Experimental Infection of Mongolian Gerbils with Wild-Type and Mutant *Helicobacter pylori* Strains

HANS-PETER WIRTH,^{1,2} MICHAEL H. BEINS,¹ MANQIAO YANG,^{1,2} KYI T. THAM,^{3,4}
AND MARTIN J. BLASER^{1,4*}

Division of Infectious Diseases, Department of Medicine,¹ and Department of Pathology,³ Vanderbilt University School of Medicine, and Department of Veterans Affairs Medical Center,⁴ Nashville, Tennessee, and Division of Gastroenterology, University Hospital, Zurich, Switzerland²

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Experimental *Helicobacter pylori* infection was studied in Mongolian gerbils with fresh human isolates that carry or do not carry *cagA* (*cagA*-positive or *cagA*-negative, respectively), multiply passaged laboratory strains, wild-type strain G1.1, or isogenic *ureA*, *cagA*, or *vacA* mutants of G1.1. Animals were sacrificed 1 to 32 weeks after challenge, the stomach was removed from each animal for quantitative culture, urease test, and histologic testing, and blood was collected for antibody determinations. No colonization occurred after ≥ 20 in vitro passages of wild-type strain G1.1 or with the *ureA* mutant of G1.1. In contrast, infection occurred in animals challenged with wild-type G1.1 (99 of 101 animals) or the *cagA* (25 of 25) or *vacA* (25 of 29) mutant of G1.1. Infection with G1.1 persisted for at least 8 months. All 15 animals challenged with any of three fresh human *cagA*-positive isolates became infected, in contrast to only 6 (23%) of 26 animals challenged with one of four fresh human *cagA*-negative isolates ($P < 0.001$). Similar to infection in humans, *H. pylori* colonization of gerbils induced gastric inflammation and a systemic antibody response to *H. pylori* antigens. These data confirm the utility of gerbils as an animal model of *H. pylori* infection and indicate the importance of bacterial strain characteristics for successful infection.

Helicobacter pylori is an important bacterial pathogen that causes chronic gastritis and is associated with gastroduodenal ulcer disease, adenocarcinoma of the distal stomach, and gastric lymphoma in humans (13). Animals used for studying experimental *H. pylori* infection (19) include monkeys, dogs, piglets, domestic cats, and rodents (14, 15, 21, 31, 32, 35, 37, 46, 47). Several models described thus far are not optimal, since the animals cannot be handled with ease or in large numbers, germ-free conditions must be used, or infection rates are rather low. Excellent models involving *Helicobacter* species other than *H. pylori* exist (4, 20, 38), but their applicability to *H. pylori* infection is limited by the differences between the organisms. Infection of conventional mice with *H. pylori* overcomes many of these problems (40), although infection is either not easily achieved and/or is transient (31, 40). In general, the gastritis induced appears less intense than in humans (29, 40, 42, 54) or is restricted to the use of highly selected mouse-adapted strains (39), limiting the utility of these models for examining *H. pylori* isolates from humans.

Published results about experimental *H. pylori* infection in Mongolian gerbils appear promising, although somewhat conflicting. Gastritis was minimal in the initial report by Yokota et al. (59) but quite pronounced in later studies (29, 41) that used similar experimental conditions, including the same *cagA*-positive *H. pylori* strain (ATCC 43504) (29) and the same source of gerbils (29, 41). The authors (29) therefore raised the question whether the differences in histopathology could be due to the inbred gerbil strain (MGS/Sea; Seiwa Experimental Animals, Fukuoka, Japan) they used. The histomorphology of the gerbil stomach corresponds closely to that of the mouse and rat stomach (24). Naturally acquired gastritis among gerbils is

rare, occurring in only 3 (2%) of 141 animals examined, in each case as a necrotizing form (6). Moreover, natural infection of gerbils with *Helicobacter* species does not appear to occur (5, 57). These points suggest that Mongolian gerbils are suitable to study experimental *H. pylori* infection. Our goals were to evaluate whether infection with wild-type and mutant *H. pylori* strains in Mongolian gerbils induces gastritis and to define parameters important for gastric colonization and inflammation.

MATERIALS AND METHODS

Animals and housing. Outbred Mongolian gerbils (*Meriones unguiculatus*) with a body weight of 30 to 50 g, corresponding to an age of approximately 4 to 8 weeks (9, 26), were purchased from Harlan Sprague-Dawley, Inc. (Indianapolis, Ind.) (Hsd:MON) or Charles River Laboratories (Wilmington, Mass.) [CrI: (MON)BR (outbred)]. Up to six animals per solid-bottom cage were housed in the Animal Care Facilities of Vanderbilt University in a room with a 12-h light-dark cycle at 21 to 22°C and fed a standard commercial rodent chow. Access to food and water was free throughout all experiments. No special pretreatments (such as acid inhibition or antibiotics) were used before orogastric *H. pylori* inoculation or before the animals were sacrificed. Since only a few experiments were carried out with female gerbils, all other experiments were done with male gerbils when not otherwise indicated. All experiments and procedures carried out on the animals had been approved by the institutional Animal Care Committee of Vanderbilt University (protocol M/95/291).

***H. pylori* wild-type and mutant strains used for inoculation.** Fresh human *cagA*-positive (B127, B128, and B129) or *cagA*-negative (SW18, B102, B108, and B120) *H. pylori* isolates were obtained from patients with duodenal ulcers (strains B127, B129, and SW18) or gastritis alone (strains B128, B102, B108, and B120) who were undergoing gastrointestinal endoscopy at the Nashville Department of Veterans Affairs Medical Center or the Gastroenterology Division, University Hospital, Zurich, Switzerland. The isolates were grown on Trypticase soy agar plates with 5% sheep blood (BBL Microbiology Systems, Cockeysville, Md.) for 3 to 5 days under microaerobic conditions and characterized as *H. pylori*, as previously described (56). The *cagA* status was determined by PCR with chromosomal DNA, as previously described (55). Aliquots of the human *H. pylori* isolates with fewer than five in vitro passages were frozen, and stock cultures were maintained at -70°C in brucella broth (BBL) supplemented with 15% glycerol. ICR mouse-passaged *cagA*-positive strain CPY3401 (31), originally isolated from a duodenal ulcer patient, was kindly provided by Satoru Matsumoto and Mikio Karita; after gerbil passages and expansion of a single colony, it was designated G1.1WT (wild type). *cagA*-positive *H. pylori* strains J223,

* Corresponding author. Mailing address: Vanderbilt University School of Medicine, A-3310 Medical Center North, Nashville, TN 37232-2605. Phone: (615) 322-2035. Fax: (615) 343-6160. E-mail: Martin.Blaser@mcmail.vanderbilt.edu.

