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Helicobacter pylori-Induced Mucosal Inflammation Is Th1 Mediated and Exacerbated in IL-4, But Not IFN-γ, Gene-Deficient Mice

Lesley E. Smythies,* Ken B. Waites, † J. Russell Lindsey, ‡ Paul R. Harris, 2* Paolo Ghiara, § and Phillip D. Smith3*¶

To elucidate the pathogenesis of Helicobacter pylori-associated gastritis, we studied immune responses of C57BL/6J wild-type (WT), SCID, and gene deficient (IFN-γ−/− and IL-4−/−) mice following infection with a pathogenic isolate of H. pylori (SPM326). During early infection in WT mice, mononuclear and polymorphonuclear cells accumulated in the gastric lamina propria, and the numbers of cells in the inflamed mucosa expressing IFN-γ, but not IL-4, mRNA rose significantly (p < 0.005), consistent with a local Th1 response. Splenic T cells from the same infected WT mice produced high levels of IFN-γ, and no detectable IL-4, and low amounts of IL-10 following in vitro H. pylori urease stimulation, reflecting a systemic Th1 response. Infected C57BL/6J SCID mice did not develop gastric inflammation despite colonization by many bacteria. Infected C57BL/10J and BALB/c mice also did not develop gastric inflammation and displayed a mixed Th1/Th2 splenic cytokine profile. These data imply a major role for the Th1 cytokine IFN-γ in H. pylori-associated gastric inflammation in C57BL/6J mice. Compared with WT animals, infected IL-4−/− animals had more severe gastritis and higher levels of IFN-γ production by urease-stimulated splenocytes (p < 0.01), whereas IFN-γ−/− mice exhibited no gastric inflammation and higher levels of IL-4 production by stimulated splenocytes. These findings establish C57BL/6J mice as an important model for H. pylori infection and demonstrate that up-regulated production of IFN-γ, in the absence of the opposing effects of IL-4 (and possibly IL-10), plays a pivotal role in promoting H. pylori-induced mucosal inflammation. The Journal of Immunology, 2000, 165: 1022–1029.

Helicobacter pylori is the most common bacterial pathogen of the gastrointestinal tract in humans worldwide. Although noninvasive, the bacterium causes gastritis and gastroduodenal ulceration and has been linked to the development of gastric carcinoma and lymphoma (1–4). The pathogenesis of these sequelae likely involves the interaction between host mucosal cells (5, 6) and bacterial products, such as urease (7, 8), vaculating cytotoxin (VacA)1 (9, 10), and cytotoxin-associated gene product (CagA)1 (11, 12), resulting in local inflammation. The recent sequencing of the H. pylori genome should facilitate the identification of other bacterial products that participate in the disease process (13).

The inflammatory response to H. pylori is less well characterized than the bacterium and its products. However, recent studies have suggested that the Th paradigm may apply to H. pylori infection. According to this paradigm, Th1 (IFN-γ, IL-2) and Th2 (IL-4, -5, -10) lymphocyte-derived cytokines regulate the resolution of intracellular and extracellular infections, respectively (14). During infection with H. pylori, an extracellular pathogen, a failure to promote Th2 relative to Th1 responses would in this context impede resolution of the infection and promote chronic inflammation. Supporting this concept are animal studies in which infection with Helicobacter felis, a very different Helicobacter species that lacks VacA as well as the entire CagA pathogenicity island, stimulated a Th1 response in mice (15, 16) and human studies in which gastric lymphocytes from H. pylori-infected subjects displayed a Th1 phenotype (17–19). To extend these findings, we characterized the Th cytokine responses of splenic and gastric lymphocytes in C57BL/6J genetically intact (wild type, WT) mice, C57BL/6J SCID mice, C57BL/6J IFN-γ−/− (20), and C57BL/6J IL-4−/− (21) animals infected with a pathogenic strain of H. pylori. In addition, we compared the responses of WT C57BL/6J animals with those of two other inbred strains of mice, C57BL/10J and BALB/c.

