

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

Hans-Peter Wirth,<sup>\*,†</sup> Manqiao Yang,<sup>\*,†</sup> Edgardo Sanabria-Valentín,<sup>‡</sup> Douglas E. Berg,<sup>§</sup> André Dubois,<sup>||</sup> and Martin J. Blaser<sup>\*,‡,1</sup>

<sup>\*</sup>Division of Infectious Diseases, Vanderbilt University School of Medicine, and VA Medical Center, Nashville, Tennessee, USA; <sup>†</sup>Division of Gastroenterology, Zurich University School of Medicine, Zurich, Switzerland; <sup>‡</sup>Departments of Medicine and Microbiology, New York University School of Medicine, and VA Medical Center, New York, New York, USA; <sup>§</sup>Departments of Molecular Microbiology and of Genetics, Washington University School of Medicine, St. Louis, Missouri, USA; and <sup>||</sup>Laboratory of Gastrointestinal and Liver Studies, Digestive Diseases Division, Department of Medicine, Uniformed Services of the Health Sciences, Bethesda, Maryland, USA

**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<sup>a/b</sup> 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<sup>a+b+</sup> animals predominantly expressed Lewis<sup>y</sup>. In contrast, J166 populations had switched to a Lewis<sup>x</sup> dominant phenotype in the two Lewis<sup>a+b-</sup> animals; a frame shift in *futC*, regulating conversion of Lewis<sup>x</sup> to Lewis<sup>y</sup>, 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.—Wirth, H.-P., Yang, M., Sanabria-Valentín, E., Berg, D. E., Dubois, A., and Blaser, M. J. Host Lewis phenotype-dependent *Helicobacter pylori* Lewis antigen expression in rhesus monkeys. *FASEB J.* 20, E812–E820 (2006)

**Key Words:** 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<sup>a</sup> and Le<sup>x</sup>) and difucosylated (Le<sup>b</sup> and Le<sup>y</sup>) Lewis antigens on surfaces of many cell types, including erythrocytes and gastric epithelial 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<sup>b</sup> and sialyl-Le<sup>x</sup> antigens, respectively (6–7), and *H. pylori* colonization is associated with increased sialyl-Le<sup>x</sup> 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<sup>x</sup> and Le<sup>y</sup>) (16, 17), but type 1 expression (Le<sup>a</sup> and Le<sup>b</sup>) 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<sup>a/b</sup> with *H. pylori* Le<sup>x/y</sup> phenotypes, we proposed that *H. pylori* populations adapt their Le expression to that of the host Le

<sup>1</sup> Correspondence: Department of Medicine, New York University School of Medicine, 550 First Ave., New York, NY 10016, USA. E-mail: martin.blaser@med.nyu.edu  
doi: 10.1096/fj.05-5529fe

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<sup>y</sup> optical density (OD) value was significantly ( $P < 0.001$ ) greater than the Le<sup>x</sup> value in patients of the Le<sup>a-b+</sup> 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<sup>a+b+</sup> and Le<sup>a-b-</sup> 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 reappraised 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<sup>a</sup> or Le<sup>b</sup> 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 supple-

mented 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% N<sub>2</sub>, 5% O<sub>2</sub>, and 5% CO<sub>2</sub> (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<sup>a</sup> and anti-Le<sup>b</sup> and Reagent Le<sup>a+b-</sup> or Le<sup>a-b+</sup> 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<sup>a</sup>, Le<sup>b</sup>, Le<sup>x</sup>, sialyl-Le<sup>x</sup>, or Le<sup>y</sup> 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<sup>a+b-</sup> or Le<sup>a-b+</sup> 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.

TABLE 1. PCR primers used in this study

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

<sup>a</sup>Gene designation and number (in parenthesis) assigned to ORF in the *H. pylori* 26695 genome. <sup>b</sup>Position refers to the localization of the 5' origin of the primer with respect to the ORF start site. <sup>c</sup>Coordinates refer to the localization in the *H. pylori* 26695 genome of the 5' origin of each primer.

## 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 AFRRRI colony, differed in the pattern of Le antigens on their erythrocytes and in their secretions. Erythrocyte typing for Le<sup>a</sup> or Le<sup>b</sup> 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<sup>a</sup> or Le<sup>b</sup> antigens in saliva and gastric juice by a hemagglutination inhibition method (45). Five animals (including A and B) were Le<sup>a+b-</sup>, and three animals (including C and D) were Le<sup>a-b+</sup> in saliva and gastric juice, and none were positive or negative for both. Therefore, test monkeys A and B were classified as Le<sup>a+b-</sup>, and C and D were classified as Le<sup>a-b+</sup>.

### 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

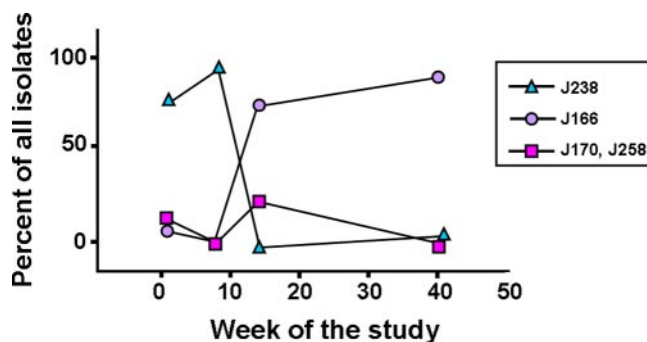


Figure 1. Relative proportion of *H. pylori* strains recovered after inoculation of seven strains into four rhesus monkeys. Early after inoculation (weeks 1 and 8) most *H. pylori* isolates were identified as strain J238, whereas strain J166 became predominant subsequently (week 14 and 40). The relative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

strains reacted with monoclonal antibodies to Le<sup>a</sup>, Le<sup>b</sup>, or sialyl-Le<sup>x</sup>. For each strain, the population inoculated expressed either or both Le<sup>x</sup> and Le<sup>y</sup>, in the following mean Le<sup>x</sup>/Le<sup>y</sup> 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<sup>x</sup> or Le<sup>y</sup> 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<sup>x</sup> and Le<sup>y</sup> varied in the sample of preinoculation strain J166 (Fig. 2A), which indicated a preexisting pool of variants available for host selection.

