Lewis Antigen Expression and Stability in Helicobacter pylori Isolated from Serial Gastric Biopsies

David A. Rasko,† Trevor J. M. Wilson,† David Zopf,‡ and Diane E. Taylor†

The expression of Lewis antigens by the gastric pathogen Helicobacter pylori in serial biopsy isolates was investigated to assess antigen distribution and stability. A total of 26 asymptomatic subjects were given various doses of 3′ sialyllactose for up to 56 days. Gastric biopsies were performed during the dosing period, as well as 30 days after dosing, which provided 127 H. pylori isolates that were examined by use of ELISA and immunoblot. A large proportion of subjects (14/26) yielded sequential H. pylori isolates, which appeared genetically identical but had variable Lewis antigen expression. The proportion of subjects with H. pylori isolates not expressing any Lewis antigens was greater than that previously reported (10/26). Thus, the expression of the Lewis antigens by H. pylori does not appear to be a requirement for colonization, whereas the antigen expression after human infection is more variable than the previously reported rate observed with in vitro cultures.

Chronic infection with Helicobacter pylori is the most common cause of recurrent gastroduodenal inflammatory disease, including gastric and duodenal ulcers [1–3]. H. pylori is now recognized as one of the most ubiquitous infectious organisms, with >50% of the world’s population being infected [4]. A causative role for H. pylori in the development of both gastric adenocarcinoma [5, 6] and lymphoma of mucosa-associated lymphoid tissue [7] has been identified, leading to the assignment of a class 1 carcinogen status to this bacterium [8]. Extensive studies of this organism in recent years have yielded significant information concerning the infectious process, especially Lewis antigen expression and its role in the infectious process [9, 10].

Lipopolysaccharide (LPS) is an essential component of the gram-negative cell envelope. LPS plays a critical role in the structure and function of the outer membrane [11] and functions as a toxin. It has been shown that H. pylori LPS contains the Lewis antigenic structures [12–15]. The Lewis structures within the LPS are thought to provide a mechanism by which the H. pylori can evade the immune system by mimicking the Lewis antigens expressed on the gastric epithelium [16, 17]. Simoons-Smit et al. [18] have identified a small population of H. pylori isolates that did not express Lewis antigens as a component of the LPS yet still caused infection. It has been recently shown that multiple isolates obtained from a single gastric biopsy can yield different LPS profiles [19, 20], which indicates that the LPS antigen expression is variable after in vivo growth.

When grown in vitro, the rate of phase variation of the Lewis antigens in H. pylori has been showed to be 0.2%–0.5% [21]. The fucosyltransferase (fucT) genes are responsible for the addition of fucose to the carbohydrate backbone of the O side chain, which is the final step in Lewis antigen production [22, 23]. A proposed mechanism of antigenic variation has recently been shown to involve slip-strand mispairing during replication at the polynucleotide tracts contained in the fucT genes [24]. A mechanism that can overcome the deleterious effects of the slip strand mispairing has recently been identified [25]. The identification of a translational frameshift cassette in the α(1,2) fucT gene of H. pylori allows the production of functional protein from a truncated open-reading frame containing a stop codon caused by slip-strand mispairing [25]. A definite biological role for antigenic variation of the Lewis antigens has not yet been identified, although the antigenic variation in other organisms allows increased persistence and/or pathogenicity [17].

It was previously shown that 3′ sialyllactose (3SL) was an epithelial cell surface receptor for H. pylori [26, 27]. A further study provided direct evidence that the free oligosaccharide, 3′SL, could inhibit H. pylori adhesion to tissue culture cells in vitro [28]. Recently, a human clinical trial was undertaken to

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All subjects involved in this study provided informed consent before participating.

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evaluate the safety and efficacy of orally administered 3SL on gastric *H. pylori* colonization in *H. pylori*-positive asymptomatic adults. In this report we describe the variability of *H. pylori* Lewis antigens among genetically identical serial *H. pylori* isolates obtained during the 86-day sampling period.