HPK127, CPY3401, 26695, and HPK5 are from the Nashville strain collection and have been described previously (55); all these strains had been passaged in vitro multiple times without documentation of the exact number of passages. Therefore, immediately after isolation from the gerbil stomach, strain G1.1 was passaged on blood plates 70 times and aliquots were frozen every 5 passages, yielding strains G1.1+0 to G1.1+70. Lewis⁺ and Lewis⁻ expression of strains before and after passage was determined by enzyme-linked immunosorbent assay (ELISA) with monoclonal antibodies, as previously described (55). Chromosomal DNA was extracted from each of the preserved strains between G1.1+0 and G1.1+70 for genotypic comparison by random amplified polymorphic DNA PCR (RAPD-PCR; see below). The frequencies of natural transformation for strains G1.1+0 and G1.1+35 were compared by using chromosomal DNA from G1.1 *ureA* (see below). Chromosomal DNA from G1.1+0 and G1.1+35 was used for natural transformation (approximately 20 ng of DNA/10⁹ cells) of cells of strains G1.1+0 and G1.1+35. Equal numbers of bacterial cells, based on the optical density (OD) at 550 nm, were then inoculated into gerbils to examine for selection of transformants able to colonize in vivo.

Isogenic G1.1 *cagA* (G1.1C⁻) or *ureA* (G1.1U⁻) mutants were obtained by natural transformation with chromosomal DNA from the respective CPY3401 mutants in which *cagA* or *ureA* had been interrupted by insertion of a cassette of *xylE* and a kanamycin resistance (*aphA*) gene (33). G1.1 *vacA* (G1.1V⁻) was obtained by natural transformation of G1.1 with pV2.2(*xylE/aphA*), which was created by blunt-end ligation of the 2.6-kb *KpnI-SacI* fragment from pMK1 containing the *xylE/aphA* cassette (33) into the unique *BglII* site (nucleotide [nt] 2950) of a PCR-amplified 2.4-kb *vacA* fragment cloned into pT7Blue (Novagen, Madison, Wis.) with selection for resistance to kanamycin (30 µg/ml). The *vacA* PCR fragment (nt 1708 to 4134) was obtained with the primers 5'-TAGTAAC AAGACTCATAT (nt 1708 to 1725) and 5'-AAGCTTGATTGATCACTCC (nt 4134 to 4116; primer 6 [12]) for 35 amplification cycles of 94°C for 1 min, 41°C for 2 min, and 72°C for 2 min. Mutants were characterized by kanamycin resistance and by *XylE* activity by using a 10 mM catechol solution in water, and the site of insertion in all mutants was confirmed by PCR with *cagA*, *ureA*, or *vacA* primers flanking the insertion site together with an outward-pointing *xylE* and *aphA* primer, respectively (33); G1.1WT served as a control.

***H. pylori* inoculation and animal sacrifice.** *H. pylori* strains for each challenge were started from freezer stocks and were grown on Trypticase soy agar plates with 5% sheep blood. After 24 to 36 h, the cells were harvested in brucella broth (BBL), the concentration was adjusted according to the OD at 550 nm, and the cells were administered to the animals immediately after harvest. The animals were orogastrically inoculated three times (days 0, 2, and 4) with 0.8 to 1.0 ml of an *H. pylori* cell suspension of 10⁹ to 10¹⁰ CFU/ml in sterile brucella broth through a feeding needle. Age- and sex-matched control animals were inoculated with identical volumes of sterile brucella broth alone. Alternatively, in one experiment, *H. pylori* G1.1+0 and G1.1+35 were grown for 24 h in brucella broth supplemented with 5% fetal calf serum for subsequent challenge of 10⁹ CFU in 0.8 to 1.0 ml.

At predetermined times, animals were anesthetized in a CO₂ chamber and sacrificed by cervical dislocation. Blood was obtained by heart puncture, the forestomach was discarded, and the glandular stomach and first part of the duodenum were removed and opened along the lesser curvature. After removal of the gross gastric contents with forceps, the stomach was inspected macroscopically and divided in half by cutting along the greater curvature. One half was fixed in 10% neutral buffered formalin for histologic testing; the other half was transferred to 1 ml of sterile 0.1 M phosphate-buffered saline (PBS; pH 7.4) and used for culture and the rapid urease test (RUT).

Isolation and characterization of *H. pylori* from gerbils. Each stomach specimen in a standardized total volume of 2 ml of PBS was homogenized, and 100-µl aliquots of undiluted and 10-fold-diluted homogenate (corresponding to 1/40 and 1/400 of the entire stomach, respectively) were plated on selective Trypticase soy agar-5% sheep blood plates containing vancomycin (20 µg/ml), nalidixic acid (10 µg/ml), bacitracin (30 µg/ml), and amphotericin B (2 µg/ml) (Sigma Chemical Co., St. Louis, Mo.) and grown for 3 to 5 days, as previously described (55). The colonies were counted after 4 and 5 days, and the results are expressed as CFU/stomach. In each case, colonies were identified as *H. pylori* based on their resistance to the above antibiotics, characteristic morphology on plates, and urease and oxidase activity of multiple colonies collected with a swab from the primary-culture plate. RUT was performed by incubating 100 µl of undiluted homogenate with 100 µl of filter-sterilized 0.05% phenol red-2% urea in 0.01 M sodium phosphate (pH 6.5) (27) at room temperature and was evaluated after 12 h. As controls, gastric homogenates of brucella broth-challenged animals were regularly included. Oxidase activity was tested by using the DrySlide oxidase test (Difco Laboratories, Detroit, Mich.). G1.1 mutant strains were additionally tested for kanamycin resistance and *XylE* activity, as described above. Chromosomal DNA extracted from the *H. pylori* challenge strain G1.1 and from isolates obtained after 1, 4, 8, 12, 16, and 24 weeks of colonization was examined by RAPD-PCR with 10-nt primers 1281, 1254, and 1247 (2).

