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Therapeutic Intragastric Vaccination against *Helicobacter pylori* in Mice Eradicates an Otherwise Chronic Infection and Confers Protection against Reinfection

PAOLO GHIARA,[1]* MICHELA ROSSI,[1] MARTA MARCHETTI,[1] ANNALISA DI TOMMASO,[1] CARLA VINDIGNI,[2] FABRIZIO CIAMPOLINI,[3] ANTONELLO COVACCI,[1] JOHN L. TELFORD,[1] MARIA TERESA DE MAGISTRIS,[1] MARIAGRAZIA PIZZA,[1] RINO RAPPUOLI,[1] AND GIUSEPPE DEL GIUDICE[1]

IRIS, Chiron Vaccines Immunobiological Research Institute Siena,[1] and Institute of Pathologic Anatomy and Histology[2] and Department of Environmental Biology,[3] University of Siena, 53100 Siena, Italy

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Chronic infection of the gastroduodenal mucosae by the gram-negative spiral bacterium *Helicobacter pylori* is responsible for chronic active gastritis, peptic ulcers, and gastric cancers such as adenocarcinoma and low-grade gastric B-cell lymphoma. The success of eradication by antibiotic therapy is being rapidly hampered by the increasing occurrence of antibiotic-resistant strains. An attractive alternative approach to combat this infection is represented by the therapeutic use of vaccines. In the present work, we have exploited the mouse model of persistent infection by mouse-adapted *H. pylori* strains that we have developed to assess the feasibility of the therapeutic use of vaccines against infection. We report that an otherwise chronic *H. pylori* infection in mice can be successfully eradicated by intragastric vaccination with *H. pylori* antigens such as recombinant VacA and CagA, which were administered together with a genetically detoxified mutant of the heat-labile enterotoxin of *Escherichia coli* (referred to as LTK63), in which the serine in position 63 was replaced by a lysine. Moreover, we show that therapeutic vaccination confers efficacious protection against reinfection. These results represent strong evidence of the feasibility of therapeutic use of VacA- or CagA-based vaccine formulations against *H. pylori* infection in an animal model and give substantial preclinical support to the application of this kind of approach in human clinical trials.

Infection of the human gastroduodenal mucosae by *Helicobacter pylori* is associated with chronic gastritis, peptic ulcers, and gastric malignancies such as adenocarcinoma and low-grade B-cell lymphoma (2, 34, 35). Eradication of chronic *H. pylori* infection of human mucosae with antibiotic therapy markedly alters the natural history of chronic gastritis, peptic ulcers, nonulcer dyspepsia, and low-grade B-cell gastric lymphoma and reduces the rate of relapse of clinical symptoms (44). Problems such as poor patient compliance and increasing occurrence of strains of *H. pylori* resistant to some of the antimicrobials used (i.e., clarithromycin and metronidazole) represent major drawbacks that may limit the efficacy of chemotherapeutic intervention on a large scale (20, 25, 45). Moreover recent evidence suggests that infections cured with antimicrobial agents in adults do not induce immunity against reinfection (39).

Experiments with animal models suggest that mucosal vaccination with either whole-cell preparations or purified antigens plus appropriate adjuvants may prevent infection (5, 16, 17, 24, 27, 28, 31). Development of efficacious strategies that exploit the therapeutic use of vaccines against an ongoing infection represents an attractive therapeutic alternative to the use of antibiotics. Successful eradication of *Helicobacter felis* infection from mice has been reported previously by others using either lysates (13) or the *H. pylori* recombinant urease (6). More recently Cuenca et al. have also reported a low but significant rate of eradication of natural *Helicobacter mustelae* infection in ferrets by therapeutic vaccination with *H. pylori* urease (10).

We (7, 8, 47) and others (3, 15, 46) have shown that severe symptomatic gastric diseases are associated with gastric colonization by a subset of *H. pylori* strains (called type I strains) expressing a potent vacuolating cytotoxin (VacA), which is cytopathic in vitro to various epithelial cells (22, 37) and causes gastric mucosal damage in vivo to mice (19, 42), and that its toxicity is epidemiologically associated with peptic ulcer (1, 9). Type I strains also bear a 40-kb pathogenicity island (PAI) that codes for several disease-associated virulence factors, including the immunodominant antigen CagA (4, 7, 8, 43).

We have recently developed a mouse model of colonization by *H. pylori* (27). This model, which is at variance with previously described animal models that use *Helicobacter* species that do not express neither VacA or CagA, allows assessment of the potential of these molecules as vaccine candidates. Since no formal evidence has yet been reported regarding the feasibility of the therapeutic use of *H. pylori* antigens as vaccines against infection with *H. pylori*, with the mouse model of persistent *H. pylori* infection we have assessed the potential use of antigen formulations that we have already shown to be effective in preventive vaccination as therapeutic vaccines (27, 28).

