Host-dependent Lewis (Le) antigen expression in *Helicobacter pylori* cells recovered from Le^b-transgenic mice

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Variation of surface antigen expression is a mechanism used by microbes to adapt to and persist within their host habitats. *Helicobacter pylori*, a persistent bacterial colonizer of the human stomach, can alter its surface Lewis (Le) antigen expression. We examined *H. pylori* colonization in mice to test the hypothesis that host phenotype selects for *H. pylori* (Le) phenotypes. When wild-type and Le^b-expressing transgenic FVB/N mice were challenged with *H. pylori* strain HP1, expressing Le^x and Le^y, we found that bacterial populations recovered after 8 mo from Le^b-transgenic, but not wild-type, mice expressed Le^b. Changes in Le phenotype were linked to variation of a putative galactosyltransferase gene (*β-(1,3)galT*); mutagenesis and complementation revealed its essential role in type I antigen expression. These studies indicate that *H. pylori* evolves to resemble the host's gastric Le phenotype, and reveal a bacterial genetic locus that is subject to host-driven selection pressure.

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Abbreviations used: Le, Lewis; MFI, mean fluorescence intensity; ORF, open reading frame; PI, propidium iodide.

For microbes that are obligatory parasites of outbred host species, an important challenge is to adapt to each new individual host (Moxon et al., 1994; Falk et al., 2000; Bayliss et al., 2004; Blaser and Kirschner, 2007). Such coevolved bacteria use multiple strategies, including stealth, variation, and antidefense (Monack et al., 2004; Blaser and Kirschner, 2007). One mechanism to generate variation is the use of contingency genes to change expression of bacterial cell-surface structures relevant to the hosts being colonized (Moxon et al., 1994; Bayliss et al., 2001; Bayliss et al., 2004).

Humans are polymorphic for the expression of the fucosylated Lewis (Le) antigens on erythrocytes and in other body compartments, including the gastric epithelium (Sakamoto et al., 1989). *Helicobacter pylori*, the dominant human gastric bacteria (Bik et al., 2006; Andersson et al., 2008), are also polymorphic for expression of Le antigens (Fig. S1; Wang et al., 2000). Most strains predominately express Le^x and Le^y (type II antigens), which are major human gastric antigens (Simoons-Smit et al., 1996), whereas <5% express Le^a and Le^b (type I antigens; Wirth et al., 1996), which are also expressed in the stomach (Sakamoto et al., 1989). *H. pylori* may vary type II Le expression using a variety of genetic mechanisms (Appelmelk et al., 1998; Wang et al., 1999; Wirth et al., 2006; Sanabria-Valentín et al., 2007; Nilsson et al., 2008).

We have hypothesized that *H. pylori* Le expression reflects host selection operating on a population of stochastically varying strains that have differential fitness in particular hosts (Webb and Blaser, 2002). Observations in humans naturally colonized with *H. pylori* (Wirth et al., 1997) and in rhesus monkeys experimentally infected with *H. pylori* (Wirth et al., 2006) support this hypothesis. However, these studies are not conclusive, because the human studies were correlative, and the monkey studies were an experimental challenge with multiple strains and a small number of study animals (Wirth et al., 1997; Wirth et al., 2006).

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Wild-type mice do not express Le^b in their stomach. The creation of transgenic mice that express a human α-1,3/4 fucosyltransferase (accession no. EC 2.4.1.65 from the IntEnz database, available at http://www.ebi.ac.uk/intenz/index.jsp) in their mucus-producing gastric pit cells led to Le^b expression (Falk et al., 1995; Guruge et al., 1998). The presence of Le^b in the gastric mucosa of these mice and its absence in their nontransgenic littermates presented an opportunity to examine whether host phenotype selects for *H. pylori* phenotypic (Le antigen) expression. We hypothesized that among *H. pylori* strains introduced into "humanized" Le^b-transgenic mice but not their isogenic Le^b-negative (wild-type) littermates, there would be selection for bacterial Le^b expression. In the present study, we verify this hypothesis, and characterize the genetic loci and mechanisms responsible for the changed *H. pylori* phenotype.

RESULTS

H. pylori colonization of wild-type and Le^b-transgenic FVB/N mice

Conventionally raised, specific pathogen-free transgenic FVB/N Le^b mice (n = 20) and wild-type littermates (n = 20) were group housed in microisolator cages, maintained on a standard chow diet, and challenged at 6 wk of age (Fig. S2) with *H. pylori* strain HP1 (98-964), which expressed both Le^x and Le^y but had no detectable Le^a or Le^b expression. At varying times after infection, mice of each genotype were sacrificed, the stomachs were homogenized, and *H. pylori* was cultured by serial dilution on selective media. *H. pylori* colonization could only be detected in three out of the five mice in each group after 4 wk but was detected in each mouse at all subsequent time points (8, 16, and 32 wk; n = 5 animals/group/time point). There were no significant differences in bacterial density observed according to mouse genotype at any of the time points surveyed (Fig. 1). In total, we achieved stable (10^3 CFU/mouse stomach), long-term (≥8 mo) *H. pylori* colonization in both wild-type and Le^b-transgenic mice (Fig. 1).

Host-dependent Le antigen expression in mice

We next addressed whether colonization of the wild-type and Le^b-transgenic mice would select for differing *H. pylori* Le phenotypes, as determined by Le-specific ELISAs. All bacterial populations recovered expressed Le^x and Le^y, and there were no significant differences in Le^x or Le^y expression between sweeps recovered from wild-type or Le^b-transgenic mice in either the early or late isolates (Fig. S3). None of the isolates recovered from the wild-type or transgenic mice before the 8-mo time point expressed Le^a or Le^b (n = 10 assayed/time point), nor were these epitopes detectable in *H. pylori* sweeps recovered from wild-type mice 8 mo after challenge (Table I). Although the ratio of Le^x to Le^y in the *H. pylori* cells recovered from the 8-mo wild-type mice was similar to the inoculum strain, their overall expression was higher. However, in *H. pylori* sweeps from the 8-mo Le^b-transgenic mice, there was expression of Le^b in addition to Le^x and Le^y in four out of the five mice (Table I), which was significantly (P = 0.001) different from the wild-type mice. In addition, overall Le^x expression in the bacterial populations isolated from the Le^b-transgenic mice was significantly (P = 0.014) lower than in populations from the wild-type mice. There was a trend toward higher colonization levels in the four mice carrying Le^b-positive *H. pylori* compared with colonization densities of the five wild-type mice (P = 0.054). This result could reflect increased adhesion to host-expressed Le^b through binding by the bacterial Le^b ligand, BabA (Ilver et al., 1998). Although this result suggests that bacterial Le^b expression enhanced colonization and, thus, may have been positively selected, future studies will be required to confirm this observation.

Mouse humoral responses to *H. pylori* challenge

One hypothesis to explain the drop in Le^x expression in the 8-mo Le^b-transgenic mice is that an increase in anti-Le^x antibodies in the transgenic mice selected for cells with reduced Le^x expression, resulting in the emergence of Le^b expression, paralleling phenomena that have been observed previously in other organisms (Bayliss et al., 2001). To test this hypothesis, we determined mouse antibody levels against both *H. pylori* whole cells and purified *H. pylori* LPS with distinct Le phenotypes. ELISAs testing serum responses to 98-964 (HP1) whole-cell antigen showed that both wild-type and Le^b-transgenic mice progressively developed anti–*H. pylori* IgG responses during the course of the challenge (Fig. S4). No IgG was detected in the uninfected (control) mice, but an IgG response appeared during the early infection period (4–8 wk), rising to the highest levels during late infection (4–8 mo).

