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Chronic *Helicobacter pylori* Infection with Sydney Strain 1 and a Newly Identified Mouse-Adapted Strain (Sydney Strain 2000) in C57BL/6 and BALB/c Mice

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The mouse model of *Helicobacter pylori*-induced disease using Sydney strain 1 (SS1) has been used extensively in *Helicobacter* research. Herein we describe the isolation and characterization of a new mouse-colonizing strain for use in comparative studies. One strain capable of persistent mouse colonization was isolated from a total of 110 clinical isolates and is named here SS2000 (Sydney strain 2000). Genome typing revealed a number of differences between SS1 and SS2000 as well as between them and the respective original clinical isolates. In particular, SS2000 lacked the entire *cag* pathogenicity island, while SS1 contained all 27 genes of the island. C57BL/6 and BALB/c mice were infected with SS1 or SS2000 or were treated with broth medium (controls). After 6 months host-specific effects were evident, including lower colonization levels in the BALB/c animals. Few pathological differences were observed between SS1- and SS2000-infected animals. However, by 15 months postinfection, SS1-infected C57BL/6 mice had developed more severe gastritis than the SS2000-infected animals. In contrast SS2000-infected BALB/c mice showed increased accumulation of mucosa-associated lymphoid tissue compared to those infected with SS1. This improved comparative model of *H. pylori*-induced disease allowed dissection of both host and strain effects and thus will prove useful in further studies.

Mouse models of *Helicobacter pylori*-induced disease have been extensively used in *Helicobacter* research and have been particularly useful in the elucidation of factors required for colonization, distribution and persistence of infection (10, 29, 31), the contribution of various virulence factors (some examples are given in references 12 and 43), and the development of vaccines (reviewed in reference 28). Early mouse model infection studies utilized *Helicobacter felis*, a close relative of *H. pylori*, because no *H. pylori* strains persistently colonized mice (5, 29). In 1997, Lee et al. isolated *H. pylori* Sydney strain 1 (SS1) by screening a number of clinical isolates for their ability to colonize mice using a novel technique where a number of strains were pooled and passaged from mouse to mouse. SS1 was found to consistently colonize multiple strains of mice to a high level and to establish infection that persisted over many months, thus providing an ideal opportunity to study the effects of chronic infection and host specificity in this small rodent model (31). The difficulty in finding additional *H. pylori* strains that persistently colonize mice has hindered studies of strain-specific effects on infection and immunity. Other groups have attempted to isolate new mouse-colonizing strains, but in most cases those strains utilized do not colonize efficiently and often do so only transiently (some examples are given in references 34, 39, and 47).

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The SS1 mouse model has provided important information regarding *H. pylori*-related disease, particularly with respect to colonization properties. However, given the lack of well-scrutinized and characterized mouse-adapted strains, the role of specific *H. pylori* virulence factors has been limited, with the exception of gene knockout studies. Additionally, it is not known whether the behavior of SS1 in mice is typical of *H. pylori* strains or if this strain is unique. It has long been appreciated that *H. pylori* strain-specific differences can influence disease progression in humans. In particular the presence of the *cag* pathogenicity island (PAI) in clinical isolates has been associated with more-severe disease (4, 37). However, studies attempting to assess the effects of deletions in the *cag* PAI on colonization and inflammation in the mouse model using SS1 have reported conflicting results (13, 33). In addition other contradictory reports have been published regarding the effect of insertional mutants on *H. pylori* virulence factors. For example, Chevalier et al. (7) reported that the gamma-glutamyltranspeptidase gene was essential for colonization; in contrast, McGovern et al. (34) found the gene to be unnecessary for colonization. Such differences may have arisen due to the use of different *H. pylori* strains or animal models.

Increasing evidence in both human populations and animal models suggests that the response to *H. pylori* is also highly host dependent. Comparative studies of *H. felis* and SS1 infection of various mouse strains have contributed greatly to the understanding of host-dependent gastritis. Significant differences were found in the host response of different mouse strains to infection with *H. felis* and *H. pylori* SS1. In particular, the concept of responder and nonresponder strains has been

TABLE 1. *H. pylori* strains used in this study

Strain	Relevant characteristic	Source (reference)
10700 (PMSS1)	Clinical isolate	UNSW (31)
SS1	Murine passaged isolate	UNSW (31)
2.1 (PMSS2000)	Clinical isolate	UNSW (this study)
SS2000	Murine passaged isolate	UNSW (this study)
10319	Clinical isolate	UNSW (31)
10217	Clinical isolate	UNSW (31)
26695	Sequenced clinical isolate	Stanford (51)
J99	Sequenced clinical isolate	Stanford (2)

suggested by Sakagami et al. (41). In their study, C57BL/6 and C3H/He mouse strains were found to develop moderate to severe chronic active gastritis 6 months postinfection (mpi) with SS1 or *H. felis* and were termed responder mice. In contrast, BALB/c and CBA mice developed only a mild form of gastritis and thus were termed nonresponder strains (41). However, long-term colonization (18 to 28 months) of the nonresponder BALB/c strain with *H. felis* caused pathology resembling mucosa-associated lymphoid tissue (MALT) lymphoma (17). The different inflammatory responses of C57BL/6 and BALB/c mice have been partially attributed to their T-helper-cell phenotype. In C57BL/6 mice the proinflammatory Th1 phenotype dominates, while in BALB/c mice the largely noninflammatory Th2 phenotype is present (19, 40). In addition to these differences, C57BL/6 mice show a high level of antral dominant colonization, while BALB/c mice show a lower overall level of colonization that is localized mainly in the antrum-body transitional zone (31). Thus, by using a combination of mouse strains, host-dependent colonization and inflammation studies can be performed.

To investigate the contributions of host- and strain-specific effects on colonization and inflammation, we aimed to isolate new mouse-colonizing strains of *H. pylori* for use in comparative studies with SS1. It was hypothesized that strains with enhanced ability to colonize mice might cause an increased inflammatory response, and thus these may represent better strains for use in vaccine studies. Following the isolation of a new mouse-colonizing strain, Sydney strain 2000 (SS2000), genome typing was performed and the rates of colonization and inflammation induced by SS2000 and SS1 were compared over long-term infection of C57BL/6 and BALB/c mice. This study design enabled the investigation of the specific contributions of both host and strain differences in *H. pylori*-induced pathology and colonization in the mouse model.

MATERIALS AND METHODS

Bacterial strains and growth. *H. pylori* strains used in this study are described in Table 1. For the isolation of new mouse-colonizing strains, a selection of 110 clinical *H. pylori* isolates collected by the University of New South Wales (UNSW) *Helicobacter* laboratory from patients undergoing endoscopy at a Sydney gastroenterology unit were used. Strains were isolated from patient biopsy specimens on *Campylobacter*-selective agar (CSA) containing Skirrow's selective supplement (31). All clinical isolates were stored in brain heart infusion broth (BHI) (Oxoid) plus 20% (wt/vol) glycerol in liquid N_2 after isolation until use in animal experimentation. Each culture was revived from liquid N_2 and passaged once on CSA plates before inoculation of animals. Reisolation of strains and determination of CFU were undertaken using previously described methods (31).

Animals. All animals used in this study were age-matched (6 to 8 weeks old) female C57BL/6 or BALB/c mice obtained from the Animal Resources Center (Canning Vale, Western Australia, Australia). The animals were housed under clean conditions and fed a commercial diet (Gordon's Specialty Stock Feed Pty. Ltd., Yanderra, New South Wales, Australia) and given water ad libitum. All animal experimentation protocols were approved by the Animal Care and Ethics Committee of the UNSW.