We report here for the first time, with an animal model of *H. pylori* infection, that an otherwise chronic infection in mice by an *H. pylori* type I strain can be successfully eradicated by intragastric therapeutic vaccination with *H. pylori* antigens (recombinant VacA or CagA) together with a nontoxic mucosal adjuvant. Moreover, we show that cured mice are resistant to reinfection.

MATERIALS AND METHODS

Preparation of *H. pylori* antigens and LTK63 mutant. *H. pylori* SPM326 was used to prepare the bacterial lysate as previously described (27). Briefly, bacteria cultured as described below were harvested from the plates and suspended in sterile saline; the suspension mixture was then pulse sonicated (Branson Ultra-

* Corresponding author. Mailing address: Department of Immunology, IRIS, Chiron Vaccines, Via Fiorentina 1, 53100 Siena, Italy. Phone: 39-577-243316. Fax: 39-577-243564. E-mail: Ghiara@iris02.biocine.it.

sonic, Danbury, Conn.) for 3 min at 50% capacity while kept in an ice bath. The protein concentrations of bacterial lysate were determined with the Bradford reagent (Bio-Rad). Aliquots of the lysate were then snap-frozen in liquid nitrogen and kept at −80°C until used. This lysate was used for both immunizations and enzyme-linked immunosorbent assay (ELISA) plates. Whole recombinant VacA molecule (TOX100) was expressed in *Escherichia coli* and purified by affinity chromatography as described elsewhere (26). We have reported previously (26) that this recombinant form of VacA is nontoxic. Recombinant native CagA antigen was expressed in *E. coli* and purified as described elsewhere (8, 28). The degrees of purity of these *H. pylori* antigens were assessed by Coomassie blue staining of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels, and the identity of the purified molecule was confirmed by Western blot analysis with specific antibodies. The LTK63 mutant of *E. coli* labile toxin (LT; bearing an Ser→Lys substitution in position 63) was obtained by site-directed mutagenesis and was purified from the periplasm of a recombinant *E. coli* strain as previously described (36).

Mouse-adapted *H. pylori* strains. The bacterium used for infection was the type I strain SPM326. This strain was adapted to colonize the gastric mucosae of the mouse stomach by repeated in vivo passages consisting of several isolation-reinoculation cycles as previously described (27). Bacteria, cultured in microaerobic conditions as described below, were harvested immediately before inoculations to mice with a sterile cotton swab and resuspended in a small volume of sterile saline. After determination of the optical density at 530 nm, bacterial cell suspensions were diluted to allow intragastric inoculation of 10^9 CFU/0.1 ml to each mouse and were kept on ice until use.

Treatments of mice. Mice were infected as previously described, with slight modifications (27). Briefly, 5-week-old CD1/SPF male mice (Charles River, Calco, Italy) at days 0, 2, and 4, after neutralization of gastric acidity with Na bicarbonate, were challenged intragastrically with *H. pylori* SPM326. The mice were sacrificed at various times after the bacterial inoculation. Colonization of gastric mucosae was assessed as previously described (27, 28). In brief, the mucosal surface of each stomach was gently streaked onto the surface of a separate Columbia agar plate containing 10% horse blood plus amphotericin B (50 μg/ml), vancomicin (100 μg/ml), polymixin B (3.3 μg/ml), bacitracin (200 μg/ml), and nalidixic acid (10.7 μg/ml) (30). The plates were then incubated under microaerobic conditions with the Anaerojar system with the Campygen atmosphere generating system (Oxoid, Basingstoke, United Kingdom) for 4 to 7 days. Growing *H. pylori* colonies were identified by morphology and confirmed by positive rapid urease reaction. Mice were considered not infected when no *H. pylori* colony on the plate on which the stomach was cultured was detected. For the assessment of the efficacy of therapeutic vaccination, mice infected for 6 weeks were treated intragastrically with three weekly doses of saline alone (control) or saline containing either (i) 10 μg of LTK63 mutant alone or (ii) 100 μg of *H. pylori* antigens (TOX100, CagA, or lysate) plus 10 μg of LTK63. At the times indicated, the mice were sacrificed by cervical dislocation, their stomachs were collected, and colonization was determined by culture as described. Data are expressed as percentages of protection. The significance of the differences observed between groups was assessed by Fisher's exact test.