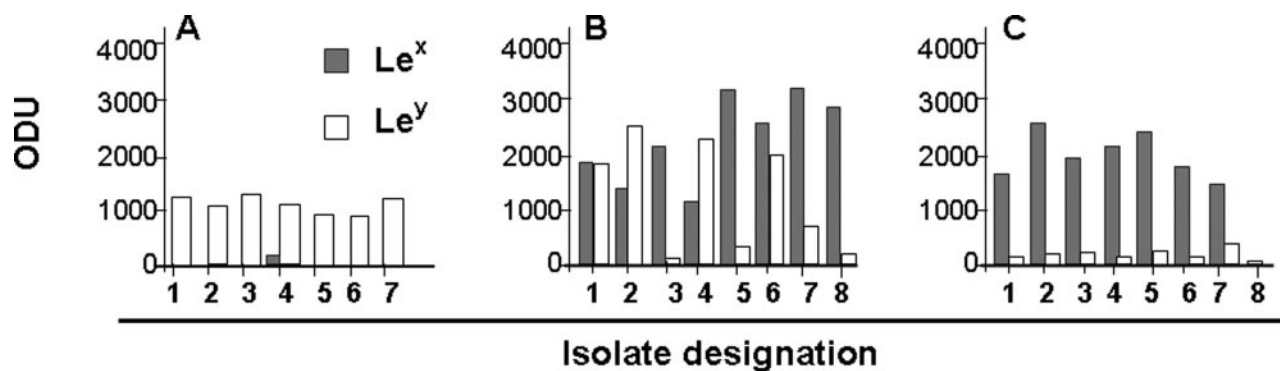
#### Lewis determination of strain J166 isolates

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<sup>a+b-</sup> vs. the Le<sup>a-b+</sup> animals (data not shown). By 40 wk, only strain J166 was recovered from animals A, B,

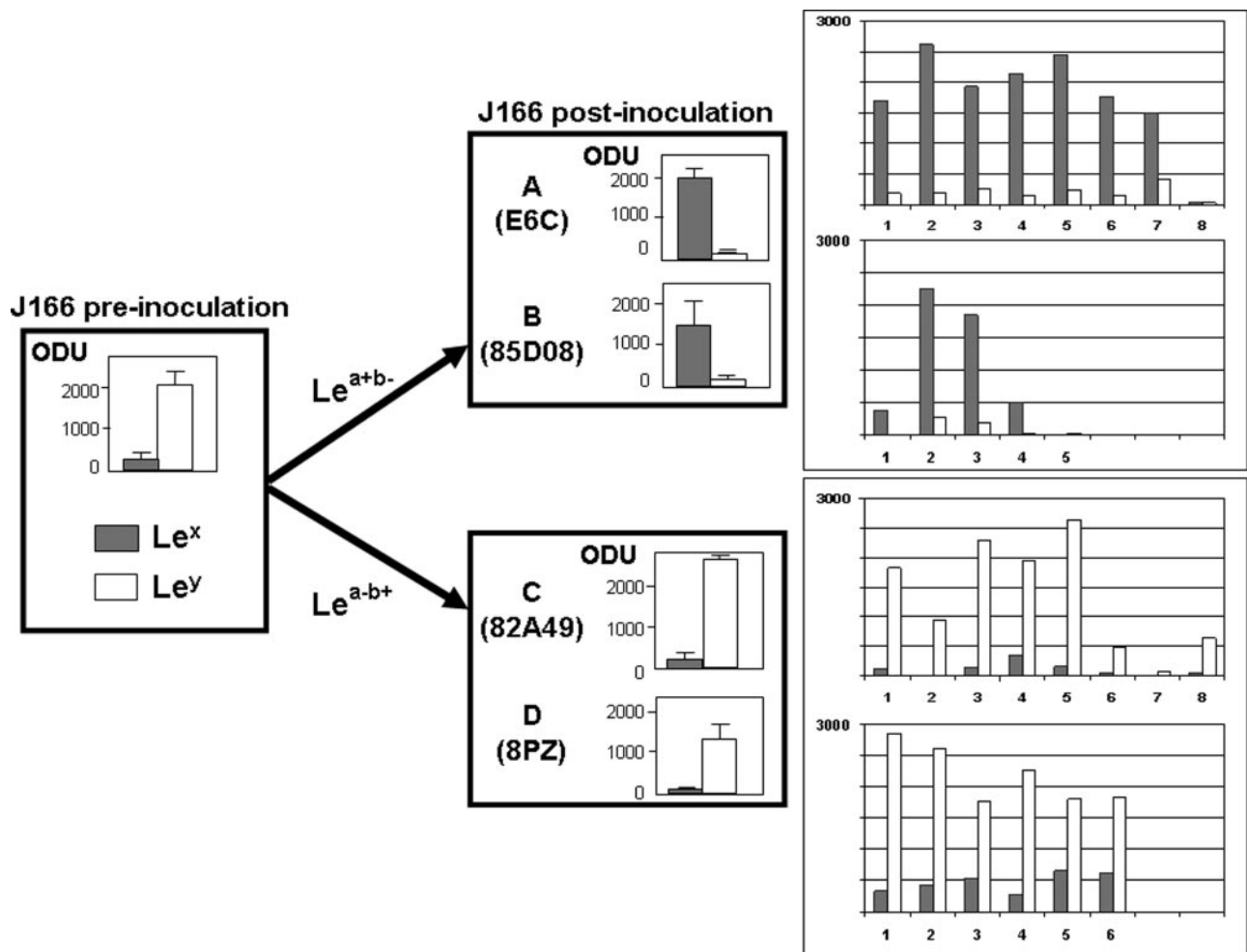
and D, and comprised 6 of the 8 isolates from animal C. Importantly, J166 Le expression at 40 wk differed substantially for the Le<sup>a+b-</sup> and Le<sup>a-b+</sup> animals (Le<sup>x</sup> 1473±922 vs. 269±220 ODU, Le<sup>y</sup> 160±122 vs. 1712±858 ODU,  $P<0.0001$  in both cases) (Fig. 3). The J166 isolates predominantly expressed Le<sup>y</sup> preinoculation and at week one in Le<sup>a+b-</sup> animal A (E6C). Le<sup>y</sup> 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<sup>x</sup>-dominant phenotype (Fig. 2C). Comparing the preinoculation strain J166 with the week-40 J166 isolates in monkeys A and B, Le<sup>x</sup> rose significantly ( $P<0.001$ , and 0.05), and Le<sup>y</sup> 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<sup>x/y</sup> 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<sup>x</sup>-expressing monkey B (85D08) at 40 wk; the Le<sup>x</sup>/Le<sup>y</sup> 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