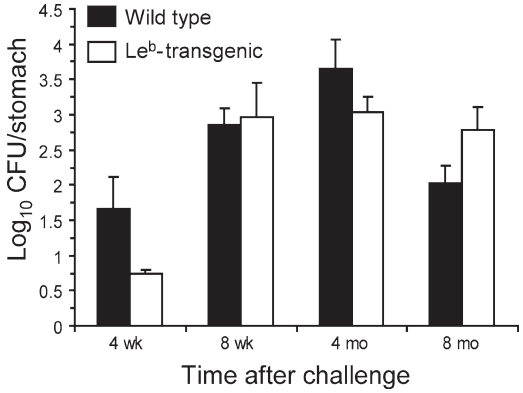

Figure 1. Quantitative *H. pylori* culture of wild-type and Le^b-transgenic FVB/N mice after experimental challenge with strain HP1. In this single experimental challenge, CFUs were determined by homogenization of a segment of the mouse stomach in sterile PBS (pH 7.4), followed by serial dilution on *H. pylori*–selective medium. At 4 wk, *H. pylori* was not detected in four mice; the means shown are the means for the *H. pylori*–positive mice. *H. pylori* was cultured from the stomachs of all of the other 36 mice in this study. Each of the 40 mice received an independent challenge with the stock culture of strain HP1 (n = 5 animals/group/time point). There were no significant differences in colonization levels between transgenic and nontransgenic animals at any of the time points as determined by a t test.

There were no significant differences in IgG levels between the wild-type and Le^b-transgenic mice (n = 10 mice/genotype/time point). These data confirm that the experimental *H. pylori* challenges induced adaptive humoral responses, as expected. The lack of difference between the groups provides evidence that unlike in previous studies (Guruge et al., 1998), the host genotype did not substantially affect the responses to the heterologous *H. pylori* antigens.

Next, we tested mouse sera with LPS preparations from wild-type strain J166 (OD values: Le^x = 0 and Le^y = 3.36) with strong Le^y expression, J166 Δ*futC* (OD values: Le^x = 2.22 and Le^y = 0) with strong Le^x expression, and J166 Δ*futA*/Δ*futB* (OD values: Le^x = 0 and Le^y = 0) with neither Le^x nor Le^y expression. Thus, we could examine the serologic response to specific Le epitopes. First, we examined responses to J166 whole-cell antigen, expecting that there would be robust responses, as observed with 98-964 whole-cell antigen (Fig. S4). The high antibody levels in 4- and 8-mo wild-type (OD value = 1.69 ± 1.48) and Le^b-transgenic mice (OD value = 1.89 ± 0.98; Table S1) confirmed that the mice were capable of responding to *H. pylori* antigens and showed no significant difference in response according to mouse genotype.

The responses to the LPS antigens were heterogeneous (Table S1), with a bimodal distribution, and roughly correlated with those to whole-cell antigen. The responses to Le^y LPS (wild type) and Le^0 LPS (Δ*futA*/Δ*futB*) were significantly (P = 0.035 and P = 0.011, respectively) higher in the 8-mo compared with the 4-mo mice, and trended in that direction (P = 0.15) for the Le^x LPS (Δ*futC*). Response to LPS also appeared to be independent of LPS Le phenotype; mice with strong anti-LPS responses showed strong responses to LPS of all three Le phenotypes. There were no significant differences in anti-LPS responses between wild-type and Le^b-transgenic mice. Finally, we asked whether there was an association between anti-LPS response and the *H. pylori* colonization density in the mice. Comparing the $\log_{10}$ CFU (2.43 ± 0.98) of the three mice with the highest anti-Le^x (J166 Δ*futC* LPS) responses (3.49 ± 0.7) to the $\log_{10}$ CFU (2.61 ± 0.92) of those with the lowest responses (0.03 ± 0.001) showed no significant differences; results were parallel for the other LPS preparations (unpublished data).

Analysis of *babA* in mouse-derived *H. pylori* isolates

One hypothesis to explain why colonization densities were higher in the Le^b-transgenic mice carrying Le^{b+} *H. pylori* could be the increased bacterial adherence to the gastric epithelia by the *H. pylori* Le^b ligand, BabA. In a previous challenge of rhesus monkeys (Solnick et al., 2004), a gene conversion event occurred that replaced a portion of *babA* with *babB* (as demonstrated by a series of PCRs), which resulted in a loss of Le^b adhesion. To examine this question, we first sought to confirm the previous findings (Solnick et al., 2004) by studying a strain (J166) that successfully colonized rhesus monkeys and comparing it with a J166-derived strain recovered from a monkey 10 mo later (98-169) from our previous monkey challenge studies (Wirth et al., 2006). Sequence analysis of the band produced by primers 834F and

Table I. Bacterial counts and Le antigen phenotypes of *H. pylori* gastric sweeps from wild-type and Le^b-transgenic FVB/N mice recovered after 8 mo

Source of *H. pylori*	Sweep designation	Log_{10} CFU/stomach[a]	Le antigen phenotype[b]			
			Le^a	Le^b	Le^x	Le^y
Inoculum strain	HP1		0	0	**0.62**	**0.65**
Wild-type mice	00-4	1.95	0.01	0.01	**1.85**	**1.39**
	00-6	1.86	0	0.01	**2.9**	**0.51**
	00-8[c]	2.43	0	0	**2.26**	**1.81**
	00-10	2.06	0	0	**1.94**	**1.82**
	00-12	1.78	0	0	**1.73**	**1.92**
Mean ± SD		2.02 ± 0.25	0 ± 0	0 ± 0	2.14 ± 0.47	1.49 ± 0.58
Leb-transgenic mice	00-14	3.68	0.02	**0.57**	**0.86**	**1.37**
	00-16[c]	4.28	0.01	**0.15**	**1.44**	**1.39**
	00-18	3.56	0.01	**0.46**	**0.52**	**1.14**
	00-20	2.1	0	**0.15**	**1.88**	**1.83**
	00-22	0.78	0.01	0	**0.19**	**1.27**
Mean ± SD		2.88 ± 1.42	0.01 ± 0	0.27 ± 0.24[d]	0.98 ± 0.69[e]	1.4 ± 0.26

[a]Bacterial counts (CFUs) were determined by homogenization of one third of the mouse stomach in sterile PBS (pH 7.4), followed by serial dilution on selective medium. The mean bacterial counts recovered from wild-type and transgenic mice were not significantly different.
[b]Phenotype was determined by standardized ELISA using monoclonal antibodies to Le^a, Le^b, Le^x, or Le^y, with values expressed as OD. ELISAs were performed in triplicate wells for each strain, and the OD values presented represent the mean of three independent experiments. An OD value ≥0.1, indicated in bold, is considered significant.
[c]Sweeps of *H. pylori* gastric isolates that were selected as sources for subculture to examine phenotypes of isolated colonies (Fig. 2).
[d]Significantly different than for populations isolated from wild-type mice (P = 0.001).
[e]Significantly different than for populations isolated from wild-type mice (P = 0.014).

AR6 amplified in 98-169 show that the middle region of *babA* was replaced by *babB*, indicating that gene replacement had occurred, providing independent confirmation of the previous findings in monkeys (Fig. S7; Solnick et al., 2004). On this basis, we turned to the mouse isolates. When we tested the inoculum strain 98-964 and one isolate each from the wild-type (03-261) and Le^b-transgenic mice (03-270), we found that the *babA* allele was intact in all cases (Fig. S7), supporting the hypothesis that there was no selection away from *babA*, and that its maintenance is important for mouse colonization, differentiating the findings in monkeys and mice.