Histology and SEM. Specimens of gastric tissue from infected or noninfected (control) mice were fixed in 4% buffered formalin and processed for histological staining with hematoxylin and eosin as described previously (19). For scanning electron microscopy (SEM) analysis, pieces of infected stomachs were fixed in 3% buffered glutaraldehyde for 20 min. After dehydration in ethanol, the samples were mounted on stubs, coated with gold, and examined at 7.2 kV under a Philips 501 scanning electron microscope (Philips, Eindhoven, The Netherlands).

Titration of *H. pylori* antigen-specific antibodies by ELISA. Briefly, detection of serum antibody titers against *H. pylori* was achieved by ELISAs on 96-well plates coated with whole SPM326 lysate (10 μg/well) or with purified native VacA (42) (0.2 μg/well). Coated wells were blocked with PBS containing 2.7% polyvinylpyrrolidone (Sigma, St. Louis, Mo.). Sera from infected mice were pooled and tested as follows. Serial dilutions of serum samples were incubated at 37°C for 2 h and then washed with PBS. Antigen-specific immunoglobulin G (IgG) titers were determined with affinity-purified, γ-chain-specific, biotin-conjugated rabbit polyclonal antibody (Sigma). Serum *H. pylori* antigen-specific IgA, IgG1, and IgG2a titers were determined with affinity-purified α- or γ1- or γ2a-chain-specific, biotin-conjugated rabbit polyclonal antibodies (Southern Biotechnology Association, Birmingham, Ala.) for 2 h at 37°C. Horseradish peroxidase-conjugated streptavidin (Sigma) was then added to the washed plates, and the plates were incubated at 37°C for 2 h. Antigen-bound antibodies were revealed by the addition of *o*-phenylenediamine as a substrate (Sigma). Antibody titers were determined as previously described (12).

RESULTS

Persistence of *H. pylori* infection in mice. We have previously reported that *H. pylori* strains freshly isolated from patient biopsies and adapted to mouse gastric mucosae by several in vivo passages can establish colonization in mice (27). Infection by type I strains causes epithelial damage and inflammation that becomes evident after 8 to 12 weeks of infection.

TABLE 1. Persistence of infection by the type I strain SPM326[a]

Time (wk)	No. of mice		% of infection
	Group total	Infected	
1	19	17	89
2	25	23	92
4	27	27	100
6	40	39	98
8	20	19	95
16	18	17	94
34	11	11	100
52	18	16	89

[a] CD1/SPF mice (n = 178) were infected with three intragastric challenges of 10^9 CFU of *H. pylori* type I strain SPM326 as described in Materials and Methods. At different time intervals, groups of mice were sacrificed and the extent of gastric colonization was assessed.

These findings have been subsequently confirmed by others (23, 38). In order to assess the persistence of *H. pylori* infection and to monitor the evolution of the disease induced by the infection, a large group of mice was inoculated intragastrically with the mouse-adapted type I strain SPM326 and monitored for as long as 1 year. At various intervals, groups of mice were sacrificed and gastric colonization was assessed by culture. As shown in Table 1, inoculation of mice with an adapted strain of *H. pylori* results in the colonization of their gastric mucosae that is persistent for as long as 1 year. The numbers of colonies that were recovered from the stomachs were also stable over the examined period of time and varied between 10^3 and 2×10^4 in the majority of the infected mice (not shown). The pathological changes induced by infection were followed by histological examination. Figure 1 shows pictures of representative histological fields of gastric mucosae of uninfected control mice (Fig. 1A) and of mice sacrificed at different times after the onset of infection (Fig. 1B through F). At 8 weeks of infection, in agreement with our previously published data (27), gastric lesions consisted mainly of focal epithelial damage. Fields showing epithelial erosive-reparative lesions with polymorphonuclear leukocytes infiltrating the lesions and the surrounding mucosae were present in most of the infected mice (Fig. 1B). However, these lesions were less frequent in the mice observed at subsequent times. Figure 1G is an SEM micrograph obtained from a mouse infected for 8 weeks showing an *H. pylori* cell adhering to the gastric epithelial surface. At 16 and 34 weeks of infection, the most frequently observed lesions consisted of inflammatory cells (mainly mononuclear cells) infiltrating the lamina propria in the superficial layers of the mucosae (chronic superficial gastritis) (Fig. 1C and D). Polymorphonuclear cells were also found to be mainly associated with the surface epithelial layer (Fig. 1D). At 34 weeks of infection, small lymphoid aggregates were also found in the laminae propriae at the base of the glands (Fig. 1E). In mice infected for 1 year or longer, the main histopathological finding consisted in a follicular gastritis, with large lymphoid follicles present in the mucosae as well as in the submucosa (Fig. 1F).