Mouse infections. (i) Isolation of new mouse-colonizing strains. For the isolation of new mouse-colonizing strains of *H. pylori*, strains grown on CSA plates for 24 to 48 h were harvested in BHI, and approximately equal concentrations of five individual strains were combined (termed "groups"). All strains grew at approximately equivalent rates over 48 h on CSA plates. A total of 22 groups of strains consisting of 110 individual clinical isolates plus three individual control strains—SS1, 10319, and 10217 (Table 1) were used. Two C57BL/6 mice per group of strains (a total of 50 mice) were inoculated by orogastric gavage, twice in a 3-day period. Individual mice in each cage were tracked using ear notching. Six weeks postinfection (p.i.) all of the infected mice were euthanized by CO_2 asphyxiation. Of the two animals in each group, the homogenized stomach of one was passaged to a new group of three C57BL/6 mice by orogastric gavage (passage A), while the infecting strains were isolated from the gastric tissue of the other mouse. Of those plates on which colonies resembling *H. pylori* grew, 12 colonies per group were subcultured individually onto new CSA plates. Genomic DNA (gDNA) was extracted from a sample of all cultures that were successfully passaged, and these individual clones were also harvested and stored in liquid N_2. Three subsequent passages were performed in a similar manner for 4 (passage B), 7 (passage C), and 10 (passage D) weeks, except that at each of these time points the homogenized stomachs of mice in each individual group were pooled before being used for mouse passage and CFU determination. Additionally, for passage D a total of 10 C57BL/6 mice received doses of homogenized stomach tissue from those groups in which there was colonization remaining after passage B. Colonizing isolates were obtained from each passage as described and stored in liquid N_2. Only two groups of mice were still colonized after passage D, the SS1 group and group 2. The new mouse-adapted strain from group 2 was termed SS2000.

(ii) Colonization of original clinical isolate and mouse-adapted SS2000. To assess the extent of mouse adaptation that may have occurred in the SS2000 strain after the multiple mouse passages described above, the colonization level of SS2000 was compared with that of the original clinical isolate of SS2000 (strain number 2.1, termed pre-mouse-adapted [premouse] SS2000 [PMSS2000]) that had been identified by random amplified polymorphic DNA (RAPD) profiling. Two groups of 10 C57BL/6 mice were inoculated intragastrically with an equal amount ($\sim 10^7$ CFU/mouse) of either SS2000 or PMSS2000. After 4 weeks, all 20 mice were euthanized and their stomachs were excised. The nonglandular portion of the stomach was removed, opened along the lesser curvature, washed briefly in saline, and then divided in half such that each section contained a portion of the cardium, body, and antrum regions. One half of each stomach was homogenized for viable CFU counts and the other was fixed in 10% buffered formalin for histology (see below).

(iii) Long-term infection with SS2000 in comparison to SS1. Ninety C57BL/6 and 90 BALB/c mice were inoculated orogastrically twice within a 3-day period with BHI, SS1, or SS2000 (30 C57BL/6 and 30 BALB/c mice in each case). Equivalent numbers of bacteria for each strain (10^8 CFU/ml) were estimated for inoculation using hemacytometer counts and checked by retrospective CFU counts. At 6 mpi, 10 animals in each group were euthanized and their stomachs were excised as described above. Half of the gastric tissue was fixed in 10% buffered formalin and embedded in paraffin, and 4-μm-thick sections were cut. Sections were stained with hematoxylin and eosin (H&E) for histopathology assessment or a modified Steiner silver stain for colonization distribution and level assessment. The other half of the stomach was homogenized for viable CFU counts. At 15 mpi, 10 mice in all groups were euthanized, the stomachs were cut in half, one half of each stomach was fixed for histology, and the remaining half was placed in a cryo-tube and snap-frozen in liquid N_2. The stomachs of the remaining animals in each group (10 mice in all groups except for the BHI-inoculated groups in which there were three deaths, unrelated to the nature of the study, during the time period of the experiment) were handled as described in the previous two sections for histology and viable counts.

Assessment of colonization level and distribution. Using the silver-stained slides from the 6- and 15-month time points, five areas of the stomach (antrum, antrum-body transitional zone, body, body-cardium transitional zone, and cardium regions) were assessed for the level and presence of bacteria using a grading system from 0 to 4 as described previously (9, 36). Briefly, a grade of 0 indicates no bacteria observed, while a grade of 4 indicates heavy colonization where all crypts were densely packed. Since we did not have log CFU/gram levels for all mice (colonization levels for half the 15-month-time-point animals were only assessed by histology) a "total colonization score" was calculated for all mice. First, data for any animal for which there was missing data due to abnormal sectioning (e.g., antrum region not represented on stained slide) were removed

for the purpose of this analysis. The sum of the grades for each section of the stomach described above was then calculated and used to assess the relationship between colonization and inflammation in individual mice.

Assessment of histopathology. The histopathological features were assessed by light microscopy in blinded H&E-stained slides using criteria previously described (16, 49). Antral and body mucosae were graded separately for the presence of neutrophils and mononuclear cells. The scoring system was as follows: 1, mild multifocal; 2, mild widespread or moderate multifocal; 3, mild widespread and moderate multifocal or severe multifocal; 4, moderate widespread; 5, moderate widespread and severe multifocal; and 6, severe widespread. The total numbers of glands with neutrophil infiltration in the crypt and lumen were also counted to assess the number of gland abscesses (GA). Atrophy was evaluated on the degree of loss of parietal cells and mucous cell hyperplasia and assessed as 0, 1 (mild), 2 (moderate), or 3 (severe). Submucosal inflammation was assessed on a scale of 0 to 6 as described above. The total numbers of lymphoid aggregates (LA) and lymphoepithelial lesions in each section were counted. In order to assess the relationship between colonization and inflammation in individual mice, data for any animal for which there was missing data due to abnormal sectioning were removed for the purpose of this analysis. An "overall histopathology score" was calculated for all mice by using the sum of the histopathology grades described above. This score was used solely to determine the relationship between colonization and inflammation in individual mice.

Statistical analysis. Significant differences in colonization levels, LA, and GA were determined using unpaired *t* tests with Welch's correction (the variances of the groups were not equal). Analysis of differences in colonization distribution grading was determined using the nonparametric Kruskal-Wallis analysis (more than two groups were compared). Nonparametric Mann-Whitney analysis was used for the comparison of the histopathological grades between two groups at a time. Standard correlation coefficient determination was used to assess the level of association between bacterial load and gastritis in individual mice.

DNA manipulations. gDNA was extracted from *H. pylori* strains using the Puregene DNA Purification Kit (Gentra Systems, Minneapolis, Minn.) or the Wizard Genomic DNA Purification Kit (Promega, Madison, Wis.) according to the manufacturers' instructions and stored at −20°C until required. RAPD profiling based on the method by Akopyanz et al. (1) was performed on gDNA from the mouse-adapted SS2000 strain and the original clinical isolates from the group of input strains (2.1 to 2.5) using two primers (1281, AACGCGCAAC, and 1290, GTGGATGCGA) as previously described (31). The profiles obtained were compared in order to elucidate from which clinical isolate SS2000 originated.

Genome typing and analysis was done on the premouse and mouse-adapted strains. gDNA labeling and hybridization to *H. pylori* microarrays was performed as described by Salama et al. (42). In each case the test gDNA sample was labeled with Cy5 (red), and this was hybridized with Cy3 (green)-labeled reference DNA. The reference DNA consisted of equal amounts of gDNA from the two *H. pylori* strains used to make the *H. pylori* microarray, 26695 and J99 (Table 1). Two microarrays were performed for each of the strains 10700 (the premouse isolate of SS1), SS1, PMSS2000, and SS2000 (eight arrays in total).

