

Oral Immunization with Recombinant *Lactobacillus acidophilus* Expressing the Adhesin Hp0410 of *Helicobacter pylori* Induces Mucosal and Systemic Immune Responses

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Helicobacter pylori infection is relatively common worldwide and is closely related to gastric mucosa-associated lymphoid tissue (MALT) lymphoma, chronic gastritis, and stomach ulcers. Therefore, a safe and effective method for preventing *H. pylori* infection is urgently needed. Given that developing an effective vaccine against *H. pylori* is one of the best alternatives, *H. pylori* adhesin Hp0410 was expressed in the food-grade bacterium *Lactobacillus acidophilus*. The recombinant live bacterial vaccine was then used to orally vaccinate mice, and the immunoprotective effects of Hp0410-producing strains were investigated. *H. pylori* colonization in the stomach of mice immunized with the recombinant *L. acidophilus* was significantly reduced, in comparison with that in control groups. Furthermore, mucosal secretory IgA antibodies were elicited in the mucosal tissue of mice immunized with the recombinant bacteria, and specific anti-Hp0410 IgG responses were also detected in mouse serum. There was a significant increase in the level of protection against gastric *Helicobacter* infection following a challenge with *H. pylori* Sydney strain 1 (SS1). Our results collectively indicate that adhesin Hp0410 is a promising candidate vaccine antigen, and recombinant *L. acidophilus* expressing Hp0410 is likely to constitute an effective, low-cost, live bacterial vaccine against *H. pylori*.

Helicobacter pylori is a Gram-negative bacterium found in different areas of the stomach, especially in the antrum. Approximately 50% of the world's population is infected by the pathogen and up to 64% of China's population (1, 2). *H. pylori* infections typically commence during childhood and last for life. The infection is transmitted within the family in childhood (3, 4), likely by fecal-oral or oral-oral transmission (5). Moreover, infection is strongly associated with the development of gastric mucosa-associated lymphoid tissue (MALT) lymphoma and gastric cancer (6, 7). In 1994, the World Health Organization classified *Helicobacter pylori* as class I human carcinogen (8). Therefore, prevention of *H. pylori* infection is highly topical and is the subject of intense debate for many researchers.

At present, the treatment for *H. pylori* infection is triple therapy consisting of two antibiotics and a proton pump inhibitor. *H. pylori* can be successfully eradicated in most situations. However, there are some problems with this treatment, such as patient compliance, antibiotic resistance, and high cost, resulting in relapse after a short period in about 15% to 30% of patients (9–12). There is some evidence of the presence of *H. pylori* in the oral cavity, which a recent meta-analysis related to gastric colonization and possible reinfection (13, 14). The presence of *H. pylori* in tonsils is controversial (15–17); if confirmed, it could help further understanding of *H. pylori* transmission and reinfection. Since antibiotic therapy is not ideal and *H. pylori* eradication therapy is designed for treatment after infection rather than prevention, vaccination against *H. pylori* would be the most effective approach.

A protective antigen is an essential part of vaccine construction. To date, many protein molecules expressed by *H. pylori* have been identified as immunogenic, including urease (UreB), cytotoxin-associated antigen (CagA), neutrophil-activating protein A (NapA), *H. pylori* adhesin A (HpaA), vacuolating toxin A (VacA), catalase, and outer membrane protein (Omp) (18, 19). Among these protein antigens, *H. pylori* adhesin A (HpaA) is a flagellar

sheath protein of *H. pylori* and also is one of the bacterium's main adherence factors (20). HpaA can bind to the many kinds of surface receptors of gastric epithelial cells and then do further damage to the gastric mucosa (21). Previous research showed that the *hpaA* gene is harbored by all *H. pylori* strains and is considerably conserved in its nucleotide and amino acid sequences (22). Furthermore, genomic studies show no significant sequence homologies of HpaA with other known proteins (23), and antibodies against HpaA could be found in the sera of almost all *H. pylori*-infected patients (24). Taken together, these data indicate that HpaA can be considered a potential vaccine antigen. *H. pylori* adhesin 0410 (Hp0410) (GenBank accession no. NC_000915.1) is a gene homologue of the *H. pylori* *hpaA* family and is highly conserved, sharing 94% to 95% of its gene sequence with standard *H. pylori* strains, such as J99 and ATCC 26695 (25). It would be a rational choice for a candidate antigen for an *H. pylori* vaccine (26, 27).

