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Regulation of Gastric B Cell Recruitment Is Dependent on IL-17 Receptor A Signaling in a Model of Chronic Bacterial Infection

Holly M. Scott Algood,‡*† Shannon Sedberry Allen,† Mary K. Washington,‡ Richard M. Peek Jr.,*†¶ Geraldine G. Miller,† and Timothy L. Cover*†¶

Th17-driven immune responses contribute to the pathogenesis of many chronic inflammatory diseases. In this study, we investigated the role of IL-17 signaling in chronic gastric inflammation induced by Helicobacter pylori, a Gram-negative bacterium that persistently colonizes the human stomach. Wild-type C57BL/6 mice and mice lacking IL-17RA (IL-17RA−/−) were orogastrically infected with H. pylori. Differences in bacterial colonization density and gastric inflammation were not apparent at 1 mo postinfection, but by 3 mo postinfection, H. pylori colonization density was higher and mononuclear gastric inflammation more severe in infected IL-17RA−/− mice than in infected wild-type mice. A striking feature was a marked increase in gastric B cells, plasma cells, and lymphoid follicles, along with enhanced H. pylori-specific serum Ab responses, in infected IL-17RA−/− mice. Fewer gastric neutrophils and lower levels of neutrophil-recruiting chemokines were detected in infected IL-17RA−/− mice than in infected wild-type mice. Gastric IL-17A and IL-21 transcript levels were significantly higher in infected IL-17RA−/− mice than in infected wild-type mice or uninfected mice, which suggested that a negative feedback loop was impaired in the IL-17RA−/− mice. These results underscore an important role of IL-17A signaling in regulating B cell recruitment. In contrast to many chronic inflammatory diseases in which IL-17A signaling promotes an inflammatory response, IL-17A signaling down-regulates the chronic mononuclear inflammation elicited by H. pylori infection. The Journal of Immunology, 2009, 183: 5837–5846.

The Gram-negative bacterium Helicobacter pylori persistently colonizes the stomachs of approximately half of the global human population. A hallmark of H. pylori infection is a gastric mucosal inflammatory response, termed superficial gastritis (1, 2). In most persons, H. pylori infection and gastritis persist for decades without any adverse effects. However, the presence of H. pylori increases the risk for development of duodenal ulcer disease, gastric ulcer disease, noncardia gastric adenocarcinoma, and B cell malignancies such as gastric mucosa-associated lymphoid tumor lymphomas and high-grade lymphomas (reviewed in Refs. 3, 4).

Previous studies have shown that H. pylori infection of humans and experimental infection of rodents typically result in a Th1 immune response (5–9). Recently, several studies have reported that IL-17A is expressed in the stomachs of H. pylori-infected humans and experimentally infected mice, which suggests that a Th17 response may also be elicited (10–13). However, the roles of IL-17 and Th17 responses in gastric immunopathology and control of H. pylori proliferation have not been fully elucidated.

The Th17 lineage develops in a pathway independent from Th1 and Th2 differentiation (14). A hallmark of Th17 cells is the production of IL-17, a proinflammatory cytokine. Th17 cells produce IL-17A and IL-17F homodimers, as well as IL-17aff heterodimers. These cytokines bind to multimeric IL-17 receptors comprised of two IL-17A subunits and one IL-17RC subunit (15). Additional IL-17 receptors also have been described, including IL-17RB, IL-17RD, and IL-17RE (reviewed in Ref. 16). The functional roles of these additional receptors are not yet completely understood. IL-17 can induce an inflammatory response by signaling a variety of cell types (including epithelial cells, endothelial cells, and fibroblasts) to express IL-8 (or KC, MIP-2, and LIX in mouse), IL-1β, TNF-α, and IL-6 (reviewed in Ref. 16).