The serum antibody response during infection was monitored by ELISA with *H. pylori* antigens. Figure 2A and C shows that with an *H. pylori* whole-cell sonicate or purified native VacA as solid-phase antigens in the ELISA, IgG and IgA titers were already detectable at 4 to 8 weeks after the onset of infection and increased slowly with time. The IgG response was further assessed by determining the patterns of IgG1 and IgG2a isotypes during infection. Figure 2B and C shows that

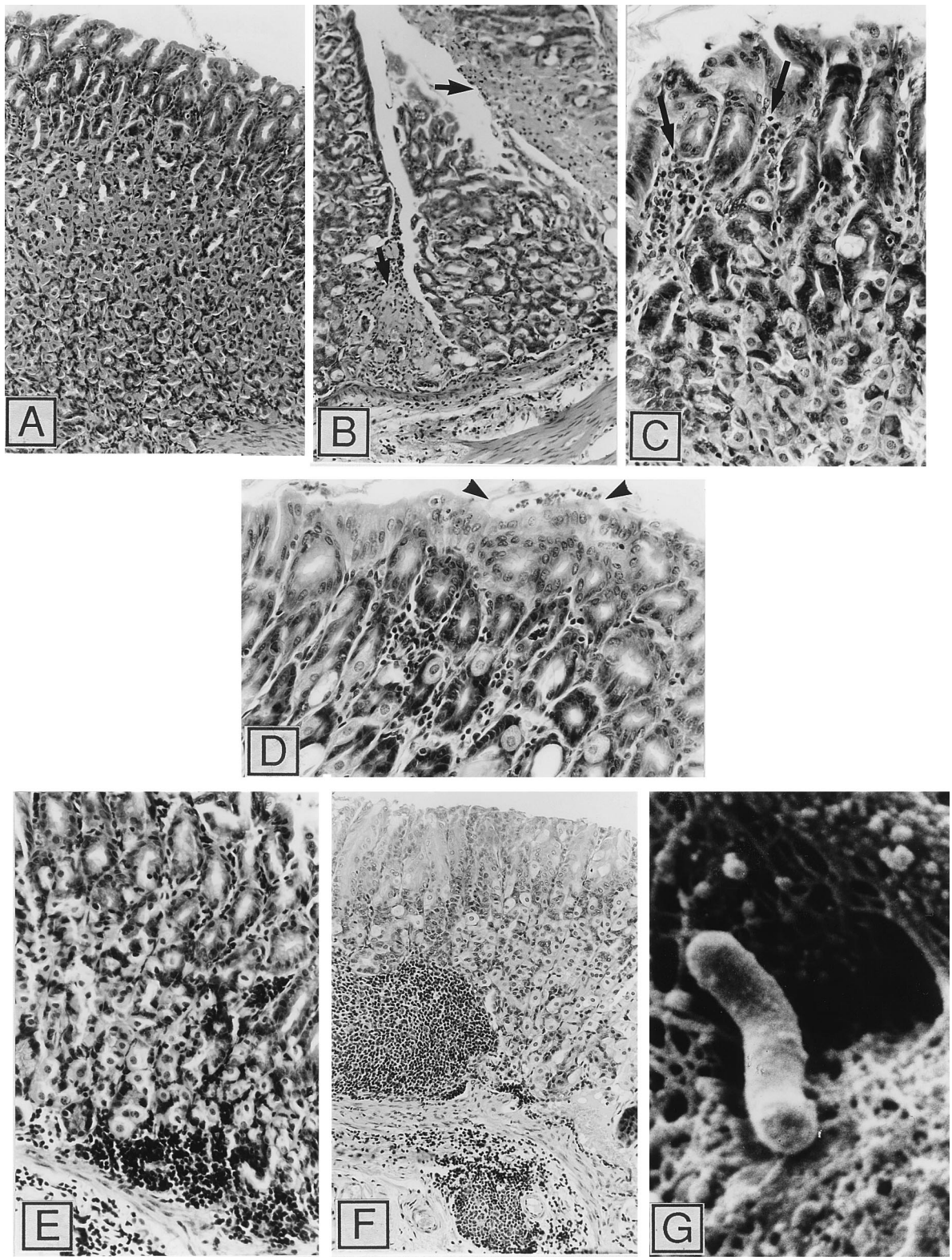

FIG. 1. Histopathology of infected mice. (A) Oxyntic mucosae of a control mouse (original magnification, ×200; (B) oxyntic mucosae of a mouse infected for 2 months showing erosive-reparative lesions (arrows) (original magnification, ×200; (C and D [original magnification, ×400]) superficial chronic inflammatory cells in the laminae propriae (arrows) of mice infected for 4 months (neutrophil polymorphs are also present [arrowheads in panel D] on the superficial epithelium and are associated with epithelial degeneration) (E) lymphocytic aggregate at the base of the glands in the oxyntic mucosae of a mouse infected for 8 months; (F) follicular gastritis with large lymphoid follicles present in the oxyntic mucosae and in the submucosae of a mouse infected for 1 year (original magnification, ×200; (G) SEM of the gastric epithelial surface of a mouse infected for 2 months showing an *H. pylori* cell adhering to the gastric cells (original magnification, ×15,000).

