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Protection Against *Helicobacter pylori* Infection Following Immunization Is IL-12-Dependent and Mediated by Th1 Cells

Ali A. Akhiani,* Jacques Pappo,† Zita Kabok,‡ Karin Schön,* Wei Gao,† Lennart E. Franzén,‡ and Nils Lycke*

The regulatory roles of Th1 and Th2 cells in immune protection against *Helicobacter* infection are not clearly understood. In this study, we report that a primary *H. pylori* infection can be established in the absence of IL-12 or IFN-γ. However, IFN-γ, but not IL-12, was involved in the development of gastritis because IFN-γ−/− (GKO) mice exhibited significantly less inflammation as compared with IL-12−/− or wild-type (WT) mice. Both IL-12−/− and GKO mice failed to develop protection following oral immunization with *H. pylori* lysate and cholera toxin adjuvant. By contrast, Th2-deficient, IL-4−/−, and WT mice were equally well protected. Mucosal immunization in the presence of coadministered rIL-12 in WT mice increased Ag-specific IFN-γ-producing T cells by 5-fold and gave an additional 4-fold reduction in colonizing bacteria, confirming a key role of Th1 cells in protection. Importantly, only protected IL-4−/− and WT mice demonstrated substantial influx of CD4+ T cells in the gastric mucosa. The extent of inflammation in challenged IL-12−/− and GKO mice was much reduced compared with that in WT mice, indicating that IFN-γ/Th1 cells also play a major role in postimmunization gastritis. Of note, postimmunization gastritis in IL-4−/− mice was significantly milder than WT mice, despite a similar level of protection, indicating that immune protection is not directly linked to the degree of gastric inflammation. Only protected mice had T cells that produced high levels of IFN-γ to recall Ag, whereas both protected and unprotected mice produced high levels of IL-13. We conclude that IL-12 and Th1 responses are crucial for *H. pylori*-specific protective immunity. The Journal of Immunology, 2002, 169: 6977–6984.

*Helicobacter pylori* is a Gram-negative spiral bacterium that colonizes the gastric mucosa of humans, causing chronic gastritis, peptic ulcers, gastric adenocarcinoma, and lymphoma (1–3). Despite the development of strong immune responses against *H. pylori* infection in humans, the bacteria are rarely eliminated from the stomach and the infection is usually lifelong. Eradication of *H. pylori* infection results in the cure of the ulcer and prevention of its recurrence (4). Studies using the *Helicobacter felis* or *H. pylori* mouse model have shown that oral or intranasal *Helicobacter* vaccines administered with a mucosal adjuvant, such as cholera toxin (CT),3 can protect against infection (5–8). It was recently shown that *H. pylori*-specific Ab responses do not play a major role in protection, as demonstrated in immunized B cell-deficient, μMT mice (9, 10). By contrast, immunized MHC class II-deficient mice were poorly protected (9, 11) indicating that CD4+ T cells are critical for protection against *H. pylori* infection.

The roles of Th1 or Th2 cells in *Helicobacter*-specific protective immunity are, however, still incompletely understood. Studies with *H. felis* infection have reported on a dominant IFN-γ-Th1-type response, which enhanced gastric inflammation without reducing bacterial load (12). In adoptive transfer studies, it was further shown that *H. felis*-specific Th1 and Th2 cell lines enhanced gastric inflammation, but only Th2 cells were associated with reduced bacterial colonization (13). Also, repeated therapeutic oral immunizations in BALB/c mice stimulated significant protection, which correlated with a gradual skewing of the T cell response toward Th2 (14). However, both Th1 and Th2 cells generated after systemic immunization with *H. felis* lysate and CFA or aluminum hydroxide, respectively, mediated protection (15). Other studies have indicated that IL-4- and IL-13-dependent Th2 type responses were, in fact, not required for protection, as immunized IL-4−/− mice were strongly protected against *H. pylori* infection (16). Finally, a replication-defective adenovirus infection, causing Th1 skewing, therapeutically reduced bacterial colonization in chronically infected WT mice, but not in IFN-γ- or IL-12-deficient mice (17).

Because of the inconsistencies in our current information on the regulatory roles of Th1 and Th2 cells in protective immunity against *Helicobacter* infection, we investigated this matter in some detail using IL-12- (IL-12−/−), IFN-γ- (GKO), or IL-4-deficient (IL-4−/−) mice and compared the results to that found in WT mice. We found that only if Th1-type responses dominated, as seen in the IL-4−/− and WT mice following oral immunizations, did we observe protection against a live challenge infection with *H. pylori* bacteria.

