

Oral Vaccination Against *Helicobacter pylori* with Recombinant Cholera Toxin B-Subunit

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ABSTRACT

Background. The innocuous pure recombinant cholera toxin B-subunit (rCTB) is very attractive as a strong adjuvant for host immunization, but little is known about rCTB's gastric mucosal immunoadjuvanticity against *Helicobacter pylori*. The immunoadjuvanticity of rCTB against *H. pylori* was tested.

Material and methods. Mice were immunized with sonicated *H. pylori* and rCTB orally or intranasally and sacrificed on day 42 after immunization. Passive cutaneous anaphylaxis (PCA) test was performed to evaluate IgE-mediated anaphylaxis with serum from mice to which *H. pylori*-antigen with rCTB had been administered. Immunoglobulin titer specific to *H. pylori* in serum, lavation of the gastrointestinal tracts and feces were examined. Gastritis in vaccinated mice after a challenge was assessed with the scoring defined from grading of gastric inflammation. *H. pylori* proliferation after immunization was investigated by

counting colony forming units (CFU) per gram of stomach tissue.

Results. PCA test exhibited no reactions against the serum from mice immunized with *H. pylori*-antigen with rCTB administered orally and intranasally. Oral and nasal coadministrations of rCTB significantly raised systemic and mucosal immunities against *H. pylori* and suppressed proliferation of *H. pylori* in gastric mucosa. The score of gastritis in mice immunized orally was significantly higher than that of mice immunized nasally due to postimmunization gastritis. Only oral administration of rCTB suppressed *H. pylori* proliferation as compared with intranasal administration and without rCTB. **Conclusions.** The present study indicated that rCTB has systemic and mucosal immunoadjuvanticities against *H. pylori* and that oral vaccination with rCTB might additively support antibiotic eradication.

Keywords. Oral vaccination, rCTB, *Helicobacter pylori*.

Helicobacter pylori, which chronically infects gastric mucosa, is well known as the major gastroduodenal pathogen of peptic ulcer disease, mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric cancer [1–4]. In 90% of *H. pylori*-infected patients treated with antibiotics and strong acid suppressor drug such as proton pump inhibitors, eradication is successful [5]. However, failure of *H. pylori* eradication treatment has recently increased due to the proliferation of antibiotic-resistant strains [5] and the inhibitory effects of activated metabolic

enzymes, such as CYP2C19, on proton pump inhibitors [6]. Thus, simple and safe vaccination against *H. pylori* is valuable and important for prophylactic or therapeutic treatment, especially for large-scale prevention of *H. pylori* infection.

Mucosal vaccination has received increasing attention with respect to the anti-infectious effects of protective immune responses at the mucosa and subsequent anti-inflammatory effects [7]. To promote mucosal immunization, it has become clear that coadministration of an appropriate adjuvant is necessary to enhance the immunogenicity of vaccine antigens [8,9]. We have recently reported that pure recombinant cholera toxin B-subunit (rCTB) is effective and attractive for stimulating and promoting mucosal immunization as an adjuvant [9–13].

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Oral and nasal mucosal immunizations have the potential to stimulate both mucosal and systemic immunities through the common mucosal immune system, which allows protective immune responses induced at one mucosal site to be expressed at other sites [14]. Intranasal administration of rCTB adjuvant together with vaccine antigen may lead to a valuable strategy for prevention of infectious diseases due to promoted immunization [13]. On the other hand, oral vaccination with other adjuvants also induced systemic and mucosal immunizations effectively [15–17]. However, the systemic and mucosal immune responses leading to suppression of *H. pylori* proliferation with administration of rCTB remain to be elucidated. The present study examines the systemic and gastric mucosal immunoadjuvant activities of pure rCTB against *H. pylori* when administered orally or intranasally.

Materials and methods

Bacterial Strain and Culture

H. pylori Sydney strain 1 (SS1) was kindly provided by Professor Adrian Lee (School of Microbiology and Immunology, University of New South Wales, Australia). This strain was grown in Brucella Broth (BBL; Cockeysville, MD) containing 3% fetal bovine serum with gentle shaking at 37 °C for 72 hours under micro-aerobic conditions with gas generator envelopes (Campy Pak Plus™ and Gas Pak™; BBL; Cockeysville, MD) in gas jars. After cultivation for 2 days, the live bacteria were collected and centrifuged at 5000 × g for 15 minutes and washed three times with phosphate-buffered saline (PBS), pH 7.4.