Th17 cells are known to have an important role in a growing list of immune-mediated diseases, including inflammatory bowel disease, experimental autoimmune encephalopathy, and collagen-induced arthritis (reviewed in Refs. 17–19). In addition, several animal models of infectious diseases, the IL-23/IL-17 axis promotes cell migration to the site of infection to kill microorganisms and activates the bactericidal activity of macrophages (reviewed in Ref. 20). The host’s ability to control the proliferation of Klebsiella pneumoniae, Citrobacter rodentium, Mycoplasma pneumoniae, Bordetella pertussis, Pseudomonas aeruginosa, Porphyromonas gingivalis, Escherichia coli, Listeria monocytogenes, Helicobacter felis, and Salmonella enterica is at least partially dependent on IL-23 and IL-17 (21–30).

In previous studies, IL-17A−/− mice have been used to study the role of IL-17 and Th17 responses in various infections and inflammatory conditions (including colitis, synovitis, arthritis, and allergic asthma) (21, 26, 31, 32–38). In this study, we used
IL-17RA<sup>−/−</sup> mice to investigate a potential role of IL-17 signaling during <i>H. pylori</i> infection.

**Materials and Methods**

**Animals**

Male and female IL-17RA-deficient (IL-17RA<sup>−/−</sup>) mice on a C57BL/6 background were obtained from Amgen for the establishment of a breeding colony. The IL-17RA<sup>−/−</sup> mice have a targeted deletion of exons 4–11 in the IL-17RA locus on mouse chromosome 6 (39). C57BL/6 mice (Tacomic Farms) were used as controls. *Helicobacter*-free male mice, 8- to 10-wk-old, were used in all experiments. The Vanderbilt University Institutional Animal Care and Use Committee approved all animal protocols used in this study. The IL-17RA<sup>−/−</sup> breeding pairs tested negative for intestinal *Helicobacter* species. Feces from sentinel mice housed in the same room were routinely tested by PCR for intestinal *Helicobacter*, pinworms, mouse parvovirus, and several other murine pathogens and consistently tested negative for each of these infections.

**Culture of <i>H. pylori</i>**

A mouse-passaged derivative of <i>H. pylori</i> strain SS1 was used in all experiments. Bacteria were grown on trypticase soy agar plates containing 5% sheep blood. Alternatively, bacteria were grown in *Brucella* broth containing 5% heat-inactivated FBS and 10 µg/ml vancomycin. Cultures were grown at 37°C in either room air supplemented with 5% CO<sub>2</sub> or under microaerobic conditions, and then live cells were counted by using a hemocytometer and trypan blue exclusion staining. The samples were stained with 2 µg/ml anti-CD4, anti-CD8, and anti-CD23 or 1.5 µg/ml anti-Gr1, 2 µg/ml anti-CD11b, and anti-B220 (BD Biosciences) in a volume of 100 µl of FACS buffer (PBS, pH 7.4) containing 0.1% sodium azide, 0.1% BSA, and 20% mouse serum. Cells were washed, resuspended in FACS buffer, and analyzed on a BD LSR II flow cytometer (BD Biosciences). The number of each cell type in a sample was calculated as previously described (13).

**One day before infection of mice, <i>H. pylori</i> were inoculated into liquid medium and were cultured for 18 h under microaerobic conditions, as described. Mice were ovariectomically inoculated with a suspension of 5 × 10<sup>10</sup> CFU <i>H. pylori</i> (in 0.5 ml of *Brucella* broth) twice over 5 days.

**Flow cytometric analysis**

To analyze gastric cellular infiltrates, whole mouse stomachs were harvested and processed. As a first step in the analysis, gastric epithelial cells were disrupted and released by incubating mouse stomachs in HBSS containing 10% FBS, 15 mM HEPES, 5 mM EDTA, and 0.014% DTT for 1 h at 37°C. The released epithelial cells were discarded, and stomachs were then digested for 30 min at 37°C in RPMI 1640 containing 10% FBS and 1 mg/ml collagenase A. The suspension was passed through a 70-µm cell strainer (BD Biosciences). Cells were harvested by centrifugation, washed, and then live cells were counted by using a hemocytometer and trypan blue exclusion staining. The samples were stained with 2 µg/ml anti-CD4, anti-CD8, and anti-CD23 or 1.5 µg/ml anti-Gr1, 2 µg/ml anti-CD11b, and anti-B220 (BD Biosciences) in a volume of 100 µl of FACS buffer (PBS, pH 7.4) containing 0.1% sodium azide, 0.1% BSA, and 20% mouse serum. Cells were washed, resuspended in FACS buffer, and analyzed on a BD LSR II flow cytometer (BD Biosciences). The number of each cell type in a sample was calculated as previously described (13).