Histopathologic testing. Formalin-fixed tissue samples were processed routinely and stained with hematoxylin and eosin. For better visualization of *H. pylori* in the tissue sections, silver staining was performed. Briefly, after the deparaffinized tissue sections were sensitized for 5 min with 1% (wt/vol) uranyl nitrate in H₂O and rinsed in distilled H₂O, staining was performed by covering the sections for 1 h at room temperature with a freshly mixed solution consisting

of 2 volumes of 50% (wt/vol) silver nitrate in H₂O and 1 volume of 1% formic acid with 2% (wt/vol) gelatin (49). Histologic evaluation was done by a single pathologist (K.T.T.) blinded to the experimental data. As semiquantitative estimates, the following parameters each were graded from 0 to 3 in the gastric antrum, body, and duodenum: acute inflammation, chronic inflammation, and spiral organisms in the gastric glands. One or no foci of inflammation per low-power field with a 10× objective was graded as 1, more than one focus but less than half of the mucosa was graded as 2, and diffuse or multifocal inflammatory infiltration of more than half the mucosa was graded as grade 3. Few single organisms or no more than two small clusters of silver-stainable organisms in the gastric glandular lumina per high-power field with a 40× objective was grade 1; three to six clusters was grade 2; and more than six clusters was grade 3. Because of the paucity of spiral silver-stainable organisms on the mucosal surface, bacteria in this location were graded from 0 to 2, where grade 0 represented no detectable organisms; grade 1 represented a few single organisms; and grade 2 represented a few small clusters of organisms. Lymphoid aggregates were graded as present or absent. In parallel with mucosal (superficial) inflammation, inflammation also was graded from 1 to 3 for density of the cellular infiltrates within the gastric wall on the serosal aspect of the muscularis mucosae and was referred to as deep inflammation. For each animal, the grading for each region was based on evaluating all tissue available for this region on one microscopic slide. The total diameters of the specimens of the duodenum, antrum, and body were 3.6 ± 2.8, 14.8 ± 6.6, and 18.0 ± 6.8 mm, respectively.

Serum antibodies to *H. pylori*. For evaluation of the time course of the systemic immune response to *H. pylori*, a group of gerbils were tail bled before and 2, 3, 4, 5, 6, 8, and 12 weeks after orogastric challenge and at sacrifice. Serum samples were stored at -20°C before being used for antibody determinations. Immunoglobulin G (IgG) or IgM antibody levels in serum were measured by ELISAs. Immulon 2 flat-bottom microtiter plates (Dynatech Laboratories, Inc., Chantilly, Va.) were coated with 100 µl of a whole-cell sonicate of *H. pylori* G1.1, corresponding to 1 µg of protein per well as determined by the bicinchoninic acid protein assay (Pierce, Rockford, Ill.). Sera were diluted 1:500 or 1:100 in PBS-1% bovine serum albumin for determination of IgG or IgM, respectively. Specific detection of gerbil Ig by goat anti-mouse Ig (25), gerbil IgG by sheep or goat anti-mouse (48, 53, 61) or goat anti-hamster (1) IgG, and gerbil IgA by goat anti-mouse IgA (61) has been described previously. Therefore, bound gerbil IgG antibodies were detected by goat anti-mouse IgG and, by analogy, gerbil IgM was detected by goat anti-mouse IgM antibodies coupled with alkaline phosphatase (Sigma) and *p*-nitrophenylphosphate as the substrate, using methods described previously (55). The results were expressed as OD units (1 OD unit = 1,000 OD₄₁₀) corrected for the OD values obtained from controls without conjugate. Serum samples from animals before challenge with *H. pylori* and after challenge with brucella broth served as negative controls and selected positive sera served as positive controls in subsequent assays. The OD variation of the controls between different assays was within a range of ±10%.

Statistical analysis. Bacterial CFU and OD units were expressed as mean ± standard error of the mean (SEM). Proportions were compared by using Fisher's exact test. ODs and CF units were compared by using Student's *t* test for a comparison of means of independent samples or one-way analysis of variance. In two-tailed analyses, *P* < 0.05 was considered significant.

RESULTS

Inoculation of *H. pylori* G1.1WT and mutant strains. To assess its ability to colonize the gerbil stomach, the mouse-passaged strain CPY3401 was first passaged through gerbils. In contrast to the multiply in vitro-passaged CPY3401, gerbils could be colonized with this strain recovered from freezer stock, and the gerbil-passaged, reisolated, and clonally expanded strain then was called G1.1 for further use.

Having available a strain that reproducibly colonizes the gerbil stomach, we then created isogenic U⁻, C⁻, and V⁻ mutants of G1.1 to examine the effect of ablation of *ureA*, *cagA*, and *vacA*, respectively, in G1.1 on gastric colonization and inflammation in Mongolian gerbils. The results of experimental infection of gerbils with G1.1WT and mutant strains are summarized in Table 1. Compared with the colonization rate of 98% with G1.1WT, ablation of *ureA* (G1.1U⁻) completely abolished colonization whereas ablation of *vacA* (G1.1V⁻) only slightly reduced the colonization rate (to 86%) (*P* = 0.024, Fisher's exact test) and ablation of *cagA* (G1.1C⁻) had no effect at all (100%). There was no evidence for spontaneous clearance of the infections for up to 32 weeks for animals infected with G1.1WT or for up to 12 weeks (maximum observation period) for animals infected with the G1.1C⁻ or G1.1V⁻ mutant. In parallel to the ratios of infect-

TABLE 1. Experimental infection of Mongolian gerbils with wild-type and isogenic mutants of *H. pylori* G1.1

Bacterial genotype	No. of infected animals/no. challenged at time (wk) postchallenge:											Total no.
	1	2	3	4	6	8	12	16	20	24	32	
Wild type	14/15	16/17	5/5	7/7	7/7	12/12	21/21	2/2	9/9	2/2	4/4	99/101
<i>cagA</i>	3/3	3/3	3/3	3/3	3/3	5/5	5/5					25/25
<i>vacA</i>	2/3	3/3	3/3	3/3	3/5	7/8	4/4					25/29 ^a
<i>ureA</i>	0/6	0/4	0/2				0/2					0/14

^a $P = 0.024$, compared with wild type (Fisher's exact test).

ed-to-challenged animals, the mean levels of colonization determined by quantitative culture remained stable between 10^4 and 10^5 CFU/stomach for both G1.1WT and the G1.1C⁻ or G1.1V⁻ mutants during the observation periods (Fig. 1). Analysis by RAPD-PCR of G1.1 isolates recovered after 1, 4, 8, 12, 16, and 24 weeks of infection showed virtually identical patterns to those of the inoculation strain G1.1.

Effect of the number of challenges on colonization. To examine whether the number of challenges necessary to induce infection could be reduced, we compared the ratios of infected-to-challenged gerbils after one, two, or three orogastric inoculations with *H. pylori* G1.1. Whereas in one experiment all four animals in each group challenged one to three times ($n = 12$) were infected 12 weeks after challenge (a total of 12 animals), in another experiment in which five gerbils per group were challenged once with 1×10^8 , 5×10^8 , 1×10^9 , or 5×10^9 CFU of G1.1, based on measurement of the OD₅₅₀, only two or three animals were infected 2 weeks after challenge with the three higher doses and one animal was infected after challenge with the lowest dose, as detected by culture (a total of 8 of 20 animals). Based on these findings, we concluded that in our hands, experimental infection of Mongolian gerbils with *H. pylori* in a single-challenge protocol would provide results that were too unpredictable. Therefore, in all future experiments, we used three challenges.

