INFECTION AND IMMUNITY, Aug. 1996, p. 2885–2891 Vol. 64, No. 8
0019-9567/96/$04.00+0

Transient and Persistent Experimental Infection of Nonhuman Primates with *Helicobacter pylori*: Implications for Human Disease

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Received 25 March 1996/Returned for modification 19 April 1996/Accepted 1 May 1996

Helicobacter pylori **can establish chronic infection in the human gastric mucosa, and it is a major cause of peptic ulcer disease and a principal risk factor for gastric cancer. This creates a need for *H. pylori* infection models that mimic the human condition. To test the suitability of rhesus monkeys as infection models, *H. pylori*-free animals were inoculated intragastrically with mixtures of *H. pylori* strains, bacteria recovered from colonized animals were typed by arbitrarily primed PCR, and host inflammatory and immunologic responses were monitored. Among five *H. pylori*-free animals inoculated with a mixture of two human strains plus one monkey strain, one became persistently infected and one became only transiently infected. The recovered bacteria matched the monkey input strain in DNA fingerprint. A subsequent trial using two new human isolates and three animals that had resisted colonization by the monkey strain resulted in persistent infection in one animal and transient infection in two others. Antral gastritis, anti-*H. pylori* serum immunoglobulin G, and atrophy all increased, but with patterns that differed among animals. We conclude that (i) rhesus monkeys can be infected experimentally with *H. pylori*, (ii) individuals differ in susceptibility to particular bacterial strains, (iii) infections may be transient, and (iv) the fitness of a particular strain for a given host helps determine the consequences of exposure to that strain.**

Helicobacter pylori, a common bacterial pathogen of humans, is the principal cause of chronic active gastritis and peptic ulcer disease and a risk factor for gastric cancer, even though most infections are asymptomatic (3, 4, 41). Once established, most infections last for years and rarely cure spontaneously, although they usually can be cured by antimicrobial therapy. Infection also increases the risk of other diseases, such as cholera (8) and persistent diarrhea (44). *H. pylori* is a very diverse species, and it is possible that the range of outcomes following infection reflects differences in bacterial genotypes, as well as human host genotypes and environmental factors.

Much of our understanding of *H. pylori*-host interactions has come from hundreds of studies of humans with well-established infections, often identified during population screens or because of persistent gastroduodenal disease symptoms. Fragmentary data indicate major differences between such chronic (established) infections and the early (acute) phase when a person is just being colonized (3, 17, 27, 29, 32–34). Acute infections are particularly difficult to analyze in people, however, because experimental human infection is unethical and early stages of natural infections do not usually receive medical attention. This emphasizes the special need for human-like *H. pylori* infection models.

The importance of *H. pylori* has spurred development of several animal models for studies of *Helicobacter* infection. Of particular interest are (i) gnotobiotic newborn piglets, which are easily infected by *H. pylori* of human origin (2, 23) but are best suited for short-term studies; (ii) mice and ferrets, which can be colonized for months and years, respectively, although most easily by *Helicobacter* species other than *H. pylori* (16, 24); (iii) certain domestic cats, which can carry *H. pylori* (20); and (iv) particular strains of mice, which can be colonized by selected *H. pylori* strains (26, 30). Each of these models, although useful, is also limited by major differences from humans in gastric anatomy, physiology, diet, immune or inflammatory responses, technical difficulty of endoscopy, and/or short-term life span. It is in this context that nonhuman primates are of particular interest, especially for studies of subtle bacterial and host-specific factors that affect colonization or the emergence and progression of gastroduodenal disease.

Of the several primate species studied (6, 7, 15, 18, 21, 42, 43), rhesus monkeys appeared most promising because of their worldwide availability, moderate size, and large repertoire of useful immunological reagents, and the knowledge base gained through years of study. *H. pylori* is enzootic in at least some rhesus monkey colonies, including that from which the animals used in this study come (10, 12, 14). More than half of such animals become colonized by 2 years of age, although some remain uninfected for many years (13). This high incidence of infant infection resembles epidemiologic patterns among the very poor in developing countries and even in the United States and Western Europe (19, 31, 38). It is also noteworthy that *H. pylori*-infected rhesus monkeys exhibit atrophy, microerosions, and loss of mucus reminiscent of those seen in hu-

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TABLE 1. Characteristics of *H. pylori* strains used in the study