**Figure 2.** Representative example of the evolution of Le expression diversity of *H. pylori* strain J166 in animal A (E6C). Le<sup>x</sup> and Le<sup>y</sup> 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<sup>y</sup> 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<sup>x</sup> predominance. By 40 wk, 7 of 8 colonies sampled showed Le<sup>x</sup> predominance, and the other colony showed low expression of both Le<sup>x</sup> and Le<sup>y</sup>. The differences between the preinoculation isolates ( $n=7$ ) and the week 40 isolates ( $n=8$ ) are highly significant for both Le<sup>x</sup> (247±76 vs. 1771±797) and Le<sup>y</sup> (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<sup>x</sup> and Le<sup>y</sup> 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<sup>a+b+</sup> animals [C (82A49), D (8PZ)] showed an aggregate predominance of Le<sup>y</sup> expression (*middle*), in the 14 isolates examined (*bottom right*). In contrast, in the two Le<sup>a+b-</sup> animals [A (E6C), B (85D08)] at 40 wk, the predominant Le expression (*middle, top*) of the J166 populations had shifted from Le<sup>y</sup> to Le<sup>x</sup>, as determined from 13 isolates (*top right*).

monkey A and 99–171 from monkey B) that had lost Le<sup>y</sup> 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<sup>y</sup> 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<sup>y</sup> levels decreased with time, whereas Le<sup>x</sup> 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<sup>x</sup> and Le<sup>b</sup> 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 subclones 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<sup>b</sup>, sialyl-Le<sup>x</sup>) 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  $\alpha$ -(1,2) fucosyltransferase gene (*futC*) in all three Le<sup>x</sup>-expressing isolates tested is sufficient to explain both the observed loss of Le<sup>y</sup> expression and the increase in Le<sup>x</sup> expression, as Le<sup>x</sup> 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 exam-

ple, 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<sup>y</sup> 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<sup>a-b+</sup> 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). F

Supported in part by R01DK53707, DK53727, AI38166, RO1 CA82312, GM63270, GH070098, and P30DK52574 from the National Institutes of Health, and by the Medical Research Service of the Department of Veterans Affairs.

## REFERENCES

1. Peek R. M., and Blaser, M. J. (2002) *Helicobacter pylori* and gastrointestinal tract adenocarcinomas. *Nat. Rev. Cancer* **2**, 28–37
2. Blaser M. J., and Atherton, J. (2004) *Helicobacter pylori* persistence: biology and disease. *J. Clin. Invest.* **113**, 321–333
3. Mollicone R., Bara J., Le Pendu J., and Oriol, R. (1985) 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.* **53**, 219–227
4. Oriol R., Le Pendu J., and Mollicone, R. (1986) Genetics of ABO, H, Lewis X, and related antigens. *Vox Sang* **51**, 161–171
5. Lindén S., Nordman H., Hedenbro J., Hurtig M., Boren T., and Carlstedt, I. (2002) Strain- and blood group-dependent binding of *Helicobacter pylori* to human gastric MUC5AC glycoforms. *Gastroenterology* **123**, 1923–1930
6. Ilver D., Arnqvist A., Ogren J., Frick I. M., Kersulyte D., Inceci E. T., Berg D. E., Covacci A., Engstrand L., and Boren, T. (1998) *Helicobacter pylori* adhesion binding fucosylated histo-blood group antigens revealed by retagging. *Science* **279**, 373–377
7. Mahdavi J., Sonden B., Hurtig M., Olfat F. O., Forsberg L., Roche N., Angstrom J., Larsson T., Teneberg S., Karlsson K. A., et al. (2002) *Helicobacter pylori* SabA adhesion in persistent infection and chronic inflammation. *Science* **297**, 573–578
8. Sakamoto J., Watanabe T., Tokumaru T., Takagi H., Nakazato H., and Lyod, K. O. (1989) Expression of Lewis<sub>x</sub>, Lewis<sub>y</sub>, sialyl-Lewis<sub>x</sub>, and sialyl-Lewis<sub>x</sub> blood group antigens in human gastric carcinoma and in normal gastric tissue. *Cancer Res.* **49**, 745–752
9. Torado J., Blasco E., Cosme A., Gutierrez-Hoyos A., and Arenas, J. I. (1989) Expression of type 1 and type 2 blood group-related antigens in normal and neoplastic gastric mucosa. *Am. J. Clin. Pathol.* **91**, 249–254
10. Davidson, J. S., and Triadafilopoulos, G. (1992) Blood group-related antigen expression in normal and metaplastic human upper gastrointestinal mucosa. *Gastroenterology* **103**, 1552–1561
11. Aspinall, G. O., Monteiro, M. A., Pang, H., Walsh, E. J., and Moran, A. P. (1994) O antigen chains in the lipopolysaccharide of *H. pylori* NCTC 11637. *Carbohydrate Lett.* **1**, 151–156
12. Sherburne R., and Taylor, D. E. (1995) *Helicobacter pylori* expresses a complex surface carbohydrate, Lewis X. *Infect. Immun.* **63**, 4564–4568
13. Chan, N. W. C., Stangier, K., Sherburne, R., Taylor, D. E., Zhang, Y., Dovichi, N. J., and Palcic, M. M. (1995) The biosynthesis of Lewis X in *Helicobacter pylori*. *Glycobiology* **5**, 683–688
14. Aspinall, G. O., Monteiro, M. A., Pang, H., Walsh, E. J., and Moran, A. P. (1996) Lipopolysaccharide of the *Helicobacter pylori* type strain NCTC 11637 (ATCC 43504): structure of the O antigen chain and core oligosaccharide regions. *Biochemistry* **35**, 2489–2497
15. Aspinall G. O., and Monteiro, M. A. (1996) Lipopolysaccharides of *Helicobacter pylori* strains P466 and MO19: structures of the O antigen and core oligosaccharide regions. *Biochemistry* **35**, 2498–2504
16. Simoons-Smit, I. M., Appelmelk, B. J., Verboom, T., Negrini, R., Penner, J. L., Aspinall, G. O., Moran, A. P., She, F. F., Shi, B., Rudnica, W., Savio, A., de Graaff, J. (1996) Typing of *Helicobacter pylori* with monoclonal antibodies against Lewis antigens in lipopolysaccharide. *J. Clin. Microbiol.* **34**, 2196–2200
17. Wirth, H. P., Yang, M., Karita, M., and Blaser, M. J. (1996) Expression of the human cell surface glycoconjugates Lewis x and Lewis y by *Helicobacter pylori* isolates is related to *cagA* status. *Infect. Immun.* **64**, 4598–4605
18. Monteiro, M. A., Chan, K. H. N., Rasko, D. A., Taylor, D. E., Zheng, P. Y., Appelmelk, B. J., Wirth, H. P., Yang, M., Blaser, M. J., Hynes, S. O., et al. (1998) Simultaneous expression of type 1 and 2 Lewis blood-group antigens by *Helicobacter pylori* lipopolysaccharides. *J. Biol. Chem.* **273**, 11533–11543
19. Monteiro, M. A., Appelmelk, B. J., Rasko, D. A., Moran, A. P., Hynes, S. O., MacLean, L. L., Chan, K. H., Michael, F. S., Logan, S. M., O'Rourke, J., et al. (2000) 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.* **267**, 305–320
20. Negrini, R., Savio, A., Poesi, C., Appelmelk, B. J., Buffoli, F., Paterlini, A., Cesari, P., Graffeo, M., Vaira, D., and Franzin, G. (1996) Antigenic mimicry between *Helicobacter pylori* and gastric mucosa in the pathogenesis of body atrophic gastritis. *Gastroenterology* **111**, 655–665
21. Appelmelk, B. J., Simoons-Smit, I., Negrini, R., Moran, A. P., Aspinall, G. O., Forte, J. G., de Vries, T., Quan, H., Verboom, T., Maaskant, J. J., et al. (1996) Potential role of molecular mimicry between *Helicobacter pylori* lipopolysaccharide and host Lewis blood group antigens in autoimmunity. *Infect. Immun.* **64**, 2031–2040
22. Wirth, H. P., Yang, M., Peek, R. M., Tham, K. T., and Blaser, M. J. (1997) *Helicobacter pylori* Lewis expression is related to the host Lewis phenotype. *Gastroenterology* **113**, 1091–1098
23. Akopyants, N., Bukanov, N. O., Westblom, T. U., and Berg, D. E. (1992) PCR-based RFLP analysis of DNA sequence diversity in the gastric pathogen *Helicobacter pylori*. *Nucleic Acids Res.* **20**, 6221–6225
24. Alm, R. A., Ling, L. L., Moir, D. T., King, B. L., Brown, E. D., Doig, P. C., Smith, D. R., Noonan, B., Guild, B. C., deJonge, B. L., et al. (1999) Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen *Helicobacter pylori*. *Nature* **397**, 176–180