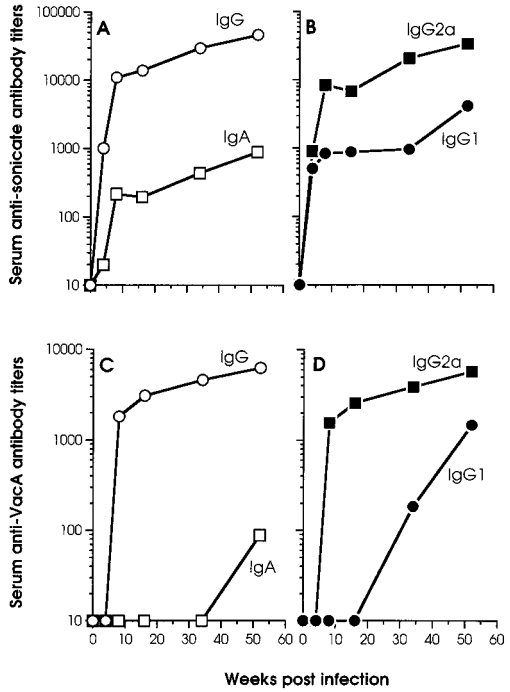

FIG. 2. Serum antibody response during infection. Anti-*H. pylori* antigen-specific titers in pooled sera from CD1/SPF mice during infection with *H. pylori* type I strain SPM326. (A and B) IgG and IgA titers and IgG1 and IgG2a serum titers, respectively, against whole-cell sonicate. (C and D) IgG and IgA titers and IgG1 and IgG2a serum titers against VacA, respectively.

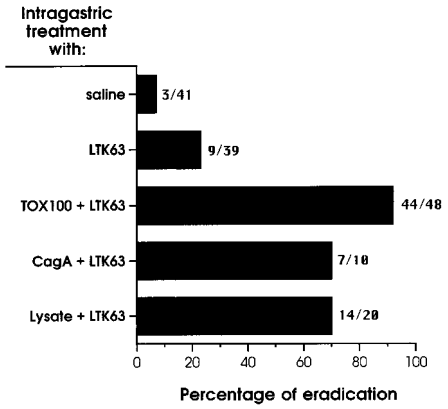

FIG. 3. Therapeutic intragastric vaccination; cumulative results of five experiments. Mice that had been infected 6 weeks previously with *H. pylori* type I strain SPM326 received three weekly intragastric treatments with the indicated vaccine formulations. Control groups received either saline alone or saline containing the adjuvant LTK63 alone. The statistical significance of the observed differences was assessed by Fisher's exact test as described in Materials and Methods. The percentages of protected (noninfected) mice in groups receiving TOX100 or lysate plus LTK63 were significantly different ($P \leq 0.0001$) compared to groups receiving saline alone or LTK63. Treatment with CagA plus LTK63 also induced highly significant eradication compared to treatment with saline alone ($P \leq 0.0001$) or with LTK63 alone ($P \leq 0.02$). The level of protection achieved by administration of lysate plus LTK63 was also significantly different from that obtained with either saline alone ($P \leq 0.0001$) or LTK63 alone ($P \leq 0.002$).

IgG2a was the prevailing IgG isotype against both the whole-cell sonicate and VacA.

Therapeutic vaccination eradicates infection. To assess the feasibility of therapeutic vaccination, mice were infected with *H. pylori* SPM326 and, 6 weeks later, received three weekly doses of 100 μg of the recombinant VacA (TOX100), CagA, or a whole SPM326 lysate together with 10 μg of the mucosal adjuvant LTK63. Control groups received either saline alone or saline containing LTK63. All mice were sacrificed 1 weeks after the last intragastric treatment, and gastric colonization by *H. pylori* was assessed by culture. Figure 3 shows the cumulative results of five experiments. Highly significant levels of eradication were achieved when infected mice were therapeutically vaccinated with TOX100, or CagA, or whole-cell lysates administered intragastrically together with LTK63 (for all vaccine groups, $P \leq 0.0001$ versus saline-treated mice), while treatment with adjuvant alone induced a slight but not statistically significant eradication. To better assess the effect of therapeutic vaccination, the numbers of colonies recovered from infected mice that received the treatments were determined. Table 2 shows the cumulative results of two experiments in which the mice were therapeutically vaccinated with TOX100 or with CagA. In the small proportion of mice in which infection resulted after the therapeutic vaccination, a marked decrease in the relative amount of *H. pylori* colonies recoverable from their stomachs was observed, compared to that for mice which received control treatments (i.e., LTK63 alone or saline).