To determine changes in the genomic content of these *H. pylori* strains, the data obtained from the microarrays described above were analyzed separately using the microarray genome analysis program GACK (26). This program generated a dynamic cutoff for assigning genes as present or divergent. Divergent genes either may be completely absent or may have significant sequence divergence from the gene used to generate the microarray. Thus, an individual cutoff was determined by GACK for each separate array hybridization. The program assigns an estimated probability of presence (EPP) according to the distribution of the $\log_2$ (red net intensity/green net intensity) ratios [$\log_2$(R/G)] for each array and thus gives an estimate of how likely a gene is to be present. The EPP range is from 0% (assigned as divergent) to 100% (assigned as present). Those genes falling in the transition region between 0% and 100% EPP are classified as "slightly divergent." The graded output of the GACK program that consists of a continuous range of values from −0.5 (divergent) to 0.5 (present) was used in these analyses. The GACK program is freely available (http://falkow.stanford.edu).

Data for the eight arrays were normalized and filtered (elements whose Cy3 net intensity was ≤100 were removed) using the Stanford Microarray Database (44). Duplicate spots representing the same open reading frame within each array were then averaged and genes with at least 90% good data were retrieved leaving a total of 1,522 unique open reading frames for analysis using the GACK program (26). If the assigned grade for a particular gene differed by more than 0.5 units (50% variation) using the graded output between duplicate arrays for the same strain, it was discarded from analysis because its hybridization was deemed irreproducible. The values reported are the average graded GACK values for each gene for the duplicate arrays for each strain. Genes that did not differ between the pre- and post-mouse-adapted (postmouse) isolates by at least 25% were not reported.

Supplementary material. Raw data for the microarrays used in the genome typing analysis can be found at http://genome-www.stanford.edu/. A table entitled "Genes differing between SS1 and SS2000" can be found at http://falkow.stanford.edu/whatwedo/supplementarydata.

RESULTS

Colonization ability of clinical isolates. The colonization levels in mice inoculated with one of the 22 groups of clinical isolates or three control strains were assessed 6 weeks p.i. The mouse-adapted SS1 strain, along with strains 10319 and 10217, which had previously been shown to colonize C57BL/6 mice for at least 1 month (31), were chosen as control strains. As expected, all three control strains were able to colonize at this early time point: 6.57 log CFU g^{-1} for SS1, 4.20 log CFU g^{-1} for 10319, and 4.94 log CFU g^{-1} for 10217. In the clinical isolate groups 18 of 22 (82%) mice were colonized to various degrees (median log CFU g^{-1}, 4.48; range, 3.00 to 7.18). The limit of detection for these experiments was 3.00 log CFU g of stomach tissue^{-1}. This suggests that at least 18 of the clinical isolates were able to colonize C57BL/6 mice for at least 6 weeks.

During the three subsequent mouse passages, the numbers of mice colonized decreased dramatically. After passage B, colonization was detected in 5 of 22 (21%) groups, and after passages C and D, colonization was detected for only one of the control strains (SS1) and for group 2. For passage C the levels for SS1 and group 2 were 7.11 and 7.18 log CFU g^{-1}, respectively. All of the strains that failed to colonize past passage B did not reach levels of >6.0 log CFU g^{-1}. For passage D 10 C57BL/6 mice were infected for 10 weeks to ensure reproducibility in the infection capability of the remaining isolates. The result for this final passage was 100% colonization in both the SS1 (7.42 ± 0.62 log CFU g^{-1})- and group 2 (7.39 ± 0.16 log CFU g^{-1})-infected animals. A single strain was isolated from this final mouse passage of group 2 and this was deemed the new mouse-colonizing strain SS2000. Thus, only one strain out of 110 tested clinical isolates was able to colonize mice for more than 2 months.

Identification of the original clinical isolate of SS2000. The original clinical isolate that gave rise to the new mouse-colonizing strain SS2000 was determined by comparing the RAPD profiles of all the input clinical isolates from group 2 (2.1, 2.2, 2.3, 2.4, 2.5) with the profiles for a number of mouse-adapted isolates of SS2000 from passages B, C, and D of group 2. Comparison of the RAPD profiles using two different primers (1281 and 1290) were in agreement and identified the clinical isolate named 2.1 as the premouse isolate of SS2000 (PMSS2000) (data not shown). This strain was originally isolated from an Australian male diagnosed with gastritis.

Colonization ability of premouse and mouse-adapted strains. Since the SS2000 strain was originally derived from a group of five strains, the colonization ability of the original clinical isolate (PMSS2000) compared to the recovered SS2000 strain was assessed. Both strains colonized 100% (10 of 10 each) of the animals 4 weeks p.i. However, the mouse-adapted strain of SS2000 was found to colonize to a statistically significant higher level than PMSS2000 (7.02 ± 1.19 and 6.80 ± 0.19 log

CFU g^{-1}, respectively; $P = 0.0003$; unpaired t test with Welch's correction).

Genomic typing of the pre- and postmouse strains. Mouse adaptation of the SS1 strain had also been observed by Lee et al. when compared to its original clinical isolate (termed PMSS1 herein) (31). Thus, genomic typing using an *H. pylori* microarray was performed on the pre- and postmouse strains of SS1 and SS2000 to determine whether any genomic changes were involved in the mouse adaptation of these strains. Analysis was performed with the GACK program that calculates an estimate of how likely a gene is to be present in the strain being tested (26). This program has been used previously for analysis of gene content in other bacterial species (6, 32).

These results revealed few modifications of genes during the process of mouse adaptation, even though the colonization abilities of these strains had changed. Only three genes were found to differ between the pre- and postmouse strains of SS2000, while 49 genes showed differences between the pre- and postmouse strains of SS1 (Table 2). These differences in SS1 represent less than 0.04% of the analyzed genes in the *H. pylori* genome. The majority of the genes that showed variation were of unknown function and many of these are located in one of the two plasticity zones in the *H. pylori* genome (2). There were also a number of changes in genes encoding outer membrane proteins, including the *babB* gene that encodes an adhesin. The hybridization capacity of *babB* increased in the SS1 strain compared to that in the PMSS1 isolate (Table 2). Interestingly the highly related *babA* gene, also coding for an adhesin, was missing from all pre- and postmouse isolates tested. Only one gene, HP0486, was found to vary between both the SS1 and SS2000 pairs of strains. This gene was deemed to be divergent in the premouse isolates but present in both SS1 and SS2000 by GACK analysis. This gene encodes a hypothetical protein with known homologues occurring only in *Helicobacter* species (http://www.tigr.org/tdb/mdb/mdbcomplete.html). Other important changes that may have occurred in the genomes of these strains during mouse adaptation may be in strain-specific genes not represented on our microarray or may have been too minor to detect using this type of microarray analysis.

To investigate possible differences in the genetic makeup of SS1 and SS2000, further analysis of the GACK output was performed. There were 96 genes that varied between SS1 and SS2000 (http://falkow.stanford.edu/whatwedo/supplementarydata). The most striking difference between these strains was the apparent absence of the entire *cag* PAI in SS2000 while all 27 genes of the island were deemed present in SS1. The majority of the other differences were in genes of unknown function mainly located in the plasticity zones.