Phenotypic diversity of single-colony *H. pylori* isolates

To analyze the diversity in Le expression within *H. pylori* populations recovered from the wild-type and transgenic mice 8 mo after challenge, single colonies were isolated from sweeps 00-8 (from a wild-type FVB/N mouse) and 00-16 (from an Le^b-transgenic mouse; Fig. S2). The single colonies isolated from sweep 00-8 only showed expression of Le^x and Le^y, with no detectable Le^a or Le^b expression, confirming results obtained from the gastric sweeps (unpublished data). In contrast, 9 out of 11 single colonies isolated from the 00-16 sweep expressed Le^a and/or Le^b in addition to Le^x and Le^y (Fig. 2 A). The phenotypic diversity within the bacterial populations derived from the gastric sweeps of these mouse stomachs is similar to that reported in humans (Wirth et al., 1999; González-Valencia et al., 2008). Expression of Le^a in five of the 00-16 single colonies, in addition to Le^b, indicates diminished *futC* activity in these isolates (Sanabria-Valentín et al., 2007; Nilsson et al., 2008), preventing substrate Le^a from being fucosylated to form Le^b (Fig. S1). The ratios of Le^a to Le^b and Le^x to Le^y expression in these single colonies are strongly correlated ($R = 0.89$; $P < 0.001$; Fig. 2 B), indicating that *futC* is governing the relative expression levels of the mono- and difucosylated antigens in tandem for the type I and II pathways (Fig. S1). From these studies, three strains were chosen for further genotypic and phenotypic analysis: HP1 (98-964, the inoculum strain from which Le^b was not detectable), 00-8B (03-261, a non-Le^b expressor isolated from a wild-type mouse), and 00-16A (03-270, an Le^b expressor isolated from an Le^b-transgenic mouse; Table I).

DNA sequence analysis of the Le antigen synthesis genes

Nucleotide sequence analysis was performed to determine the genetic basis for the phenotypic differences in Le antigen expression in the selected representative mouse-derived *H. pylori* strains. A β-(1,4) galactosyltransferase involved in Le^x and Le^y synthesis is encoded by *galT* (Fig. S1), which does not contain polynucleotide repeat regions; however, the upstream intergenomic region varies in length between strains (not depicted). Because sequence analysis showed that all three strains share an identical upstream region (unpublished data), their different Le antigen phenotypes were not attributed to *galT* locus variation.

Sequence analysis of the α-(1,3/4) fucosyltransferase gene (*futA*; Fig. S1), which affects both the type I and II Le synthesis pathways, showed that all three strains had identical in-frame *futA* alleles. Sequence analysis of *futB* (Fig. S1) revealed extensive intrastrain poly-C tract length variation among the three strains (Fig. S5), but overall there were no significant differences between the three strain populations. Thus, differential activity of the α-(1,3/4) fucosyltransferases in these strains does not correlate with the Le^b phenotypic differences observed but is consistent with the overall phenotypic variation in Le expression within the populations of cells, as reflected in the Le phenotypes of single-colony isolates (Fig. 2 A).

The poly-C tract of *futC* was cloned and sequenced for each strain (10 clones/strain) and, as expected, revealed

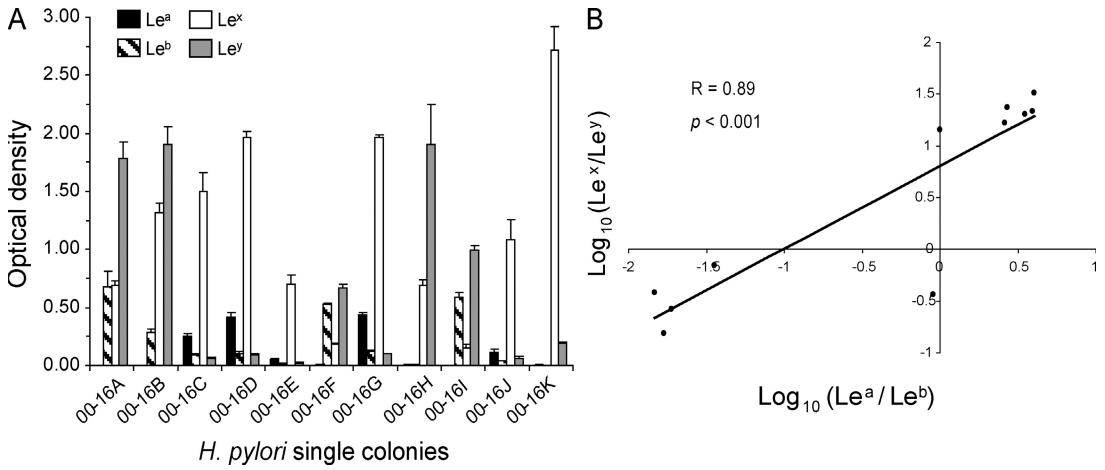

Figure 2. Le phenotypes of *H. pylori* single colonies isolated from wild-type and Le^b-transgenic FVB/N mice recovered after 8 mo. (A) 11 colonies, each independently picked, were isolated from *H. pylori* gastric sweep 00-16, recovered 8 mo after challenge from Le^b-transgenic mouse 16 (Table I), and studied. Standardized ELISAs, performed three independent times using monoclonal antibodies to Le^a, Le^b, Le^x, or Le^y, were performed in triplicate for each isolate, and the OD values presented from one representative experiment are shown (means ± SD). OD values ≥0.1 are considered significant. (B) Log_{10} Le mono- and difucosylated antigen expression ratios of 00-16–derived *H. pylori* strains (single colonies were picked and expanded for assay).

extensive intrastrain variation (Sanabria-Valentín et al., 2007). The primary sequence data suggest that only 2 (61C6 and 70C7) out of 30 intrastrain variants tested would produce a full-length α-(1,2) fucosyltransferase (Fig. S6 B). Although multiple mechanisms can be present (Wang et al., 1999), some α-(1,2) fucosyltransferase activity must exist in strains 98-964, 03-261, and 03-270, because all three produce Le^y and 03-270 also expresses Le^b. The distribution of poly-C tract lengths in Le^b-expressing 03-270–derived clones was significantly ($P < 0.01$ and $P < 0.001$) different from that in clones derived from 98-964 and 03-261, respectively, consistent with enhanced selection for Le^b expression in the Le^b-transgenic mice.

Nucleotide sequence analysis of *β-(1,3)galT*

Sequence analysis of *β-(1,3)galT* showed that the non-Le^b–expressing strains 98-964 and 03-261 were identical in sequence (Fig. 3 A). Both contain C_{14} poly-C tracts, whereas Le^b-expressing strain 03-270 has a C_{16} tract (Fig. 3 B). Based on the predicted (ATG) start codon of *β-(1,3)galT* in sequenced strain J99 (Fig. 3 A; Alm et al., 1999) as the putative translation start site, all three strains appear to have an out-of-frame *β-(1,3)galT* (Fig. 3 D). Thus, primary sequence data would not be sufficient to explain differences in Le antigen expression between these strains. However, if the alternate translation initiation codon (TTG), active in ∼9% of *H. pylori* genes (Alm et al., 1999), is used in these strains at the same position as the GTG start codon (∼8% of *H. pylori* genes) of *β-(1,3)galT* in reference strain 26695 (Fig. 3 A; Tomb et al., 1997), then frame status is determined by poly-C tract length. Strains 98-964 and 03-261 encode seven *β-(1,3)galT* heptad repeats versus four in strain 03-270 (Fig. 3 C); similar changes were observed in a previous mouse gastric challenge (Salaün et al., 2005).