In order to assess whether the observed eradication persisted with time, 80 mice were infected with *H. pylori*. Six weeks later, half of them were treated intragastrically with three weekly doses of 100 μg of TOX100 together with the adjuvant LTK63. The remaining 40 mice received LTK63 alone. Groups of 10 mice in the control and in the vaccine groups were sacrificed at different times after the intragastric treatment, and gastric colonization by *H. pylori* was assessed. Figure 4 shows that eradication achieved with intragastric therapeutic vaccination was stable and that as many as 70% of the mice that were treated intragastrically with TOX100 plus the adjuvant LTK63 remained noninfected for at least 3 months after therapeutic vaccination.

Therapeutic vaccination confers protection against reinfection. Then, we asked whether mice in which infection had been eradicated by therapeutic vaccination were protected from reinfection. Mice were infected with strain SPM326 as described above and, 6 weeks later, received three weekly intragastric treatments with LTK63 alone (control) or with LTK63 together with TOX100 (vaccine). A group of mice from both the control and vaccine groups were sacrificed 1 week after the last intragastric treatment, and gastric colonization by *H. pylori* was assessed by culture. Figure 5A shows that as expected from data shown above, 80% of the mice that received the thera-

TABLE 2. Number of *H. pylori* colonies recovered in plates from infected-vaccinated mice[a]

Treatment	No. of mice		No. of mice with the following no. of colonies:		
	Total	Infected	1–100	101–1,000	>1,000
Saline	18	17		2	15
LTK63	8	6			6
TOX100 + LTK63 or CagA + LTK63	28	5	3	1	1

[a] Mice were infected with an *H. pylori* type I strain and, 6 weeks later, received three weekly doses of intragastric therapeutic vaccination with TOX100 or CagA plus the adjuvant LTK63. Control groups consisted of mice that received either saline alone or the LTK63 adjuvant alone. One week after the final treatment, the mice were sacrificed and the extent of gastric infection by *H. pylori* was assessed as described. *H. pylori* colonies were detected by visual inspection of the plates and were counted. Data pooled from two separate experiments are shown.

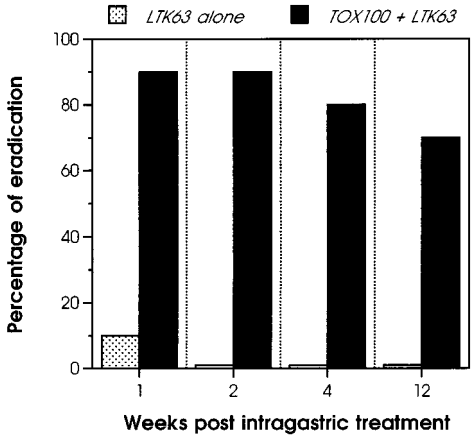

FIG. 4. Persistence of *H. pylori* eradication induced by therapeutic vaccination. A group of 80 mice was infected with strain SPM326. Six weeks later, half of them received three weekly intragastric treatments with TOX100 plus LTK63 (vaccine), while the remaining mice received LTK63 alone (control). At 1, 2, 4, and 12 weeks after the last treatment, 10 mice per group were sacrificed to assess colonization as described in Materials and Methods. Eradication in mice receiving the vaccine was significantly higher than that in mice receiving control treatment at 1 week ($P \leq 0.003$), 2 weeks ($P \leq 0.0001$), 4 weeks ($P \leq 0.002$), and 12 weeks ($P \leq 0.01$) after intragastric treatments. On the other hand, no statistically significant difference was observed among groups of mice that received TOX100 plus LTK63 and that were sacrificed at different times after treatment.

peutic intragastric vaccination were cured. At the same time, the remaining mice from both the control and vaccine groups were rechallenged intragastrically with three doses of 10^9 CFU of strain SPM326 per mouse over a week. Two months after the rechallenge, the mice were sacrificed to assess gastric infection by *H. pylori*. Figure 5B shows that as many as 70% of the mice that were cured by intragastric therapeutic vaccination with TOX100 plus LTK63 were resistant to a subsequent challenge with *H. pylori*. These results clearly show that therapeutic vaccination not only successfully eradicates an otherwise chronic infection but also prevents a subsequent reinfection with *H. pylori* in the majority of vaccinated animals.