25. Aras R. A., Kang, J., Tschumi, A., Harasaki, Y., and Blaser, M. J. (2003) Extensive repetitive DNA facilitates prokaryotic genome plasticity. *Proc. Natl. Acad. Sci. USA* **100**, 13579–13584
26. Kuipers, E. J., Israel, D. A., Kusters, J. G., Gerrits, M. M., Weel, J., van der Ende, A., van der Hulst, R. W. M., Wirth, H. P., Höök-Nikanne, J., Thompson, S. A., *et al.* (2000) Quasispecies development of *Helicobacter pylori* observed in paired isolates obtained years apart from same host. *J. Infect. Dis.* **81**, 273–282
27. Falush, D., Kraft, C., Taylor, N. S., Correa, P., Fox, J. G., Achtman, M., and Suerbaum, S. (2001) 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* **98**, 15056–15061
28. Salama, N., Guillemin, K., McDaniel, T. K., Sherlock, G., Tompkins, L., and Falkow, S. (2000) A whole-genome microarray reveals genetic diversity among *Helicobacter pylori* strains. *Proc. Natl. Acad. Sci. USA* **97**, 14668–14673
29. Israel, D. A., Salama, N., Krishna, U., Rieger, U. M., Atherton, J. C., Falkow, S., and Peek, R. M. Jr. (2001) *Helicobacter pylori* genetic diversity within the gastric niche of a single human host. *Proc. Natl. Acad. Sci. USA* **98**, 14625–14630
30. Aras, R. A., Lee, Y., Kim, S.-K., Israel, D., Peek, R. M., and Blaser, M. J. (2003) Natural variation in populations of persistently colonizing bacteria affect human host cell phenotype. *J. Infect. Dis.* **188**, 486–496
31. Wirth, H. P., Yang, M., Peek, R. M., Hook-Nikanne, J., and Blaser, M. J. (1999) Phenotypic diversity in Lewis expression of *Helicobacter pylori* from the same host. *J. Lab. Clin. Med.* **133**, 488–500
32. Gibson, J. R., Chart, H., and Owen, R. J. (1998) Intra-strain variation in expression of lipopolysaccharide by *Helicobacter pylori*. *Lett. Appl. Microbiol.* **26**, 399–403
33. Appelmelk, B. J., Martín, S. L., Monteiro, M. A., Clayton, C. A., McColm, A. A., Zeng, P., Verboom, T., Maaskant, J. J., van den Eijnden, D. H., Hokke, C. H., *et al.* (1999) Phase variation in *Helicobacter pylori* lipopolysaccharide due to changes in the lengths of poly(C) tracts in  $\alpha$ 1,3-fucosyltransferase genes. *Infect. Immun.* **67**, 5361–5366
34. Appelmelk, B. J., Shiberu, B., Trinks, C., Tapsi, N., Yheng, P. Y., Verboom, T., Maaskant, J., Hooke, C. H., Schiphorst, W. E. C. M., Blanchard, D., *et al.* (1998) Phase variation in *Helicobacter pylori* lipopolysaccharide. *Infect. Immun.* **66**, 70–76
35. Wang, G., Rasko, D. A., Sherburne, R., and Taylor, D. E. (1999) Molecular genetic basis for the variable expression of Lewis Y antigen in *Helicobacter pylori*: analysis of the  $\alpha$ -(1,2) fucosyltransferase gene. *Mol. Microbiol.* **31**, 1265–1274
36. Martin, S. L., Erdbrooke, M. R., Hodgman, T. C., van den Eijnden, D. H., and Bird, M. I. (1997) Lewis X biosynthesis in *Helicobacter pylori*: Molecular cloning of an  $\alpha$ -(1,3)-fucosyltransferase gene. *J. Biol. Chem.* **272**, 21349–21356
37. Ge, Z., Chan, N. W. C., Palcic, M. M., and Taylor, D. E. (1997) Cloning and heterologous expression of an  $\alpha$ 1, 3-fucosyltransferase gene from the gastric pathogen *Helicobacter pylori*. *J. Biol. Chem.* **272**, 21357–63
38. Tomb, J.-F., White, O., Kerlavage, A. R., Clayton, R. A., Sutton, G. G., Fleischmann, R. D., Ketchum, K. A., Klenk, H. P., Gill, S., Dougherty, B. A., *et al.* (1997) The complete genome sequence of the gastric pathogen *Helicobacter pylori*. *Nature* **388**, 539–547
39. Rasko DA, Wang G, Palcic MM, and Taylor, D. E. (2000) Cloning and characterization of the alpha (1,3/4) fucosyltransferase of *Helicobacter pylori*. *J. Biol. Chem.* **275**, 4988–4994
40. Takata, T., El-Omar, E., Camorlinga, M., Thompson, S. A., Minohara, Y., Ernst, P. B., and Blaser, M. J. (2002) *Helicobacter pylori* does not require Lewis X or Lewis Y expression to colonize C3H/HeJ mice. *Infect. Immun.* **70**, 3073–3079
41. Webb, G. F., and Blaser, M. J. (2002) Dynamics of bacterial phenotype selection in a colonized host. *Proc. Nat. Acad. Sci. USA* **99**, 3135–3140
42. Wang, G., Ge, Z., Rasko, D. A., and Taylor, D. E. (2000) Lewis antigens in *Helicobacter pylori*: biosynthesis and phase variation. *Mol. Microbiol.* **36**, 1187–1196
