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Effect of Oral Immunization with Recombinant Urease on Murine *Helicobacter felis* Gastritis

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The ability of oral immunization to interfere with the establishment of infection with *Helicobacter felis* was examined. Groups of Swiss Webster mice were immunized orally with 250 μg of *Helicobacter pylori* recombinant urease (rUrease) and 10 μg of cholera toxin (CT) adjuvant, 1 mg of *H. felis* sonicate antigens and CT, or phosphate-buffered saline (PBS) and CT. Oral immunization with rUrease resulted in markedly elevated serum immunoglobulin G (IgG), serum IgA, and intestinal IgA antibody responses. Challenge with live *H. felis* further stimulated the urease-specific intestinal IgA and serum IgG and IgA antibody levels in mice previously immunized with rUrease but activated primarily the serum IgG compartment of PBS-treated and *H. felis*-immunized mice. Intestinal IgA and serum IgG and IgA anti-urease antibody responses were highest in rUrease-immunized mice at the termination of the experiment. Mice immunized with rUrease were significantly protected ($P \leq 0.0476$) against infection when challenged with *H. felis* 2 or 6 weeks post-oral immunization in comparison with PBS-treated mice. Whereas *H. felis*-infected mice displayed multifocal gastric mucosal lymphoid follicles consisting of $CD45R^+$ B cells surrounded by clusters of $Thy1.2^+$ T cells, gastric tissue from rUrease-immunized mice contained few $CD45R^+$ B cells and infrequent mucosal follicles. These observations show that oral immunization with rUrease confers protection against *H. felis* infection and suggest that gastric tissue may function as an effector organ of the mucosal immune system which reflects the extent of local antigenic stimulation.

Infection with *Helicobacter pylori* is associated with the development of gastritis, peptic ulceration, and gastric carcinoma (1, 27). Gastric tissues infected with *Helicobacter* spp. harbor lymphoid follicles (12, 32) driven by local antigen stimulation (40). Populations of $CD3^+$, $CD4^+$, and αβ T-cell receptor (αβTCR)-positive T cells recruited into gastric mucosa may regulate B-cell function and local immunoglobulin A (IgA) antibody secretion (12, 35). While *H. pylori* antigens activate peripheral blood T cells and B cells in vitro (20), the antigen specificity of lymphocytes resident in gastric mucosae has not been examined directly. Serum IgG and local mucosal IgA antibody responses are developed in response to infection with *Helicobacter* spp., although these appear to be insufficient for clearance (5, 12, 30, 37).

The urease molecule from *H. pylori* is an essential determinant of pathogenicity. Because urease-negative mutants fail to colonize gastric tissue of gnotobiotic piglets (8) and nude mice (36), urease may represent an important target for immunization and prevention of disease. Urease is a multimeric enzyme composed of two structural subunits of 29.5 (UreA) and 66 (UreB) kDa (7, 18) and is localized in both the cytoplasm and on the surface of *H. pylori* (15). The *H. pylori* urease molecule exhibits a high degree of sequence conservation in comparison with that of *Helicobacter felis* urease (11).

A model of chronic persistent gastritis using mice infected with *H. felis* has allowed the study of immune responses and disease progression in chronically infected animals (12, 23). Using this model, recent studies have shown that oral immunization with *H. felis* antigens results in protection against subsequent challenge with *H. felis* organisms (2, 6, 22). While mucosal immunization strategies which prevent *Helicobacter* infection generate mucosal IgA and serum IgG and IgA antibodies (2, 6), parenteral immunization yielding high levels of serum IgG does not confer protection against challenge (3, 9). The experiments presented herein examined the ability of orally administered recombinant urease (rUrease) antigen to protect mice from *H. felis* infection and *H. felis*-associated gastritis. We show the mucosal IgA and serum IgG and IgA antibody responses in immunized mice subsequently challenged with live *H. felis* and the gastric lymphocyte cytoarchitecture in infected and protected mice.

MATERIALS AND METHODS

Animals. Twenty-eight germfree 4-week-old female Swiss Webster mice were obtained from Taconic Farms (Germantown, N.Y.). The mice were maintained in a germfree isolator and subsequently housed in barrier conditions (12) for the duration of the experimental treatments. All materials for the germfree unit were sterilized by peracetic acid, and the mice were fed an autoclaved pelleted diet and given sterile water ad libitum.