Materials and Methods

**Mice**

IL-12-deficient (IL-12−/−; Ref. 18), IFN-γ-deficient (GKO; Ref. 19), and IL-4-deficient (IL-4−/−; Ref. 20) mice on a C57BL/6 background, were bred in ventilated cages under pathogen-free conditions at the University of Gothenburg Animal Facility, Department of Medical Microbiology and Immunology (Gothenburg, Sweden). Wild-type (WT) C57BL/6 mice were obtained from B & K Universal AB (Sollentuna, Sweden) or from Taconic Farms (Germantown, NY). All mice were seronegative for *Helicobacter*.
spp. Ags before infection or immunization. Age- and sex-matched animals were used throughout the study.

**Immunization**

Groups of 7–10 mice were immunized orally with a blunt feeding needle (Popper & Sons, New Hyde Park, NY) four times at weekly intervals with 500 μg of *H. pylori* whole cell lysate Ags and 10 μg of CT adjuvant (List Biological Laboratories, Campbell, CA) as described elsewhere (11). For experiments assessing the role of IL-12, wild-type mice were immunized once by the intranasal route with 100 μg of *H. pylori* lysate plus 1 μg of CT adjuvant in 20 μl via a pipette tip. Control groups received *H. pylori* lysate alone, or CT adjuvant alone, as indicated. Murine rIL-12 (R&D Systems, Minneapolis, MN) at 1 μg per dose was administered orally in 50 μl, followed by live challenge infection with *H. pylori* bacteria.

**H. pylori growth conditions and challenge**

*H. pylori* SS1 was grown on tryptic soy agar (TSA) plates (BD Biosciences, Cockeysville, MD) containing 5% sheep blood and 100 μg of vancomycin, 3.3 μg of polymyxin B, 200 μg of bacitracin, 10.7 μg of nalidixic acid, and 50 μg of amphotericin B (Sigma-Aldrich, St. Louis, MO) per milliliter. The plates were incubated for 48 h at 37°C under microaerobic conditions (10% CO2, 5% O2, and 85% N2). The bacteria were then harvested and inoculated into Brucella broth (BD Biosciences) supplemented with 5% heat-inactivated FCS (Biochrom, Berlin, Germany). A gas mixture consisting of 5% O2, 10% CO2, and 85% N2 was injected directly into culture flasks containing the bacteria before they were sealed and shaken at 120 rpm at 37°C for 24 h. Cultures were grown to an OD600 of 0.3 (~5 × 10^9 CFU/ml), and diluted in Brucella broth for inoculation.

Before use, *H. pylori* cells were analyzed in wet mounts to assess motility and morphology, and subjected to urease, catalase, and oxidase tests. To establish a primary *H. pylori* infection, mice were challenged intragastrically with 3 × 10^8 CFU of *H. pylori* on two consecutive days. To assess protection after immunization, the mice were challenged with 5 × 10^7 CFU of *H. pylori* 2 wk after the last immunization. Mice were killed 2 wk postchallenge, and the gastric tissue was processed for urease activity, quantitative *H. pylori* culture, histopathology, and immunohistochemical analyses as described below.

**Preparation of *H. pylori* whole cell lysate Ags**

*H. pylori* was grown on selective blood agar plates for 48 h at 37°C under microaerobic conditions (10% CO2, 5% O2, and 85% N2) and suspended in PBS. The cells were then washed three times in PBS by centrifugation at 6000 × g for 10 min at 4°C before being disrupted by freeze–pressing with X-press (21). Briefly, the bacterial suspensions were frozen at −35°C in the presence of celite or sucrose rods, which fit into the pressure chamber and are extruded by a piston forced back and forth by means of a hydraulic pump. After centrifugation (6000 × g) to remove cell fragments, the preparation was filtered through a 0.2-μm membrane filter (Schleicher & Schuell, Dassel, Germany). The protein content was determined by the Bio-Rad protein assay (Hercules, CA), and aliquots were frozen at −70°C until used.