Strain no. [other name] (reference)	Source	Phenotype[a]	Trial(s) in which used
H_1 [92-26][b]	Patient with non-ulcer dyspepsia	$CagA^+$ Tox^+	1 and 2
H_2 [88-23 or ATCC-49503] (9)	Patient with non-ulcer dyspepsia	$CagA^+$ Tox^+	1 and 2
H_3 [U-1][c]	Patient with duodenal ulcer	Unknown	3
H_4 [U-2][c]	Patient with gastric ulcer	$CagA^+$ Tox^-	3
M_1 [788-2][c]	Monkey with gastritis	$CagA^+$ Tox^+	1 and 2

[a] Production of the *cagA*-encoded high-molecular-weight protein and of the vacuolating cytotoxin (9, 45).
[b] Isolated at Vanderbilt University School of Medicine, Nashville, Tenn.
[c] Isolated at the Uniformed Services University of the Health Sciences, Bethesda, Md.

mans (12, 14) and that peptic ulcers and gastric cancer also have been reported in them (36, 37).

Experimental infection of rhesus monkeys has been tried several times. In one trial, one of five animals inoculated with a strain of human origin was reported to have become infected, but the recovered strain did not match the input strain in its DNA fingerprint (15). In another trial, each of five monkeys inoculated with a strain of monkey origin became infected, and the recovered strains were said to match the input strain in DNA fingerprint, but no data supporting this interpretation were presented (15). Experimental infection of Japanese monkeys and chimpanzees have been reported, but without DNA fingerprinting data to show whether the recovered strains matched those used for inoculation (21, 42).

Here we report experimental infection of colony-raised *H. pylori*-free rhesus monkeys, with DNA fingerprinting that identifies input and recovered strains, and describe immediate and long-term inflammatory and immune responses to infection.

MATERIALS AND METHODS

Inocula. Five different *H. pylori* strains were used in these experiments (Table 1). Primary isolates were stored at −70°C in saline or brucella broth with 20% glycerol. Two days before inoculation of monkeys, the strains were transferred to flasks containing 25 ml of brain heart infusion broth plus 4% fetal calf serum, which were then incubated with shaking for 2 days in an atmosphere of 90% N_2, 5% O_2, and 5% CO_2. On the day of inoculation, the cultures were centrifuged at 4°C and resuspended in brucella broth at 10^8 to 10^9 *H. pylori* CFU/ml and mixed when indicated.

Animals. The experiments reported herein were conducted according to the principles set forth in *Guide for the Care and Use of Laboratory Animals* (21a). All experiments were approved by the Armed Forces Radiobiology Research Institute Institutional Animal Care and Use Committee and monitored and reapproved yearly. Eight domestic male rhesus monkeys (*Macaca mulatta*) that were 2 to 5 years old, weighed 2 to 5 kg, had not been in previous research protocols, and were free of *H. pylori* by culture and histology were used. They had been bred, reared, and socially housed either in indoor gang cages, in outdoor corrals, or in a large free-ranging colony on a sea island (Laboratory Animals Breeders and Services, Yemassee, S.C.).

Upon arrival at our facility in Bethesda, Md., monkeys were quarantined for 90 days in individual stainless steel cages in conventional holding rooms of an animal facility approved by the American Association for Accreditation of Laboratory Animal Care and were provided with tap water ad libitum, commercial primate chow, and fruits. They were tested by three intradermal tuberculin injections at 2-week intervals; all were negative. After release from quarantine, they were kept in equivalent individual housing. Endoscopies were performed between 8:00 and 12:00 a.m. after overnight fasts (see below).

Endoscopic procedures and biopsies. Monkeys underwent gastroduodenal endoscopic examination under general anesthesia (atropine sulfate, 0.02 mg/kg intramuscularly followed by ketamine HCl, 10 mg/kg intramuscularly), using an EG2700 Pentax (Orangeburg, N.Y.) videogastroscope with an outer diameter of 9.0 mm. After each endoscopy, the equipment was rinsed with water and then disinfected by soaking for 10 min in an activated dialdehyde solution of a 2% glutaraldehyde solution (Cidex; Johnson & Johnson Medical, Inc., Arlington, Tex.); the instruments were then rinsed sequentially with sterile water and 70% alcohol and air dried. Six pinch mucosal biopsies each of the gastric corpus and antrum were taken from each animal.