DISCUSSION

The results shown in this paper demonstrate that an otherwise chronic gastric infection of *H. pylori* in mice can be successfully cured with oral administration of either an *H. pylori* sonicate or purified recombinant nontoxic VacA or CagA plus a nontoxic mutant of LT as an adjuvant. It has been reported that oral administration of either sonicates or purified recombinant urease B subunit plus cholera toxin (CT) eradicates infection in mice previously infected with *H. felis* (6). Use of the mouse *H. felis* model has also allowed establishment of the first evidence of the feasibility of preventive vaccination against a chronic gastric infection by a *Helicobacter* species (5). However, *H. felis* is not a human pathogen and, more importantly, does not express some *H. pylori* pathogenic determinants (i.e., VacA and antigens encoded by the *cag* PAI, including CagA) that are thought to be involved in human disease (47). In fact, the gastric mucosae of symptomatic infected patients most frequently harbor type I *H. pylori* strains that bear the *cag* PAI, which contains several genes involved in pathogenesis, including the immunodominant disease marker product of the gene *cagA* (4, 8). These type I strains also secrete the toxin VacA, which is hypothesized to be involved in the genesis of ulcerative lesions (1, 9, 19, 22, 37, 41, 42). Infection with type I strains is also strongly associated with the increased risk of occurrence of gastric adenocarcinoma (3) and low-grade gastric B-cell lymphoma (15). The use of the mouse model of *H. pylori* infection that we have developed has allowed us to support the concept that type I strains have enhanced virulence compared to Type II strains that do not bear the *cag* PAI and do not express cytotoxic activity (4, 27). Therefore, this animal model of infection is a powerful tool to study the pathogenesis of infection and to develop vaccination strategies with, as vaccine candidates, molecules such as VacA and CagA, which are expressed only by type I *H. pylori* strains, play a major role in human disease, and cannot be tested in the *H. felis* model.

In the present paper, we have shown that the type I strain SPM326 can establish a chronic infection in mice that is stable for at least 1 year. The infection elicits evident gastric lesions, i.e., epithelial erosions and infiltration of inflammatory cells in the laminae propriae of the gastric mucosae. Evident chronic gastritis has also been reported for long-term infection of mice with *H. felis* (33); however, in that model, no evidence of overt mucosal erosions has been shown. We have shown here that long-term infection of mice by *H. pylori* also elicits the appearance of lymphoid follicles in the mucosae, a type of lesion that is also frequently present in chronically infected humans and that in some patients precedes the emergence of low-grade gastric B-cell lymphoma (35).

Our results show that infection of CD1 mice with *H. pylori* induces serum IgG and IgA antibodies which persist for the duration of infection (Table 1 and Fig. 2). It is interesting to note that although both IgG1 and IgG2a isotypes are produced, IgG2a antibodies represent the prevailing isotype. This was even more evident when titers of VacA-specific IgG were determined. This result suggests that chronic *H. pylori* infection in CD1 mice is associated with a predominant activation of

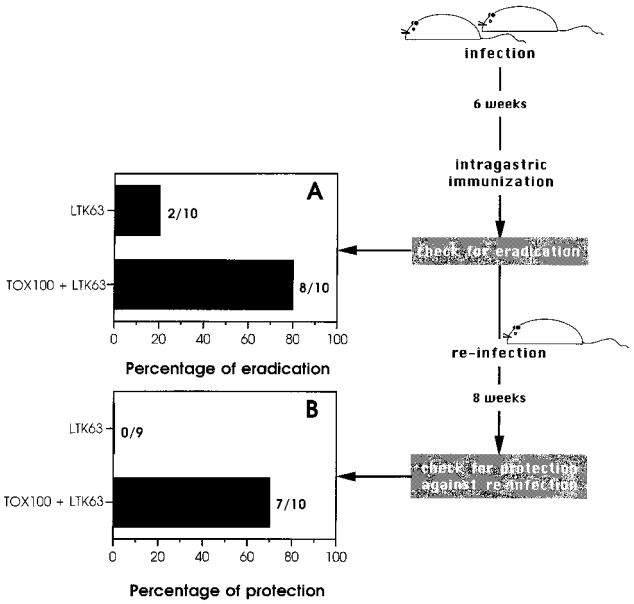

FIG. 5. Therapeutic vaccination confers protection against reinfection. A group of 80 mice were infected with strain SPM326. Six weeks later, half of the mice received three weekly therapeutic vaccinations with TOX100 plus LTK63 (vaccine), while the remaining mice received LTK63 alone (control). (A) At 1 week after the last treatment, 10 mice in the control group and 10 mice in the vaccine groups were sacrificed to assess the level of eradication achieved. (B) The remaining mice were reinfected as described, and 8 weeks later protection was assessed. Both therapeutic eradication and protection against rechallenge obtained in mice that received vaccine treatment were statistically significant ($P \leq 0.03$ and ≤ 0.01, respectively) compared to groups that received control treatment.