Bacteria. *H. pylori* ATCC 43505 was cultured on Mueller-Hinton agar plates supplemented with 5% defibrinated sheep blood and 10 μg of vancomycin per ml, 10 μg of trimethoprim per ml, and 10 μg of polymyxin B (Sigma Chemical Co., St. Louis, Mo.) per ml. *Escherichia coli* BL21-DE3 (Novagen, Madison, Wis.) harboring plasmid pORV154 was cultured in liquid medium in Luria broth (Difco Laboratories, Detroit, Mich.) containing 50 μg of ampicillin (Sigma) per ml or on Luria broth plates containing 1.5% agar and 100 μg of ampicillin per ml. The *H. felis* ATCC 49179 used for oral challenge was grown under microaerobic conditions on 5% lysed horse blood agar supplemented with 10 μg of vancomycin per ml, 5 μg of trimethoprim lactate per ml, 3 μg of polymyxin B (Sigma) per ml, and 2.5 μg of amphotericin per ml as described elsewhere (23). The bacteria were harvested, inoculated in brain heart infusion agar with 30% glycerol, and frozen at −70°C. Prior to use, aliquots were thawed, analyzed for motility, and cultured for evidence of aerobic or anaerobic bacterial contamination.

Preparation of *H. felis* antigens and native *H. pylori* urease. *H. felis* sonicate antigens were prepared as described elsewhere (13). In brief, *H. felis* was grown for 48 h in brucella broth (Difco) containing 5% fetal calf serum. Cultures were incubated at 37°C in a microaerobic environment and shaken at 120 rpm. The cultures were centrifuged at 10,000 rpm (Sorvall RC-5B, Newtown, Conn.) for 10 min, the pellet was washed in phosphate-buffered saline (PBS), and the cells were disrupted by sonication (Artec K System, Inc., Farmingdale, N.Y.). After

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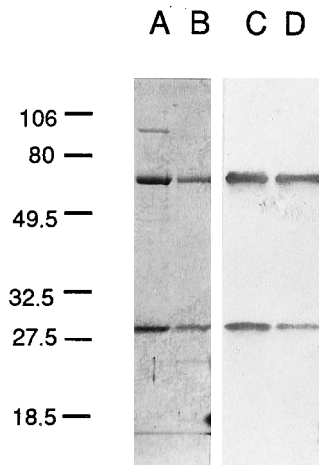

FIG. 1. Characterization of *H. pylori* native urease and rUrease. Purified urease (5 μg) derived from *E. coli* containing the UreA and UreB genes of *H. pylori* (lanes A and C) or from *H. pylori* ATCC 43505 (lanes B and D) was electrophoresed on 10% sodium dodecyl sulfate–polyacrylamide gels (lanes A and B) or transferred to nitrocellulose for Western blotting (lanes C and D) with mouse polyclonal antibody raised against *H. pylori* apoenzyme. Molecular size standards are shown in kilodaltons.

centrifugation at 10,000 rpm (Sorvall RC-5B; Dupont) for 10 min, the protein content was determined (25) and aliquots were frozen at −70°C until used. Urease from *H. pylori* was purified from organisms harvested from blood agar plates. The *H. pylori* organisms were lysed, centrifuged, and subjected to chromatography on DEAE-Sepharose (Pharmacia, Piscataway, N.J.) (18). The bound material was eluted with 150 mM NaCl, concentrated, and applied to a Sephacryl 300 sizing column (Pharmacia). Eluted fractions were assayed for the presence of urease activity (16). Fractions containing urease activity were then bound to Mono-Q Sepharose (Pharmacia) and eluted with a 0 to 1 M NaCl gradient. The fractions containing urease activity were pooled, concentrated, and stored in 50% glycerol at −20°C.

Cloning and purification of *H. pylori* rUrease. *H. pylori* rUrease was derived from *E. coli* ORV154 expressing the structural genes for the A (UreA) and B (UreB) subunits (4, 21) required for assembly (17). ORV154 was constructed as described elsewhere (24). rUrease was expressed constitutively from the T7 promoter (33) of pGEM3Z and purified as follows. ORV154 was cultured overnight at 37°C in shake flasks. The organisms were harvested by centrifugation, washed in PBS, and lysed by sonication. Contaminating proteins were removed from cell extracts by chromatography on DEAE-Sepharose (Pharmacia) and then by a 50 mM NaCl wash. The eluate was diluted 10- to 20-fold and then bound to DEAE-Sepharose. Urease was eluted with 150 mM NaCl, and fractions containing urease were identified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blotting (immunoblotting) (34) using a mouse polyclonal antibody raised against *H. pylori* urease. The urease-containing fractions were pooled, concentrated, and subjected to Sephacryl 300 sizing chromatography. This procedure yielded enzymatically inactive rUrease exhibiting two major bands of 60 and 30 kDa, corresponding to the B and A subunits, respectively (Fig. 1), with >90% purity, as assessed by scanning densitometry (Pharmacia-LKB UltroScan).

Experimental protocol for oral immunization. Mice were divided into three groups and immunized per os on days 0, 10, 20 and 30 with a blunt feeding needle (Popper & Sons, Inc., New Hyde Park, N.Y.) as follows: group 1, PBS and 10 μg of cholera toxin (CT; Calbiochem, La Jolla, Calif.; $n = 8$); group 2, 1 mg of *H. felis* sonicate and 10 μg of CT ($n = 10$); and group 3, 250 μg of *H. pylori* rUrease plus 10 μg of CT ($n = 10$). Groups of mice were challenged with live *H. felis* three times at 2-day intervals (12) either 2 weeks (day 45) or 6 weeks (day 73) after the last oral immunization.