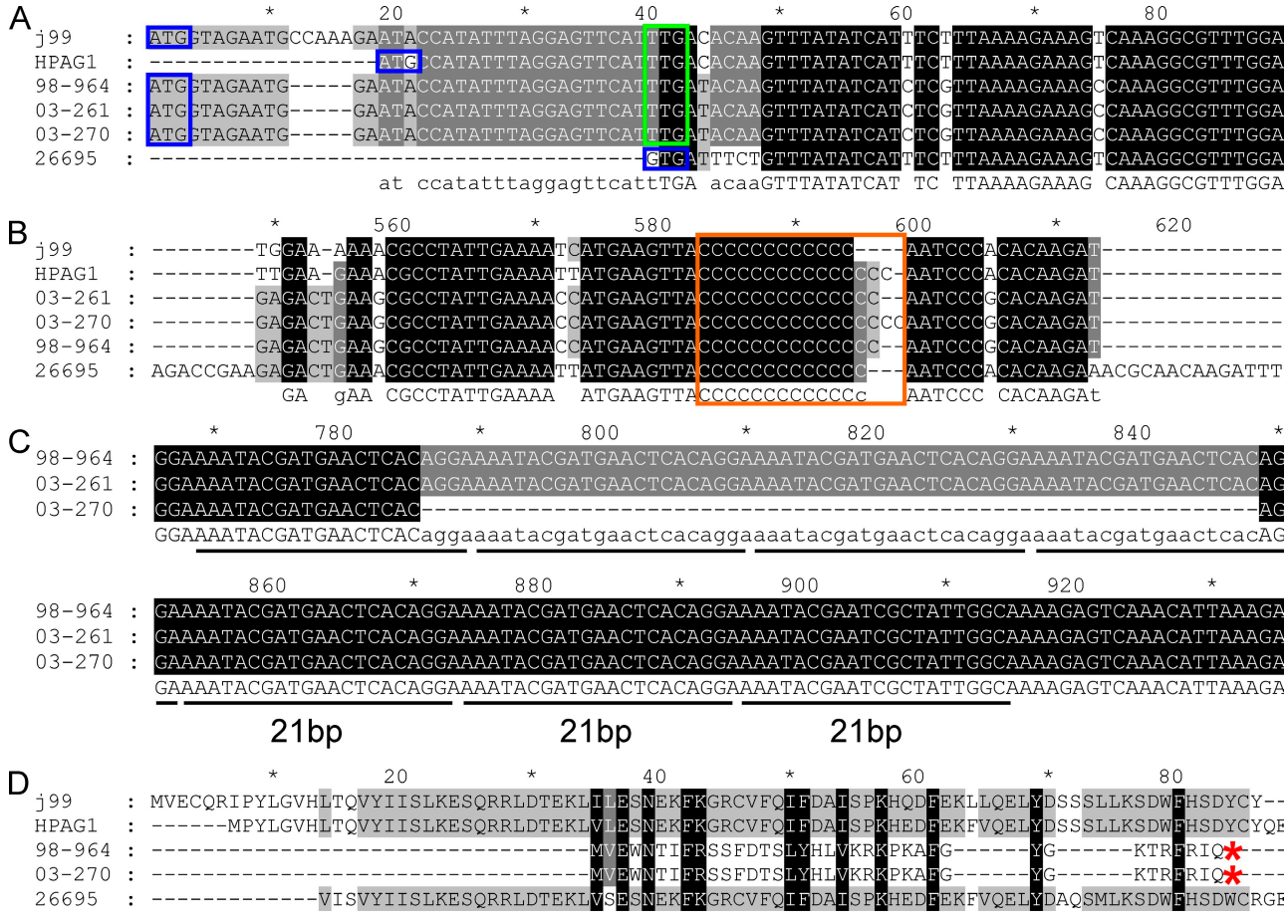

Figure 3. Nucleotide sequence alignments of informative *β-(1,3)galT* regions in three *H. pylori* reference strains and in three studied strains related to mouse infection. (A) For *H. pylori* reference strains J99, HPAG1, and 26695, and for the mouse-derived *H. pylori* strains 98-964 (inoculum), 03-261 (recovered from a wild-type mouse), and 03-270 (recovered from an Le^b-transgenic mouse), the 5′ regions including the putative translational initiation codons of *β-(1,3)galT* are boxed in blue. An alternative TTG start site (boxed in green and present in 8.1% of 26695 ORFs) aligned with the *H. pylori* 26695 *β-(1,3)galT* GTG start site (present in 9.7% of 26695 ORFs). (B) Homocytosine tract region, boxed in orange. (C) 21-bp repeat region, encoding KYDELTG repeats. (D) Predicted amino acid sequence of the 5′ end of *β-(1,3)galT*. Compared with strain J99, the *H. pylori* strains obtained from these mouse experiments contain a 5-bp deletion resulting in premature termination (red asterisks). The *β-(1,3)galT* sequence for strain 03-261 was identical to strain 98-964 (not depicted).

Mutagenesis of *β-(1,3)galT*

Because of these uncertainties, allelic replacement mutagenesis was performed to determine whether *β-(1,3)galT* is essential for *H. pylori* type I Le antigen expression (Fig. S8 B). A plasmid containing a copy of *β-(1,3)galT* interrupted by a nonpolar kanamycin resistance (*aphA-3*) cassette (p98B13k) was introduced into test *H. pylori* strains via natural transformation (Fig. S8 B), and insertion of the plasmid-encoded sequences was confirmed by PCR analysis of chromosomal DNA from transformed cells. Strains 99-8 and JP26 were included as positive controls for Le^a and Le^b phenotypic expression, respectively, and strain JP26 was transformed with pCTB8 (Cover et al., 1994) as a positive control for transformation. Introduction of p98B13k resulted in loss of Le^b expression in strains JP26 and 03-270 (Fig. 4 A), and loss of Le^a expression in strain 99-8 (not depicted). Also as expected, introduction of control plasmid pCTB8 into JP26 had no effect on Le antigen phenotype (unpublished data). In total, these studies provide evidence that open reading frame (ORF) *jhp0563* encoding the presumed *β-(1,3)galT* is essential for synthesis of type I Le antigens.

Complementation of *β-(1,3)galT* null mutants

To determine whether the loss of type I Le antigen expression was specifically caused by *β-(1,3)galT* inactivation and not an adventitious event, complementation studies were performed. To accomplish this, *jhp0563* from strain 03-270 was introduced in trans (p70B13comp) into *H. pylori* strains in which the native ORF had been interrupted (Fig. S8 C), and its placement was confirmed by PCR. Introduction of *β-(1,3)galT* at the *ureA* locus resulted in restoration of type I Le antigen expression in the JP26 and 03-270 *β-(1,3)galT* null mutants (Fig. 4 A). Complementation of strain 03-261 led to a low level but new expression of Le^b, which had not been observed in either the wild-type or mutant strains (Fig. 4 A). However, there was no complementation of Le^a expression in the 99-8 *β-(1,3)galT* null mutant and no de novo synthesis of Le^a or Le^b in strain 98-964 (unpublished data). Collectively, these results provide evidence that *β-(1,3)galT* is necessary, but not sufficient, for type I antigen expression.

Sequence analysis of complemented *β-(1,3)galT*

Because colonies of *H. pylori* represent a mixture of cells of varying genotypes (Appelmelk et al., 1998; Kuipers et al., 2000; Sanabria-Valentín et al., 2007), especially in reference to metastable loci such as homopolymeric tracts (Salaün et al., 2004; Sanabria-Valentín et al., 2007), we analyzed nucleotide sequences from the complemented strains. Analysis of the 5′ 500 nucleotides of the complemented copy of *β-(1,3)galT* revealed sequences identical to 03-270 wild-type *β-(1,3)galT* in all five complemented mutants tested. These results indicate that sequence variation in the 5′ region is not responsible for the phenotypic variation observed. However, as determined by direct PCR sequencing, poly-C tract lengths varied among the complemented mutant strains. Therefore, Le antigen phenotypes were determined by ELISA for 10 single colonies isolated from strain 03-270::p98B13k::p70B13comp. The Le^b expression in these strains ranged from the level of 03-270 wild type to no detectable expression (Fig. 4 B). The *β-(1,3)galT* heptad repeat region was identical in length in these single-colony isolates, indicating that variation in Le^b expression is not caused by variation in this region (unpublished data). To identify the basis of this variation, we examined genomic DNA from isolate 70C1A with high Le^b expression (Le^b = 0.74), from isolate 70C1E with an intermediate Le^b phenotype (Le^b = 0.25), and from isolate 70C1F with no detectable Le^b expression to PCR amplify the poly-C tract regions (OD values are shown). After cloning the products, sequence analysis of the recombinant pGEM-T Easy plasmids revealed extensive *β-(1,3)galT* poly-C tract length variation (Fig. 4 C). 4 out of 10 70C1A-based sequences contained C_{16} poly-C tracts, identical to 03-270 wild type, versus 2 out of 10 from the 70C1E sequences and 0 out 10 from the 70C1F sequences. The number of clones with a *β-(1,3)galT* C_{16} tract, identical to the 03-270 poly-C tract, correlated with the level of Le^b expression in each isolate. The 70C1A poly-C tract lengths (16.2 ± 1.48 nucleotides) and the 70C1E poly-C tract lengths (15.8 ± 2.04 nucleotides) were significantly ($P < 0.001$) longer than those in 70C1F (11.8 ± 0.79 nucleotides). Thus, within populations of *H. pylori* cells, *β-(1,3)galT* frame status as well as homopolymeric tract length vary extensively, critically affecting Le^b expression.

