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Host Determinants of Expression of the *Helicobacter pylori* BabA Adhesin

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Expression of the *Helicobacter pylori* blood group antigen binding adhesin A (BabA) is more common in strains isolated from patients with peptic ulcer disease or gastric cancer, rather than asymptomatic colonization. Here we used mouse models to examine host determinants that affect *H. pylori* BabA expression. BabA expression was lost by phase variation as frequently in WT mice as in RAG2^{-/-} mice that do not have functional B or T cells, and in MyD88^{-/-}, TLR2^{-/-} and TLR4^{-/-} mice that are defective in toll like receptor signaling. The presence of other bacteria had no effect on BabA expression as shown by infection of germ free mice. Moreover, loss of BabA expression was not dependent on Le^b expression or the capacity of BabA to bind Le^b. Surprisingly, gender was the host determinant most associated with loss of BabA expression, which was maintained to a greater extent in male mice and was associated with greater bacterial load. These results suggest the possibility that loss of BabA expression is not driven by adaptive immunity or toll-like receptor signaling, and that BabA may have other, unrecognized functions in addition to serving as an adhesin that binds Le^b.

Helicobacter pylori infects the gastric mucosa of about 50% of the world's population¹. The majority of those infected have only asymptomatic gastritis, but about 10% develop peptic ulcer and 1–3% develop gastric cancer^{1–3}, which is the third most common cause of cancer death worldwide (~1 million cases per year). Given the large number of infected individuals, increasing development of antibiotic resistance^{4,5}, and accumulating evidence that in some people *H. pylori* may be beneficial^{6–9}, treatment of all infected individuals may not be warranted. Therefore, it is important to determine the elements of the host-*H. pylori* interaction that influence whether an individual will develop clinical disease or asymptomatic infection. One risk factor associated with more severe disease outcomes is the virulence factor, blood group antigen binding adhesin (BabA), which belongs to a family of *H. pylori* outer membrane proteins¹⁰ that also includes LabA¹¹, SabA¹², and the recently characterized HopQ^{13,14}. BabA is a well-characterized adhesin^{15–18} that binds to ABO blood group antigens, fucosylated carbohydrates expressed on the gastric epithelium and the protective mucus layer. BabA exhibits highest affinity for Lewis b (Le^b)¹⁹, owing to a polymorphic, three-pronged carbohydrate binding domain identified recently by X-ray structural analysis^{20,21}. Epidemiologic studies of an association of BabA with disease^{22,23} are supported by *in vitro* evidence that BabA-mediated attachment to host gastric epithelium facilitates translocation of the CagA oncoprotein into host cells²⁴. Translocation occurs via the type IV secretion system encoded on the cytotoxin associated gene pathogenicity island (*cagPAI*), itself a well-recognized risk factor for disease^{25–29}.

BabA mediated attachment and development of disease are influenced by host expression of Lewis antigens, which is determined by a number of factors, including ABO blood type and secretor status^{30–33}. The risk of ulcer is increased in individuals with blood group O, and in non-secretor individuals who do not express Le^b and ABO antigens on gastric epithelial cells or on mucins^{30–34}. Thus, disease outcome is related to both bacterial expression of the BabA adhesin and to ABO glycosylation on gastric epithelial cells and gastric mucins.

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Strain	Description	Antibiotic Resistance ^a	Source (Reference)
J166	Wild Type		35
J99	Wild Type		78
26695	Wild Type		36,78
J166Δ <i>babA</i> _{upstream}	J166 with the upstream portion of <i>babA</i> replaced by CAT_rpsL (transformed with pBabA)	Cm	This study
J166 _{sc}	Single colony isolate of J166	Str	This study
J166 _{7CT}	J166 engineered with 7 CT repeats in the 5' end of <i>babA</i>	Str	This study
J166 _{8CT}	J166 engineered with 8 CT repeats in the 5' end of <i>babA</i>	Str	This study
J166Δ <i>babA</i>	J166 with <i>babA</i> replaced by CAT_rpsL	Cm	20
J166 BabACL2	J166 with Cys 189 and 197 in BabA replaced with Ala	Str	20
J166 BabACL2 _{8CT}	J166BabACL2 engineered to have 8 CT repeats in the 5' end of <i>babA</i> , non-binding BabA is expressed	Str	This study
J166 BabACL2 _{9CT}	J166BabACL2 engineered to have 9 CT repeats in the 5' end of <i>babA</i> , BabA is not expressed	Str	This study

Table 1. Bacterial strains. ^aCm, chloramphenicol; Str, streptomycin.

Previous studies have shown that *H. pylori* expression of BabA is lost during the first 2–12 weeks of infection using animal models such as rhesus macaques, gerbils, and mice^{35–37}. Loss of BabA expression has been observed to occur by two mechanisms. In rhesus macaques, recombination between the *babA* gene and its *babB* paralog can result in duplication of all or part of the *babB* gene into the *babA* locus, resulting in loss of BabA expression and reciprocal overexpression of BabB. Alternatively, in mice and in macaques, slipped strand mispairing of a CT repeat region in the 5' portion of the *babA* open reading frame (ORF) can lead to a frame shift, resulting in an early stop codon within the *babA* ORF and loss of BabA protein expression. That this phenomenon occurs across several animal models, and that loss of BabA expression can also be seen in human clinical isolates³⁸, suggests that modulation of BabA expression is an important component of the *H. pylori*-host relationship.

Loss of BabA expression might occur as a bacterial mechanism of persistence to adapt to changing levels of inflammation, glycosylation patterns, or a combination of both. Here we used knockout and transgenic mouse models to determine the host factors that affect BabA expression. Surprisingly, the results suggest that adaptive immune responses and toll-like receptor signaling play little if any role in loss of BabA expression, and that the capacity to bind Le^b is not required, but that gender-specific physiological differences may be important.

Methods

Bacterial strains and growth conditions. A complete list of bacterial strains used in this study is provided in Table 1. *H. pylori* cultures were maintained on Brucella agar (Becton, Dickinson and Company, Sparks, MD) supplemented with 5% heat inactivated newborn calf serum (Gibco, Grand Island, NY) and antibiotics (Sigma-Aldrich, Inc., St. Louis, MO), either TVPA (5 μg/mL trimethoprim, 10 μg/mL vancomycin, 2.5 units/mL polymyxin B and 2.5 μg/mL amphotericin B) for laboratory adapted cultures or ABPNV (100 μg/mL vancomycin, 3.3 μg/mL polymyxin B, 200 μg/mL bacitracin, 10.7 μg/mL nalidixic acid and 10 μg/mL amphotericin B) for primary cultures from infected mice. All *H. pylori* cultures were maintained at 37 °C in a CO₂ incubator or in an AnoxomatTM jar (Advanced Instruments, Inc., Norwood, MA) adjusted to contain 5% oxygen, 7.6% carbon dioxide, and 7.6% hydrogen³⁹.

Site directed mutagenesis. Isogenic mutants of *H. pylori* J166 were engineered with 7 (no ORF) or 8 (ORF) CT repeats in the 5' coding region of *babA* using a contraselection approach modified from that previously described³⁶. Briefly, 244 base pairs upstream of the *babA* translational start site and 39 base pairs of 5' *babA* gene sequence were replaced with a CAT_rpsL cassette in a streptomycin resistant strain of *H. pylori* J166, using the plasmid pBabA as previously described³⁹. The CAT_rpsL cassette was amplified using primers RpsLF and CamR (Table 2)³⁶. The pBabA plasmid was constructed by ligating CAT_rpsL sequence between two PCR amplicons. The first was a 1211 bp amplicon containing *hypD* and intergenic sequence 244 bp upstream of *babA* in *H. pylori* J166 (primers HypDF and BabApmR, Table 2), and the second amplicon contained 996 bp of *babA* starting 39 bp downstream of the *babA* start site (primers BabAF and BabAR, Table 2). The resulting *babA* knockout strain was then transformed using genomic DNA from primary mouse output strains containing either 7 or 8 CT repeats in the 5' coding region of *babA*. Transformants containing the desired number of CT repeats were selected by plating on streptomycin followed by replica plating to confirm loss of chloramphenicol resistance. The same approach was used to generate 8 (ORF) or 9 (no ORF) CT isogenic variants of J166 BabACL2, a site directed mutant of J166 in which Cys to Ala replacements at residues 189 and 197 result in a BabA protein that is expressed but cannot bind Le^b²⁰. All mutants were sequenced using the method of Sanger to confirm the correct CT repeat structure.