**Analysis of cytokine secretion by primary gastric epithelial cells**

Primary gastric epithelial cells were isolated using a modification of a previously published protocol (44). The glandular portion of the stomach was opened via dissection, and after washing the stomach in cold PBS, the tissue was placed in 0.04% sodium hypochlorite for 15 min at room temperature. The stomach was then washed in PBS and placed in PBS containing 0.5 mM DTT and 3 mM EDTA for 1.5 h at room temperature. The EDTA/DTT solution was then replaced with PBS, and the tissue was disrupted using manual shaking. Cells in the supernatant were collected by centrifugation, resuspended in Ham’s F-12 medium containing 5% FBS and penicillin/streptomycin, and plated into collagen coated 24-well plates (BD Biosciences). After 24 h in culture, nonadherent cells were removed. Cells were then washed with PBS and fresh medium was added. Primary gastric epithelial cells isolated from infected wild-type mice and IL-17RA<sup>−/−</sup> mice were stimulated with IL-17 (10 ng/ml; R&D Systems). For all experiments, the medium overlying epithelial cells was collected, centrifuged, and the supernatant filtered. Supernatants were analyzed for expression of GM-CSF, IL-1β, IL-6, MIP-2, KC by using the Searchlight system (PerkinElmer) for FACS, followed by using Miliplex technology (Millipore). These are ELISA-based systems in which capture Abs are spotted in arrays within microplate wells, which allows multiple cytokines to be detected simultaneously.

**H. pylori-specific serum Ab responses**

Mouse serum Ab responses to <i>H. pylori</i> were analyzed by ELISA. To generate an Ag preparation for use in ELISA, <i>H. pylori</i> strain SS1 was cultured overnight in liquid medium, and the bacteria were collected by centrifugation at 2100 × g and washed with cold PBS. Subsequently, the bacterial suspension was sonicated. The sonicate was then centrifuged at 10,000 × g, and the soluble fraction was used for subsequent experiments. Influenza 2HB plates (Thermo Fisher Scientific) were coated with 10 µg/ml of the <i>H. pylori</i> Ag preparation overnight at 4°C. Plates were washed with 0.05% Tween 20-PBS and blocked with 5% skim milk-PBS for 1 h at 37°C. Following incubation with mouse serum samples, plates were washed and detection Abs were added (anti-mouse IgG conjugated to HRP (Pierce), goat anti-mouse IgG1-HRP, goat anti-mouse IgG2a-HRP, or goat anti-mouse IgA-HRP (Santa Cruz Biotechnology)). Following another series of washes, <i>H. pylori</i>-specific Abs were detected using 1-Step Ultra TMB ELISA substrate (Pierce). Titers are expressed as the reciprocal of the maximum dilution of sera that yielded an OD (450 nm) three times higher than the background signal generated by sera from uninfected mice.

**Statistical analysis**

From 4 to 10 mice per group per time point were used for all of the experiments. To compare results obtained with different groups of mice, statistical analysis was performed using one-way ANOVA, followed by a Student-Neuman-Keuls posthoc test. For analyses of bacterial and cell
numbers, data were normalized by log transformation before statistical analysis. Histology scores for wild-type and IL-17RA−/− mice were compared using the Mann-Whitney U test.