**Gastric tissue analyses**

The stomach was dissected along the greater curvature and divided into four longitudinal strips for assessment of urease activity, *H. pylori* culture, histopathology, or immunohistochemical analyses. For histopathology, longitudinal segments including the antrum and corpus plus a piece of the lamina propria and into the submucosa. For evaluation of epithelial changes, H&E-stained sections were scored based on the degree of infiltrating lymphocytes, plasma cells, and neutrophils (22). Grades were defined as follows: 0, none; 1, a few leukocytes scattered in the deep mucosa; 2, moderate numbers of leukocytes in the deep to mid mucosa and occasional neutrophils in gastric glands (microabscesses); 3, dense infiltrates in the deep to mid mucosa, a few microabscesses, and one or two lymphoid aggregates; and 4, dense, diffuse infiltrates throughout the lamina propria and into the submucosa, frequent microabscesses, and prominent lymphoid aggregates.

**Epithelial changes**

For evaluation of epithelial changes, H&E-stained sections were scored based on the degree of infiltrating lymphocytes, plasma cells, and neutrophils (22). Grades were defined as follows: 0, none; 1, a few leukocytes scattered in the deep mucosa; 2, moderate numbers of leukocytes in the deep to mid mucosa and occasional neutrophils in gastric glands (microabscesses); 3, dense infiltrates in the deep to mid mucosa, a few microabscesses, and one or two lymphoid aggregates; and 4, epithelial changes throughout the mucosa plus one to three microabscesses or cystic glands; or 4, epithelial changes throughout the mucosa plus four or more microabscesses or cystic glands.

**Immunohistochemistry of gastric tissue**

Frozen sections from gastric tissue were fixed in 50% acetone for 30s followed by 100% acetone for 5 min at 4°C. After washing in PBS, sections were blocked with avidin-biotin blocking reagents (Vector Laboratories, Burlingame, CA) and endogenous peroxidase activity blocked with 0.3% H2O2 in PBS. Sections were then incubated with 5% horse serum in PBS. Sections were then incubated with 5% horse serum in PBS. Sections were incubated with biotinylated rat mAbs against mouse CD4 or CD8 (BD Pharmingen, San Diego, CA). For detection of CD3 cells, sections were incubated with rat mAb against mouse CD3 (BD Pharmingen) followed by incubation with biotinylated rabbit anti-rat IgG (H + L; Vector Laboratories). Sections were then incubated with HRP-conjugated to an avidin-biotin complex (ABC-Elite kit; DAKO, Glostrup, Denmark). Cell-bound peroxidase was detected with 3-amino-9-ethylcarbazole (AEC) (Vector Laboratories) and H2O2. Sections were counterstained with hematoxylin. Control sections were incubated without specific mAb. Stained sections were scored from 0 to 4 based on the extent of infiltrating T cells. Scores were defined as follows: 0, none; 1, a few T cells scattered in the mucosa; 2, moderate numbers of T cells in the gastric mucosa; 3, dense infiltrates in the deep to mid mucosa; 4, dense, diffuse infiltrates throughout the lamina propria and into the submucosa.

**Serum and gastric samples**

Blood was obtained from the axillary plexus of the mouse at sacrifice. Gastric secretions were collected with absorbent wicks positioned longitudinally in the gastric lumen (24), after extensive rinsing with PBS containing 0.2 mM 4-(2-aminoethyl)-benzenesulfonyl fluoride (Calbiochem, La Jolla, CA), 1 μg of aprotinin per ml, 10 mM leupeptin (Sigma-Aldrich), and 3.25 mM Bestatin (Boehringer Mannheim Biochemicals, Indianapolis, IN) protease inhibitors. For extraction of gastric secretions from the wick, 0.5 ml of protease inhibitor containing 5% nonfat dry milk was added to each sample tube containing two wicks, vortexed extensively, and then frozen at −70°C until analyzed.

**Serum and gastric anti-*H. pylori* Ab determinations by ELISA**

Flat-bottom 96-well microtiter plates (Nunc, Roskilde, Denmark) were incubated with 10 μg/ml *H. pylori* lysate in PBS. After washing and blocking with PBS containing 0.1% BSA, the wells were incubated with serial dilutions of sera or gastric secretions. The wells were then incubated with the appropriate dilutions of alkaline phosphatase (AP)-conjugated goat-anti-mouse IgG1, anti-mouse IgG2a, or anti-mouse IgA (Southern Biotechnology Associates, Birmingham, AL), followed by the phosphatase substrate P-nitrophenyl phosphate (NPP; Sigma-Aldrich) in ethanolamine buffer (pH 9.8). Abs were defined on the linear portion of the curve as the interpolated dilution of a sample giving rise to an absorbance of 0.4 U above background.
In vitro stimulation of spleen cells