Culture for *H. felis* and urease tests. From 31 to 32 days (2-week postimmunization groups) or 41 to 42 days (6-week postimmunization groups) post-*H. felis* challenge, the mice were euthanized with an overdose of carbon dioxide. Two-millimeter cubes of gastric mucosa from the antrum, fundus, and duodenum were collected aseptically for culture or for the tissue urease test (12, 16).

Determination of antibody levels to urease and *H. felis* antigens in serum and feces. Blood was obtained from the retro-orbital sinus 7 days after the second and fourth immunizations, 13 to 14 days after *H. felis* challenge, and by cardiac puncture at the termination of the experiment. Secretory IgA was extracted from fecal pellets (14) by incubation in PBS containing 5% nonfat dry milk, 0.2 mM 4-(2-aminoethyl)-benzenesulfonylfluoride (Calbiochem), 1 μg of aprotinin per ml, 10 μM leupeptin (Sigma), and 3.25 μM bestatin (Boehringer Mannheim Biochemicals, Indianapolis, Ind.). After extensive vortexing, the fecal material was centrifuged (16,000 × *g* for 10 min), and the supernatants were used for determination of antibody. An enzyme-linked immunosorbent assay (ELISA) was used for antibody measurement. In brief, triplicate wells of microtiter plates (Dynatech, Chantilly, Va.) were incubated with purified *H. pylori* urease or with *H. felis* sonicate preparations (100 μg/ml) in carbonate buffer. After washing with PBS–0.5% Tween 20, the wells were blocked with PBS-Tween containing 2.5% nonfat dry milk and incubated for 1 h at 37°C with serial dilutions of sera or fecal extracts. The wells were then incubated with biotinylated goat-anti mouse IgG or goat-anti-mouse IgA (Southern Biotechnology, Birmingham, Ala.) and then with streptavidin-alkaline phosphatase (Calbiochem). Negative control sera and fecal extracts and positive serum controls with known anti-*H. felis* activity were included in each assay.

TABLE 1. Effect of oral immunization on the presence of *H. felis* in gastric tissue[a]

Treatment group	Time postimmunization of *H. felis* challenge (wk)	No. of mice positive/total no. by:		
		Urease test	Culture	Warthin-Starry histology
PBS	2	3/4	4/4	3/4
H. felis sonicate	2	0/5[b]	1/5[c]	0/5[b]
H. pylori rUrease	2	0/5[b]	0/5[d]	0/5[b]
PBS	6	1/4	3/4	2/4
H. felis sonicate	6	0/5	0/5[b]	0/5
H. pylori rUrease	6	0/5	0/5[b]	0/5

[a] Groups of germfree Swiss Webster mice were orally immunized and challenged per os three times at 2-day intervals with 10^8 *H. felis* organisms. After 31 to 32 days (2-week postimmunization groups) or 41 to 42 days (6-week postimmunization groups), gastric tissue was examined for the presence or absence of *H. felis* infection as indicated.

[b] $P = 0.0476$ by Fisher's exact test compared with value of corresponding PBS group.

[c] $P = 0.0238$ by Fisher's exact test compared with value of corresponding PBS group.

[d] $P = 0.0079$ by Fisher's exact test compared with value of corresponding PBS group.

Histopathology. Longitudinal sections of gastric tissue, from the esophageal junction through the duodenum, were fixed in 10% neutral buffered formalin. Stomachs were processed for routine histology and embedded in paraffin, and 5-μm sections were stained with hematoxylin and eosin. Sections of the fundus, antrum, and duodenal-pyloric junction were examined in a coded fashion for histological changes and for the presence of *H. felis* in Warthin-Starry-stained specimens.

Immunohistochemistry. Longitudinal sections of gastric tissue including the corpus and antrum were mounted in O.C.T. compound (Miles Scientific, Naperville, Ill.) and frozen in liquid-nitrogen-cooled Freon 22 (12). Tissue sections (7 μm) were fixed with acetone, and biotin-avidin-binding sites were blocked for 30 min (Vector Laboratories, Burlingame, Calif.). Tissue sections were incubated with biotinylated monoclonal antibodies (MAb; see below) and then with avidin conjugated to biotinylated horseradish peroxidase (ABC; Vector Laboratories) as described elsewhere (12). Controls included incubation with a MAb of unrelated specificity. Cell-bound peroxidase was visualized with 0.05% diaminobenzidine tetrahydrochloride (Organon Teknika, Durham, N.C.) and 0.01% H_2O_2 in PBS, and sections were counterstained with methyl green. Baseline values of infiltrating leukocytes were established with groups of known PBS-treated and challenged mice. The tissue sections from the remaining experimental groups for immunohistology were scored by a code. The degree of gastric infiltration and/or expression of antigens defined by the MAb was scored as mild (+), moderate (++), or severe (+++).