Inoculation of fresh human *H. pylori* isolates. After establishing colonization of gerbils with an *H. pylori* strain (G1.1) that had been both mouse and gerbil passaged and with mutants derived from this strain, and after finding no colonization with a multiply in vitro-passaged version of the same strain (see below), we examined whether gerbils could be infected with fresh clinical *cagA*-positive or *cagA*-negative isolates of human

origin. All 15 gerbils challenged with one of three fresh human *cagA*-positive *H. pylori* isolates (B127, B128, and B129) became infected. Only 6 (23%) of 26 gerbils challenged with one of four fresh *cagA*-negative isolates (B108 [2 of 5], SW18 [2 of 5], B120 [2 of 6], B102 [0 of 10]) became infected, a significant ($P < 0.001$, Fisher's exact test) difference from the results of challenges with fresh human *cagA*-positive *H. pylori* isolates.

Infection of gerbils with *cagA*-positive *H. pylori* B127, B128, or B129 has been documented for up to 8 weeks thus far. Parallel to G1.1WT and the G1.1V⁻ mutant, comparison of colonization levels at up to 6 weeks and after 6 weeks of infection for *H. pylori* B127, B128, and B129 did not show a significant tendency of the mean CFU/stomach to decrease with time (data not shown), but long-term experiments with these strains are required. Next, we determined whether other in vitro-passaged strains were able to colonize gerbils or whether this phenomenon was specific for G1.1.

Effect of in vitro passaging of G1.1 on colonization. Since for several of the strains used in our animal studies, the exact number of in vitro passages after isolation from human gastric biopsy specimens was not documented, we studied the effect of sequential in vitro passaging of strain G1.1 on its ability to colonize the gerbil stomach. Comparison of gerbils infected with strain G1.1 that was freshly isolated from the gerbil stomach with those infected with strains obtained after an additional 10 to 35 in vitro passages on blood plates showed a progressive diminution of colonization ability, until after 20 passages, colonization was lost. The ratio of infected to challenged animals decreased from 10 of 10 animals challenged with G1.1+0 to 7 of 10 challenged with G1.1+10, 1 of 5 challenged with G1.1+15 ($P = 0.004$), and 0 of 20 challenged with G1.1+ ≥ 20 ($P < 0.0001$), resulting in a continuous decrease of the average CFU per challenged animal with increasing in vitro passage (Fig. 2). In contrast to the results obtained with the freshly isolated human strains, none of five animals challenged in each group with one of five multiply in vitro-passaged human *cagA*-positive *H. pylori* strains (J223, HPK5, HPK127, CPY3401, and 26695) became colonized.

Attempts to reverse the effect of in vitro passaging on colonization ability. Having defined for G1.1 the exact number of in vitro passages that abolishes its ability to colonize the gerbil stomach, we tested whether this in vitro-induced inability to colonize could easily be overcome. First, rechallenge with the G1.1+15 isolate that was recovered from the one infected animal induced colonization in three of five animals (mean log₁₀ of 4.08 CFU/stomach), in contrast to one of five animals infected with the original G1.1+15 strain (log₁₀ of 3.71 CFU [not significant]). Second, to determine whether the in vitro-induced changes were due to the growth of *H. pylori* on solid medium, gerbils were challenged three times with G1.1+35 grown in brucella broth supplemented with 5% fetal bovine serum. However, none of five animals was found to be infected by 2 weeks after challenge, whereas all five control animals

FIG. 1. Gastric colonization of Mongolian gerbils infected with *H. pylori* wild-type or mutant strains. Solid boxes, G1.1WT; open circles, *cagA* mutant; open triangles, *vacA* mutant. Animals were sacrificed 1 to 32 weeks after bacterial inoculation, and the number of CFU per stomach was determined by quantitative culture, as described in the text. The numbers of animals included are shown in Table 1. Results are expressed as mean and SEM for the infected animals.

FIG. 2. Effect of in vitro passage of *H. pylori* G1.1 on gastric colonization of Mongolian gerbils. A total of 5 or 10 animals in each group were challenged with strain G1.1 after 0 to 35 in vitro passages, and the number of CFU per stomach was determined by quantitative culture 1 and 2 weeks after challenge, as described in the text. Results are expressed as mean CFU per stomach of the originally challenged animals of the 1- and 2-week determinations and SEM. Student's *t* test was used to calculate significance.

inoculated with broth-grown G1.1+0 were infected. Third, since *H. pylori* G1.1 is naturally competent for transformation in vitro by chromosomal DNA from itself with an approximate frequency of 10^{-6} to 10^{-7} for the occurrence of G1.1U⁻ and 10^{-8} to 10^{-9} for G1.1A⁻ or G1.1V⁻, we examined whether the ability to colonize could be transferred from the colonizing strain (G1.1+0) to the multiply in vitro-passaged strain (G1.1+35) by transforming growing G1.1+35 cells with chromosomal DNA from G1.1+0. In contrast to direct selection in vitro for a marker in a conventional natural transformation experiment, the G1.1+35 recipients were cultured to expand the population and then 10^{10} CFU was inoculated three times at 2-day intervals into gerbils to potentially select for colonizing transformants in vivo. Inocula of G1.1+0 transformed with chromosomal DNA extracted from G1.1+0 or G1.1+35 and inocula of G1.1+35 transformed with DNA from G1.1+35 were used as controls. In an additional control experiment, G1.1U⁻ transformants were found for G1.1+0 and G1.1+35 at a similar frequency of 10^{-6} to 10^{-7} , indicating that the natural competence of *H. pylori* G1.1 remained unchanged during extended in vitro passage for more than 1 month. Cells of G1.1+0 or G1.1+35 were equally urease positive in the RUT. Similarly, cells of G1.1 after 0 to 70 in vitro passages were indistinguishable in Lewis^x and Lewis^y expression and in RAPD-PCR with chromosomal DNA (results not shown). None of five animals in each group became infected with strain G1.1+35 transformed with DNA from either G1.1+0 or G1.1+35, whereas all five animals in each group challenged with G1.1+0 transformed with DNA from G1.1+0 or G1.1+35 became infected. Similarly, none of the G1.1+35 isolates after passage through different mouse strains (BALB/cBy, C57BL/10, and C3H/HeJ) and freshly recovered from the mouse stomach was able to colonize the gerbils (54). Together, these data indicate that once lost, the ability of in vitro-passaged *H. pylori* G1.1 to colonize Mongolian gerbils cannot easily be regained in vitro or even by in vivo passage through mice.