Preparation of *H. pylori*-antigen and rCTB

Live bacteria (10⁹ bacteria/mL) were incubated in 10 mL of PBS containing 0.5% glutaraldehyde at 4 °C overnight and were subsequently annihilated. The killed bacteria were washed three times and re-suspended in PBS at a final concentration of 10⁹ bacteria/mL, and ethylenediaminetetraacetic acid (EDTA) and phenylmethylsulfonyl fluoride (PMSF) were also added at final concentrations of 5 mmol/L and 1 mmol/L, respectively, to inhibit the activities of proteases. Ten milliliters of suspension including the killed bacteria was crushed with an ultrasonicator (INSONA-

TOR Model 200, KUBOTA, Tokyo) at 2–4 °C for 30 minutes and used as *H. pylori*-antigen.

Expression vector of recombinant cholera toxin B-subunit (CTB) was constructed as previously described [12,18]. The rCTB gene was inserted into the plasmid with the promoter domain and the signal peptide sequence of the major extracellular protein to obtain pNU212-CTB.

Bacillus brevis transfected with pNU212-CTB was cultured at 30 °C for 5 days and pNU212-CTB was purified from the culture supernatant. After ammonium sulfate precipitation of the supernatant, affinity chromatography on a D-galactose-divinylsulfone-agarose column [18], which is a receptor analog for GM1 ganglioside, was performed. The rCTB preparation was eluted with a D-galactose gradient. The purity of the rCTB preparation was electrophoretically confirmed.

Passive Cutaneous Anaphylaxis (PCA) Test

Serum IgE antibodies specific to *H. pylori* were determined by passive cutaneous anaphylaxis (PCA) tests with Sprague–Dawley rats, adding partial modifications of the previous method [9]. The serum of mice immunized with *H. pylori*-antigen with or without rCTB or aluminum, under an oral, a nasal, or a subcutaneous administration was titrated in serial 2-fold dilutions in PBS. The rats were injected intradermally with 0.1 mL of each diluted (×40 to 160) serum into shaved dorsal skin sites. Two days later, 1 mL of PBS containing 200 µL of *H. pylori*-antigen and 2.5 mL of Evans blue was injected intravenously via the dorsal penile vein. After 30 minutes, the rats were sacrificed. The dorsal skin of the rats was removed for measurement of the pigment area. The diameters of blue spots on the internal surface of the skin were measured.

Immunization and Sample Collection

Female C57/BL6 mice (Japan SLC, Inc., Shizuoka, Japan) aged 7 weeks were used in this study. Before immunization, blood samples were taken from the retro-orbital plexus of ether-anesthetized mice. For oral immunization, 500 µL of PBS containing 1 mg of *H. pylori*-antigen in the presence or absence of 100 µg of rCTB was administered through gastric intubation on days 0, 7, 14, and 21. For nasal immunization, 30 µL of PBS containing 50 µg of *H. pylori*-antigen in

the presence or absence of 10 µg of rCTB was administered into the nasal cavity on days 0, 7, 14, and 21. Mice were sacrificed on day 42. Blood, lavage fluids of the stomach and small and large intestines, and feces were collected. Briefly, the digestive tracts were removed and peristaltically washed three times with 1 mL of cold wash solution consisting of 100 µg/mL of soybean trypsin inhibitor (Sigma Chemical Co.), 50 mmol/L EDTA, 1 mmol/L PMSF, 1% bovine serum albumin, and 0.05% NaN₃ in PBS (total wash fluid, 3 mL). The lavage fluid was collected, homogenized twice for 30 seconds at 0 °C and centrifuged at 12,000 × g at 4 °C for 1 hour. Feces were suspended in the wash solution containing 1 mmol/L PMSF at final concentration of 1 mg/mL and centrifuged at 12,000 × g at 4 °C for 1 hour [10].

