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Host Lewis phenotype-dependent *Helicobacter pylori* Lewis antigen expression in rhesus monkeys

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Abstract

Both human and *H. pylori* populations are polymorphic for the expression of Lewis antigens. Using an experimental *H. pylori* challenge of rhesus monkeys of differing Lewis phenotypes, we aimed to determine whether *H. pylori* populations adapt their Lewis phenotypes to those of their hosts. After inoculation of four monkeys with a mixture of seven strains identified by RAPD-polymerase chain reaction, *H. pylori* Lewis expression was followed in 86 isolates obtained over 40 wk. Host Lewis^{a/b} secretion status was characterized by immunological assays. Fingerprints of the predominating strain (J166) were identical in all four animals after 40 wk, but its Lewis phenotype had substantial variability in individual hosts. At 40 wk, J166 populations from two Lewis^{a-b+} animals predominantly expressed Lewis^y. In contrast, J166 populations had switched to a Lewis^x dominant phenotype in the two Lewis^{a+b-} animals; a frame shift in *futC*, regulating conversion of Lewis^x to Lewis^y, accounted for the phenotypic switch. The results indicate that individual cells in *H. pylori* populations can change Lewis phenotypes during long-term colonization of natural hosts to resemble those of their hosts, providing evidence for host selection for bacterial phenotypes.

Keywords

bacterial pathogenesis; evolution; genetics; colonization; phase variation

GASTRIC COLONIZATION of humans with *Helicobacter pylori* increases the risk for development of gastroduodenal ulcers, gastric adenocarcinoma, and MALT lymphoma, but *H. pylori* persists in most hosts for decades without clinical consequences (1). Acquisition of *H. pylori* is common, especially in developing countries, despite human diversity, and persistence occurs despite host responses to *H. pylori* and physiological changes with age in the gastric milieu, but the bases for persistence are not well understood (2).

Humans express both monofucosylated (including Le^a and Le^x) and difucosylated (Le^b and Le^y) Lewis antigens on surfaces of many cell types, including erythrocytes and gastric epithelial

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cells, as well as on the highly glycosylated proteins (mucins) comprising the mucus layer (3-5). *H. pylori* cells may express BabA and SabA that specifically adhere to the host Le^b and sialyl-Le^x antigens, respectively (6-7), and *H. pylori* colonization is associated with increased sialyl-Le^x expression (7). Importantly, humans are polymorphic for the individual Le antigens expressed (5,8-10). Conversely, ~90% of *H. pylori* isolates express human-type Le antigens in their LPS (11-15), preferentially, the type 2 antigens (Le^x and Le^y) (16,17), but type 1 expression (Le^a and Le^b) also has been noted (17-19). Although initial attention examined the role of these *H. pylori* antigens in autoimmunity (20,21), an alternative, but not exclusive, possibility is that the Le antigens expressed contribute to the adaptation of *H. pylori* to individual hosts (22). *H. pylori* strains are highly diverse (23-25), in part, reflecting continued evolution during persistent colonization of individual hosts (26-30). Even within a single gastric biopsy, the *H. pylori* cells present can show extensive diversity in Lewis expression, due to the existence of antigenic variants within single clones (31). Studies of the variability of *H. pylori* LPS *in vivo* (31-33) and *in vitro* (34,35) show that *H. pylori* Lewis expression is complex, polygenic, and dynamic (36-41) and represents an incompletely understood phenotype (38,42).

Comparing human host Le^{a/b} with *H. pylori* Le^{x/y} phenotypes, we proposed that *H. pylori* populations adapt their Le expression to that of the host Le phenotype (22). That hypothesis was challenged in two other clinical reports (43,44). However, the studies addressing this question (43,44) were not directly comparable to the earlier report (22), because of differences in definitions, sample size, study populations, strain collections, assays used, and validation (22,43,44). Nevertheless, Heneghan *et al.* (44) found that the mean *H. pylori* Le^y optical density (OD) value was significantly ($P < 0.001$) greater than the Le^x value in patients of the Le^{a-b+} erythrocyte phenotype, which is directly consistent with our hypothesis.

Monkeys are human-like in polymorphisms for, and expression of, Lewis antigens (45,46), and gastric colonization by *H. pylori* is highly similar in the two primate species (47-50). When monkeys were challenged with mixtures of *H. pylori* strains isolated from humans, one or a few strains eventually predominated (47). Changes in bacterial populations have been shown to be accompanied by variation in host gastric Lewis expression, differing in Le^{a+b+} and Le^{a-b-} monkeys; thus, host Le phenotype appears dynamic in response to *H. pylori* (51). As such, experimental challenge of rhesus monkeys allows a direct test of the hypothesis that after *in vivo* inoculation, *H. pylori* Le expression changes to adapt to that of the host.

MATERIALS AND METHODS

Animals and inoculation

All animal experiments had been approved by the Armed Forces Radiobiology Research Institute Institutional Animal Care and Use Committee and monitored and reapproved at yearly intervals. All experiments were conducted according to the principles set forth in the *Guide for the Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, National Research Council, (Washington DC: National Academy Press, 1996).

From 13 male rhesus monkeys (*Macaca mulatta*) studied (age 4 to 13 yr), four [A: (E6C), B: (85D08), C: (82A49), and D: (8PZ)] were subjected to an inoculation study, as described (49,52). In brief, they had been cleared of *H. pylori* by antimicrobial therapy and had remained negative for *H. pylori* for 6 mo. They then were inoculated with a mixture of seven different human *H. pylori* strains (J166, J170, J178, J238, J254, J258, J282). Each strain represented a sweep of the colonies from the primary isolation plate from a human clinical specimen; thus each strain represented a heterogeneous group of clonal variants (26,31). After esophagogastroduodenoscopy, a mixture of the seven *H. pylori* strains was sprayed onto the antral mucosa, as described (47). Re-endoscopies with mucosal biopsies for culture and

histology were performed at 1, 8, 14, and 40 wk after inoculation (52). Saliva and gastric juice samples were collected from each animal for determination of soluble Le^a or Le^b antigen (see *Antibodies*).

