Contribution of Secretory Antibodies to Intestinal Mucosal Immunity against *Helicobacter pylori*

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The natural immune response to *Helicobacter pylori* neither clears infection nor prevents reinfection. However, the ability of secretory antibodies to influence the course of *H. pylori* infection has not been determined. We compared the natural progression of *H. pylori* infection in wild-type C57BL/6 mice with that in mice lacking the polymeric immunoglobulin receptor (pIgR) that is essential for the secretion of polymeric antibody across mucosal surfaces. *H. pylori* SS1-infected wild-type and pIgR knockout (KO) mice were sampled longitudinally for gastrointestinal bacterial load, antibody response, and histological changes. The gastric bacterial loads of wild-type and pIgR KO mice remained constant and comparable at up to 3 months postinfection (mpi) despite SS1-reactive secretory IgA in the intestinal contents of wild-type mice at that time. Conversely, abundant duodenal colonization of pIgR KO animals contrasted with the near-total eradication of *H. pylori* from the intestine of wild-type animals by 3 mpi. *H. pylori* was cultured only from the duodenum of those animals in which colonization in the distal gastric antrum was of sufficient density for immunohistological detection. By 6 mpi, the gastric load of *H. pylori* in wild-type mice was significantly lower than in pIgR KO animals. While there was no corresponding difference between the two mouse strains in gastric pathology results at 6 mpi, reductions in gastric bacterial load correlated with increased gastric inflammation together with an intestinal secretory antibody response in wild-type mice. Together, these results suggest that naturally produced secretory antibodies can modulate the progress of *H. pylori* infection, particularly in the duodenum.

Carriers of the human gastric bacterial pathogen *Helicobacter pylori* develop a substantial immune response manifested by cellular infiltration of the gastric mucosa and the development of an antibody response. While this natural immune response is not protective against gastric infection (1) and can even facilitate *H. pylori* pathogenesis, vaccine-induced immunity may reduce gastric loads of *H. pylori* in animal models of infection when used either prophylactically (2) or therapeutically (3). Investigations of the mechanism(s) of vaccine-induced immunity suggest that cellular immunity, perhaps in concert with innate factors, is responsible for the observed protection (4).

Studies of the contribution of antibodies to vaccine-induced immunity suggest that *H. pylori*-specific IgA may suppress the development of vaccine-induced protective inflammatory responses (5). Several studies have reported similar or enhanced vaccine-mediated protection in IgA- and B-cell-deficient animals, suggesting that antibodies are dispensable for protective immunity against *H. pylori* (6–9). However, the compensatory contribution of enhanced IgM production and significant Th1 skewing in IgA knockout (KO) (7) and μMT (10, 11) mouse strains, respectively, remains to be examined as a factor contributing to the maintenance of vaccine efficacy in the absence of IgA in these animals. Nevertheless, the lack of any correlation between *H. pylori*-specific antibody-mediated immune response and vaccine-induced immunity is in agreement with the apparent inefficacy of antibody-mediated immunity following natural infection. As *H. pylori* resides within or beneath the mucous-gel layer that protects the gastric mucosa from acid and digestive enzymes, the failure of antibodies to protect against *H. pylori* is not fully explained by antibody degradation in the gastric lumen.

Antibodies destined for mucosal translocation are polymeric structures composed of multiple covalently linked immunoglobulin molecules associated with a joining (J)-chain protein (12). In mice, J-chain expression is regulated by interleukin-2 (IL-2)-induced downregulation of the negative regulatory element, BSAP (B-cell-specific activator protein) (13). The J-chain facilitates attachment of polymeric immunoglobulin to the polymeric immunoglobulin receptor (pIgR), which is expressed at the basolateral surface of the mucosal epithelium. Epithelial cell expression of pIgR is constitutive in the intestine, although this expression can be upregulated by gamma interferon (IFN-γ) binding to its receptor on the epithelial cell surface (14). Following attachment to pIgR, the polymeric antibody/receptor complex is internalized by endocytosis, translocated through the epithelial cell, and released from the apical mucosal surface following proteolytic cleavage of pIgR, leaving a remnant known as the secretory component attached to the secreted antibody.

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In contrast to the lungs, vagina, and most of the gastrointestinal tract, the healthy mammalian stomach produces no to little plgR (15, 16). In fact, plgR expression in the gastric mucosa is a marker of intestinal metaplasia (17, 18) and IgA in gastric mucus from healthy humans is predominantly nonsecretory (19, 20). Studies in H. pylori-infected humans have shown that baseline plgR expression by the gastric epithelium can be upregulated in response to gastric inflammation (15, 21–23) due to increased local IFN-γ production (24). This is accompanied by downregulation of J-chain synthesis in local plasma cells (25), probably as a consequence of reduced IL-2 production in H. pylori-infected gastric tissue (24) due to the effects of the VacA cytotoxin on T cells (26). However, despite significantly increased plgR expression and IgA plasma cell infiltration in response to H. pylori infection (21, 27), there is no concomitant increase in IgA secretion into the stomach, and it is nonsecretory monomeric IgA which predominates in the stomach of H. pylori-infected individuals (25, 28). This observation is consistent with the leakage of serum antibodies across the infected gastric mucosa. Together, these findings suggest that, although inflammation upregulates plgR expression, cytokines released in gastric tissue as a consequence of H. pylori-induced gastritis dysregulate the expression of genes encoding effectors required for the efficient translocation of secretory antibodies. Antibodies in the stomach may also be sourced from extragastric sites, namely, from the mouth via saliva and the duodenum via reflux. Salivary IgA is swallowed continually and may contribute to vaccine-mediated protection against H. pylori infection (29). In humans, peak IgA levels in the gastric lumen coincide with gastroduodenal reflux resulting from retrograde peristalsis arising from the interdigestive migrating motor complex (MMC) (30).

Although substantial evidence suggests insufficient quantities of antibody are translocated across the gastric mucosa to mediate immunity and that the bulk of gastric antibody is intermittently and distantly sourced, these factors have not been accounted for in any previous studies investigating the contribution of antibodies to H. pylori immunity. In particular, the contribution of intestinal antibodies to H. pylori immunity has been poorly examined. In this study, we compared the progression of gastric H. pylori infection in naïve wild-type C57BL/6 mice with that in plgR KO C57BL/6 mice which lack the ability to secrete antibodies across mucosal surfaces (31). We also examined duodenal colonization, gastric inflammation, and humoral immune responses in both groups of mice following infection with H. pylori.

METHODS AND MATERIALS

Mice and bacterial infections. C57BL/6 wild-type and plgR knockout mice (31) were bred and housed in the Department of Microbiology and Immunology Animal Facility at the University of Melbourne as described previously (32). All animal experiments were compliant with the Australian National Health and Medical Research Council guidelines and approved by the University of Melbourne Animal Ethics Committee. A total of 82 wild-type and 82 plgR KO female mice were used in this study. The number and age of the animals used were dictated by the availability of the plgR KO mice from two consecutive breeding cycles. The animals derived from each breeding were handled as independent cohorts in two experiments staggered approximately 3 weeks apart for inoculation and subsequent sample collection (Table 1). H. pylori strain SS1 was used for all experiments and cultured as described previously (32). Mice were inoculated at 6 to 8 weeks of age with 0.1 ml of a suspension of H. pylori SS1 in brain heart infusion broth (BHI) (10⁹ CFU/ml), or BHI alone, by gavage using a stainless-steel mouse-feeding needle (Cole-Parmer) (20 gauge; 1.5 in. long; curved). Two SS1-inoculated wild-type mice required euthanasia due to reasons unrelated to the experiment (malocclusion and eye tumor).