Results

H. pylori proliferation in mice lacking IL-17RA

To investigate a potential role of IL-17RA signaling in H. pylori-host interactions, wild-type C57BL/6 mice and IL-17RA−/− mice were orogastrically infected with H. pylori. Mice were sacrificed at serial time points, and bacterial colonization of the stomach was quantified as described in Materials and Methods. At 1 mo postinfection, there was no significant difference in the density of H. pylori colonization in the two populations of mice (Fig. 1A). By 3 mo postinfection, significantly higher numbers of H. pylori were cultured from the stomachs of IL-17RA−/− mice than from stomachs of wild-type mice, and this difference also was observed at 6 mo postchallenge (Fig. 1A). Despite the higher burden of H. pylori in the stomachs of the IL-17RA−/− mice, these mice did not exhibit weight loss or other physical signs of illness during an observation period of 6 mo postinfection.

Increased severity of gastric mononuclear inflammation in IL-17RA−/− mice following H. pylori infection

The severity of gastric inflammation in H. pylori-infected IL-17RA−/− and wild-type mice was examined at 1, 3, and 6 mo postinfection. Total gastric inflammation was analyzed using a scoring system that evaluates both acute inflammation and chronic inflammation in the gastric corpus and antrum of each animal. At 1 mo postinfection, there was no significant difference in the severity of total gastric inflammation in the two populations of infected mice (Fig. 1B). At 3 and 6 mo postinfection, however, the severity of total gastric inflammation was significantly greater in infected IL-17RA−/− mice compared with wild-type mice (Fig. 1B). Both antral and corpus chronic (mononuclear) inflammation scores were significantly higher in infected IL-17RA−/− mice than in wild-type mice at 3 and 6 mo postinfection (Tables I and II). At 3 mo postinfection, plasma cell infiltration of the antrum was detected in all of the H. pylori-infected IL-17RA−/− mice, but not in the infected wild-type mice (Table I). At 6 mo postinfection, gastric lymphoid follicles with germinal centers were detected in nearly all of the infected IL-17RA−/− mice, but not in the infected wild-type mice (Table II). In addition to the results shown in Tables I and II, wild-type and IL-17RA−/− mice were experimentally infected with H. pylori in three other independent experiments, and the IL-17RA−/− mice consistently exhibited increased severity of chronic inflammation at 3 mo postinfection compared with wild-type mice (data not shown). There were no significant differences in acute (polymorphonuclear) gastric inflammation scores when comparing the infected wild-type and IL-17RA−/− mice (Tables I and II). At 3 and 5 mo of age, uninfected IL-17RA−/− mice and uninfected wild-type mice did not exhibit detectable gastric inflammation (data not shown).

Fig. 2 illustrates representative histology of the transition zone between the gastric corpus and the antrum, a region of the mouse stomach that typically exhibits relatively severe inflammation in response to H. pylori. By 3 mo postinfection, infected IL-17RA−/− mice had increased gastric mononuclear cell infiltration compared with infected wild-type mice, and at 6 mo postinfection, thickening of the gastric mucosa (gastric mucosal hyperplasia) was observed in infected IL-17RA−/− mice (Fig. 2). Of great interest, a focus of gastric adenocarcinoma invasive into the submucosa was detected in one of the infected IL-17RA−/− mice at 6 mo postinfection (data not shown).

A hallmark of H. pylori is its ability to persistently colonize the stomach and, therefore, all of the experiments described focused on analysis of bacterial density and inflammation in the setting of chronic infection. We also analyzed these parameters in the setting of acute infection. Wild-type mice and IL-17RA−/− mice were infected with H. pylori, and bacterial density and gastric histology were analyzed 3 days later. The density of H. pylori infection was relatively low at this early time point compared with later time points, but there was no significant difference in the density of H. pylori colonization in the two populations of mice (data not shown).
Consistent with a previously published study of acute *H. pylori* infection in wild-type mice (13), minimal gastric inflammation was detected at this early time point, and there was no significant difference in the severity of inflammation in the two groups of *H. pylori*-infected mice (data not shown).