Growth conditions and characterization of isolates

At the time of each endoscopy, biopsies were immediately placed in 0.1 ml of iced sterile 0.9% NaCl on ice. Within a maximum of 3 h, they were homogenized, and an aliquot was streaked on *Campylobacter* chocolate agar plates supplemented with TVPA (5 µg/ml trimethoprim, 10 µg/ml vancomycin, 5 µg/ml amphotericin B and 10 U/ml polymyxin B; Remel, Lenexa, KS) and incubated at 37°C in an atmosphere of 90% N₂, 5% O₂, and 5% CO₂ (52). *H. pylori* isolates from monkeys were identified as forming pinhead-sized colonies with urease, oxidase, and catalase activity, and by microscopy as Gram-negative, curved rods, as described (52). Single colony-derived bacterial populations of the isolates, as well as the inoculated strains then were cultured on Trypticase soy agar/5% sheep blood plates (BBL Microbiology Systems, Cockeysville, MD), as described (49), and used for determination of Le expression heterogeneity as described below. Chromosomal DNA was extracted for use in DNA fingerprinting by random amplified polymorphic DNA (RAPD)–polymerase chain reaction with 10 nucleotide (nt) primers 1247, 1254, 1281, and 1283, as described (31,53).

Antibodies

Murine monoclonal anti-Le^a and anti-Le^b and Reagent Le^{a+b-} or Le^{a-b+} red blood cells (Ortho Diagnostic Systems, Raritan, NJ) were used for monkey saliva and gastric juice hemagglutination inhibition assays. Monoclonal antibodies T174, T218, P-12, CSLEX1, or F3 with specificity for Le^a, Le^b, Le^x, sialyl-Le^x, or Le^y antigen (Signet Laboratories, Dedham, MA) were used for determination of Le expression of *H. pylori* cells.

Detection of Lewis antigens in secretions

Soluble Lewis antigens in saliva and gastric juice were detected by hemagglutination inhibition assays using standard conditions (54). Samples were boiled for 10 min and centrifuged; supernatants were then preserved at -20°C before use. Saliva and gastric juice from humans of known erythrocyte Le^{a+b-} or Le^{a-b+} phenotype (19) were used as controls.

Determination of *H. pylori* Lewis antigens

Expression of Lewis antigens on whole *H. pylori* cells was determined by enzyme-linked immunosorbent assays, as described (17). In brief, microtiter plates were coated with *H. pylori* cells grown for 48 h and subsequently incubated with the above monoclonal antibodies, then goat antimouse IgM or IgG antibodies coupled to alkaline phosphatase (Sigma), and then with p-nitrophenylphosphate, as substrate. The results were expressed as OD at 410 nm × 1000 U (ODU), and controls were included as described (17,22). ODU values of Le expression were expressed as mean and sd.

Glycosyltransferase genotyping of *H. pylori* isolates

Genetic analyses of selected *H. pylori* isolates of specified Lewis phenotypes was done based on sequences of galactosyl and fucosyltransferases, as reported for genomic strains 26695 and J99 (24,38). In brief, genomic DNA was prepared, polymerase chain reaction (PCR) was performed using primers flanking each of the five relevant ORFs (Table 1), and nucleotide sequences of the PCR products determined on both strands.

Statistics

Statistical comparisons were done with Student's *t* test. Two-tailed *P* values < 0.05 were considered statistically significant.

RESULTS

Lewis determination in host secretions

We first assessed whether the four monkeys used in the present colonization studies, as well as additional animals in the AFRR colony, differed in the pattern of Le antigens on their erythrocytes and in their secretions. Erythrocyte typing for Le^a or Le^b by direct hemagglutination, as done for humans (22) was not useful; 12 of 13 animals tested (including animals A and D) were negative for both determinants. These results agree with findings in *M. mulatta* (W. Socha, personal communication), indicating that direct hemagglutination does not allow detection of rhesus monkey erythrocyte Le antigens or ABO-blood group antigens (55). We then tested for Le^a or Le^b antigens in saliva and gastric juice by a hemagglutination inhibition method (45). Five animals (including A and B) were Le^{a+b-}, and three animals (including C and D) were Le^{a-b+} in saliva and gastric juice, and none were positive or negative for both. Therefore, test monkeys A and B were classified as Le^{a+b-}, and C and D were classified as Le^{a-b+}.

DNA-fingerprinting of *H. pylori* strains

RAPD-PCR had been performed on the seven *H. pylori* strains used for monkey inoculation to allow identification of recovered subsequent isolates, primarily using primers 1247 and 1281 (49). In pilot studies of *H. pylori* strain G1.1, we found virtually identical DNA-fingerprinting patterns using primers 1247 and 1281 after 70 *in vitro* passages, corresponding to ~5 mo *in vitro* culture, and after 6 mo of colonization in Mongolian gerbils (31,56), illustrating that RAPD-PCR provides accurate strain identification. The strain types revealed through DNA-fingerprinting of the 86 isolates recovered at one, 8, 14, or 40 wk postinoculation have been described (49), and are summarized in aggregate (Fig. 1). Strain identification using RAPD-PCR was unequivocal in all cases and was consistent with a lack of major genetic changes during the 40 wk of colonization. In total, four (J166, J170, J238, and J258) of the original seven inoculated strains were recovered on different occasions. Although up to four different strains were isolated from each animal at the week-one sampling, the tendency to detect several strains decreased with time, and the relative proportions of the strains changed substantially (Fig. 1). Although in the short term, strain J238 was predominant in the mixed bacterial populations, eventually all strains in each animal were out-competed by J166.