![Figure](http://iai.asm.org/)

**TABLE 1 Experimental design with respect to the numbers of animals in each experimental group and sampled at each time point**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals sampled at each time point postinoculation</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 11</td>
<td>Mo 1</td>
</tr>
<tr>
<td>SS1 inoculated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cohort I</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Cohort II</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
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<td>10</td>
</tr>
<tr>
<td>BHI inoculated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cohort I</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Cohort II</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

²See also Fig. S1 in the supplemental material. WT, wild type.
phosphate-buffered saline (PBS) to recover the intestinal contents, and the recovered lavage fluid was supplemented immediately thereafter with 40 μl 25× Complete protease inhibitor Cocktail (Roche). The intestine was then bisected longitudinally, with one half used for culture and the other used for histology. All tissue recovered for culture was washed twice with saline solution and blotted before weighing. It was then homogenized in 2 ml sterile saline solution and used for quantitative culture on lysed horse blood agar plates supplemented with Dent selective supplement (Oxoid) and 20 μg/ml bacitracin (Sigma-Aldrich) as described previously (32). *H. pylori* was identified by colony morphology; randomly selected colonies from intestinal tissues were confirmed by measuring urease activity and by colony PCR for species determination as described previously (33).

**Preparation and histological analysis of gastric and intestinal tissues.** Gastric tissue was rinsed in 10% neutral buffered formalin and allowed to flatten against the tube wall for several minutes before being returned to the formalin. Unrinsed intestinal tissue was gently scrunched around a cylindrical wooden stick coated with silicone (Sigma-Arote, Sigma-Aldrich) and was pinned in place before immersion in formalin. Formalin-fixed tissues were transferred to 70% ethanol within 24 h of fixation. Following ethanol equilibration, gastric tissues were further bisected longitudinally and embedded internal-cut-side down to provide 2 samples per section for analysis. An additional longitudinal strip of tissue (2 mm wide) was recovered from the internal cut side of samples from animals killed at 12 mpi for determination of bacterial load by quantitative PCR (qPCR). Sections (3 μm thick) of gastric and intestinal tissue were stained with hematoxylin and eosin and then assessed by a clinical pathologist who was blinded to details of the study design.

For gastric pathology, the 4-grade scoring scale (0 to 3) of the updated Sydney system (34) was used. Separate scores were assigned to the distal antrum, proximal antrum (corpus-antrum transitional zone), and corpus regions of the stomach for each of the following key pathological features: neutrophil infiltration, mononuclear cell infiltration, atrophy, and intestinal metaplasia. The scoring system was modified such that tissues containing grade 3 inflammation or atrophy of extensive breadth across a specific region were assigned a grade of 4. Any additional observations, including lymphoid follicle development and lymphocytic gastritis, were also recorded. Neutrophil and mononuclear cell infiltration scores were combined to produce summary inflammation scores for the proximal antrum and corpus (0 to 8). For assessment of intestinal pathology, sections were examined for any architectural changes, lymphoid follicle development, gastric metaplasia, and cellular infiltration.

**qPCR to assess gastric bacterial load.** Because quantitative culture data were not available for the 12-month time point of cohort I due to the inadvertent inclusion of a nonpermissive antibiotic supplement in the culture plates, the bacterial loads of all mice killed at this time point were assessed by qPCR using previously reported primers specific for *H. pylori* rRNA (35). Genomic DNA was purified from formalin-fixed (cohorts I and II) or fresh (cohort II only) gastric tissue, or from broth-grown SS1, using a blood and tissue DNaseasy kit (Qiagen) according to the manufacturer’s protocol for purification of DNA from mammalian tissue. For DNA extracted from fresh tissue, 300 μl of the same homogenate used for culture was centrifuged (10 min, 20,000 × g, 4°C) and the pelletted tissue was resuspended in Qiagen ATL buffer and proteinase K solution supplied in the kit. To extract DNA from formalin-fixed samples, the tissue was sliced finely before incubation in ATL buffer and proteinase K. After overnight incubation, an additional 0.1× volume of ATL buffer and proteinase K was added to each sample and further incubated with frequent vortexing for 3 h before resuming the manufacturer’s protocol for purification of genomic DNA from mammalian tissue (Qiagen). To generate template for the qPCR standard curve, genomic DNA was purified from *H. pylori* SS1 cells pelleted from a broth culture. The bacterial density of this culture, as determined by quantitative culture and by counting in a hemocytometer chamber, was used to confirm the estimation of 1.1 × 10⁸ *H. pylori* genomes/ng DNA as predicted from the molecular weight of the *H. pylori* genome.

For qPCR of DNA from fixed tissue, all DNA, including that used to construct the standard curve, was preincubated (98°C, 10 min) before addition to the reaction mix to destroy any formalin-induced DNA cross-linking that might have interfered with the early cycles of amplification. qPCR was conducted using Brilliant II master mix (Stratagene) according to the manufacturer’s protocol. All reactions were performed in duplicate in a Mx3005P real-time thermal cycler (Stratagene). PCR cycling conditions were 10 min at 95°C followed by 40 cycles of 95°C for 30 s, 60°C for 1 min (read fluorescence at end of hold), and 72°C for 30 s and 1 cycle of 95°C for 1 min and 55°C for 30 s followed by dissociation curve measurements. Total gastric DNA was used in reactions at 10 and 1 ng, and the number of genomes was estimated against a standard curve ranging from 2.0 to 0.00016 ng SS1 genomic DNA. qPCR conducted using DNA extracted from formalin-fixed tissue recovered from animals in cohort II showed good agreement with matched fresh tissue qPCR and quantitative culture results (Pearson r = 0.88 and 0.90 compared with fresh tissue and r = 0.84 and 86 compared with viable count, in duplicate experiments). As the DNA purification procedure yielded approximately 500 ng DNA/gram stomach tissue, the number of *H. pylori* genomes/500 μg DNA was used as a proxy for CFU/gram stomach to present the qPCR data.