**Materials and Methods**

*Study subjects.* The study subjects were nonrelated, asymptomatic adults (ages 22–65 years) who scored as *H. pylori*-positive on a 13C-urea breath test (Meretek, Houston) at screening and were shown by subsequent exploratory endoscopy to be free of gastric or duodenal ulcer disease. Two biopsies taken at endoscopy—one from the antrum and the other from the greater curvature of the corpus—were submitted for histologic analysis and culture. The subjects were enrolled in the study if either test result was positive. Demographic characteristics of the 26 study subjects are shown in table 1. Subjects were randomized to be mock treated, receiving microcrystalline cellulose as a placebo (6 subjects), or 3 g SL 4 times per day for 28 days. The remaining 7 subjects received 5 g of 3SL 4 times per day for 28 days.

Subjects in the 56-day dosing groups were monitored with urea breath tests on study days 0, 14, 27, 42, 55, and 86 (30 days after treatment), and endoscopic biopsies were performed on days 0, 28, 56, and 86. Subjects in the 28-day dosing group were monitored with urea breath tests on days 0, 14, 27, and 55 and with endoscopic biopsies on days 0, 28, and 56. Incomplete data are included from 1 subject in the 5-g dosing group who withdrew for personal reasons but returned to the clinic for a follow-up endoscopic biopsy. Biopsy samples taken from the 2 distant sites within the stomach were sent to the University of Alberta for *H. pylori* culture.

*H. pylori isolation.* Strains were cultured by standard methods described by Taylor et al. [29]. Biopsy samples were plated out on brain heart infusion agar plates (BHI-YE agar) containing 0.5% yeast extract, 15 µg/mL of both vancomycin and amphotericin B, as well as 50 µL/F of fetal bovine serum. These plates were incubated at 37°C under microaerobic conditions for 2–4 days. Positive *H. pylori* cultures were confirmed by urease test and light microscopy. A total of 127 culture-positive biopsy samples were identified from the 194 samples obtained.

**ELISA for Lewis antigens.** Isolated *H. pylori* were examined for Lewis antigenic expression patterns. The conditions for the ELISA were described elsewhere [15, 30]. The primary antibodies used were anti-Lea (monoclonal antibody [MAb] BG-5, clone T174), anti-Leb (MAb BG-6, clone T218), anti-Lee (MAb BG-7, clone P12), anti-Le’ (MAb BG-8, clone F3), and anti-sialyl Le’ (MAb BG-9) from Signet Laboratories (Dedham, MA). The primary antibodies were diluted 1 : 100, whereas the secondary antibody, goat anti-mouse IgG + IgM conjugated to horseradish peroxidase (HRP; Biocan #115 035 068, Mississauga, Ontario, Canada), was diluted 1 : 2000. The absorbance was recorded at 405 nm by use of a Titerekt Multiscan MC (Helsinki, Finland) microtiter plate reader. Absorbance values are an average of triplicate wells with blanks subtracted. Values <0.1 absorbance units were considered negative. Strains found not to express Lewis antigens were assayed on 2 separate occasions to confirm their status.

### Table 1. Characteristics of study subject population.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Male</th>
<th>Female</th>
</tr>
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<tbody>
<tr>
<td>No.</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>Mean age, years (range)</td>
<td>45 (22–65)</td>
<td>51 (35–65)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Black</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>White</td>
<td>6</td>
<td>7</td>
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**Statistical analysis.** Statistical analysis was done by use of Fisher’s exact test to compare the significance of proportions. A probability value of *P* < 0.05 was considered significant.

**Polyacrylamide gel electrophoresis and immunoblots.** Whole-cell extracts of the *H. pylori* strains were treated with proteinase K and processed as described by Hitchcock and Brown [31]. For the analysis of the LPS, a 15% polyacrylamide separating gel containing urea and a 5% polyacrylamide stacking gel was used. Electrophoresis was conducted with a constant current of 35 mA for 1 h. These gels were either stained with zinimidazole, according to the method of Hardy et al. [32], or transferred to nitrocellulose membrane (Micron Separations, Inc., Westboro, MA; pore size, 0.22 µm) according to the methods described by Towbin et al. [33]. Nitrocellulose membranes, with transferred LPS, were probed with the primary antibodies described earlier, diluted 1 : 500, and bound antibodies detected with goat anti-mouse antibodies conjugated to HRP. Blots were developed by use of an enhanced chemiluminescence kit (Amersham Pharmacia Biotech, Baie d’Urfé, Quebec, Canada) according to the manufacturer’s specifications, and images were visualized on BioMax BM film (Kodak, Rochester NY).