MAb. The following MAb recognizing lymphocyte cell surface structures were used in this study: anti-Thy1.2 (clone 30-H12), anti-CD4 (clone GK1.5), anti-CD8 (clone 53-6.7) (Becton Dickinson, San Jose, Calif.), anti-CD3 (clone 145-2C11), anti-CD5 (clone 53-7.3), anti-CD49d (VLA-4; clone MFR 4.B), anti-CD62L (L-selectin; clone MEL-14), anti-αβTCR (clone H57-597), anti-γδTCR (clone GL3), anti-I-A^d (clone AMS-32.1), and anti-I-A^p (clone 7-16.17) (Pharmingen, San Diego, Calif.). The MAb anti-CD45R (B220; clone RA3-3A1/6.1), anti-IgM (clone 331.12), and anti-CD11b (M1/70.15) were obtained from the American Type Culture Collection (Rockville, Md.). The MAb 10-4.22 was used to identify IgA-positive B cells (13, 31).

RESULTS

Effect of oral immunization on *H. felis* infection. The outcome of gastric urease tests, bacterial cultures, and histological identification of *H. felis* organisms by Warthin-Starry stain of

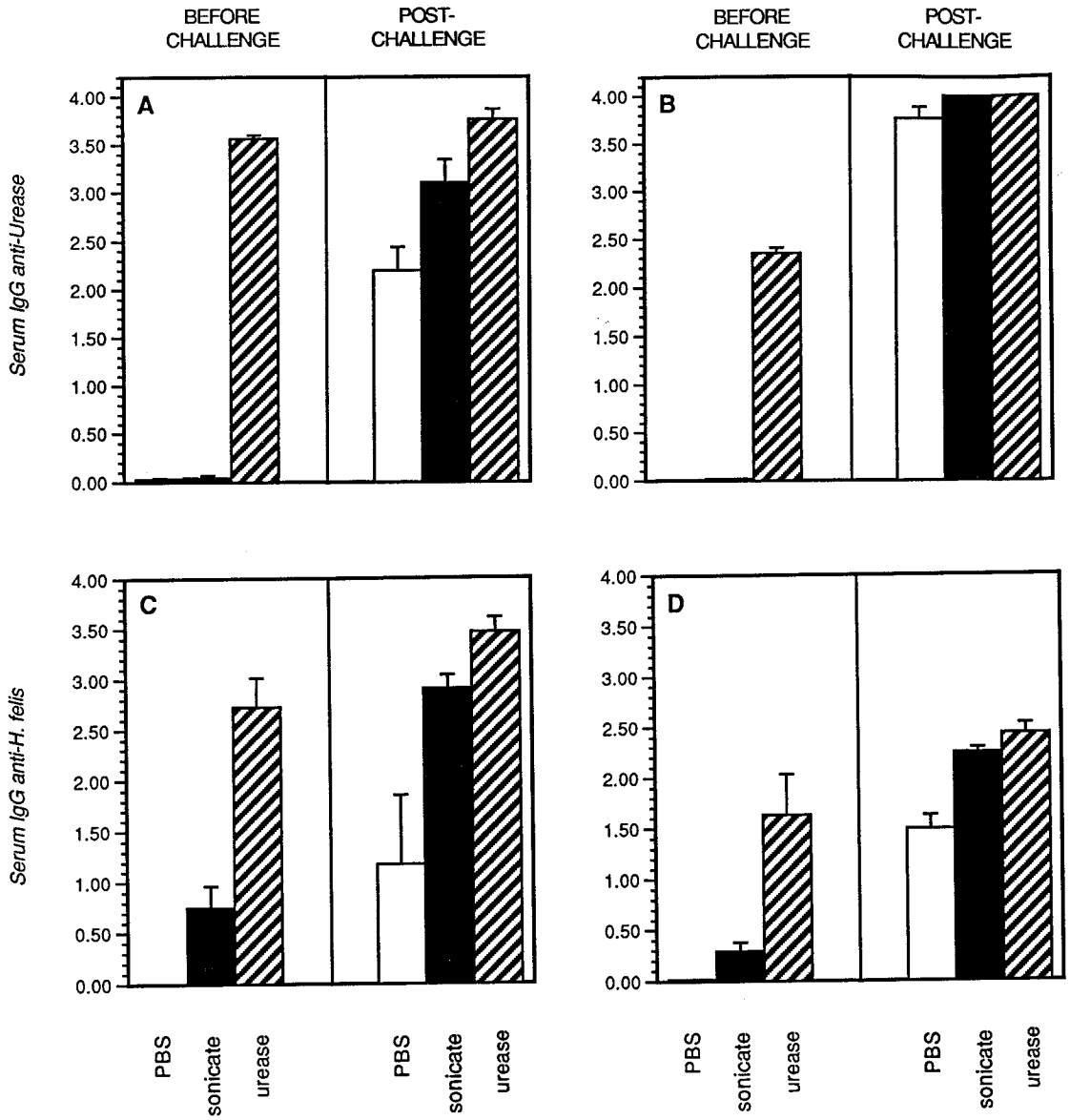

FIG. 2. Antibody responses in sera of immunized mice. Groups of Swiss Webster mice were immunized orally with PBS and 10 μg of CT (open bars; $n = 4$), 1 mg of *H. felis* sonicate and 10 μg of CT (filled bars; $n = 5$), or 250 μg of *H. pylori* rUrease and 10 μg of CT (striped bars; $n = 5$). The mice were challenged with live *H. felis* 2 weeks (A and C) or 6 weeks (B and D) later, and serum IgG anti-urease antibody (A and B) or serum IgG anti-*H. felis* antibody (C and D) levels were quantitated by ELISA. Values from immunized animals before challenge with *H. felis* were derived from samples taken 7 days after the last immunization. Values from postchallenge mice were obtained by analysis of samples harvested 13 to 14 days after *H. felis* challenge. The bars show the mean optical density at 405 nm for each group, and the brackets enclose 1 standard error of the mean.