**Immunohistochemistry and immunofluorescence.** For immunohistochemical staining, all antibodies were diluted in 2% (vol/vol) fetal bovine serum (FBS)–PBS, and slides were washed between steps with PBS. Dewaxed tissue sections (3 μm thick) were blocked with serum-free protein block (30 min; Dako) before incubation (1 h) with one of the following primary antibodies: goat anti-mouse pIgR antibody (R&D Systems) (2 μg/ml); rabbit anti-*H. pylori* SS1ΔureAB outer membrane vesicle (OMV) polyclonal sera (Sigma) (diluted 1 in 500) produced by hyperimmunization of a rabbit with 3 doses (125 μg protein per dose in Freund’s incomplete adjuvant) of OMVs purified from SS1ΔureAB spent culture media as described previously (32); or horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG (KPL Inc.) (diluted 1 in 200). Following washes (performed once for 5 min and once for 30 min), endogenous peroxidase was blocked by immersing the slides in 0.05% (vol/vol) peroxide (30 min), and the slides were washed (three times for 5 min each time). Bound anti-pIgR or anti-*H. pylori* antibodies were revealed by incubation with HRP-conjugated rabbit anti-goat (Sigma-Aldrich) or HRP-conjugated sheep anti-rabbit (Chemicon) antibodies, respectively, each diluted 1 in 200. After washes (performed once for 5 min and once for 30 min), bound antibodies were visualized with DAB/H₂O₂ substrate prepared from DAB tablets (Dako) followed by 3 washes with distilled water and counterstaining with hematoxylin before dehydration and mounting in DePeX (Sigma).

For colocalization of *H. pylori* and pIgR by two-color immunofluorescence, antibodies were diluted in 2% FBS–PBS supplemented with 0.02% (vol/vol) Tween 20 (PBS-T₀.₀₂; Sigma-Aldrich). All washes were performed three times for 5 min each time with PBS-T₀.₀₂, unless otherwise stated. Dewaxed sections were blocked with 20% normal donkey serum (Jackson ImmunoResearch Laboratories) before incubation for 1 h with goat anti-pIgR and rabbit anti-SS1ΔureAB OMV polyclonal sera diluted as described above. Autofluorescence of the formalin-fixed tissue was quenched by incubation in 0.1% (wt/vol) Sudan Black–70% (vol/vol) ethanol for 15 min followed by washes. Bound primary antibodies were detected by incubation with Alexa Fluor 594-conjugated donkey anti-goat Ig and Alexa Fluor 488-conjugated donkey anti-rabbit Ig (Invitrogen) (1 h; both diluted 1 in 500). Following washes, sections were counterstained with DAPI (4′,6-diamidino-2-phenylindole) (Invitrogen) (5 min, 400 ng/ml) followed by PBS washes before mounting with fluorescent mounting medium (Dako).

**Quantification of *H. pylori*-specific IgG and IgA in serum and intestinal contents.** Serum was assayed for *H. pylori* SS1-reactive IgG and IgA by endpoint titration enzyme immunoassay (EIA). Intestinal washings were assayed for total IgA using a mouse IgA-specific EIA (Bethyl Laboratories) and for SS1-reactive IgG and IgA using a standardized EIA as described previously (32). For the standardized EIA of intestinal contents,
EIA units were assigned from a standard curve using pooled serum from mice hyperimmunized with *H. pylori* SS1. This serum, which had an endpoint titer of 1 in 64,000, was assigned an arbitrary value of 1,000 units and used to allocate units of reactivity to the unknown samples. Intestinal contents were clarified by centrifugation and diluted independently 1 in 4 and 1 in 20 for assays. Optical densities (ODs) falling within the linear range of the curve were assigned units which subsequently were adjusted for their dilution factor and averaged for each sample. For samples where the highest OD was below the lower limit of the curve, units were assigned from the nonlinear region. For comparison of intestinal SS1-reactive antibody levels of individual C57BL/6 wild-type mice, SS1-reactive antibody levels were corrected for total IgA content. However, because of the lack of secretory antibody in the intestinal contents of pIgR KO mice, uncorrected SS1-reactive antibody levels were used to compare samples from wild-type and pIgR KO animals.

**Immunoblot analysis of SS1-reactive IgA and IgG in serum and intestinal contents.** Total membrane and soluble fractions of *H. pylori* SS1ure were prepared as described previously (32). Lipopolysaccharide (LPS) was purified from cells of plate-grown SS1 using the method described by Hitchcock and Brown (36). Antigen preparations were separated using preparative-well Novex 4% to 12% Bis-Tris gels with NuPAGE MOPS (morpholinepropanesulfonic acid) SDS running buffer for membranes and soluble fractions and NuPAGE MES SDS running buffer for LPS, and separated antigens were transferred onto polyvinylidene fluoride (PVDF) membranes, all following standard NuPAGE protocols (Life Technologies). Strips of each membrane were blocked with 10% skim milk powder–PBS and incubated with 2.5 ml 5% skim milk powder–PBS–Tg0 containing serum (1 in 500) or intestinal contents (1 in 10) from individual animals at room temperature for 2 h and then overnight at 4°C. Bound mouse IgA and IgG were detected sequentially and visualized as described previously (32).

**Statistical analysis.** Statistical analysis of quantitative data was performed using Prism v6.01 (GraphPad Software Inc.) and included the following tests as indicated in the text and/or figure legends: the D’Agostino & Pearson omnibus normality test was used to test individual groups for normal distribution; graphs illustrating populations that were normally distributed ions are shown as the mean ± 95% confidence interval (CI) or standard deviation (SD); data in graphs with one or more nonparametric populations are shown as the median ± interquartile range (IQR); two groups of data were compared by using Student’s t test (parametric) or the Mann-Whitney rank sum test (nonparametric); comparisons of three or more groups with one variable were done by one-way analysis of variance (ANOVA) with the Bonferroni posttest (parametric) or the Kruskal-Wallis test with Dunn’s multiple comparison posttest (nonparametric); comparisons of two or more groups with two variables were done by two-way ANOVA with the Bonferroni posttest (parametric); contingency tables were analyzed by using Fisher’s exact test; and correlations were calculated using Pearson’s correlation coefficient (parametric) or Spearman’s correlation coefficient (nonparametric). Where applicable, tests were unpaired and two tailed. P values of <0.05 were considered significant.

**RESULTS**

C57BL/6 wild-type and isogenic pIgR KO mice were inoculated with *H. pylori* SS1 or sterile BHI as outlined in Methods and Materials, Table 1, and Fig. S1A in the supplemental material. Experimental cohorts I and II represent two independent experiments that started approximately 3 weeks apart, and samples from all subsequent time points were collected and processed with a 3-week interval between the two cohorts. Quantitative and qualitative data sets arising from each cohort were combined for analysis of bacterial load in the stomach and duodenum, serum and intestinal secretory antibody levels, gastrointestinal pathology, and pIgR expression (see Fig. S1B). The contribution of secretory antibodies to immunity against *H. pylori* infection in these animals was examined in two ways: (i) comparison of the course of gastrointestinal infection in C57BL/6 wild-type and pIgR KO mice and (ii) comparison of the course of gastrointestinal *H. pylori* infection in chronically infected responder and nonresponder wild-type and pIgR KO mice (see Fig. S1C).

