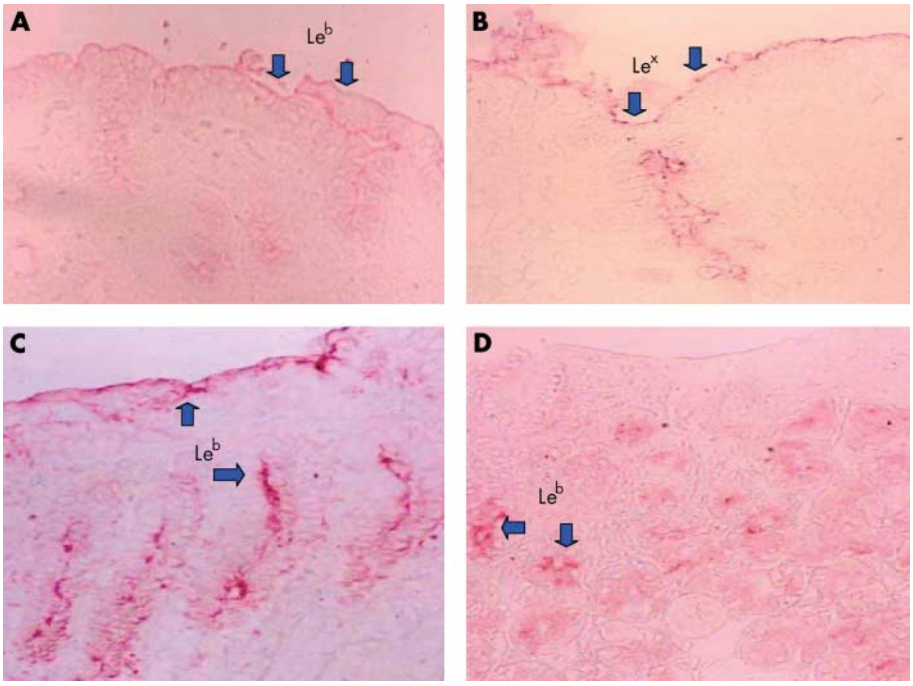

Figure 1 (A, B) Gastric immunohistochemical stains of Lewis antigen Le^b expression. (A) Positive staining over the surface epithelium only. (B) Diffuse staining over the intercryptal epithelium. (C, D) Gastric immunohistochemical stains of Le^x expression. (C) Positive staining over the surface epithelium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

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

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

Values are mean (SD).
*Significant difference by paired *t* test with two tailed analysis ($p<0.05$).
A, antrum; B, body; C, cardia.

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

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

AIS, acute inflammatory score; CIS, chronic inflammatory score; AT, antral atrophy; IM, intestinal metaplasia; HPD, total density of *H pylori*.
Significant difference ($p<0.05$): *between Le^a (+) and Le^a (–) patients; †between Le^b (+) and Le^b (–) patients; ‡between Le^x (+) and Le^x (–) patients; §between Le^y (+) and Le^y (–) patients.

TG, corresponding to bp 369 to 391 of AF033654) designed by Ilver and colleagues[9] 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 $MgCl_2$, and 1 unit of DyNAzyme II DNA polymerase (Finnzymes OY, Espoo, Finland). Amplification was carried out over 30 cycles consisting of 94°C for one minute, 45°C for one minute, and 72°C for one minute in a thermal cycler (Perkins-Elmer Cooperation, Norwalk, Connecticut, USA). The two primers achieved a 391 bp product (by primers designed by Ilver *et al*) and a 271 bp product (using the self designed primers in this study), respectively. The sequences of these two PCR products were