Flow cytometric analysis of *H. pylori β-(1,3)galT* mutants

To better understand the distribution of Le^b phenotypic expression within a population of *H. pylori* cells, wild-type strains and their *β-(1,3)galT* mutants were examined by flow cytometry. Cells were incubated with an anti-Le^b monoclonal antibody and anti–*H. pylori* polyclonal antibodies as a positive control. Cells of all three *H. pylori* strains (98-964, 03-261, and 03-270) were detectable with anti–*H. pylori*, essentially to the same extent (Fig. 5 B). As expected, when stained with antibodies directed against Le^b, the 03-270 wild-type strain showed the greatest levels of fluorescence, with a bimodal distribution, indicating that most but not all cells were Le^b positive (Fig. 5 C). In contrast, also as expected, the 03-270 *β-(1,3)galT* mutant showed fluorescence levels similar to the background level of cells stained with secondary antibodies alone (Fig. 5 C). The 03-270 *β-(1,3)galT*–complemented mutant was positive for Le^b, but the percentage of Le^b-positive cells was lower than in the wild type (Fig. 5, C and F), indicating that the complementation was partial. The net mean fluorescence intensity (MFI; region M1) for the Le^b-positive cell populations in the wild-type and complemented strains was similar, indicating that a proportion of the cells had been fully complemented (Fig. 5 G). Similar flow cytometry experiments performed on JP26 and 03-261 indicated that the complemented mutant populations were a mixture of Le^{b+} and Le^{b-} cells (Fig. 5, D and E). The Le^b positivity in the JP26-complemented cells was significantly ($P < 0.001$) lower than in the wild-type strains, confirming that complementation was partial and less efficient than in

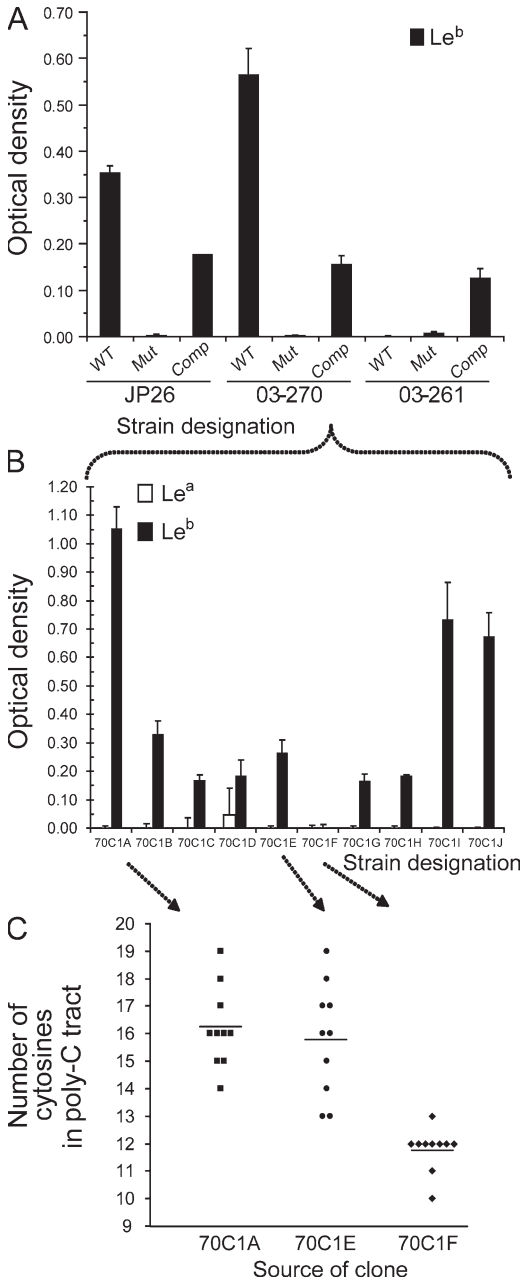

Figure 4. Le phenotypes and genotypes of *H. pylori* β-(1,3)galT mutant and complemented strains. (A) Le phenotypes of *β-(1,3)galT* mutant (Mut) and complemented (Comp) strains. Wild-type (WT) strains were JP26 (Le^b expressing), 03-270 (Le^b-transgenic mouse isolate), and 03-261 (wild-type mouse isolate). Phenotype was determined via standardized ELISAs using monoclonal antibodies to Le^a, Le^b, Le^x, or Le^y, and expressed as OD values. ELISAs were performed in triplicate for each strain, and the OD values represent the means of the triplicates. An OD value ≥0.1 is considered significant. (B) Le phenotypes of single colonies derived from *H. pylori* strain 03-270 *β-(1,3)galT*–complemented mutant. 10 independent colonies of the 03-270 *β-(1,3)galT*–complemented mutant were picked from a plate streaked for isolation and phenotyped by ELISA. ELISAs were independently performed three times, and one representative experiment is presented in A and B. (C) *β-(1,3)galT* poly-C tract lengths of isolates 70C1A (Le^b = 0.74), 70CC1E (Le^b = 0.25), and 70C1F (Le^b = 0). The *β-(1,3)galT* poly-C tract was PCR amplified and cloned, and 10 independent clones per isolate were chosen for sequence analysis. The mean poly-C tract length for each strain is depicted as a horizontal bar.

strain 03-270 (Fig. 5 F). However, as in strain 03-270, net MFI levels showed full complementation in a portion of the population (Fig. 5 G). Collectively, these results paralleled phenotypes determined by ELISA (Fig. 4 A) and define the range of Le^b phenotypes exhibited by wild-type *H. pylori* strains and their *β-(1,3)galT* mutants.

DISCUSSION

These studies showed that experimentally challenged wild-type and Le^b-transgenic FVB/N mice can stably maintain *H. pylori* gastric colonization for up to 8 mo, regardless of Le antigen expression, consistent with studies in other mouse genetic backgrounds (Takata et al., 2002; Lozniewski et al., 2003). That *H. pylori* populations expressing Le^b were somewhat more efficient than non-Le^b expressors in colonizing Le^b-transgenic mice (Table I) may reflect greater host tolerance to self-antigens, although future studies are needed to confirm this observation. This phenomenon also could be attributed to enhanced adhesion of bacterial cells to the gastric epithelia via BabA binding to host Le^b, because unlike in monkeys (Solnick et al., 2004), *babA* recovered from the mice 8 mo after challenge showed intact copies of *babA* and no evidence of gene conversion (Fig. S7). The host Le^b–bacterial BabA interaction also could be a target of selection. Enhanced bacterial Le^b expression could result in BabA-mediated bacterial aggregation, contributing to the higher colonization densities observed in Le^b-transgenic mice; further experimentation is required to test this hypothesis.