Mouse strains, housing and breeding. The local Institutional Animal Care and Use Committee approved all mouse experiments performed at Should read, University of California, Davis and the University of Michigan. Experiments performed at Umeå University, Umeå, Sweden were approved by Umeå Ethical Committee on Animal Research. All methods involving mice were performed according to the locally approved guidelines. Mice (Supplementary Table S1) were housed in sterilized, ventilated microisolator cages and given

Primer	Restriction Site	Sequence (5'-3') ^a
Construction of pBabA		
RpsLF	SacI	AAC <u>GAGCTC</u> GAT GCT TTA TAA CTA TGG ATT AAA CAC
CamR	BamHI	AAC <u>GGATCC</u> TTA TCA GTG CGA CAA ACT GGG AT
HypDF	NotI	AAC <u>GCGGCCGC</u> AGC CAC AAA ACC TCT AAA GA
BabApromR	SacI	AAC <u>GAGCTC</u> GGG GTA TTT TGA AAT AAC TCT C
BabAF	BamHI	AAC <u>GGATCC</u> TTG CTC CAC GCT GAA GAC
BabAR	XhoI	AAC <u>CTCGAG</u> GAC GCT CGT TTG ATT GAC CA
Le ^b genotyping		
hGh-F		AGC TGG CCT TTG ACA CCT ACC AGG
hGh-R		TCT GTT GTG TTT CCT CCC TGT TGG
CT repeat length determination		
BabAF14		GCA TCA AGC AAG CGA TAA CTT TAC TAA
BabARJC2		TTT GCC GTC TAT GGT TTG G

Table 2. Primer sequences. ^aRestriction sites are underlined.

autoclaved water and irradiated food *ad libitum* as previously described³⁶. FVB/N mice heterozygous for human α -1,3/4-fucosyltransferase gene (Le^b transgenic mice)⁴⁰ were bred to FVB/N WT mice and genotyped using tail snips obtained at weaning. DNA was extracted from tail snips by digestion in lysis buffer (50 mM KCl, 10 mM Tris, pH 8.5; 2 mM EDTA, Sigma-Aldrich, St. Louis, MO; 0.45% NP-40, Roche Diagnostics, Indianapolis, IN; 0.45% Tween-20, Bio-Rad, USA; and 1 mg/mL proteinase K, Roche Diagnostics, Indianapolis, IN) for 2 hr or overnight. Undigested fragments were removed by centrifugation (8,500 g for 10 min) and proteinase K was heat inactivated at 98 °C for 10 min. The resulting digest was used as template for PCR detection of human α -1,3/4-fucosyltransferase gene using primers hGh-F and hGh-R (Table 2), which were previously described⁴¹. Germ free C57BL/6 mice were raised and housed in soft-sided bubble isolators at the germ free mouse facility at the University of Michigan. Germ-free status was verified by aerobic and anaerobic cultures at least weekly, and all mice remained free of bacteria (other than the inoculated *H. pylori* strain) throughout the experiment.

Experimental *H. pylori* challenge and sample collection. *H. pylori* harvested from agar plates grown overnight (18–24 hr) was used to inoculate liquid cultures to an optical density A₆₀₀ (OD) of 0.05 to 0.1. Liquid cultures were grown in Brucella broth (Becton, Dickinson and Company, Sparks, MD) supplemented with 5% NCS and TVPA shaking at 60–100 rpm overnight (18–24 hr) to an OD of 0.3 to 0.7. Bacteria from overnight liquid cultures were centrifuged (7,000 g for 10 min) and suspended in Brucella broth at approximately 10¹⁰ CFU/mL. For competition experiments where the inoculum contained equal parts of BabA expressing and deficient *H. pylori*, liquid cultures were grown to matching OD values prior to centrifugation and resuspension. In a subset of experiments, dilutions of the inoculum mixture were plated and 16 colonies were selected and sequenced at the 5' end of the *babA* locus to empirically determine the ratio of BabA expressing bacteria in the inoculum. On average the inoculum contained 45% BabA expressing bacteria with a standard deviation of 11%. This variability reflects both variation in the growth and survival of the cultures and technical variation in the detection of BabA expression in the output colonies. Mice were challenged at 10 to 14 weeks of age with 250 μ L of suspension by oral gavage with a 20 gauge, 38mm animal feeding needle (Fisher Scientific)³⁶. Mice were euthanized by intraperitoneal injection of 5 mg pentobarbital-Na and 0.6 mg phenytoin-Na (Beuthanasia-D) and stomachs were removed and dissected into 2 to 4 longitudinal sections^{36,42}. One quarter of the stomach tissue was placed in 10% phosphate buffered formalin (Fisher Scientific) for histological analysis and one half to one quarter was weighed and homogenized in Brucella broth for *H. pylori* culture and calculation of *H. pylori* colony forming units per gram of stomach tissue (CFU/g). The comparison between male and female mice was performed twice, as indicated in the figure legends and supplementary files. The remainder of the experiments was conducted exclusively in female mice.

Detection of BabA expression. In *H. pylori* J166, 8 CT repeats at the 5' end of *babA* yield an ORF, with expression of BabA and attachment to Le^b³⁶; 7 or 9 CT repeats does not because it produces a stop codon at position 49 or 79, respectively. Therefore, the proportion of BabA-expressing *H. pylori* in each mouse was determined by sequencing the CT repeat region of *babA*, which was selectively confirmed by RIA analysis using methods previously described^{36,43}. Briefly, for CT analysis, a DNA fragment was sequenced from multiple individual colonies from each mouse after PCR amplification with primers BabAF14 and BabARJC2 (Table 2), which yields a 1046 bp product containing the CT repeats. On average 8 colonies were collected per mouse and analyzed in this way (minimum of 3 and maximum of 20). The percentage of the total colonies collected per mouse with a *babA* ORF is displayed as a single point in each figure describing BabA expression in mice. For RIA analysis, total cultured dilutions from mouse stomach homogenate (sweeps) were collected and 1 mL of an OD₆₀₀ = 0.1 suspension of the mixture was incubated with a cocktail of ¹²⁵I radiolabeled Le^b conjugated to human serum albumin (Le^b-HSA). The ratio of bound Le^b-HSA (radioactivity measured in the bacterial pellet) to free Le^b-HSA (radioactivity measured in the supernatant) was used to estimate BabA expression of the *H. pylori* community⁴³.

In vitro analysis of BabA attachment to Le^b. *H. pylori* expression of functional BabA protein was assayed by *in vitro* attachment to Le^b using an ELISA as previously described^{22,35,43}. Briefly, digoxigenin (Roche Applied Biosciences) labeled *H. pylori* cells were applied to Le^b-HSA coated wells in a 96 well polystyrene plate. Unbound bacteria were removed by washing and bound bacteria were detected with anti-digoxigenin Fab fragments conjugated to horseradish peroxidase (POD) (Roche Applied Biosciences) followed by incubation with 2,2'-azino-di(3-ethyl-benzthiazoline-6-sulfonate) (ABTS). Color change was measured by subtraction of absorbance at 490 nm from 405 nm. Attachment ratio values were reported as an average of readings from two Le^b positive wells divided by two Le^b negative wells.