Materials and Methods

Bacteria

VacA+ /CagA− H. pylori strain SPM326 (22) was cultured on Brucella agar (Remel Laboratories, Lenexa, KS) supplemented with 5% sheep blood, trimethoprim, polymyxin B, and vancomycin in a microaerophilic, humidified atmosphere at 35°C. The bacteria were confirmed to be H. pylori by Gram stain morphology and the presence of urease, oxidase, and catalase activity (see below) and stored at -70°C in Brucella broth plus sterile glycerol 20% v/v. To infect mice, low-passaged organisms were thawed, cultured for 72 to 96 h on Brucella agar, enumerated by nephelometry, and administered by gavage within 20 min of harvesting to prevent bacterial transition from infective spiral to noninfective coccoid forms (23, 24).
Animals

Six- to 8-wk-old male C57BL/6J WT, C57BL/6J IL-4−/− (C57BL/6J-Il4tm1Nnt), C57BL/6J IFN-γ−/− (C57BL/6J-Ifngtm1It), C57BL/6J SCID (C57BL/6J-Pkd1tm1sSm), C57BL/10J, and BALB/c mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Representative mice of each strain were monitored in each experiment for the presence of murine pathogens using our previously described comprehensive battery of tests (25), including virus serologies, bacterial cultures, endo- and ectoparasite exams, and histopathology of all major organs, by one of us (J.R.L., Animal Health Surveillance/Diagnostic Laboratory, Department of Comparative Medicine, University of Alabama, Birmingham, AL), and PCR on cecal contents for Helicobacter hepaticus and Helicobacter bilis (Annmed Biosafe, Rockville, MD). All test results were negative.

Experimental design

Mice fasted overnight were inoculated three times by gavage with 250 µl of *H. pylori* bacteria (1–5 × 10^8 CFU/ml) with 1 day separating each inoculation. Age-matched control animals were mock-inoculated with PBS. Before inoculation (15 min), all animals were gavaged with 250 µl 0.1 M sodium bicarbonate. Four inoculated and one control C57BL/6J, C57BL/10J, and BALB/c mouse (five per strain) were sacrificed at each 2, 5, and 10 wk time point (n = 2 longitudinal experiments). In two additional experiments, C57BL/6J IL-4−/−, IFN-γ−/−, and SCID animals were sacrificed 5 wk after inoculation.

Confirmation of infection

Gastric tissue specimens from each animal (n = 120) were examined using microbiological, histological, and molecular techniques with the investigator(s) blinded in each experiment as to the mouse strain and results from the companion techniques.

**Microbiology.** Resected gastric tissue (one-third of the stomach dissected longitudinally) from each animal was streaked directly onto a *Brucella* agar plate and then incubated within 30 min of harvest at 35°C in a microaerophilic atmosphere for 72–96 h until colonies became visible. Colonies were judged to be *H. pylori* based on three criteria: 1) characteristic pinhead-sized, yellow appearance; 2) typical Gran-negative and spiral rod morphology of the colony bacteria; and 3) the presence of urease (Rapid Urea Broth; Becton Dickinson, Cockeysville, MD), catalase, and oxidase (BACTIDROP Oxidase, Remel Laboratories).

**Histology.** Two serial sections of formalin-fixed gastric tissue from each animal were treated with either Warthin-Starry silver stain or hematoxylin and eosin (H&E) and examined for the presence of *H. pylori* and bacteria-associated pathology. Bacteria were identified as *Escherichia coli* when PCR were run with DNA from *H. pylori* infected and uninfected C57BL/6 mice were bound to silanized slides, deparaffinized, and hydrated in graded alcohol solutions. After treatment with 0.2 M HCl (30 min) and permeabilization with proteinase K (0.1 mg/ml, 15 min) (Sigma, St. Louis, MO), the slides were treated with 0.1 M succinic anhydride in 0.1 M triethanolamine buffer (pH 8.0) followed by 0.1 M iodoacetamide in triethanolamine buffer to remove background and block eosiophilic myelocyte basic protein and then derivatized with acetic anhydride. The primary antibody, the slides were incubated overnight with *35S*-labeled riboprobes (2 × 10^5 counts/section) produced from subcloned DNA encoding IFN-γ and IL-4 in the antisense (complementary) configuration (Lofstrand Laboratories, Gaithersburg, MD). After extensive washing and removal of single-stranded RNA with ribonuclease A followed again by washing, the slides were dipped in emulsion, exposed for 4 days, developed, and stained with H&E. Cells were considered positive when ≥10 grains above background were detected overlying a cell in a stellate pattern in an area of 200 mm^2^.