It is also important to choose an antigen delivery system. Recently, lactic acid bacteria (LAB) have been widely studied as mucosal surface vaccine delivery vehicles (28). This system has an advantage over traditional vaccines, in that LAB can colonize the respiratory tract, gastrointestinal tract, urinary tract, and other mucosal epithelial cells and induce a strong mucosal immune response (29, 30). To date, several bacterial and viral antigens have

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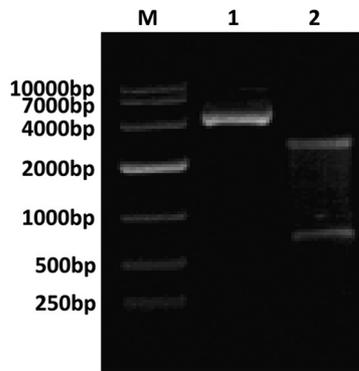


FIG 1 Identification of the plasmid digested with XbaI/HindIII. Lane 1, the recombinant pMG36e-hp0410 plasmid; lane 2, the recombinant pMG36e-hp0410 plasmid digested with XbaI and HindIII, showing the 750-bp Hp0410 and the bone of the plasmid; lane M, DNA marker DL2k.

been produced in *Lactococcus lactis* (31–33), and immunization with these strains elicits immune responses specific to heterologous antigens (34–37). These reports indicate that recombinant *Lactobacillus acidophilus* strains used as oral vaccination strains can prevent gastric infection and allow direct contact between the antigen and the immune system. In addition, if *Lactobacillus acidophilus* is chosen as a vaccine vector, then there is no need to culture pathogens and to purify antigenic components (38). However, no effective safe vaccine against *H. pylori* is currently available for humans.

In this study, we successfully constructed a recombinant *Lactobacillus acidophilus* GIM 1.208/hp0410 strain that expresses the foreign *H. pylori* protein adhesin Hp0410. In order to explore a safe and convenient oral mucosal vaccine candidate against *H. pylori*, we measured and evaluated the effects on immunity in a mouse model with oral administration of pathogenic *H. pylori* Sydney strain 1 (SS1) strains.

MATERIALS AND METHODS

Bacterial strains, vector plasmid, and growth conditions. The *Escherichia coli*-*Lactobacillus* shuttle vector pMG36e plasmid was provided by J. Kok (University of Groningen, the Netherlands). *L. acidophilus* GIM 1.208 was grown at 30°C, without agitation, in MRS medium supplemented with glucose. The pMD19-T plasmid containing the Hp0410 gene of *H. pylori* was constructed by our laboratory. pMG36e-based plasmids were maintained in *L. acidophilus* on glucose-enriched MRS agar plates supplemented with 1.25 µg/ml erythromycin. *Escherichia coli* was grown at 37°C in Luria-Bertani medium with 250 µg/ml erythromycin. The *H. pylori* strain was cultured for 3 to 5 days at 37°C, in a microaerophilic atmosphere (85% N₂, 10% CO₂, and 5% O₂), on Columbia agar supplemented with 8% (vol/vol) defibrinated sheep blood and Dent selective supplement (Oxoid).

Construction of recombinant plasmid pMG36e-hp0410. To insert the Hp0410 gene into the XbaI/HindIII site of the pMG36E plasmid, PCR was performed using pMD19-T-S-hp0410 templates. The Hp0410 gene was amplified using the following primers (XbaI/HindIII sites are underlined): 5'-GAGAAAGCTTCTACTTTTCGTTTTTCATT-3' (forward primer) and 5'-AGCGTCTAGAGATGAAAAAAGGTAGTTTGGC-3' (reverse primer). The amplified Hp0410 (750 bp) was cloned into the XbaI/HindIII site of pMG36e. The recombinant plasmid pMG36eH-hp0410 was sequenced to ensure that the Hp0410 open reading frame was correct. Additionally, the recombinant plasmid pMG36e-hp0410 was digested with the restriction enzymes SalI and HindIII for verification (Fig. 1). Finally, the plasmids pMG36e and pMG36e-hp0410 were transformed

into *L. acidophilus* GIM 1.208 by electroporation, as described previously (39).

Cell lysates and Western blot analysis. Bacterial cell lysates were prepared as described elsewhere (40). Briefly, *L. acidophilus* bacterial cells were suspended in phosphate-buffered saline (PBS), disrupted by sonication, and then incubated at 4°C for 30 min. After centrifugation at 16,000 × g for 20 min, the supernatant and the pellet were obtained as the soluble fraction and the inclusion body fraction of the cell lysates, respectively. Equivalent amounts of cell extracts (15 µg) were subjected to 4 to 12% gradient sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and electrotransferred to an Immobilon-P membrane (Millipore Corp., Bedford, MA). The membrane was probed with a primary antibody and then with a horseradish peroxidase-linked secondary antibody. Detection of proteins was performed using the ECL Western blotting detection system, according to the manufacturer's instructions.