Lewis determination of inoculated *H. pylori* strains

We then examined populations of the seven *H. pylori* input strains to determine whether the mixture contained a broad spectrum of Le expression. None of the strains reacted with monoclonal antibodies to Le^a, Le^b, or sialyl-Le^x. For each strain, the population inoculated expressed either or both Le^x and Le^y, in the following mean Le^x/Le^y ratios in ODU: J166, 268/2016; J170, 60/2278; J178, 133/1542; J238, 112/425; J254, 767/654; J258, 1818/308; and J282, 23/532. In total, the mixture contained phenotypically varied strains, including those with strong expression of Le^x or Le^y with little or no expression of the other, or strong expression of both. Therefore, use of this mixture could permit testing the hypothesis that host phenotypes select for *H. pylori* Le expression (22). Because of the considerable changes in Le expression of the recovered 4 strains, J166, J170, J238, and J258, during the first few weeks after challenge (see below), we also analyzed their Le expression diversity preinoculation. Since Le expression of *H. pylori* cells from a single biopsy often is diverse (31) and because these strains originally had been picked as sweeps from primary cultures of human gastric

biopsies, we hypothesized that these minimally *in vitro* passaged strains were substantially diverse before the monkey inoculation. Le determination of 8 single colony-derived populations of each strain confirmed considerable diversity (data not shown). In particular, expression of both Le^x and Le^y varied in the sample of preinoculation strain J166 (Fig. 2A), which indicated a preexisting pool of variants available for host selection.

Lewis determination of strain J166 isolates

We then determined Le expression of the isolates at different time intervals after inoculation. For the first 14 wk, a mixture of 2 to 4 strains was detected in three of the four animals, with too few individual isolates to permit reliable comparisons of Le expression in the Le^{a+b-} vs. the Le^{a-b+} animals (data not shown). By 40 wk, only strain J166 was recovered from animals A, B, and D, and comprised 6 of the 8 isolates from animal C. Importantly, J166 Le expression at 40 wk differed substantially for the Le^{a+b-} and Le^{a-b+} animals (Le^x 1473±922 vs. 269±220 ODU, Le^y 160±122 vs. 1712±858 ODU, $P<0.0001$ in both cases) (Fig. 3). The J166 isolates predominantly expressed Le^y preinoculation and at week one in Le^{a+b-} animal A (E6C). Le^y expression then significantly decreased from week 14 (Fig. 2B) to week 40 (1298±1040 to 203±112 ODU, $P<0.01$) resulting in an Le^x-dominant phenotype (Fig. 2C). Comparing the preinoculation strain J166 with the week-40 J166 isolates in monkeys A and B, Le^x rose significantly ($P<0.001$, and 0.05), and Le^y declined significantly ($P<0.001$ in both). In total, these results indicate that *H. pylori* populations can undergo substantial changes in Le phenotype *in vivo* within 40 wk and that this is related to predominant host Le phenotypes.

Genetic mechanisms for altered *H. pylori* Lewis expression

H. pylori Le^{x/y} expression reflects the action of products of at least four genes, including the β -(1,4) galactosyltransferase β -(1,4) *galT*, two α -(1,3) fucosyltransferases (*futA/futB*), and the α -(1,2) fucosyltransferase (*futC*). All of these genes, except β -(1,4) *galT*, are metastable as a result of frame shift-prone repetitive sequences (35-38). We examined genotypes in strain J166 preinoculation and in strain 98-169, a J166 descendant, isolated from Le^x-expressing monkey B (85D08) at 40 wk; the Le^x/Le^y phenotype of this strain was 1990/10 compared with 480/1180 ODU for a single colony of J166 pre-inoculation. In *futC* of J166, there was a poly-C tract with 9 cytosines, and the full gene was in frame. In contrast, for *futC* of 98-169, the poly-C tract contained 10 cytosines, and the ORF was truncated. The alleles of the other three genes tested showed no frame shifts. Examination of *futC* in two other isolates (98-149 from monkey A and 99-171 from monkey B) that had lost Le^y expression showed the identical frame shift as in 98-169, but 98-208, a single colony recovered from monkey D, which did not have a changed phenotype, did not have the frame shift. In total, these data provide evidence that a +1 frame shift in the poly-C tract of *futC* resulted in the *in vivo* change in phenotype (loss of Le^y expression).

DISCUSSION

Using rhesus monkeys, we provide direct evidence supporting the hypothesis that host Le phenotype is a determinant for particular Le phenotypes within an *H. pylori* population (22). Our interpretation assumes that the *H. pylori* Le expression *in vitro* and the fractions of recovered Le types accurately reflect *in vivo* conditions. In the absence of selection, the potential bias due to *in vitro* phase variation during the 2 or 3 passages until Le types are determined should be relatively small (~0.5 to 1.5%) (34). Similarly, *H. pylori* Le expression *in vitro* has been relatively constant in our prior experiments (17,31), with essentially no differences whether Le types of gerbil-derived isolates were determined directly or after 2 or 3 *in vitro* passages (31).

One limitation of the present report is that our data are derived from a relatively small number of Rhesus monkeys, which is usual with this animal model (49). Because *H. pylori* inoculation of humans has been considered unethical, we carefully considered available models and selected the Rhesus monkey. These are the only animals that have the natural occurrence of *H. pylori* (47), natural expression of human-like gastric Le antigens (45,46), and are suitable for periodic upper gastrointestinal endoscopy (49). As illustrated below, we assessed the population patterns over time and obtained relevant results using only four animals. This was possible because we selected two animals from each of the two pertinent Le groups and studied a large number of serial specimens with multiple *H. pylori* isolates from each animal.