Enzyme-linked Immunosorbent Assay (ELISA)

Titer of antibodies, IgG and IgA, specific to *H. pylori* in serum and lavage fluids were measured with an enzyme-linked immunosorbent assay (ELISA) adding partial modifications of the previous method [19]. The killed bacterial suspension (10⁷ bacteria/mL) was used as an antigen for ELISA. Fifty microliters of the suspension was added to each well of ELISA plates (PVC Microtiter Flat Bottom Plates™, DYNEX Technologies, Inc., Chantilly, VA) and the plates were then centrifuged at 200 × g for 10 minutes at room temperature. After washing three times with PBS(-), 200 µL of blocking buffer, containing 100 mmol/L glycine, 2% gelatin, and 0.1% bovine serum albumin, was added to each well, and the plates were incubated at 25 °C overnight and washed again three times with PBS(-). Subsequently, 50 µL of each diluted sample solution was added to the wells and incubated for 2 hours at room temperature; serial dilutions of serum (10-fold) or each lavage fluid (4-fold) were made with the dilution buffer containing 0.1% BSA in PBS(-). The plates were then washed with PBS containing 0.05% Tween 20 (PBS-T). Horseradish peroxidase (HRP)-labeled goat antimouse IgG or IgA (Southern Biotechnology Assoc., Birmingham, AL), diluted 8000-fold with 25 mmol/L Tris-buffered saline (TBS), pH 7.4 containing 0.1% BSA, together with 50 µL of the diluted antibody solution was added to each well, and the plates were incubated for 2 hours at room temperature. After washing with PBS-T, the color was developed for 30 minutes with

50 µL of tetramethylbenzidine-H₂O₂ (Bio-Rad Laboratories, Richmond, CA), stopped by adding 50 µL of 1 N H₂SO₄ and the immunoglobulin titer was determined from the absorbance at 450 nm using an automatic microplate reader (MODEL 550™, Bio-Rad Laboratories). Each sample was assayed in duplicate. The mean and standard deviation (SD) of values of six unimmunized control mice were calculated, and antibody-positive cut-off values were set as the mean + 2 SD. The titer of anti-*H. pylori* antibodies was expressed as the highest endpoint dilution of each sample resulting in positive reaction.

Challenge Tests

Female C57/BL6 mice (Japan SLC, Inc., Shizuoka, Japan) aged 4 weeks were used. Five weeks after the last immunization, as described above, mice were infected once with 10⁸ *H. pylori* SSI cells. Four months after infection, mice were sacrificed and *H. pylori* infection and gastritis in the stomach of each mouse were assessed histologically.

Assessment of *H. pylori* Colonization and Histology

Half of the stomach was homogenized in Brucella Broth, and spread on *H. pylori* selective agar plates (Eliken Chemical Co., Tokyo, Japan). After incubation for 5 days, colonies were counted to determine the colony forming units (CFU) per gram of stomach tissue. The residual half of the stomach was fixed in 10% buffered formalin and 5 µm thick paraffin embedded sections were stained with hematoxylin and eosin (H&E) to assess the presence of inflammation. The degree of antral gastritis was scored using a scale of 0–3 points modified from Marshall and Warren's grading of gastric inflammation [20] as follows; briefly, 0: intact mucosal lining and essentially no infiltration of the lamina propria with monocytes, 1: mild increase in mononuclear infiltration localized in the upper half of the mucosa, 2: mononuclear infiltration extending from the surface into the lamina propria resulting in atrophy, 3: marked mononuclear infiltration extending from the surface into the lamina propria and disrupting the structure of the glands and leading to marked atrophy, and/or polymorphonuclear leukocyte infiltration in glands and surface erosions. Each stained specimen was examined by a pathologist unaware of the experiment.

Immunoblot Assays

H. pylori-antigen mixed with a sample buffer [62.5 mmol/L Tris-HCl buffer (pH 6.8) containing 0.1 mol/L sucrose, 3% SDS, 5% 2-mercaptoethanol, and 0.05% bromophenol blue] was applied to a gel after boiling for 5 minutes. Electrophoresis was performed in a 10% gel (READY GELS J™, Bio-Rad Laboratories). Samples containing 20 µg of protein were loaded on the gels, and migrated under a constant current of 150 V. Proteins separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) were transferred onto prewetted polyvinylidene difluoride (PVDF) membranes (Immobilon, Millipore, Bedford, MA) using a semidry blotting apparatus for 45 minutes under a constant current of 180 mA. The membranes were blocked with 5% skimmed milk in PBS(–) for 2 hours at room temperature. The PVDF membranes were blocked with 3% skimmed milk in TBS containing 0.05% Tween 20 (TBS-T) for 1 hour, then incubated with the serum for 1 hour, and washed three times in TBS-T. They were then incubated for 1 hour with HRP-labeled antimouse IgG (Southern Biotechnology Assoc.) diluted 2500-fold with blocking buffer. After washing, the membranes were treated with BM blue POD substrate™ (Boehringer, Mannheim, Germany) at room temperature before exposure to scientific imaging films (Eastman Kodak Co, Rochester, NY).