Stability of pMG36e-hp0410 in recombinant *L. acidophilus*. Frozen aliquots of recombinant *L. acidophilus* GIM 1.208 vaccine preparations were diluted (1:100) in MRS medium and grown to an optical density at 600 nm (OD₆₀₀) of 0.8. A 1:100 dilution of this preliminary culture was grown a further 20 times under the same conditions. Both cultures were used as inoculants, at suitable dilutions, for MRS agar plates with or without erythromycin (25 µg/ml). The stability of pMG36e/hp0410 in recombinant *L. acidophilus* GIM 1.208 was evaluated by comparing the numbers of CFU recovered on erythromycin-containing plates versus non-erythromycin-containing plates.

Immunization. Eighty-four specific-pathogen-free (SPF), 6- to 8-week-old, female C57BL/6 mice (weighing 18 to 22 g) were obtained from the Southern Medical University Laboratory Animal Center (Guangzhou, China) and housed in a pathogen-free environment. All experiments involving animals were approved by the Institutional Animal Care and Use Committee of the Southern Medical University. Animals were randomly divided into three groups ($n = 28$ each) and were orally immunized with approximately 1×10^9 CFU of recombinant *L. acidophilus* strain GIM 1.208/pMG36e-hp0410 or strain GIM 1.208/pMG36e or with phosphate-buffered saline (PBS) as a negative control. All mice were immunized on days 1, 2, 3, 7, 10, 14, and 20, seven times in total. No boosting immunization was performed.

One week after the last immunization, 10 randomly selected mice from each group were used to collect blood from the orbital vein and their sera were harvested for evaluation. At 10, 21, and 35 days after the last immunization, six mice from each group were killed by cervical dislocation and their gastrointestinal fluid was harvested. The intestinal tissue was then incised along the longitudinal axis of the intestinal tract and washed with PBS containing protease inhibitors. The resulting fluid was centrifuged at 3,000 × g for 10 min, and the supernatant was collected and stored at -20°C until use.

ELISA for detection of Hp0410-specific serum antibodies. Enzyme-linked immunosorbent assay (ELISA) plates were coated overnight at 4°C with 2 µg/ml recombinant purified Hp0410. Threefold serial dilutions of each serum sample, starting from 1:100, were applied to the plates, and the plates were incubated for 2 h at 37°C. Serum IgG and IgA were detected with peroxidase-labeled goat anti-mouse IgG (1:1,000, 100 µl; Amersham Biosciences) and goat anti-mouse IgA (1:5,000, 100 µl; Sigma), respectively. The reaction was stopped by the addition of 2 M H₂SO₄, and absorbance was measured at 450 nm using an ELISA reader (Bio-Tek EL 9800).

ELISA for detection of Hp0410-specific mucosal IgA. Hp0410-specific IgA and total IgA in the gastric mucosa were quantified by ELISA. Portions of each plate were coated with an anti-mouse IgA monoclonal antibody (Sigma) and Hp0410, respectively. In addition to the diluted samples, a dilution series of purified IgA was applied to each plate to provide a standard curve. After incubation with peroxidase-labeled anti-mouse IgA (1:5,000, 100 µl), the concentrations of Hp0410-specific IgA and total IgA were determined using the standard curve. In order to address the possibility that increased IgA concentrations were the result of a

nonspecific polyclonal response to mucosal stimulation, responses were expressed as the ratio of specific IgA to total IgA.

Survival time of recombinant *Lactobacillus acidophilus* in mice. Twenty SPF, 6-week-old, female C57BL/6 mice were divided into two groups ($n = 10$ each) and were orally immunized with 1×10^9 CFU of recombinant *L. acidophilus* strain GIM 1.208/pMG36e-hp0410 or strain GIM 1.208/pMG36e as a negative control. Then, feces were collected from mice in the two groups, at the same time each day. The Hp0410 gene and the pMG36e plasmid were amplified from fecal genomic DNA using a TIANamp Bacteria DNA kit (Qiagen, Germany) to detect the presence of recombinant *L. acidophilus* GIM 1.208/pMG36e-hp0410 or GIM 1.208/pMG36e in mice. The pMG36e plasmid primers used were 5'-pMG36e (5'-GAGTTATGATTTCTCTGA-3') and 3'-pMG36e (5'-CGTTGGCGA TTGAAAACC-3'), in addition to the Hp0410 gene primers described in "Construction of recombinant plasmid pMG36e-hp0410."