Statistical Analysis. Unless otherwise indicated, Mann-Whitney U, Fisher's exact test or analysis of variance was performed using Graphpad Prism Software. Bacterial colonization (CFU/gram) was log transformed prior to analysis. The proportion of BabA expressing colonies was logit transformed using R software⁴⁴ and 0 values were remapped to a proportion of 0.025 prior to analysis of variance. If no bacteria were isolated from a given mouse, the colonization level was shown at the limit of detection. $P < 0.05$ was considered statistically significant.

Results

Adaptive immunity does not affect *H. pylori* BabA expression. Loss of *H. pylori* BabA expression by phase variation might result from adaptive immune pressure directed against BabA. When loss of BabA expression occurs by a gene conversion event in which *babB* is duplicated into the *babA* locus^{35,36}, this might represent a form of antigenic variation. Both occur commonly in bacteria and other pathogens to avoid adaptive immunity^{45–48}. To test this hypothesis, we measured BabA expression in output strains after inoculation of *H. pylori* J166 into wild type (WT) and RAG2^{-/-} mice, which do not develop mature B or T lymphocytes⁴⁹. *H. pylori* colonized RAG2^{-/-} mice at a significantly higher level than WT (Fig. 1a), supporting previous evidence that adaptive immunity, including mature T lymphocytes^{50,51}, is important for control of *H. pylori* infection. To examine BabA expression, 3 to 6 *H. pylori* colonies were isolated from the stomachs of RAG2^{-/-} and WT mice (N = 4–7) at 2 and 8 weeks post infection (PI). Since we previously showed that loss of BabA expression in mice occurs only by phase variation and not by gene conversion³⁶, we determined BabA expression by DNA sequence analysis of the 5' region that contains the CT dinucleotide repeats, where 8 repeats corresponds to a *babA* open reading frame (ORF) and attachment to Le^b³⁶. Although there was considerable variability among individual mice 2 weeks PI, by 8 weeks PI BabA expression was lost in 8 of 8 mice (Fig. 1b). No differences were found between RAG2^{-/-} and WT mice. The experiment was repeated with similar results (Fig. 1c,d). These data indicate that although the adaptive immune response controls *H. pylori* infection, it does not play a role in selection against BabA expressing bacteria in mice.

***H. pylori* BabA expression is lost in both conventional and germ free mice.** It is now recognized that *H. pylori* infection occurs in the context of a gastric microbial community, which is less complex than that in the gut, but is probably autochthonous^{52–55} and may affect the outcome of infection. For example, in INS-GAS mice, which overexpress gastrin under the insulin promoter⁵⁶, *H. pylori* infection induces more severe gastrointestinal intraepithelial neoplasia when animals have conventional microbiota compared to germ free counterparts⁵⁷. Additionally, differences in intestinal microbiota composition in mice from different vendors have been shown to impact the immune response to pathogens⁵⁸. Since adaptive immunity did not select for loss of BabA expression, and there was substantial variability among individual mice during the first two weeks of infection, we considered the possibility that the gastric microbial community could affect BabA expression. To determine whether microbiota could influence *H. pylori* expression of BabA, we infected germ free and conventionally raised C57BL/6 mice with *H. pylori* J166, and examined BabA expression in output colonies from mice sacrificed 3 weeks PI. Similar to the results 2 weeks PI (Fig. 1), there was marked variability, but no statistical differences in loss of BabA expression between conventional and germ free C57BL/6 mice (Fig. 2). These results indicate that the microbiota does not affect *H. pylori* expression of BabA, nor influence the variability among individual mice.

The *H. pylori* J166 inoculum is heterogeneous at the BabA locus. The marked variability in BabA expression among output colonies (Figs 1 and 2) might result from stochastic or bottleneck effects if the inoculum contains a significant population of phase variants with no *babA* ORF. This possibility was supported by the finding that sequence of 5–6 individual colonies from 3 instances of liquid culture grown from the same *H. pylori* J166 stock contained variable percentages of BabA expressing clones, ranging from 67 to 100%. We therefore re-isolated a J166 single colony (designated J166_{SC}) with a *babA* ORF (8 CT repeats) and compared the outcome of infection with J166_{SC} to that with the original J166 inoculum containing a mixture of phase variants. After infection for between 2 and 8 weeks, loss of BabA expression was observed in 6 of 11 (55%) mice infected with the original stock of J166, but only 1 of 12 (8%) mice infected with J166_{SC} ($P < 0.05$, Fisher's exact test). Since J166_{SC} might differ from J166 in other ways, in addition to the number of CT repeats in *babA*, we used contraselection to generate isogenic clones of J166_{SC} that had either 8 CT repeats (J166_{8CT}), encoding a *babA* ORF, or 7 CT repeats (J166_{7CT}), which was out of frame and led to loss of BabA expression. J166_{8CT} and J166_{7CT} showed similar growth rates in liquid culture (Supplementary Figure S1), but, as expected, only J166_{8CT} attached to Le^b (Supplementary Figure S2). For all subsequent experiments, *H. pylori* inoculation was performed as a competition experiment in which mice were infected with an equal mixture of J166_{8CT} and J166_{7CT} (designated J166_{8CT:7CT}).

BabA expression decreases within 24 hours during acute infection. Previous experiments indicated that loss of BabA expression can occur within the first two weeks of *H. pylori* infection (Fig. 1). We next examined this more precisely by inoculating WT C57BL/6 mice with J166_{8CT:7CT} and examining BabA expression