**Genomic analysis.** Genomic DNA was isolated from *H. pylori* strains by the method of Ge and Taylor [34]. A Perkins-Elmer DNA Thermal Cycler 480 was used for 35 cycles of amplification under the following conditions: 95°C for 1 min, 36°C for 1 min, and 72°C for 2 min. The reaction conditions used were previously described by Taylor et al. [35]. The 4 random primers used were: primer 1, 5’-AAGAAGCCGCT; primer 2, 5’-C CGACGCCAA, primer 3, 5’-AACGGCCAAC, and primer 4, 5’-GCGATTCCCCA. These primers were originally developed by Akopyants et al. [36]. Polymerase chain reaction (PCR) products were subjected to electrophoresis on 1% agarose gels with 1× Tris acetate running buffer and photographed under ultraviolet light [37]. Isolates with no more than 1 band difference were considered to be identical.

### Results

*Lewis antigen expression of *H. pylori* isolates.* *H. pylori* strains were isolated from 127 endoscopic biopsy samples. Isolates were limited to a single passage on BHI-YE agar, and then the LPS expression was maximized by passage in liquid media before Lewis antigen status was determined [38, 39]. The Lewis antigens expressed by each *H. pylori* clinical isolate were determined by ELISA (table 2). Lea was expressed by 69.3% (88/127) of isolates and was the only Lewis antigen expressed by 31.5% (40/127). Leb was produced by 40.2% (51/127) of isolates and was the only Lewis antigen expressed by 2.4% (3/127). No Lewis antigen could be identified on 28.3% (36/127).
of *H. pylori* isolates. No isolate was identified that expressed Le©, Le©, or sialyl-Le©.

The distribution of Lewis antigen expression by *H. pylori* changes slightly when examined on a per subject basis. The Le© antigen was detected on at least 1 serial isolate from 73.1% (19/26) of subjects and was the only antigen detected in isolates from 7.7% (2/26) of subjects. Le© was present on at least 1 of the serial isolates from 61.5% (16/26) of subjects, but no subject produced isolates expressing Le© exclusively. At least 1 serial isolate with no detectable Lewis antigens was obtained from 38.5% (10/26) of the study subjects, and 23% (6/26) of subjects produced isolates that were consistently negative for Lewis antigen expression.

All the subjects in this study were infected with *H. pylori* at pretreatment screening, as determined by positive urea breath test plus histology or culture of endoscopic biopsy samples, and remained positive at the end of the treatment period and 30-day posttreatment evaluation period. A formal report of clinical results will be presented elsewhere.

**Stability of Lewis antigen expression.** A switch in antigen expression is defined as an alteration of the Lewis antigen expression pattern (i.e., a change from Le© alone to Le© and Le©) of the *H. pylori* isolate when compared with a previous isolate from the same subject at the same gastric site—that is, corpus or antrum, as measured by ELISA. In our study population, Lewis antigen expression frequently differed between *H. pylori* isolates cultured from the 2 distant biopsy sites, but no trend was observed between the expression of any 1 antigen and the gastric location. We also did not detect a bias toward expression of specific Lewis antigens by *H. pylori* with respect to sex, age, or ethnic origin of subjects from which the organisms were isolated. We did observe single subjects expressing the Le© antigen exclusively in both the 1-g and 5-g dosing groups, whereas the simultaneous expression of the Le© and Le© antigens was observed in isolates from single subjects in the placebo, 2-g, and 5-g groups. Isolates from 2 subjects in the placebo group, 3 subjects in the 1-g group, and a single subject in the 2-g group were consistently negative for the Lewis antigens tested. There were 3 subjects with isolates containing switches in the placebo group, 2 subjects in the 1-g group, and 5 subjects with this phenotype in the 2-g and 5-g groups. Although we observe an increase in the number of strains switching antigens with the increased 3SL dose, no statistically significant trend could be identified (Fisher’s exact test, *P > .3*).