Spleen cells were obtained by mechanical dissociation and filtering through a nylon mesh. RBCs were subjected to lysis by osmotic shock using hypotonic Tris-ammonium chloride. After washing in HBSS (Life Technologies, Paisley, Scotland), the cells were resuspended in Iscove’s medium (Biochrom) supplemented with 10% heat-inactivated FCS, 50 μM 2-ME (Sigma-Aldrich), 1 mM L-glutamine (Biochrom), and 50 μg/ml gentamicin. Spleen cells (10⁵ cells/well) were then cultured (8% CO₂, 37°C) in round-bottom, 96-well microtiter plates (Nunc) in the presence or absence of varying concentrations of H. pylori lysate Ags or anti-CD3 by adding 10% supernatant from the 145-2C11 cell line (25). Cell-free supernatants were collected 96 h after incubation, and stored at −70°C until assayed for cytokine concentration.

Cytokine assays

The concentrations of IFN-γ, IL-4, IL-5, IL-10, or IL-13 in the supernatants were assessed by ELISA. Briefly, 96-well round bottom microtiter plates (Dynatech Laboratories, Chantilly, VA) were incubated with 2.5 μg/ml rat anti-mouse IFN-γ (BD PharMingen) or 1–5 μg/ml anti-mouse IL-4 (Endogen, Woburn, MA), IL-5, or IL-10 (BD PharMingen). The sample supernatants, or recombinant mouse IFN-γ, IL-4, IL-5, or IL-10 (R&D Systems) standards were then added to the appropriate wells. Bound IFN-γ, IL-4, IL-5, or IL-10 were detected by sequential incubations with a polyclonal rabbit anti-IFN-γ antiserum or biotinylated mAb to mouse IL-4 (Endogen), IL-5, or IL-10 (BD PharMingen), followed by AP-conjugated goat anti-rabbit Ig (Southern Biotechnology Associated) for IFN-γ, or HRP-conjugated anti-biotin (Vector Laboratories) for the IL-4, IL-5, and IL-10 assays. Finally, the AP substrate NPP in ethanolamine buffer (pH 9.8) or the HRP substrate 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid (ABTS; Sigma-Aldrich) in 0.1 M citrate buffer (pH 4.5) was added to each well and the extent of the reaction was read at 405 nm using a Titertek multiscan spectrophotometer. The cytokine concentrations in stimulated culture supernatants were estimated from the standard curves generated with each cytokine. IL-13 was determined using a mouse-specific IL-13 ELISA kit according to the manufacturer’s instructions (R&D Systems).

The sensitivity of detection for the respective cytokine was 0.5 ng/ml for IFN-γ, 40 pg/ml for IL-4, 0.05 ng/ml for IL-5, 0.5 ng/ml for IL-10, and 1.5 pg/ml for IL-13.

ELISPOT

Single cell suspensions were prepared from the spleens of WT mice immunized with lysate plus CT in the presence or absence of rIL-12 followed by challenge infection with H. pylori bacteria. Ag-specific IFN-γ-producing T cells were then determined by ELISPOT assay as described by Gottwein et al. (15).

Statistical analysis

Wilcoxon rank sum test was used for independent samples for analysis of significance in all experimental groups, except for the cytokine values which were compared by the one-tailed Student’s t test.

Results

Differential effects of a primary infection with H. pylori in IL-12⁻/⁻ and GKO mice

Previous studies in mice have shown that a primary Helicobacter infection results in persistent colonization and gastric inflammation associated with a predominantly Th1-type response (12, 26, 27). Because IL-12 plays a central role in Th1 development, and IFN-γ is an important Th1-effector molecule, we investigated the susceptibility for H. pylori colonization in the Th1-deficient IL-12⁻/⁻ and GKO mouse strains. We found that the level of colonization in IL-12⁻/⁻ and GKO mice was comparable to that of C57BL/6 WT mice at 2 and 8 wk postinfection (Fig. 1). At 2 wk postinfection, inflammatory changes in IL-12⁻/⁻, GKO, and WT mice were minimal, but at 8 wk more severe gastritis had developed in both IL-12⁻/⁻ and WT mice compared with that seen in GKO mice (Table I). Thus, a primary H. pylori infection can be established in the absence of the proinflammatory cytokines IL-12 or IFN-γ, but it appeared that IFN-γ, more than IL-12, was directly involved in the development of chronic gastritis in the course of the infection.