To assess the levels of IFN-γ and IL-4 mRNA-expressing cells in gastric mucosa, we measured IFN-γ and IL-4 mRNA using an inverse sampling technique previously described in detail (32). With the reader blinded as to the source of the tissue section, nonoverlapping microscopic fields of the corpus and antrum were analyzed until a field containing one or more positive cells was found. The number of negative fields and the number of positive cells in the last field were used to calculate the prevalence of IFN-γ and IL-4 mRNA-expressing cells in the gastric mucosa (see footnote to Table I). To compliment the prevalence analysis, the frequency of IFN-γ- and IL-4 mRNA-expressing cells in the gastric mucosa of infected and naive mice was also determined by counting the numbers of positive cells/mm of gastric tissue, also in a blinded protocol.

**Measurement of splenic T lymphocyte production of IFN-γ, IL-4, and IL-10**

The optimal culture conditions for cytokine production were established in preliminary experiments using spleen cells from infected C57BL/6J WT mice at 5 wk postinoculation. In these experiments, splenocytes were cultured in 1:1 37°C, 48, 72, and 96 h serial experiments (Lofstrand, Gaithersburg, MD). After extensive wash, the slides were dipped in emulsion, exposed for 4 days, developed, and stained with H&E. Cells were considered positive when ≥10 grains above background were detected overlying a cell in a stellate pattern in an area of 200 mm^2^.

To validate the levels of IFN-γ and IL-4 mRNA-expressing cells in the gastric mucosa, we measured IFN-γ and IL-4 mRNA using an inverse sampling technique previously described in detail (32). With the reader blinded as to the source of the tissue section, nonoverlapping microscopic fields of the corpus and antrum were analyzed until a field containing one or more positive cells was found. The number of negative fields and the number of positive cells in the last field were used to calculate the prevalence of IFN-γ and IL-4 mRNA-expressing cells in the gastric mucosa (see footnote to Table I). To compliment the prevalence analysis, the frequency of IFN-γ- and IL-4 mRNA-expressing cells in the gastric mucosa of infected and naive mice was also determined by counting the numbers of positive cells/mm of gastric tissue, also in a blinded protocol.

**Molecular analysis.** RT-PCR was used to detect *H. pylori* mRNA in gastric tissue specimens. Briefly, 60–100 µg of gastric tissue was homogenized in 1 ml guanidinium isothiocyanate lysis buffer, and RNA was purified using the Qiagen RNeasy kit (Qiagen, Chatsworth, CA). RNA (1 µg) from individual mouse was unoiled (65°C, 5 min) and reverse transcribed into cDNA using as primers random hexamers (Pharmacia Biotech, Piscataway, NJ). The cDNA was amplified using primers for the *H. pylori* 16S ribosome gene: forward primer, 5′-GCTAAAGATCAGCCTAT GTCC-3′ and reverse primer, 3′-TTGCAATCGTCGTCCTATG-5′ (27); *CaqA* forward primer, 5′-GTAACAGGGCCAGTTCCTTTGGAG-3′ and reverse primer, 3′-GTTGCGAACCTTGAGGCTCTTG-5′; and the control gene GAPDH: forward primer, 5′-GTTCTTCACCCATCG GAGAAGGCT-3′ and reverse primer, 3′-CTGGCCCATGAGGTT CCSCTCA-5′ (29). PCR products were visualized by 2% agarose gel electrophoresis in the presence of ethidium bromide (0.5 µg/ml), and a 1-kb DNA ladder (Bethesda Research Laboratory, Gaithersburg, MD) was included in each gel. Band intensities for the 16S ribosome and *CaqA* genes (but not those for the adenase gene (30) were used to be specific for *H. pylori* when PCR were run with DNA from *H. pylori* and *Escherichia coli* (data not shown).

