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Article

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Helicobacter pylori strain-specific differences in genetic content, identified by microarray, influence host inflammatory responses

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Helicobacter pylori enhances the risk for ulcer disease and gastric cancer, yet only a minority of *H. pylori*–colonized individuals develop disease. We examined the ability of two *H. pylori* isolates to induce differential host responses in vivo or in vitro, and then used an *H. pylori* whole genome microarray to identify bacterial determinants related to pathogenesis. Gastric ulcer strain B128 induced more severe gastritis, proliferation, and apoptosis in gerbil mucosa than did duodenal ulcer strain G1.1, and gastric ulceration and atrophy occurred only in B128$^+$ gerbils. In vitro, gerbil-passaged B128 derivatives significantly increased IL-8 secretion and apoptosis compared with G1.1 strains. DNA hybridization to the microarray identified several strain-specific differences in gene composition including a large deletion of the *cag* pathogenicity island in strain G1.1. Partial and complete disruption of the *cag* island in strain B128 attenuated induction of IL-8 in vitro and significantly decreased gastric inflammation in vivo. These results indicate that the ability of *H. pylori* to regulate epithelial cell responses related to inflammation depends on the presence of an intact *cag* pathogenicity island. Use of an *H. pylori* whole genome microarray is an effective method to identify differences in gene content between *H. pylori* strains that induce distinct pathological outcomes in a rodent model of *H. pylori* infection.

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Introduction

Chronic gastritis induced by *Helicobacter pylori* enhances the risk for a diverse spectrum of diseases including duodenal and gastric ulceration and noncardia gastric cancer (1–3). However, the majority of *H. pylori*–colonized individuals remain asymptomatic; propensity to develop disease may depend on host characteristics, particular bacterial factors, or to the specific interactions between host and microbe. One histological pattern induced by *H. pylori* is pangastritis, which may subsequently progress to gastric ulceration (GU), atrophy, and intestinal metaplasia and lower the threshold for distal gastric cancer (4, 5). In contrast, patients with antral-predominant gastritis are more likely to develop duodenal ulcer (DU) disease, yet less likely to develop distal gastric cancer, underscoring the dichotomy of these two histological pathways (5).

Given that *H. pylori* is the most common etiologic agent of chronic gastritis, it is likely that this organism plays a causative role early in the progression to neoplasia. Therefore, attention has focused on mechanisms by which *H. pylori* may alter cellular processes with premalignant potential, such as apoptosis and proliferation (6, 7). *H. pylori* infection of Mongolian gerbils induces gastritis (8, 9), which then may lead to distal gastric adenocarcinoma (10), and experimental *H. pylori* infection alters gastric epithelial cell apoptosis and proliferation in this model of carcinogenesis (9).

In addition to differences in host response, bacterial determinants also contribute to disease outcome. One strain-specific *H. pylori* gene is *cagA*, a component of the *cag* pathogenicity island; several genes within this island encode products that are homologs of proteins of the type IV bacterial secretion pathway (11, 12). In vivo, *cagA*$^+$ strains augment the risk for peptic ulcer disease, atrophic gastritis, and distal gastric cancer compared with *cagA*$^-$ strains (13–16). A second polymorphic *H. pylori* locus is *vacA*, which encodes the vacuolating cytotoxin. *H. pylori* strains of the s1a *vacA* subtype are usually strongly toxigenic and *cagA*$^+$ (17) and also have

been associated with more severe clinical outcomes (17). Recently, another *H. pylori* gene, *iceA*, has been identified that is induced after contact with epithelial cells (18). The *iceA* gene exists as two distinct genotypes, *iceA1* and *iceA2*, and carriage of *iceA1* strains has been associated with peptic ulceration in some (18, 19), but not all (20), studies.

Although *H. pylori cagA*$^+$ *vacA* s1a *iceA1* strains are more frequently associated with disease, important geographic differences in susceptibility to disease exist, as clear-cut markers for *H. pylori* strains that affect Western populations have little or no predictive power in East Asian populations (20, 21). *H. pylori* isolates exhibit extensive genetic diversity involving point mutations and intragenic variation of larger segments (22, 23), which hinders identification of strain-specific, pathogenesis-related genes using molecular fingerprinting techniques such as RFLPs or random arbitrarily primed PCR. A more comprehensive approach to understanding the different pathogenic effects of these strains requires that each gene within the bacterial genome be interrogated simultaneously. The availability of complete genome sequences for two different *H. pylori* strains (22, 23) and development of microarrays containing representations of each of these genes (24) provide both of the prerequisites for this approach. Therefore, we sought to determine whether *H. pylori* isolates from patients with divergent cancer risks (i.e., GU versus DU) differentially affected epithelial cellular responses in vivo and in vitro and to identify and characterize genetic differences responsible for these events.

Methods

Animals, housing, and H. pylori challenge. Mongolian gerbils 4–8 weeks of age were purchased either from Harlan Sprague Dawley Inc. (Hsd:MON; Indianapolis, Indiana, USA) or Charles River Laboratories (Crl:[MON]BR[outbred]; Wilmington, Massachusetts, USA). All procedures were approved by the Institutional Animal Care Committee of Vanderbilt University. Gerbil-passaged *cagA*$^+$ *vacA* s1a *iceA1* strains B128 (18) and G1.1 (8) were originally isolated from patients with GU and those with DUs, respectively. Isogenic *picB*$^-$ mutants used for inoculation were generated within strain B128 as described later. Gerbils were orogastrically challenged with sterile Brucella broth or *H. pylori* and sacrificed between 1 and 52 weeks after inoculation, as described previously (8). One half of the glandular stomach was fixed in 10% neutral buffered formalin for histological examination, and the other half was homogenized in sterile PBS (pH 7.4); plated on selective Trypticase soy agar plates containing vancomycin (20 μg/ml), nalidixic acid (10 μg/ml), bacitracin (30 μg/ml), and amphotericin B (2 μg/ml); and grown for 4–5 days, as described previously (8). Colonies were identified as *H. pylori* based on their characteristic morphology, and by urease and oxidase activities; colony counts were expressed as log CFU per stomach (8). B128 *picB*$^-$ mutant strains were also tested for kanamycin resistance, as described elsewhere (8).