Selection for Le^b expression among *H. pylori* cells persistently colonizing Le^b-positive transgenic but not Le^b-negative wild-type mice is consistent with increased fitness of *H. pylori* variants that match the Le phenotype of their host, extending previous studies in rhesus monkeys (Wirth et al., 2006) and in some (Wirth et al., 1997) but not all (Taylor et al., 1998; Heneghan et al., 2000) human studies. One hypothesis to explain the appearance of Le^b expression in *H. pylori* recovered from the transgenic mice 8 mo after challenge is the development of an anti-Le^x response in these mice, which is consistent with the lower expression of Le^x in the *H. pylori* cells recovered from the Le^b-transgenic mice after 8 mo. Such a response would provide selection for the expansion of Le expression variants, a phenomenon that has been observed previously in *Neisseria meningitidis* (Bayliss et al., 2001). To test this hypothesis, we determined levels of anti-Le antibodies by ELISA using purified LPS with well-defined Le antigen phenotypes. The results showed a wide range in responses among the mice, with no correlation between mouse genotype and antibody response. Further, antibody responses were independent of both LPS Le phenotype and colonization density of the mice. These results

provide evidence that serum antibody pressure was not the driving force behind the emergence of bacterial Le^b expression. However, our observations are limited by a small sample size and an inability to directly test for anti-Le^a and anti-Le^b antibody levels. Future studies are needed to further investigate this hypothesis.

Sequence analysis showed that selection of Le^b-positive *H. pylori* variants in the transgenic mice was mediated by phase variation of *β-(1,3)galT*. Solnick et al. (2004) provided evidence that phase variation of *babA* occurred in vivo, supporting the hypothesis that this is a gene regulation mechanism used by *H. pylori* to adapt to changing host environments.

Experimental challenge of wild-type mice and gerbils for up to 5 mo with *H. pylori* isolates led to no substantial Le antigen expression diversification (Wirth et al., 1999). Similarly, when wild-type FVB/N and Le^b-transgenic mice were experimentally challenged for 8 or 16 wk, no change in Le antigen expression was detected (Guruge et al., 1998). One explanation for those results is that there was no phenotypic selection, or alternatively, that the *H. pylori* founding populations were too small and/or colonization periods too short for detection of differential fitness (Webb and Blaser, 2002). That no changes were observed before 8 mo in the present studies may reflect both the relatively low level of *H. pylori* colonization of conventionally raised mice compared with humans (Atherton et al., 1996) and/or relatively small differential fitness (Webb and Blaser, 2002) because of bacterial Le^b expression in the Le^b-transgenic mice.

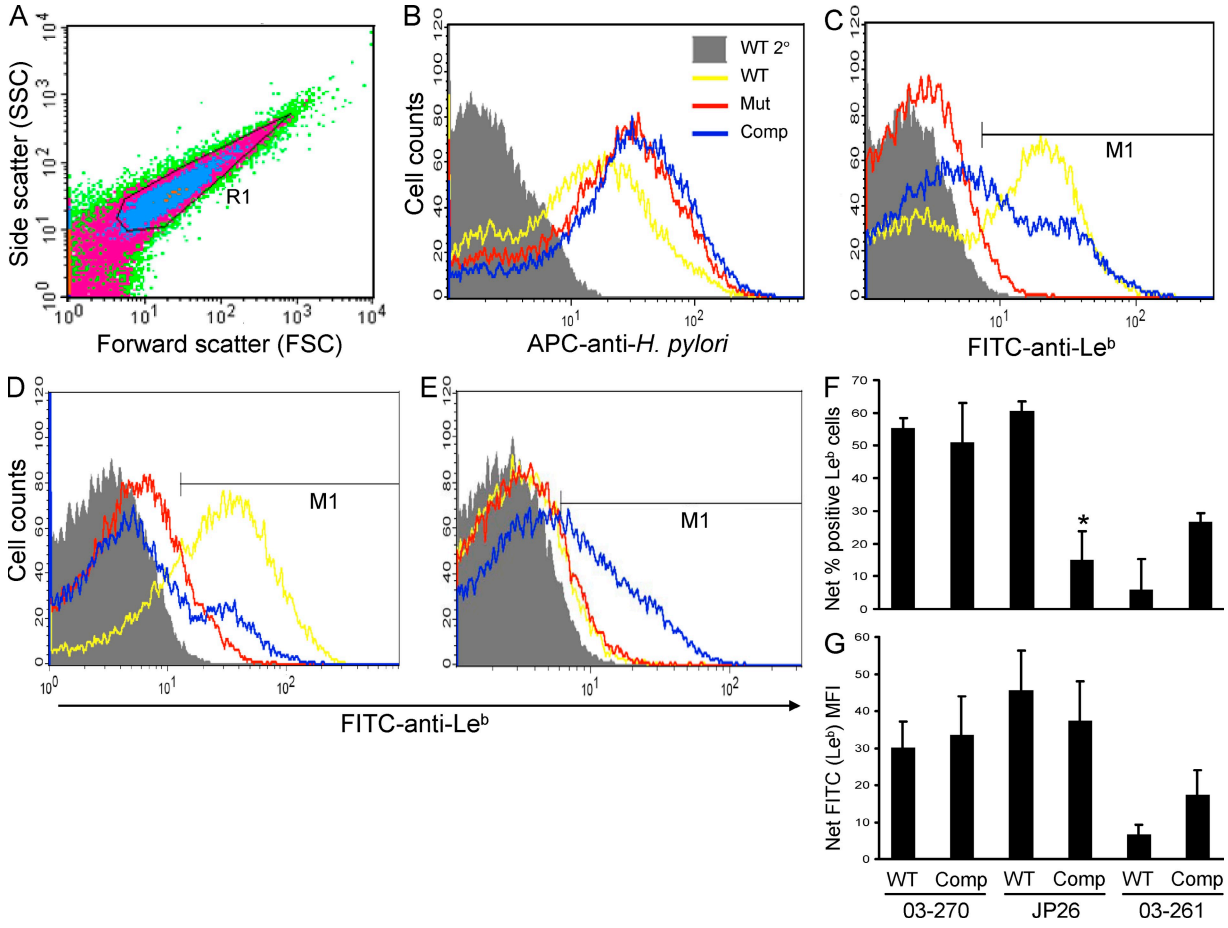

Figure 5. Le^b expression by *H. pylori* wild-type and *β-(1,3)galT* mutants. *H. pylori* cells were incubated with mouse anti-Le^b IgM and human anti–*H. pylori* IgG and detected with fluorescent secondary (2°) antibodies. Irrelevant staining in the absence of anti-Le^b or anti–*H. pylori* are displayed for cells incubated with 2° antibodies alone. (A) Dot plot displaying forward and 90° angle scattered laser light intensities of PI-labeled bacterial cells. Intact bacteria were selected using region R1. For each sample 20,000 R1-gated events were counted (green, 30% log density; pink, 9% log density; blue, 2.7% log density; orange, 0.81% log density). (B) Histograms comparing wild-type *H. pylori* strain 03-270 (WT), *β-(1,3)galT* null mutant (Mut), and *β-(1,3)galT*–complemented mutant (Comp) bacteria labeled with allophycocyanin–anti–*H. pylori*. (C–E) Histograms of *H. pylori* strains 03-270 (C), JP26 (D), and 03-261 (E) labeled with FITC–anti-Le^b. Region M1 denotes the Le^b-positive cell populations, as determined by the crossover point between the wild-type and *β-(1,3)galT* null mutant plots. (F) Net percentage of Le^b-positive cells in *β-(1,3)galT* mutant strains. Net values were calculated by subtracting the percentage of *β-(1,3)galT* null mutant cells in region M1 from the percentage of wild-type and complemented cells in M1. There were significantly (*, $P < 0.001$) fewer Le^b-positive cells in the JP26 *β-(1,3)galT*–complemented mutant cell population than the JP26 wild-type cell population. (G) Net FITC MFI. Values were normalized based on the number of Le^b-positive cells, and net values were calculated as in F (means ± SD). C and F represent three independent experiments.