In situ hybridization for IFN-γ and IL-4 mRNA-expressing cells

To assess the levels of IFN-γ and IL-4 mRNA-expressing cells in the gastric mucosa of *H. pylori*-infected and naive mice, the expression of IFN-γ and IL-4-specific mRNA in gastric tissue was analyzed by in situ hybridization according to our previously described protocol (31–33). Briefly, formalin-fixed, paraffin-embedded sections of gastric tissue from *H. pylori*-infected and uninfected C57BL/6 mice were bonded to silanized slides, deparaffinized, and hydrated in graded alcohol solutions. After treatment with 0.2 M HCl (30 min) and permeabilization with proteinase K (0.1 mg/ml, 15 min) (Sigma, St. Louis, MO), the slides were treated with 0.1 M succinic anhydride in 0.1 M triethanolamine buffer (pH 8.0) followed by 0.1 M iodoacetamide in triethanolamine buffer to remove background and block eosiophilic myelocyte basic protein and then derivatized with acetic anhydride. The primary antibody, the slides were incubated overnight with *35S*-labeled riboprobes (2 × 10^5 counts/section) produced from subcloned DNA encoding IFN-γ and IL-4 in the antisense (complementary) configuration (Lofstrand Laboratories, Gaithersburg, MD). After extensive washing and removal of single-stranded RNA with ribonuclease A followed again by washing, the slides were dipped in emulsion, exposed for 4 days, developed, and stained with H&E. Cells were considered positive when ≥10 grains above background were detected overlying a cell in a stellate pattern in an area of 200 mm^2^.

In situ hybridization for IFN-γ and IL-4 mRNA-expressing cells in gastric tissue from 1) *H. pylori*-infected animals with the cytokine-specific sense (noncomplementary) probe, 2) naive animals with the cytokine-specific antisense probe, and 3) infected animals with an irrelevant (HV-1) probe (32). The prevalence of IFN-γ and IL-4 mRNA-expressing cells in gastric mucosa was measured using an inverse sampling technique previously described in detail (32). With the reader blinded as to the source of the tissue section, nonoverlapping microscopic fields of the corpus and antrum were analyzed until a field containing one or more positive cells was found. The number of negative fields and the number of positive cells in the last field were used to calculate the prevalence of IFN-γ and IL-4 mRNA-expressing cells in the gastric mucosa (see footnote to Table I). To compliment the prevalence analysis, the frequency of IFN-γ- and IL-4 mRNA-expressing cells in the gastric mucosa of infected and naive mice was also determined by counting the numbers of positive cells/mm of gastric tissue, also in a blinded protocol.

**Statistical analysis**

Results are expressed as the mean ± SEM. Data were compared using Student’s or paired t test and considered significant at values of p < 0.05.
mRNA-expressing cells in inflamed gastric mucosa of H. pylori
To define the role of Th cytokines in murine infected WT C57BL/6 mice
Increased numbers of IFN-γ detected in any of the control mice.
H. pylori served among the infected mouse strains, and mucosal invasion by columnar (glandular) and stratified squamous epithelium. No clear pus and antrum but most commonly at the junction between the epithelium. Infected crypts were present throughout the corpus and antrum but most commonly at the junction between the columnar (glandular) and stratified squamous epithelium. No clear differences in the distribution or numbers of bacteria were observed among the infected mouse strains, and mucosal invasion by H. pylori was not observed in any animal. H. pylori were not detected in any of the control mice.

**FIGURE 1.** Histology of the gastric antrum in C57BL/6 WT mice infected with H. pylori. A, Gastric antrum from a representative C57BL/6 mouse 10 wk after inoculation with H. pylori SPM326 shows moderate infiltration of polymorphonuclear and mononuclear cells between the bases of the glands and the muscularis mucosae. B, Gastric antrum from a control C57BL/6J mouse 10 wk after mock-inoculation shows no inflammation. (H&E; magnification, ×200; magnification of inset, ×320).

Increased numbers of IFN-γ mRNA-expressing cells but not IL-4 mRNA-expressing cells in inflamed gastric mucosa of H. pylori-infected WT C57BL/6 mice

To define the role of Th cytokines in murine H. pylori infection, we first enumerated the IFN-γ and IL-4 mRNA-expressing cells in gastric mucosa of H. pylori-infected and control (uninfected) WT C57BL/6 mice (Fig. 2 and Table I). Cells that expressed IFN-γ or IL-4-specific mRNA displayed lymphocyte morphology (Fig. 2A, inset) and were detected exclusively in the lamina propria, usually at the bases of crypts but also throughout the lamina propria (Fig. 2A). The prevalence of IFN-γ mRNA-expressing cells in the gastric mucosa of H. pylori-infected C57BL/6J animals (8.84 ± 1.42) was significantly greater than the prevalence of such cells in uninfected mice (2.30 ± 1.19; p < 0.002) (Table I). In contrast, the prevalence of IL-4 mRNA-expressing cells in the infected mice (5.74 ± 1.48) was not significantly different from that of uninfected animals (3.98 ± 0.75; p < 0.179). Moreover, in the infected mice, the prevalence of mucosal cells that expressed IFN-γ mRNA (8.84 ± 1.42) was significantly greater than the prevalence of cells that expressed IL-4 mRNA (5.74 ± 1.48; p < 0.030) (Table I).