Histological examination and serum antibodies to H. pylori. Hematoxylin and eosin sections were examined by a single experienced pathologist (KT). For semiquantitative estimates, the following parameters were graded from 0 to 3: acute and chronic inflammation, epithelial cell degeneration, and erosions (9). To quantitate parietal cell mass, total thickness of the gastric corpus glandular layer, which is composed predominantly of parietal cells, was measured in all gerbils (9). Gerbils were tail bled before sacrifice and by cardiac puncture at sacrifice, and serum Immunoglobulin G (IgG) antibody levels were measured by ELISA (8).

Immunohistochemistry. Proliferation and apoptosis were quantitated in situ using immunohistochemical stains for the proliferating cell nuclear antigen (PCNA) or terminal uridine deoxynucleotidyl nick end-labeling (TUNEL), respectively, as described (9). At least 20 well-oriented gastric glands were scored, and results are expressed as the mean number of PCNA-positive or TUNEL-positive cells per gland. Apoptosis and proliferation scores were determined on antral sections from 97 animals (77 *H. pylori*–infected and 20 controls), and corpus sections from 41 animals (36 infected and five controls).

H. pylori strains examined in vitro. Experiments were performed with *H. pylori* strains B128 and G1.1 already described here, or with their respective gerbil-passaged derivatives. Isogenic *picB*/*cagE* (HP0544) null mutants were constructed within GU strain B128 by insertional mutagenesis, using *aphA* (conferring kanamycin resistance) as described previously (25). The *cag* island deletion mutants were generated within strain B128 by allelic exchange, using the chloramphenicol acetyl transferase (*cat*) gene (25). Briefly, the 5′ and 3′ flanking regions of the *cag* island were amplified from strain 26695 *cag*$^-$ (26) in which the *cag* island has been replaced with *cat* (25) using the primers 5′ CCAATTTCACTCGCTATGACGGCATG 3′ and 5′ AAGCTTTGTCTATTCTAAAATGCAAC 3′, and *H. pylori* B128 Δ*cag* mutants were generated by natural transformation and allelic exchange using this purified PCR product, as described elsewhere (26). Isogenic *picB* and Δ*cag* mutants were selected with either kanamycin (25 μg/ml) or chloramphenicol (10 μg/ml), respectively.

Assessment of AGS cell viability, apoptosis, and IL-8 production. AGS human gastric epithelial cells (ATCC CRL 1739) were grown in RPMI 1640 (Life Technologies Inc., Rockville, Maryland, USA) supplemented with 10% FBS (Sigma Chemical Co., St. Louis, Missouri, USA) and 20 μg/ml gentamicin in an atmosphere of 5% CO_2 at 37°C (25). For coculture experiments, *H. pylori* were grown in Brucella broth with 5% FBS for 48 hours, harvested by centrifugation (2,000 *g*), and resuspended in antibiotic-free RPMI 1640 with 10% FBS. For quantitation of apoptosis and IL-8, *H. pylori* were added to cells at bacteria/cell concentrations of 100:1 and

1,000:1, respectively, based on reports that *H. pylori* reproducibly induce apoptosis and IL-8 secretion in AGS cells at these ratios (12, 25). For viability, AGS cells were seeded to a density of 5×10^4 cells per well in 24-well plates, incubated overnight, and inoculated with *H. pylori* (5×10^6 CFU per well) or media alone for up to 48 hours, as described elsewhere (25). Viability was determined by trypan blue exclusion using phase-contrast microscopy. DNA fragmentation, reflecting apoptosis, was quantified using a commercially available ELISA (Roche Molecular Biochemicals, Indianapolis, Indiana, USA); AGS cells (5×10^3 per well) in 96-well plates were incubated in triplicate with *H. pylori* or media alone for 24–48 hours and lysed, and after centrifugation supernatants were used for ELISA (25). For quantitation of IL-8, AGS cell monolayers in six-well plates were cocultured with or without *H. pylori* for 24 hours in triplicate, and supernatants removed from the wells were centrifuged at 15,000 *g* before freezing at –70°C as described previously (12). IL-8 protein was measured by ELISA (R&D Systems Inc., Minneapolis, Minnesota, USA) on supernatants (12).

Microarray analysis of strains B128 and G1.1. The genomic content of GU strain B128 and DU strain G1.1 was determined using an *H. pylori* whole genome microarray containing 1,660 unique *H. pylori* genes (24). Briefly, Cy5-labeled probes were prepared for each strain from 2 μg of genomic DNA using Klenow fragment, and a Cy3-labeled reference probe was prepared from a mixture of 1 μg of DNA each from strains 26695 and J99, the strains used for construction of the microarray (24). Cy5 and Cy3 probes were mixed, hybridized to the microarrays, and washed (27), and arrays were scanned using an Axon scanner and further processed using Genepix 3.0 software (Axon Instruments Inc., Foster City, California, USA). Data for each channel were normalized using the Stanford Microarray Database (28). The geometric mean of the normalized red/green (R/G) ratio was computed using data from two arrays yielding one to four readings per gene (each array contains two spots per gene). Spots were excluded due to slide abnormalities or low signal (<100 arbitrary units in the green channel). The cutoff for absence of a gene was a normalized R/G ratio < 0.5 based on test hybridizations (24). Data were simplified into a binary score (gene present in a given strain = 1, absent = 0) and analyzed by average hierarchical clustering using Cluster software and displayed using TreeView (29, 30).

Statistical analysis. The Mann-Whitney *U* test for nonparametric data was used to compare scores between samples, whereas scores for inflammation, apoptosis, and proliferation within the same animals were compared by linear regression analysis. Apoptosis, proliferation, inflammation, and IL-8 data are presented as mean ± SD. Significance was defined as $P \leq 0.05$.