Effect of selected host factors on experimental infection of gerbils. In parallel with the gerbils normally purchased from Harlan Sprague-Dawley, 15 of 15 animals from Charles River Laboratories challenged with G1.1 were colonized with an

average of 9.7×10^4 CFU per stomach for up to 20 weeks, and the results indicate that both the systemic immune response and gastric histopathology were the same as described for the Harlan animals, consistent with the common origin of gerbils available in the United States (see below).

To examine whether the sex of Mongolian gerbils affects experimental infection, female gerbils (from Harlan) were challenged with *cagA*-positive (G1.1, B128) or *cagA*-negative (B108) *H. pylori* strains that had been previously passaged through male gerbils. In ongoing experiments, 2 of 3, 5 of 6, and 3 of 3 female gerbils in each group were still infected at 1, 2, 4, 8, 12, and 16 weeks, respectively, after challenge with G1.1 (19 of 21); 3 of 3, 2 of 2, 2 of 2, and 2 of 2 were still infected after challenge with B108 (9 of 9); and 2 of 2 were still infected at each of 1, 2, 4, 6, 8, and 12 weeks after challenge with B128 (12 of 12). Together, these preliminary results indicate that neither the origin of the Mongolian gerbils purchased from different U.S. sources nor the sex of the animals markedly influences their susceptibility to the early stage of experimental infection with *H. pylori*.

RUT. Of 99 gerbils infected with G1.1WT, the RUT after 12 h was positive in 84 (85%), equivocally positive in 10 (10%), and negative in 5 (5%) of the animals. The mean $\log_{10}$ CFU $\pm$ SEM/stomach determined by quantitative culture of samples from the RUT-positive animals (4.60 ± 0.07) was higher than in the equivocally RUT-positive animals (4.31 ± 0.14 ; not significant), and the RUT-negative animals (3.85 ± 0.35 ; $P = 0.013$ by Student's *t* test, $P = 0.021$ by one-way analysis of variance). Of the 25 gerbils infected with G1.1V⁻, the number of infected animals with truly positive RUT was smaller (15 animals [60%]; $P = 0.012$ by Fisher's exact test) and the number with only equivocal RUT was larger (8 animals [32%]; $P = 0.009$ by Fisher's exact test) compared with those infected with G1.1WT, and the remaining 2 (8%) animals both were RUT negative. In contrast to the infection with G1.1WT, where the lack of RUT positivity was related to lighter colonization of the animals, for the G1.1V⁻-infected animals the mean $\log_{10}$ CFU/stomach of the RUT-positive (4.70 ± 0.07) and the RUT-equivocal animals (4.54 ± 0.24) were similarly high. Of the 25 animals infected with G1.1C⁻, 23 (92%) had a positive RUT and 2 (8%) had an equivocally positive RUT. The gastric homogenates of a total of 40 control animals challenged with brucella broth alone, as well as those of 131 animals that did not become infected after challenge with either the original or the in vitro-passaged strain G1.1WT, mutant G1.1V⁻ or G1.1U⁻, or various *cagA*-negative wild-type strains, were consistently negative, indicating the absence of urease-positive organisms in gastroduodenal homogenates of uninfected gerbils.

These results indicate that the RUT allowed rapid detection of *H. pylori* infection in Mongolian gerbils, with a sensitivity of approximately 90%, and that low colonization density or infection with G1.1V⁻ was chiefly responsible for decreased sensitivity. Since the RUT was evaluated only after 12 h, a correlation between the CFU and the time elapsed until test positivity could not be made.

Histopathologic testing. Having established colonization of gerbils with several *H. pylori* wild-type and mutant strains, we asked whether colonization induced gastroduodenal inflammation. To exclude the possibility that gastric histopathologic changes were induced by the inoculation procedure itself, a total of 40 (control) animals challenged with brucella broth alone in different experiments were examined for histopathologic changes (the number of animals is given in parentheses) 0 (5 animals), 1 (2), 2 (7), 3 (3), 4 (5), 6 (6), 8 (5), 12 (1), 16 (2), 20 (1), 24 (1), or 32 (2) weeks after inoculation. None of these

FIG. 3. Histologic findings in the gastric antrum mucosa in uninfected (A and B) or *H. pylori* G1-1-infected (C to I) Mongolian gerbils. (A and B) Neither inflammatory infiltration (A) nor silver-stainable spiral microorganisms (B) are visible in a 10-week-old uninfected Mongolian gerbil challenged three times with brucella broth alone 2 weeks before sacrifice. The arrows in panel B indicate a narrow band of amorphous material at the mucosal surface, which was present equally in *H. pylori*-infected and uninfected animals. (C) The mucosa of an animal infected for 4 weeks shows a mild polymorphonuclear infiltrate with minimal cryptitis (neutrophils in glandular lumina) and mild chronic inflammation. (D) Mucosal erosion (arrow) where the superficial mucosa is focally infiltrated by polymorphonuclear inflammatory cells in an animal infected for 6 weeks. (E) Ulcer in a gerbil infected for 8 weeks. The surface epithelium is interrupted (arrows) by the ulceration, and the wall is heavily infiltrated by inflammatory cells. The muscularis mucosae is indicated by a star. (F) Same gerbil as in panel E. Silver-stainable dark brown curved or spiral-shaped microorganisms are visible in the ulcer floor and in glandular lumina at the ulcer edges. (G) Transmurial inflammation in an animal infected for 8 weeks, involving the gastric wall from the mucosa (right) to the serosa (left). The muscularis mucosae is indicated by a star. (H) Mucosal infiltration of an animal infected for 32 weeks with focal replacement of gastric glands. (I) Antral lymphoid aggregates, some with germinal centers, were found after 4 weeks of infection in animals with dense mucosal (top) inflammation, which could extend beyond the muscularis mucosae (bottom; arrows), as in this gerbil after 8 weeks of infection. Magnifications: $\times 150$ (A), $\times 195$ (B), $\times 194$ (C), $\times 195$ (D), $\times 59$ (E), $\times 208$ (F), $\times 59$ (G), $\times 91$ (H), $\times 65$ (I). Hematoxylin and eosin stain (A, C to E, and G to I); silver stain (B and F).