gastric tissue is shown in Table 1. Immunization with *H. pylori* rUrease or with *H. felis* sonicate antigens interfered ($P \leq 0.0476$) with the establishment of infection upon challenge with live *H. felis* 2 or 6 weeks after the last immunization dose. The gastric tissues of PBS-treated mice challenged with *H. felis* remained colonized during the time periods examined.

Serum antibody levels to *H. pylori* urease and *H. felis* antigens in orally immunized mice. Oral immunization with rUrease resulted in the development of urease-specific serum IgG in 9 of 10 mice after two immunizations and in marked elevation of serum IgG anti-urease antibody in 10 of 10 mice following four immunizations (Fig. 2A and B). Serum IgA antibody was assayed consistently in rUrease-immunized mice only after the fourth oral immunization. Specific antibody responses directed against urease were not measurable in PBS-treated mice nor in mice immunized with *H. felis* sonicate. Oral immunization with rUrease also elicited a greater serum IgG antibody against *H. felis* sonicate antigens than that generated in mice immunized with *H. felis* sonicate (Fig. 2C and D). Challenge of mice with *H. felis* either 2 or 6 weeks postimmunization further stimulated the serum IgG anti-urease antibody response of rUrease-immunized mice and activated the serum IgG antibody compartment of PBS-treated and *H. felis*-immunized animals. Whereas *H. felis* challenge similarly stimulated the urease-specific serum IgA of rUrease-immunized mice, these levels constituted about 40% of the serum IgG antibody level (not shown). The highest level of serum IgA antibody against *H. felis* was measured in mice challenged 6 weeks post-