**The course of natural *H. pylori* infection in C57BL/6 wild-type and pIgR KO mice.** (i) Quantitative analysis of *H. pylori* infection in the stomach of wild-type and pIgR KO mice. Gastric bacterial loads of wild-type and pIgR KO C57BL/6 mice inoculated with *H. pylori* SS1 were determined 11 days (d) and 1, 3, 6, and 12 months (m) postinoculation (mpi) (Table 1). All SS1-challenged animals of both mouse strains were culture positive at each of the 5 time points sampled. There was no difference in the gastric bacterial loads of wild-type and pIgR KO mice at up to 3 mpi (Fig. 1A; P > 0.05; Mann-Whitney test). However, the median gastric bacterial load of wild-type mice determined by quantitative culture at 6 mpi (Fig. 1A) and by qPCR at 12 mpi (Fig. 1B) was significantly reduced compared to that of matched pIgR KO mice (P = 0.036 and 0.032, respectively; Mann-Whitney test). Moreover, while the median viable gastric bacterial load in pIgR KO mice remained constant for 12 mpi, the median loads of wild-type mice were significantly lower at 6 and 12 mpi, but not at 3 mpi, than in acutely infected wild-type mice (both P < 0.05; Kruskal-Wallis test, Dunn’s posttest).

(ii) Quantitative culture of *H. pylori* from the duodenum of wild-type and pIgR KO mice. We also determined the number of cultivatable *H. pylori* bacteria that remained attached to the first 5 cm of intestine following lavage of the intestinal lumen with PBS and two PBS washes of the longitudinally bisected tissue. Although the duodenal loads of *H. pylori* were comparable in the two mouse strains at up to 1 mpi (Fig. 1C), duodenal colonization in wild-type mice declined significantly over time (P = 0.0015; Kruskal-Wallis test). Conversely, intestinal colonization persisted in mice lacking secretory antibodies (P > 0.05; Kruskal-Wallis test) such that pIgR KO mice had significantly greater intestinal colonization at 3, 6, and 12 mpi than similarly infected wild-type mice (P = 0.002, 0.002, and 0.02, respectively; Mann-Whitney test). In addition, the density of *H. pylori* colonization was higher in the intestine of more pIgR KO mice than wild-type mice (Fig. 1D), with more than 1,000 CFU/gram intestine cultured from a significantly greater number of pIgR KO mice (27/45 pIgR KO mice versus 4/43 wild-type mice; P < 0.0001; Fisher’s exact test). Intestinal tissue from all animals gavaged with BHI was culture negative.

(iii) Distribution of *H. pylori* in the stomach of infected wild-type and pIgR KO mice. We performed qualitative analysis of the distribution of *H. pylori* throughout the stomach of infected animals by using silver staining and immunohistochemical and immunofluorescent detection of *H. pylori* in formalin-fixed tissue. The specificity of *H. pylori* antiserum was confirmed by using gastric tissue sections from BHI-inoculated mice (see Fig. S2 in the supplemental material). At 11 dpi, *H. pylori* was detected along the entire length of duplicate glandular stomach sections of all SS1-inoculated animals. Of the infected mice sampled at 3, 6, and 12 mpi, both duodenal culture and gastric immunohistological *H. pylori* analysis data were available for 19 mice of each strain. In these animals, only mice colonized in the distal antrum by *H. pylori* at a sufficient density for immunohistological visualization were duodenum culture positive (see Fig. S3). *H. pylori* coloniz-
tion of the distal antrum was visualized in 5/19 wild-type mice and 17/19 plgR KO mice (P = 0.0002; Fisher’s exact test) at these later time points, and 4 and 16 were culture positive for duodenal infection, respectively.

Similar analysis of the corpus region of mice sampled at 3, 6, and 12 mpi showed visible \textit{H. pylori} colonization in the corpus of 14/24 wild-type and 23/24 plgR KO mice examined (P = 0.0001; Fisher’s exact test), including 5 wild-type mice in which the bacterial density in the distal antrum was below the level required for immunodetection. All animals lacking visible \textit{H. pylori} colonization of the corpus had gastric bacterial loads below 10^7 CFU/gram.

(iv) Cellular infiltration and atrophic changes in response to \textit{H. pylori} infection of wild-type and plgR KO mice. Significantly more C57BL/6 mice (83%) than plgR KO mice (48%) developed corpus inflammation as a consequence of chronic \textit{H. pylori} infection (Table 2). This difference was chiefly seen in animals sampled at 3 mpi (see Fig. S4 in the supplemental material) (9 of 10 wild-type versus 2 of 10 plgR KO mice with corpus inflammation; P = 0.006; Fisher’s exact test) rather than at the 6 and 12 mpi time points. In contrast, similar numbers of plgR KO and C57BL/6 mice showed cellular infiltration in the proximal antrum (Table 2). Apart from underlying lymphocytic infiltration of the duodenum in both infected and uninfected plgR KO mice, inflammation was not observed in the distal antrum or duodenum of any animal examined.

Atrophic changes, scored primarily on clear cell metaplasia of parietal cells, were also observed in the corpus of some animals at 3, 6, and 12 mpi (Table 2), but there was no difference in the proportions of affected plgR KO and wild-type mice.

The many animals with no pathology made the data nonparametric and skewed the extent of inflammation toward zero. This
made statistical analysis of differences in the extent of inflammation or atrophy between the two mouse strains difficult. However, exclusion of unaffected animals from the populations normalized the data. Comparison of only affected mice showed no difference between C57BL/6 and pIgR KO mice in the degree of gastric inflammation or atrophy in response to chronic H. pylori infection (Fig. 2). In addition, this comparison showed that although the numbers of animals with inflammation of the proximal antrum were similar for infected and uninfected mice of both types (Table 2), the extent of inflammation was significantly greater for infected mice than uninfected mice (Fig. 2).

Taken together, these data suggest that the inflammatory response in the gastric corpus of wild-type mice from 3 mpi onwards may contribute to the reduction in gastric bacterial load of these mice at 6 mpi compared to pIgR KO mice. However, comparable cellular infiltration in the proximal antrum, and the absence of H. pylori-related inflammation in the distal antrum and intestine of all animals, suggests that it is unlikely that the inflammatory response is responsible for differences between immunocompetent and secretory antibody-deficient mice in the antral and duodenal localization of H. pylori infection.

(v) Antibody response of wild-type and pIgR KO mice to infection with H. pylori. Serum and intestinal contents from all mice were assayed for H. pylori SS1-reactive IgG and IgA (Fig. 3). In contrast to IgG seroconversion from 1 mpi (Fig. 3A), SS1-reactive IgA was detected in serum samples from both infected and uninfected pIgR KO mice from 11 dpi (Fig. 3B). This antibody most likely included the broadly reactive innate secretory IgA that protects mucosal surfaces before the development of an adaptive immune response (37) and had accumulated in the blood of pIgR KO animals due to continual mucosal IgA production in the absence of active antibody translocation. These preexisting SS1-reactive IgA antibodies continued to accumulate in the serum of these animals to the extent that uninfected pIgR KO mice had SS1-reactive serum IgA titers similar to those of infected wild-type mice by 3 mpi. This increasing IgA baseline for the pIgR mice, together with the wide spread of IgG and IgA titers for mice of both strains, precluded accurate statistical analysis of the antibody titers of the different mouse strains. Instead, we determined the numbers of animals of each strain that seroconverted ≥4-fold and ≥10-fold in response to infection compared to the geometric mean titers of matched uninfected animals sampled at the same time point after inoculation. IgG and IgA seroconversion rates of pIgR KO mice were not different from those of matched wild-type mice at any individual time point (see Table S1 in the supplemental material); however, there was a trend toward poorer IgA seroconversion rates for pIgR mice. Accordingly, the number of chronically infected (3, 6, and 12 mpi, combined) pIgR KO mice with ≥10-fold IgA seroconversion was significantly lower than that of the wild-type mice (10 of 29 pIgR KO mice compared with 20 of 29 wild-type mice; P = 0.02; Fisher’s exact test).