Histologic examination. Two biopsies each from the corpus and antrum were fixed in neutral 10% buffered formalin and embedded in paraffin. Five-micrometer-thick sections were stained with hematoxylin and eosin (H&E) and viewed under ×100 to ×1,000 magnification. Antral gastritis was scored on coded slides, using a scale of 0 to 3 as modified from reference 28 (0, intact mucosal lining and essentially no infiltration of the lamina propria with lymphocytes and plasma cells; 1, mild increase of mononuclear infiltration, localized in the upper half of the mucosa; 2, mononuclear infiltration extending from the surface into the lamina propria; 3, marked mononuclear infiltration extending from the surface into the lamina propria and disrupting the structure of the glands and leading to atrophy, and/or polymorphonuclear leukocytes in glands and surface erosions). Slides also were scored for the presence of *H. pylori* infection after Warthin-Starry or H&E plus Gram staining.

Microbiological methods. Two other biopsies each from the corpus and antrum were immediately placed in 0.1 ml of sterile 0.9% NaCl on ice, coded, and homogenized with a sterile ground-glass cone-shaped pestle fitting a tapered 1.5-ml Eppendorf tube. An aliquot (1 to 2 μl) was streaked on *Campylobacter* chocolate agar plates supplemented with trimethoprim, vancomycin, amphotericin B, and polymyxin B (Remel, Lenexa, Kans.) and incubated at 37°C in an atmosphere of 90% N_2, 5% O_2, and 5% CO_2. *H. pylori* isolates were identified as forming pinhead-sized colonies that grew within 7 to 10 days and had urease, oxidase (Becton Dickinson, Cockeysville, Md.), and catalase activities and by microscopy as gram-negative and curved or "gull-wing" rods. The two remaining biopsies from each region of the stomach were immediately placed in sterile 20% glycerol in 0.9% NaCl and frozen at −70°C for further analyses.

DNA fingerprinting. To distinguish among *H. pylori* strains, the arbitrarily primed PCR or random amplified polymorphic DNA (RAPD) fingerprinting method was used as described previously (1, 5) with DNAs purified by phenol extraction from cultures of individual single colony isolates. Four arbitrary primers were used: 1247 (5′-AAGAGCCCGT), 1254 (5′-CCGCAGCCAA), 1281 (5′-AACGCGCAAC), and 1283 (5′-GCGATCCCCA) (1). After PCR, 8-μl aliquots were electrophoresed in 2% agarose gels in 1× Tris acetate running buffer containing 0.5 μg of ethidium bromide per ml and photographed under UV light. The 1-kb DNA ladder (Gibco-BRL, Gaithersburg, Md.) was used as a size marker in all gels.

Measurement of *H. pylori*-specific plasma IgG. Five milliliters of blood was drawn from each monkey at the time of each endoscopy into 7-ml tubes containing 10.5 mg of EDTA and centrifuged, and the supernatant plasma was frozen at −70°C. Anti-*H. pylori* immunoglobulin G (IgG) levels in the plasma were determined blindly, using a modification of a previously described enzyme-linked immunosorbent assay (ELISA) with >95% sensitivity and specificity for human infection (11, 39). This modified ELISA used anti-monkey antibody conjugates, and established thresholds provided 92% specificity and 85% sensitivity for infection in monkeys (12). Results were corrected for day-to-day variation of the ELISA and expressed as optical density ratios. All assays were done at least in duplicate.

Inoculation protocol. Monkeys were treated with famotidine (Pepcid; Merck, Inc., West Point, Pa.; 2 mg/kg, given intramuscularly 14 and 1 h before inoculation) to suppress acid output. After an overnight fast, the animals were endoscoped as described above, phenol red was sprayed to estimate the pH of the gastric mucosa (generally a pH of between 2 and 7), and 5 ml of 0.25 M $NaHCO_3$ was introduced onto the antrum to neutralize gastric acid. A suspension of 10^8 to 10^9 CFU of *H. pylori* (1 ml of each strain) was then sprayed onto the gastric antrum. The monkeys were reendoscoped 5 to 7 days after inoculation, and generally at 3- to 5-week intervals thereafter, at which time biopsies were collected and plasma was also obtained for measurement of IgG levels. To use these animals most efficiently, some that had been inoculated, but in which *H. pylori* had not become established, were enrolled in a subsequent trial.