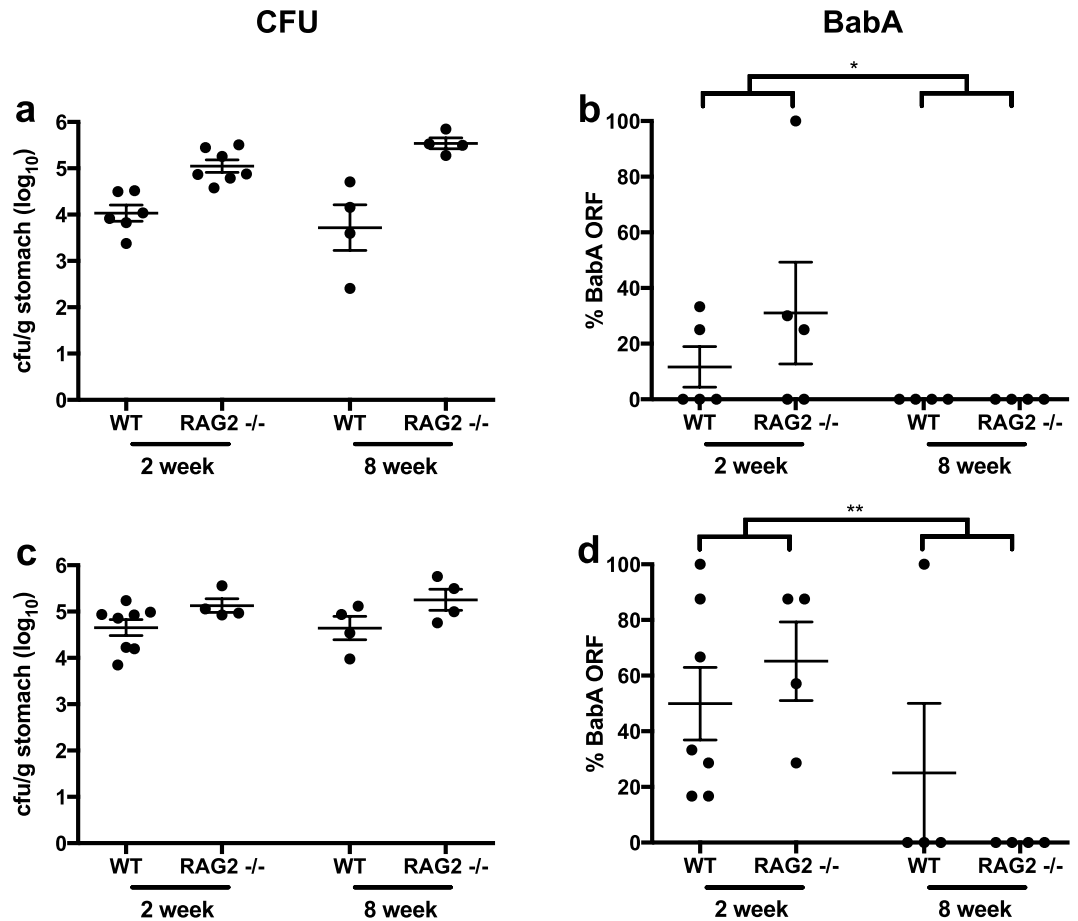


Figure 1. Loss of BabA expression in mice is not affected by B or T cell response. *H. pylori* colonization was significantly greater in female RAG2^{-/-} compared to WT mice at 2 and 8 weeks PI when the results were pooled over time, $P < 0.0001$ (a), but loss of the *babA* ORF was unaffected (b). Each point represents the percentage of colonies (average of 8 colonies total) from a single mouse determined by sequencing to express a full length *babA* ORF. Similar results were obtained when the experiment was repeated (c,d), although the difference in colonization level was less significant, $P < 0.05$ (c). Data were analyzed by two-way ANOVA of log (a,c) or logit (b,d) transformed values. * $P < 0.05$, ** $P < 0.01$.

in output colonies recovered from mice sacrificed between 6 hrs and 14 days PI. Loss of BabA expression was detected as early as 6 hrs PI, was dominant by 3 days PI, and nearly uniform at 2 weeks PI (Fig. 3a). To confirm that this was not specific to a particular mouse strain, we performed the same experiment in WT FVB/N mice. Loss of BabA expression was similar in FVB/N mice (Fig. 3b) to what we previously observed in C57BL/6 mice (Fig. 3a), with colonies from 10 of 11 mice (91%) showing no BabA expression by 2 weeks PI. To confirm that sequencing the *babA* CT repeat region in a limited number of output colonies is representative, we performed RIA analysis, which detects Le^b binding in the total *H. pylori* population cultured from the stomach. Le^b binding analyzed by RIA correlated closely with CT analysis (Pearson $R^2 = 0.57$, $P < 0.0001$), and demonstrated a similar loss in BabA expression over time (Fig. 3c). Together, these data suggest that there is a strong selection against BabA expression in mice that occurs within hours to a few days after inoculation.

Role of toll like receptor (TLR) signaling in BabA expression. Early selection, hours to days post infection, against BabA expression is consistent with the absence of a role for adaptive immunity in selecting against BabA expression (Fig. 1), and suggests that innate immunity might play a role. Attachment to host epithelial cells has been shown to induce expression of IL-8²⁴, suggesting that attachment increases host innate inflammatory responses and perhaps selects against strains expressing BabA. Moreover, we previously found that BabA expression was retained to a greater extent in C3H/HeJ mice³⁶, which express a defective toll like receptor 4 (TLR4)⁵⁹. Together, these data suggest the possibility that TLR signaling could be involved in driving loss of BabA expression. To examine this, BabA expression was analyzed 2 weeks PI in C57BL/6 WT mice and compared to mice with a homozygous deletion of MyD88 (MyD88^{-/-}), toll like receptor 2 (TLR2^{-/-}), or TLR4^{-/-}. By 2 weeks PI, no BabA expressing bacteria were detected in the large majority of mice from each experimental group (Fig. 4), indicating that loss of BabA expression is under strong negative selection even in the absence of TLR signaling.

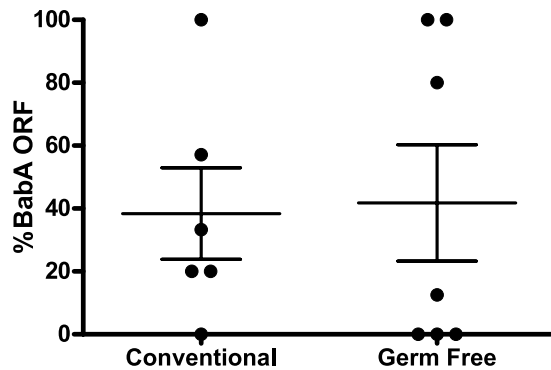


Figure 2. *H. pylori* loss of BabA expression occurs equally in germ free and conventionally raised mice. Percent BabA expressing *H. pylori* is shown for conventional and germ free female C57BL/6 mice inoculated with *H. pylori* J166 and sacrificed 3 weeks PI. No significant differences were found between the experimental groups.

***H. pylori* BabA expression is greater in male mice.** Glycosylation levels and patterns are altered early during *H. pylori* infection, with loss of fucosylation and increase in sialylation due to inflammation-induced changes in glycosyltransferases^{12,36,60–62}. Specific glycans can affect not only adherence, but also *H. pylori* growth and gene expression⁶³. We therefore compared BabA expression in WT mice, which do not express the Le^b antigen, to transgenic mice that express Le^b in the gastric mucosa⁴⁰. Male and female Le^b transgenic mice and their wild type littermates were infected with J166_{8CT:7CT} and sacrificed 2 weeks PI. *H. pylori* colonization levels were greater in males (Fig. 5a, $P < 0.001$), but there was no difference between WT and Le^b mice. BabA expression was also similar in WT and Le^b mice, but again there was a significant effect of gender (Fig. 5b, $P < 0.0001$). Female mice were predominantly colonized by *H. pylori* lacking BabA expression, similar to what we observed previously (Figs 3 and 4), while BabA expression was greater in male mice (Fig. 5b). Expression of BabA in *H. pylori* isolated from male Le^b mice was significantly greater than that from either female Le^b mice or female WT mice (Tukey's post test, $P = 0.002$ and $P = 0.001$, respectively). Increased colonization and a small increase in Le^b binding were also observed in male Le^b relative to female Le^b mice 4 weeks PI using *H. pylori* strain J99 (Supplementary Figure S3), indicating that this phenomenon is robust across *H. pylori* strains that express functional BabA.

Loss of *H. pylori* BabA expression is not dependent on BabA attachment to Le^b. Since loss of BabA expression in mice is not significantly affected by expression of Le^b, we hypothesized that it might also be unaffected by the capacity of BabA to bind Le^b. We recently demonstrated that BabA residues Cys189 and Cys197 form a redox-sensitive disulfide-clasped loop designated CL2, which is essential to bind the Le^b α -1-2-linked fucose residue²⁰. Cys to Ala replacement at BabA residues 189 and 197 in *H. pylori* J166 (designated BabACL2) was sufficient to eliminate all Le^b binding activity, though the protein was expressed on the cell surface at levels similar to WT²⁰. To examine the effect of Le^b binding on BabA expression in mice, we used contraselection to generate isogenic strains of BabACL2 with either 8 (J166 BabACL2_{8CT}) or 9 (BabACL2_{9CT}) CT repeats. The BabA expression status predicted by the CT repeat number for each strain was confirmed by western blot (Supplementary Figure S4). The elimination of Le^b binding activity in all strains was confirmed by ELISA (Supplementary Figure S4). These strains were combined in equal proportions (BabACL2_{8CT:9CT}) and used to infect female Le^b transgenic mice and their WT littermates, which were sacrificed 2 weeks PI. For comparison, WT and Le^b mice were also infected with J166_{8CT:7CT}. There were no differences in colonization levels between BabACL2_{8CT:9CT} compared to J166_{8CT:7CT} (Fig. 6a) in WT or in Le^b mice. Strikingly, there was strong selection for loss of BabA expression, even in the absence of the capacity to bind Le^b (Fig. 6b).