Results

H. pylori strains induce distinct patterns of inflammation and injury in Mongolian gerbils. In total, 104 gerbils were challenged either with *H. pylori* DU strain G1.1 ($n = 60$), GU strain B128 ($n = 24$), or Brucella broth alone ($n = 20$). Of 84 gerbils challenged with *H. pylori*, 80 (57 G1.1, 23 B128) were successfully infected, and there were no significant differences in colonization efficiency ($P = 0.4$).

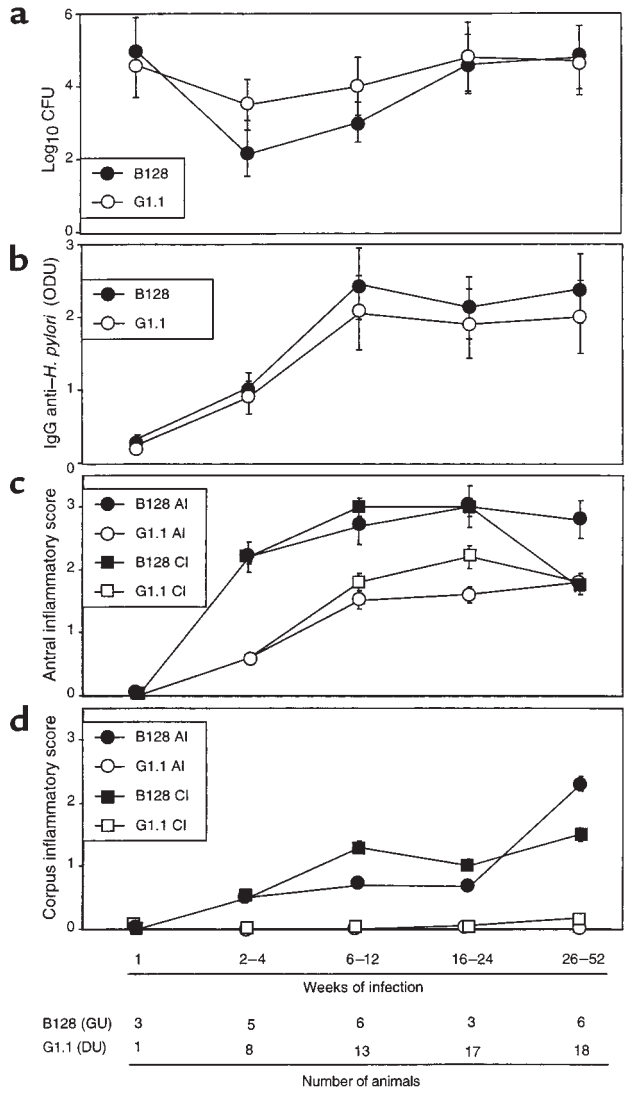
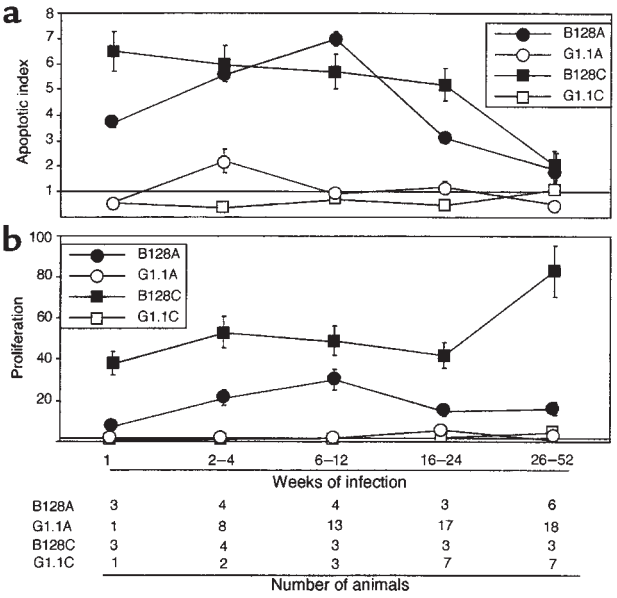


Figure 1
Comparison of colonization density (**a**), serum antibody response (**b**), and gastric inflammation in the antrum (**c**) and corpus (**d**) in gerbils infected with *H. pylori* GU strain B128 or DU strain G1.1. (**a**) Animals were sacrificed 1–52 weeks after bacterial inoculation with B128 (solid symbols) or G1.1 (open symbols), and the number of CFU per stomach was determined by quantitative culture (8). Results are expressed as mean $\log_{10}$ CFU ± SD. (**b**) Anti–*H. pylori* IgG antibody levels in serum were determined by ELISA (8). Mean absorbance (ODU) values ± SD are shown. (**c**) Acute (AI) and chronic (CI) inflammation in the antrum of animals infected with B128 (solid symbols) or G1.1 (open symbols) was determined by histological testing, as described in the text. (**d**) Corpus inflammation for gerbils challenged with B128 or G1.1 was scored from 0 to 3, and results are expressed as mean histological scores ± SD.

Figure 2
H. pylori strains differentially alter gastric epithelial cell apoptosis (**a**) and proliferation (**b**) in Mongolian gerbil gastric mucosa. Apoptosis and proliferation scores at each time point are expressed as mean number of positive cells per gland in infected gerbils divided by mean scores in uninfected gerbils. Therefore, a value of 1 represents baseline. (**a**) Apoptosis scores were significantly higher in animals infected with GU strain B128 in the antrum (B128A; filled circles) and corpus (B128C; filled squares) 1–24 weeks after challenge compared with DU strain G1.1 (open symbols) or controls ($P = 0.01$ and 0.009, respectively); scores subsequently decreased, and by 26–52 weeks, they were not different from controls. Compared with uninfected animals, apoptosis was also significantly increased in gerbils infected with DU strain G1.1 but only in the antrum (G1.1A, open circles) and only at 2–4 weeks after inoculation ($P = 0.01$). (**b**) Proliferation scores increased shortly following challenge with GU strain B128 (filled symbols) and were significantly higher at all time points than either G1.1-infected (open symbols) or control animals ($P < 0.001$ for each).