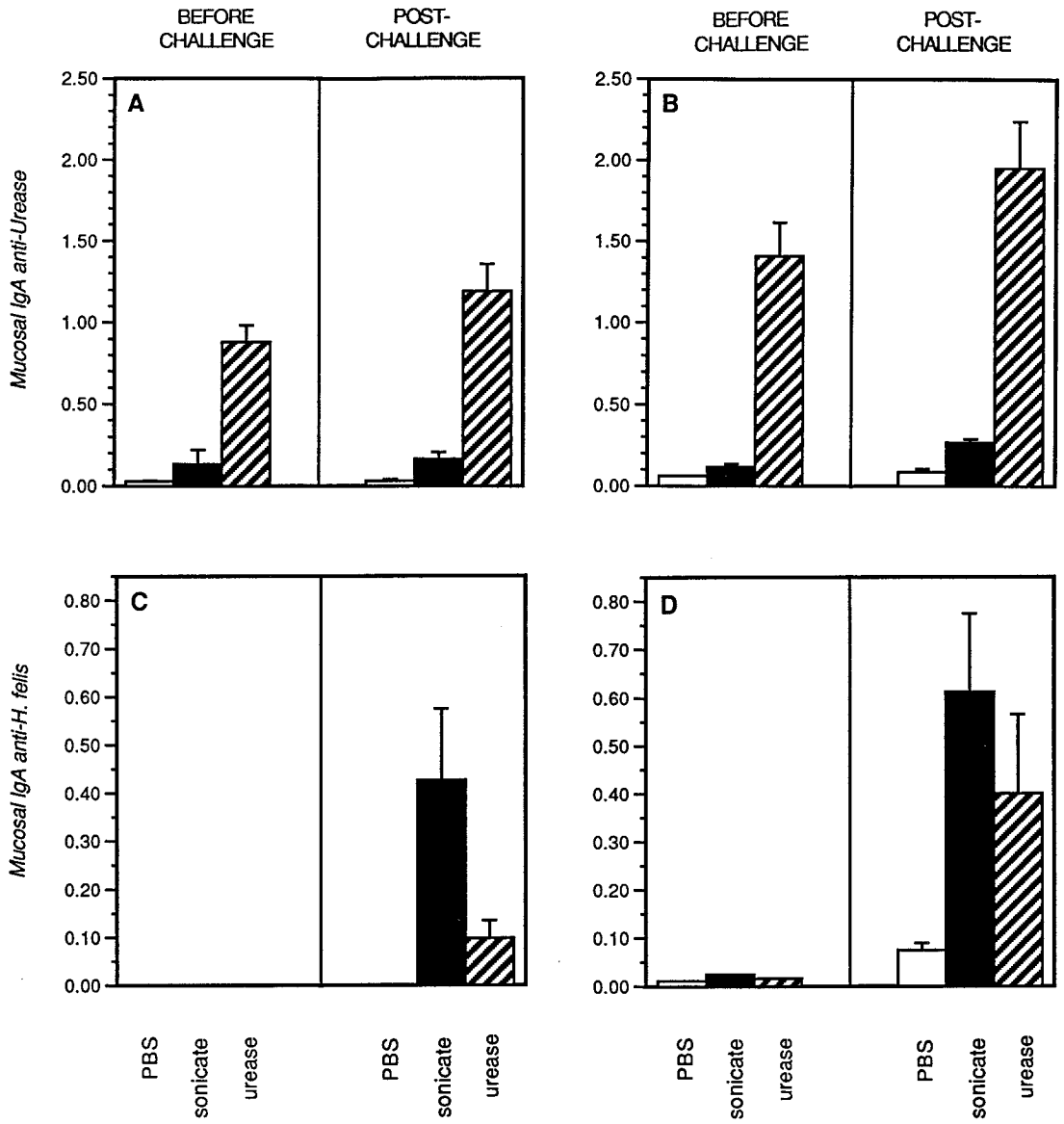

FIG. 3. Secretory IgA antibody levels in the intestinal compartment of Swiss Webster mice immunized orally with PBS (open bars; $n = 4$), *H. felis* sonicate (filled bars; $n = 5$), or *H. pylori* rUrease (striped bars; $n = 5$). The mice were challenged with live *H. felis* 2 weeks (A and C) or 6 weeks (B and D) after the last immunization, and the fecal IgA antibody levels against urease (A and B) or *H. felis* (C and D) were quantitated by ELISA. The bars show the mean optical density at 405 nm for each group, and the brackets enclose 1 standard error of the mean.

rUrease immunization (optical density at 405 nm = 0.337 ± 0.117). The magnitude of both serum IgG and IgA antibody responses against rUrease and *H. felis* antigens at the termination of the experiment was greatest in mice immunized with rUrease.

Intestinal antibody responses. The specific fecal IgA antibody response against urease and *H. felis* antigens before and after *H. felis* challenge is shown in Fig. 3. Oral immunization with rUrease generated much greater IgA anti-urease antibody levels than did immunization with *H. felis* sonicate antigens. Whereas all mice immunized with rUrease developed anti-urease IgA, fecal urease-specific antibody responses were low (optical density at 405 nm < 0.16) or were not measurable in groups of mice immunized with *H. felis* sonicate. Challenge of rUrease-immunized mice with *H. felis* 2 or 6 weeks postimmunization increased the urease-specific intestinal IgA antibody level. However, *H. felis* challenge of PBS-treated control mice or *H. felis* sonicate-immunized mice had little effect on the intestinal IgA anti-urease antibody concentration (Fig. 3A and B). Although the fecal IgA antibody levels against *H. felis* resulting from oral delivery of antigen were very low, oral immunization of mice with rUrease or with *H. felis* antigens effectively primed the mucosal IgA compartment, as shown by the generation of much greater IgA responses in antigen-immunized animals after inoculation with live *H. felis* (Fig. 3).

Histopathology and immunohistopathology of gastric tissue. Inflammatory changes were observed in all mice in both the body and the antral regions in the subglandular portion of the mucosa and frequently extended into the underlying submucosa. The cell infiltrates were characterized as active chronic inflammation and were present as multifocal, relatively diffuse aggregates of mononuclear leukocytes and neutrophils.