RESULTS

Colonization. We tested whether rhesus monkeys that had somehow evaded natural infection despite 2 to 3 years in a high-risk environment could be experimentally infected with *H. pylori* strains of rhesus monkey (M_1) or human (H_1, H_2, H_3, and H_4) origin in three trials (Table 2). Eight monkeys that had

TABLE 2. Results of *H. pylori* inoculation

Monkey no.[a]	Colonization		Gastritis score >2	Serum IgG >cutoff	RAPD fingerprint
	By culture	By histology			
Trial 1					
7N1	None	None	None	None	
7MG	None	None	None	None	
8R1	None	None	None	None	
8RC	None	None	None	None	
8V5	None	None	None	None	
9A5	None	None	None	2 mo only	
Trial 2[b]					
8RC	1–12 mo	1–12 mo	1.5–12 mo[c]	2.5–12 mo	M_1
8V5	None	None	1 wk–3 mo	1 wk–6.5 mo	
9A5	1 wk only	1–7 wk	2–3 mo only	1–4 mo only	M_1
E0E	None	None	3 mo only	3–4 mo only	
KJ2	None	None	None	None	
Trial 3[d]					
8V5	1 wk only	None	1 wk only	1–2 mo only	H_4
9A5	1 wk only	None	None	1–2 mo only	H_4
KJ2	1 wk–9 mo	2 and 9 mo	8–9 mo only	3.5–9 mo	H_4

[a] Ages of monkeys in years: E0E, 2; E5V, 2 to 3; 8R1, 3; 9A5, 8V5, and KJ2, 3 to 4; 7N1 and 7MG, 4; 8RC, 4 to 5.
[b] 8RC, 8V5, and 9A5 were rechallenged 3 months after the beginning of trial 1; E0E and KJ2 were used for the first time.
[c] Gastric corpus and antral atrophy starting at 9 months.
[d] KJ2 was used 7 months after the beginning of trial 2; 8V5 and 9A5 were used 14 months after the beginning of trial 2. Inoculation of an animal with a natural low-grade infection led to establishment of the inoculum strain and also emergence of new, apparently recombinant strains.

evaded natural infection were inoculated with various *H. pylori* strains in these trials, and the following results were obtained.

(i) Trial 1. Six animals were inoculated with human strain H_1. None of the animals was judged to have become colonized by it, given the failure to culture *H. pylori* from any of 32 biopsies taken at 3- to 5-week intervals from day 7 through 3 months, and also given the failure to detect *H. pylori* by microscopy in Warthin-Starry-, or H&E-, and/or Gram-stained histologic sections from 16 other biopsies taken at the same times. In consequence, this trial was discontinued.

(ii) Trial 2. To more efficiently assess whether any *H. pylori* strain could colonize a monkey after experimental challenge, we decided to use a mixture of several strains, each of which was readily distinguishable by DNA fingerprinting. Five animals were each inoculated with a mixture of H_1 (used in trial 1), H_2 (another human isolate), and M_1 (an isolate from a monkey). Three animals (8RC, 8V5, and 9A5) were from trial 1, which had started 3 months earlier, and the other two (E0E and KJ2) had not been used previously.

We recovered *H. pylori* from biopsies taken a week after inoculation from two of the three animals that had resisted infection in the first trial. One of them (8RC) remained infected throughout the 12 months that he was studied. The other (9A5) was judged to have spontaneously cured his infection by 3 months after inoculation, as judged from the inability to detect *H. pylori* organisms histologically in four biopsies or to culture them from any of eight other biopsies. However, light infection was still evident in him by histologic examination, but not by culture, at 4 and 7 weeks. In contrast, neither the third animal (8V5) nor either of the two new animals (E0E and KJ2) showed any sign of having been colonized during the 3 months of observation.

Since each input strain was readily distinguishable from the others by RAPD fingerprinting (Fig. 1), eight representative isolates taken at 1 month from each colonized animal were fingerprinted. Each isolate tested matched the input strain of monkey origin; none matched either input human strain (Fig. 1). These results indicate that while some monkeys could be infected experimentally with an enzootic, monkey-adapted *H. pylori* strain, other animals were resistant to this same strain. Left unsettled, however, was the issue of whether colonization of monkeys by *H. pylori* strains of human origin is possible or is blocked by lack of appropriate host specificity determinants.