Th1-type cell populations. This is in full agreement with previous results obtained by others with a mouse model of infection with *H. felis* (32) and also for humans with *H. pylori*-associated peptic disease (11).

Therapeutic vaccination of *H. pylori*-infected mice with TOX100 or CagA together with the adjuvant LTK63 induces a high level of eradication that persists for at least 12 weeks after the vaccination. The fate of gastric pathology following therapeutic vaccination could not be optimally assessed after eradication, since in most of the experiments the mice were vaccinated at 6 weeks of infection, i.e., when the gastric pathology was not yet fully evident in all mice. Furthermore, in these experiments the mice were sacrificed 1 week after the end of the treatment. However, in the experiment in which the persistence of eradication was assessed, vaccinated mice that were sacrificed at times later than 1 week did not develop gastric pathology and, in particular, no signs of epithelial erosions could be found compared to infected LTK63-treated controls (not shown).

In the experiments reported in the present paper, we did not observe any major change in the serum antibody responses of infected mice after they were immunized with *H. pylori* antigens in the presence of LTK63 as a mucosal adjuvant (not shown). These data are in agreement with those reported with the mouse model of infection with *H. felis*, in which therapeutic immunization with recombinant *H. pylori* urease was studied (6). Considering that as discussed above, chronic *H. pylori* infection seems to be associated with a preferential activation of Th1 cell subpopulations, it is tempting to speculate that immunization procedures leading to a substantial activation of Th0- or Th2-type cell populations at the local (mucosal) and/or systemic level may favor eradication of an otherwise chronic infection with *H. pylori*. This hypothesis is sustained by recent experimental evidence obtained both in vivo (17) and in vitro (21). In our experimental procedure, this may have been mediated by the mucosal adjuvant, the nontoxic LT mutant LTK63. Previous work (14, 29, 40, 48) has clearly shown that nontoxic mutants of both LT and CT coadministered with antigens at the mucosal level drive preferential activation of Th0- or Th2-type $CD4^+$ cells, depending on the experimental model employed. This hypothesis may explain the finding that approximately 20% of the mice receiving the LTK63 adjuvant alone exhibited a nonspecific eradication of *H. pylori* infection. However, this nonspecific effect was transient, since it was not observed when gastric colonization was assessed more than 1 week after the last intragastric treatment with LTK63.

There is no full agreement on whether previous infection eradicated by antibiotic therapy in infected humans could confer immunity against a subsequent reinfection. Although most of the reinfections in eradicated adult patients seem to be explained by a recurrence of the infection with the same strain due to failure in the antibiotic regimen used, a recent report has shown that a previous state of chronic infection by *H. pylori*, which had been successfully eradicated by antibiotic therapy, did not confer immunity against reinfection (39). It has also been recently reported that ongoing chronic infection does not protect from superinfection with a new strain (18). In agreement with previous data obtained by others using the mouse model of infection with *H. felis* and treatment with recombinant urease (29), the results presented in the present paper show that mice cured by therapeutic vaccination with recombinant VacA and CagA are resistant to a challenge with three doses of 10^9 CFU of *H. pylori*. These data demonstrate that vaccination may play an important role, either alone or in association with currently available antibiotic chemotherapy, in reducing the rate of reinfection and/or recrudescence in previously infected individuals.

Taken together, our results provide strong evidence that an otherwise experimental chronic infection by a type I *H. pylori* strain, which is more frequently isolated from patients with severe diseases, can be successfully cured by therapeutic vaccination with purified recombinant VacA and CagA coadministered with the nontoxic LT mutant LTK63 as a mucosal adjuvant. Previously, this conclusion could be inferred only by evidence obtained from animal models that employed infections with surrogate *Helicobacter* species, such as *H. felis* and *H. mustelae*, that are not human pathogens and, more importantly, do not express the type I *H. pylori*-specific set of pathogenic determinants (i.e., VacA, CagA, and the other factors encoded by the *cag* PAI) that have a pivotal role in the induction of disease in humans. Finally, these data strongly support the concept of developing therapeutic vaccine formulations based on these antigens for safe use in human clinical trials.

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