We next asked whether colonization density, serum antibody response, or gastric inflammation and injury differed between the gerbil populations. There were no differences in colonization density (Figure 1a) or anti–*H. pylori* IgG antibody responses (Figure 1b) between B128- and G1.1-infected animals. All gerbils infected for more than 2–4 weeks developed acute and chronic antral gastritis, and scores increased until 16–24 weeks (Figure 1c). Similar to the antrum, 14 (93%) of 15 gerbils infected with GU strain B128 for more than 2–4 weeks developed corpus gastritis, and scores steadily increased throughout the observation period (Figure 1d). In contrast, only 3 (5%) of 57 gerbils infected with DU strain G1.1 had any corpus inflammation ($P \leq 0.001$ compared with B128). Acute and chronic inflammatory scores (Figure 1, c and d), and the degree of epithelial cell degeneration (data not shown) were significantly ($P \leq 0.03$) higher at all gastric sites in animals infected with B128 compared with G1.1. Thus, GU strain B128 induced a more severe and global inflammatory response than did DU strain G1.1.

Because gastric ulcer disease is associated with an increased risk for gastric adenocarcinoma (5), we next sought to determine whether GU strain B128 enhanced the development of premalignant mucosal lesions. Antral erosions and glandular displacement each were significantly ($P \leq 0.03$) more frequent among gerbils infected with GU strain B128 (19/23 and 10/23 animals, respectively) compared with DU strain G1.1 (3/57 and 0/57 animals, respectively). Gastric ulcers developed in 4 (17%) of 23 and 0 (0%) of 57 animals infected with GU strain B128 and DU strain G1.1, respectively ($P < 0.001$), and all ulcers were located within the gastric antrum. Given that parietal cell loss can lead to aberrant epithelial cellular differentiation (31), we determined whether the respective *H. pylori* strains variably altered parietal cell mass. Infection with GU strain B128 for more than 26 weeks led to parietal cell loss and glandular atrophy in 5 (83%) of 6 gerbils, but these changes did not occur at any time point in the G1.1-infected animals.

H. pylori GU strain B128 induces more severe mucosal cellular imbalances. Because the severity and pattern of gastritis differed among B128- and G1.1-infected animals and because mucosal inflammation may contribute to cell turnover (7, 9), we next addressed whether apoptosis or proliferation was differentially affected by these *H. pylori* strains. Gerbils infected with GU strain B128 had significantly ($P \leq 0.05$) higher antral and corpus apoptotic indices 1–24 weeks after inoculation than did broth- or G1.1-challenged animals (Figure 2a). In contrast, antral apoptosis scores in G1.1-infected animals were significantly increased only at 2–4 weeks, and corpus apoptosis scores were no different from controls (Figure 2a). We have previously shown that *H. pylori*–infected Mongolian gerbils develop hyperproliferation, although with different timing and morphological location than apoptosis (9). After challenge with GU strain B128, mucosal proliferation indices increased rapidly and were significantly higher at all time points than either G1.1-infected or control animals (Figure 2b; $P < 0.001$ for each). Thus, GU strain B128 induces a more intense tissue response than G1.1 as reflected by enhanced mucosal inflammation, cell proliferation, and cell death.

H. pylori GU strain B128 enhances cytokine production and apoptosis in vitro. Given that GU strain B128 altered inflammation and cellular turnover more profoundly in vivo, we examined whether similar effects could be observed in vitro. Levels of IL-8, a proinflammatory cytokine that has potent chemoattractive and neutrophil-activating properties, are significantly related to severity of gastric inflammation among *H. pylori*–positive persons (32, 33). Comparing IL-8 production in AGS cells after coculture with our prototype

H. pylori strains, GU strain B128 stimulated significantly ($P = 0.003$ versus G1.1) higher levels of IL-8 (Figure 3a), results that parallel the in vivo response to this strain. AGS cells cocultured with either *H. pylori* strain had significantly ($P < 0.05$) reduced viability compared with controls (Figure 3a); however, GU strain B128 significantly ($P < 0.001$) decreased viability compared with DU strain G1.1 (Figure 3a). To determine whether the reduction in viability reflected apoptosis, we quantitated DNA fragmentation in AGS cells after bacterial coculture. *H. pylori* significantly increased cytoplasmic oligonucleosomal fragments compared with AGS cells alone ($P < 0.001$ for each time point; Figure 3a), and again, GU strain B128 significantly enhanced this effect ($P < 0.001$; Figure 3a).

The ability of strains B128 and G1.1 to differentially alter IL-8 secretion and apoptosis in AGS cells reflected the responses to these same strains in vivo. Therefore, we next asked whether the bacterial properties responsible for these changes had been affected by prolonged in vivo growth. Similar to the results obtained with the parental strains (Figure 3a), all B128 strains after prolonged gerbil colonization induced significantly higher levels of apoptosis and IL-8 production than G1.1 derivatives ($P \leq 0.01$; Figure 3b), although no consistent intrafamilial strain differences were identified. These data indicate that the properties necessary for induction of epithelial cell responses remain relatively stable during persistent *H. pylori* colonization of the gastric niche.

H. pylori whole genome microarray reveals a partial cag island deletion in strain G1.1. Having established that strains B128 and G1.1 have different phenotypes, we next used an *H. pylori* whole genome microarray to examine differences in gene content between these prototype strains. A total of 153 strain-specific genes (98 B128-specific and 55 G1.1-specific; Figure 4) were identified in the two isolates, and 44 (29%) of these (Table 1) were predicted to encode gene products with known, diverse functions such as recombination (*xerD*) and basic cellular metabolism (*yxjE*, *trpA*, *topA3*). A substantial proportion of these ORFs had significant homology to genes encoding putative restriction-modification (R-M) enzymes (Table 1), findings that substantiate recent reports (34–36) demonstrating the presence of unique complements of R-M systems within individual *H. pylori* strains. The microarray also identified major differences in gene content that localize to regions of linked genes, one of which includes a portion of the *cag* pathogenicity island (Figure 4). GU strain B128 contains 26 of 27 *cag* island genes, whereas DU strain G1.1 possesses only seven of the 27 *cag* genes, indicating a strain-specific deletion of this region (Figure 4; Table 1). This dele-