SS1-reactive IgA and IgG were not detected in intestinal contents from any animals sampled at 11 dpi and 1 mpi, which was consistent with the lack of serum IgA seroconversion in all wild-type animals at those time points. From 3 mpi onwards, measurable SS1-reactive IgA was detected in the intestine of the majority of infected wild-type mice (21 of 27 animals) compared to few infected pIgR KO mice (7 of 26 animals) and uninfected animals of both strains (1 of 42 animals) (Fig. 3C). We also observed a strong correlation between the amount of SS1-reactive IgA translocated into the intestine of wild-type animals and their serum SS1-reactive IgA titer (Fig. 3D; Spearman r = 0.93; P < 0.0001). Interestingly, SS1-reactive IgA was detected in the intestinal contents of a small number of infected pIgR KO mice at 3, 6, and 12 mpi (Fig. 3C). All of these animals had extremely high SS1-reactive serum IgA titers (≥64,000) compared to other pIgR KO mice, as well as atrophy in the gastric corpus and inflammation in both the corpus and proximal antrum. These findings agree with the underlying mucosal leakiness previously reported for pIgR KO mice (38).

Immunoblot analysis of paired serum samples and intestinal contents from wild-type mice confirmed that IgG was the dominant isotype of SS1-reactive serum antibody in these animals and that IgA was the dominant isotype in the intestinal contents (Fig. 4). Some wild-type animals showed restricted profiles of IgA re-

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**TABLE 2** Histopathological changes in the stomach of C57BL/6 and pIgR KO mice 3 to 12 months after inoculation with H. pylori SS1 or BHI

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Mouse strain</th>
<th>Total no.</th>
<th>Corpus inflammation</th>
<th>Proximal antrum inflammation</th>
<th>Corpus atrophy</th>
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<tr>
<td>SS1</td>
<td>C57BL/6*</td>
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<td>21</td>
<td>2 (9)*</td>
<td>10 (48)</td>
<td>0 (0)*</td>
</tr>
<tr>
<td></td>
<td>pIgR KO*</td>
<td>21</td>
<td>3 (14)*</td>
<td>12 (57)</td>
<td>2 (9)</td>
</tr>
</tbody>
</table>

* *, statistically significant difference by Fisher’s exact test for corpus inflammation for a versus b data (P = 0.01), a versus c data (P < 0.0001), and b versus d data (P = 0.02) and for corpus atrophy for a versus c data (P = 0.0002). All other comparisons were not statistically significant (P > 0.05).

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**FIG 2** Histopathological grading of gastric mucosa for pathology-positive H. pylori-inoculated and control (BHI-inoculated) wild-type (WT) and pIgR KO C57BL/6 mice sampled at 3, 6, and 12 months after inoculation. Pathology scores (mean ± 95% CI) for inflammation of the gastric corpus (SS1-inoculated animals only) and gastric proximal antrum (SS1-inoculated and BHI-inoculated animals) represent combined scores for mononuclear and neutrophil infiltration. Pathology scores for corpus atrophy represent parietal cell clear cell metaplasia. The numbers of animals in each group correspond to those in Table 2 and are reported in parentheses. All statistical comparisons of corpus inflammation and corpus atrophy (Student’s t test) were not significant (P > 0.05). Statistical analysis of proximal antrum inflammation by two-way ANOVA with Bonferroni’s posttest showed significant differences between mice inoculated with H. pylori and controls. *, P < 0.05; **, P < 0.01.
activity to SS1 lipopolysaccharide (see Fig. S5 in the supplemental material; 3 mpi). We did, however, observe some differences in the reactivity profiles of intestinal IgA compared with serum IgA from wild-type mice, suggesting that the intestinal antibody was produced locally (Fig. 4 and S5; C57BL/6, 6 mpi). In contrast, intestinal SS1-reactive IgG, where present, reflected the serum IgG profile (Fig. 4; C57BL/6, 6 mpi). For pIgR KO mice, IgA was the dominant SS1-reactive serum isotype and mainly recognized SS1 lipopolysaccharide (see Fig. S5). EIA-positive intestinal contents from pIgR KO animals showed no reactivity to the tested antigens by immunoblot analysis, supporting the reportedly high level of broadly reactive, low-specificity IgA in these mice.

Previous studies have suggested that the IgA response to \textit{H. pylori} infection may be anti-inflammatory (6). However, we found there was a positive correlation between the serum IgA titer and the corpus inflammation score for both wild-type mice (see FIG 3).

**FIG 3** \textit{H. pylori} SS1-reactive IgG and IgA in serum and intestinal contents from wild-type and pIgR KO C57BL/6 mice inoculated with \textit{H. pylori} or BHI. (A and B) Serum \textit{H. pylori}-reactive IgG (A) and IgA (B) titers were determined by EIA using endpoint titration. Symbols represent individual animals from cohort I for 11 dpi and 1 mpi and from cohorts I and II for 3, 6, and 12 mpi. Each sample was assayed once in duplicate. Error bars denote median ± IQR of the results for each group. The numbers of animals shown above each group in panel A also apply to panel B. (C) SS1-reactive IgA in intestinal contents. Animal numbers are shown beneath the data for each group. EIA units were derived by using standard curves prepared from pooled \textit{H. pylori}-immune mouse serum. Symbols represent individual animals, and intestinal SS1-reactive antibody levels were averaged from duplicate independent EIAs for 3- and 6-mpi animals and from a single EIA for 12-mpi animals (each assay included independently diluted duplicates). Error bars denote the median ± IQR of the results for each group. *P < 0.05; ** P = 0.01; *** P = 0.001. (D) Correlation analysis of serum and intestinal SS1-reactive IgA. The linear regression (solid line) ± 95% CI (dotted line) is shown.

**FIG 4** Immunoblot analyses of \textit{H. pylori}-reactive IgG and IgA in serum and intestinal contents from \textit{H. pylori} SS1-infected C57BL/6 wild-type and pIgR KO mice. NuPAGE-separated \textit{H. pylori} total membrane fraction antigens were transferred to PVDF membranes and probed with serum (S) or intestinal contents (IC) of animals killed at 3 and 6 mpi. Data from one representative animal of each strain are shown per time point. All 4 animals represented in the figure had intestinal contents that were EIA positive for SS1-reactive IgA and had gastric bacterial loads of <10^7 CFU/g stomach. Lane M contains Magic-Mark markers (Bio-Rad; estimated sizes are indicated in kDa).
Acute infections, despite technically seroconverting to their infection. These fully colonized (nonresponder) animals contrasted with a subset of animals (responders) that had reduced bacterial loads together with strong humoral and inflammatory responses. These differences provided a unique opportunity to investigate immune responses that may contribute to the decline in the gastric bacterial load of wild-type mice compared with plgR KO mice. More importantly, the responder and nonresponder wild-type mice allowed us to examine a potentially protective immune response to natural infection in naive, immunocompetent animals.