To compliment the prevalence studies, we also determined the frequency of cytokine mRNA-positive cells in coded tissue sections. In naive animals, the frequency of cells expressing mRNA for IFN-γ per millimeter of tissue (2.05 ± 0.60) and IL-4 (4.11 ± 0.13) was not significantly different (p < 0.06). Following infection, the frequency of IFN-γ mRNA-positive cells per millimeter tissue (11.23 ± 2.94) and IL-4 mRNA-positive cells (6.17 ± 3.08) increased, but only the frequency of cells expressing IFN-γ mRNA was significantly higher than that of naive mice (p < 0.008). Furthermore, in infected mice, the frequency of cells expressing mRNA for IFN-γ was significantly greater (p < 0.007) than that of cells expressing IL-4 mRNA. Thus, both prevalence and frequency analyses indicated the number of IFN-γ mRNA-expressing cells significantly exceeded that of IL-4 mRNA-expressing cells in the inflamed gastric mucosa of H. pylori-infected mice.

Increased IFN-γ and reduced IL-4 and IL-10 production by splenic T lymphocytes in H. pylori-inoculated mice

To correlate the level of IFN-γ mRNA-expressing cells in the gastric mucosa with systemic T cell-mediated responses, we next determined the ability of splenocytes from individual H. pylori-inoculated and control mice to produce IFN-γ, IL-4, and IL-10 in vitro following stimulation with H. pylori recombinant urease.

Preliminary experiments revealed that the production of IFN-γ and IL-4 by splenic T lymphocytes from H. pylori-infected C57BL/6 WT mice at 5 wk postinoculation was proportional to the amount of recombinant urease (1–10 μg/ml) used to stimulate the cells, with slightly lowered cytokine production above 10 μg/ml urease (Fig. 3A). Unstimulated cells did not produce IFN-γ or IL-4. Differing kinetics of IFN-γ and IL-4 release were observed in urease-stimulated splenic T cell cultures (Fig. 3B); production of IFN-γ was detected after 24 h, with maximum levels at 48–72 h, but production of IL-4 was not observed until 72 h, with increased levels detected at 96 h. Therefore, in subsequent experiments splenic T lymphocytes were cultured in the presence or absence of 5 μg/ml recombinant urease for 72 h.

Cells from H. pylori-infected WT C57BL/6 mice produced markedly elevated levels of IFN-γ, which increased gradually over the 10-wk study period (Fig. 4). In sharp contrast, IL-4 production was below the level of detection at each time point (2 pg/ml). Because IL-10, like IL-4, down-regulates Th1 lymphocyte and macrophage activity, we also assayed urease-stimulated splenocytes for IL-10 production. Coincident with increasing production of IFN-γ, IL-10 levels decreased over the time course (Fig. 4), becoming undetectable at 10 wk postinoculation when gastric inflammation was most intense. Urease-stimulated splenic lymphocytes from uninfected control mice and unstimulated splenocytes from infected animals produced no detectable IFN-γ or IL-4 and nearly undetectable IL-10 (data not shown). These findings indicate that the elevated IFN-γ production by splenocytes from H. pylori-infected WT C57BL/6J mice mirror the increased number of IFN-γ mRNA-expressing cells in the inflamed gastric mucosa of these animals. Thus, both systemic and local cell-mediated responses to H. pylori are dominated by the Th1 cytokine IFN-γ.