**Immunoblot analysis of Lewis antigen expression.** Immunoblots of proteinase K–treated whole cell lysates were done for all *H. pylori* strains that had undergone an antigenic switch. The staining patterns confirmed the results of the Lewis antigen expression profile obtained by ELISA and revealed gel mobility changes of the *H. pylori* LPS in serial isolates that had undergone an antigenic switch (figure 1). One subject’s isolates show the most extreme example of this variation (figure 1, lanes 1–3). The first isolate from this subject does not express an O side chain, and thus it does not have the potential to express the Lewis antigens (figure 1, lane 1). The second isolate from this subject expresses an O side chain that contains Le© (figure 1, lane 2), whereas the final isolate from this subject had an O side chain that expressed both Le© and Le© (figure 1, lane 3). Most of the switches (11/15) involved a variation of the Lewis status of the isolate and not a switch to non-Lewis antigen production. The Lewis status switches also show some minor changes in the gel mobility of the LPS (figure 1, lanes 4 and 5). Similar LPS gel mobility shifts were observed on immunoblots of strains from all 15 subjects in which an antigenic switch occurred (data not shown). No Le©, Le©, or sialyl-Le© was detected on any isolate by immunoblot.

**Analysis of nontypable *H. pylori* strains.** Ten (38.5%) of the 26 subjects yielded at least 1 *H. pylori* isolate during the sampling period, which did not express any of the Lewis antigens included in the screening assay. These isolates were designated nontypable *H. pylori* (NTHp). A greater proportion of NTHp isolates, within this study population, was identified when compared with previous reports [18, 30]. A NTHp *H. pylori* LPS sample from each of the 10 subjects is shown in figure 2. The results of the immunoblots (not shown) confirmed the results obtained by ELISA, that the LPS from these isolates do not contain any Lewis antigens. All NTHp strains investigated, except 1 (figure 2, lane 5), expressed LPS containing an O side chain, which may contain alternate carbohydrate structures or precursors to Lewis antigens. It should be noted that not all of the LPS examined from the isolates designated NTHp were of the same mobility, indicating that, although grouped together in this and other studies, the LPS from these isolates most likely varies in carbohydrate composition.

**Genomic analysis of *H. pylori* isolates.** To investigate the possibility that individual subjects may have been colonized by more than 1 *H. pylori* strain and that the presence of a mixture of *H. pylori* strains in the biopsy samples could explain the changes in Lewis antigen expression, we performed genomic analyses on the serial isolates obtained. We observed an antigenic switch in *H. pylori* serial isolates from 15 (57.7%) of 26 subjects. Genomic DNA was isolated from all *H. pylori* serial isolates from any subject expressing an antigenic switch—a total of 78 *H. pylori* DNA samples. In addition, DNA samples were obtained from several randomly chosen isolates in which ex-
expression of LPS antigens (Le^a, Le^e, or NTHp) were stable over the sampling period. The DNA from these strains was investigated by randomly amplified polymorphic DNA (RAPD) PCR. The RAPD-PCR pattern obtained from 14 of the 15 sets of H. pylori serial isolates that exhibited an antigenic switch, and all serial isolates with stable LPS antigen expression showed that each group of serial isolates was genetically identical, irrespective of gastric site of isolation (an example is shown in figure 3B). In addition, each group of serial isolates produced a RAPD-PCR pattern that was distinct from the RAPD-PCR patterns of all other isolates from different subjects (data not shown). Only 1 subject had evidence for coccolonization with more than 1 H. pylori strain, because the RAPD-PCR pattern between isolates was different (figure 3A). These observations imply that each subject, with 1 exception, was colonized throughout the entire gastric mucosa with a genetically distinct H. pylori strain, each with the potential to express variable LPS antigens.

**Discussion**

In the present study, 3' SL was administered to H. pylori-positive asymptomatic subjects to evaluate safety, tolerance, and efficacy of the drug when administered over a range of doses and by use of a variety of regimens. Two potentially significant differences must be noted when comparing this study with previous studies that examined the expression of Lewis antigens. First, the subjects involved in this study were asymptomatic, which provided an opportunity to identify possible alterations in the distribution of H. pylori Lewis antigens among this population. Secondly, in contrast to most previous studies, where H. pylori clinical isolates are obtained from a single endoscopic biopsy per subject, this study provided an opportunity to examine multiple biopsy isolates obtained over a short period of time (86 days) from 2 distinct gastric sites.

When examining the H. pylori isolates obtained from the endoscopic biopsies, we noted an unexpectedly high rate of switching of Lewis antigen expression. This antigenic switch occurred in 57.7% (15/26) of the subjects investigated. The effect was evident in both placebo and dosing groups, and no statistically significant difference could be identified (P > 3). Coccolonization with 2 genetically distinct H. pylori strains was observed in only 1 of 26 study subjects as determined by RAPD-PCR. The 2 distinct isolates were present at an early time point.