### Analysis of Th17 signature proteins

The histologic analysis shown in Fig. 2 demonstrated that there were significant differences in the chronic gastric mucosal inflammatory responses in *H. pylori*-infected IL-17RA−/− mice compared with infected wild-type mice. To investigate differences in the gastric mucosal cytokine milieu that might contribute to these differences, we analyzed the gastric expression of several proinflammatory cytokines and CXC neutrophil chemoattractant chemokines that are considered to be Th17 signature proteins (reviewed in Ref. 16). Real-time RT-PCR was performed on RNA isolated from gastric tissue of *H. pylori*-infected wild-type mice and IL-17RA−/− mice at 3 and 6 mo postinfection. KC, LIX, and GM-CSF were expressed at significantly lower levels in the stomachs of *H. pylori*-infected IL-17RA−/− mice than in stomachs of infected wild-type mice (Fig. 3), which suggests that IL-17 is an important factor driving expression of these cytokines and chemokines in the mouse stomach. At the 3-mo time point, there was no significant difference in levels of IL-1 and IL-6, but at the 6-mo time point, IL-1 and IL-6 were expressed at higher levels in *H. pylori*-infected IL-17RA−/− mice than in wild-type mice (Fig. 3A). There were no significant differences in the gastric transcript levels of these cytokines or chemokines in the two groups of uninfected mice (data not shown).

We next tested the hypothesis that gastric epithelial cells might be one source of the Th17-signature proinflammatory cytokines and chemokines detected in the mouse stomach. Primary gastric epithelial cells were isolated from wild-type mice and IL-17RA−/− mice, and then were stimulated with recombinant IL-17a.

### Table I. Gastric histology of *H. pylori*-infected mice at 3 mo postinfection

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Inflammation Grade</th>
<th>Antrum</th>
<th>Corpus</th>
</tr>
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<tr>
<td></td>
<td></td>
<td>Acute (0–3)</td>
<td>Chronic (0–3)</td>
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<td>2</td>
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<td>3</td>
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<tr>
<td></td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IL-17RA−/−</td>
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<td>2</td>
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<td></td>
<td>2</td>
<td>0</td>
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<tr>
<td></td>
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<td></td>
<td>4</td>
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<td>5</td>
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</tr>
<tr>
<td>Significance</td>
<td>NS</td>
<td>NS</td>
<td>**</td>
</tr>
</tbody>
</table>

* Comparison between IL-17RA−/− mice and wild-type mice.

NS, Nonsignificant.

*, *p* < 0.05 and **, *p* < 0.01 indicates statistical significance.

### Table II. Gastric histology of *H. pylori*-infected mice at 6 mo postinfection

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Inflammation Grade</th>
<th>Antrum</th>
<th>Corpus</th>
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<tr>
<td></td>
<td></td>
<td>Acute (0–3)</td>
<td>Chronic (0–3)</td>
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<td>6</td>
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<td>IL-17RA−/−</td>
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<td></td>
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<td>1</td>
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<td>2</td>
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<tr>
<td>Significance</td>
<td>NS</td>
<td>*</td>
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</tr>
</tbody>
</table>

* Comparison between IL-17RA−/− mice and wild-type mice.

NS, Nonsignificant.

*, *p* < 0.05 indicates statistical significance.
or left unstimulated. Supernatants were collected, and concentrations of selected Th17 signature proteins were quantified by multiplex ELISA. In response to stimulation with recombinant IL-17a, wild-type gastric epithelial cells secreted significantly higher levels of KC (CXCL1), LIX (CXCL5), MIP2 (CXCL2), and GM-CSF compared with IL-17-stimulated gastric epithelial cells derived from IL-17RA−/− mice (Fig. 4). These results are consistent with the pattern of gastric cytokine expression detected in vivo (Fig. 3).