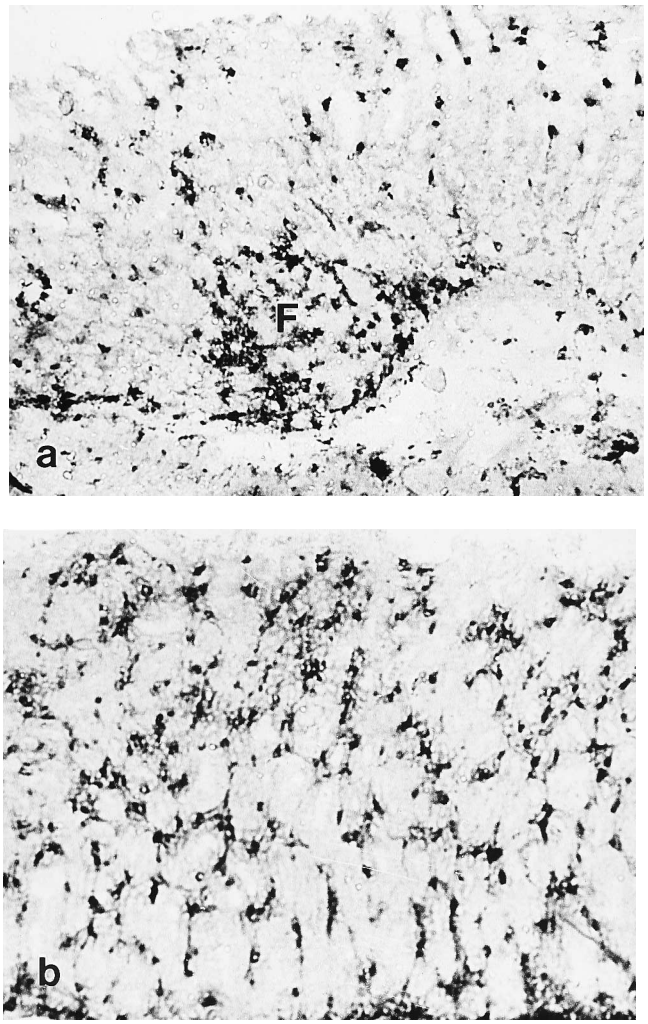

FIG. 4. Immunohistochemical localization of infiltrating Thy1.2$^+$ T cells in cross sections of gastric mucosa approximately 40 days post-oral inoculation with *H. felis*. (a) Gastric tissue from *H. felis*-infected mice immunized with PBS showing lymphoid follicles (F) delimited by scattered mucosal Thy1.2$^+$ T cells. (b) The gastric mucosa from rUrease-immunized mice subsequently challenged with *H. felis* exhibiting predominant clusters of mucosal and submucosal Thy1.2$^+$ T cells. Magnification, ×500.

Immunohistochemical analyses of PBS-treated control animals challenged with *H. felis* revealed multifocal CD45R$^+$ and IgM$^+$ B cells assembled into mucosal and submucosal lymphoid follicles frequently surrounded by aggregates of Thy1.2$^+$ T cells (Fig. 4). In contrast, the gastric tissue from rUrease- or *H. felis* sonicate-immunized animals contained scattered CD45R$^+$ B cells and occasional mucosal follicles but exhibited mild to moderate infiltration by Thy1.2$^+$ T cells distributed as single cells or as clusters in the gastric mucosa and submucosa (Fig. 4 and Table 2). All mice showed a similar distribution of CD3$^+$ CD4$^+$ αβTCR-positive T cells. CD8$^+$ or γδ T cells were infrequently observed in gastric mucosae. Populations of CD5$^+$, CD11b$^+$, and IgA$^+$ cells occurred as isolated cells or as small clusters of cells in the gastric pits, lamina propria, and epithelium. The gastric epithelium and infiltrating mononuclear leukocytes were substantially I-A^{p+} (Table 2). The architectural compositions of cell populations infiltrating gastric tissue were similar whether mice were challenged with *H. felis* 2 or 6 weeks after the last oral immunization.

TABLE 2. Relative phenotypic distributions of mononuclear leukocytes infiltrating gastric tissue after oral immunization and challenge with *H. felis*

Antigen structure or CD designation	Findings for experimental group immunized with[a]:		
	PBS	*H. felis* sonicate	rUrease
Thy1.2	+	+	+
CD3	+	+	+
CD4	+	+	+
CD5	+	+	+
CD8	−[b]	−[b]	−[b]
αβTCR	+	+	+
γδTCR	−[b]	−[b]	−[b]
CD45R	+++	+	+
IgA	+[c]	+[d]	+[d]
IgM	++[e]	+[d]	+[d]
CD11b	+	+	+
CD49d (VLA-4)	+	+	+
CD62L (L-selectin)	−[b]	−[b]	−[b]
I-A^p	+++[f]	+++[f]	+++[f]

[a] The degree of gastric infiltration and/or expression of antigens was scored as mild (+), moderate (++), or severe (+++).
[b] Few scattered cells in mucosa.
[c] Weakly stained cells in follicle coronas.
[d] Scattered cells in mucosa, lamina propria, and epithelium.
[e] Densely labeled cells in lymphoid follicles.
[f] Positive reaction in epithelium and infiltrating leukocytes.