Statistics

The Mann–Whitney *U*-test was used to compare the mean anti-*H. pylori* antibody titer values of different groups. Results with *p* < .05 were considered statistically significant. Data were expressed as mean ± s.e.m.

Results

Vaccination with rCTB is Safe Treatment Without Anaphylaxis

First, to evaluate whether IgE-mediated anaphylaxis occurred when *H. pylori*-antigen with rCTB is administered for immunization, we performed PCA with Sprague–Dawley rats (Fig. 1). The skin of rats injected subcutaneously with the serum from mice immunized with *H. pylori*-antigen with aluminum as adjuvant exhibited positive reactions at dilutions of 1 : 10, 1 : 20, and

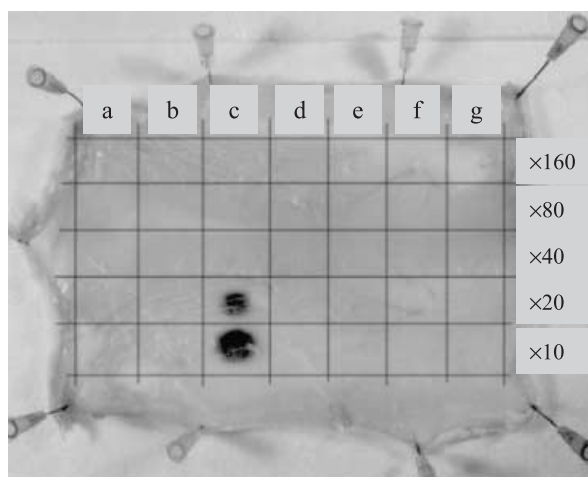


Figure 1 Passive cutaneous anaphylaxis (PCA) reaction of rats treated with mouse sera. The sensitized serum of mice subcutaneously immunized with *Helicobacter pylori* (HP) alone (a), HP and recombinant cholera toxin B-subunit (rCTB) (b), or with HP and aluminum (c), orally immunized with HP alone (d) or with HP and rCTB (e), and nasally immunized with HP alone (f) or with HP plus rCTB (g), were injected intradermally into shaved dorsal skin sites of Sprague–Dawley rats. Two days later, a mixture of PBS (1 mL) containing *H. pylori*-antigen (200 µL) and Evans blue (2.5 mL) was injected into dorsal penile vein of these rats. After 30 minutes, the skin was removed and blue spots on the internal surface of the skin were measured.

1 : 40. No reactions were observed in the skin of the back of rats injected subcutaneously with the serum from mice to which *H. pylori*-antigen with rCTB had been administered orally and intranasally (Table 1).

rCTB Induces Serum Immunoadjuvant Activity

To evaluate adjuvant activity, we assessed the immunization status of mice after oral and intranasal administrations of *H. pylori*-antigen with or without rCTB. Oral administration of *H. pylori*-antigen in the absence of rCTB did not induce production of serum IgG antibody specific to *H. pylori*, whereas, in the presence of rCTB, oral immunization with *H. pylori*-antigen induced significant production of serum IgG antibody specific to *H. pylori* in all mice examined, with about a 5-fold induction of the logarithmic scale as compared to mice without administration of rCTB. Serum IgA antibody specific to *H. pylori* was not induced in all mice with oral administration of *H. pylori*-antigen in the absence of rCTB. In contrast, significantly high levels of serum IgA specific to *H. pylori* were detected in mice immunized with rCTB (Fig. 2).

Table 1 The number of rats positive responses to passive cutaneous anaphylaxis (PCA) test. Rats were treated with the indicated dilutions of serum from mice immunized with *H. pylori*-antigen (HP) with or without recombinant cholera toxin B-subunit (rCTB) or aluminum (Al) by the indicated route. SI stands for immunized subcutaneously, OI for immunized orally, NI for immunized nasally

		Dilution rate				Total no.
		$\leq \times 10$	$\times 20$	$\times 40$	$\times 160$	
SI	HP alone	6	0	0	0	6
	HP + rCTB	6	0	0	0	6
	HP + Al	1	1	2 ^a	0	4
OI	HP alone	6	0	0	0	6
	HP + rCTB	6	0	0	0	6
NI	HP alone	4	0	0	0	4
	HP + rCTB	4	0	0	0	4
Unimmunized		10	0	0	0	10

^aResults with $p < .05$ were considered statistically significant between mice immunized together with aluminum and unimmunized mice.