Colonization of monkeys for 40 wk is relatively brief, compared with adult humans who usually have carried their *H. pylori* strains for decades (2). Whether the predominant Le phenotypes we observed would be maintained indefinitely is not known and could be the subject of future studies. In any event, the J166 strain outcompeted the other inoculated strains in every monkey, and in monkeys A and B, its Le phenotype changed substantially within 40 wk of inoculation. These variations parallel the changes detected in expression of *H. pylori* outer membrane proteins involved in host ligand binding (7,50). Among a small number of paired *H. pylori* isolates obtained from humans 7 to 10 yr apart (26), Le^y levels decreased with time, whereas Le^x levels remained constant, paralleling the expected changes in human gastric Le expression with age-dependent increases in gastric intestinal metaplasia (57), and consistent with the observed Le expression changes following inoculation of monkeys (51). Taken together, these observations suggest that this early period is important for the long-term persistence of *H. pylori* in the human and nonhuman primate stomach.

Because the animals used here had been cured of their resident *H. pylori* strains six months before our experimental inoculation (49,51), prior exposure to *H. pylori* antigens could have influenced the strain selection observed. However, the six-month interval had been sufficient for the anti-*H. pylori* immunoglobulins, gastric cellular infiltration, and gastrin levels each to significantly decline (49). That the J166 phenotypes that emerged were polar in terms of Le expression, and corresponded to the host Le phenotypes, suggests a lack of obvious bias in the direction of selection, although the prior exposure could have affected intensity or tempo (41). In contrast to previous hypotheses (20,21), host anti-Le antibodies appear unrelated to colonizing *H. pylori* Le phenotypes (data not shown). Components other than Le antigens are responsible for most of the immunogenicity of *H. pylori* LPS in humans (58,59), and anti-Le antibodies also may be present in *H. pylori*-negative persons (60,61). Although *H. pylori*-specific immunoglobulins peaked within the first two months after challenge (49), selection for specific Le phenotypes did not become evident until after 14 wk. Thus, if preexisting host responses affected the fitness of Le types, we would have expected the major effects to become evident during the early stages of the experiment.

When hosts are exposed to several *H. pylori* strains, both bacterial and host characteristics may influence which strain is most successful (41). Whereas short-term J238 dominance clearly seemed strain-determined and independent of the host colonized (49), long-term dominance by J166 appeared to be both strain- and host-related, as reflected by the variation in bacterial Le phenotypes in the different animals. That the dichotomy of bacterial Le phenotypes observed at 40 wk could have developed at random is improbable (41) but could have been subject to periodic selection (62). If random, then mixtures of different Le phenotypes also would have been expected, rather than the relatively homogeneous populations observed at 40 wk. The consistency of the Le changes in each of the 4 animals and the intermediate state of J166 Le expression in animal A at week 14 suggests a dynamic selection process, as modeled mathematically (41).

Insights into the possible selective pressures behind such host-adaptation by *H. pylori* are provided by the recent demonstration that *H. pylori* colonization induces changes in host mucosal sialyl-Le^x and Le^b expression, consequently affecting binding site availability (7, 51). In contrast, Le variation occurring *in vivo* at similar frequency to that demonstrated *in vitro* (33) probably would not be sufficient to explain the magnitude of changes seen in our experiment, in the absence of selection (41). The uniformity of Le epitopes of bacteria recovered from each animal late after challenge is consistent with selection for better-adapted phenotypes (2). These might have preexisted in sub-clones in the preinoculation J166 population, but could also have arisen often during persistent colonization, given the metastability of *H. pylori* genes controlling Le epitope synthesis. In this, we are drawn to the possibility that host gastric Le antigens contributed importantly to the implied selection pressure, although other possible explanations also merit testing.

For epithelial attachment, host Le antigens (Le^b, sialyl-Le^x) interacting with *H. pylori* adhesins (BabA, SabA) appear more important than bacterial Le antigens (7,63-66), yet multiple factors could contribute. In contrast to standard microbiological practice to examine single colony-derived bacterial populations, future comparisons for bacterial and host Le expression should be based on representative (multicolony) *H. pylori* isolates (22), as human hosts are colonized by polymorphic *H. pylori* populations (25,26,28,30).

A frame shift in the α -(1,2) fucosyltransferase gene (*futC*) in all three Le^x-expressing isolates tested is sufficient to explain both the observed loss of Le^y expression and the increase in Le^x expression, as Le^x is no longer substrate for the inactive FutC; changes in the other three genes that affect upstream steps in Le antigen synthesis are not required. These genotypic studies suggest that the capability for host-dependent Lewis phenotypic adaptation has been selected and maintained at particular loci in *H. pylori*. This model is both stochastic, with the host selecting for differential fitness of variants in the population (2,41), yet “programmed” in that only certain types of genes contain hot-spots for frame shift (ON-OFF) variation; for example, such hotspots are not found in genes whose expression is likely always needed (such as those for ribosomal proteins or metabolic enzymes) (25,30,38, 67). Consistent with the known heterogeneity within *H. pylori* populations colonizing an individual host (26,29), the initial J166 inoculums may have included 10-cytosine-*futC* cells. However, with strong overall Le^y expression, such cells likely represented a minor constituent of the overall population, if present at all. Although such “founding” cells may have had ultimate fitness advantage (41) in the Le^{a-b+} monkeys, even in their absence, phase variation could have generated an appropriate population from among the cells proliferating within the monkeys.