Sequence analysis of *β-(1,3)galT* in HP1 and its descendants revealed a 5′ 5-bp deletion compared with *H. pylori* reference strain J99, suggesting either a truncated ORF or that the annotated 5′ ATG is not the initiation codon in these strains. Because Le^b is expressed in strain 03-270, a more downstream start codon may be used for translation, translational frameshifting is occurring, or *β-(1,3)galT* is undergoing recombination with its upstream homologue, *jhp0562*, in some *H. pylori* cells within the population. TTG present in some strains at the same position as the 26695 initiation GTG (Fig. 3 A) may be an alternative initiation codon (Alm et al., 1999), representing another translational control locus of Le synthesis gene expression.

Our findings indicate that as with *H. pylori futA*, *futB*, and *futC*, poly-C tract length likely regulates *β-(1,3)galT* translation, with C_{16} in 03-270 critical for Le^b expression. The three strains tested also differed at the *β-(1,3)galT* heptad repeat region, paralleling observations in another mouse challenge experiment (Salaün et al., 2005), potentially explaining *β-(1,3)galT* enzymatic activity variation, paralleling that shown in *futA* and *futB* (Ge et al., 1997; Lin et al., 2006; Ma et al., 2006; Nilsson et al., 2006).

Although the inoculum strain HP1 expressed Le^x and Le^y in relatively equal proportions, the gastric sweeps and single colonies varied greatly in their Le antigen phenotypes. That several single colonies isolated from within the Le^b-expressing bacterial populations also expressed Le^a, which was not detected in the parental strain, indicates *futC* phase variation among the strains, consistent with the extensive sequence variation observed (Fig. S6). Diversity in *futC* sequences in differing strain backgrounds (Alm et al., 1999; Wang et al., 1999; Salaün et al., 2004; Salaün et al., 2005; Sanabria-Valentín et al., 2007) may reflect strain-specific adaptations that have evolved to facilitate Le expression control, particularly the tension between mono- and difucosylated forms (Wang et al., 1999; Rasko et al., 2000; Kang and Blaser, 2006a).

Loss of type I Le antigen expression in a *β-(1,3)galT* mutant confirms a previous report (Appelmelk et al., 2000) that in trans complementation of *β-(1,3)galT* restored the type I pathway phenotype lost in null mutants, and introduced Le^b expression into a strain previously negative for type I antigens expands on these previous studies. The lower type I phenotypes in the complemented strains compared with wild type probably reflects lower *β-(1,3)galT* expression from the heterologous *ureA* promoter than from the wild-type promoter. Alternatively, the complemented strains are cell populations mixed in *β-(1,3)galT* frame status because of poly-C tract phase variation, as was indicated by sequence analysis.

Flow cytometric analysis of Le^b phenotypes in wild-type and mutant *H. pylori* strains confirmed results determined by ELISA, providing an alternative phenotyping method. Although ELISA determines the overall phenotype of a cell population, flow cytometry determines the phenotype of each individual *H. pylori* cell, providing an indication of the population structure, which is highly advantageous for studying genetically diverse organisms. The results indicate that the descendants of the complemented strain vary in their Le^b phenotype, and as indicated by the sequence data, generated by phase variation of the metastable poly-C tracts of *β-(1,3)galT*, *futA*, *futB*, and *futC*.

In conclusion, we have developed a tractable animal model to examine the hypothesis that changes in Le phenotypes are a mechanism used by *H. pylori* to adapt to specific host milieus. Our studies provide evidence that *H. pylori* can change Le phenotype to the type I pathway in vivo to match the Le phenotype of its host, extending previous studies in humans and rhesus monkeys (Wirth et al., 1997; Wirth et al., 2006). That the pathway involves phase-variable Le antigen synthesis genes provides a mechanistic explanation for the observed phenotypic changes, and can be harnessed to allow quantitative analysis of the force of selection.

MATERIALS AND METHODS

Animals. Wild-type and Le^b-transgenic FVB/N mice breeding pairs were provided by the Washington University Animal Facility, and were bred and maintained under specific pathogen-free conditions in microisolators in a barrier facility, as previously described (Falk et al., 1995; Guruge et al., 1998). All experiments using mice were performed using protocols approved by the Animal Studies Committee of Washington University. DNA was extracted from the mouse tails using the QIAamp Tissue Kit (Promega) and used as a template in two PCR reactions to determine the mouse genotype: one with primers specific for α-actin (control; gene amplified in all mice), and one with primers specific for *hGH* (only present in transgenic animals; Table S2).

Challenge of mice with *H. pylori* strain HP1. Mice were challenged with *H. pylori* strain HP1, isolated from a Peruvian patient with gastritis (Guruge et al., 1998). Before inoculation, *H. pylori* strain HP1 was grown for 24 h and harvested in Brucella broth. Cell concentration was adjusted to an OD of 2 ($\sim 6 \times 10^7$ cells) at 550 nm, and 0.4 ml of cell suspension was used to inoculate, via orogastric gavage, 20 wild-type FVB/N and 20 Le^b-transgenic FVB/N mice. Orogastric challenge was repeated for three consecutive days. At 4 wk, 8 wk, 4 mo, and 8 mo, five mice of each genotype were sacrificed and their stomachs were removed. One third of each stomach was homogenized in 1× PBS (pH 7.4), and undiluted and 1:10 diluted suspensions were plated on Skirrow's medium agar plates (BBL Microbiology Systems) and incubated for ∼72 h at 37°C and 5% CO_2. CFUs were determined for each gastric sweep and cell populations were harvested; 10–11 single colonies were picked from each gastric sweep and expanded for further analysis.

Bacterial strains and growth conditions. *H. pylori* strains used in this study were routinely grown on Trypticase soy agar/5% sheep blood plates (BBL Microbiology Systems) or Brucella agar (BA) with 10% newborn calf serum (NCS) supplemented with the appropriate antibiotic (e.g., vancomycin, kanamycin, or chloramphenicol). *H. pylori* strains JP26, a wild-type Le^b-positive strain isolated in Japan, and 99-8, an Le^a-positive strain, were included as controls in mutagenesis and complementation experiments.

Determination of Le antigen phenotypes. *H. pylori* Le antigen phenotypes were determined by ELISA using monoclonal antibodies to Le^a, Le^b, Le^x, or Le^y (Signet Laboratories, Inc.) and protocols described previously (Wirth et al., 1996). ODs at 410 nm were determined on a microplate reader (MRX; Dynatech Laboratories Inc.). Corrected OD values were determined by averaging the OD values of two or three wells per sample and subtracting the blank (*Escherichia coli* strain HB101).

Determination of anti–*H. pylori* antibody levels in mice. Levels of anti–*H. pylori* antibodies in mouse sera were determined by ELISA, essentially as previously described (Wirth et al., 1997). Mouse sera were tested against *H. pylori* strains 98-964 and J166 whole-cell antigens, as well as the following

LPS antigens: wild-type strain J166 ($Le^x = 0$; $Le^y = 3.36$), J166 Δ*futA*/Δ*futB* ($Le^x = 0$; $Le^y = 0$), and J166 Δ*futC* ($Le^x = 2.22$; $Le^y = 0$; a gift of E. Sanabria-Valentín, New York University School of Medicine, New York, NY).

DNA sequence analysis of Le antigen synthesis genes. Three *H. pylori* isolates were chosen for sequence analysis: HP1 (98-964; inoculum strain), 00-8B (03-261; non-Le^b expressor, isolated from a wild-type mouse), and 00-16A (03-270; Le^b expressor, isolated from an Le^b-transgenic mouse). Isolates were grown for 48 h, and harvested in 1ml sterile PBS. Cells were pelleted for 5 min at ∼4,300 *g*, and the extracts were prepared for genomic analysis with the Wizard Genomic DNA Purification Kit (Promega).