Immunoprotective effect of recombinant *L. acidophilus* expressing adhesin Hp0410. *H. pylori* Sydney strain 1 (SS1) (41) was recovered from storage at -80°C and resuspended in PBS to yield a concentration of 1×10^9 CFU/ml. Twenty-six vaccinated mice (10 immunized with recombinant *L. acidophilus* strain GIM 1.208/pMG36e-hp0410, 10 immunized with strain GIM 1.208/pMG36e, and six given PBS as a control) were given 0.2 ml (2×10^8) *H. pylori* SS1 via the orogastric route (using a feeding tube). Animals were challenged with an equal dose of *H. pylori* SS1 four times, at 24-h intervals. Food and water were given 4 h after the challenge. Four weeks after receiving the challenge, mice were sacrificed by cervical dislocation. Their stomachs were washed twice in sterile 0.9% NaCl, and the gastric antrum from each stomach was assessed for *H. pylori* colonization by testing for urease activity, catalase testing, quantitative culture, and histological analyses of bacterial cultures. First, 50 μl of the succus gastricus was placed in 500 μl of urease reagent. Urease positivity was determined by an increase in pH, as indicated by a color change from yellow to red within 24 h at 37°C . Strongly positive samples became red within 2 h, and negative samples showed no change within 10 h. For the catalase test, 3% H_2O_2 solution was dropped onto a plate containing gastric tissue washing fluid. The formation of bubbles indicated a positive result. For *H. pylori* SS1 quantitative culturing, the stomach samples were weighed, homogenized in thioglycolate medium, serially diluted in PBS, and plated onto Columbia agar plates with antibiotics. Bacterial counts were expressed as CFU per gram of gastric tissue. To histologically assess bacterial colonization, 5- μm -thick sections of the gastric antrum were cut and stained with hematoxylin and eosin (H&E). The degree of inflammation was analyzed on blinded slides by two pathologists, by assessing lymphocyte and monocyte densities and grading the depth of the invasion of gastritis from 0 to 3 (0, no infiltration; 1, mild; 2, moderate; 3, marked). Scores were expressed according to the updated Sydney system (42).

Statistical analysis. Statistical analysis was carried out using SPSS 13.0 computer software, and data were expressed as the mean \pm standard deviation (SD). Differences among the three groups were determined by one-way analysis of variance using the Tukey *post hoc* test. Differences in rates were analyzed by the chi-square test. *P* values of less than 0.05 were considered significant.

RESULTS

Expression of adhesin Hp0410 by recombinant *Lactobacillus acidophilus*. To express the Hp0410 protein of *H. pylori* in the *L. acidophilus* delivery plasmid, the Hp0410 gene was inserted into the *E. coli*-*Lactobacillus* shuttle plasmid pMG36e, which was designated pMG36e-hp0410. The recombinant plasmid was constructed and confirmed by restriction enzyme digestion with XbaI and HindIII (Fig. 1). Two plasmids, i.e., the recombinant plasmid pMG36e/hp0410 and the control plasmid pMG36e, were introduced into *L. acidophilus* GIM 1.208, resulting in strains GIM 1.208/pMG36e-hp0410 and GIM 1.208/pMG36e, respectively. Proteins were extracted and assayed by Western blotting after