43. Taylor, D. E., Rasko, D. A., Sherburne, R., Ho, C., and Jewell, L. D. (1998) Lack of correlation between Lewis antigen expression by *Helicobacter pylori* and gastric epithelial cells in infected patients. *Gastroenterology* **115**, 1113–1122
44. Heneghan, M. A., McCarthy, C. F., and Moran, A. P. (2000) Relationship of blood group determinants on *Helicobacter pylori* lipopolysaccharide with host Lewis phenotype and inflammatory response. *Infect. Immun.* **68**, 937–941
45. Socha, W. W., and Ruffie J. (1983) *Blood groups of primates: theory, practice, evolutionary meaning*. Alan R. Liss, Inc, New York, pp. 128–129
46. Lindén, S., Borén, T., Dubois, A., and Carlstedt, I. (2004) Rhesus monkey gastric mucins: Oligomeric structure, glycoforms and *Helicobacter pylori* binding. *Biochem. J.* **379**, 765–775
47. Dubois, A., Berg, D. E., Fiala, N., Incecik, E. T., Fiala, N., Heman-Ackah, L. M., Perez-Perez, G. I., and Blaser, M. J. (1996) Transient and persistent experimental infection of nonhuman primates with *Helicobacter pylori*: implications for human disease. *Infect. Immun.* **64**, 2885–2891
48. Solnick, J. V., Hansen, L. M., Canfield, D. R., and Parsonnet, J. (2001) Determination of the infectious dose of *Helicobacter pylori* during primary and secondary infection in rhesus monkeys (*Macaca mulatta*). *Infect. Immun.* **69**, 6887–6892
49. Dubois, A., Berg, D. E., Incecik, E. T., Fiala, N., Heman-Ackah, L. M., Del Valle, J., Yang, M., Wirth, H. P., Perez-Perez, G. I., and Blaser, M. J. (1999) Host specificity of *Helicobacter pylori* strains and host responses in experimentally challenged non-human primates. *Gastroenterology* **116**, 90–96
50. Solnick, J. V., Hansen, L. M., Salama, N. R., Boonjakuakul, J. K., and Syvanen, M. (2004) Modification of *Helicobacter pylori* outer membrane protein expression during experimental infection of rhesus macaques. *Proc. Natl. Acad. Sci. USA* **101**, 2106–2111
51. Linden, S., Mahdavi, J., Walker, M., Borén, T., Carlstedt, I., and Dubois, A. (2003) 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* **124**, W885
52. Dubois, A., Berg, D. E., Fiala, N., Heman-Ackah, L. M., Perez-Perez, G. I., and Blaser, M. J. (1998) Cure of *Helicobacter pylori* infection by omeprazole-clarithromycin-based therapy in non-human primates. *J. Gastroenterol.* **33**, 18–22
53. Akopyants, N., Bukanov, N. O., Westblom, T. U., Kresovich, S., and Berg, D. E. (1992) DNA diversity among clinical isolates of *Helicobacter pylori* detected by PCR-based RAPD fingerprinting. *Nucleic Acids Res.* **20**, 5137–5142
54. Harris, P. A. (1991) *Immunohematology special techniques*. Baxter Diagnostics Inc., Miami, Florida. Monograph by Dade education, pp. 22–25
55. Socha, W. W., Blancher, A., and Moor-Jankowski, J. (1995) Red cell polymorphisms in nonhuman primates: a review. *J. Med. Primatol.* **24**, 282–305
56. Wirth, H. P., Beins, M. H., Yang, M., Tham, K. T., and Blaser, M. J. (1998) Experimental infection of Mongolian gerbils with wildtype and mutant *Helicobacter pylori* strains. *Infect. Immun.* **66**, 4856–4866
57. Murata, K., Egami, H., Shibata, Y., Sakamoto, K., Misumi, A., and Ogawa, M. (1992) 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.* **98**, 67–75
58. Yokota, S., Amano, K., Hayashi, S., Kubota, T., Fujii, N., and Yokochi, T. (1998) Human antibody response to *Helicobacter pylori* lipopolysaccharide: presence of an immunodominant epitope in the polysaccharide chain of lipopolysaccharide. *Infect. Immun.* **66**, 3006–3011
59. Claeys, D., Faller, G., Appelmelk, B. J., Negrini, R., ad Kirchner, T. (1998) The gastric H<sup>+</sup>, K<sup>+</sup>-ATPase is a major autoantigen in chronic *Helicobacter pylori* gastritis with body mucosa atrophy. *Gastroenterology* **115**, 340–347
60. Amano K, Hayashi S, Kubota T, Fujii N, and Yokota, S. (1997) 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.* **4**, 540–544
61. Kurtenkov, O., Klaamas, K., Miljukhina, L., Shljapnikova, L., Ellamaa, M., Bovin, N., and Wadstrom, T. (1999) 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.* **24**, 227–232

