Host Adaptation and Immune Modulation Are Mediated by Homologous Recombination in Helicobacter pylori

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Rearrangement of genomic DNA via homologous recombination provides an alternative mechanism of gene regulation that is essential for successful colonization of the gastric mucosa by Helicobacter pylori. Inoculation of outbred mice with the H. pylori SS1 wild-type strain elicited a T helper (Th) 2 response and established a persistent infection. In contrast, inoculation with an isogenic H. pylori strain defective for homologous recombination elicited a Th1-mediated immune response and clearance of infection within 70 days. We, therefore, demonstrate that recombination is critical for mediating persistence of a microbial pathogen through the induction of ineffective immune responses.

Helicobacter pylori colonizes the stomachs of approximately half of the world’s population and is associated with peptic ulceration and gastric malignancy [1]. Despite inducing strong humoral and cellular immune responses, H. pylori is rarely eliminated from the gastric mucosa, and, in the absence of treatment, infection is usually lifelong. H. pylori is able to elicit an ineffective immune response that contributes to both persistence and disease [2]. Although the mechanisms of immune subversion are unclear, protection is thought to depend on a Th1 response [3–8]. It would be advantageous to have an immune-based strategy to reprogram the natural response to H. pylori and to induce protection and eradication.

Persistent colonization depends on the ability to respond to changing environmental conditions and circumvent host defense mechanisms initiated during infection. Rearrangement of genomic DNA (e.g., through transposition and inversion) allows a variety of pathogens to adapt expression of surface antigens and evade host immunity [9, 10]. H. pylori has the highest rate of genetic recombination of any known bacterial species [11], suggesting that this process confers a selective advantage in colonization. Our earlier studies showed that H. pylori mutants defective for homologous recombination were spontaneously cleared from the murine gastric mucosa, whereas the H. pylori SS1 wild-type (wt) strain established a persistent high level of colonization [12]. We hypothesized that clearance of these mutant strains was accomplished via an immune-mediated mechanism and not merely because of their increased susceptibility to oxidative stress and phagocytosis.

To test whether immune evasion by H. pylori is dependent on recombination-dependent rearrangement of genes, we examined the humoral and cellular immune responses in mice infected with the wt strain [13] or a recombination-deficient isogenic mutant in which the ruvC gene, which encodes a Holliday junction resolvase [14], had been inactivated [12]. The present study was designed to use outbred mice because the most commonly used inbred strains (C57BL/6 and BALB/c) are known to have a genetically determined bias in their T helper (Th) responsiveness [15]. It was...
anticipated that intragroup variation would be large but that the model would be robust and more relevant for human infection than if genetically identical mice were used. We found that the wt strain and the ruvC mutant elicited oppositely polarized Th cell responses that correlated with persistence and clearance, respectively. Temporary colonization by the ruvC mutant conferred significant protection against subsequent challenge with the wt strain.

**MATERIALS AND METHODS**

**Bacterial strains, cell lines, and growth conditions.** *H. pylori* SS1 wt strain [13] and an isogenic strain in which the ruvC gene had been disrupted [12] were used. Inactivation of ruvC reduced the frequency of homologous recombination of *H. pylori* by ~30-fold [12]. *H. pylori* strains were cultured under microaerobic conditions at 37°C on blood agar base 2 plates (Oxoid) supplemented with 10% horse blood and 10 μg/mL vancomycin (Sigma-Aldrich), 2.5 IU/L polymyxin (Sigma), 5 μg/mL trimethoprim (Sigma), and 4 μg/mL amphotericin B (Sigma). Liquid cultures of *H. pylori* were grown in brain-heart infusion (BHI) broth containing 0.2% cyclodextrin and an antibiotics-fungicide mixture.

Mucormysm spleen-cell suspensions were grown in complete medium (RPMI 1640 medium [Invitrogen] with 10% fetal calf serum [FCS], 10 mmol/L HEPES, 2 mmol/L l-glutamine, 100 IU/mL penicillin, and 100 μg/mL streptomycin [all from Sigma]), at 37°C in 5% CO₂.