In summary, these results support the hypothesis that *H. pylori* populations are selected on the basis of their Le phenotype, which is dependent on that of the host colonized. We propose that the ability of *H. pylori* populations to adapt their predominant Le expression to that of the host contributes to their persistence in the gastric niche. That such selection is apparent suggests that the “host” gastric phenotype can determine “guest” phenotype, possibly through differential bacterial adherence and/or evasion of local immune responses. Le expression is not necessary for successful long-term colonization of mice by *H. pylori* (40), illustrating the desirability of primate models for mirroring human conditions (48). That local gastric Lewis phenotypes change in response to *H. pylori* (7,51) indicates the dynamic and complex interactions between persistent *H. pylori* and its host (2,26,41).

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REFERENCES

1. Peek RM, Blaser MJ. *Helicobacter pylori* and gastrointestinal tract adenocarcinomas. Nat. Rev. Cancer 2002;2:28–37. [PubMed: 11902583]
2. Blaser MJ, Atherton J. *Helicobacter pylori* persistence: biology and disease. J. Clin. Invest 2004;113:321–333. [PubMed: 14755326]
3. Mollicone R, Bara J, Le Pendu J, Oriol R. Immunohistologic pattern of type 1 (Lea, Leb) and type 2 (X, Y, H) blood group-related antigens in the human pyloric and duodenal mucosae. Lab. Invest 1985;53:219–227. [PubMed: 2410664]
4. Oriol R, Le Pendu J, Mollicone R. Genetics of ABO, H, Lewis X, and related antigens. Vox Sang 1986;51:161–171. [PubMed: 2433836]
5. Lindén S, Nordman H, Hedenbro J, Hurtig M, Boren T, Carlstedt I. Strain- and blood group-dependent binding of *Helicobacter pylori* to human gastric MUC5AC glycoforms. Gastroenterology 2002;123:1923–1930. [PubMed: 12454849]
6. Ilver D, Arnqvist A, Ogren J, Frick IM, Kersulyte D, Incecik ET, Berg DE, Covacci A, Engstrand L, Boren T. *Helicobacter pylori* adhesion binding fucosylated histo-blood group antigens revealed by retagging. Science 1998;279:373–377. [PubMed: 9430586]
7. Mahdavi J, Sondén B, Hurtig M, Olfat FO, Forsberg L, Roche N, Angstrom J, Larsson T, Teneberg S, Karlsson KA, et al. *Helicobacter pylori* SabA adhesion in persistent infection and chronic inflammation. Science 2002;297:573–578. [PubMed: 12142529]
8. Sakamoto J, Watanabe T, Tokumaru T, Takagi H, Nakazato H, Lyod KO. Expression of Lewis_x, Lewis_y, sialyl-Lewis_x, and sialyl-Lewis_x blood group antigens in human gastric carcinoma and in normal gastric tissue. Cancer Res 1989;49:745–752. [PubMed: 2910493]
9. Torado J, Blasco E, Cosme A, Gutierrez-Hoyos A, Arenas JJ. Expression of type 1 and type 2 blood group-related antigens in normal and neoplastic gastric mucosa. Am. J. Clin. Pathol 1989;91:249–254. [PubMed: 2923092]
10. Davidson JS, Triadafilopoulos G. Blood group-related antigen expression in normal and metaplastic human upper gastrointestinal mucosa. Gastroenterology 1992;103:1552–1561. [PubMed: 1426874]
11. Aspinall GO, Monteiro MA, Pang H, Walsh EJ, Moran AP. O antigen chains in the lipopolysaccharide of *H. pylori* NCTC 11637. Carbohydrate Lett 1994;1:151–156.
12. Sherburne R, Taylor DE. *Helicobacter pylori* expresses a complex surface carbohydrate, Lewis X. Infect. Immun 1995;63:4564–4568. [PubMed: 7591106]
13. Chan NWC, Stangier K, Sherburne R, Taylor DE, Zhang Y, Dovichi NJ, Palcic MM. The biosynthesis of Lewis X in *Helicobacter pylori*. Glycobiology 1995;5:683–688. [PubMed: 8608270]
14. Aspinall GO, Monteiro MA, Pang H, Walsh EJ, Moran AP. Lipopolysaccharide of the *Helicobacter pylori* type strain NCTC 11637 (ATCC 43504): structure of the O antigen chain and core oligosaccharide regions. Biochemistry 1996;35:2489–2497. [PubMed: 8652593]
15. Aspinall GO, Monteiro MA. Lipopolysaccharides of *Helicobacter pylori* strains P466 and MO19: structures of the O antigen and core oligosaccharide regions. Biochemistry 1996;35:2498–2504. [PubMed: 8652594]
16. Simoons-Smit IM, Appelmeik BJ, Verboom T, Negrini R, Penner JL, Aspinall GO, Moran AP, She FF, Shi B, Rudnica W, Savio A, de Graaff J. Typing of *Helicobacter pylori* with monoclonal antibodies against Lewis antigens in lipopolysaccharide. J. Clin. Microbiol 1996;34:2196–2200. [PubMed: 8862584]
17. Wirth HP, Yang M, Karita M, Blaser MJ. Expression of the human cell surface glycoconjugates Lewis x and Lewis y by *Helicobacter pylori* isolates is related to *cagA* status. Infect. Immun 1996;64:4598–4605. [PubMed: 8890213]
18. Monteiro MA, Chan KHN, Rasko DA, Taylor DE, Zheng PY, Appelmeik BJ, Wirth HP, Yang M, Blaser MJ, Hynes SO, et al. Simultaneous expression of type 1 and 2 Lewis blood-group antigens by *Helicobacter pylori* lipopolysaccharides. J. Biol. Chem 1998;273:11533–11543. [PubMed: 9565568]
19. Monteiro MA, Appelmeik BJ, Rasko DA, Moran AP, Hynes SO, MacLean LL, Chan KH, Michael FS, Logan SM, O'Rourke J, et al. Lipopolysaccharide structures of *Helicobacter pylori* genomic strains 26695 and J99, mouse model *H. pylori* Sydney strain, *H. pylori* P466 carrying sialyl Lewis