FIG. 3—Continued.

animals, which covered the entire age range of animals evaluated for histopathologic changes in this study, developed gastric mucosal inflammation (Fig. 3A) or silver-stainable spiral organisms at the mucosal surface and particularly in gastric glandular lumina (Fig. 3B) 1 to 32 weeks after challenge. Two control animals had chronic mucosal inflammation (grade 1) of the duodenum. Similarly, the gastric histologic findings of 14 animals challenged with G1.1U⁻ and sacrificed after 1 to 12 weeks were normal. In both infected and uninfected animals, a band of amorphous material that was slightly silver stainable was visible on the mucosal surface and could occasionally hamper the identification of silver-stainable spiral organisms in this location. In contrast, silver-stainable spiral organisms evaluated as *H. pylori* were seen on the mucosal surface only when the underlying glands were infected.

Although the animals were not fasted before or after challenge, to further assess whether acute infection with *H. pylori* is stressful to the animals, the weight changes of the animals from 2 weeks before challenge with brucella broth or G1.1 until 6 weeks after challenge were compared. The increase in body weight of the infected and uninfected animals was similar and, in both groups, indistinguishable (data not shown) from the standard weight curve of unchallenged male gerbils obtained from the same supplier (26) or from male gerbils in the United States (9), which all are derived from the same few breeding pairs introduced into the United States in 1954 (9). Consistent with the normal weight increase, observation of the animals did not provide any evidence of distress from the inoculation procedure or infection. Consistent with these findings, no gastric

macropathologic changes were evident at necropsy, except for an ulcer at the antrocorpal border in one animal after 8 weeks of infection with G1.1 (Fig. 3E).

The histopathologic changes in the gastric antrum of gerbils infected with strain G1.1 for up to 32 weeks are shown in Fig. 4. All the antral histopathologic scores increased gradually from 2 weeks after challenge. Whereas acute inflammation (Fig. 3C) scores essentially remained constant after 4 weeks, chronic inflammation scores steadily increased until 16 weeks. In addition to the predominant superficial inflammation of the antrum mucosa (Fig. 3D), deep (i.e., reaching beyond the muscularis mucosae) gastric wall inflammation (Fig. 3E, G, and I) occurred in the gerbils, which is in contrast to the *H. pylori*-induced gastric inflammation in humans. In parallel with the deep inflammation, *H. pylori* organisms were seen mainly in the glandular lumina (Fig. 3F), in contrast to their usual confinement to the surface epithelium and the gastric pits in humans (10). Although the total gastric bacterial counts for G1.1 as determined by culture did not change significantly over the first 32 weeks of infection (Fig. 1), the mucosal distribution of

FIG. 4. Evolution of gastric inflammation and colonization over time in Mongolian gerbils infected with *H. pylori* G1.1. Parameters were determined by histologic testing as described in the text, and scored from 0 to 3. Solid triangles, chronic inflammation; open triangles, acute inflammation. Upright and downward triangles (solid or open) represent superficial and deep (beyond the lamina muscularis mucosae) inflammation, respectively. Curved organisms detectable by silver staining in the glandular lumina are represented as open boxes. The numbers of animals included are shown in Table 1, except for weeks 2 and 3, when only three animals for each time were evaluated histologically. The results shown are mean values of the histologic scores and SEM.

FIG. 5. Comparison of gastric antrum inflammation in gerbils infected with G1.1WT and mutant strains. Acute (A) and chronic (B) superficial mucosal inflammation of animals after 1 to 12 weeks of infection with G1.1WT (solid squares), G1.1C⁻ (circles), or G1.1V⁻ (triangles) were determined by histologic testing, as described in the text. The results are expressed as mean histologic scores and SEM. The numbers of animals included are shown in Table 1. None of the differences was statistically significant.

organisms, reflected by the numbers visualized by histologic testing, varied, with a peak in the gastric gland score in the first 3 to 4 weeks (Fig. 4). In contrast to uninfected gerbils, only 2 (3%) of 62 animals infected for ≥ 4 weeks with G1.1 did not show antral inflammatory changes, indicating the nearly universal induction of inflammation by experimental *H. pylori* infection. Antral lymphoid aggregates (Fig. 3H) occurred after 6 weeks of infection and were observed in 30 (79%) of 38 animals by 12 weeks. A few large lymphoid aggregates contained germinal centers (Fig. 3I). Whereas the histologic scores for *H. pylori* on the mucosal surface were unrelated to the mean CFU determined by quantitative culture, gastric gland scores of 1, 2, and 3 were associated with increasing $\log_{10}$ CFU in culture of 4.22 ± 0.24 , 4.56 ± 0.11 , and 4.65 ± 0.17 , respectively; however, these differences were not significant because of the large variability of the CFU. In contrast to the antrum, no silver-stainable organisms or inflammatory changes were detectable in the duodenum of gerbils infected with *H. pylori* G1.1 and only two animals also had gastric body inflammation (after 8 and 32 weeks of infection, including the animal with a gastric ulcer).

Gastric histopathologic changes induced by G1.1WT did not differ substantially from those induced by the G1.1C⁻ or G1.1V⁻ mutant for up to 12 weeks after challenge (Fig. 5); long-term comparative studies will show whether or not differences appear later. Also, preliminary results indicate that gas-

FIG. 6. Variability of the serum antibody response to *H. pylori* in six representative infected gerbils during the first 12 weeks following challenge with strain G1.1. (A) IgM. (B) IgG. Blood specimens were obtained prechallenge, weekly from weeks 2 to 6, and at 8 and 12 weeks. Antibody levels in serum were determined by ELISA with a sonicate of G1.1 cells as the antigen. The results shown for each gerbil represent the mean of two duplicate determinations. The symbols for each gerbil are the same in both panels. (C) Mean values for IgG (circles) or IgM (squares) and SEM in 21 animals.

tritis induced by fresh human clinical *cagA*-positive or *cagA*-negative *H. pylori* isolates is similar to that induced by strain G1.1WT or its mutants (54).

Serum antibody response to *H. pylori*. The serum antibody response to *H. pylori* G1.1 was monitored from prechallenge to 12 weeks post-challenge in 21 animals (Fig. 6). *H. pylori* infection was documented by culture and histologic testing in all animals. As detected by using goat anti-mouse IgG or IgM conjugates, both the serum IgG and IgM antibody responses to *H. pylori* whole-cell sonicate varied substantially among individual animals (Fig. 6A and B). There was no correlation of the antibody responses with bacterial CFU at 12 weeks or with the scores for chronic superficial or deep gastric inflammation.