**Animal colonization.** Specific pathogen–free CD1 mice (6–8 weeks old; Charles River) were housed in isolators and fed a commercial pellet diet with water ad libitum. Guidelines of the UK Home Office and those of the authors’ institution were followed in the conduct of animal studies. One hundred microliters containing 10⁵–10⁶ *H. pylori* in BHI broth supplemented with 0.2% cyclodextrin was administered to mice orogastrically [12]. For all experiments, suspensions of wt and ruvC mutant bacteria used for inoculations were prepared from identical low-subculture stocks (8–12 in vitro passages). Mice were killed up to 70 days after infection. Colonization with *H. pylori* was assessed by quantitative culture, as described elsewhere [16]. The stomach tissue was washed in PBS; divided so that each fragment contained cardia, body, and antrum; and placed in BHI broth/0.2% cyclodextrin. Tissue fragments were homogenized by use of disposable plastic grinders (PolyLabo), and homogenates were serially diluted and plated onto blood agar plates.

**Detection of antibodies by ELISA.** Serum samples were tested for *H. pylori* antigen–specific IgA, IgM, IgG, IgG1, and IgG2A antibodies by an ELISA technique [16]. Ninety-six–well Maxisorp plates (Nunc) were coated with 25 μg of a sonicated whole-cell extract of *H. pylori* SS1. Serum samples were diluted 1:100 and were added in 100-μL aliquots to coated microtiter wells. Bound *H. pylori*–specific antibodies were detected by use of biotinylated goat anti–mouse immunoglobulin and streptavidin-peroxidase conjugate (Amersham). The readings for uncoated wells were subtracted from those for the respective test samples.

**T cell proliferation assay.** Splenocytes were individually placed into tubes containing RPMI 1640 medium supplemented with 2% FCS on ice. Tissues were rubbed through 100-μm nylon mesh cell strainers (BD Biosciences), and red blood cells were lysed with 0.85% ammonium chloride solution [17]. The cells were washed, counted, and resuspended at 10⁶ cells/mL in complete medium. Proliferation was determined by measuring [³H]-thymidine incorporation [17]. Quadruplicate aliquots (0.2 mL) of spleen cells (SPLCs) from each mouse were incubated in complete medium, in 96-well plates, for 5 days at 37°C in 5% CO₂ in the presence or absence of either a 5 μg/mL sonicated preparation of *H. pylori* SS1, 5 μg/mL recombinant *H. pylori* urease B subunit, or 5 μg/mL concanavalin A (ConA) (optimal stimulatory antigen concentrations were determined in pilot experiments; data not shown). One microcurie of [³H]-thymidine (Amersham) was added to each well for the final 18 h of culture, and [³H]-thymidine incorporation was measured by use of a scintillation counter. Results are expressed as the change in counts per minute (Δcpm) of stimulated cells, compared with unstimulated cells.

**Immunostaining and flow-cytometric analysis.** Expression of CD4, interferon (IFN)–γ, interleukin (IL)–4, and IL-10 by freshly isolated SPLCs was determined by flow-cytometric analysis, after intracellular staining with fluorochrome-conjugated antibodies [18]. Suspensions of 5 × 10⁵ SPLCs in 1 mL of complete medium were inoculated into sterile culture tubes (Elkay Laboratory Products), before a sonicated preparation of either *H. pylori* SS1, phorbol myristate acetate (Sigma), or ionomycin (Sigma) (Sigma) was added, to final concentrations of 5 μg/mL, 20 ng/mL, and 1 μmol/L, respectively. No antigens or mitogens were added to negative control tubes. The cultures were incubated for 2 h at 37°C in 5% CO₂, before 10 μg/mL brefeldin A (Sigma) was added. Cultures were incubated for a further 18 h (test samples and negative controls) or 4 h (positive controls) (optimal incubation times were determined in pilot experiments; data not shown). The cells were first stained with anti–CD4-biotin (Serotec), followed by streptavidin conjugated to R-phycocerythrin–Texas Red (Serotec), in accordance with the manufacturer’s instructions. After washing and fixation in 1 mL of 0.5% formaldehyde in PBS at 4°C, the cells were washed, permeabilized, and stained with anti–IFN-γ–fluorescein isothiocyanate–biotin (Beckman Coulter), anti–IL-4–phycoerythrin (Beckman Coulter), and anti–IL-10–allophycocyanin (BD Bioscience) antibody conjugates [18]. Flow-cytometric analysis was performed by use of a Coulter EPICS XL-MCL (Beckman Coulter) flow cytometer, and data on 100,000 events were acquired. In all ex-
periments, unstained cells and cells stained separately with each fluorochrome-labeled antibody were included, to optimize compensation settings. Data analysis was performed by use of WinMDI (version 2.8; available at: http://facs.scripps.edu).