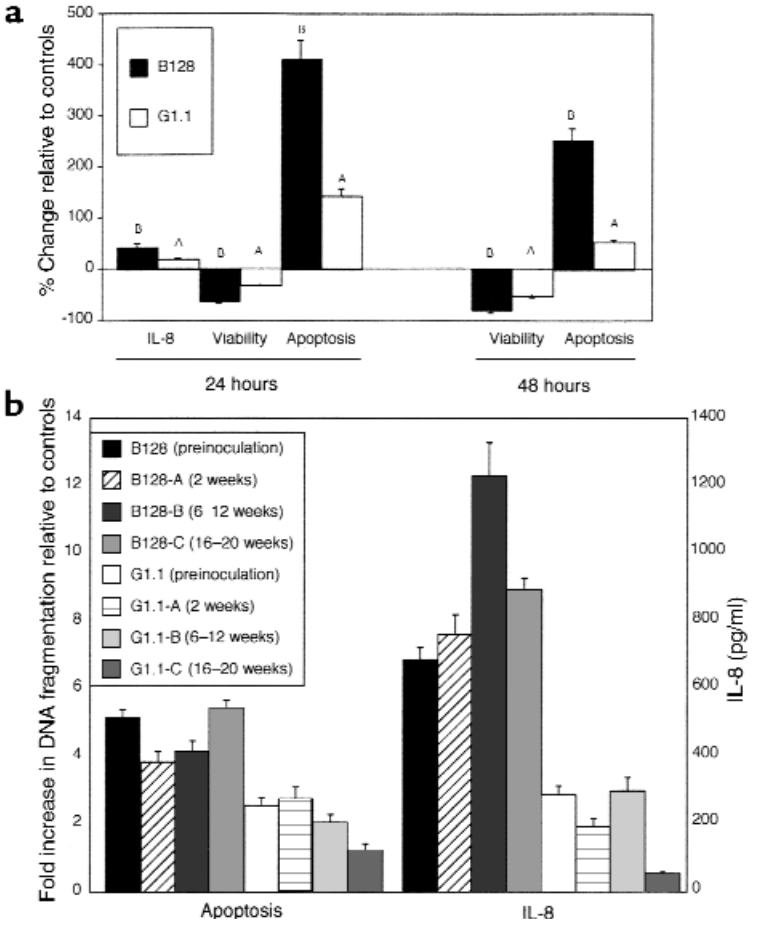


Figure 3
Quantitation of IL-8 and analysis of apoptosis among preinoculation (parental) strains B128 and G1.1 (**a**) and their derivative progeny after gerbil passage (**b**). (**a**) AGS cells were grown alone or in the presence of *H. pylori* GU strain B128 (filled columns) or DU strain G1.1 (open columns). IL-8 concentrations were determined 24 hours after inoculation by ELISA. No 48-hour samples were analyzed due to the significant loss of AGS cell viability that had occurred by this time point. Cell viability and apoptosis were assessed by trypan blue exclusion and DNA fragmentation ELISA, respectively, 24 and 48 hours following inoculation. Results are expressed as the percent change in IL-8, viability, or apoptosis relative to controls. Data represent mean ± SD of three independent experiments. $^{A}P \leq 0.05$ compared with AGS cells alone. $^{B}P \leq 0.05$ compared with controls or with DU strain G1.1. (**b**) Six gerbil-passaged B128 and G1.1 isolates were selected (B128-A and G1.1-A, 2 weeks; B128-B and G1.1-B, 6–12 weeks; B128-C and G1.1-C, 16–20 weeks after challenge, respectively). Apoptosis results are expressed as levels of nucleosomal release relative to controls. IL-8 concentrations are expressed as pg/ml. Bars, SD.