Th1-deficient mice fail to develop protection against Helicobacter infection

To investigate the protective role of Th1-type immune responses against Helicobacter bacteria, we undertook studies in Th1-defective, IL-12⁻/⁻, or GKO mice and compared the results with those obtained in WT mice or Th2low, IL-4⁻/⁻ mice. The mice were

Table I. Dependency of IFN-γ or IL-12 on gastric inflammation

<table>
<thead>
<tr>
<th>Mouse Strain</th>
<th>Weeks Post-H. pylori Infection</th>
<th>Gastritis Score⁵</th>
</tr>
</thead>
<tbody>
<tr>
<td>GKO</td>
<td>2</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>IL-12⁻/⁻</td>
<td>2</td>
<td>0.6 ± 0.05</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>2</td>
<td>0.6 ± 0.07</td>
</tr>
<tr>
<td>GKO</td>
<td>8</td>
<td>0.7 ± 0.08⁶</td>
</tr>
<tr>
<td>IL-12⁻/⁻</td>
<td>8</td>
<td>1.0 ± 0.15⁶</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>8</td>
<td>1.1 ± 0.13</td>
</tr>
</tbody>
</table>

⁴ Groups of 10 mice were challenged with H. pylori, and the extent of histological gastritis was assessed 2 or 8 wk postchallenge.

⁵ Mean gastritis score ± SEM of pooled data from two to three experiments.

⁶ p < 0.05 by Wilcoxon rank sum test compared with infected C57BL/6 WT mice.

⁷ NS compared with infected WT mice.
immunized orally with bacterial lysate plus CT adjuvant and subsequently challenged with live *H. pylori* bacteria. Whereas immunized Th1-deficient IL-12−/− and GKO mice failed to show protection against infection, immunized IL-4−/− and WT mice were significantly \( p < 0.01 \) protected (Fig. 2, A–B). Thus, while immune protection exhibited a strict requirement for IL-12 and IFN-γ, IL-4-dependent Th2-type responses appeared to be dispensable for the development of protective immunity following mucosal vaccination. Of particular note, the immunized and well-protected IL-4−/− and WT strains both exhibited significantly stronger infiltration of CD4+ T cells in the gastric mucosa as compared with that observed in immunized IL-12−/− and GKO mice (Fig. 2C). Thus, protection was associated with an increased level of CD4+ T cells in the gastric mucosa.

The requirement for IL-12 for immune protection was further examined in WT mice, which were immunized by the intranasal route in the presence or absence of murine rIL-12. A single mucosal immunization with lysate plus CT adjuvant afforded significant protection \( p = 0.0003 \) from challenge (Table II). However, coadministration of rIL-12 increased IFN-γ-producing splenic T cells by 5-fold (ELISPOT, not shown) and further reduced the bacterial colonization by 4-fold subsequent to a live challenge infection, clearly demonstrating the importance of Th1-type responses for *Helicobacter*-specific protective immunity (Table II). Delivery of rIL-12 to CT only or PBS-treated mice had no statistically significant effect on reduction of the bacterial burden upon challenge with live bacteria (Table II). Also mice immunized with lysate alone with or without rIL-12 were unprotected (not shown). Thus, not only does oral immunization, as seen in the previous experiments, convey protection, but also intranasal immunization is effective and can be further improved by the addition of rIL-12 to the immunization regimen.

**Postimmunization gastritis appears to be a Th1- as well as a Th2-dependent phenomenon**

Next, the relationship between protective immunity and the development of gastritis under the influence of Th1 or Th2 functions was examined. Protective mucosal immunization with lysate and CT adjuvant resulted in pronounced gastritis in WT mice subsequent to a challenge infection as compared with the gastric inflammatory response observed in CT only treated control mice (Fig. 3) or unchallenged immunized mice (not shown). Despite a similar level of protection, immunized IL-4−/− mice displayed significantly lower \( p < 0.05 \) gastritis than WT mice upon challenge with live bacteria (Fig. 3). Therefore, IL-4/Th2 cells may, in fact, contribute to the severity of the gastritis seen after immunization.