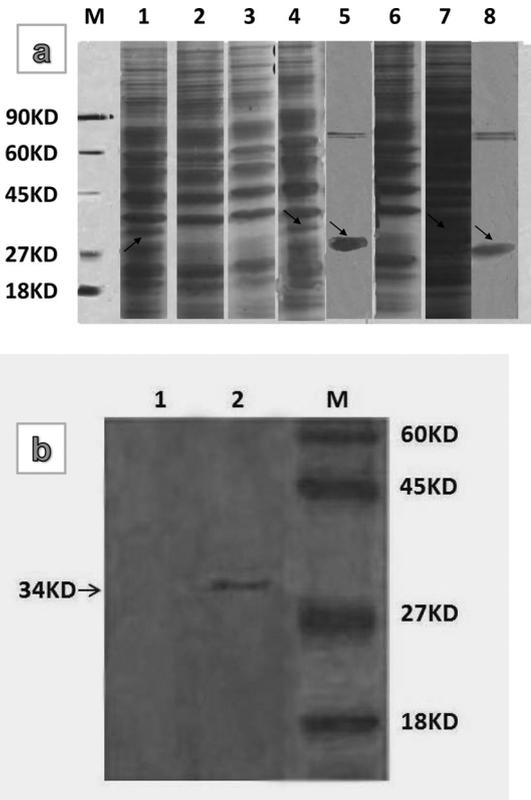


FIG 2 Detection of the Hp0410 protein by SDS-PAGE and Western blotting. (a) SDS-PAGE of recombinant *L. acidophilus* lysate and supernatant. Lane M, markers; lanes 1, 4, and 7, recombinant *L. acidophilus* GIM 1.208 lysate with pMG36e-hp0410; lanes 2, 3, and 6, recombinant *L. acidophilus* GIM 1.208 lysate with pMG36e; lanes 5 and 8, recombinant *L. acidophilus* GIM 1.208 supernatant with pMG36e-hp0410. (b) Western blot of the Hp0410 protein expressed by recombinant *L. acidophilus*. A 34-kDa band representing the Hp0410 protein is visible in lane 2 but not in lane 1. Lane M, molecular mass markers; lane 1, recombinant *L. acidophilus* GIM 1.208 containing pMG36e; lane 2, recombinant *L. acidophilus* GIM 1.208 containing pMG36e-hp0410.

SDS-PAGE (Fig. 2a and b), using polyclonal antiserum to Hp0410. The results indicated that rHp0410 was produced in the supernatant of GIM 1.208/pMG36e-hp0410 and was detected as a 34-kDa band on Western blots. In contrast, no band corresponding to the Hp0410 protein was detected in the supernatant of GIM 1.208 carrying an empty plasmid (Fig. 2b).

Stability of pMG36e-hp0410 in recombinant *Lactobacillus acidophilus*. The stability of pMG36e-hp0410 in recombinant *L. acidophilus* was 97% with erythromycin and 90% without erythromycin (data not shown). We have therefore constructed a stable recombinant *L. acidophilus* vaccine containing pMG36e expression vectors.

Serum anti-Hp0410 antibody responses after mucosal immunization. Groups of mice were immunized orally with recombinant *L. acidophilus* GIM 1.208 expressing Hp0410 protein in the culture supernatant. Control mice were vaccinated in the same way with pMG36e strains of *L. acidophilus* GIM 1.208. One week after the final vaccination with GIM 1.208/pMG36e-hp0410, the levels of Hp0410-specific IgG in the serum were significantly greater than those in the control samples ($P < 0.001$), while Hp0410-specific IgA levels in the serum were lower and did not

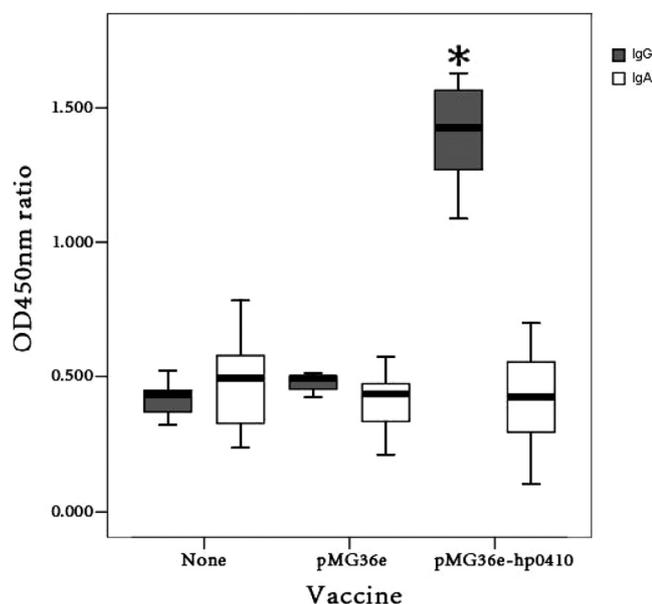


FIG 3 Serum anti-Hp0410 IgG and IgA levels elicited by recombinant *L. acidophilus*. Groups of six mice were immunized orally with recombinant *L. acidophilus* expressing Hp0410 (pMG36e-hp0410) or a control strain (pMG36e). On day 35 after the initial treatment, blood samples were collected and Hp0410-specific serum IgG and IgA titers were measured by ELISA as OD values at 450 nm. Significantly higher levels of Hp0410-specific IgG but not IgA were seen in the pMG36e-hp0410 group but not in the control groups. Bars, mean ratios; error bars, SD. *, $P < 0.001$.

differ significantly from those observed for the vector control strain (pMG36e) or unvaccinated mice (Fig. 3).