**SDS-PAGE and immunoblot analysis.** As described elsewhere [19], protein concentrations in *H. pylori* preparations were estimated by use of a Bradford assay (Sigma). Equivalent concentrations of solubilized protein preparations from the wt strain and the *ruvC* mutant were loaded onto 12%–15% SDS-PAGE gels [20]. Electrophoresis was performed, and proteins were either stained with Coomassie brilliant blue, to confirm equivalent loading, or transferred to nitrocellulose membrane (Bio-Rad). The transfer of proteins was verified by Ponceau red staining. After blocking with 5% skim milk powder in PBS/1% Tween 20 (Sigma), membranes were reacted overnight with polyclonal antisera specific for urease A (UreA), urease B (UreB), flagellin A (FlaA), flagellin B (FlaB), cytotoxicity-associated immunodominant antigen (CagA), vacuolating cytotoxin A (VacA), neutrophil-activating protein A (NapA), and the heat-shock proteins GroES and GroEL diluted in 5% skim milk powder in PBS/0.2% Tween 20 [19]. After washing with PBS/0.2% Tween 20, immunoreactants were detected by use of anti–rabbit immunoglobulin conjugated to horseradish peroxidase (Amersham). Reaction products were visualized by use of the ECL Western blotting detection system (Amersham).

**Statistical analysis.** Statistical analysis was performed by use of the Mann-Whitney *U* test. *P* ≤ .05 was considered to be significant.

**RESULTS**

**Protection study.** To examine whether temporary colonization by the *ruvC* mutant protected mice from subsequent colonization by *H. pylori*, a prime-challenge experiment was performed (table 1). Groups containing 10 mice were primed by inoculation with either BHI broth (groups 3 and 6), the wt strain (groups 1 and 4), or the *ruvC* mutant (groups 2, 5, and 7). Three priming doses of 10^2–10^8 cfu were administered on alternate days. Seventy-seven days after infection with the mutant strain, and after confirmation of clearance of the *ruvC* mutant from the stomachs of the mice in group 7, the mice were challenged with either the wt strain (groups 1, 2, and 3) or BHI broth (groups 4, 5, and 6). Colonization by the challenge inoculum was assessed after 28 days, by quantitative culture.

None of the mice in group 6 (primed and challenged with the sham inoculum) were found to be infected with *H. pylori* at the completion of the experiment (table 1). All mice in group 4 (primed with wt and challenged with BHI broth) were found to be infected, with a median bacterial load of 4.25 × 10^6 cfu/g (95% confidence interval [CI], 1.62 × 10^6 cfu/g). Of the mice in group 2 (primed with the *ruvC* mutant and challenged with the wt strain), only 7 were found, by quantitative culture of gastric tissue, to be infected with *H. pylori* (median, 2.67 × 10^4 cfu/g [95% CI, 1.15 × 10^4 cfu/g]). The bacterial load in this group was significantly lower than that in similarly challenged control mice (groups 1 and 3) that had not been primed with the *ruvC* mutant (*P* < .01). These control mice were all found to be infected, with bacterial counts of 2.6 × 10^3–8.69 × 10^6 cfu/g of tissue (group 1: median, 6.64 × 10^6 cfu/g [95% CI, 1.44 × 10^6 cfu/g]; group 3: median, 7.60 × 10^5 cfu/g [95% CI, 1.77 × 10^5 cfu/g]). A second experiment provided reproducible results: the colonization densities after priming with the *ruvC* mutant and challenge with the wt strain (median, 5.7 × 10^4 cfu/g) were again significantly lower than those in mice given 2 inoculations of the wt strain (median, 1.1 × 10^5 cfu/g; *P* < .001). These experiments demonstrate that mice that have been temporarily colonized by the *ruvC* mutant are either resistant to challenge with the wt strain or become

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**NOTE.** Groups of 10 mice were inoculated on day 0 with a priming infection of either the *H. pylori* wt strain (SS1) or the *ruvC* mutant (SS1 *ruvC* Km). Control groups were administered an equivalent volume of brain-heart infusion (BHI) broth. An additional group of 10 mice (group 7) was inoculated with only SS1 *ruvC* Km on day 0. These mice were killed on day 70, and their gastric tissues were tested to determine whether the infection had been cleared. On day 77, the remaining mice were challenged with either the wt strain or BHI broth, and gastric colonization densities were determined 28 days later.

a Two mice in group 3 died of causes unrelated to the treatment protocols.

b The limit of sensitivity of quantitative culture was 10^3 cfu/g.

c Significantly lower bacterial load than that in group 1 (*P* < .01).

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colonized at a significantly lower density of infection than do naive mice. This finding provides strong evidence that temporary colonization by the ruvC mutant confers significant protection against subsequent reinfection, implying the induction of acquired immunity.