**Neutrophil migration into the stomach following H. pylori infection is regulated by IL-17RA signaling**

In further studies, we specifically analyzed gastric neutrophil infiltration in H. pylori-infected mice. At 3 mo postinfection, significantly fewer gastric neutrophils (Gr1−, CD11b+ cells) were present in the stomachs of the IL-17RA−/− mice than in wild-type mice (Fig. 5). The failure to detect this difference in neutrophil density in an analysis of histological sections (Table I) may be attributable to the fact that neutrophils represent a minority of the gastric inflammatory cells identified by histologic assessment and are intimately admixed with mononuclear inflammatory cells; in this setting, light microscopy may be insufficiently sensitive to detect a difference in the neutrophil populations of the two groups. There was no significant difference in the number of gastric macrophages (CD11b−, Gr1−, F4/80+ cells) in H. pylori-infected IL-17RA−/− mice compared with infected wild-type mice (data not shown). As expected based on previous studies of IL-17RA−/− mice (39), there were significantly fewer neutrophils present in the spleens of H. pylori-infected IL-17RA−/− mice than wild-type mice at 1, 3, and 6 mo postinfection; the number (or percentage) of macrophages present in the spleens of H. pylori-infected IL-17RA−/− mice and wild-type mice was not significantly different (data not shown).

**Absence of IL-17RA signaling affects T lymphocyte response to H. pylori infection**

Next we analyzed CD4+ and CD8+ T cells in the mouse stomachs. A significantly higher number of gastric CD4+ T cells were detected in H. pylori-infected IL-17RA−/− mice than in wild-type mice at 3 mo postinfection (Fig. 6A). There was no significant difference in the number of CD8+ T cells (Fig. 6A). To define the type of Th responses present in the stomachs of infected mice, we analyzed the expression of several signature cytokines by real-time

![FIGURE 2. Representative gastric histology of wild-type and IL-17RA−/− mice. At 1, 3, and 6 mo postinfection, gastric tissue was collected and sections were stained with H&E. Gastric tissue from uninfected mice was also analyzed. Representative sections of the transition zones between the corpus and the antrum are shown. At 3 and 6 mo postinfection, chronic (mononuclear) inflammation was more severe in the IL-17RA−/− mice than in wild-type mice. Sections are representative of 15–20 animals in each group. Images are at magnification ×200 original.](http://www.jimmunol.org/)

![FIGURE 3. Gastric expression of proinflammatory cytokines and chemokines in H. pylori-infected wild-type mouse and IL-17RA−/− mouse. A, Real-time RT-PCR for KC (CXCL1), LIX (CXCL5), and MIP-2 (CXCL2) was performed on RNA isolated from the gastric transition zones of C57BL/6 and IL-17RA−/− mice at 3 and 6 mo after H. pylori infection. Data represent mean ± SE for each group (n = 5 per group). Similar results were also observed in another independent experiment. *, p < 0.05; **, p < 0.01. B, Real-time RT-PCR comparing GM-CSF, IL-1α, and IL-6 message in stomachs of H. pylori-infected C57BL/6 and IL-17RA−/− mice at 3 and 6 mo postinfection; Data represent mean ± SE for each group (n = 5–6 per group). *, p < 0.05; **, p < 0.01.](http://www.jimmunol.org/)

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RT-PCR. Th1 and Th2 cytokines (IFN-γ and IL-4, respectively) were expressed at similar levels in infected IL-17RA−/− mice and infected wild-type mice (Fig. 6B). To examine cytokines known to be produced by Th17 cells, we analyzed gastric expression of IL-17a, IL-17f, IL-21, and IL-22 (Fig. 6C). IL-17a and IL-21 were expressed at significantly higher levels in the stomachs of infected IL-17RA−/− mice than in infected wild-type mice (Fig. 6C), and levels of IL-17a were significantly higher in *H. pylori*-infected IL-17RA−/− mice than in uninfected IL-17RA−/− mice (Fig. 6D). This suggests that a negative feedback loop might be impaired in the IL-17RA−/− mice. In contrast to the pattern observed with expression of IL-17a and IL-21, there was no significant difference in gastric expression of IL-17f in infected IL-17RA−/− mice compared with infected wild-type mice (Fig. 6C).