62. Levin, B. R. (1981) Periodic selection, infectious gene exchange and the genetic structure of *E. coli* populations. *Genetics* **99**, 1–23
63. Boren, T., Falk, P. G., Roth, K. A., Larson, G., and Normark, S. (1993) Attachment of *Helicobacter pylori* to human gastric epithelium mediated by blood group antigens. *Science* **262**, 1892–1895
64. Falk, P. G., Bry, L., Holgersson, J., and Gordon, J. I. (1995) Expression of a human  $\alpha$ -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* **92**, 1515–1519
65. Guruge, J. L., Falk, P. G., Lorenz, R. G., Dans, M., Wirth, H. P., Blaser, M. J., Berg, D. E., and Gordon, J. I. (1998) Epithelial attachment alters the outcome of *Helicobacter pylori* infection. *Proc. Natl. Acad. Sci. USA* **95**, 3925–3930
66. Salaun, L., Linz, B., Suerbaum, S., and Saunders, N. J. (2004) The diversity within an expanded and redefined repertoire of phase-variable genes in *Helicobacter pylori*. *Microbiology* **150**, 817–30

*Received for publication December 29, 2005.*

*Accepted for publication March 3, 2006.*

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

Hans-Peter Wirth,<sup>\*,†</sup> Manqiao Yang,<sup>\*,†</sup> Edgardo Sanabria-Valentín,<sup>‡</sup> Douglas E. Berg,<sup>§</sup> André Dubois,<sup>||</sup> and Martin J. Blaser<sup>\*,‡,1</sup>

\*Division of Infectious Diseases, Vanderbilt University School of Medicine, and VA Medical Center, Nashville, Tennessee, USA; †Division of Gastroenterology, Zurich University School of Medicine, Zurich, Switzerland; ‡Departments of Medicine and Microbiology, New York University School of Medicine, and VA Medical Center, New York, New York, USA; §Departments of Molecular Microbiology and of Genetics, Washington University School of Medicine, St. Louis, Missouri, USA; and ||Laboratory of Gastrointestinal and Liver Studies, Digestive Diseases Division, Department of Medicine, Uniformed Services of the Health Sciences, Bethesda, Maryland, USA

 To read the full text of this article, go to <http://www.fasebj.org/cgi/doi/10.1096/fj.05-5529fje>

## SPECIFIC AIMS

Both human cells and *H. pylori* populations are polymorphic for the expression of the fucosylated Lewis antigens. In an animal model using rhesus monkeys that differed in the major Lewis phenotypes, we asked whether *H. pylori* populations adapt their Lewis phenotypes to those of the host. Because *H. pylori* cells have hypermutable homopolymeric tracts in genes that regulate Lewis expression, we sought to determine whether variation was generated *in vivo* that could explain the phenotypic changes.

## PRINCIPAL FINDINGS

### 1. Rhesus monkeys represent a model system to study Lewis phenotypic variation in the stomach

Humans express both monofucosylated (including Le<sup>a</sup> and Le<sup>x</sup>) and difucosylated (Le<sup>b</sup> and Le<sup>y</sup>) Lewis antigens on surfaces of many cell types, including erythrocytes and gastric epithelial cells, as well as on the highly glycosylated proteins (mucins) comprising the mucus layer. Conversely, ~90% of *H. pylori* isolates express human-type Le antigens in their LPS, preferentially the type 2 antigens (Le<sup>x</sup> and Le<sup>y</sup>). *H. pylori* strains are highly diverse, in part reflecting continued evolution during persistent colonization of individual hosts. Even within a single gastric biopsy, the *H. pylori* cells present can show extensive diversity in Lewis expression, because of the existence of antigenic variants within single clones.

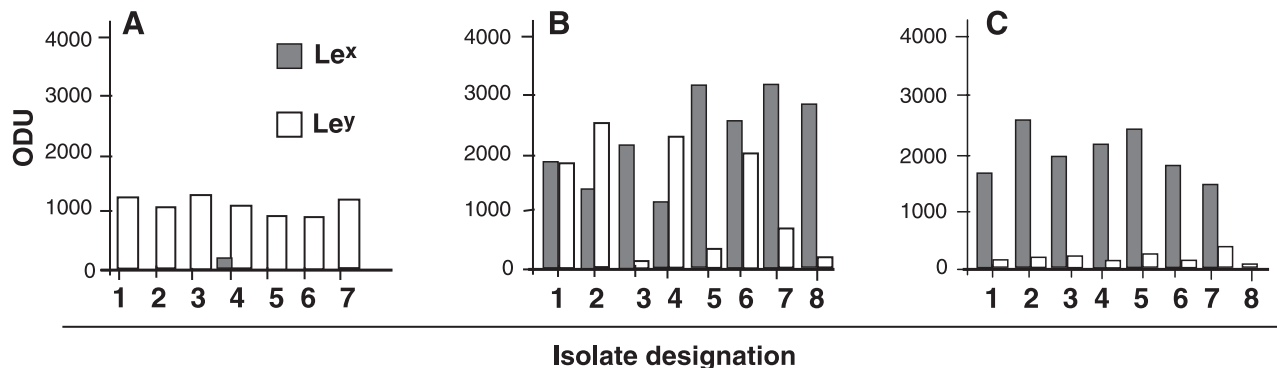
We have proposed that *H. pylori* populations contain cells with differing Lewis phenotypes and that host phenotypes select the predominant populations. Rhesus monkeys are human-like in polymorphism for, and expression of, Lewis antigens, and gastric colonization