**Humoral and cellular immune responses.** The immune responses in mice infected with either the ruvC mutant or the wt strain were determined in 2 independent experiments. In the first experiment, mice were killed 14 and 28 days after infection, and, in the second experiment, mice were killed 14, 28, and 70 days after infection. Colonization was assessed by quantitative culture, and serum samples were tested by ELISA for *H. pylori*-specific IgA, IgM, IgG, IgG1, and IgG2a antibodies. Proliferation of SPLCs and production of cytokines were determined by [3H]-thymidine incorporation and flow-cytometric analysis.

There was no significant difference in the levels of *H. pylori*-specific IgG in the serum from mice infected with the wt strain and in serum from mice infected with the ruvC mutant (figure 1A). At all time points, a trend toward increased production of *H. pylori* antigen–specific IgG1 antibody was observed in mice infected with the wt strain, compared with that in mice infected with the ruvC mutant, and, by 70 days after infection, this had reached statistical significance (figure 1B; *P* < .01). In contrast, mice that had been infected with the ruvC mutant for 70 days produced more IgG2a (figure 1C), and the IgG1:IgG2a ratio was significantly lower in these mice, compared with mice infected with the wt strain (figure 1D; *P* < .01). There was no significant difference between the magnitude of the IgM and IgA serological responses mounted to the wt strain or the ruvC mutant at any of the time points examined.

Significant cellular proliferation of SPLCs from infected mice (but not from control mice) was observed on days 14 (*P* < .005, for wt; *P* < .001, for ruvC) and 28 (*P* < .01, for wt; *P* < .05, for ruvC) after infection, in response to the wt sonicate antigen (figure 2). A 5-fold higher proliferative response was observed on day 14 in mice infected with the ruvC mutant, compared with that in mice infected with the wt strain (*P* < .01). Similar results were obtained when a recombinant form of the *H. pylori* antigen UreB was used as stimulating antigen, with a 7-fold higher response (*P* < .01). There was no significant difference between the groups in the response to ConA, with Δcpm values always >20,000.

SPLCs from mice infected with the ruvC mutant for 14 days gave a significantly higher frequency of CD4+ IFN-γ+ events (median, 622.0 events/10^6 cells) than those from uninfected control mice (median, 251.5 events/10^6 cells; *P* < .01) (figures 3A and 4). The difference between mice infected with the mutant and mice infected with the wt strain (median, 531.0 events/10^6 cells), however, was not significant. In contrast, infection with the wt strain resulted in a significantly higher frequency
of CD4+ IL-4+ events (median, 8102.0 events/10^6 cells), compared with those in both uninfected control mice (median, 1778.5 events/10^6 cells; P < .005) and mice infected with the ruvC mutant (median, 3786.0 events/10^6 cells; P < .005) (figures 3B and 4). SPLCs from mice infected with the wt strain also gave a higher frequency of CD4+ IL-10+ events (median, 3089.0 events/10^6 cells) than did those from uninfected control mice (median, 1608.5 events/10^6 cells; P < .05) (figures 3C and 4), but the IL-10 responses of ruvC mutant–infected (median, 1971.0 events/10^6 cells) and uninfected mice were not significantly different. Similar polarized CD4 T cell cytokine profiles were observed after 28 days of infection. Significantly higher frequencies of CD4+ IFN-γ+ events were induced by infection with the ruvC mutant, compared with those induced by infection with the wt strain (P < .005), and an increased frequency of CD4+ IL-4+ events was induced by infection with the wt strain, compared with that induced by infection with the ruvC mutant (P < .05). By 70 days after infection, however, the profile of cytokine-positive CD4+ events was similar in infected and uninfected mice (data not shown).

Unstimulated cells from wt-infected mice produced median responses of 245, 2336, and 487 IFN-γ, IL-4, and IL-10–positive CD4 events/10^6 cells, respectively. Mitogen-stimulated positive control cultures produced 3748, 16,556, and 1308 IFN-γ, IL-4, and IL-10–positive CD4 events/10^6 cells, respectively.

**DISCUSSION**

Persistent colonization of the gastric mucosa depends on the ability to respond to changing environmental conditions and circumvent host immunity during infection. Although sequence analysis of the H. pylori genomes has revealed a low abundance of transcriptional regulators [21, 22], it appears that other regulatory networks facilitate adaptation to an otherwise hostile environmental niche [19]. Recombinational genetic exchange provides an alternative mechanism of gene regulation that is essential for the long-term survival of H. pylori within the stomach [12]. We have demonstrated here that this process is also critical for mediating immune evasion, presumably through programmed rearrangement of genomic DNA [9, 10, 23].