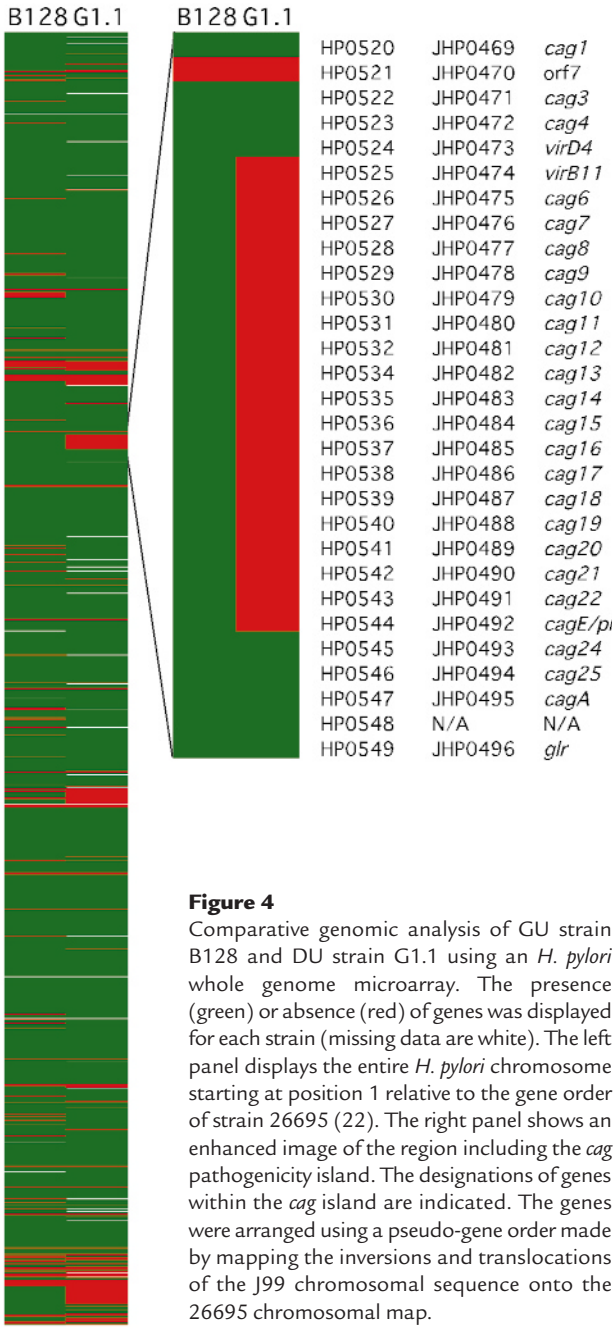


Figure 4
Comparative genomic analysis of GU strain B128 and DU strain G1.1 using an *H. pylori* whole genome microarray. The presence (green) or absence (red) of genes was displayed for each strain (missing data are white). The left panel displays the entire *H. pylori* chromosome starting at position 1 relative to the gene order of strain 26695 (22). The right panel shows an enhanced image of the region including the *cag* pathogenicity island. The designations of genes within the *cag* island are indicated. The genes were arranged using a pseudo-gene order made by mapping the inversions and translocations of the J99 chromosomal sequence onto the 26695 chromosomal map.

tion consisted of contiguous genes that were located in the midregion of the island and included *picB*/*cagE* (HP0544, JHP0492), but not *cagA* (HP0547, JHP0495) (22, 23) (Figure 4; Table 1).

Inactivation of components of the cag island attenuates IL-8 induction and development of gastritis. Because previous studies have demonstrated that genes within the *cag* island contribute to induction of IL-8 and apoptosis in vitro (11, 12, 25), we next disrupted *picB*/*cagE* and also deleted the entire *cag* island in GU strain B128 to determine whether these components were required for the phenotypes observed in the current study. Compared to parental strain B128, loss of *picB*/*cagE* and/or the entire *cag* island significantly reduced IL-8 secretion ($P \leq 0.001$ versus B128) to levels that were similar to those induced by DU strain G1.1 (Figure 5a). Disruption of *picB*/*cagE* alone was sufficient to produce the phenotype obtained by deletion of the entire *cag* island. In contrast, the ability of these same mutant strains to induce apoptosis was not significantly different from the wild-type strain ($P = 0.32$ and 0.20 versus B128, respectively; Figure 5a).

We next sought to corroborate these in vitro findings by infecting gerbils with either wild-type B128 or its isogenic *picB*$^-$ derivative. Of 44 gerbils challenged with either B128 ($n = 26$) or B128 *picB*$^-$ ($n = 18$), 43 (25 B128 and 18 *picB*$^-$) were successfully colonized. All strains recovered from mutant-challenged animals were kanamycin-resistant and contained the *aphA* cassette as determined by PCR using primers that flanked the insertion site within *picB*, indicating genomic stability of the antibiotic resistance cassette during in vivo colonization. There were no differences in colonization levels between the populations (data not shown). However, compared with the parental strain B128, infection with the *picB*$^-$ mutant significantly attenuated development of antral inflammation ($P \leq 0.03$ versus B128; Figure 5b) as early as 2 weeks after challenge, and corpus inflammation by 4 weeks after infection ($P \leq 0.04$ versus B128; Figure 5c). Within the antra of *picB*$^-$-challenged gerbils, neutrophilic infiltration was reduced to a greater degree compared with mononuclear cell infiltration (Figure 5b). In addition to inflammation, the severity of epithelial cell degeneration and erosion was significantly ($P \leq 0.04$ for each) decreased in gerbils infected with the *picB*$^-$ mutant compared with the wild-type strain (data not shown). These findings confirm our in vitro results (Figure 5a) and support the utility of whole genome microarray to identify strain-specific *H. pylori* components relevant to pathogenesis in vivo.

Discussion

Although certain *H. pylori* strains are associated with pathological outcomes, the specific mechanisms that lead to these relationships have not been fully delineated. One strain-specific *H. pylori* constituent associated with an augmented risk for ulcer disease and distal gastric cancer is the *cag* pathogenicity island (13–16). After *H. pylori* adherence to host cells, CagA is transported into the epithelial cell, where it becomes phosphorylated (37–39), and these events trigger changes in cellular morphology similar to those induced by hepatocyte growth factor (40). A CagA-independent consequence of *cag* island-mediated *H. pylori*–epithelial cell contact is activation of intracellular signaling pathways including NF-κB, which may contribute to IL-8 secretion (41). Thus, *cagA*$^+$ strains appear to be disproportionately represented among persons who develop serious sequelae of *H. pylori* carriage, and *cag* island genes are necessary for induction of epithelial cell responses relevant to pathogenesis.

In the current study, we examined two strains that possessed similar virulence genotypic profiles (*cagA*$^+$

vacA s1a *iceA1*), but which induced distinct phenotypic responses. GU strain B128 induced more severe inflammation within gerbil mucosa and significantly higher levels of IL-8 in vitro. Using an *H. pylori* whole genome microarray, we found that although both prototype strains possessed the *cagA* gene and hence were *cagA*$^+$, their gene content within the *cag* island differed substantially. GU strain B128 contains a near complete copy of the island (26 of 27 genes), whereas in DU strain G1.1, a large deletion is present. These results prompted us to re-examine epithelial cell responses without the influence of *cag* gene products, and we confirmed the findings of previous investigators (11, 12) that inactivation of *picB*/*cagE* or deletion of the entire *cag* island significantly attenuated the ability of GU strain B128 to induce cytokine release in vitro. Infection of gerbils with an isogenic *picB*$^-$ derivative recapitulated these events in vivo as mucosal inflammation, but not colonization, was significantly decreased in animals challenged with mutant strains compared with those infected with parental B128. The inflammatory component most profoundly affected was polymorphonuclear cell infiltration, which is consistent with the demonstration that loss of *picB* is associated with decreased production of IL-8, a cytokine with potent chemotactic and activating properties for neutrophils. Our previous studies have shown that neither CagA nor VacA is required for induction of inflammation in this model of *H. pylori*–induced gastritis (9), and genomic profiling of strains B128 and G1.1 support these observations, as both strains contained *cagA* and *vacA*. These findings underscore and confirm the importance of the entire *cag* island as a bacterial locus related to high-grade host responses