Mucosal antibody responses. Significantly elevated Hp0410-specific IgA responses could be detected in fresh intestinal mucus specimens from mice immunized orally with GIM 1.208/pMG36e-hp0410 but not in specimens from mice given the control strain (Fig. 4). On day 21 after oral inoculation, the mean ratios of specific IgA to total IgA detected in the group given pMG36e-hp0410 were about 3 times those in unvaccinated controls ($P < 0.01$). On days 10 and 35, the ratios in the pMG36e-hp0410 group were also significantly higher than those in the control groups ($P < 0.05$). No significant differences could be detected between mice vaccinated with the pMG36e control strain and the unvaccinated control group at any of the time points.

Survival time of recombinant *Lactobacillus acidophilus* in mice. As shown in Table 1, recombinant *L. acidophilus* GIM 1.208/pMG36e-hp0410 was detected in all mice on the first and second days after immunization, through amplification of the pMG36e plasmid and the Hp0410 gene; GIM 1.208/pMG36e was also detected with the pMG36e plasmid. On the third day, the presence of recombinant bacteria could still be detected in the intestines of 70 to 80% of the mice. On the fourth day, however, no recombinant bacteria were detectable in any of the mice. These results indicate that recombinant *L. acidophilus* can survive for at least 72 h in mice, and they suggest that *L. acidophilus* may be a suitable LAB-based delivery vehicle for human vaccines.

Immunoprotective effect of recombinant *Lactobacillus acidophilus*. Three weeks after immunization, mice were challenged with 2×10^8 *H. pylori* SS1. Four weeks later, all of the 10 stomach biopsy specimens from the C57BL/6 mice immunized with live

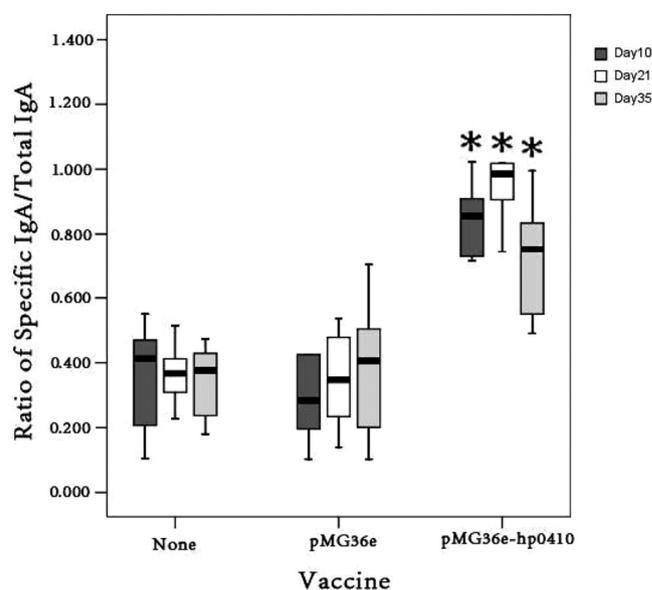


FIG 4 Comparison of the specific IgA/total IgA ratios in the intestinal mucus of mice immunized orally with *L. acidophilus* expressing Hp0410 (pMG36e-hp0410) or the control strain (pMG36e). Intestinal mucus was collected from groups of six mice on days 10, 21, and 35 after the initial treatment. The samples were tested by ELISA for Hp0410-specific and total IgA, and the results are expressed as the ratio of OD values at 450 nm. The ratios were significantly higher in the pMG36e-hp0410 group than in the pMG36e group or unvaccinated controls at all time points but were greatest on day 21. Bars, mean ratios; error bars, SD. *, $P < 0.05$.

vaccine expressing Hp0410 were negative by the urease assay and the catalase test, but all were positive as assessed by colony counts. These 10 immunized mice had a range of *H. pylori* colonization loads, from 10^4 to 10^5 bacteria per gram of stomach tissue. However, a significant difference in the level of protection was seen between the immunized mice and the control groups, which had higher bacterial loads ($P < 0.05$). Similarly, examination of histological sections of gastric tissue showed that, in comparison with the control group, the histopathological changes in the recombinant *L. acidophilus* GIM 1.208/pMG36e-hp0410 group were relatively minor (Fig. 5), being limited to the lamina propria without reaching the serosa and leaving the glands largely intact. This difference was quantified and shown to be statistically significant, and the numbers of inflammatory cells and their exudates were also significantly reduced (Table 2). There was no significant dif-

TABLE 1 Survival times of recombinant *L. acidophilus* GIM 1.208/pMG36e-hp0410 in the mouse gastrointestinal tract after immunization

Treatment group	Origin primer	No. of mice with gene ^a			
		Day 1	Day 2	Day 3	Day 4
<i>L. acidophilus</i> GIM 1.208/pMG36e-hp0410	Hp0410 gene	10	10	8	0
	pMG36e plasmid	10	10	7	0
<i>L. acidophilus</i> GIM 1.208/pMG36e	Hp0410 gene	0	0	0	0
	pMG36e plasmid	10	10	7	0

^a Results are expressed as the number of mice of the total of 10 immunized in which the Hp0410 gene and pMG36e plasmid could be detected in fecal DNA using a TIANamp Bacteria DNA kit.