by *H. pylori* is highly similar in the two primate species. When monkeys were challenged with mixtures of *H. pylori* strains isolated from humans, one or a few strains eventually predominated. Testing four rhesus monkeys (designated A–D) for Le<sup>a</sup> or Le<sup>b</sup> antigens in saliva and gastric juice, by a hemagglutination inhibition method, we found that two (A and B) were Le<sup>a+b-</sup> and two (C and D) were Le<sup>a-b+</sup>. Therefore, in the former monkeys, the predominant gastric epithelial expression was Le<sup>a</sup> and Le<sup>x</sup>, whereas in the latter monkeys, Le<sup>b</sup> and Le<sup>y</sup> were predominantly expressed. The four monkeys were inoculated with seven individual *H. pylori* strains, with a broad range of Le phenotypes, but only four strains could be recovered over the next 40 wk from the test animals. Although in the short term, strain J238 was predominant in the mixed bacterial populations, eventually all strains in each animal were outcompeted by J166. Thus, these experimental conditions permitted exploration of the evolution of *H. pylori* Le phenotype in a strain (J166) that was successful in colonizing animals that varied in their predominant Le phenotype.

### 2. Predominant Lewis phenotypes in the *H. pylori* population resemble those of the host by 40 wk

In the sample of preinoculation strain J166, Le<sup>y</sup> expression was variable but predominated, whereas some Le<sup>x</sup>-expressing cells were present in the population (Fig. 1A). In Le<sup>a+b-</sup> monkey E6C (animal A), by 14 wk, Le<sup>y</sup> expression was highly variable, and Le<sup>x</sup> expression was substantially increased (B). By week 40, Le<sup>x</sup> expression predominated (C). Despite the same initial J166

<sup>1</sup> Correspondence: Department of Medicine, New York University School of Medicine, 550 First Ave., New York, NY 10016, USA. E-mail: martin.blaser@med.nyu.edu  
doi: 10.1096/fj.05-5529fje



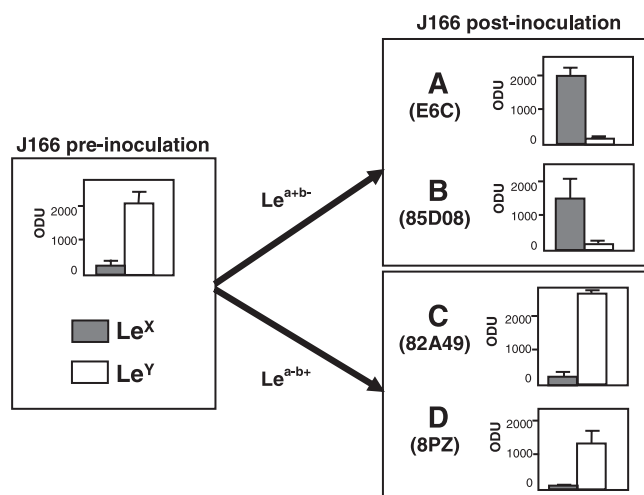
**Figure 1.** Representative example of the evolution of Le expression diversity of *H. pylori* strain J166 in animal A (E6C). Le<sup>x</sup> and Le<sup>y</sup> expression was determined in single colony-derived cell populations preinoculation (A), and at week 14 (B), and week 40 postinoculation (C). The preinoculation cells showed predominant Le<sup>y</sup> 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<sup>x</sup> predominance. By 40 wk, 7 of 8 colonies sampled showed Le<sup>x</sup> predominance, and the other colony showed low expression of both Le<sup>x</sup> and Le<sup>y</sup>. The differences between the preinoculation isolates ( $n=7$ ) and the week 40 isolates ( $n=8$ ) are highly significant for both Le<sup>x</sup> ( $247 \pm 76$  vs.  $1771 \pm 797$ ) and Le<sup>y</sup> ( $2113 \pm 257$  vs.  $203 \pm 112$ ) expression ( $P < 0.001$ ).

inoculum, by 40 wk of colonization of the two Le<sup>a+b-</sup> monkeys, Le<sup>x</sup> expression had become significantly higher, whereas in the two Le<sup>a-b+</sup> monkeys, Le<sup>y</sup> expression remained higher (Fig. 2). In total, these results indicate that within 40 wk *H. pylori* populations can undergo substantial changes in Le phenotype *in vivo* and that this variation is related to predominant host Lewis phenotype in the predicted pattern.

### 3. Genetic mechanisms for altered *H. pylori* Lewis expression

*H. pylori* Le<sup>x/y</sup> expression reflects the action of products of at least four genes, including the  $\beta$ -(1,4) galactosyl-

transferase ( $\beta$ -(1,4) *galT*), two  $\alpha$ -(1,3) fucosyltransferases (*futA/futB*) and the  $\alpha$ -(1,2) fucosyltransferase (*futC*). Most of these genes are metastable due to repetitive sequences with high frequency frame shifts. We examined genotypes in strain J166 preinoculation and in strain 98-169, a J166 descendant, isolated from Le<sup>x</sup>-expressing monkey B at 40 wk; the Le<sup>x</sup>/Le<sup>y</sup> phenotype of this strain was 1990/10 compared with 480/1180 ODU for the population in a single colony of J166 tested preinoculation. In *futC* of J166, there was a poly-C tract with 9 cytosines and the full gene was in frame (Fig. 3). 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 and 99-171) that had lost Le<sup>y</sup> 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 with loss of Le<sup>y</sup> expression.