(iii) Trial 3. Two of the monkeys that had resisted infection in the second trial (KJ2 and 8V5), plus the monkey that had sustained a transient infection (9A5), were again challenged with *H. pylori*, this time using a mixture of two other human strains (H_3 and H_4). *H. pylori* was recovered from all three animals at 1 week. Histology and culture tests indicated that two of the animals had spontaneously cleared their infections within a month of inoculation, whereas the other one remained colonized for the duration of the study. By RAPD fingerprinting, all 62 isolates from both the transiently and persistently infected animals matched input strain H_4 (Fig. 2). This trial establishes that an *H. pylori* strain of human origin can colonize

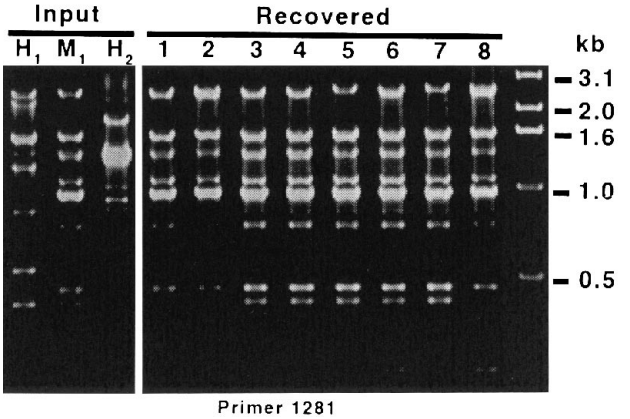

FIG. 1. RAPD fingerprinting with primer 1281 of representative isolates recovered after inoculation of monkey 8RC with a mixture of two human strains, H_1 (=92-26) and H_2 (=88-23), and one monkey strain (M_1). Data showing that all eight recovered isolates matched the monkey input strain M_1 also were obtained with primers 1254 and 1283.

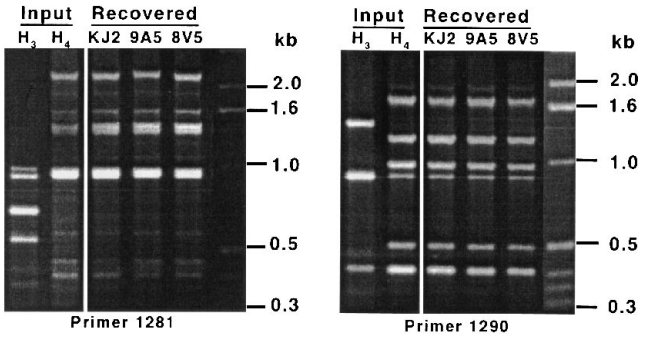

FIG. 2. RAPD fingerprinting of representative isolates recovered after inoculation of monkeys KJ2, 9A5, and 8V5 with a mixture of two human strains (H_3 and H_4). All recovered isolates matched strain H_4, which was obtained from a patient with a gastric ulcer.

rhesus monkeys either persistently or transiently and can infect animals resistant to an *H. pylori* strain of monkey origin that is enzootic in their colony.

Histological findings. The two animals with persistent infection (8RC in trial 2; KJ2 in trial 3) had normal gastric mucosae just prior to infection but developed chronic-active antral gastritis within a month of inoculation. The gastritis increased progressively to grade >2 by 1.5 months in monkey 8RC and by 8 months in monkey KJ2 and then persisted for the duration of observations (9 to 12 months). In addition, each monkey developed marked atrophy of the previously normal stomach mucosae and microerosions and loss of mucus from superficial epithelial cells beginning 6 to 8 months after inoculation (Fig. 3 and 4). Interestingly, each animal also had developed transient gastritis in the earlier trial that had not led to their becoming colonized (Fig. 4). In animals with either no colonization or only transient colonization, a gastritis score of >2 either was not observed or lasted only 1 to 3 months (Fig. 5 and data not shown).

Serological immune responses. The two animals with long-term infection (8RC and KJ2) exhibited increased anti-*H. pylori* plasma IgG, beginning 2.5 to 3.5 months postinoculation. The levels peaked at 4 to 5 months and then declined, but remained greater than the cutoff for positivity for the remaining 2 to 6 months of the study (Fig. 4). In contrast, the two transiently colonized monkeys (8V5 and 9A5) exhibited rapid increases in levels of anti-*H. pylori* IgG within 1 week of inoculation and then gradual decreases following elimination of the bacteria (Fig. 5). The four monkeys that were not colonized in trial 1 or 2 (7MG, 8R1, 7N1, and E0E) did not exhibit any consistent change in anti-*H. pylori* IgG levels (Table 2 and Fig. 5).