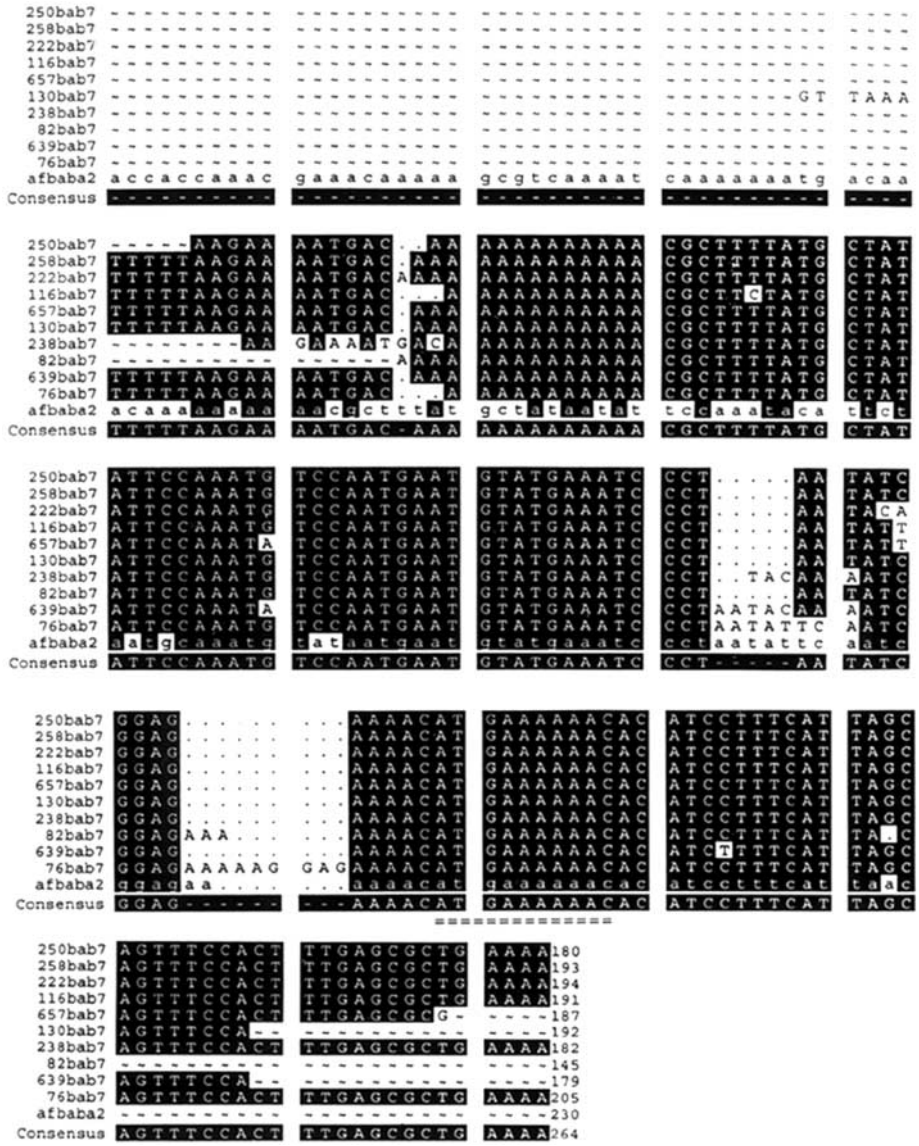

Figure 2 The nucleotide sequence of the 271 bp polymerase chain reaction product gained from the self designed *babA2* primers. The nucleotide sequence of the 10 randomly selected domestic strains (hp250, hp258, hp222, hp116, hp657, hp130, hp238, hp82, hp 639, and hp76) was confirmed to be *babA2* in origin with >90% homology to the published sequence of the *babA2* gene (afbabA2) of CCUG 17875. The 10 nucleotides (ATG AAA AAA C), representative of the *babA2* gene of *Helicobacter pylori*, are indicated by "=".

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

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

Analysis of *H pylori* related histology

The same pathologist, unaware of the endoscopic and culture results, analysed the gastric histology. *H pylori* density for each specimen was scored according to Yang and colleagues[17]: score 0, no bacteria; score 1, one or two small clusters with less than 10 bacteria; score 2, less than half the superficial crypt area with less than 10 bacteria in each crypt; score 3, less than half the area but with more than 10 bacteria, or more than half the area with less than 10 bacteria in each crypt; score 4, >10 bacteria in forvelae with some free area; and score 5, >10 bacteria without a free area. Total *H pylori* density (HPD) was defined as the sum of the densities from the three biopsy samples, obtained from the antrum, corpus, and cardia. Thus the HPD score ranged from 1 to 15. The acute inflammatory score (range 0–3), chronic inflammation score (range 0–3), atrophic change (absent, 0; present, 1), and intestinal metaplasia (IM) (absent, 0; presence, 1) were graded using the updated Sydney system.[18] The total acute (AIS) and chronic (CIS) inflammatory scores were also a sum of the three specimens (range 0–9).