Long-term infection with SS1 and SS2000: colonization. To compare the abilities of the two mouse-colonizing strains, SS1 and SS2000, to persist in mice and to produce pathology, C57BL/6 and BALB/c mice were infected with these individual strains and assessed 6 and 15 mpi. All control animals were found free from gastric *Helicobacter* infection. The colonization levels of SS1 and SS2000 after 6 months were similar in both mouse strains and in both cases the level of colonization in the C57BL/6 mice was significantly higher ($P < 0.02$) than in the BALB/c mice. The distribution of SS1 and SS2000 throughout the stomach was similar in C57BL/6 mice, with the bacteria having a preference for the non-acid-secreting regions (antrum and cardium) and low-acid regions of the stomach (transitional zones [TZs] between the antrum and body, and between the cardium and body) (Fig. 1). In BALB/c mice the distribution of the two *H. pylori* strains was similar, with the highest levels detected in the two TZs. However, the level of colonization of SS2000 in the TZs and the cardium region were greater than in the SS1-infected BALB/c mice and this difference was statistically significant.

The colonization levels 15 mpi were statistically increased compared to the 6-month levels in the SS2000-infected groups (Tables 3 and 4). The level of SS2000 in the C57BL/6 mice was also higher than the level of SS1 and this was statistically significant (Table 3). At this later time point, both strains retained a statistically significant higher level of colonization in the C57BL/6 mice than in the BALB/c mice. The distribution patterns of both strains in the C57BL/6 mice were similar, although the grade in each section of the stomach was statistically higher in the SS2000-infected mice than in the SS1-infected mice (Fig. 1A and B). This reflects the higher colonization level of SS2000. In the BALB/c mice the higher level of colonization of SS2000 was reflected in a statistically significant higher grade in the antrum compared only with the SS1 group (Fig. 1C and D).

Interestingly, when the level of colonization of both SS1- and SS2000-infected C57BL/6 animals 6 mpi was compared with the level detected 10 weeks p.i. in passage D of the isolation experiment, it was found that the bacterial loads were statistically lower in the animals infected for the longer time (SS1-infected animals, $P < 0.01$; SS2000-infected animals, $P < 0.001$). This remained the case 15 mpi in the SS1-infected group compared with 10 weeks p.i. However, as mentioned earlier, the level of SS2000 increased by 15 mpi back to a level similar to that in the mice infected for 10 weeks. Thus, it appears that in SS1-infected C57BL/6 animals the bacterial load decreased significantly after 10 weeks of infection and then remained at a steady lower level during the later stages of infection. In contrast, in the SS2000-infected C57BL/6 animals, although the bacterial load was reduced between 10 weeks and 6 months, this level rose again by 15 mpi. We did not perform any shorter-term colonization experiments with the BALB/c mice so it is not known whether the same trends exist in this animal type with these *H. pylori* strains.

Histopathology. Infection of BALB/c and C57BL/6 mice with both SS1 and SS2000 for 6 months induced moderate to severe multifocal lymphoplasmacytic and follicular gastritis (Tables 3 and 4). Overall, the inflammatory response induced by SS1 and SS2000 was similar in both mouse types at this time point; however, the monocytic cell infiltration in response to SS2000 tended to be slightly more severe (Tables 3 and 4). Only a mild influx of neutrophils was observed in animals infected with either *H. pylori* strain, which was statistically indistinguishable from the control animals. The follicular gastritis, characterized by LA and follicles, was most notable in the cardium equivalent region of BALB/c mice infected with SS2000 (Table 4). One striking difference between the inflammatory responses observed between mouse strains in response to *H. pylori* infection was functional atrophy distinguished primarily by loss of parietal cells. By 6 mpi, 60% of both SS1- and SS2000-infected C57BL/6 mice showed signs of atrophy ranging from moderate to severe (grade range of 2 to 3) (Table 3).

TABLE 2. Genes that vary between pre- and postmouse SS2000 and SS1 strains

Strain and unique identification no.	Gene	Putative function(s)	Category	GACK score[a]	
				Premouse	Mouse passaged
SS2000					
JHP0562	Put.[b]	Lipopolysaccharide biosynthesis protein	Cell envelope	−0.025	−0.5
HP0486	OMP	Outer membrane protein	Cell envelope	0.25	0.5
JHP0929		Unknown		0.5	−0.025
SS1					
HP1265	*nuoF*	Putative NADH oxidoreductase	Energy metabolism	−0.5	0.3
HP0079	*omp3*	Outer membrane protein	Cell envelope	−0.5	0.275
HP1432		Histidine- and glutamine-rich protein	Transport and binding	−0.5	0.275
HP1298	*infA*	Translation initiation factor EF-1	Translation	−0.4	0.5
HP0843	*thiB*	Thiamine phosphate pyrophosphorylase	Biosynthesis cofactors	0.25	0.5
HP1342	*omp29*	Outer membrane protein	Cell envelope	0.025	0.5
HP0486		Outer membrane protein	Cell envelope	0.025	0.5
HP0896	*babB*	Outer membrane protein	Cell envelope	0.05	0.5
HP0536	*cag15*	*cag* PAI protein	Cellular processes	−0.375	0.5
HP0979	*ftsZ*	Cell division protein GTPase	Cellular processes	0.225	0.5
HP1452	*tdhF*	Thiophene and furan oxidizer	Cellular processes	0.2	0.5
HP0599	*hylB*	Methyl-accepting chemotaxis protein (MCP)	Cellular processes	−0.35	0.5
HP1370	*mod*	Type III restriction enzyme M	DNA metabolism	−0.25	0.5
HP0464	*hsdR*	Type I restriction enzyme R	DNA metabolism	0.125	0.5
HP1347	*ung*	Uracil-DNA glycosylase	DNA metabolism	0.2	0.5
HP1383		Restriction modification system S subunit	DNA metabolism	0.225	0.5
JHP1297	*res_1*	Putative type III restriction enzyme	DNA metabolism	0.225	0.5
HP1269	*nuoJ*	NADH-ubiquinone oxidoreductase, NQO10	Energy metabolism	−0.1	0.5
HP1212	*atpE*	ATP synthase F_0, subunit c energy metabolism	Energy metabolism	0.15	0.5
HP0961	*gpsA*	Glycerol-3-phosphate dehydrogenase	Energy metabolism	0.075	0.5
HP0690	*fadA*	Acetyl-coenzyme A acetyltransferase (thiolase)	FA metabolism	0.125	0.5
HP0557	*accA*	Acetyl-coenzyme A carboxylase	FA metabolism	−0.025	0.5
HP0919	*carB*	Carbamoyl-phosphate synthase (glutamine hydrolyzing)	Pyrimidine ribonucleotide biosynthesis	0.225	0.5
HP1572	*dniR*	Regulatory protein DniR	Regulator	−0.175	0.5
HP0835	*hup*	Histone-like DNA-binding protein	Translation	−0.075	0.5
HP1295	*rps11*	Ribosomal protein S11, 30S	Translation	0.025	0.5
HP1153	*valS*	Valyl-tRNA synthetase	Translation	−0.3	0.5
HP0686	*fecA*	Iron(III) dicitrate transport protein	Transport and binding	−0.275	0.5
HP1341	*tonB*	Siderophore-mediated iron transport protein	Transport and binding	−0.175	0.5
HP0853	*yheS*	ABC transporter, ATP-binding protein	Transport and binding	0.05	0.5
HP0710		Cons. hypoth.[c] protein, putative outer membrane protein		−0.025	0.5
HP1337		Cons. hypoth. protein		0.2	0.5
HP1484		Cons. hypoth. integral membrane protein		0.075	0.5
HP0102		Cons. hypoth. protein		0.175	0.5
HP0105		Cons. hypoth. protein		−0.3	0.5
HP0186				−0.325	0.5
HP0720				0.075	0.5
HP0189		Cons. hypoth. integral membrane protein		−0.225	0.5
HP0408				−0.225	0.5
HP1353				−0.125	0.5
HP0594				−0.075	0.5
HP0057				−0.3	0.5
HP0924	*dmpI*	4-Oxalocrotonate tautomerase		−0.35	0.5
HP0783				−0.275	0.5
HP0311				−0.45	0.5
HP0823		Cons. hypoth. protein		0.5	0.225
JHP0950				0.425	−0.5
HP1097				0.5	−0.35

[a] The numbers in these columns refer to the average across duplicate arrays of the values assigned by the graded GACK analysis to each of the genes in each microarray (described in Materials and Methods).
[b] Put., putative function.
[c] Cons. hypoth., conserved hypothetical.