Whereas IgG antibody levels steadily rose for up to 12 weeks after challenge, IgM antibody levels in most animals showed a biphasic course with an early peak at 2 weeks and then a progressive rise until 12 weeks. Compared with the prechallenge antibody levels, levels from ≥ 2 weeks after challenge all were significantly ($P < 0.01$) higher for both IgG and IgM (Fig. 6C). This phenomenon allows confirmation of *H. pylori* infection after 2 weeks of challenge without the need to sacrifice the animals.

DISCUSSION

Our results confirm (29, 30, 41, 59, 60) that experimental infection of Mongolian gerbils with *H. pylori* is possible and indicate that stable colonization occurs for at least 32 weeks, which reproducibly induces gastric inflammation and a systemic immune response. For the first time, isogenic mutants were studied in this rodent model. The strains that can infect gerbils include several *cagA*-positive and *cagA*-negative human *H. pylori* isolates that now are available for in vivo studies; the availability of multiple strains is a prerequisite for the study of mixed infections.

Reproducible induction of gastritis in conventional mice thus far is restricted to selected and adapted *H. pylori* strains such as the Sydney strain (39), and several reports describe minimal or no inflammation with *H. pylori* strains that have not been preselected (29, 40, 42). Gastritis in animals infected for 4 weeks to 5 months with *H. pylori* G1.1 was significantly greater in gerbils than in several commonly used mouse strains (including C57BL/10, C57BL/6, BALB/c, C3H/HeJ, C3H/HeOuJ, and C3H/HeSnJ) (54). This finding suggests that gastritis induced in gerbils, at least in the short term, is more pronounced than in mice infected with strain G1.1. Induction of gastritis in gerbils infected with strains other than G1.1 (54) supports the view that gerbils are suitable and are potentially superior to mice for studying experimental *H. pylori* gastritis, provided that host genotypic characteristics are not the focus of interest. The longer life span of gerbils than of mice (9) and the larger amount of gastric tissue available for histologic examination may represent additional advantages.

Consistent with previous reports from Japan (29, 30, 41), the *H. pylori* viable counts per gerbil stomach were around 10^5 CFU, gastric inflammation affected mainly or exclusively the antrum, and inflammation increased with increasing time after *H. pylori* inoculation. Both in our study and in the cited reports, gastric histopathologic findings were confined to *H. pylori*-infected animals as detected by culture, histologic testing, and RUT. In contrast, in gerbils that were not challenged or were unsuccessfully challenged, neither *H. pylori* nor other urease-positive organisms could be cultured from the gastroduodenal mucosa, no spiral organisms were found in silver stains in histologic examinations, RUT results were consistently negative, and no serum antibodies to *H. pylori* whole-cell sonicates were detected. In attempts to recover *H. pylori* from small intestinal, cecal, or distal colonic contents of *H. pylori*-infected gerbils by using selective and nonselective media, we could detect neither *H. pylori* nor other urease-positive bacteria. Culture of gastric homogenates from infected gerbils on nonselective blood agar plates on average yielded fewer colonies that were morphologically consistent with *H. pylori*, compared with the yield for the selective medium normally used (54). Although cultures on nonselective media usually were more heavily contaminated, the diminished *Helicobacter* growth is evidence against the concomitant presence of *Helicobacter* species other than *H. pylori*. In gnotobiotic gerbils carrying a defined six-member murine microflora, the gastrointestinal

flora did not differ from that of conventional gerbils, except for the presence of larger numbers of gastric *Bacteroides* spp. in the former (5). Because gerbils, like other rodents, are coprophagic, their lower intestinal microflora could be of importance for the gastric microflora. However, in a survey of anaerobic and aerobic bacteria in the lower intestinal tract of Mongolian gerbils, organisms consistent with *Helicobacter* species were not identified (57). Although these studies were not carried out with the specific aim of identifying *Helicobacter* species, these data and the low prevalence of naturally occurring gastritis in gerbils reported in the literature (6) indicate that even if natural colonization of gerbils with *Helicobacter* species other than *H. pylori* does occur, its relevance for induction of gastritis is limited. Clearly, with the future use of the gerbil model of *H. pylori* infection in mind, further characterization of the gastrointestinal microflora of gerbils with special emphasis on *Helicobacter* species should be performed (22).

In contrast to studies from Japan, the gerbils in our experiments were challenged three times to reproducibly achieve colonization, the prevalence of gastric ulcers seemed to be lower, and intestinal metaplasia was not a prominent finding. Whether these differences are related to genetic host differences (43) and/or bacterial factors will have to be addressed in future experiments, but they suggest the possibility of establishing different disease models by appropriate selection of the gerbils and/or the colonizing *H. pylori* strains (30). The undefined genetic background of our outbred gerbils is a disadvantage compared to the use of genetically defined inbred mouse strains, which are superior for examining the effect of differences in host genotype, provided that the animals can be reproducibly infected with *H. pylori*. Although three orogastric challenges represent a greater effort during the experimental procedures, this protocol is well tolerated by the animals, never limited the number of animals included in different types of experiments, and, more importantly, ensured that lack of colonization reflects the characteristics of the bacterial strain challenge rather than slight technical differences in the challenge procedure. Whereas Matsumoto et al. (41) reported that two of their four animals had gastric ulcers after 16 weeks of infection, the ulcer incidence in the report by Hirayama is not indicated and ulcers occurred after 6 months of infection or more. Therefore, comparison of the ulcer incidences is difficult on the basis of the available data. Induction of intestinal metaplasia during *H. pylori* infection does not seem to be a general phenomenon. In contrast to the one positive report by Hirayama et al. (30), occurrence of intestinal metaplasia was not evident in our animals, and it is not mentioned in the report of Matsumoto et al. (41), who used animals from the same source as that used by Hirayama et al. Because gastric goblet cells normally occur in our uninfected animals without gastritis (54), it would be important to know whether the earlier diagnosis of intestinal metaplasia (30) was based mainly on the occurrence of goblet cells or on other criteria.

In vitro passage of *H. pylori* strains progressively reduces their ability to colonize the gerbil stomach, and the phenotypes necessary for colonization, although undefined at present, are not easily restored. However, animals could successfully be colonized with several isogenic mutants of strain G1.1 despite the in vitro time necessary to construct these from their wild-type parental strain. In contrast, six of seven unselected fresh human *H. pylori* isolates colonized the gerbil stomach. These results indicate that gerbils represent a suitable rodent model for in vivo comparisons of fresh human *H. pylori* isolates. Urease is essential for establishment of *H. pylori* colonization in gerbils, similar to gnotobiotic piglets (16), primates (28), nude mice (51), and ferrets (3). The exact contribution of urease is

unknown, but effects on local pH could be tested by attempting to establish infection in animals treated with H₂-blockers or proton pump inhibitors that would elevate gastric pH (18).