- X, and of *H. pylori* UA915 expressing Lewis B. Eur. J. Biochem 2000;267:305–320. [PubMed: 10632700]
20. Negrini R, Savio A, Poiesi C, Appelmek BJ, Buffoli F, Paterlini A, Cesari P, Graffeo M, Vaira D, Franzin G. Antigenic mimicry between *Helicobacter pylori* and gastric mucosa in the pathogenesis of body atrophic gastritis. Gastroenterology 1996;111:655–665. [PubMed: 8780570]
 21. Appelmek BJ, Simoons-Smit I, Negrini R, Moran AP, Aspinall GO, Forte JG, de Vries T, Quan H, Verboom T, Maaskant JJ, et al. Potential role of molecular mimicry between *Helicobacter pylori* lipopolysaccharide and host Lewis blood group antigens in autoimmunity. Infect. Immun 1996;64:2031–2040. [PubMed: 8675304]
 22. Wirth HP, Yang M, Peek RM, Tham KT, Blaser MJ. *Helicobacter pylori* Lewis expression is related to the host Lewis phenotype. Gastroenterology 1997;113:1091–1098. [PubMed: 9322503]
 23. Akopyants N, Bukanov NO, Westblom TU, Berg DE. PCR-based RFLP analysis of DNA sequence diversity in the gastric pathogen *Helicobacter pylori*. Nucleic Acids Res 1992;20:6221–6225. [PubMed: 1361982]
 24. Alm RA, Ling LL, Moir DT, King BL, Brown ED, Doig PC, Smith DR, Noonan B, Guild BC, deJonge BL, et al. Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen *Helicobacter pylori*. Nature 1999;397:176–180. [PubMed: 9923682]
 25. Aras RA, Kang J, Tschumi A, Harasaki Y, Blaser MJ. Extensive repetitive DNA facilitates prokaryotic genome plasticity. Proc. Natl. Acad. Sci. USA 2003;100:13579–13584. [PubMed: 14593200]
 26. Kuipers EJ, Israel DA, Kusters JG, Gerrits MM, Weel J, van der Ende A, van der Hulst RWM, Wirth HP, Höök-Nikanne J, Thompson SA, et al. Quasispecies development of *Helicobacter pylori* observed in paired isolates obtained years apart from same host. J. Infect. Dis 2000;81:273–282. [PubMed: 10608776]
 27. Falush D, Kraft C, Taylor NS, Correa P, Fox JG, Achtman M, Suerbaum S. Recombination and mutation during long-term gastric colonization by *Helicobacter pylori*: estimates of clock rates, recombination size, and minimal age. Proc. Natl. Acad. Sci. USA 2001;98:15056–15061. [PubMed: 11742075]
 28. Salama N, Guillemin K, McDaniel TK, Sherlock G, Tompkins L, Falkow S. A whole-genome microarray reveals genetic diversity among *Helicobacter pylori* strains. Proc. Natl. Acad. Sci. USA 2000;97:14668–14673. [PubMed: 11121067]
 29. Israel DA, Salama N, Krishna U, Rieger UM, Atherton JC, Falkow S, Peek RM Jr. *Helicobacter pylori* genetic diversity within the gastric niche of a single human host. Proc. Natl. Acad. Sci. USA 2001;98:14625–14630. [PubMed: 11724955]
 30. Aras RA, Lee Y, Kim S-K, Israel D, Peek RM, Blaser MJ. Natural variation in populations of persistently colonizing bacteria affect human host cell phenotype. J. Infect. Dis 2003;188:486–496. [PubMed: 12898434]
 31. Wirth HP, Yang M, Peek RM, Hook-Nikanne J, Blaser MJ. Phenotypic diversity in Lewis expression of *Helicobacter pylori* from the same host. J. Lab. Clin. Med 1999;133:488–500. [PubMed: 10235132]
 32. Gibson JR, Chart H, Owen RJ. Intra-strain variation in expression of lipopolysaccharide by *Helicobacter pylori*. Lett. Appl. Microbiol 1998;26:399–403. [PubMed: 9717308]
 33. Appelmek BJ, Martin SL, Monteiro MA, Clayton CA, McColm AA, Zeng P, Verboom T, Maaskant JJ, van den Eijnden DH, Hokke CH, et al. Phase variation in *Helicobacter pylori* lipopolysaccharide due to changes in the lengths of poly(C) tracts in α 1,3-fucosyltransferase genes. Infect. Immun 1999;67:5361–5366. [PubMed: 10496917]
 34. Appelmek BJ, Shiberu B, Trinks C, Tapsi N, Yheng PY, Verboom T, Maaskant J, Hooke CH, Schiphorst WECM, Blanchard D, et al. Phase variation in *Helicobacter pylori* lipopolysaccharide. Infect. Immun 1998;66:70–76. [PubMed: 9423841]
 35. Wang G, Rasko DA, Sherburne R, Taylor DE. Molecular genetic basis for the variable expression of Lewis Y antigen in *Helicobacter pylori*: analysis of the α -(1,2) fucosyltransferase gene. Mol. Microbiol 1999;31:1265–1274. [PubMed: 10096092]
 36. Martin SL, Erdbrooke MR, Hodgman TC, van den Eijnden DH, Bird MI. Lewis X biosynthesis in *Helicobacter pylori*: Molecular cloning of an α -(1,3)-fucosyltransferase gene. J. Biol. Chem 1997;272:21349–21356. [PubMed: 9261148]