(i) Gastric bacterial load of responder and nonresponder C57BL/6 mice infected with \textit{H. pylori}. All acutely infected animals (i.e., sampled at 11 dpi and 1 mpi) had bacterial loads of greater than 10^7 CFU/g stomach, and the median bacterial load of the wild-type cohort during this period was 10^{7.5} (IQR, 10^{7.2} to 10^{7.7}) CFU/g stomach. Therefore, we selected 10^7 CFU/g stomach as an arbitrary threshold for full colonization, and any animal with a gastric bacterial load less than this threshold was considered to have reduced colonization. Examination of bacterial load data determined by using both qPCR (genomes/500 μg DNA) and culture (indicated in Fig. 1A and B) methodologies demonstrated that 10^7 genomes/500 μg DNA was also a suitable threshold for categorization of animals whose bacterial load had been determined by qPCR. Consequently, bacterial loads determined by qPCR were considered equally with those determined by culture because the relationship between the bacterial loads of individual animals was highly reproducible between the two methodologies.

In all, 27 wild-type mice were included in this comparison, of which 13 were fully colonized. Two additional mice from cohort II were excluded: intestinal contents were inadvertently not collected from one animal; the other animal sampled at 3 mpi had a reduced gastric bacterial load by viable count contradicted by very strong \textit{H. pylori}-specific labeling throughout the stomach by immunohistochemistry and immunofluorescence. Comparison of the bacterial loads of mice with full and reduced colonization showed a greater than 10-fold difference in the median bacterial load between the cohorts (10^7.6 [IQR 10^7.4 to 10^7.6] versus 10^6.0 [IQR 10^5.5 to 10^6.5] CFU/g stomach, respectively; Fig. 6A). Of the mice for which duodenal culture data were available, 4 of 10 and 0 of 12 with full and reduced colonization, respectively, were culture positive (P = 0.03; Fisher’s exact test).

(ii) Immune responses correlating with full and reduced gastric bacterial load. Animals with a reduced bacterial load had greater cellular infiltration into the mucosa of the gastric corpus than fully colonized animals (Fig. 6B). The negative correlation between inflammation and bacterial load was highly significant (Pearson r = –0.77; P < 0.0001; Fig. 6C). For comparisons of intestinal antibody levels from individual wild-type animals, SS1-reactive IgA was standardized against total IgA in the intestinal contents. Standardized SS1-reactive IgA was 8 times more abundant in the intestinal contents of animals with a reduced bacterial load than in fully colonized mice (Fig. 6D) and also showed a significant negative correlation with bacterial load (Pearson r = –0.69; P < 0.0001; Fig. 6E). However, animals with reduced gastric infection also had significantly higher levels of total intestinal IgA than fully colonized and uninfected mice (see Fig. S7 in the supplemental material). This indicates an even greater discor-

![Fig. 5: Body weights of \textit{H. pylori}-infected and -uninfected wild-type C57BL/6 and plgR KO mice. Symbols represent individual animals combined from independent experimental cohorts I and II as detailed in the text and Table 1; numbers in each group are shown in parentheses. Error bars denote the mean ± 95% CI of the results for each group. P values were determined by using two-way ANOVA (Bonferroni’s posttest) with a two-way comparison of infection status and mouse strain for each time point. *, P < 0.05; ***, P < 0.0001.](http://iai.asm.org/)

Fig. S6A in the supplemental material) (Spearman r = 0.72; P < 0.0001) and plgR KO mice (see Fig. S6B) (Spearman r = 0.58; P < 0.001). These findings suggest that the smaller number of plgR KO mice with inflammation in the gastric corpus was not a consequence of significantly higher IgA titers in the gastric tissue of these animals.

Of additional interest was the small number of animals sampled at 3, 6, and 12 mpi that failed to seroconvert following infection with \textit{H. pylori}. In total, there were 6 mice (4 wild-type and 2 plgR KO mice) with no detectable SS1-reactive serum IgG and 5 wild-type mice with no detectable SS1-reactive serum IgA (these included the 4 IgG nonresponders). All of these animals showed no gastric inflammation, despite being fully colonized, as determined by viable count and/or qPCR together with immunostaining of gastric tissue.

(vi) Impact of \textit{H. pylori} infection on body weight of plgR KO mice. Although body weight was not included as a monitored parameter in the initial study design, it was evident by 3 months postchallenge that plgR KO mice infected with \textit{H. pylori} were smaller than their wild-type counterparts. Consequently, the animals sampled at 6 and 12 mpi were weighed immediately postmortem. Analysis of the body weights showed that \textit{H. pylori} infection significantly slowed the growth of the plgR KO mice but not that of the wild-type C57BL/6 mice (Fig. 5). Although the difference in body weights of infected and uninfected plgR KO mice was only slight at 6 mpi, by 12 mpi, the mean body weight of \textit{H. pylori}-infected plgR KO mice was only 70% that of mice of the BHI-challenged plgR KO cohort (P < 0.0001; two-way ANOVA).

The course of gastric \textit{H. pylori} infection in responder and nonresponder mice. As stated above, we observed that some chronically infected wild-type mice failed to seroconvert following infection with \textit{H. pylori}. These mice did not mount an inflammatory response to infection. Some of these animals also had persistent duodenal colonization, and all had a gastric bacterial load similar to that in wild-type mice sampled during acute infection (at up to 1 mpi). Interestingly, there were other chronically infected wild-type mice with bacterial loads similar to those of acutely infected animals, despite technically seroconverting to their infection. These fully colonized (nonresponder) animals
dance in the amounts of SS1-reactive IgA in the intestine of responder versus nonresponder mice.

The inflammatory response of individual animals was also assessed qualitatively with respect to histological changes. Apart from the standard grading of neutrophil and mononuclear cell infiltration, specific disease-associated histological changes, including lymphocytic gastritis and gastric lymphoid follicle formation, were identified. These changes, which can also occur in humans infected with *H. pylori*, were not present in tissue from fully colonized animals but were identified in 6 of 14 animals with reduced bacterial loads (*P* < 0.02; Fisher’s exact test), all of which had bacterial loads of less than $10^6$ CFU/g stomach.

We also examined the corelationship of both inflammation and antibody with gastric bacterial load. Taking 1 standard deviation from the mean of the inflammatory and intestinal IgA responses of animals with reduced bacterial loads as the minimum protective threshold (mean minus 1 SD = 3.6 corpus inflammation score and 436 SS1-reactive IgA/μg total IgA, respectively), all animals with both cellular and secretory antibody responses above these thresholds had reduced gastric colonization (Fig. 6F). Conversely, the majority of fully colonized animals had attenuated responses with respect to both corpus inflammation and intestinal IgA.