Th cytokine production by splenic T lymphocytes from infected C57BL/10J and BALB/c mice displayed a mixed Th1/Th2 profile with the Th2 cytokines IL-4 and IL-10 more prominent at the earlier time points than for the C57BL/6J animals (Fig. 4, inset). Although the magnitude of the IFN-γ response was similar in all three strains, no recruitment of either lymphocytes or polymorphonuclear cells was observed in the gastric mucosa of infected C57BL/10J or BALB/c animals in the first 10 wk after infection.
H. pylori-associated gastric lesions in IL-4−/− mice

To extend the above findings that IFN-γ played a key role in mediating H. pylori-associated inflammation, we next inoculated C57BL/6J WT, SCID, IFN-γ−/−, and IL-4−/− mice with H. pylori and evaluated their stomachs for H. pylori colonization and mucosal inflammation. All mice became colonized with H. pylori. However, despite the presence of many bacteria on the epithelium and in gastric crypts, infected SCID and IFN-γ−/− mice did not develop any gastric inflammation (Fig. 5A). However, in infected WT mice, gastric inflammation was significantly greater than in SCID or IFN-γ−/− animals (p ≤ 0.01), with mononuclear and polymorphonuclear cells present throughout the corpus and antral regions. Moreover, in infected IL-4−/− mice, higher levels of gastric inflammation was reflected in a higher gastritis score compared with WT mice (p < 0.2), SCID mice (p < 0.06), and IFN-γ−/− mice (p < 0.06) (Fig. 5A), and the gastric inflammation in the infected IL-4−/− mice was associated with the presence of gastric lesions (Fig. 5B).

Coincident with the enhanced gastric inflammation in the IL-4−/− mice, urease-stimulated splenic lymphocytes from the same animals produced log-fold higher levels of IFN-γ compared with splenic lymphocytes from genetically intact mice (p ≤ 0.01) (Fig. 6). As expected, no urease-stimulated IL-4 production was detected in cultures from IL-4−/− mice, but urease-stimulated spleen cells of IFN-γ−/− mice produced substantially more IL-4 than cultures from WT C57BL/6 splenocytes, reflecting the absence of

Table I. Prevalence of cells expressing IL-4 and IFN-γ mRNA in gastric tissue from H. pylori-infected* and control C57BL/6 mice

<table>
<thead>
<tr>
<th>Cytokine mRNA</th>
<th>Treatment</th>
<th>Average No. Cells/Quadrant (Q)</th>
<th>Average No. Negative Fields Counted (N)</th>
<th>Average No. Positive Cells/Field (M)</th>
<th>Prevalence (×10^6) (P)^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-4</td>
<td>Infected</td>
<td>96.95 ± 9.95</td>
<td>2.60 ± 0.47</td>
<td>6.05 ± 2.74</td>
<td>5.74 ± 1.48*</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>93.30 ± 9.22</td>
<td>4.17 ± 2.43</td>
<td>3.48 ± 0.95</td>
<td>3.98 ± 0.75</td>
</tr>
<tr>
<td></td>
<td>Infected</td>
<td>96.95 ± 9.95</td>
<td>1.69 ± 0.25</td>
<td>6.64 ± 0.94</td>
<td>8.84 ± 1.42*</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>93.30 ± 9.22</td>
<td>6.60 ± 2.45</td>
<td>2.93 ± 0.56</td>
<td>2.30 ± 1.19?</td>
</tr>
</tbody>
</table>

* Ten weeks postinoculation.

P = 1/(Q [N + 1/(M + 1)]), where Q is the average number of cells per quadrant, N is the average number of negative fields, and M is the average number of positive cells per first positive field (31). Infected and control animal prevalence values were derived from two or three sections, each section analyzed in triplicate, from four animals per group.

†, p = 0.03.

‡, p = 0.002.

FIGURE 2. Identification of IFN-γ and IL-4 mRNA-expressing cells in mouse gastric tissue by in situ hybridization with IFN-γ- and IL-4-specific antisense probes. Consecutive sections of gastric antrum from an H. pylori-infected and an uninfected control C57BL/6J mouse 10 wk after inoculation shows cells expressing mRNA for (A–D) IFN-γ and (E and F) IL-4. Sections hybridized with IFN-γ and IL-4 sense probes showed no positive cells (data not shown). (See Table I for statistical analysis of the prevalence of the IFN-γ and IL-4 mRNA-expressing cells in H. pylori-infected and uninfected control mice.) A and B, Bright field illumination and stained with H&E. C–F, Dark field illumination. Magnification, ×100; magnification of inset, ×200 (n = 2).
the counterregulatory effect of IFN-γ. In addition, spleen cells from IL-4−/− and IFN-γ−/− mice produced little IL-10 in response to urease stimulation (Fig. 6). Cells from uninfected control animals did not produce cytokines in response to urease stimulation (Fig. 6), although they responded well to stimulation with Con A (data not shown). Thus, local and systemic inflammatory responses were exacerbated in IL-4−/− mice and reduced in IFN-γ−/− animals, implying a counterregulatory balance between these two cytokines in inducing inflammation in this model.