37. Ge Z, Chan NWC, Palcic MM, Taylor DE. Cloning and heterologous expression of an α 1, 3-fucosyltransferase gene from the gastric pathogen *Helicobacter pylori*. J. Biol. Chem 1997;272:21357–63. [PubMed: 9261149]
38. Tomb J-F, White O, Kerlavage AR, Clayton RA, Sutton GG, Fleischmann RD, Ketchum KA, Klenk HP, Gill S, Dougherty BA, et al. The complete genome sequence of the gastric pathogen *Helicobacter pylori*. Nature 1997;388:539–547. [PubMed: 9252185]
39. Rasko DA, Wang G, Palcic MM, Taylor DE. Cloning and characterization of the alpha (1,3/4) fucosyltransferase of *Helicobacter pylori*. J. Biol. Chem 2000;275:4988–4994. [PubMed: 10671538]
40. Takata T, El-Omar E, Camorlinga M, Thompson SA, Minohara Y, Ernst PB, Blaser MJ. *Helicobacter pylori* does not require Lewis X or Lewis Y expression to colonize C3H/HeJ mice. Infect. Immun 2002;70:3073–3079. [PubMed: 12011000]
41. Webb GF, Blaser MJ. Dynamics of bacterial phenotype selection in a colonized host. Proc. Nat. Acad. Sci. USA 2002;99:3135–3140. [PubMed: 11867714]
42. Wang G, Ge Z, Rasko DA, Taylor DE. Lewis antigens in *Helicobacter pylori*: biosynthesis and phase variation. Mol. Microbiol 2000;36:1187–1196. [PubMed: 10931272]
43. Taylor DE, Rasko DA, Sherburne R, Ho C, Jewell LD. Lack of correlation between Lewis antigen expression by *Helicobacter pylori* and gastric epithelial cells in infected patients. Gastroenterology 1998;115:1113–1122. [PubMed: 9797366]
44. Heneghan MA, McCarthy CF, Moran AP. Relationship of blood group determinants on *Helicobacter pylori* lipopolysaccharide with host Lewis phenotype and inflammation response. Infect. Immun 2000;68:937–941. [PubMed: 10639467]
45. Socha, WW.; Ruffié, J. Blood groups of primates: theory, practice, evolutionary meaning. Alan R. Liss, Inc; New York: 1983. p. 128-129.
46. Lindén S, Borén T, Dubois A, Carlstedt I. Rhesus monkey gastric mucins: Oligomeric structure, glycoforms and *Helicobacter pylori* binding. Biochem. J 2004;379:765–775. [PubMed: 14736333]
47. Dubois A, Berg DE, Fiala N, Incecik ET, Fiala N, Heman-Ackah LM, Perez-Perez GI, Blaser MJ. Transient and persistent experimental infection of nonhuman primates with *Helicobacter pylori*: implications for human disease. Infect. Immun 1996;64:2885–2891. [PubMed: 8757808]
48. Solnick JV, Hansen LM, Canfield DR, Parsonnet J. Determination of the infectious dose of *Helicobacter pylori* during primary and secondary infection in rhesus monkeys (*Macaca mulatta*). Infect. Immun 2001;69:6887–6892. [PubMed: 11598063]
49. Dubois A, Berg DE, Incecik ET, Fiala N, Heman-Ackah LM, Del Valle J, Yang M, Wirth HP, Perez-Perez GI, Blaser MJ. Host specificity of *Helicobacter pylori* strains and host responses in experimentally challenged non-human primates. Gastroenterology 1999;116:90–96. [PubMed: 9869606]
50. Solnick JV, Hansen LM, Salama NR, Boonjakuakul JK, Syvanen M. Modification of *Helicobacter pylori* outer membrane protein expression during experimental infection of rhesus macaques. Proc. Natl. Acad. Sci. USA 2004;101:2106–2111. [PubMed: 14762173]
51. Linden S, Mahdavi J, Walker M, Borén T, Carlstedt I, Dubois A. Effect of persistent and transient *H. pylori* infection on H,K-ATPase and sialylated Lewis antigens expression: Role of the host Lewis gastric phenotype. Gastroenterology 2003;124:W885.
52. Dubois A, Berg DE, Fiala N, Heman-Ackah LM, Perez-Perez GI, Blaser MJ. Cure of *Helicobacter pylori* infection by omeprazole-clarithromycin-based therapy in non-human primates. J. Gastroenterol 1998;33:18–22. [PubMed: 9497216]
53. Akopyants N, Bukanov NO, Westblom TU, Kresovich S, Berg DE. DNA diversity among clinical isolates of *Helicobacter pylori* detected by PCR-based RAPD fingerprinting. Nucleic Acids Res 1992;20:5137–5142. [PubMed: 1408828]
54. Harris, PA. Immunohematology special techniques. Baxter Diagnostics Inc.; Miami, Florida: 1991. p. 22-25. Monograph by Dade education
55. Socha WW, Blancher A, Moor-Jankowski J. Red cell polymorphisms in nonhuman primates: a review. J. Med. Primatol 1995;24:282–305. [PubMed: 8750505]
56. Wirth HP, Beins MH, Yang M, Tham KT, Blaser MJ. Experimental infection of Mongolian gerbils with wildtype and mutant *Helicobacter pylori* strains. Infect. Immun 1998;66:4856–4866. [PubMed: 9746590]