DISCUSSION

In the present study, we have shown that mice orally immunized with *H. pylori*-derived rUrease were protected from infection upon challenge with living *H. felis* organisms. Oral immunization with rUrease resulted in the generation of secretory IgA antibody, and high levels of intestinal IgA antibody against urease were associated with protection of mice against infection when challenged with living *H. felis*. The high degree of conservation at the amino acid level between *H. pylori* and *H. felis* urease (11) may account for the immunological basis of cross-protection with UreB subunit containing protective epitopes (11a, 26). The ability of rUrease antigen to confer protection in immunized mice against infection with *H. felis* was similar to that afforded by *H. felis* sonicate antigens, as shown in this study and others (2, 6, 22). However, while the protective effect of oral immunization with *H. felis* sonicate antigens has been observed in mice challenged with *H. felis* 3 to 14 days after the last immunization (2, 22), we have found the protective response to last up to 6 weeks postimmunization. The findings reported herein are consistent with previous studies showing induction of mucosal IgA and absence of infection in mice immunized with *H. felis* sonicate (6). Furthermore, the present observations indicate that intestinal anti-urease IgA antibody activated by immunization with rUrease may be required to interfere with the establishment of *H. felis* infection and suggest that low levels of urease-specific IgA, as assayed in *H. felis* sonicate-immunized mice, may be protective as well. However, it is not clear whether IgA antibody responses directed against *H. felis* surface structures other than urease may also interfere with colonization. Detailed analyses of antibody responses to *H. felis* surface antigens in protected mice and studies with urease-negative *Helicobacter* mutants (8, 36) may help answer this question. Because IgA may function in the mucosal environment by inhibition of microbial adherence to epithelial cells (39), the mucosal anti-urease IgA antibody response generated in animals immunized with rUrease or *H.*

felis sonicate would have the potential to interfere with the *H. felis*-gastric epithelium interactions which result in colonization. The findings that high levels of IgG antibody induced by parenteral immunization did not protect against infection (3, 9) and that an orally administered anti-*H. felis* MAb protected mice against *H. felis* infection (6) support an important role for local IgA antibody in the prevention of infection in target gastric tissue. The ability of oral immunization to prime the mucosal compartment for a greater IgA antibody response upon deliberate challenge with live *H. felis*, presumably via recognition of urease epitopes displayed at the *H. felis* surface or present as luminal antigen, suggests that protective levels of IgA antibody may be generated in uninfected, immunized hosts as a function of reexposure to the organism.

The continued presence of *H. felis* in the gastric mucosa of PBS-treated mice, as shown in this study, and in gastric tissue of chronically infected mice (12) may signal persistent antigenic stimulation by *H. felis* which gives rise to gastric germinal-center reactions, whereas the reduction or absence of gastric lymphoid follicles in immunized animals may reflect clearance of the organisms and down-regulation of B-cell function. The observation of regression of B-cell gastric lymphoma after eradication of *H. pylori* (40) supports the notion that formation of organized lymphoid tissue in gastric mucosa may be antigen driven. Whether the gastric epithelium overlying mucosal follicles harbors M cells specialized for antigen uptake found in intestinal lymphoid tissues (29) or whether the follicular architecture is maintained by stimulation with luminal *H. felis* antigens which gain access to gastric mucosa is not known at present. However, the finding of scattered $CD45R^+$ B cells and IgM^+ and IgA^+ B cells in gastric tissue of protected animals raises the possibility that oral immunization with rUrease may result in the accumulation and proliferation in gastric tissue of IgA antibody-secreting cells activated in mucosa-associated lymphoid tissue (38).

Recent studies have suggested that T-cell populations from infected hosts are sequestered into gastric mucosa (20) and may regulate local B-cell function and IgA antibody secretion (12). The finding of discrete T-cell populations in murine gastric mucosa after inoculation with live *H. felis* suggests the recruitment and/or local proliferation of T cells dominated by the $CD3^+$, $CD4^+$ $CD8^-$, and $\alpha\beta TCR^+$ phenotypes. Although the gastric TCR specificities for *H. felis* antigens in infected or protected mice have not been probed, recent findings have shown the antigen-specific activation of gastric T cells (19). That gastric $CD4^+$ $CD8^-$ T cells may play a role in local IgA antibody production is also suggested by findings of increased gastric IgA antibody after oral immunization with *H. felis* antigens (6) or with rUrease (28).

Previous studies have found up-regulation of class II major histocompatibility complex expression by gastric epithelial cells in infected patients (10). Whereas I-A antigen expression by gastric epithelial cells and infiltrating mononuclear leukocytes was shown in the present work, these observations suggest that interaction with, but not necessarily colonization by, *H. felis* may be sufficient for induction of I-A, since no differences in the immunohistochemical expression of I-A antigen were seen between infected and protected mice. While the effects of oral immunization on the activation of immunological effector functions by gastric T cells and B cells are not well understood, the current observations indicate that oral immunization with rUrease antigen interferes with the establishment of infection with *H. felis.*

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