DISCUSSION

The experiments presented here (i) establish conditions for experimental infection of rhesus monkeys by *H. pylori*; (ii) help develop monkeys as human-like infection models; (iii) exploit mixtures of bacterial strains that are readily distinguished by DNA fingerprinting to assess whether a given host can be colonized; and (iv) lead to three major conclusions that are particularly relevant to human infection.

First, individuals differ in susceptibility to particular *H. pylori* strains. This conclusion emerges from the finding that two of six monkeys tested in trial 2 were susceptible, and four were resistant, to an *H. pylori* strain that had been circulating in their colony. This large fraction of animals apparently resistant to *H. pylori* infection may be related to the fact that these animals had been selected as being *H. pylori* free despite several years in group housing with infected animals. The spontaneous cure of infection in some colonized animals, but not others, in trials 2 and 3 also indicates diversity among individual hosts in factors important for maintaining a given strain.

Second, *H. pylori* strains differ in the ability to grow in different hosts. This conclusion is based on findings in trial 3 that three animals that had been resistant to a monkey strain were colonized by a human isolate, H_4.

Third, *H. pylori* infection can be of short duration, as demonstrated by the observation that two monkeys that seemed to be well colonized by *H. pylori* when examined 1 to 7 weeks after

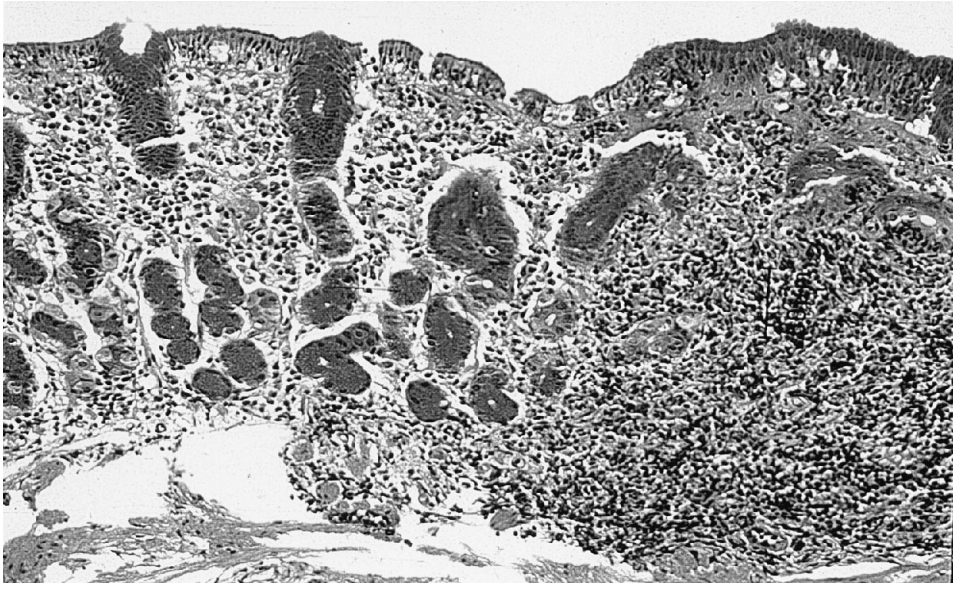

FIG. 3. Illustration of chronic-active (grade 3) antral gastritis in animal 8RC at 10 months postinoculation, demonstrating marked atrophy, microerosions, and loss of mucus (H&E stain). *H. pylori* was isolated from this animal throughout a 12-month period.