Discussion
This is the first in-depth report of cell-mediated responses in the mouse model of gastric Helicobacter infection using H. pylori, the bacterial species that infects humans. H. pylori infection in C57BL/6J mice induced a polymorphonuclear and mononuclear cell infiltrate in the gastric antrum and corpus, the mononuclear...
cells containing predominantly IFN-γ mRNA-expressing cells. This local Th1 response was mirrored systemically by abundant IFN-γ production and undetectable or low IL-4 and IL-10 production by H. pylori urease-stimulated splenic T lymphocytes. These data implicate a Th1 lymphocyte response in the pathogenesis of the gastric inflammation associated with H. pylori infection. In contrast to C57BL/6d mice, C57BL/10J and BALB/c mice infected with H. pylori did not develop gastric inflammation and displayed a mixed Th cytokine profile, including early production of IL-4 and persistent production of IL-10. Interestingly, Ag-stimulated splenocytes from C57BL/10J and BALB/c mice produced equivalent amounts of IFN-γ compared with cells from C57BL/6J mice. The absence of gastritis in these mice underscores the role of IL-4 and possibly IL-10 in down-modulating inflammation mediated by IFN-γ.

The predominance of IFN-γ vs IL-4 mRNA-expressing cells in the gastric mucosa of infected C57BL/6d mice reported here concurs with the results of Goto et al. (34), who found IFN-γ but not IL-4 message in gastric tissue homogenates of H. pylori-infected mice. The origin of these IFN-γ mRNA-expressing cells is not clear. However, studies of murine infection with H. felis, the feline Helicobacter species, have demonstrated that Ag-stimulated lymphocytes recovered from gastric mucosa and spleens have similar cytokine profiles when stimulated with Ag in vitro (15). Also, transfer of splenic T cells from H. felis- or H. pylori-infected mice enhance gastric pathology in recipient infected animals (16, 35). Moreover, Ibraghimov et al. (36) have shown that gastric intraepithelial lymphocytes isolated from SS1-infected C57BL/6d mice display a CD4+ memory T cell phenotype typical of peripheral T cells recruited from the circulation. These data are consistent with the concept that Ag released from bacteria that have colonized the stomach is carried from the gastric mucosa, perhaps by mucosal dendritic cells, to the draining lymph nodes where clonal expansion of H. pylori-specific T cells occurs. Memory T cells exiting the lymph nodes could then recruit to the gastric mucosa or repose in the spleen (37). This sequence of events would permit splenic and lamina propria T cells to produce cytokines whose profiles mirror one another, as shown by our data using H. pylori-infected C57BL/6d WT mice and by others using the H. felis model (15). The importance of T cells in mediating gastric mucosal inflammation during H. pylori infection is underscored by our observation that H. pylori-infected SCID mice, which lack T cells but have NK cells, developed no gastric inflammation. Interestingly, our studies with C57BL/10J and BALB/c mice revealed that the H. pylori-specific splenic T cells in these animals did not recruit to the gastric mucosa. Whether this was due to some feature inherent to the cells themselves or to the gastric tissue influencing cell recruitment is not known. However, our data suggest a possible role for IL-4 (or a cytokine not measured here), because inflammation occurred only in the absence of this cytokine in our model.

Infection of IL-4−/− and IFN-γ−/− mice by the Helicobacter species that infects humans, as reported here, reveals a direct correlation between induction of a dominant H. pylori-specific Th1-mediated immune response and the development of gastric inflammation. Infected IL-4−/− mice displayed the highest levels of IFN-γ in Ag-stimulated splenic T lymphocyte cultures and the most intense H. pylori-associated gastric inflammation, whereas IFN-γ−/− mice displayed a predominantly IL-4 splenic T cell response to H. pylori urease and no gastric inflammation. Moreover, we observed significantly less gastritis in IFN-γ−/− mice compared with WT and IL-4−/− animals, further supporting a role for IFN-γ in inducing inflammation, particularly in the absence of IL-4. Our results also concur with those of Sawai, et al. (38), who found no inflammation in long-term infected IFN-γ−/− mice. However, because IFN-γ−/− mice have several other defects that potentially could reduce H. pylori-associated inflammation, including impaired macrophage function, reduced class II Ags, and reduced NK cell activity, the down-regulation of gastritis reported here in IFN-γ−/− mice cannot be attributed exclusively to the lack of IFN-γ.