**Table II. IL-12 promotes the development of immune protection against *H. pylori* infection in WT mice**

<table>
<thead>
<tr>
<th>Immunization</th>
<th>IL-12</th>
<th>H. pylori Infection (CFU × 10^−3 ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. pylori</em> lysate</td>
<td>−</td>
<td>0.20 ± 0.07b</td>
</tr>
<tr>
<td>IL-12</td>
<td>+</td>
<td>0.05 ± 0.03c</td>
</tr>
<tr>
<td>CT</td>
<td>−</td>
<td>7.20 ± 1.50</td>
</tr>
<tr>
<td>CT+CT</td>
<td>+</td>
<td>5.41 ± 1.18</td>
</tr>
<tr>
<td>PBS</td>
<td>−</td>
<td>6.86 ± 1.95</td>
</tr>
<tr>
<td>PBS+CT</td>
<td>+</td>
<td>5.74 ± 1.09</td>
</tr>
</tbody>
</table>

Groups of C57BL/6 WT mice were immunized once intranasally and challenged with *H. pylori* 2 wk later. IL-12 (n = 5 mice/group) or PBS (n = 10 mice/group) was administered before challenge as described in Materials and Methods. The infection density was assessed 2 wk postchallenge by quantitative bacterial culture.

\( p = 0.0003 \) by Wilcoxon rank sum analysis compared with CT-treated mice.

\( p = 0.0112 \) by Wilcoxon rank sum analysis compared with CT + IL-12-treated mice.

**FIGURE 2.** *H. pylori* colonization (A and B) and CD4+ T cell infiltration (C) in IL-12−/−, GKO, IL-4−/−, and C57BL/6 WT mice after mucosal immunization. Groups of 9–10 mice were immunized orally with 500 μg of *H. pylori* lysate (Ly) together with 10 μg of CT (■) or CT alone (□), and then challenged with *H. pylori* bacteria. The presence of *H. pylori* in gastric tissue was assessed by quantitative culture (A) and urease activity (B) at 2 wk after challenge. The bars represent the mean ± SEM of each group. *, \( p < 0.05 \) and **, \( p < 0.01 \) by Wilcoxon rank sum analysis compared with CT-immunized IL-4−/− mice. ***, \( p < 0.001 \) compared with CT-immunized wild-type mice. One representative experiment of three is shown. C, CD4+ T cells were detected in the gastric tissue by immunohistochemistry. Sections were scored from 0 to 4 as described in Materials and Methods. The bars represent the mean ± SEM of each group. *, \( p < 0.05 \) and **, \( p < 0.01 \) by Wilcoxon rank sum analysis as compared with IL-12−/− or GKO mice immunized with Ly + CT. One representative experiment of two is shown. A–C: We used both CT alone and lysate alone (not shown) as negative controls. Similar results were observed when we compared the Ly + CT group with Ly alone (not shown).
Table III. Cytokine production by spleen cells from the immunized challenged mutant and WT mice

<table>
<thead>
<tr>
<th>Mice</th>
<th>Immunization</th>
<th>Lysate</th>
<th>Anti-CD3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IFN-γ (ng/ml)</td>
<td>IL-13 pg/ml</td>
</tr>
<tr>
<td>IL-12−/−</td>
<td>Lysate + CT</td>
<td>0.13 ± 0.12</td>
<td>459.3 ± 101.2</td>
</tr>
<tr>
<td>CT</td>
<td>0</td>
<td>0.00 ± 0.00</td>
<td>300.4 ± 74.2</td>
</tr>
<tr>
<td>GKO</td>
<td>Lysate + CT</td>
<td>0.13 ± 0.12</td>
<td>1054.3 ± 327.9b</td>
</tr>
<tr>
<td>CT</td>
<td>0</td>
<td>0.00 ± 0.00</td>
<td>180.7 ± 107.8</td>
</tr>
<tr>
<td>IL-4−/−</td>
<td>Lysate + CT</td>
<td>0.13 ± 0.12</td>
<td>221.3 ± 69.6a</td>
</tr>
<tr>
<td>CT</td>
<td>0</td>
<td>0.00 ± 0.00</td>
<td>210.4 ± 4.6</td>
</tr>
<tr>
<td>WT</td>
<td>Lysate + CT</td>
<td>11.6 ± 3.2a</td>
<td>175.0 ± 30.6a</td>
</tr>
<tr>
<td>CT</td>
<td>5.0 ± 0.6</td>
<td>0.00 ± 0.00</td>
<td>38.3 ± 1.8</td>
</tr>
</tbody>
</table>

*Groups of 9–10 mice were immunized orally with *H. pylori* lysate together with CT or CT alone and then challenged with *H. pylori* bacteria. At 2 wk after challenge, spleen cells from the mutant and WT mice were cultured in the presence or absence of 2 μg/ml of *H. pylori* lysate Ags or anti-CD3. Cell-free supernatants were collected 96 h later and analyzed in triplicate for the concentration of IFN-γ or IL-13 by ELISA. The results are given as mean ± SEM of three pairs of mice per group.