The vacuolating cytotoxin (VacA) is not essential for colonization, confirming results obtained with gnotobiotic piglets (17), although the infection rate was slightly lower with the *vacA* mutant than with the wild-type strain. The results of the RUT suggest that the lower infection rate could be associated with decreased urease activity of the *vacA* mutant in vivo or with decreased levels of colonization. *H. pylori* VacA is heterogeneous (12), and some strains produce little protein in vitro. The role of VacA in vivo is unknown, and it is not certain that the induction of vacuoles in eukaryotic cells is relevant to its biological role in vivo. We do not know the levels of VacA expression in vivo in humans; vacuole production in vitro may be induced at doses many times those occurring in vivo, for example. Nevertheless, the presence of two copies of *vacA* homologs in the *H. pylori* genome (50) suggests that VacA has an important conserved function. The association between toxigenic *H. pylori* strains and ulcer disease in humans (7) and the animal data (obtained with piglets and gerbils) indicating that VacA is not necessary for short-term colonization and does not influence gastric inflammation in the first 3 months suggest to us that VacA may be involved in long-term rather than acute events. The degree to which results from experimental mouse challenge with sonicates from *H. pylori* wild-type or *vacA* mutant strains (23) are applicable to the induction of gastric histopathologic changes during natural infection is not known.

Colonization of gerbils with the isogenic *cagA* mutant indicates that *cagA* itself is not essential for colonization. Since many *H. pylori* strains do not possess *cagA* (11, 52) or the complete *cag* island (8), it clearly is not necessary for the establishment of colonization in humans. However, reduced colonization of gerbils with fresh wild-type *cagA*-negative compared with *cagA*-positive isolates suggests that *cagA* could be a marker for genes in the *cag* island or elsewhere that enhance colonization ability in gerbils. In vivo passage through gerbils appeared to increase the ability of *cagA*-negative *H. pylori* strains to colonize the gerbil stomach. Therefore, reduced colonization may be restricted to the interspecies (human-to-gerbil) transfer of *cagA*-negative *H. pylori* strains but may not be true for the intraspecies (gerbil-to-gerbil) transfer of these organisms. That inflammation during the first 3 months of infection induced by both the *cagA*- and *vacA*-ablated *H. pylori* mutants was similar to that from the wild-type strain also is consistent with the induction of gastritis in mice by *H. felis*, which does not express CagA or VacA (43). Our model provides the basis for studying the in vivo effect of ablation of essentially any *H. pylori* gene, provided that the gene product is not crucial for colonization.

Gerbils from different U.S. sources tolerate colonization with *H. pylori* well, with no change in their expected weight gain in captivity. In total, only one animal infected with strain G1.1 (2% of 48 animals observed for ≥ 8 weeks) developed a gastric ulcer. Whereas strain G1.1 was originally isolated from a patient with a duodenal ulcer (31), no duodenal ulcers were observed in the gerbils. This low rate of ulcerogenicity may be analogous to ulcerogenesis in persistently colonized humans, in contrast to the findings in gnotobiotic piglets (36). The histopathologic response to colonization also shows some notable similarities in the antrum between humans and gerbils, with both acute polymorphonuclear leukocyte and chronic lymphoplasmacytic cell infiltration. That not only were 99 of 101 gerbils challenged with strain G1.1 infected, but that also 97% of the animals infected for ≥ 4 weeks had antral gastritis shows the potential of this animal model. The interindividual varia-

tion in the degree of inflammation may be related in part to the use of outbred animals. Selection and mating of animals with strong responses could lead to inbred gerbil strains with enhanced gastric inflammation during *H. pylori* infection. The gradual increase of the chronic deep inflammation during the first 24 weeks suggests that these deep lesions may persist for the entire duration of *H. pylori* infection, whereas the scores for chronic superficial inflammation reach a plateau after about 12 weeks. The deep inflammation extending beyond the muscularis mucosae is not observed in humans and may relate to the presence of *H. pylori* in the lumina of the gastric glands, in contrast to their usual absence in human gastric glands. The histopathologic changes induced by several human clinical *H. pylori* isolates are virtually identical to those induced by strain G1.1 (54).

The variability in antibody responses to *H. pylori* infection in gerbils parallels that observed in humans (45), and, similar to humans, chronic infection develops despite a host immune response. The present lack of gerbil-specific reagents represents a limitation of this rodent model of *H. pylori* infection; however, due to interspecies cross-reactions, mouse-specific antibodies can be used (25, 48, 53, 61) until gerbil-specific immune reagents are developed. The bacterial antigen preparation used for the ELISA to detect antibodies can be individualized based on the challenge *H. pylori* strain, with inclusion of many if not all of the relevant antigens to which immune responses may occur. Experimental infection with particular strains also excludes the possibility of mixed infection with several *H. pylori* strains differing in their antigenicity. The variability in antibody responses in gerbils may therefore reflect true heterogeneity of the host immune response to *H. pylori* antigens rather than lack of representativeness of the antigens used in the ELISA. Despite the marked variability in antibody responses between different hosts, and although isotype-specific detection of gerbil IgG by antibodies to mouse IgG isotypes may not be completely accurate, several clear trends emerge, as shown in Fig. 6C. The time course of the response is similar to that after experimental viral infection (34) or parenteral parasitic infection with *Dipetalonema viteae* or *Leishmania major*, where elevated antibody levels occur in serum after 1 week of infection (1, 58). The early peak in the IgM response is similar to that observed in a human volunteer who ingested *H. pylori* (44), although the secondary rise differs; long-term studies should indicate whether there is parallelism with phenomena in humans. That the early IgM peak immediately precedes the peak of the histologic score for *H. pylori* in gastric glands suggests the existence of at least a temporal relationship. The steady rise in IgG levels also parallels human responses; the decreasing numbers of bacteria observed in the gastric glands may reflect the development of an immune response that limits the bacterial population in this location. The increase in the IgG levels in serum and in the number of mononuclear cells in the lamina propria, after the numbers of neutrophils have reached a plateau, suggests that a mature immunological response to the organism is developing.

In conclusion, specific advantages of the gerbil model outweigh its present limitations and suggest applications that are complementary to those of existing rodent models. Limitations include the lack of information about host genetics, gastric physiology, and gastrointestinal microflora and the lack of gerbil-specific immune reagents. Experimental infection of Mongolian gerbils with *H. pylori* represents a versatile model, since it offers the technical advantage of a small laboratory animal with good susceptibility to experimental *H. pylori* infection, a brisk gastric inflammatory and systemic immune response, and

prolonged colonization. For colonization, bacterial strain characteristics appeared more important than host characteristics.

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