Our results, together with those of others (16, 35, 39), indicate that H. pylori-associated gastric inflammation in mice is a consequence of both the Helicobacter isolate and the host’s T cell-mediated response to the isolate. Both responses contribute to the variation in the intensity of gastric inflammation that we observed here and reported by others (35, 39, 40). The Helicobacter used in this study (H. pylori SPM326) was derived from a person with chronic gastritis and produced VacA (type 1 bacteria) (22). Type 1 strains of H. pylori are more relevant to human disease, because in humans these strains are implicated in H. pylori-associated peptic ulceration (22, 41–45). Moreover, infection of mice with type 1 H. pylori strains is associated with active gastritis (22, 46), and the administration of purified VacA and lysates of type 1, but not type 2, bacteria induce gastric mucosal damage (10). In contrast, previous studies of Th responses in mice used the feline-derived species H. felis, which does not produce VacA and lacks the CagA pathogenicity island. Although H. felis has been shown to induce Th1-mediated gastric inflammation in genetically intact mice (15), Mohammadi et al. showed that infection of IL-4−/− animals resulted in less inflammation and greater numbers of colonizing bacteria than in IL-4−/− mice (16). The difference between these findings and the present study is likely due to differences in bacterial phenotype (e.g., VacA− vs VacA+) of the two Helicobacter

FIGURE 6. IFN-γ, IL-4, and IL-10 production by splenocytes from H. pylori-infected and uninfected control mice. C57BL/6 WT, IL-4−/−, and IFN-γ−/− mice (four per group) were sacrificed at 5 wk postinoculation, and splenic T lymphocytes (4 × 10^6 cells/ml) from individual mice were cultured 72 h with and without H. pylori urease (5 µg/ml) in triplicate wells. Values are the mean ± SEM of duplicate ELISA determinations from each animal from a representative experiment (n = 2).
strains (H. felis vs H. pylori), although other differences, including the ages of the mice and the duration of infection, may also be important.

Study of the inflammatory response to H. pylori in humans has been limited to persons with established infection. Using the mouse model described here, we investigated the early events in H. pylori infection. Together with previous observations, the results suggest the following sequence of events in early H. pylori infection. In susceptible hosts, ingested bacteria colonize the stomach and interact with gastric epithelium, causing up-regulation of MHC class II and costimulatory molecules (47, 48), facilitating epithelial cell presentation of H. pylori Ags to T cells. Interaction between H. pylori (or their products) and the epithelium also induces epithelial cell release of IL-8 (49–52), which initiates the local recruitment of neutrophils. Simultaneously, the bacteria release VacA, causing increased epithelial permeability (53) to allow immunoreactive H. pylori molecules to enter the lamina propria (54).

One such molecule is urease, which has potent chemotactic activity for acute and chronic inflammatory cells (8) and activates macrophages for the production of proinflammatory products (6, 55–57). In addition, H. pylori activate NK cells for IFN-γ production (58). As shown here, H. pylori also activate gastric mucosal and splenic T cells in vivo, which, depending on the genetic background of the host, also leads to up-regulation of IFN-γ and possibly IL-12 (18) and down-regulation of IL-4 and IL-10, driving the inflammatory lesion, resulting in symbiotic homeostasis (61, 62).

The Th1 cytokines, particularly IFN-γ, which promote macrophage activation, likely amplify local tissue destruction (60). The inflammation becomes chronic as the gastric antrum remains colonized with bacteria, whose survival is promoted by nutrient release from the inflammatory lesion, resulting in symbiotic homeostasis (61, 62).

Further elucidation of the cascade of interactions between H. pylori and mucosal cells will provide additional insights into the pathogenesis of H. pylori-induced gastric inflammation. Identification of the cytokines that regulate this cascade and the bacterial proteins involved in local cytokine induction will play a pivotal role in devising effective therapeutic and preventive vaccines for this remarkable pathogen.

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References