In contrast, no atrophy was noted in any BALB/c mice, regardless of infection (Table 4).

By 15 mpi clear differences in the host response to the two strains of *H. pylori* were apparent (Tables 3 and 4; Fig. 2). SS1 infection induced a more severe widespread lymphoplasmacytic gastritis in C57BL/6 mice than SS2000 infection (grade range of 2 to 5) (Fig. 2). Follicular gastritis, characterized by LA, was also observed in the SS1-infected BALB/c mice. Submucosal infiltration of inflammatory cells in both strains of mice had increased from a mild or moderate multifocal influx to a more moderate widespread infiltration of cells (grade, 2 to 4) (Fig. 2). However, the functional atrophy in C57BL/6 animals was less severe than at 6 months, and it remained virtually absent in the BALB/c animals. Neutrophil involvement had

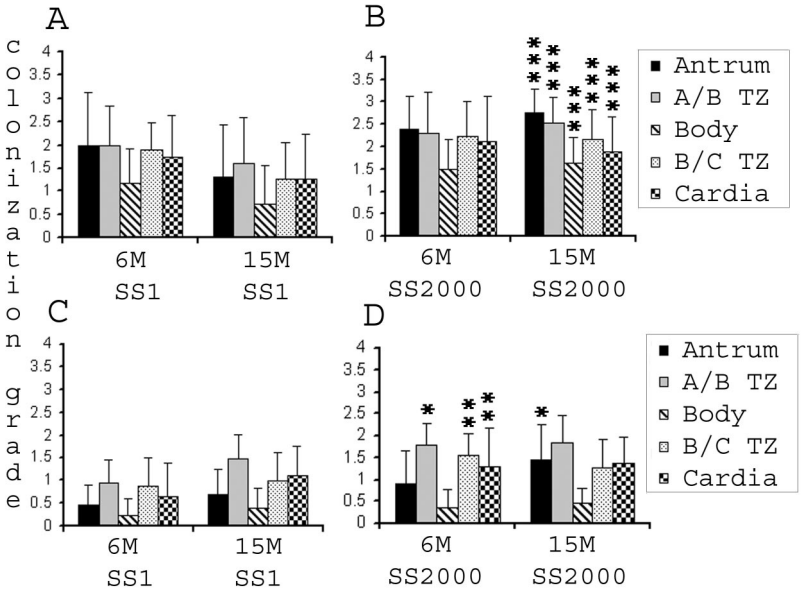

FIG. 1. Histograms showing the grades of colonization of SS1 and SS2000 after 6 and 15 months of infection in each part of the stomach. (A) SS1-infected C57BL/6 mice; (B) SS2000-infected C57BL/6 mice; (C) SS1-infected BALB/c mice; (D) SS2000-infected BALB/c mice. Levels are represented as the grade of colonization from 1 to 3. A/B TZ and B/C TZ are the antrum-body and body-cardium TZs, respectively. The mean grade is used and error bars represent 1 standard deviation. The colonization grade of SS2000 is statistically significant compared to that of SS1 in the indicated regions at the same time point. Level of significance (Kruskal-Wallis unpaired *t* test) is indicated as follows: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

increased such that the levels were statistically significant compared with the control animals.

In contrast to the deteriorating gastritis apparent in SS1-infected animals 15 mpi, in SS2000-infected C57BL/6 mice, gastritis was largely reduced or remained at a similar level. The lymphoplasmacytic gastritis had decreased in severity since the 6-month time point and was statistically less severe than in SS1-infected C57BL/6 mice at the 15-month time point (Table 3). Similar to the SS1-infected animals, functional atrophy in the SS2000-infected C57BL/6 animals was at a statistically significant lower level at 15 months compared with the level at 6 mpi. Despite the overall reduction in inflammation in these animals, gastritis was still more severe than that observed in the uninfected control animals (Fig. 2).

The host response to SS2000 observed in C57BL/6 mice was in stark contrast to that observed in the BALB/c mice. In the latter group of mice, SS2000 induced a moderate to severe neutrophilic lymphoplasmacytic follicular gastritis that was widespread throughout the stomach (grade, 2 to 5) (Table 4 and Fig. 2). The number of LA and follicles had increased (range, 0 to 6 per section), as had the number of GA (range, 0 to 6 per section). A mild-to-severe widespread influx of inflammatory cells was observed in the submucosa (grade, 1 to 5). In contrast to that seen in the C57BL/6 mice, the severity of atrophy had increased compared with the level at 6 months and was also more severe than that seen with SS1 infection.

Association between inflammation and bacterial load. The relationship between colonization level and inflammation was assessed for individual mice in all groups. In the C57BL/6 mice there was an inverse correlation between the calculated total colonization score and total histopathology score (described in Materials and Methods) in the mice infected with SS1 (corre-

TABLE 3. Histopathology grades and colonization levels for long-term-infected C57BL/6 mice[a]

C57BL/6 group	Time point (mo)	Antrum		Body		LA	GA	Atrophy	Submucosal inflammation	Colonization (log CFU g^{-1})
		Neutrophils	Monocytes	Neutrophils	Monocytes					
Noninfected	6	0.1 (0–1)	0.6 (0–2)	0 (0)	0.4 (0–1)	0 (0)	0 (0)	0 (0)	0 (0)	0
	15	0.3 (0–1)	1.2 (0–2)*	0.1 (0–1)	0.4 (0–2)	0 (0)	0 (0)	0 (0)	0 (0)	0
SS1 infected	6	0.2 (0–2)	1.5 (1–3)	0.1 (0–1)	2 (1–3)	1.3 (0–8)	0.3 (0–2)	1.2 (0–3)	1.2 (0–3)	6.36 ± 0.88
	15	1.2 (0–2)*	2.6 (1–4)*†	1.4 (0–3)*	3 (1–5)*†	1 (0–3)	1.4 (0–6)	0.5 (0–2)	2.4 (0–4)*†	6.48 ± 0.86
SS2000 infected	6	0.5 0–2)	2.1 (1–3)	0.5 (0–2)	2.3 (1–4)	1.7 (0–8)	0.8 (0–4)	1.5 (0–3)₱†	1.5 (0–3)	6.40 ± 0.54
	15	0.9 (0–2)	1.6 (1–3)	1 (0–2)	1.7 (0–3)	0.8 (0–3)	1 (0–3)	0.3 (0–2)	1.5 (0–5)	7.45 ± 0.14*¥

[a] All scores show the mean (range) or mean ± standard deviation. Symbols: *, significantly greater than corresponding value at 6 months; †, significantly greater in SS1-infected mice compared with SS2000-infected mice after 15 months; ¥, significantly greater in SS2000 than in SS1 at the same time point; ₱, significantly greater than corresponding value at 15 months. All significance tests involving neutrophil or monocyte infiltration, atrophy, and submucosal inflammation used $P < 0.05$ (Mann-Whitney), while tests for the counts of the number of LA or GA and for the colonization level determinations used $P < 0.05$ (unpaired *t* test with Welch's correction).