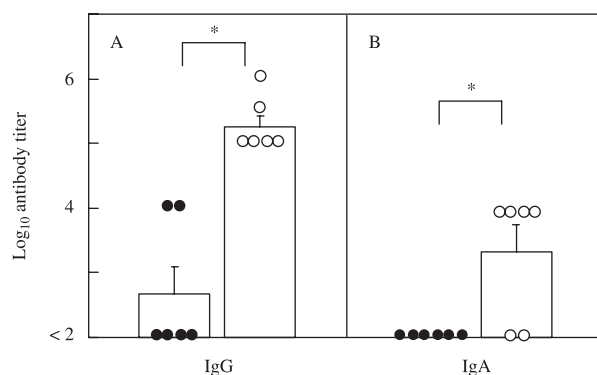


Figure 2 Serum (A) IgG and (B) IgA antibody responses to *Helicobacter pylori*-antigen coadministered orally with or without recombinant cholera toxin B-subunit (rCTB). Mice were immunized with 100 µg of *H. pylori*-antigen together with 10 µg of rCTB (○), or without rCTB (●), on days 0, 7, 14, and 21, and sacrificed on day 42. IgG and IgA antibodies specific to *H. pylori* in serum were measured with an ELISA. Open bars represent the means of antibody titer on a logarithmic scale. * $p < .05$, mice immunized orally with *H. pylori* and rCTB vs. unimmunized mice.

Similarly, intranasal administration of *H. pylori*-antigen together with rCTB induced remarkable serum IgG production specific to *H. pylori*, as well as serum IgA production (Fig. 3).

rCTB Induces Mucosal Immunoadjuvant Activity

To assess the mucosal adjuvant activity of rCTB, we measured the titer of IgA antibody specific to *H. pylori* in the mucosa of stomach and small and large intestines, and in feces from mice immunized, by oral and intranasal administrations, with *H. pylori*-antigen with or without rCTB. Oral immunization with *H. pylori*-antigen with-

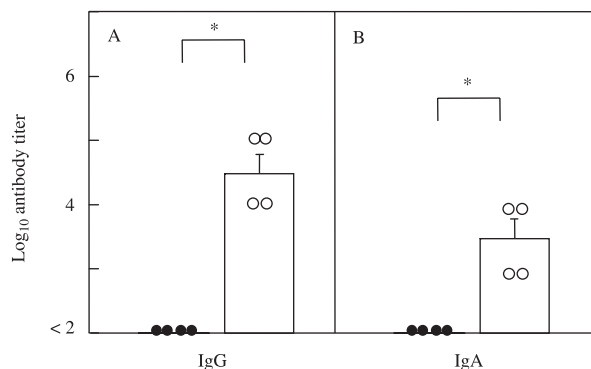


Figure 3 Serum (A) IgG and (B) IgA antibody responses to *Helicobacter pylori*-antigen coadministered intranasally with or without recombinant cholera toxin B-subunit (rCTB). Mice were immunized with 50 µg of *H. pylori*-antigen together with 10 µg of rCTB (○), or without rCTB (●), on days 0, 7, 14, and 21, and sacrificed on day 42. *H. pylori* IgG and IgA antibodies in serum were measured by an ELISA. Open bars represent the means of antibody titer on a logarithmic scale. * $p < .05$, mice immunized intranasally with *H. pylori* and rCTB vs. unimmunized mice.

out rCTB did not induce mucosal IgA antibody specific to *H. pylori* in any gastrointestinal mucosa examined, whereas coadministration of rCTB exhibited significant levels of IgA antibody titer specific to *H. pylori* in all gastrointestinal mucosa. The titer of IgA antibody in the mucosa of stomach and small intestine and in feces increased to logarithmic values of 1, 2, and 0.85, respectively (Fig. 4A). When administered intranasally, significant increase in the titer of IgA antibody specific to *H. pylori* was observed in the mucosa of stomach and small and large intestines from almost all mice examined. The titer of IgA antibody in mucosa of small and large intestines and feces increased logarithmic values of 1.5, 1.5, and 1.75, respectively (Fig. 4B). Our studies also showed that nasal immunization with 10 µg of rCTB and 50 µg of *H. pylori*-antigen induced the mucosal and systemic immune responses to a similar degree to that of oral immunization with 100 µg of rCTB added to 1 mg of *H. pylori*-antigen.