Discussion

We previously found that expression of BabA was lost either by phase variation or by gene conversion within the first 2–12 weeks during experimental *H. pylori* infection of non-human primates, mice, and gerbils^{35,36}. Similar observations have been made by others^{37,64}. Clinical isolates of *H. pylori* also show remarkable diversity at the *babA* locus, which may encode a “specialist” adhesin that binds only blood group O/Le^b, or a “generalist” that also binds blood group A/A/Le^b and B/BL/Le^b^{19,20}. In other cases, BabA may be expressed but not bind any known blood group antigen, may be present as a pseudogene, or even be absent from the genome altogether³⁶. We recently suggested that in order to persist in the human stomach, *H. pylori* must face what we called an “attachment dilemma”, in which the benefits of adherence to the gastric epithelium such as escape from luminal acid and nutrient acquisition must be balanced with the costs, particularly interaction with the host immune response⁶⁵. This is likely a dynamic process where BabA expression can be lost but also regained, either by phase variation, or perhaps by reintroduction of a copy of *babA* that has been archived in a small proportion in the population⁶⁶. The diversity and dynamic nature of host glycosylation further adds to the complexity. With this perspective, we hypothesized that modulation of BabA expression and attachment to Le^b would be driven by the host immune response and glycan expression. Here we set out to test this hypothesis.

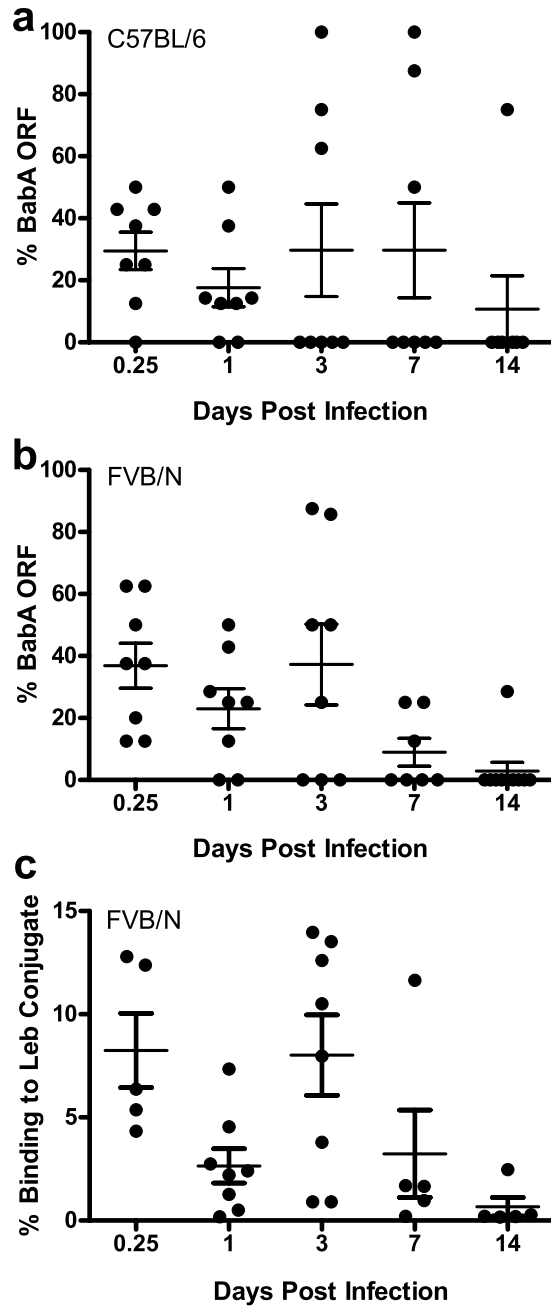


Figure 3. *H. pylori* loss of BabA expression occurs early during infection. Percentage of BabA expressing *H. pylori* detected by CT repeat analysis in female C57BL/6 (a) and female FVB/N (b) mice inoculated with J166_{8CT:7CT} and sacrificed from 6 hrs to 14 days PI. (c) *H. pylori* populations cultured from the stomachs of FVB/N mice shown in B, analyzed for binding to Le^b-HSA conjugate by RIA analysis.

Surprisingly, our results suggest that neither adaptive immunity, toll like receptor mediated host immune responses, Le^b expression, nor even the capacity of BabA to bind Le^b affected loss of BabA expression in mouse models. BabA expression was lost by phase variation equally in WT mice as in RAG2^{-/-} mice that do not have functional B or T cells, and in MyD88^{-/-} mice that cannot signal via all TLRs except TLR 3. Initial experiments suggested that loss of BabA expression required up to 8 weeks of colonization (Fig. 1), which might suggest involvement of host immunity, but competition experiments demonstrated selection for loss of BabA expression as early as one day PI (Fig. 3). Although we have not specifically examined the role of innate immune cells such as polymorphonuclear leukocytes (PMNs) and macrophages, our results suggest that adaptive immunity and TLR mediated immune responses do not select for loss of BabA expression. Moreover, loss of BabA expression is not dependent on Le^b expression or the capacity of BabA to bind Le^b, which suggests the possibility that BabA may have other, unrecognized functions.

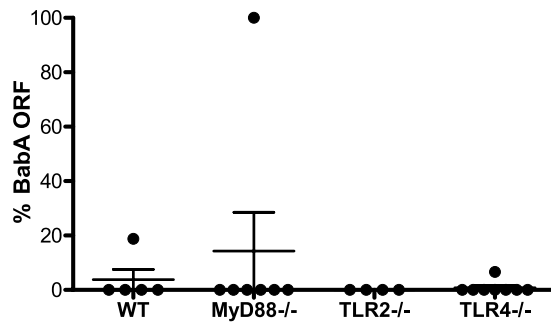


Figure 4. TLR signaling does not influence *H. pylori* BabA expression. Percentage of BabA expressing *H. pylori* detected in female C57BL/6 WT, MyD88^{-/-}, TLR2^{-/-} and TLR4^{-/-} mice inoculated with J166_{8CT:7CT} and sacrificed 2 weeks PI. No significant differences were found between any of the experimental groups.

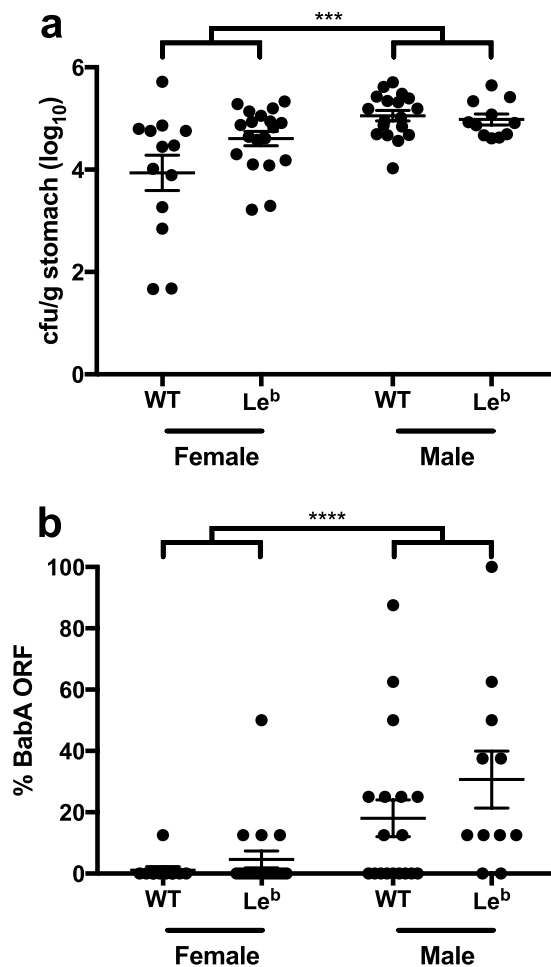


Figure 5. *H. pylori* colonization and BabA expression are greater in male than female FVB/N mice. Male and female Le^b transgenic FVB/N mice and their WT littermates were inoculated with J166_{8CT:7CT} and sacrificed 2 weeks PI. *H. pylori* colonization density (a) and expression of BabA (b) were greater in male than in female mice. *** $P < 0.001$, **** $P < 0.0001$ (Two-way ANOVA of logit (a) or log (b) transformed values).