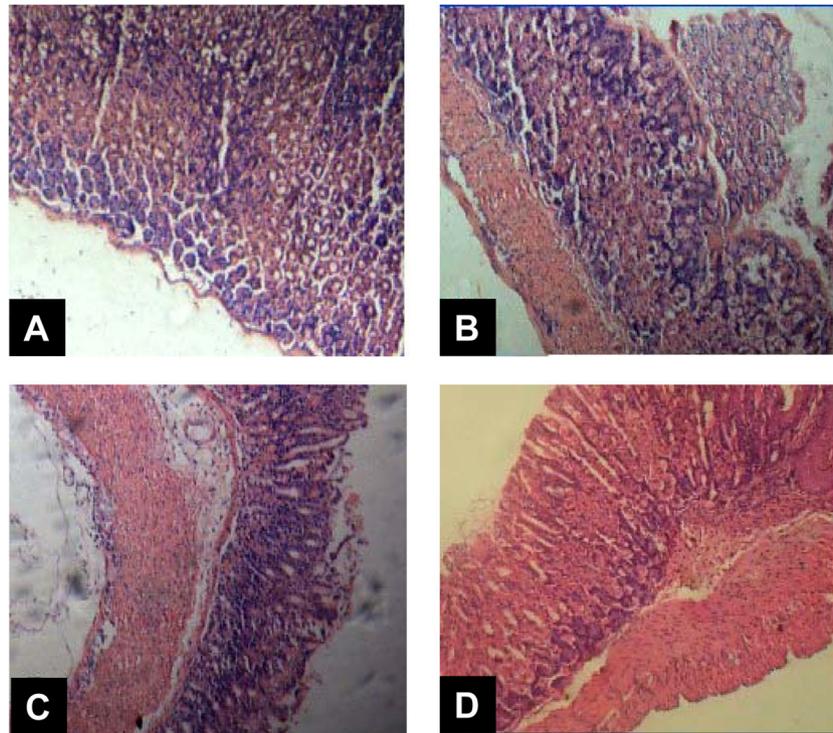


FIG 5 Histological analysis of gastric specimens obtained from mice after challenge with *H. pylori* (H&E stain; magnification, $\times 100$). (A) Gastric tissue from a control mouse vaccinated with PBS, showing severe inflammatory infiltrates in the mucosa and submucosa. (B) Gastric mucosa from a mouse immunized with recombinant *L. acidophilus* GIM 1.208/pMG36e. (C) Gastric mucosa from a mouse immunized with recombinant *L. acidophilus* GIM 1.208/pMG36e-hp0410. (D) Histologically normal gastric mucosa specimen.

ference in histological scores between the two LAB-treated groups.

DISCUSSION

H. pylori is a microorganism that has been linked to diseases such as gastritis and peptic ulcers. It has a high incidence of infection, especially in developing countries, and antibiotic treatment not only is expensive but also is likely to induce antibiotic resistance. Taking these issues into account, vaccination appears to be a promising intervention. To reduce the risk of *H. pylori* infection, it

is important to induce protective immune responses in the mucosal surfaces.

In this study, our main objective was to develop an effective vaccine against *H. pylori* using Hp0410 as the antigen and *L. acidophilus* as the antigen delivery system. Our study showed that mucosal immune responses in the intestine could be elicited by oral vaccination with a *L. acidophilus* vaccine. We tested the effect of immunization with the recombinant *L. acidophilus* strain on protective immunity to *H. pylori* in C57BL/6 mice. Following oral immunization, although all animals were positive by culture (Table 2), the quantity of *H. pylori* in the mice immunized with pMG36e-hp0410 was significantly less than that in the control groups. Furthermore, all 10 of the mice in the experimental group were negative by the catalase and urease tests. Similar results have been obtained with immunization with whole bacterial sonicates and cholera toxin (43) or urease subunit B (44). Meanwhile, examination of histological sections of gastric tissue showed that the density of *H. pylori* in the gastric mucosa in the recombinant *L. acidophilus* GIM 1.208/pMG36e-hp0410 group was significantly lower than those in the control groups. These findings all suggest that recombinant *L. acidophilus* can significantly ameliorate the inflammatory responses caused by *H. pylori* infection in immunized mice. However, no significant difference in gastric tissue lesions was found between the recombinant *L. acidophilus* GIM 1.208/pMG36e-hp0410 group and the GIM 1.208/pMG36e group. The reason is likely to be that *L. acidophilus* itself may also be able to improve the degree of inflammation caused by *H. pylori* infection. Possible mechanisms for this are as follows: (i) *L. acidophilus* may adhere to and colonize the gastric mucosa in ad-