![Figure 1](image1.png)

**Figure 1.** Zinc-imidazole stain and immunoblot of proteinase K-digested whole Helicobacter pylori cell lysates. Lanes 1–3 are samples from H. pylori isolates of one subject, whereas lanes 4 and 5 are from another, and lane 6 contains strain 26695, which expresses Le^a and Le^e [25]. A. Zinc-imidazole stained gel of the lipopolysaccharide from the H. pylori isolates. B. Immunoblot of the same samples probed with anti-Le^e monoclonal antibody (MAb). C. Immunoblot of the same samples probed with anti-Le^a MAb.

![Figure 2](image2.png)

**Figure 2.** Zinc-imidazole-stained 15% polyacrylamide gel of proteinase K-treated whole cell lysates, showing 1 Helicobacter pylori sample from each of the subjects with a non-Lewis antigen expressing strain. All samples except 1 (lane 5) express smooth O side chains.
is more commonly reported to be the phase-variation rate of the LPS in most other microorganisms switching at rates as high as 12% ± 16% [40, 41], whereas the studies of Appelmelk et al. [21] were operative, one could have expected to observe only a single antigenic switch among the isolates obtained from the 26 subjects sampled in this study, rather than the 15 switches (57.7%) that were actually observed.

Interestingly, 2 groups have recently showed differences in LPS phenotype depending on which primary colony of Helicobacter pylori isolated from 2 subjects. A. RAPD-PCR samples from 3 isolates of a single subject demonstrating a mixed infection. B. RAPD-PCR samples from 4 isolates of another subject. The serial isolates appear to be identical and are representative of the pattern observed for the serial isolates of other subjects.

Figure 3. Randomly amplified polymorphic DNA (RAPD) polymerase chain reaction (PCR) samples of Helicobacter pylori strains isolated from 2 subjects. A. RAPD-PCR samples from 3 isolates of a single subject demonstrating a mixed infection. B. RAPD-PCR samples from 4 isolates of another subject. The serial isolates appear to be identical and are representative of the pattern observed for the serial isolates of other subjects.

(day 0), but only 1 of these strains could be isolated later in the study (day 86). It is not apparent whether 1 strain emerged as a consequence of natural competition between the H. pylori isolates or whether 1 strain may have been eliminated because of the effect of the carbohydrate treatment. In any case, results from analysis of genomic DNA from serial H. pylori isolates indicate that a large proportion of H. pylori strains may switch LPS phenotype during the course of infection while maintaining the same genetic profile. Some bacterial species have been shown to undergo antigenic switching at rates as high as 12%–16% [40, 41], whereas the phase-variation rate of the LPS in most other microorganisms is more commonly reported to be ~1% [42]. Appelmelk et al. have shown that lab-adapted isolates of H. pylori can alter their Lewis antigen phenotype at a rate of 0.2%–0.5% [21]. It should be noted that this rate was measured with a laboratory-adapted culture and may not accurately depict what occurs after growth in vivo. In fact, if the phenotypic switch frequency calculated by Appelmelk et al. [21] were operative, one could have expected to observe only a single antigenic switch among the isolates obtained from the 26 subjects sampled in this study, rather than the 15 switches (57.7%) that were actually observed. Interestingly, 2 groups have recently showed differences in LPS phenotype depending on which primary colony of H. pylori was chosen for investigation, indicating variability of recently isolated H. pylori [19, 20]. Both groups noted that the gel mobility, staining characteristics [19], and Lewis antigen expression [20] of LPS obtained from different H. pylori isolates from same gastric biopsy sample were variable. Also, Janvier et al. [43] have shown that the LPS profile of the Sydney strain (SS1), adapted for mouse colonization, was altered by passage through mice. All of the mentioned studies characterized LPS from organisms cultured in vitro after growth in vivo. We may have identified a similar phenomenon by isolating multiple “variants” of the same strain over a period of time rather than from a single primary culture plate. Phase variation appears to be a common mechanism used by all H. pylori during colonization or under certain conditions, and it appears as though these in vivo conditions or signals are not duplicated by in vitro growth conditions.