Instead, we found that gender is the host determinant most associated with loss of BabA expression, which was maintained to a greater extent in male mice than in females, and was also associated with greater bacterial load (Fig. 5 and Supplementary Figure S3). These data highlight the importance of conducting host-pathogen interaction studies in both genders when using a mouse model, which is a common experimental design problem that has gained increased interest in recent years⁶⁷. Male gender is a well-known risk factor for *H. pylori*-associated disease, including gastric adenocarcinoma⁶⁸ and peptic ulcer, though the male predominance in ulcers appears to be declining as its prevalence decreases⁶⁹. Animal models also provide support for male predominance of

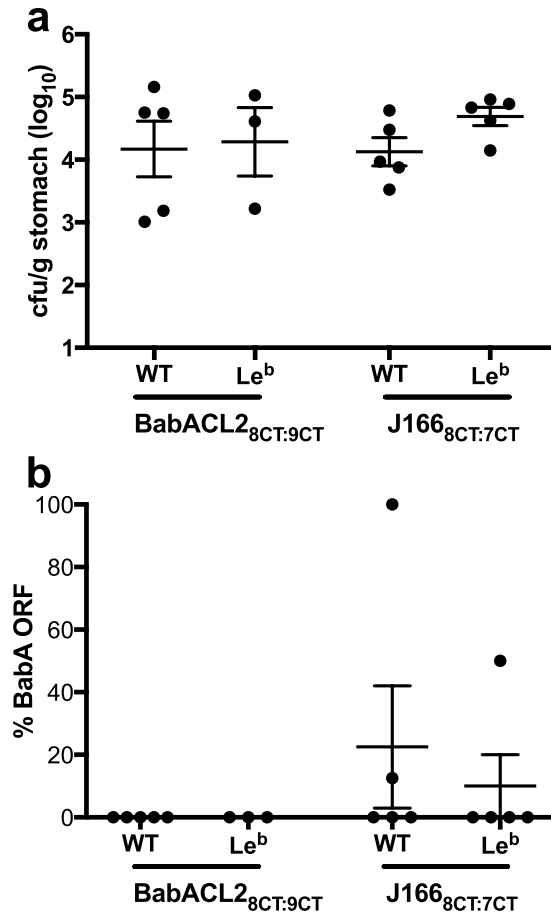


Figure 6. Loss of BabA expression in mice occurs even in the absence of capacity to bind Le^b. Female Le^b transgenic FVB/N mice and their WT littermates were inoculated with BabACL2_{8CT:9CT} or J166_{8CT:7CT} and sacrificed 2 weeks PI. Colonization density (a) and selection for loss of BabA expression (b) were similar in WT and Le^b mice inoculated with J166 and BabACL2, which expresses BabA but does not attach to Le^b. No significant differences were found between any of the experimental groups.

gastric cancer⁷⁰. Although less studied, there is also epidemiologic evidence that male gender is a risk factor for *H. pylori* infection⁷¹, and that, as we observed, *H. pylori* bacterial load in mouse models is higher in males than in females^{72,73}.

The mechanistic link between host gender and *H. pylori* expression of BabA remains to be determined. There are numerous differences in gastric physiology between males and females⁷⁴, including pH, transit time, and enzyme expression, though it is unclear how these or other differences might affect relative fitness of *H. pylori* BabA expression. Similarly, gender dependent differences in glycosylation patterns have been described in humans^{75–77}, suggesting the possibility that differences in mucosal glycan expression between male and female mice might participate in selection against BabA expression. However, since capacity to bind Le^b is not required for loss of BabA expression, the effect of gender differences in glycosylation may involve as yet uncharacterized lectin functions of BabA.

References

- Kusters, J. G., van Vliet, A. H. & Kuipers, E. J. Pathogenesis of *Helicobacter pylori* infection. *Clin. Microbiol Rev.* **19**, 449–490, doi: 10.1128/CMR.00054-05 (2006).
- Sipponen, P. *et al.* Cumulative 10-year risk of symptomatic duodenal and gastric ulcer in patients with or without chronic gastritis. A clinical follow-up study of 454 outpatients. *Scand. J. Gastroenterol.* **25**, 966–973, doi: 10.3109/0036552900897621 (1990).
- Basso, D., Plebani, M. & Kusters, J. G. Pathogenesis of *Helicobacter pylori* infection. *Helicobacter* **15** Suppl 1, 14–20, doi: 10.1111/j.1523-5378.2010.00781.x (2010).
- Hsiang, J. *et al.* Increasing primary antibiotic resistance and ethnic differences in eradication rates of *Helicobacter pylori* infection in New Zealand—a new look at an old enemy. *N. Z. Med. J.* **126**, 64–76 (2013).
- Vakil, N. & Vaira, D. Treatment for *H. pylori* infection: new challenges with antimicrobial resistance. *J. Clin. Gastroenterol.* **47**, 383–388, doi: 10.1097/MCG.0b013e318277577b (2013).
- Arnold, I. C. *et al.* *Helicobacter pylori* infection prevents allergic asthma in mouse models through the induction of regulatory T cells. *J. Clin. Invest.* **121**, 3088–3093, doi: 10.1172/JCI45041 (2011).
- Amedei, A., Codolo, G., Del Prete, G., de Bernard, M. & D'Elia, M. M. The effect of *Helicobacter pylori* on asthma and allergy. *J. Asthma Allergy* **3**, 139–147, doi: 10.2147/JAA.S8971 (2010).
- Luther, J., Dave, M., Higgins, P. D. & Kao, J. Y. Association between *Helicobacter pylori* infection and inflammatory bowel disease: a meta-analysis and systematic review of the literature. *Inflamm. Bowel Dis.* **16**, 1077–1084, doi: 10.1002/ibd.21116 (2010).