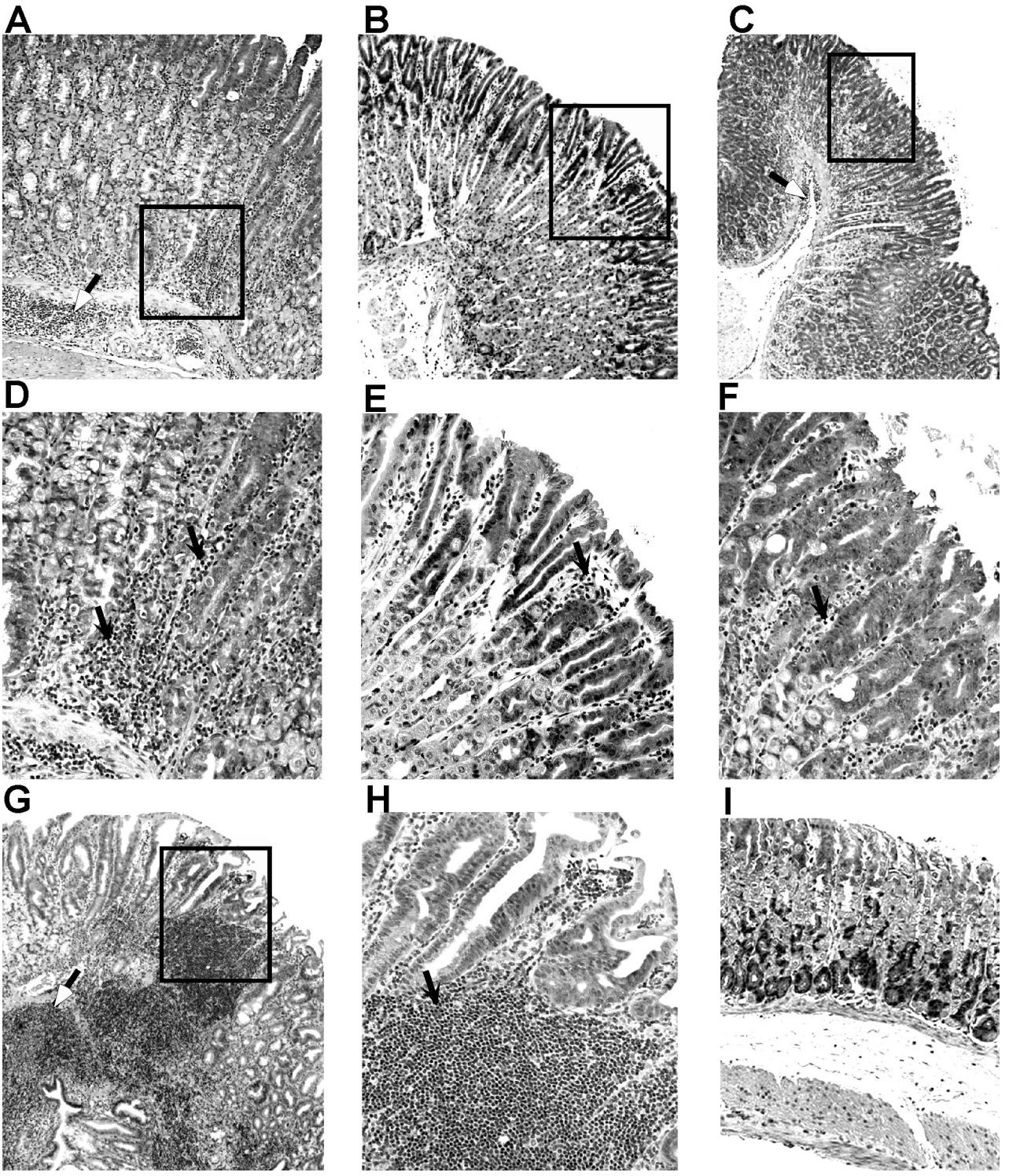

FIG. 2. Representative regions of the gastric body mucosae of C57BL/6 and BALB/c mice following a 15-month infection with *H. pylori*. Significant widespread lymphoplasmacytic gastritis of the entire mucosa and parts of the submucosa can be seen in the SS1-infected C57BL/6 animal (A and D) that is significantly less severe in the SS2000-infected C57BL/6 animal (B and E) and in the SS1-infected BALB/c animal (C and F). (G and H) Severe follicular gastritis in this SS2000-infected BALB/c animal, characterized by the large LA destroying the mucosa. (I) Uninfected C57BL/6 animal. Original magnifications: ×50 (A, B, C, G, and I) and ×200 (D, E, F, and H). The boxes in panels A, B, C, and G are represented in the higher magnification in panels D, E, F, and H, respectively. Closed arrows indicate infiltrating inflammatory cells in the mucosa and open arrows indicate inflammatory cells in the submucosal regions. There are no inflammatory cells evident in the control panel I. Staining was done with hematoxylin and eosin.

TABLE 4. Histopathology grades and colonization levels for long-term-infected BALB/c mice[a]

BALB/c group	Time point (mo)	Antrum		Body		LA	GA	Atrophy	Submucosal inflammation	Colonization (log CFU g^{-1})
		Neutrophils	Monocytes	Neutrophils	Monocytes					
Noninfected	6	0 (0)	1.1 (0–2)	0 (0)	1 (0–2)	0 (0)	0 (0)	0 (0)	0.1 (0–1)	0
	15	0.2 (0–1)	1.2 (1–2)	0.1 (0–1)	1.4 (1–2)	0 (0)	0 (0)	0 (0)	0.1 (0–1)	0
SS1 infected	6	0.6 (0–2)	1.9 (1–3)	0.5 (0–2)	2.3 (2–3)	0.6 (0–2)	0.2 (0–1)	0.1 (0–1)	1.3 (0–3)	4.97 ± 0.39
	15	1.3 (0–2)*	2.5 (0–2)*	1.1 (0–2)*	3 (2–4)*	2.1 (0–5)*	0.5 (0–3)	0.2 (0–1)	2.7 (1–4)*	5.38 ± 0.70
SS2000 infected	6	0.6 (0–1)	2.1 (1–3)	0.2 (0–1)	2.4 (2–4)	1.8 (1–3)¥	0.7 (0–3)	0.2 (0–1)	1.9 (1–3)	5.28 ± 0.47
	15	1.3 (0–2)*	2.6 (1–4)	1.4 (0–3)*	3.6 (2–5)*	3.7 (0–6)*†	1.9 (0–6)†	1 (0–3)*†	3.1 (1–5)*	6.06 ± 0.37*

[a] All scores show the mean (range) or mean ± standard deviation. Symbols: *, significantly greater than corresponding value at 6 months ($P < 0.05$); †, significantly greater in SS2000-infected mice compared with SS1-infected mice after 15 months ($P < 0.05$; Mann-Whitney); ¥, significantly greater in SS2000 than in SS1 at the same time point. All significance tests involving neutrophil or monocyte infiltration, atrophy, and submucosal inflammation used $P < 0.05$ (Mann-Whitney), while tests for the counts of the number of LA or GA and for the colonization level determinations used $P < 0.05$ (unpaired t test with Welch's correction).