Table 1
Strain-specific B128 and G1.1 genes with putative functions

Strain[A]				Present[D]	
26695	J99	Gene designation[B]	Gene description[C]	B128	G1.1
	JHP0045		Putative type II DNA modification enzyme	+	–
	JHP0046		Putative type II restriction enzyme	+	–
	JHP0585		Putative 3-hydroxyacid dehydrogenase	+	–
	JHP0931	*topA3*	Topoisomerase I	+	–
	JHP1422	*hsdS3a*	Putative type I restriction enzyme (specificity subunit)	+	–
HP0459		*virB4*	*virB4* homolog	+	–
HP0483	JHP0435		Type II DNA modification enzyme	+	–
HP0525	JHP0474	*virB11*	*virB11* homolog	+	–
HP0526	JHP0475	*cag6*	*cag* pathogenicity island protein	+	–
HP0527	JHP0476	*cag7*	*cag* pathogenicity island protein	+	–
HP0528	JHP0477	*cag8*	*cag* pathogenicity island protein	+	–
HP0529	JHP0478	*cag9*	*cag* pathogenicity island protein	+	–
HP0530	JHP0479	*cag10*	*cag* pathogenicity island protein	+	–
HP0531	JHP0480	*cag11*	*cag* pathogenicity island protein	+	–
HP0532	JHP0481	*cag12*	*cag* pathogenicity island protein	+	–
HP0534	JHP0482	*cag13*	*cag* pathogenicity island protein	+	–
HP0535	JHP0483	*cag14*	*cag* pathogenicity island protein	+	–
HP0536	JHP0484	*cag15*	*cag* pathogenicity island protein	+	–
HP0537	JHP0485	*cag16*	*cag* pathogenicity island protein	+	–
HP0538	JHP0486	*cag17*	*cag* pathogenicity island protein	+	–
HP0539	JHP0487	*cag18*	*cag* pathogenicity island protein	+	–
HP0540	JHP0488	*cag19*	*cag* pathogenicity island protein	+	–
HP0541	JHP0489	*cag20*	*cag* pathogenicity island protein	+	–
HP0542	JHP0490	*cag21*	*cag* pathogenicity island protein	+	–
HP0543	JHP0491	*cag22*	*cag* pathogenicity island protein	+	–
HP0544	JHP0492	*cag23/cagE/picB*	*cag* pathogenicity island protein	+	–
HP0962		*acpP*	acyl carrier protein	+	–
HP0995	JHP0941	*xerD*	integrase/recombinase	+	–
HP1000			PARA protein	+	–
HP1193			aldo-keto reductase	+	–
HP1370		*mod*	type III restriction enzyme M protein	+	–
HP1371	JHP1285		type III restriction enzyme R protein	+	–
HP1517		*Eco57IR*	type IIS restriction enzyme R and M protein	+	–
	JHP0164		putative restriction enzyme	–	+
HP0091	JHP0084	*hsdR*	type II restriction enzyme R protein	–	+
HP0289	JHP0274		toxin-like outer membrane protein	–	+
HP0315		*vapD*	virulence associated protein D	–	+
HP0317		*omp9*	outer membrane protein	–	+
HP0428			phage/colicin/tellurite resistance cluster	–	+
HP0692	JHP0636	*yxjE/scoB*	3–oxoadipate coA-transferase subunit B	–	+
HP0896	JHP1164	*babB*	outer membrane protein	–	+
HP1009			site-specific recombinase	–	+
HP1277	JHP1198	*trpA*	tryptophan synthase	–	+
HP1404		*hsdS*	type I restriction enzyme S protein	–	+

[A]Unique identifier from *H. pylori* genes in annotated sequences of 26695 and J99. [B]Putative gene name if available. [C]Description of putative gene function. [D]Presence (+) as determined by an average fluorescence ratio of greater or equal to 0.5 from two independent hybridizations. Absence (–) was defined as an average fluorescence ratio of less than 0.5.