9. Higgins, P. D. *et al.* Prior *Helicobacter pylori* infection ameliorates *Salmonella typhimurium*-induced colitis: mucosal crosstalk between stomach and distal intestine. *Inflamm. Bowel. Dis.* **17**, 1398–1408, doi: 10.1002/ibd.21489 (2011).
10. Alm, R. A. *et al.* Comparative genomics of *Helicobacter pylori*: analysis of the outer membrane protein families. *Infect. Immun.* **68**, 4155–4168 (2000).
11. Rossez, Y. *et al.* The lacdiNAc-specific adhesin LabA mediates adhesion of *Helicobacter pylori* to human gastric mucosa. *J. Infect. Dis.* **210**, 1286–1295, doi: 10.1093/infdis/jiu239 (2014).
12. Mahdavi, J. *et al.* *Helicobacter pylori* SabA adhesin in persistent infection and chronic inflammation. *Science* **297**, 573–578, doi: 10.1126/science.1069076 (2002).
13. Koniger, V. *et al.* *Helicobacter pylori* exploits human CEACAMs via HopQ for adherence and translocation of CagA. *Nat. Microbiol.* **2**, 16188, doi: 10.1038/nmicrobiol.2016.188 (2016).
14. Javaheri, A. *et al.* *Helicobacter pylori* adhesin HopQ engages in a virulence-enhancing interaction with human CEACAMs. *Nat. Microbiol.* **2**, 16189, doi: 10.1038/nmicrobiol.2016.189 (2016).
15. Boren, T., Falk, P., Roth, K. A., Larson, G. & Normark, S. Attachment of *Helicobacter pylori* to human gastric epithelium mediated by blood group antigens. *Science* **262**, 1892–1895, doi: 10.1126/science.8018146 (1993).
16. Ilver, D. *et al.* *Helicobacter pylori* adhesin binding fucosylated histo-blood group antigens revealed by retagging. *Science* **279**, 373–377 (1998).
17. Bjornham, O., Bugaytsova, J., Boren, T. & Schedin, S. Dynamic force spectroscopy of the *Helicobacter pylori* BabA-Lewis b binding. *Biophys. Chem.* **143**, 102–105, doi: 10.1016/j.bpc.2009.03.007 (2009).
18. Subedi, S. *et al.* Expression, purification and X-ray crystallographic analysis of the *Helicobacter pylori* blood group antigen-binding adhesin BabA. *Acta crystallographica. Section F, Structural biology communications* **70**, 1631–1635, doi: 10.1107/S2053230X14023188 (2014).
19. Aspholm-Hurtig, M. *et al.* Functional adaptation of BabA, the *H. pylori* ABO blood group antigen binding adhesin. *Science* **305**, 519–522, doi: 10.1126/science.1098801 (2004).
20. Moonens, K. *et al.* Structural Insights into Polymorphic ABO Glycan Binding by *Helicobacter pylori*. *Cell Host Microbe* **19**, 55–66, doi: 10.1016/j.chom.2015.12.004 (2016).
21. Hage, N. *et al.* Structural basis of Lewis(b) antigen binding by the *Helicobacter pylori* adhesin BabA. *Sci. Adv.* **1**, e1500315, doi: 10.1126/sciadv.1500315 (2015).
22. Gerhard, M. *et al.* Clinical relevance of the *Helicobacter pylori* gene for blood-group antigen-binding adhesin. *Proc. Natl. Acad. Sci. USA* **96**, 12778–12783, doi: 10.1073/pnas.96.22.12778 (1999).
23. Prinz, C. *et al.* Key importance of the *Helicobacter pylori* adherence factor blood group antigen binding adhesin during chronic gastric inflammation. *Cancer Res.* **61**, 1903–1909 (2001).
24. Ishijima, N. *et al.* BabA-mediated adherence is a potentiator of the *Helicobacter pylori* type IV secretion system activity. *J. Biol. Chem.* **286**, 25256–25264, doi: 10.1074/jbc.M111.233601 (2011).
25. Aviles-Jimenez, F. *et al.* *In vivo* expression of *Helicobacter pylori* virulence genes in patients with gastritis, ulcer, and gastric cancer. *Infect. Immun.* **80**, 594–601, doi: 10.1128/IAI.05845-11 (2012).
26. Backert, S. *et al.* Translocation of the *Helicobacter pylori* CagA protein in gastric epithelial cells by a type IV secretion apparatus. *Cell Microbiol.* **2**, 155–164, doi: 10.1126/science.287.5457.1497 (2000).
27. Blaser, M. J. *et al.* Infection with *Helicobacter pylori* strains possessing cagA is associated with an increased risk of developing adenocarcinoma of the stomach. *Cancer Res.* **55**, 2111–2115 (1995).
28. Odenbreit, S. *et al.* Translocation of *Helicobacter pylori* CagA into gastric epithelial cells by type IV secretion. *Science* **287**, 1497–1500, doi: 10.1126/science.287.5457.1497 (2000).
29. Segal, E. D., Cha, J., Lo, J., Falkow, S. & Tompkins, L. S. Altered states: involvement of phosphorylated CagA in the induction of host cellular growth changes by *Helicobacter pylori*. *Proc. Natl. Acad. Sci. USA* **96**, 14559–14564, doi: 10.1073/pnas.96.25.14559 (1999).
30. Linden, S. *et al.* Role of ABO secretor status in mucosal innate immunity and *H. pylori* infection. *PLoS Pathog.* **4**, e2, doi: 10.1371/journal.ppat.0040002 (2008).
31. Azevedo, M. *et al.* Infection by *Helicobacter pylori* expressing the BabA adhesin is influenced by the secretor phenotype. *J. Pathol.* **215**, 308–316, doi: 10.1002/path.2363 (2008).
32. Magalhaes, A. *et al.* Fut2-null mice display an altered glycosylation profile and impaired BabA-mediated *Helicobacter pylori* adhesion to gastric mucosa. *Glycobiology* **19**, 1525–1536, doi: 10.1093/glycob/cwp131 (2009).
33. Guruge, J. L. *et al.* Epithelial attachment alters the outcome of *Helicobacter pylori* infection. *Proc. Natl. Acad. Sci. USA* **95**, 3925–3930, doi: 10.1073/pnas.95.7.3925 (1998).
34. Ball, P. A. Influence of the secretor and Lewis genes on susceptibility to duodenal ulcer. *Br. Med. J.* **2**, 948–950, doi: 10.1136/bmj.2.5310.948 (1962).
35. Solnick, J. V., Hansen, L. M., Salama, N. R., Boonjakuakul, J. K. & Syvanen, M. Modification of *Helicobacter pylori* outer membrane protein expression during experimental infection of rhesus macaques. *Proc. Natl. Acad. Sci. USA* **101**, 2106–2111, doi: 10.1073/pnas.0308573100 (2004).
36. Styer, C. M. *et al.* Expression of the BabA adhesin during experimental infection with *Helicobacter pylori*. *Infect. Immun.* **78**, 1593–1600, doi: 10.1128/IAI.01297-09 (2010).
37. Ohno, T. *et al.* Effects of blood group antigen-binding adhesin expression during *Helicobacter pylori* infection of Mongolian gerbils. *J. Infect. Dis.* **203**, 726–735, doi: 10.1093/infdis/jiq090 (2011).
38. Colbeck, J. C., Hansen, L. M., Fong, J. M. & Solnick, J. V. Genotypic profile of the outer membrane proteins BabA and BabB in clinical isolates of *Helicobacter pylori*. *Infect. Immun.* **74**, 4375–4378, doi: 10.1128/IAI.00485-06 (2006).
39. Moore, M. E., Lam, A., Bhatnagar, S. & Solnick, J. V. Environmental determinants of transformation efficiency in *Helicobacter pylori*. *J. Bacteriol.* **196**, 337–344, doi: 10.1128/JB.00633-13 (2014).
40. Falk, P. G., Bry, L., Holgersson, J. & Gordon, J. I. 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, doi: 10.1073/pnas.92.5.1515 (1995).
41. Pohl, M. A. *et al.* Host-dependent Lewis (Le) antigen expression in *Helicobacter pylori* cells recovered from Leb-transgenic mice. *J. Exp. Med.* **206**, 3061–3072, doi: 10.1084/jem.20090683 (2009).
42. Sigal, M. *et al.* *Helicobacter pylori* Activates and Expands Lgr5(+) Stem Cells Through Direct Colonization of the Gastric Glands. *Gastroenterology* **148**, 1392–1404 e1321, doi: 10.1053/j.gastro.2015.02.049 (2015).
43. Aspholm, M. *et al.* *Helicobacter pylori* adhesion to carbohydrates. *Method Enzymol* **417**, 293–339, doi: 10.1016/S0076-6879(06)17020-2 (2006).
44. R Core Team. R: A language and environment for statistical computing. *R Foundation for Statistical Computing, Vienna, Austria*, available at <http://www.R-project.org/> (2015).
45. van Belkum, A., van Leeuwen, W., Scherer, S. & Verbrugh, H. Occurrence and structure-function relationship of pentameric short sequence repeats in microbial genomes. *Res Microbiol* **150**, 617–626, doi: 10.1016/s0923-2508(99)00129-1 (1999).
46. Stern, A. & Meyer, T. F. Common mechanism controlling phase and antigenic variation in pathogenic neisseriae. *Mol Microbiol* **1**, 5–12, doi: 10.1111/j.1365-2958.1987.tb00520.x (1987).
47. Telford, J. L. Bacterial genome variability and its impact on vaccine design. *Cell Host Microbe* **3**, 408–416, doi: 10.1016/j.chom.2008.05.004 (2008).