lation coefficient for 6 mpi was −0.8, and that for 15 mpi was −0.6) and SS2000 (correlation coefficient for 6 mpi was −0.7, and that for 15 mpi was −0.8). This result indicates that in individual animals high levels of gastritis were associated with lower levels of colonization. This occurred in spite of the fact that when the entire groups were compared no statistically significant reduction in colonization level was observed in any group of animals between 6 and 15 mpi, while there was a statistical increase in gastritis scores. The reason for this apparent discrepancy can be explained by the highly variable responses in individual mice with regard to colonization level and gastritis induction, particularly at the 15-month point (data not shown). Further investigation revealed that the association between low bacterial load and high gastritis level could be attributed to the presence or absence of LA. Thus, the total colonization scores were compared in animals with <1 LA and those with ≥1 LA utilizing Student's t test with Welch's correction. At 6 mpi the total colonization score had reached a statistically significant higher level in SS2000-infected ($P = 0.03$) but not in SS1-infected ($P = 0.13$) animals with no detectable LA, compared to those with ≥1 LA. However, by 15 mpi this level was statistically significant in both animals infected with SS1 and SS2000 ($P = 0.01$ in both cases).

In contrast, no association between colonization level and gastritis was found when the total colonization score and the total histopathology score were compared in individual BALB/c mice in any group (data not shown). This is likely due to the lower overall numbers of bacteria in the BALB/c mice and to the fact that a MALT-type inflammatory response was induced in these animals, resulting in only 12% of infected BALB/c mice at 6 and 15 mpi having no detectable LA, compared with 56% of the infected C57BL/6 mice.

DISCUSSION

A new strain of *H. pylori* with mouse colonization abilities superior to those of the commonly used SS1 strain was isolated through sequential mouse passages from a large number of clinical isolates. Whole-genome typing using microarrays revealed only a small number of differences in gene content between the premouse and mouse-adapted isolates of SS1 and SS2000. However, the differences in gene content between SS1 and SS2000 were significant as microarray analysis revealed that SS2000 lacks the entire *cag* PAI in comparison to SS1, which contains all 27 genes of the island. Both SS1 and SS2000 colonized at high levels for at least 15 months in two strains of mice (C57BL/6 and BALB/c). Significant differences between SS1- and SS2000-induced inflammation in both mouse types were found only 15 mpi, indicating that inflammation induced in the mouse model after long-term colonization is dependent on host and *H. pylori* strain differences.

A new mouse model for chronic *H. pylori* infection. Colonization by a number of individual *H. pylori* strains for 1 to 2 months has been shown by several researchers (for examples, see references 39 and 50). However, the majority of these studies do not report longer-term colonization, and in many cases less than 100% of the inoculated mice remain infected over the short time frame (45). Short-term infection experiments in mice using genetically modified *H. pylori* strains can be useful for determining factors required for colonization (13, 36, 43, 52). However, given that *H. pylori* infection in humans is chronic, studies in animal models in which the effects of long-term colonization can be assessed are desirable. Herein we show that many of the clinical isolates (at least 20) were able to colonize mice to various degrees for up to 6 weeks. However, only one strain, SS2000, was able to colonize for longer than 2 months. Since none of the strains that colonized transiently reached a level greater than 6.0 log CFU g^{-1} in C57BL/6 mice, this may indicate a minimum bacterial load required for long-term persistence in mice. Another previous study has investigated the colonization and inflammation induced by SS1 and two other *H. pylori* strains, 119p and G50, over a 2-year period in C57BL/6 and BALB/c mice. While both the SS1 and 119p strains were able to colonize 100% of mice for 10 weeks, by the 23-month point only SS1 remained able to colonize all infected mice (53). Thus, difficulties arise when attempting to interpret host and strain-specific effects due to nonequivalent colonization levels. Hoffman et al. have taken a different approach to establish a new robust model for *H. pylori* infection by using C57BL/6 mice deficient in interleukin-12p40 (IL-12p40) (22). These knockout mice are more permissible to infection with both SS1 and the sequenced strain 26695 that typically does not colonize wild-type C57BL/6 mice. This model may be useful for investigating *H. pylori* virulence factors in vivo; however, it has limitations for studying immune responses to infection since IL-12 plays a dominant role in the immune response. In contrast, the model described in the

present study provides the opportunity to improve our understanding of the contribution of strain and host factors to disease development during chronic infection.

Mouse adaptation of *H. pylori* isolates. The mouse-adapted SS2000 strain colonized to a level approximately 0.5 log unit higher than the pre-mouse-adapted strain (PMSS2000) in C57BL/6 mice; thus, some host adaptation appears to have occurred during the isolation of this strain. Host adaptation of *H. pylori* has been reported to occur in mice (9, 31, 39), gerbils (23), primates (11), piglets (15), and humans (18, 24, 25). The mechanisms of these adaptations are largely unknown, although they are likely to involve genomic variations (3, 18). Genomic variation may induce changes in phenotype such as *cag* PAI functionality, lipopolysaccharide expression and Lewis antigen expression (35, 39, 54). In an effort to understand host adaptive mechanisms, we compared PMSS1 and PMSS2000 to the postmouse isolates SS1 and SS2000 using an *H. pylori*-specific microarray.

The recently described GACK microarray analysis program was used to assign genes in each of the strains tested as present, slightly divergent, or highly divergent or absent (26). GACK analysis of the pre- and postmouse isolates revealed few changes in the genetic content of the strains after adaptation to colonization of C57BL/6 mice in both cases. Many of these possible changes in both strains occurred in genes of unknown function that reside in the plasticity zones of the genome. This finding is in agreement with other host adaptation studies in *H. pylori* that have also shown that most changes occur in the plasticity zones (42).

Only one consistent change was detected in the two pairs of strains studied here, in a gene encoding a hypothetical protein, HP0486. This *Helicobacter*-specific protein may thus be crucial for host-specific adaptation. A previous report by Solnick et al. reported genomic changes occurring between the input and output strains in a rhesus macaque model of *H. pylori* infection (46). In that study the *babA* gene was deleted in the output strains, while the *babB* gene appeared to be duplicated during infection. Interestingly we found that the *babA* gene was missing from both the premouse and postmouse isolates we tested, supporting the notion that this gene is not required for animal colonization (46). In contrast, the *babB* gene was present in both the pre- and postmouse SS2000 strains and its hybridization capacity appeared to increase during host adaptation. This may also be associated with gene duplication in this strain, although this hypothesis requires further investigation.

To our knowledge no previous report has investigated genetic changes in multiple strains in parallel during such host adaptation on a genome-wide level. Thus, the present study is the first to indicate that genomic adaptations in *H. pylori* may be largely strain specific and that there appear to be few specific genomic changes required for host adaptation. There is also the distinct possibility that some of the genetic changes occurring in these strains during mouse adaptation may occur in strain-specific genes that are not represented on the microarray. This suggests that newly discovered strain-specific genes should be added to the *H. pylori* microarray to help elucidate the importance of these genes in multiple strains adapted to different hosts. Other genetic changes that are too small to detect using the microarray, such as point mutations or frame shifts, may dramatically affect transcription or translation of the affected protein, and thus other methods may prove useful to elucidate important consistent changes that occur during host adaptation.