Analysis of gastric B cell infiltration and humoral immune responses

To further analyze the mononuclear cells observed infiltrating the mouse stomachs following *H. pylori* infection (Tables I and II and Fig. 2), gastric B cells were enumerated using flow cytometry. At 3 mo postinfection, B cells (B220+ cells) were significantly more abundant in the stomachs of *H. pylori*-infected IL-17RA−/− mice than in infected wild-type mice (Fig. 7A). Based on these data, in conjunction with the visualization of plasma cells and lymphoid follicles in histologic analysis (Tables I and II and Fig. 7D), we hypothesized that there might be enhanced production of anti-*H. pylori* Abs in the *H. pylori*-infected IL-17RA−/− mice. As shown in Fig. 7B, the levels of *H. pylori*-specific serum IgG and IgA Abs were significantly higher in the IL-17RA−/− mice compared with wild-type control mice at 3 mo postinfection. We analyzed the expression of several B cell-recruiting chemokines, including two that are known to be up-regulated in the stomachs of *H. pylori*-infected humans (45), using real-time RT-PCR on RNA isolated from the mouse stomachs. Expression of CCL28 and CXCL12 did not correlate with the observed recruitment of B cells in IL-17RA−/− mice (Fig. 7C). However, CXCL13, also known as B lymphocyte chemoattractant and a mediator of B cell organization in lymphoid tissues and the gut, was expressed at significantly higher levels in *H. pylori*-infected IL-17RA−/− mice than in infected wild-type mice (Fig. 7C).
for optimal host defense against several other pathogenic bac-

previous reports indicating that IL-17RA signaling is required

A striking finding in the current study was that

Discussion

In this study, we investigated a potential role of IL-17 signaling in

Role of IL-17RA signaling in regulating B cell responses

A striking finding in the current study was that H. pylori-infected IL-17RA−/− mice exhibited increased severity of mononuclear gastric inflammation compared with infected wild-type mice. The mononuclear gastric inflammation in infected IL-17RA−/− mice was largely attributable to the recruitment of B cells, and gastric lymphoid follicles with germinal centers were detected in these mice at 6 mo postinfection (Fig. 7 and Tables I and II). We also detected an increased number of gastric plasma cells and higher levels of specific serum Abs to H. pylori in infected IL-17RA−/− mice than in infected wild-type mice. The strong humoral Ab response to H. pylori elicited in IL-17RA−/− mice clearly was not sufficient to eradicate the bacteria (Fig. 1). Similarly, a large body of literature indicates that humoral immune responses typically fail to eradicate H. pylori infection in humans or in experimentally infected animals (1, 2). Potentially Abs are ineffective in the gastric environment due to the low pH, or they may fail to facilitate opsonization due to limited entry of phagocytic cells into the gastric mucus layer. In murine models, T cell immunity rather than humoral immunity appears to be required for protection (47, 48). One previous study reported that μMT−/− mice (B lymphocyte-deficient) harbored a decreased number of H. pylori compared with wild-type mice, and suggested that the humoral immune response might enhance H. pylori colonization (49). Therefore, it is of interest that in the current study we also observed a correlation between strong humoral anti-H. pylori immune responses and increased H. pylori colonization of the stomach.

This report is the first to our knowledge on B cell-predominant chronic inflammation in IL-17RA−/− mice. Because B cell recruitment has not been detected in other studies in which IL-17RA−/− mice were infected with various bacterial pathogens, and gastric B cell infiltration was not observed in uninfected IL-17RA−/− mice, this suggests that the B cell recruitment observed in H. pylori-infected IL-17RA−/− mice is driven by the presence of H. pylori. The mechanisms underlying the striking recruitment of B cells into the gastric mucosa of H. pylori-infected IL-17RA−/− mice are not yet entirely understood, but several scenarios can be considered. One possibility is that elevated levels of a B cell chemokine such as CXCL13 contribute to the recruitment of B cells (Fig. 7). Another possibility is that B cells in wild-type and IL-17RA−/− mice might differ in responsiveness to chemotactic signals. In support of this hypothesis, a recent study reported that, in the presence of intact IL-17 signaling, B cells exhibit attenuated responsiveness to chemokines such as CXCL12 and CXCL13 (50). The increased plasma cells and germinal center formation observed in the IL-17RA−/− mice might also be attributable to increased expression of IL-21 (Fig. 6C). IL-21 has been shown to play a role in Ab
production in humans and mice by inducing differentiation of B cells into plasma cells (51, 52).