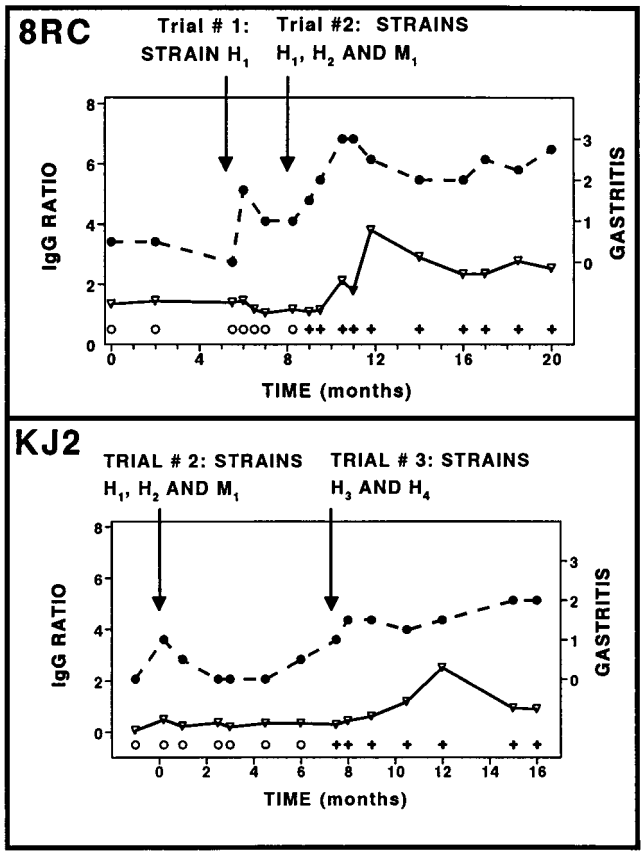

FIG. 4. Time course of antral gastritis score (●) and plasma IgG ratio (▽) before and after successful colonization. Absence of infection is indicated by ○, and positivity for infection by culture and/or histology is illustrated by +. Animal 8RC, also illustrated in Fig. 1 and 3, became persistently infected in trial 2, and plasma IgG and gastritis score increased after a 3-month delay. Animal KJ2 was not included in trial 1, did not become infected in trial 2, but was colonized by one of the strains isolated from a patient with gastric ulcer used in trial 3, as illustrated in Fig. 2.

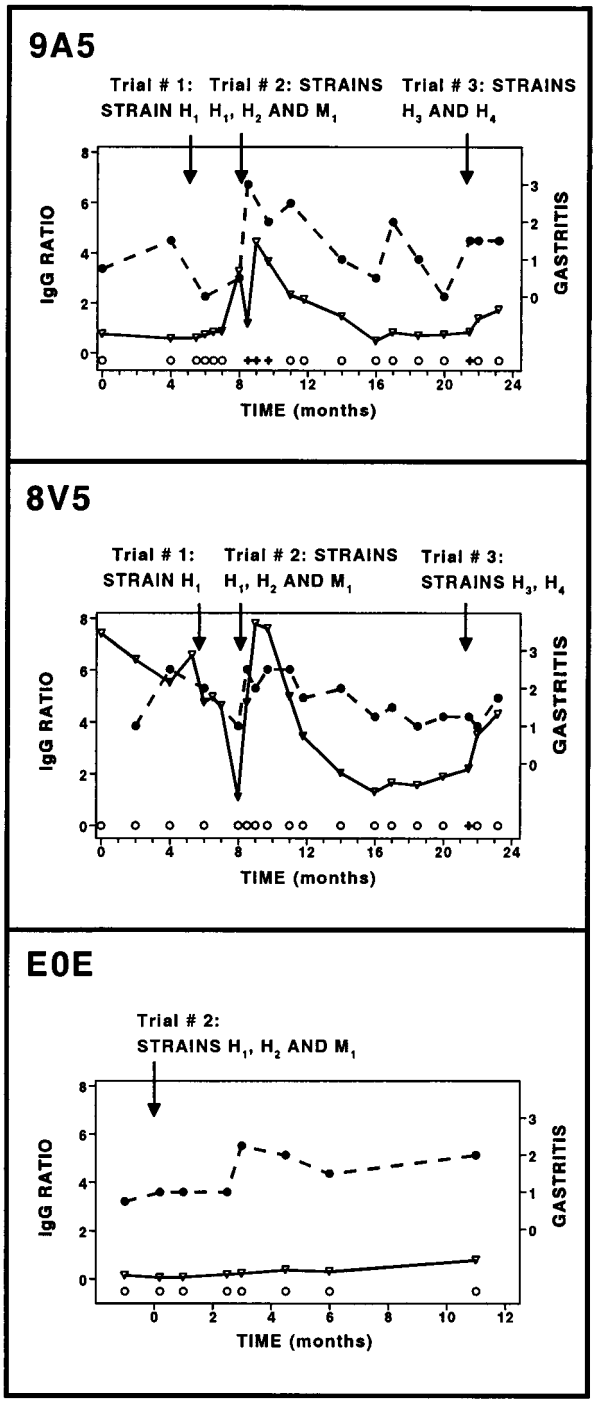

FIG. 5. Time course of antral gastritis score (●) and plasma IgG ratio (▽) before and after transient or unsuccessful colonization. Absence and presence of infection are indicated by ○ and +, respectively, as in Fig. 4. Animal 9A5 was not colonized in trial 1 but developed transient infection during trials 2 and 3. Animal 8V5 was not colonized during trials 1 and 2 but developed transient infection during trial 3. Animal E0E was included only in trial 2 and did not become colonized by *H. pylori*.