The fucosyltransferase (fucT) genes encode enzymes responsible for the final steps in the production of Lewis antigens in H. pylori. All fucT genes contain polynucleotide tracts and imperfect repeats of different size and sequence, depending on the copy of the gene and strain from which it is isolated [9, 44, 45]. It has recently been shown by Appelmelk et al. [24] that the phase variation of some H. pylori Lewis antigens can be directly attributed to slip-strand mispairing at fucT polynucleotide tracts. A recent study by Wang et al. [25] has also identified a translational frameshift mechanism that can overcome apparent stop codons, in the α(1,2)fucT gene, caused by slip-strand mispairing, to produce functional protein. This mechanism prevents the accurate determination of the status of the α(1,2)FucT enzyme activity directly from the nucleotide sequence, because a sequence that does not contain a full-length open-reading frame may still produce functional protein. Thus, in these studies, we have not assessed glycosyltransferase activity by sequence analysis but instead by measuring the end product of these enzymes, the Lewis antigens. The strains that express the Lewis antigens must contain functional levels of glycosyltransferases that synthesize the LPS core structures, as well as the FucT proteins that form the Le′ and Le′ antigens. In the strains where the Lewis antigen expression is altered, we infer that the FucT enzyme levels are also altered. The increased rate of antigenic variation observed after in vivo passage suggests that control of the H. pylori fucT genes may not be entirely under slip-strand mispairing control but may also be subject to control by environmental signal(s) and/or conditions that contribute to the variability of the Lewis antigens.

The present study identified a larger proportion of NTHp and a smaller proportion of H. pylori isolates expressing Le′ than has been identified elsewhere [18, 30]. In a study by Wirth et al. [30], the incidence of strains expressing Le′ was 70%, and the incidence of Le′ expressed as the only antigen was 19%. Similarly, Simoons-Smit et al. [18] found that Le′ was expressed by 77.1% of isolates and was solely expressed by 9.9%. In contrast, in the present study, we observed that 40.2% of isolates express Le′, and it is exclusively expressed by only 2.4% of
isolates. If a subject’s set of isolates is treated as a single isolate and categorized as Le^ or Le^ and/or NTHp, as if there was only a single biopsy, a difference in Le^ expression is still evident, with only 61.5% of the subjects yielding at least 1 isolate that expressed Le^; none of the subjects produced serial isolates that exclusively expressed the Le^ antigen. The overall population expression value is more similar to studies described earlier for the of Le^ antigen, but the proportion of isolates that expressed Le^ exclusively is still decreased.

There were 28.3% (36/127) of H. pylori isolates that were designated NTHp in this study, whereas 38.5% (10/26) of subjects yielded at least 1 NTHp isolate, and 23.0% (6/26) of the subjects harbored H. pylori isolates that expressed only this phenotype. Simoons-Smit et al. [18] reported a NTHp prevalence of 15.1% and also noted that most of the nontypable isolates were from subjects of Chinese origin. The subjects that harbored the NTHp strains in our study did not belong to any single ethnic, sex, or age group.

The relatively high frequency of NTHp strains observed in this study calls into question the biological importance of the Lewis antigens in the H. pylori LPS during infection, because these antigens are thought to be beneficial for evading the immune system by molecular mimicry [10, 46]. Clinical prevalence and persistence of NTHp strains show that there is no absolute requirement for LPS to contain Lewis antigens during human infection. In fact, recent work by Claeyss et al. [47] and Amano et al. [48] has shown that the role of Lewis antigens in H. pylori infection may not be critical. Both studies show that autoimmune antibodies produced during H. pylori infection are not directed toward the Lewis antigens but toward the core LPS oligosaccharide. It is possible that the pathobiology of H. pylori infection in asymptomatic individuals differs from that in symptomatic patients. If so, the increased NTHp prevalence and even the decrease in the number of strains expressing Le^ observed in this study may represent adaptations by H. pylori to the host in whom disease symptoms do not develop. Conversely, the Lewis phenotypes may be characteristic of H. pylori that colonize patients who become symptomatic. A thorough understanding of the incidence and biological significance of phenotypic switching of Lewis antigens in various human hosts awaits further study.

In summary, our results indicate that the rate of change of expression of LPS antigens after growth in human subjects appears to be higher than was estimated from previous studies [21] and clinical reports [18, 30]. The increased rate of switching of Lewis antigens and NTHp that was observed does not appear to be an effect of drug treatment but may be the result of exposure to as-yet-undefined environmental signal(s) encountered during human infection. This study provides the first definite evidence that a single strain of H. pylori may alter its LPS antigenic phenotype during the course of infection.

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