*p < 0.05.
*p < 0.005.
*p < 0.003.
*p < 0.0005.
*p < 0.05.
*p < 0.005.
*p < 0.003.
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*p < 0.0005.
to develop protection, whereas IL-4−/− and WT mice were significantly protected against a live challenge infection (Fig. 2). Furthermore, coadministration of rIL-12 to intranasal immunizations in WT mice substantially improved protection and reduced the bacterial load subsequent to a live challenge infection. Contrary to the work of others, we found that a Th2-type response did not play a major role in protection, but interestingly appeared to contribute to the development of the postimmunization gastritis, as IL-4−/− mice had significantly less gastritis than WT mice (Fig. 3). Protection was strongly associated with postimmunization gastritis and local infiltration of CD4+ T cells in the gastric mucosa. Only the well-protected strains, IL-4−/− and WT mice, showed strong influx of CD4+ T cells to the gastric mucosa. Splenic T cells, isolated from the well-protected mice, demonstrated strong Th1 activity and, therefore, we assume that all Th1 cells dominated the infiltrating CD4+ T cells in the gastric mucosa. Although not formally proven, it is reasonable to believe that these cells were responsible for reducing bacterial load and enhancing gastric inflammation as both unprotected GKO and IL-12−/− mice exhibited reduced T cell infiltration and gastritis. Thus, immune protection involves a certain level of gastric inflammation, but we do not believe that the inflammation alone is mediating protection. Rather, postimmunization gastritis is usually found in well-protected strains as a consequence of Th1 cells invading the mucosa, but apart from inflammation, immune protection also has other components in it as revealed in the IL-4−/− mice, which exhibited less postimmunization gastritis but were equally well-protected compared with WT mice.

We used IFN-γ, IL-12, and IL-4 gene-disrupted mice to assess the contribution of T cell subsets in protective immunity against H. pylori infection. IL-12 has been shown to be important for IFN-γ production by a variety of immune cells, including CD4+ T cells, NK cells, activated B cells, and macrophages (28, 29). IFN-γ production and delayed-type hypersensitivity responses are markedly reduced in IL-12 p40−/− mice (18). A major function of IL-12 is to promote the differentiation of Th1 cells (30, 31) and to serve as a costimulus for maximum IFN-γ secretion by already committed Th1 cells (32). In the present study, splenocytes from immunized and challenged IL-12−/− mice produced no IFN-γ after stimulation with recall Ag in vitro. This was not a result of poor priming of CD4+ T cells in IL-12−/− mice, as high levels of IL-13 were observed in cultures with recall Ag. Moreover, IL-12−/− mice exhibited comparable levels of specific IgG1, IgG2a, and IgA to WT mice after oral immunization, arguing against the idea that IL-12−/− mice were poor responders to H. pylori Ags. However, IL-13 was not associated with protection as T cells from unprotected GKO mice produced large amounts of IL-13 but were completely unprotected against a live challenge infection.

Gastric colonization with H. pylori bacteria was not influenced by the ability to respond with inflammation and a Th1 response, because at 2 and 8 wk after inoculation with live bacteria all strains exhibited comparable levels of colonizing bacteria. These findings are at variance with those of Sawai et al. (33), who reported that a strain of H. pylori (KP48a) without the ability to colonize C57BL/6 mice was able to colonize GKO mice, suggesting that the ability to produce IFN-γ could impair H. pylori colonization of the gastric epithelium. Contrary to these findings, Eaton et al. (34) reported that H. pylori colonization was not significantly different in SCID recipients of IFN-γ-deficient splenocytes than in recipients of normal splenocytes, indicating that the absence of IFN-γ did not affect the ability to colonize the gastric mucosa. In the present study, we could not detect any influence of either IFN-γ or IL-12 on the extent of primary colonization of the gastric mucosa. The GKO mice exhibited less gastritis than IL-12−/− and WT mice at 8 wk after infection, but were equally strongly colonized, demonstrating that IFN-γ is important for the development of gastritis.