inoculation had eliminated these bacteria by 3 months postinoculation. This observation contrasts with the traditional view that *H. pylori* infections persist for years, which is based primarily on patients with infections that were established long before diagnosis and that the bacteria, therefore, must have been well adapted to their hosts. There are, however, at least three reports of studies of human *H. pylori* infection in which the patients also were monitored closely from the time of exposure, and two of these infections were also transient (27, 34), whereas the third persisted until it was cured with antibiotic therapy (35). Interestingly, transient infection might also underlie the reported "spontaneous" clearance of *H. pylori* in a patient receiving placebo (46). Further evidence comes from recent studies of infants in Peru and in The Gambia, using urea breath tests, which demonstrated dramatic fluctuations over time in gastric urease levels (high, low, and then high again) in many children in the study group (22, 44). If the urea breath test is an accurate indicator of *H. pylori* infection in this population, as it is in adults in other societies (40), these results would support the hypothesis that cycles of transient infection are common in humans when first exposed to *H. pylori*.

Cases of spontaneous clearance have been ascribed to the patient immune response, which may reflect host genotype or physiology (27, 34). Consistent with this view are correlations of particular genotypes in humans and apparent resistance to infection (25). This explanation is also compatible with our finding that the three animals that were only transiently infected had rapid immune responses, whereas the two animals

(8RC and KJ2) that became persistently infected did not develop peak IgG levels until 3 to 4 months after inoculation (Fig. 4). This delay in antibody response is similar to that in the only reported human case of persistent infection after experimental inoculation (35). Thus, this rapid response may reflect protective immunity (although the serum antibodies measured here only approximate the more relevant gastric mucosal immune responses). Since gastritis appeared more rapidly after inoculation in animals that did not develop long-term infection than in those that did, we propose that a strong early tissue response can help the host eventually repel the infecting strain. Taken together, these considerations support the prospect for development of effective anti-*H. pylori* vaccines (26).

We propose that there is a link between the phenomenon of transient infection and differences among strains in the ability to colonize a given host and in susceptibility of potential hosts. Differences in susceptibility in a potential host population are, of course, a familiar theme in infectious disease. In the case of *H. pylori*, it probably reflects genetically determined traits, in combination with environmental cofactors and age of acquisition. In this context, the extensive genetic diversity among *H. pylori* strains may be adaptive, reflecting continuous selection for variants that are increasingly fit for a given infected host, even as it may change over time. A strain with such a history might often be imperfectly suited to the next host to ingest it and, in the extreme, either fail to colonize that host or result in only transient infection, much as has been seen here.

In conclusion, our results show that rhesus monkeys can be experimentally infected with strains of *H. pylori* of human or of monkey origin and suggest that monkeys may serve as particularly important models for studies of gastric physiology and immune responses. Our analysis also shows that rhesus monkeys vary in susceptibility to infection by a given *H. pylori* strain, that *H. pylori* strains also vary in traits that may be important in colonization of individual hosts, and that some infections are very short-lived. We propose that much of the remarkable diversity among *H. pylori* strains reflects selection by the different genotypes, physiologic states, and immunologic experience of billions of different human hosts. Thus, future inoculation of rhesus monkeys with sets of strains that are genetically well characterized, and DNA level analysis of recovered strains as here, should provide new insights into the mechanisms by which *H. pylori* causes gastroduodenal disease and into host-specific adaptation of the pathogen during years of chronic infection.

ACKNOWLEDGMENTS

This work was supported by NIH grants AI38166, DK48029, and DK50837 and American Cancer Society grant VM-121. Engin T. Incecik is enrolled at the Universität Regensburg, Regensburg, Germany, and was supported by the Deutscher Akademischer Austauschdienst exchange in molecular biology between Washington University and the Universität Regensburg.

We thank Natalia S. Akopyants for initial strain typing; E. Susan Drazek and Richard I. Walker (MicroCarb, Inc.) for growing strains for the present experiments; and N. S. Akopyants, E. S. Drazek, and R. I. Walker for many stimulating discussions.

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Editor: B. I. Eisenstein