Vaccination with rCTB Induces Gastric Inflammation to Promote Successful Mucosal Immunization

We examined whether *H. pylori*-associated gastritis was really induced when vaccinated mice were infected with *H. pylori*. A mean of 2 points as a defined gastric inflammation score was observed in the gastric mucosa of mice immunized orally with rCTB 4 months after infection.

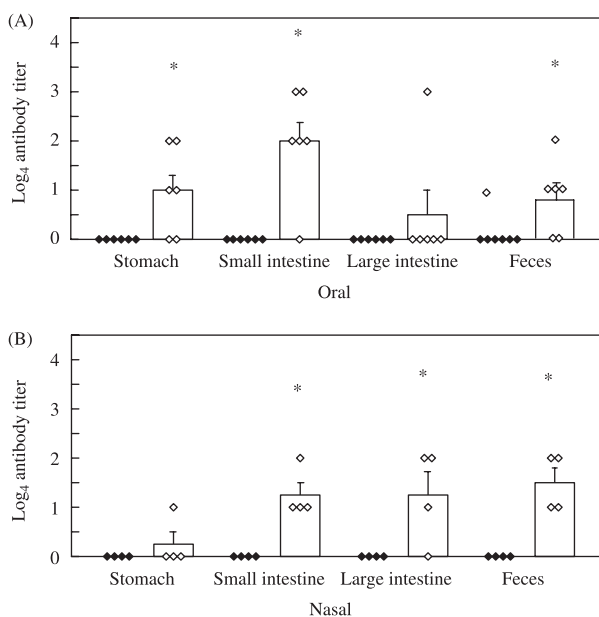


Figure 4 Mucosal IgA antibody responses in the stomach, small and large intestines and feces of mice vaccinated with or without recombinant cholera toxin B-subunit (rCTB) (A) orally and (B) intranasally. Mice were immunized with 50 µg of *H. pylori*-antigen together with 10 µg of rCTB or without rCTB on day 0, 7, 14 and 21, and sacrificed on day 42. Mucosal IgA antibodies in the stomach, small and large intestines and feces of mice vaccinated in the presence (◇) and absence (◆) of rCTB were measured by an ELISA. Open bars represent the means of antibody titer on a logarithmic scale. **p* < .05, mice immunized with *H. pylori* and rCTB vs. unimmunized mice.

In contrast, the mean inflammation scores of unimmunized mice, mice immunized intranasally with or without rCTB, and mice immunized orally without rCTB were 0.4, 0.4, 0.1, and

0.6, respectively. The gastritis score in mice immunized orally with rCTB was significantly higher than that of mice immunized orally without rCTB and that of mice immunized intranasally with or without rCTB (Fig. 5A).

Oral Vaccination with rCTB Blocks *H. pylori* Proliferation

Finally, we tested whether immunization against *H. pylori* really suppressed adherence and infection of *H. pylori*. Based on the colony formation after intubation of live *H. pylori* into the stomach, we assessed the effect of vaccination. The colony forming units (CFUs) of *H. pylori* in stomachs were measured 4 months after infection. CFUs in mice immunized orally with rCTB were significantly fewer than those in the other groups (Fig. 5B).

Discussion

In the present study, evidence indicated that coadministration of pure rCTB induced systemic and gastrointestinal mucosal immunizations, and suppressed proliferation of *H. pylori* in gastric mucosa.

The PCA test showed positive reactions against the serum from mice administered *H. pylori*-antigen with aluminum adjuvant, whereas, no reactions were observed against the serum from mice immunized with *H. pylori*-antigen and rCTB intranasally and orally. These results suggest that oral and intranasal administrations of *H. pylori*-antigen with rCTB are safe and do not cause an anaphylactic reaction.