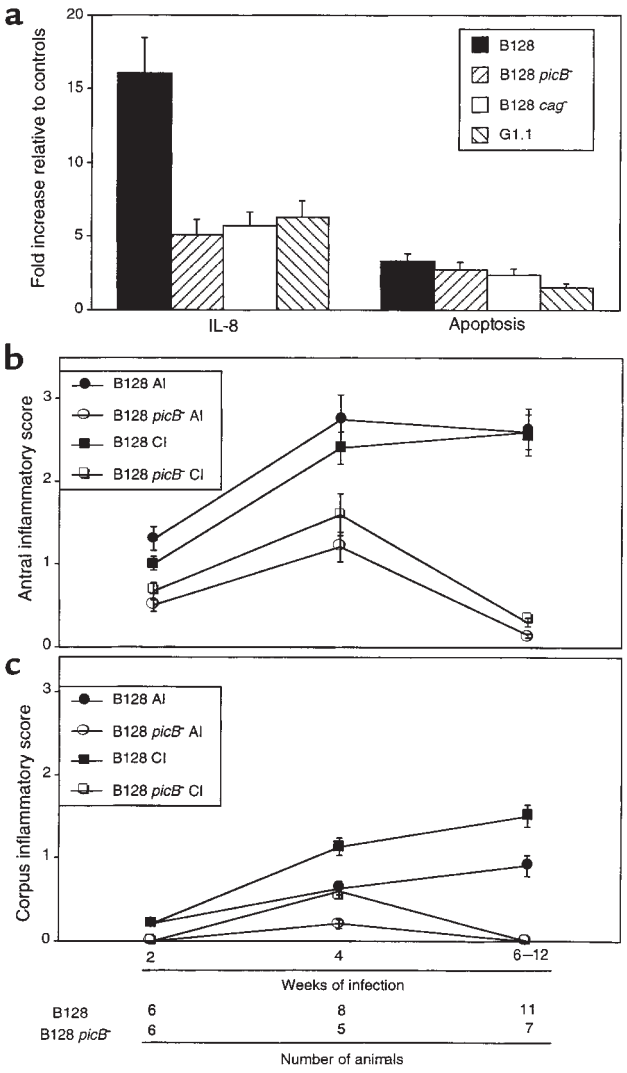


Figure 5
Inactivation of *cag* pathogenicity island components attenuates induction of IL-8 (**a**) and gastric inflammation (**b** and **c**). (**a**) Quantitation of IL-8 production and apoptosis in AGS cells incubated with parental GU strain B128, an isogenic *picB*⁻ (*cagE*⁻) mutant of B128, B128 *cag*⁻, which lacks the entire *cag* island, and DU strain G1.1. IL-8 concentrations and apoptosis were determined 24 hours after *H. pylori* inoculation and are expressed as levels of induction relative to controls. Bars, SD. (**b**) Acute (AI) and chronic (CI) inflammation in the antrum of animals after 2–12 weeks of infection with B128 (filled symbols) or B128 *picB*⁻ (open symbols) was determined by histological testing, as described in the text. (**c**) Corpus inflammation for gerbils challenged with B128 or B128 *picB*⁻ was scored from 0 to 3 by a single pathologist, and results are expressed as mean histological scores ± SD.

and provide further evidence that strain-specific bacterial components identified by microarray are relevant to pathology within the gastric niche.

Many of the genetic differences identified between the two studied strains involved the *cag* island; however, deletion of this locus did not significantly affect the ability of GU strain B128 to induce apoptosis in vitro. Other *H. pylori* components that may induce apoptosis include urease, a highly conserved *H. pylori* constituent, which can induce apoptosis in vitro by binding to MHC class II molecules expressed on the surfaces of gastric epithelial cells (42). However, although *H. pylori* can directly induce apoptosis in isolated in vitro systems, apoptosis in vivo is likely to be regulated by host mediators present within inflamed mucosa. The immune response elicited by *H. pylori* in humans and by *Helicobacter felis* in mice is Th1-predominant (43, 44), and IFN-γ, a Th1 lymphocyte–derived cytokine that is increased within colonized mucosa, is synergistic with *H. pylori* in inducing Fas-Fas ligand–regulated (FasL-regulated) apoptosis of gastric epithelial cells in vitro (45, 46). *H. pylori* infection of IFN-γ–deficient mice leads to decreased levels of gastric inflammation compared with wild-type mice (47, 48), and in vivo neutralization of IFN-γ in mice infected with *H. felis* similarly reduces the severity of gastritis (43). Adoptive transfer of Th1 cells from *H. pylori*–infected mice into infected recipients increases the severity of gastritis (49), and concurrent helminth infection modulates a proinflammatory Th1-mediated murine gastritis and atrophy response to *H. felis* toward a less injurious Th2-type response (50). *H. felis* infection of Fas-deficient mice is also associated with reduced levels of inflammation compared with infected wild-type mice, and mucosal apoptosis scores are decreased in parallel (51).

One hypothesis suggested by the previous findings described here is that Th1-derived cytokines (i.e., IFN-γ) within inflamed mucosa may increase apoptosis by regulating Fas-FasL interactions between inflammatory cells and gastric epithelial cells. However, additional host genetic factors also may contribute to the ability of *H. pylori* to alter apoptosis in vivo. Mice lacking secretory phospholipase A_2 that are infected by *H. felis* develop increased epithelial cell apoptosis (52). Apoptosis in response to *H. pylori* also may reflect heterogeneity of class II MHC host genotypes, as binding of *H. pylori* to these IFN-γ–inducible molecules can directly stimulate apoptosis (42). El-Omar et al. have recently identified particular polymorphisms of the human IL-1β gene promoter that are genetic risk factors for both precursor lesions of gastric cancer (atrophic gastritis) as well as gastric adenocarcinoma per se among *H. pylori*–infected persons (53). IL-1β can stimulate multiple intracellular signaling pathways involved in apoptosis, including Fas and NF-κB (41, 45, 46), and thus differing levels of IL-1β expression within *H. pylori*–colonized gastric mucosa may regulate levels of apoptosis in vivo.

IL-1β is also a potent inhibitor of gastric acid secretion, and polymorphisms within its promoter region may differentially influence additional physiological events that dictate disease outcomes among *H. pylori*–colonized populations. Two recent investigations have revealed that acid outputs in gerbils chronically infected with *H. pylori* are either no different (54) or reduced (55) compared with uninfected controls. Further, mucosal IL-1β mRNA levels reciprocally increase as acidity decreases and administration of recombinant

IL-1 receptor antagonist normalizes acid outputs among gerbils carrying *H. pylori* (55). Collectively, these data indicate that the host inflammatory response is an important determinant of specific pathological outcomes among *H. pylori*–infected populations.

Understanding *H. pylori* diversification and adaptation during long-term colonization is crucial toward delineating mechanisms by which these organisms may lead to disease. As evidenced by our results, the use of microarray-based comparative genomics provides a powerful tool for identifying strain-specific factors that may contribute to phenotypic variability among *H. pylori* isolates assumed to have similar genetic composition. One limitation of this technique, however, is that it cannot detect small deletions, point mutations, deletions within intergenic regions, rearrangements, ORFs that are not present in either strain 26695 or J99, or deletions of homologous repetitive elements. However, as microarrays of greater resolution are developed and new strain-specific ORFs are identified, it should soon be possible to determine the relative frequency and importance of these additional sources of genetic variability. Genotypic markers could then be developed to not only to identify individuals at risk for specific clinical sequelae of infection, but also to permit selective targeting of therapy for disease prevention.

In conclusion, we have shown that *H. pylori* strains with similar virulence markers can induce distinct patterns of gastric inflammation and injury in an animal model of *H. pylori*–induced carcinogenesis and that these findings can be corroborated in vitro and in vivo. DNA hybridization to an *H. pylori* whole genome microarray revealed that differences in the ability of *H. pylori* strains to induce epithelial cell responses related to inflammation are dependent on the presence of an intact *cag* pathogenicity island, although the specific function of numerous additional strain-specific loci and their significance in pathogenesis remain to be determined.

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