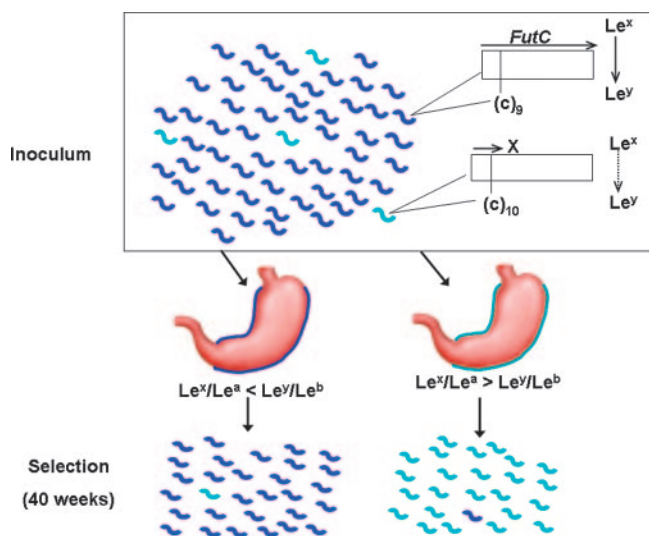


**Figure 2.** Evolution of Le expression of *H. pylori* strain J166 in four rhesus monkeys of different Le phenotypes. Le<sup>x</sup> and Le<sup>y</sup> expression of strain J166 preinoculation was determined in eight single colony-derived cell populations. At 40 wk, the Le phenotype of the strains recovered from the two Le<sup>a-b+</sup> animals [C (82A49), D (8PZ)] showed predominance of Le<sup>y</sup> expression (bottom). In contrast, in the two Le<sup>a+b-</sup> animals [A (E6C), B (85D08)] at 40 wk, the predominant Le expression of the J166 populations had shifted from Le<sup>y</sup> to Le<sup>x</sup>.

### CONCLUSIONS AND SIGNIFICANCE

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. Monkeys are the only animals other than humans that have the natural occurrence of *H. pylori*, natural expression of human-like gastric Le antigens, and they are suitable for periodic upper gastrointestinal endoscopy.

Colonization of monkeys for 40 wk can be considered relatively brief, compared with adult humans, who usually have carried their *H. pylori* strains for decades. Whether the predominant Le phenotypes we observed would be maintained indefinitely or not is not known and could be the subject of future studies. In any event,



**Figure 3.** Proposed generation of host-specific *H. pylori* Lewis antigen expression diversity. The original inoculum contains a mixed population of *H. pylori* cells, with the predominant cells having an 9-cytosine homopolymeric in-frame tract in *futC*, permitting the conversion of Lewis<sup>x</sup> into Lewis<sup>y</sup>. The mixed population also includes a small proportion of cells that each have a 10-cytosine *futC* tract, leading to a frame shift, and an inactive  $\alpha$ -(1,2) fucosyltransferase. The *H. pylori* inoculum was introduced into the stomachs of monkeys that predominantly expressed gastric epithelial Le<sup>a</sup> and Le<sup>x</sup> significantly greater than Le<sup>b</sup> and Le<sup>y</sup> (left stomach), or vice versa (right stomach). Over the ensuing 40 wk, cells with the 9-cytosine in-frame *futC* that catalyzed Lewis<sup>y</sup> production were selected in the monkeys that had the Le<sup>b</sup>/Le<sup>y</sup> predominant stomach, whereas in the Le<sup>a</sup>/Le<sup>x</sup> stomach, there was selection for the 10-cytosine out-of-frame *futC*, in which no Lewis<sup>y</sup> was produced. In this model, the stoichiometry of the slipped-strand mispairing affects the speed at which phenotypic conversion can occur, but it is the homogeneity of the host environment and the differential fitness of the competing cellular forms that determine the ultimate location of the population equilibrium between the two molecular forms.

the fact that the overall Le phenotype of the J166 strain changed substantially within 40 wk, and that it out-competed the other inoculated strains in every monkey implies that this early period is important for study. The variation we observed parallels the changes detected in expression of *H. pylori* outer membrane proteins, which are involved in host ligand binding. Among a small number of paired *H. pylori* isolates obtained from humans 7 to 10 yr apart, Le<sup>y</sup> levels decreased with time, whereas Le<sup>x</sup> levels remained constant, paralleling the expected changes in human gastric Le expression with age-dependent increases in gastric intestinal metaplasia and confirming observations of Le expression changes after our inoculation of monkeys (Fig. 2).

When hosts are exposed to several *H. pylori* strains, both bacterial and host characteristics may influence which strain is most successful. Whereas short-term J238 dominance clearly seemed strain-determined and independent of the host colonized, 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, but it could have been subject to periodic selection. 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 relatively ordered selection process, as modeled mathematically.

Insights into the possible selective pressures behind such host-adaptation by *H. pylori* are provided by the recent demonstration that *H. pylori* colonization induces dynamic changes in host mucosal sialyl-Le<sup>x</sup> and Le<sup>b</sup> expression, consequently affecting binding site availability. In contrast, Le variation occurring *in vivo* at similar frequency to that demonstrated *in vitro* probably would not be sufficient to explain the magnitude of changes seen in our experiment, in the absence of selection. Because Le expression diversity already was present in J166 preinoculation, the uniformity of Le epitopes of bacteria recovered from each animal late after challenge is consistent with selection for better-adapted phenotypes.

A frame shift in the  $\alpha$ -(1,2) fucosyltransferase gene (*futC*) in all three Le<sup>x</sup>-expressing isolates tested is sufficient to explain both the observed loss of Le<sup>y</sup> expression and the increase in Le<sup>x</sup> expression, as Le<sup>x</sup> is no longer the substrate for the inactive FutC; changes in the three other 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, yet “programmed” in that the genes with hotspots for variation are present in highly specific loci, not randomly distributed in the genome. Consistent with the known heterogeneity within *H. pylori* populations colonizing an individual host, the initial J166 inoculum likely included 10-cytosine *futC* cells; however, with the strong overall Le<sup>y</sup> expression observed in that culture, such 10-c cells likely represented a minor constituent of the overall population. Nevertheless, they would represent the “founding” cells that ultimately had fitness advantage in the Le<sup>a-b+</sup> 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 Le expression is not necessary for successful long-term *H. pylori* colonization of mice illustrates the desirability of primate models for mirroring human conditions. That local gastric Lewis phenotypes change in response to *H. pylori* indicates the dynamic and complex interactions between persistent *H. pylori* and its host. [F]