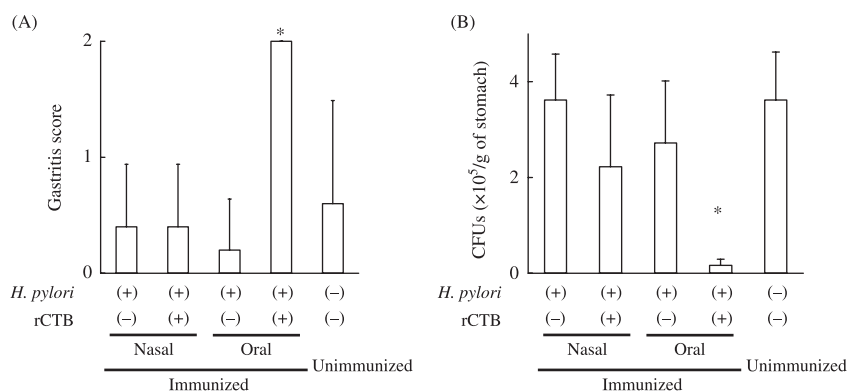


Figure 5 (A) Gastritis scores in immunized and unimmunized mice 4 months after infection. HE-stained gastric sections were scored based on the overall grade of inflammation (see Materials and methods). Open bars represent the means of gastritis scores. **p* < .05, mice immunized orally with *Helicobacter pylori*-antigen and recombinant cholera toxin B-subunit (rCTB) vs. unimmunized mice. (B) Colony forming units (CFUs) of *H. pylori* in the stomach of immunized and unimmunized mice 4 months after infection. Mice were infected once with 10⁸ *H. pylori* 5 weeks after last immunization. Four months after infection, mice were sacrificed and stomachs were removed. Half of the stomach was homogenized for 5 days and colony forming units (CFUs) of *H. pylori* per gram of stomach were counted. Open bars represent the means of CFUs. **p* < .05, mice immunized orally with *H. pylori*-antigen and rCTB vs. unimmunized mice.

Previous reports have noted the valuable additive action of rCTB as an adjuvant for systemic and mucosal immunizations [9,11]. In the present study, we showed that administration of *H. pylori*-antigen with rCTB significantly induced not only high levels of serum IgG and IgA specific to *H. pylori* but also high levels of IgA specific to *H. pylori* in the mucosa of gastrointestinal tracts. These results, which demonstrate that coadministration of rCTB and *H. pylori*-antigen is beneficial for systemic and mucosal immunization, are compatible with these previous reports.

The titers of IgA in the mucosa of stomach induced by *H. pylori*-antigen with rCTB were shown to be higher under oral administration than under intranasal administration, suggesting induction of IgA from the stomach and small intestine directly immunized with rCTB. The titers of IgA in the mucosa of small and large intestines were higher than that in the stomach, since little lymphoid tissue, such as lymphoid follicles and Peyer's patches, is detected in the mucosa of native stomach [21], and lymphoid tissues in the effector mucosal sites of small and large intestines distant from the inductive site of nasal mucosa were activated. This result was compatible with previous reports [22]. Our results therefore suggest that oral administration might be better than intranasal administration for inducing useful mucosal immunization.

Moreover, severe inflammation caused by *H. pylori* infection, significantly high levels of IgA (Fig. 4A), and a bacteriostatic effect on *H. pylori* infection, but not perfect eradication, were detected in the stomach of mice immunized orally with rCTB and *H. pylori*-antigen suggesting that the higher level of mucosal immunization induced by oral administration of rCTB with *H. pylori*-antigen causes considerable infiltration of inflammatory cells, by which a higher level of production of IgA occurred and proliferation of *H. pylori* was suppressed. These phenomena, showing that the temporary development of severe gastric inflammation under *H. pylori* infection after vaccination and inflammation were necessary to protect the host against infection, are defined as postimmunization gastritis [15,17]. This step is thought to play an important role in steady induction of mucosal immunization. Similarly, Goto et al. demonstrated that the high level of IgA observed in the stomach of mice vaccinated orally protects against infection [17].

On the other hand, less gastric inflammation and suppression of *H. pylori* proliferation were observed (Fig. 5A,B). These suggested that antigenicity of the Sydney strain 1 (SS1) *H. pylori* employed against the host may be too weak for induction of high levels of production of IgA or complete eradication of *H. pylori*. From our assessments, mucosal immunization by oral administration may be a better way of suppressing proliferation of *H. pylori*.

In conclusion, we have shown that rCTB is effective as an adjuvant for immunization against *H. pylori* infection since mucosal immunization induced by oral administration of rCTB suppressed proliferation of *H. pylori*. Oral vaccination with rCTB may be a new strategy additively supportive of conventional antibiotic eradication therapy against *H. pylori* infection.

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