The regulatory roles of Th1 and Th2 cells in immune protection against H. pylori infection are incompletely understood. Only a few reports have addressed the roles of Th subsets in Helicobacter immunity and immunopathology (12, 13, 16). The study by Mohammadi et al. (12) argued against a protective role of Th1 cells and IFN-γ, because in vivo neutralization of IFN-γ had no effect on the level of colonizing H. felis bacteria after challenge, but rather resulted in a significant reduction of gastric inflammation. They also reported that adoptive transfer of H. felis-specific Th2 cell lines resulted in the reduction of H. felis colonization (13). Thus, it was concluded that Th1 cells were more responsible for the pathogenesis of Helicobacter infection than protection, whereas the Th2 cells were involved in protection. By contrast, the present data clearly demonstrates that Th1 cells and the cytokines IFN-γ and IL-12 are crucial for the development of immune protection against H. pylori infection. These factors are also responsible for promoting the development of gastric inflammation. Th2 cells, in contrast, as demonstrated by our findings in the IL-4−/− mice, are not important for protection. These mice developed significant protection comparable to that in WT mice. However, Th2

![Graph](http://www.jimmunol.org/Downloadedfromhttp://www.jimmunol.org/)

FIGURE 4. Anti-H. pylori Ab titers in sera and gastric secretions of immunized challenged mice. Sera (A) and gastric secretions (B) from IL-12−/−, GKO, IL-4−/−, and WT mice immunized with lysate plus CT were analyzed for H. pylori lysate-specific IgG1 (■), IgG2a (□), and IgA (□) at 2 wk after challenge using ELISA. The results are given as mean ± SEM of 9–10 mice per group. One representative experiment of two is shown.
cells, or IL-4 itself, may contribute to the development of inflammation because the protected IL-4−/− mice exhibited significantly less postimmunization gastritis. Consistent with the notion that Th2 cells are dispensable for protection, Aebischer et al. (16) reported that IL-4Rα−/− mice (35, 36) were strongly protected against H. pylori infection. Furthermore, adoptive transfer of Helicobacter-specific spleen CD4+ T cells into the IL-4Rα−/− mice induced significant protection (37). Gottwein et al. (15), in contrast, reported that both Th1 and Th2 cells could induce protection. They found that systemic immunization with lystate, together with either CFA or alum, resulted in Helicobacter-specific immunity dominated by Th1 and Th2 cells, respectively, and both induced significant protection. In support of the results of the present study, Jiang et al. (17) showed that skewing toward a Th1-dominated environment using a replication-defective adenosine resulted in a significant reduction of an established H. felis infection in WT, but not in IFN-γ and IL-12-deficient, mice.

The mechanism(s) by which Th1 cytokines induce protective immunity or reduce Helicobacter colonization is poorly understood. The lack of IFN-γ and IL-12 may have some effect on the production of specific Abs as, for instance, in GKO mice, IgG2a is reduced (Fig. 4) (17). In contrast, Abs did not correlate with protection in the present study and in previous studies using IgA−/− and μMT mice significant protection developed following immunization (9, 10). The CD4+ T cells that were recruited into the gastric mucosa of protected IL-4−/− and WT mice affected the microenvironment by driving the development of gastritis, and this could have direct implications for the ability to colonize the epithelium. Postimmunization gastritis appears to be a consistent finding in all models showing protection against Helicobacter infection (8, 9, 23, 38, 40). Equally possible though, Th1 cells in the gastric mucosa could affect other mechanisms that would affect bacterial survival. In this context, the ability of macrophages to phagocytose and control bacterial infection is under the strong influence of IFN-γ and IL-12 and may play a role in the control also of gastric Helicobacter infection. Moreover, IFN-γ could enhance APC functions of gastric epithelial cells, thereby, focusing specific T cells to the epithelium (41, 42). Such a mechanism coupled with an increased apoptosis of epithelial cells, induced by the bacteria, could provide unfavorable growth conditions for the bacteria to survive in the gastric mucosa (43–45). However, the mild inflammation in the protected IL-4−/− mice argues that other more direct effects of Helicobacter-specific gastric CD4+ T cells may exist. It is not yet clear what these effects could be and extended studies in the mouse model are, therefore, much needed. Clearly, the immune response and gastritis seen in the chronically infected mice and the postimmunization gastritis appear to be very similar, but a fundamental difference is that the latter hosts components that result in bacterial clearance. This was recently demonstrated in an elegant study by Garhart et al. (46), who found that immunized and protected mice exhibited a transient bacterial colonization associated with gastritis, but which subsides over time as the bacterial load diminishes. Thus, prophylactic immunization does not prevent colonization by H. pylori completely, but it adds a quality to the resistance against infection that is not observed in unimmunized infected animals. Understanding the fundamental difference between these two types of responses is necessary for the development of effective prevention and therapy against diseases caused by H. pylori infection.

References