Although evident in only a few wild-type animals, our data suggested that having either an inflammatory response or a secretory antibody response alone was insufficient to reduce the gastric bacterial load. To investigate this possibility further, we examined the relationship between inflammation and the gastric bacterial load of pIgR KO mice, which are incapable of mounting a secretory antibody response. Responder pIgR KO
mice were defined as those animals with corpus inflammation scores greater than 3.6 (i.e., \( \geq 4 \)), which was the minimum protective score observed in chronically infected responder wild-type mice. Responder pIgR KO mice with significant corpus inflammation had reduced median bacterial loads compared to animals with corpus inflammation scores of 3 or less (\( P = 0.004; \) Mann-Whitney test; see Fig. S8A in the supplemental material). However, this 4-fold reduction in gastric bacterial load was significantly smaller (\( P = 0.01; \) Mann-Whitney test; Fig. S8A) than the 40-fold reduction observed with the same analysis of wild-type mice (\( P = 0.0004; \) Mann-Whitney test). In agreement with this, there were fewer corpus responder pIgR KO mice with a gastric bacterial load below \( 10^7 \) CFU/g stomach than corpus responder wild-type mice (4 of 9 pIgR KO mice compared with 13 of 14 wild-type mice; \( P = 0.018; \) Fisher’s exact test). These data suggest that corpus inflammation in conjunction with secretory antibodies has a greater impact on gastric bacterial load than inflammation alone.

In contrast to gastric bacterial load, comparative analysis of corpus inflammation and duodenal colonization showed no correlation. Chronically infected pIgR KO mice with significant corpus inflammation and reduced median gastric bacterial load had duodenal colonization equivalent to that seen with mice with corpus inflammation scores < 4 and maximal gastric colonization (see Fig. S8B in the supplemental material). Chronically infected wild-type mice also showed no correlation between inflammation, gastric bacterial load, and duodenal bacterial load, as both the responder and nonresponder populations (with reduced and full median gastric bacterial loads, respectively) showed negligible duodenal colonization.

(iii) Gastric pIgR expression in responder and nonresponder wild-type mice. Gastric tissue from C57BL/6 mice sampled at 3, 6, and 12 mpi was immunostained for pIgR. Colorimetric and fluorescent immunohistological detection of pIgR expression in responder and nonresponder animals was compared to that in uninfected control animals and in animals sampled during acute infection at 11 dpi.

At 11 dpi, pIgR expression was detected in gastric tissue in only 1 of 9 infected wild-type animals and was not detected in gastric tissue from any uninfected animal. In contrast, pIgR expression was readily visible in the intestinal tissue from all animals sampled at 11 dpi. We observed an increasing gradient of expression along the 5 cm of intestine examined such that pIgR was barely detectable adjacent to the pyloric sphincter but was strongly expressed in the small intestine further from the stomach (Fig. 7G to J). Increased intestinal pIgR expression in all animals was associated with increasing age and was possibly a consequence of establishment of the intestinal microbiota. By 3 mpi, we also observed infection-associated induction of pIgR in the gastric mucosa insofar as pIgR expression was evident only in chronically \( H. \) pylori-infected wild-type mice that responded to their gastric infection but not in animals that did not respond to infection (Fig. 7A to D; see also Fig. S9A and B in the supplemental material), pIgR KO mice (see Fig. S9C and D), or uninfected animals. Moreover, in contrast to the results from the gastric corpus and proximal antrum, pIgR expression was observed only infrequently and sparsely in the distal antrum of some responder animals (Fig. 7E and F).

**DISCUSSION**

In this study, we examined the natural course of \( H. \) pylori infection in naive wild-type and pIgR KO C57BL/6 mice. We observed two major effects on the \( H. \) pylori load in wild-type mice that were not observed in pIgR KO mice lacking secretory antibody: (i) clearance of transient duodenal colonization by 3 mpi and (ii) a significant reduction in gastric bacterial load by 6 mpi compared to that measured during acute infection. A time-dependent reduction in the gastric load of \( H. \) pylori infection in naive mice has been reported previously (2). However, because the present study showed that the immune responses mounted by pIgR KO mice to \( H. \) pylori were not protective, the capacity of the immune response to influ-
ence *H. pylori* chronicity in wild-type mice became evident, and measurable, for the first time.

The most striking feature of the protective immune response to *H. pylori* in wild-type mice was the eradication of transient *H. pylori* colonization from the intestine upon IgA seroconversion and secretion of *H. pylori*-reactive antibody into the intestinal lumen. Conversely, duodenal *H. pylori* colonization was sustained in chronically infected plgR KO mice that inherently lack secretory antibody, and the small number of wild-type mice defective for duodenal clearance all showed no or minimal seroconversion.

The second important feature was the reduction of bacterial load in the distal antrum of mice with *H. pylori*-reactive intestinal secretory IgA, an event that is evidently linked to the reduction in number of viable bacteria in the duodenum. However, these two features, which occurred by 3 mpi, were not sufficient to cause a measurable reduction in the overall gastric bacterial load. By 6 mpi, greater inflammation in the gastric mucosa correlated with a significant reduction in gastric bacterial load in wild-type animals. However, a significantly less pronounced reduction occurred in plgR KO animals with similar inflammatory changes. Therefore, our observations indicate that effective immunity to *H. pylori* may rely on an underlying and fully functional secretory antibody response, even though this is not sufficient in itself to have an impact on overall gastric *H. pylori* infection.

These findings suggest that the amount of secretory antibody available to the stomach can contribute to protective immunity around the gastroduodenal junction and that the altered gastric distribution of *H. pylori* colonization in wild-type mice is consistent with protective antibody being sourced from the duodenum rather than being translocated locally. This hypothesis is supported by the paucity of plgR expression in the distal antrum of all animals, including those mice that had efficiently cleared *H. pylori* from the distal antrum. One should also consider the fact that this study was conducted in C57BL/6 mice which are Th1 skewed (39). As such, our findings illustrate a significant capacity for antibody-mediated immunity even in the context of a dominant cell-mediated immune response.

There are several possible explanations for the failure of antibody-mediated immunity to protect fully against gastric *H. pylori* infection. In the vast majority of the gastric mucosa, antibody levels are insufficient to influence *H. pylori* infection. This is because the machinery for translocating antibodies, i.e., plgR, across the gastric mucosa is not expressed in the normal, healthy gastric mucosa (15). We also observed in this study that when plgR expression was upregulated during gastric inflammation, it occurred predominantly in the corpus and proximal antrum, which is likely to have been a consequence of local IFN-γ production. In contrast, there was no to very little expression of plgR in the distal antrum, where clearance of *H. pylori* colonization to below histological detection levels correlated with the presence of *H. pylori*-specific secretory antibodies in the duodenum of wild-type mice. Given that IgA production is suppressed by IFN-γ (40), upregulation of gastric plgR expression does not necessarily lead to increased IgA secretion by the gastric mucosa. This is in agreement with studies showing no increase in gastric secretory IgA levels during infection of humans with *H. pylori* (25, 28) and the observation that *H. pylori*-infected, inflamed gastric epithelium stains with similar intensities for secretory components and IgA regardless of the presence of local IgA-secreting plasma cells (16). Although *H. pylori*-induced IFN-γ production may reduce IgA production in the stomach despite its contribution to plgR upregulation, infection with *H. pylori* did not significantly suppress intestinal IgA secretion in animals in this study. In fact we measured increased total IgA in the intestinal contents of *H. pylori*-infected mice with reduced gastric bacterial load compared to fully colonized and uninfected animals.