Mouse colonization and the *cag* PAI. We found that the SS2000 strain lacks the entire *cag* PAI but is still capable of inducing inflammation in BALB/c mice in excess of the SS1 strain (which contains the entire island). However, in the C57BL/6 mice the SS2000 strain induced less inflammation than the SS1 strain. This suggests the unexpected possibility that the effect of the *cag* PAI is host specific. These data contrast with that in humans where strains with the *cag* PAI are associated with more extensive inflammation; however, this response may also be host dependent (20). The absence of the PAI in SS2000 illustrates that these genes are not required for colonization or gastritis in the mouse model. This finding is supported by previous findings by Eaton et al. who have shown that deletion of the *cag* PAI from SS1 did not have any effect on colonization and inflammation in C57BL/6 mice (13). It is important to note that the SS1 strain used by Eaton et al. was shown to have a nonfunctional *cag* PAI that was unable to induce IL-8 in cell culture (13). Other investigators have also reported a similar phenomenon (8). The functionality of the *cag* PAI in the SS1 strain used in the present study was not tested, but it is likely that our strain also harbors a nonfunctional PAI despite the fact that it has retained all of its genes. Additionally, it has previously been suggested by Philpott et al. and others that the absence of the *cag* PAI genes or mutations rendering them nonfunctional may provide an advantage for persistent infection of mice (39). Clearly the *cag* PAI is not the only factor affecting colonization ability in the mouse model, as many *cag* PAI-negative strains have been shown to be unable to persist in mice (39, 48). Considering that there is an incomplete correlation between the severity of disease in an individual and the presence of the *cag* PAI in the infecting *H. pylori* strain, this new mouse model should be useful in determining the importance of *cag*-independent virulence factors.

Comparison of the long-term colonization and inflammation properties of two mouse-adapted strains. Regardless of any genetic differences in the SS1 and SS2000 strains, 6 mpi the colonization levels of these two strains in both BALB/c and C57BL/6 mice were comparable. For both strains, the colonization level was higher in C57BL/6 animals than in BALB/c mice, suggesting that host-dependent factors previously described in these mouse types are important (27, 29). The inflammation produced by the two *H. pylori* strains after 6 months was almost indistinguishable with the exception of an increased number of LA induced in BALB/c mice by SS2000. The inflammatory response after 6 months of infection with SS1 observed here is similar to previously published data (36, 49).

However, by 15 mpi, more-distinct differences in colonization and inflammation produced by the two bacterial strains were apparent. While the level of SS1 colonization remained nearly identical to the 6-month point, the level of SS2000 colonization increased significantly in both mouse strains. Interestingly, however, the level of inflammation induced by SS2000 did not increase concomitantly in the C57BL/6 mice. The ability of SS2000 to increase an already high bacterial load in these animals without an increase in gastritis suggests that this strain may have an enhanced ability to evade the murine

immune system and thus may be inherently less inflammatory. In contrast, the level of inflammation in the C57BL/6, SS1-infected mice was statistically increased from the level at 6 months resulting in a moderate to severe neutrophilic lymphoplasmacytic gastritis at the 15-month point.

In the BALB/c mice, as was indicated at the 6-month point, SS2000 induced a significantly higher level of inflammation than that seen in the SS1-infected animals. This inflammation was characterized by a large number of LA in the body and cardium regions, indicating a MALT-type inflammatory response. Since a small proportion of *H. felis*- and "*H. heilmannii*"-infected BALB/c animals with a similar MALT-type inflammatory response have been shown to develop a pathology resembling MALT lymphoma by 24 to 28 mpi (17, 30, 38), it is possible that SS2000 may also induce a number of these lymphomas after longer-term infection. Interestingly, the *cag* PAI is thought to be missing from both *H. felis* and "*H. heilmannii*." Thus, it is possible that the lack of the *cag* PAI in SS2000 may be a requirement for the induction of MALT-type inflammation produced by this strain in BALB/c mice.

The mechanisms underlying the differences in host immune response between the C57BL/6 and BALB/c mice are largely unknown. However, an important difference is that a proinflammatory Th1 response dominates in the C57BL/6 mice, while the noninflammatory Th2 phenotype is present in the BALB/c mice. This has been used to explain the general lack of inflammation seen in the BALB/c mice after moderate-length infections (6 months) (19). A more thorough understanding of these polarized phenotypes in these mice may help explain the differences seen in their response to *H. pylori* infection and we are currently examining these possibilities.

Association between gastritis and bacterial load. Assessment of the relationship between bacterial load and gastritis in infected C57BL/6 mice revealed that in individual mice in which more severe gastritis had developed, colonization had been significantly reduced. This result is in agreement with a number of previous studies in which the colonization level of SS1 in C57BL/6 mice has been shown to be lower in mice with severe gastritis after infection for at least 16 weeks (14, 21). In a study by Garhart et al. (21) a number of early time points were investigated showing that the bacterial load was very high for up to 8 weeks p.i. and then dropped off after 16 weeks. A similar result was reported by Eaton et al. (14), in whose work colonization by SS1 was shown to decrease after 8 weeks of infection in C57BL/6 mice, while gastritis increased over the course of the experiment. Despite the colonization trends in the aforementioned studies, a statistical difference in colonization level was only reported between the very early time points ($\leq$5 weeks p.i.) and the very late time points ($\geq$51 weeks p.i.), with average levels changing little after 32 weeks ($\sim$8 months). In the present study we found no statistical difference in colonization levels between 6 months (24 weeks) and 15 months (60 weeks) in the SS1-infected C57BL/6 group. However, comparing these later colonization levels to those detected in our first experiment describing the isolation of SS2000 after only 10 weeks of infection in passage D, we found that the bacterial loads were indeed lower at 6 months than at 10 weeks p.i., thus supporting the findings of these previous studies.

In our study we have further shown that reduction in colonization levels could be related specifically to the presence of LA, rather than gastritis per se as has been previously reported (14, 21). Thus, it is feasible that MALT-type inflammation may be more effective in reducing bacterial colonization than diffuse lymphocytic infiltration. One explanation for this could be that the accumulated MALT may culminate in a larger number of lymphocytes in the mucosa than the diffuse type of infiltration. In support of this notion, we also found that the level of *H. pylori* colonization in BALB/c mice was consistently lower than that in C57BL/6 animals, confirming a number of previous observations (27, 31). This is interesting given that the type of *H. pylori*-induced pathology in BALB/c mice is predominantly lymphocytic aggregation rather than diffuse infiltration of lymphocytes. The difference in overall colonization levels and type of inflammation in the BALB/c mice may also explain why we did not find any correlation between bacterial load and gastritis in these animals. Alternatively this could be explained by a change in tissue type that accompanies LA formation. *H. pylori*, like *H. felis*, is unable to survive inside an aggregate but can be found on the periphery of the aggregates. Thus, a large number of LA may have reduced the stomach area available for colonization.

Summary. These experiments have provided the opportunity to dissect some of the factors contributing to the host- and strain-specific effects on disease in *H. pylori* infection of mice. *H. pylori* strain-specific effects were evident through the differing levels of colonization and inflammation produced by the two strains. Host-specific effects were shown in the different level of colonization occurring in C57BL/6 mice compared with BALB/c mice and in the type of pathology induced. Interestingly, the strain-specific effects were not evident until the 15-month point, indicating the utility of long-term infection experiments in this animal model. These results have also extended the previous observations that increasing inflammation is associated with reduction in bacterial load and that the specific aspect of inflammation involved in this process may be the formation of lymphocytic aggregates in the stomach mucosa. Further studies investigating the specific factors involved in these different host responses in the mouse model may shed light on the mechanisms for host-specific responses seen in human *H. pylori*-induced disease.

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