57. Murata K, Egami H, Shibata Y, Sakamoto K, Misumi A, Ogawa M. Expression of blood group-related antigens, ABH, Lewis a, Lewis b, Lewis x, Lewis y, CA19–9, and CSLEX1 in early cancer, intestinal metaplasia, and uninvolved mucosa of the stomach. *Am. J. Clin. Pathol* 1992;98:67–75. [PubMed: 1615929]
58. Yokota S, Amano K, Hayashi S, Kubota T, Fujii N, Yokochi T. Human antibody response to *Helicobacter pylori* lipopolysaccharide: presence of an immunodominant epitope in the polysaccharide chain of lipopolysaccharide. *Infect. Immun* 1998;66:3006–3011. [PubMed: 9596783]
59. Claeys D, Faller G, Appelmek BJ, Negrini R, Kirchner T. The gastric H⁺, K⁺-ATPase is a major autoantigen in chronic *Helicobacter pylori* gastritis with body mucosa atrophy. *Gastroenterology* 1998;115:340–347. [PubMed: 9679039]
60. Amano K, Hayashi S, Kubota T, Fujii N, Yokota S. Reactivities of Lewis antigen monoclonal antibodies with the lipopolysaccharides of *Helicobacter pylori* strains isolated from patients with gastroduodenal diseases in Japan. *Clin. Diagn. Lab. Immunol* 1997;4:540–544. [PubMed: 9302202]
61. Kurtenkov O, Klaamas K, Miljukhina L, Shljapnikova L, Ellamaa M, Bovin N, Wadstrom T. IgG antibodies to Lewis type 2 antigens in serum of *H. pylori*-infected and non-infected blood donors of different Lewis (a,b) blood-group phenotype. *FEMS Immunol. Med. Microbiol* 1999;24:227–232. [PubMed: 10378425]
62. Levin BR. Periodic selection, infectious gene exchange and the genetic structure of *E. coli* populations. *Genetics* 1981;99:1–23. [PubMed: 7042453]
63. Boren T, Falk PG, Roth KA, Larson G, Normark S. Attachment of *Helicobacter pylori* to human gastric epithelium mediated by blood group antigens. *Science* 1993;262:1892–1895. [PubMed: 8018146]
64. Falk PG, Bry L, Holgersson J, Gordon JI. Expression of a human α -1,3/4-fucosyltransferase in the pit cell lineage of FVB/N mouse stomach results in production of Leb-containing glycoconjugates: A potential transgenic mouse model for studying *Helicobacter pylori* infection. *Proc. Natl. Acad. Sci. USA* 1995;92:1515–1519. [PubMed: 7878011]
65. Guruge JL, Falk PG, Lorenz RG, Dans M, Wirth HP, Blaser MJ, Berg DE, Gordon JI. Epithelial attachment alters the outcome of *Helicobacter pylori* infection. *Proc. Natl. Acad. Sci. USA* 1998;95:3925–3930. [PubMed: 9520469]
66. Salaun L, Linz B, Suerbaum S, Saunders NJ. The diversity within an expanded and redefined repertoire of phase-variable genes in *Helicobacter pylori*. *Microbiology* 2004;150:817–30. [PubMed: 15073292]

Figure 1.

Relative proportion of *H. pylori* strains recovered after inoculation of seven strains into four rhesus monkeys. Early after inoculation (weeks 1 and 8) most *H. pylori* isolates were identified as strain J238, whereas strain J166 became predominant subsequently (week 14 and 40). The relative proportion of the two other strains identified (J170, J258) remained <25% at all times.

Figure 2.

Representative example of the evolution of Le expression diversity of *H. pylori* strain J166 in animal A (E6C). Le^x and Le^y expression was determined in single colony-derived cell populations preinoculation (A), and at week 14 (B), and week 40 postinoculation (C). The preinoculation cells showed predominant Le^y expression. Fourteen weeks after inoculation, the cells recovered from the stomach of monkey E6C were mixed in their Le expression, including colonies (3,5,7,8) with Le^x predominance. By 40 wk, 7 of 8 colonies sampled showed Le^x predominance, and the other colony showed low expression of both Le^x and Le^y . The differences between the preinoculation isolates ($n=7$) and the week 40 isolates ($n=8$) are highly significant for both Le^x (247 ± 76 vs. 1771 ± 797) and Le^y (2113 ± 257 vs. 203 ± 112) expression ($P < 0.001$).

Figure 3.

Evolution of Le expression of *H. pylori* strain J166 in four rhesus monkeys of different Le phenotypes. Le^x and Le^y expression of strain J166 preinoculation was determined in 8 single colony-derived cell populations. At 40 wk, the Le phenotype of the strains recovered from the two Le^{a-b+} animals [C (82A49), D (8PZ)] showed an aggregate predominance of Le^y expression (*middle*), in the 14 isolates examined (*bottom right*). In contrast, in the two Le^{a+b-} animals [A (E6C), B (85D08)] at 40 wk, the predominant Le expression (*middle, top*) of the J166 populations had shifted from Le^y to Le^x, as determined from 13 isolates (*top right*).

TABLE 1

PCR primers used in this study

Gene designation ^a	Position ^b	5' Coordinate ^c	Sequence (5'→ 3')	PCR product length (bp)
β -(1,4) <i>galT</i> (HP0826)				1469
Forward	-391	877971	TATGCAAATGCGATGAATAC	
Reverse	+1078	879439	TTTATGGGCAGAACGATTAGG	
<i>futA</i> (HP0379)				1554
Forward	-200	388634	GCGTGCTAGGGTTTATTTCGG	
Reverse	+1355	390187	ATTAGGGGCCAATATCGCTGG	
<i>futB</i> (HP0651)				1631
Forward	-112	698203	AGAGGTTTAAAAACAGCACGC	
Reverse	+1519	696573	ACATGCTCAAAAACCCACGC	
<i>futC</i> (Hp0093-0094)				1126
Forward	-140	98854	GAACACTCACACGCTCTT	
Reverse	+985	99979	TAGAATTAGACGCTCGCTAT	

^a Gene designation and number (in parenthesis) assigned to ORF in the *H. pylori* 26695 genome.

^b Position refers to the localization of the 5' origin of the primer with respect to the ORF start site.

^c Coordinates refer to the localization in the *H. pylori* 26695 genome of the 5' origin of each primer.