Genomic DNA recovered from these strains was used as a template for PCR amplification of the Le antigen synthesis genes using primers specific for each known gene (Table S2). PCR amplification was verified with agarose gel electrophoresis, and products were purified using a PCR purification kit (QIAGEN), quantified, and subjected to sequence analysis (SeqWright, Inc.). Because of the inherent difficulties of analyzing long homonucleotide repeat regions and regions with a high GC content (Stirling, 2003), the poly-C tract regions of *β-(1,3)galT*, *futB*, and *futC* were amplified with primers specific to these regions (Table S2). PCR products underwent direct sequence analysis or were cloned into pGEM-T Easy (Promega), and recombinant plasmids were analyzed by standard sequencing methods (SeqWright, Inc.) and by using a protocol for GC-rich DNA (GeneWiz).

To further characterize the intrastrain variation in the homonucleotide regions of *futB* and *futC*, the pGEM-T Easy plasmids containing PCR fragments of these regions were transformed into competent *E. coli* DH5α (Invitrogen) and plated on Luria-Bertani agar plates with 50 μm/ml X-gal (Thermo Fisher Scientific) and 100 μm/ml ampicillin. For each strain, 5–10 transformants were selected, and the recombinant plasmids were purified and subjected to sequence analysis with the universal primers T7F and SP6R (Promega). To determine the nucleotide sequence of the 5′ region of the complemented copy of *β-(1,3)galT*, PCR amplification was performed using primers A17476 and jhp0563(+482)R (Table S2), and products were purified and sequenced as described. To determine the length of the poly-C tract in the complemented mutants, 10 single colonies were isolated from 03-270::p98B13k::p70B13comp and their Le antigen phenotypes were determined. Subsequently, the isolates with the highest, lowest, and intermediate Le^b expression provided template genomic DNA for PCR amplification of the *β-(1,3)galT* poly-C tract region using primers jhp0563(+351)F and Gal(1,3)R(+770). PCR products were cloned into pGEM-T Easy, recombinant plasmids were transformed into *E. coli* as described, recombinant plasmids were purified from 10 single transformants per strain, and DNA sequence analysis was performed using primers jhp0563(+351)F and Gal(1,3)R(+770).

Construction of *β-(1,3)galT* null mutants. To disrupt *β-(1,3)galT*, a knockout plasmid, p98B13k, was constructed. The *β-(1,3)galT* ORF was amplified from strain 98-964 (HP1) with primers Jhp0562(+630)F and Jhp0564(−10)R (Table S2), and the 1.8-kb product was cloned into pGEM-T Easy. A nonpolar kanamycin resistance cassette (*aphA-3*) was amplified from pUCK18K2 (a gift from H. de Reuse, Institut Pasteur, Paris, France; Ménard et al., 1993) and inserted into a unique *HindIII* site to interrupt *β-(1,3)galT*. Orientation of the insertion was confirmed by restriction digestion using *EcoRI* and *EcoRV*. Transformation of *H. pylori* strains with p98B13k was performed essentially as previously described (Israel et al., 2000), and transformation with pCTB8 (Cover et al., 1994) was included as a positive control. Transformants were selected on BA plus 10% NCS containing 10 μg/ml vancomycin and 25 μg/ml kanamycin. Confirmation of insertion of the plasmid into the chromosome was confirmed by PCR using primers Jhp0562(+630)F and Jhp0563(+482)R (Table S2). The Le antigen phenotypes of all mutants were determined by ELISA as described.

Complementation of *β-(1,3)galT* null mutants. To restore *β-(1,3)galT* activity in *H. pylori*, a complementation plasmid, p70B13comp, was introduced into the *β-(1,3)galT::aphA-3* strains via natural transformation (Israel et al., 2000). The complementation plasmid was created using pADC::*uvrD* (pHPuD), a plasmid containing the *H. pylori ureA/ureB* genes interrupted by a chloramphenicol resistance cassette (Kang and Blaser, 2006b). A copy of *β-(1,3)galT*, amplified from strain 03-270 (Le^b positive) using primers B(1,3)compF1 and B(1,3)compR2 (Table S2), was inserted just downstream of the *ureA* promoter. Transformants were selected based on chloramphenicol resistance and were rapidly screened for disruption of the *ureA/ureB* locus by the urease test. Insertion of *β-(1,3)galT* into the *ureA/ureB* locus was confirmed by PCR using chromosomal DNA from the transformed strains that had urease-negative, chloramphenicol-resistant phenotypes, and the Le antigen phenotypes were determined by ELISA. Transformants were not recovered for any strains tested when p70B13comp was used as donor DNA, with the exception of JP26. Thus, to increase the efficiency of transformation, first a streptomycin-resistant derivative of 98-964 transformed with p98B13k and p70B13comp was selected, and after verification of genotype, chromosomal DNA from this mutant was used as donor DNA in a subsequent transformation with the remaining *β-(1,3)galT* null mutants.

Immunofluorescent labeling and flow cytometry. For each *H. pylori* strain examined by flow cytometry, one plate of cells was harvested and resuspended in 1 ml of sterile saline and pelleted at 8,000 krpm for 5 min, washed, pelleted, and resuspended in 1.2 ml saline. The cell suspension was then passed through a 40-μm cell strainer (Falcon; BD), and 50-μl aliquots were pelleted and resuspended in 100 μl 1% Tween 20 in PBS buffer (pH 7.4) to prevent cell aggregation. Cells were incubated for 30 min with 10 μl of 1:25 diluted anti-Le^b monoclonal antibody (mouse IgM) as described, washed in Tween-PBS buffer to remove unbound antibody, and resuspended in buffer. As a positive control, cells were also incubated for 30 min with 10 μl of 1:25 diluted high titer serum from an *H. pylori*–positive patient (Blaser et al., 1995). Cells were washed again, resuspended, and stained for 20 min simultaneously with 10 μl of 20 μg/ml Alexa Fluor 488 goat anti–mouse IgM (μ chain specific) and Alexa Fluor 647 goat anti–human IgG (human specific; Invitrogen). The cells were washed again and resuspended in 100 μl of buffer. Then, 400 μl of 1.25% formaldehyde in saline was added, followed by 5 μl of 0.1 mg/ml propidium iodide (PI). Cells were allowed to fix overnight at 4°C. As controls, cells were reacted with the secondary antibodies alone. Flow cytometric analyses were performed on a FACSCalibur (BD). PI fluorescence intensity was used to discriminate cells from inorganic particulates. Forward scatter and 90° angle scattered laser light intensities were used to distinguish intact bacteria (R1) from cellular debris (Fig. 5 A). For each sample, 20,000 R1-gated events were acquired in listmode and subsequently analyzed using CellQuest Pro software (BD).

Statistical analyses. The Welch two-sample *t* test and the Mann-Whitney test were used where appropriate, with $P < 0.05$ considered significant. Ratios of Le^a to Le^b expression of 0 for 00-16–derived isolates were adjusted to 0.01 to calculate $\log_{10}$ values in Fig. 2 B.

Online supplemental material. Fig. S1 outlines *H. pylori* Le antigen synthesis pathways. Fig. S2 is a schematic of the experimental challenge of wild-type and Le^b-transgenic mice with *H. pylori*. Fig. S3 shows the Le^x and Le^y phenotypes of *H. pylori* populations recovered 4 wk to 8 mo after challenge of wild-type and Le^b-transgenic mice. Fig. S4 shows serum levels of anti–*H. pylori* IgG in mice after challenge with strain HP1 (98-964). Table S1 shows serum levels of anti-LPS antibodies after challenge with strain HP1 (98-964). Fig. S5 shows the variation in *futB* in clones derived from *H. pylori* strain 98-964 and progeny. Fig. S6 shows the variation of *futC* in clones derived from *H. pylori* strain 98-964 and progeny. Fig. S7 shows PCR amplification of the *babA* locus in monkey- and mouse-derived *H. pylori* isolates. Fig. S8 shows the complementation strategy for *β-(1,3)galT*. Table S1 shows serum levels of anti-Le antigen IgG in mice 4 or 8 mo after challenge with *H. pylori* strain HP1. Table S2 lists the oligonucleotide primers used in this study. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20090683/DC1.

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