Immunochemical staining for gastric Lewis expression

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

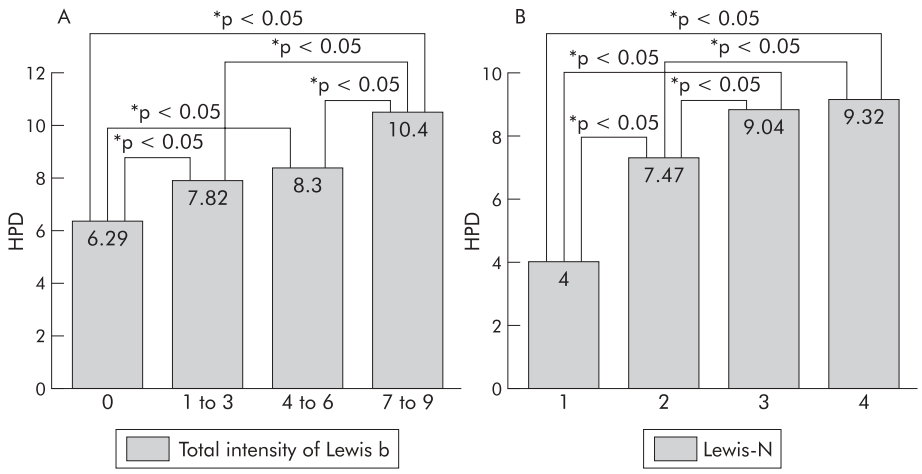

Figure 3 (A) Total *Helicobacter pylori* density (HPD) was positively correlated with an increase in Le^b intensity (*p<0.05, significant difference analysed using one way ANOVA). (B) Total Lewis number (Lewis-N) of each patient was also positively correlated with higher HPD in the stomach (one way ANOVA,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

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

Statistics

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

RESULTS

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

Fifty per cent (94/188) of *H pylori* isolates had a positive *babA2* genotype by PCR, applying the primers used by Ilver *et al* to obtain a band of 371 bp. However, the nucleotide sequence of this 391 bp PCR product from the Taiwanese isolates was confirmed as not being *babA2* in origin but had >90% homology with the published sequence of adenine specific DNA methyltransferase in *H pylori* 26695. To detect the *babA2* genotype for the domestic strains, we self designed a pair of primers and achieved a 271 bp PCR product whose nucleotide sequence was confirmed with >95% homology to the *babA2* gene of CCUG 17875 (fig 2). Based on PCR using these primers to obtain a 271 bp band, the prevalence rate of the *babA2* genotype was 100% in all 188 Taiwanese *H pylori* isolates. Western blotting also confirmed that the 30 randomly selected 271 bp genopositive strains had a uniformly positive phenotype.

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

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

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

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

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

Factors correlating with HPD in non-Le^b patients

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

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

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

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

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

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

Body+cardia, the summation of the data from the body and cardia; Total, the sum of antrum, body, and cardia,
*Significant difference between patients with (AT) and without (non-AT) antral atrophy, analysed by the two tailed Student's *t* test ($p<0.05$).

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

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

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

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

DISCUSSION

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

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

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

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

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

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

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

ACKNOWLEDGEMENTS

The study was supported by grants NHRI-EX91-9041SC from the National Health Research Institute, and NSC90-2320-B-006-048 and NSC90-2320-B-006-091 from the National Science Council, Taiwan. The authors also appreciate the assistance of Ms Hunt-Wei Wu, and the anti-BabA serum from Drs Thomas Boren and Stefan Odenbreit.

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