48. Vink, C., Rudenko, G. & Seifert, H. S. Microbial antigenic variation mediated by homologous DNA recombination. *FEMS Microbiol Rev* **36**, 917–948, doi: 10.1111/j.1574-6976.2011.00321.x (2012).
49. Shinkai, Y. *et al.* RAG-2-deficient mice lack mature lymphocytes owing to inability to initiate V(D)J rearrangement. *Cell* **68**, 855–867, doi: 10.1016/0092-8674(92)90029-c (1992).
50. Muller, A. & Solnick, J. V. Inflammation, immunity, and vaccine development for *Helicobacter pylori*. *Helicobacter* **16** Suppl 1, 26–32, doi: 10.1111/j.1523-5378.2011.00877.x (2011).
51. Taylor, J. M., Ziman, M. E., Canfield, D. R., Vajdy, M. & Solnick, J. V. Effects of a Th1- versus a Th2-biased immune response in protection against *Helicobacter pylori* challenge in mice. *Microb Pathog* **44**, 20–27, doi: 10.1016/j.micpath.2007.06.006 (2008).
52. Martin, M. E. & Solnick, J. V. The gastric microbial community, *Helicobacter pylori* colonization, and disease. *Gut microbes* **5**, 345–350, doi: 10.4161/gmic.28573 (2014).
53. Gu, S. *et al.* Bacterial community mapping of the mouse gastrointestinal tract. *PLoS one* **8**, e74957, doi: 10.1371/journal.pone.0074957 (2013).
54. Martin, M. E. *et al.* The impact of *Helicobacter pylori* infection on the gastric microbiota of the rhesus macaque. *PLoS one* **8**, e76375, doi: 10.1371/journal.pone.0076375 (2013).
55. Martin, M. E., Dieter, J. A., Luo, Z., Baumgarth, N. & Solnick, J. V. Predicting the outcome of infectious diseases: variability among inbred mice as a new and powerful tool for biomarker discovery. *mBio* **3**, e00199–00112, doi: 10.1128/mBio.00199-12 (2012).
56. Wang, T. C. *et al.* Synergistic interaction between hypergastrinemia and *Helicobacter* infection in a mouse model of gastric cancer. *Gastroenterology* **118**, 36–47, doi: 10.1016/s0016-5085(00)70412-4 (2000).
57. Lofgren, J. L. *et al.* Lack of commensal flora in *Helicobacter pylori*-infected INS-GAS mice reduces gastritis and delays intraepithelial neoplasia. *Gastroenterology* **140**, 210–220, doi: 10.1053/j.gastro.2010.09.048 (2011).
58. Ivanov, I. I. *et al.* Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* **139**, 485–498, doi: 10.1016/j.cell.2009.09.033 (2009).
59. Poltorak, A. *et al.* Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science* **282**, 2085–2088, doi: 10.1126/science.282.5396.2085 (1998).
60. Cooke, C. L. *et al.* Modification of gastric mucin oligosaccharide expression in rhesus macaques after infection with *Helicobacter pylori*. *Gastroenterology* **137**, 1061–1071, 1071 e1061–1068, doi: 10.1053/j.gastro.2009.04.014 (2009).
61. Navabi, N., Johansson, M. E., Raghavan, S. & Linden, S. K. *Helicobacter pylori* infection impairs the mucin production rate and turnover in the murine gastric mucosa. *Infect Immun* **81**, 829–837, doi: 10.1128/IAI.01000-12 (2013).
62. Marcos, N. T. *et al.* *Helicobacter pylori* induces beta3GnT5 in human gastric cell lines, modulating expression of the SabA ligand sialyl-Lewis x. *J Clin Invest* **118**, 2325–2336, doi: 10.1172/JCI34324 (2008).
63. Skoog, E. C. *et al.* Human gastric mucins differently regulate *Helicobacter pylori* proliferation, gene expression and interactions with host cells. *PLoS one* **7**, e36378, doi: 10.1371/journal.pone.0036378 (2012).
64. Liu, H. *et al.* Analysis of a single *Helicobacter pylori* strain over a 10-year period in a primate model. *Int J Med Microbiol* **305**, 392–403, doi: 10.1016/j.ijmm.2015.03.002 (2015).
65. Moore, M. E., Boren, T. & Solnick, J. V. Life at the margins: modulation of attachment proteins in *Helicobacter pylori*. *Gut microbes* **2**, 42–46, doi: 10.4161/gmic.2.1.14626 (2011).
66. Backstrom, A. *et al.* Metastability of *Helicobacter pylori* bab adhesin genes and dynamics in Lewis b antigen binding. *Proc Natl Acad Sci USA* **101**, 16923–16928, doi: 10.1073/pnas.0404817101 (2004).
67. Clayton, J. A. & Collins, F. S. Policy: NIH to balance sex in cell and animal studies. *Nature* **509**, 282–283, doi: 10.1038/509282a (2014).
68. Anderson, W. F. *et al.* Age-specific trends in incidence of noncardia gastric cancer in US adults. *JAMA* **303**, 1723–1728, doi: 10.1001/jama.2010.496 (2010).
69. Kurata, J. H. & Nogawa, A. N. Meta-analysis of risk factors for peptic ulcer. Nonsteroidal antiinflammatory drugs, *Helicobacter pylori*, and smoking. *J Clin Gastroenterol* **24**, 2–17 (1997).
70. Fox, J. G. *et al.* *Helicobacter pylori*-associated gastric cancer in INS-GAS mice is gender specific. *Cancer Res* **63**, 942–950 (2003).
71. de Martel, C. & Parsonnet, J. *Helicobacter pylori* infection and gender: a meta-analysis of population-based prevalence surveys. *Dig Dis Sci* **51**, 2292–2301, doi: 10.1007/s10620-006-9210-5 (2006).
72. Aebischer, T., Laforsch, S., Hurwitz, R., Brombacher, F. & Meyer, T. F. Immunity against *Helicobacter pylori*: significance of interleukin-4 receptor alpha chain status and gender of infected mice. *Infect Immun* **69**, 556–558, doi: 10.1128/IAI.69.1.556-558.2001 (2001).
73. Every, A. L. *et al.* Localized suppression of inflammation at sites of *Helicobacter pylori* colonization. *Infect Immun* **79**, 4186–4192, doi: 10.1128/IAI.05602-11 (2011).
74. Freire, A. C., Basit, A. W., Choudhary, R., Piong, C. W. & Merchant, H. A. Does sex matter? The influence of gender on gastrointestinal physiology and drug delivery. *Int J Pharm* **415**, 15–28, doi: 10.1016/j.ijpharm.2011.04.069 (2011).
75. Pucic, M. *et al.* Changes in plasma and IgG N-glycome during childhood and adolescence. *Glycobiology* **22**, 975–982, doi: 10.1093/glycob/cws062 (2012).
76. Knezevic, A. *et al.* Effects of aging, body mass index, plasma lipid profiles, and smoking on human plasma N-glycans. *Glycobiology* **20**, 959–969, doi: 10.1093/glycob/cwq051 (2010).
77. Brinkman-Van der Linden, C. M. *et al.* Oral estrogen treatment induces a decrease in expression of sialyl Lewis x on alpha 1-acid glycoprotein in females and male-to-female transsexuals. *Glycobiology* **6**, 407–412, doi: 10.1093/glycob/6.4.407 (1996).
78. Alm, R. A. *et al.* Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen *Helicobacter pylori*. *Nature* **397**, 176–180, doi: 10.1038/16495 (1999).

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Author Contributions

M.E.K., J.V.S. and T.B. wrote the main text of the manuscript. L.M.H. edited text, designed primers and contributed ideas. C.M.S. contributed Figure 1. S.L.D. collected data and performed a preliminary analysis for Figure 5. O.R. contributed Supplementary Figure S3. AS performed the RIA analysis throughout. K.A.E. contributed Figure 2. M.E.M. bred and characterized the transgenic Lewis B mouse colony for Figure 5. P.G. developed strain J166 BabACL2 and contributed ideas.

Additional Information

Supplementary information accompanies this paper at <http://www.nature.com/srep>

Competing Interests: TB is founder of Helicure and a member of its scientific advisory board.

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