Another important factor contributing to the failure of gastric IgA to convey immunity to *H. pylori* is the reliance of gastric immunity on extragastric secreted antibody. As stated earlier, gastric IgA levels peak during transition of the phase III migrating motor complex (MMC) from the stomach to the duodenum. The MMC, consisting of progressive phase I, II, or III, occurs between meals, and MMC phase III is responsible for the mechanical and chemical cleansing of the empty stomach in preparation for the next meal (41). Approximately 50% of MMC activity originates in the stomach (42), and its transition through to the intestine results in the stomach entering the predominantly idle phase I as the duodenum enters the highly active phase III (30), during which duodenal IgA secretion is maximal (43). Retrograde peristalsis is a normal consequence of this transition and facilitates pH restoration of the duodenal bulb and the antrum following gastric emptying (44). Duodenal retroperistalsis is also a normal consequence of gastroduodenal motility in mice (45). The resulting reflux delivers freshly translocated antibody in the duodenal contents to the gastric lumen, and in humans this is a specific event that occurs in the absence of bile reflux (30). Individuals lacking retrograde duodenal reflux during phase III of the MMC show a higher prevalence of *H. pylori* colonization (46). In contrast, IgA-deficient humans have no increased risk of infection (47). This difference may be due to immune benefits being conferred by secretory antibody of any isotype, and IgM compensation may be sufficient in IgA-deficient individuals.

The evidence in this study for a correlation between duodenal secretory IgA level and gastric *H. pylori* distribution is intriguing. In the antrum, *H. pylori* is mainly found close to the epithelium in the firmly adherent mucus layer (48), the integrity of which is significantly compromised during *H. pylori* infection (49, 50). Secretory IgA has a high affinity for mucin glycoproteins and is more soluble in mucus than other antibody isotypes (51). It has recently been hypothesized that the loosely adherent mucus layer overlying the firmly adherent mucus could be sheared from the antrum during the powerful MMC phase III contractions, thus exposing the firmly adherent mucus layer in which *H. pylori* resides to freshly delivered duodenal fluid containing secretory IgA (52). This hypothesis is readily conceivable given the ease with which the loosely adherent mucus layer can be displaced (49).

In this study, we also observed that mice which failed to seroconvert to *H. pylori* also failed to develop an inflammatory response or pathological changes. This is in agreement with work showing that tolerance of *H. pylori* mediates protection against *H. pylori*-mediated disease (53). We also found that animals which developed similar inflammatory and systemic antibody responses to *H. pylori* showed a reduced gastric bacterial load only when *H. pylori*-reactive antibodies were detected in the intestinal lumen. Together with the finding that duodenal colonization persisted in plgR KO animals, our findings suggest that *H. pylori*-reactive antibodies may be beneficial in controlling *H. pylori* infection. More specifically, our findings suggest that secretory antibody contributes to protective immunity against *H. pylori* in the distal antrum and duodenum.
In humans infected with _H. pylori_, duodenal colonization correlates with the development of gastric metaplasia and with the production of _H. pylori_-permissive gastric mucus in the duodenum (54–56). Given that we did not observe gastric metaplasia in the duodenum of any _H. pylori_-colonized animals in this study, it is not surprising that duodenal bacterial loads were low compared to those in the stomach. In contrast to studies of intestinal colonization in humans that rely on duodenal biopsy specimens, however, we were able to examine bacterial loads in the entire duodenum of mice. From this analysis we reproducibly detected duodenal _H. pylori_ colonization in infected mice, but only in the absence of _H. pylori_-reactive secretory antibody. This observation was not due to differences in the ability of _H. pylori_ to colonize the intestine of the mouse strains, as duodenal colonization results were indistinguishable between the two strains prior to seroconversion. Moreover, no differences have been observed in overall intestinal normal flora colonization of pgR KO and wild-type mice (57). In an infected human, the duodenum would be continually exposed to _H. pylori_ in the absence of an effective secretory antibody response, low levels of _H. pylori_ may closely associate with the duodenal mucosa. Persistent duodenal colonization correlated with detectable _H. pylori_ in the distal antrum, suggesting that a continual supply of bacteria was required. Over a prolonged period, the persistent presence of _H. pylori_ in the human duodenum may transform areas of the duodenal mucosa, thus facilitating the development of a gastric metaplasia which harbors the more florid patches of _H. pylori_ colonization in duodenal ulcer patients. Given the limited capacity of the gastric mucosa to secrete IgA, the development of gastric metaplasia in the intestine may suppress the intestinal immune response. Indeed, cytokine responses in the duodenal mucosa of duodenal ulcer patients have been reported to be suppressed compared to those of asymptomatic carriers of _H. pylori_ (58). Our findings suggest that intestinal secretory IgA may exert a protective effect in two ways: (i) by keeping bacterial numbers in the distal antrum at a low level, thus reducing the capacity for _H. pylori_ to amass in the normal duodenum, and (ii) by neutralizing any bacteria that reach the duodenum. It would be interesting to examine the contribution of intestinal secretory antibody to natural and vaccine-mediated _H. pylori_ immunity in the duodenum during human infection. Such studies might provide a valuable insight into the protective immunity and/or tolerance associated with life-long asymptomatic infection. This area is becoming increasingly important as the debate surrounding the protective capacity of _H. pylori_ infection against other diseases, such as chronic inflammatory and atopic disorders (59), esophageal malignancies (60–62) and tuberculosis (63), continues.

An interesting observation in our study was that pgR KO mice infected with _H. pylori_ were visibly smaller than their BHI-challenged siblings or _H. pylori_-infected wild-type mice. Cohort numbers were too small in this study to ascertain whether this body-weight effect on pgR KO animals was a consequence of uncontrolled _H. pylori_ infection in general or was specifically associated with intestinal colonization. While weight differences between _H. pylori_-infected and uninfected experimental animals are not generally reported, humans display a variety of weight-associated changes in relation to _H. pylori_ infection. Of most importance is the impact of _H. pylori_ infection on childhood development (64, 65). However, while weight loss is not a factor in adult _H. pylori_ disease, weight gain in adults following _H. pylori_ eradication has been reported (66). Our findings suggest for the first time that the humoral immune response may protect against failure-to-thrive-related sequelae resulting from _H. pylori_ infection of children.

In summary, our data suggest that the infection-induced humoral immune response to infection with _H. pylori_ can protect the duodenum against long-term colonization by _H. pylori_. Moreover, antibodies to _H. pylori_ may contribute to the maintenance of low bacterial loads in the gastric antrum, which in turn may facilitate normal gastric endocrine function for acid production and thus indirectly protect the gastric corpus. While it is highly unlikely that antibodies are sufficiently present or functional in the acid-secreting regions of the stomach to mediate direct immunity in these regions, stimulation of an effective secretory antibody response may be a necessary property of any vaccine destined to prevent _H. pylori_-related disease or to mediate sterilizing immunity.

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