**IL-17RA signaling and neutrophil recruitment**

In comparison to *H. pylori*-infected wild-type mice, *H. pylori*-infected IL-17RA−/− mice had significantly fewer gastric neutrophils and reduced levels of gastric CXC neutrophil chemotactant chemokines (KC and LIX). Neutrophil deficiency has been detected in IL-17RA−/− mice compared with wild-type mice in several other models of inflammation. For example, neutrophil recruitment to the lungs and airways was impaired in IL-17RA−/− mice compared with wild-type mice in models of allergic asthma and *K. pneumoniae* pulmonary infection (21, 37); neutrophil recruitment to the cornea was transiently impaired in IL-17RA−/− mice in response to HSV-1 infection (35); and neutrophil infiltration was reduced in IL-17RA−/− mice compared with wild-type mice in a model of acute trinitrobenzenesulfonic acid-induced colitis (36). The reduced or delayed neutrophil recruitment observed in IL-17RA−/− mice has been attributed to impaired IL-17A signaling and a consequent reduction in the production of neutrophil chemoattractant CXC chemokines.

Recently it was reported that *H. pylori*-infected IL-17−/− mice had lower levels of gastric neutrophil infiltration compared with what was observed in infected wild-type mice (53). Thus, both IL-17RA−/− mice and IL-17−/− mice have impaired neutrophil responses to *H. pylori* compared with the responses observed in wild-type mice. Several previous studies have demonstrated that neutrophils contribute to the control of *H. pylori* proliferation (54–56), and therefore, a paucity of gastric neutrophils in infected IL-17RA−/− mice may account for the observed increase in bacterial colonization density in these mice. Interestingly, levels of *H. pylori* colonization were reported to be significantly lower in the IL-17−/− mice compared with wild-type mice (53), a finding that contrasts the increased levels of *H. pylori* colonization detected in IL-17RA−/− mice in the current study. The reasons for this difference are not clear at present.

**Elevated gastric IL-17a levels in H. pylori-infected IL-17RA−/− mice**

As shown in Fig. 6, IL-17a was expressed at very high levels in the stomachs of infected IL-17RA−/− mice compared with infected wild-type mice. The increase in IL-17a levels in infected IL-17RA−/− mice was dependent on the presence of *H. pylori* because gastric IL-17a levels were markedly higher in infected IL-17RA−/− mice than in uninfected IL-17RA−/− mice (Fig. 6D). One explanation for these findings may be the loss of a negative feedback loop; for example, in wild-type mice, IL-17a may signal through IL-17RA to down-regulate IL-17a expression. Support for this hypothesis comes from a recent study that reported higher levels of IL-17a production by T cell populations from IL-17RA−/− mice than by T cell populations from wild-type mice, when the T cell populations were cultured under conditions known to induce Th17 cell differentiation (57). An additional factor accounting for the high IL-17a levels may be that increased levels of IL-21 expression (Fig. 6C) drive Th17 cell differentiation and IL-17a expression (58, 59).
In summary, this study demonstrates an important role of IL-17RA signaling in regulating H. pylori-induced gastritis, a model of inflammation induced by a chronic bacterial infection. The results of this current study highlight the importance of IL-17RA signaling in regulating B cell migration and function. In contrast to many chronic inflammatory diseases in which IL-17RA signaling promotes an inflammatory response, IL-17RA signaling down-regulates the chronic mononuclear inflammation elicited by H. pylori infection. Such down-regulation may be an important factor that allows H. pylori to persistently colonize the human gastric environment.

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