TABLE 2 Protection of mice from *Helicobacter pylori* SS1 infection following immunization with live recombinant *L. acidophilus*

Vaccine	No. of mice colonized by <i>H. pylori</i> (of 10 mice immunized)		Hp0410 in mouse gastric mucosa (mean \pm SD) (log CFU/g)	Chronic gastric inflammation score ^a (mean \pm SD)
	Urease test	Catalase test		
None ^b	10	10	6.02 \pm 0.01	2.22 \pm 0.21
pMG36e ^c	10	10	6.28 \pm 0.60	1.49 \pm 0.64
pMG36e-hp0410 ^d	0	0	4.55 \pm 0.17 ^{e,f}	1.21 \pm 0.17 ^e

^a Histopathological findings for infected mice were assessed in H&E-stained gastric mucosal sections. Gastric inflammation was graded on a scale of 0 to 3 for each group.

^b Orally immunized with PBS.

^c Orally immunized with recombinant *L. acidophilus* containing pMG36e.

^d Orally immunized with recombinant *L. acidophilus* containing pMG36e-hp0410.

^e $P < 0.05$ versus control group.

^f $P < 0.05$ versus pMG36e group.

vance and thus partially inhibit adhesion and colonization by *H. pylori* (45), and (ii) *L. acidophilus* itself as a LAB may stimulate the gastric mucosa to produce nonspecific secretory IgA (sIgA) antibodies, thereby enhancing mucosal immune responses (46–48).

Compared with traditional vaccine vectors, LAB have several advantages, including being safer and nontoxic, being able to adhere to and colonize the respiratory tract, digestive tract, and other mucosal epithelial cell layers, and functioning to express and to secrete heterologous proteins and to induce strong mucosal immune responses (29, 49). To date, several exogenous proteins have been expressed in LAB. For example, the expression of ScFv, a fragment of the anti-streptococcal adhesion molecule antibody single-chain variable region in *Lactobacillus* strain ATCC 393, has been reported by Krüger et al. (50). Gu et al. (44) showed that the urease UreB of *H. pylori* was expressed in *L. lactis* MG1363. Recombinant lactic acid bacteria, used to immunize mice through the oral and nasal routes, can induce humoral and cellular immune responses in the gastrointestinal mucosa, resulting in the secretion of sIgA antibodies (33, 44, 51) that can protect the mice from a related infection. *L. acidophilus*, used in this study as an oral vaccine antigen delivery vector, is safe, acid-resistant, and bile-tolerant and can survive in the gastrointestinal tract for more than 72 h. Compared with other lactic acid bacteria, more-prolonged gastrointestinal mucosal immune responses can be stimulated with *L. acidophilus* (52), resulting in the production of specific sIgA antibodies, which are necessary to achieve the goal of preventing *H. pylori* infection (44). Oral administration is also more convenient as a safe and effective vaccination pathway for some special groups, especially children.

Preliminary studies have used bioinformatic analysis and phage display to obtain the three B-cell epitopes of Hp0410, which all show a high degree of antigenicity (53). By using purified Hp0410 to screen serum samples from gastric ulcer patients and normal healthy controls, they showed that the body produces high titers of anti-Hp0410 antibody, with positive rates of 94.1% and 74.4%, respectively, consistent with the *H. pylori* detection rates in gastric ulcer patients and normal healthy controls *in vivo* (54). The Hp0410 monoclonal antibody specifically prevented the adhesion of *H. pylori* to the stomach mucosa of gastric cancer patients as well as gastritis patients (25). Hp0410 does not participate in inflammatory responses, as its function is only to mediate adhesion (22).

In summary, recombinant *L. acidophilus* GIM 1.208 expressing the adhesin Hp0410 was successfully constructed, and the recombinant *L. acidophilus* GIM 1.208 induced the secretion of high levels of mucosal sIgA antibody. This vaccine was effective in giving some immunoprotection against gastric *H. pylori* infection in mice. No safe vaccine against *H. pylori* is currently available for humans, so further development of existing food-grade expression systems is necessary.

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