Inactivation of ruvC in H. pylori dramatically altered the immunogenic potential of the bacteria to induce Th subset responses. This was a very important finding, especially considering that genetically diverse mice were used. The most significant differences in T cell responses were observed at early time points, at which infection with the wt strain elicited a Th2 response dominated by IL-4 and IL-10. In contrast, infection with the ruvC mutant induced IFN-γ, which is associated with a Th1 response appropriate for clearance [4, 24, 25]. These responses were confirmed by serum IgG subclass assays, in which the wt strain predominantly elicited IgG1 (associated with Th2 responses) and the ruvC mutant elicited IgG2a (associated with Th1 responses) [26]. After 70 days of infection, however, the responses of SPLCs from infected mice were no longer significantly different from the responses of SPLCs from control mice. During induction of a primary immune response in mice, an early peak in the number of antigen–specific CD4 T cells in the spleen occurs [27, 28]. This is followed by a rapid decline, as activated cells migrate under the control of homing receptors [29]. It is hypothesized that H. pylori–specific T cells proliferate in the spleens of infected mice before migrating to the site of infection. A close correlation between the murine gastric and splenic Th subset responses to H. pylori infection has been reported [30, 31]; therefore, we anticipate that we will observe similar gastric cytokine responses, and we will test this in future experiments.

We chose to use flow-cytometric analysis to measure Th subset responses in the present study since ELISA and ELISPOT tech-

**Figure 2.** Antigen-specific proliferation of spleen cells (SPLCs) from infected mice. SPLCs from mice infected 14 days before with the wt strain (spotted bars) or the ruvC mutant (solid bars) or from uninfected control mice (white bars) were incubated for 5 days with 5 μg/mL bacterial sonicate, recombinant urease B (UreB), concanavalin A (ConA), or medium alone. [3H]-thymidine was added for the final 18 h. The change in counts per minute due to stimulation of proliferation was calculated by subtracting the mean counts per minute for unstimulated cells from the mean counts per minute obtained after stimulation. The bars represent the geometric mean Δcpm for each group, and error bars depict 95% confidence intervals. Significant greater response than that in infected mice with the wt strain (P < .01).
Figure 3. The frequencies of cytokine-positive CD4 events among antigen-stimulated splenic lymphocytes from mice infected with either the wild-type (wt) strain (spotted bars) or the ruvC mutant (black bars). On day 14 after either infection or sham inoculation (white bars), spleen cells were cultured for 20 h with 5 μg/mL whole-cell sonicate of the *Helicobacter pylori* SS1 wt strain or medium alone. Positive control cultures were incubated for 6 h with 20 ng/mL phorbol myristate acetate and 1 μmol/L ionomycin, for polyclonal cellular activation. After the first 2 h of incubation, the CD4 surface marker and intracellular cytokines interferon (IFN)-γ (A), interleukin (IL)-4 (B), and IL-10 (C) were stained by use of fluorochrome-labeled antibodies, and cellular staining was examined by flow-cytometric analysis. The responses of each mouse are represented by white triangles, bars represent the geometric means for each group, and error bars depict 95% confidence intervals. *Significantly greater response than that in uninfected control mice (P < .05); † significantly lower response than that in mice infected with the wt strain (P < .005).

Techniques do not take into account the cellular source of cytokines. In addition, calculating the ratio of cytokine concentrations may be misleading if the biological potencies of factors are different. We have previously used several methods for quantification of Th subsets [18, 32, 33] but found that flow-cytometric analysis provides more detailed information. In agreement with our data, the *H. pylori* SS1 wt strain elicits a Th2 response in outbred Swiss mice [34]. Several groups, however, have shown that mice usually generate a Th1 response to *H. pylori* [6, 8, 30]. The majority of murine studies have used the C57BL/6 strain, but the Th response is strongly influenced by host genetics [35, 36]. Smythies et al. [30] found that, although C57BL/6 mice responded to *H. pylori* with a polarized Th1 response, other mouse strains produced a mixed Th1/Th2 profile.

Immunization against *H. pylori* depends on CD4+ T cells [37], but there are conflicting data concerning the immune correlates of protection. Immunized, protected mice demonstrate a Th1 response [4, 6], but an increase in gastric Th2 cells has been reported, indicating that these cells may also be important [38]. Other investigators have indicated a protective
role for Th1 and/or Th2 responses [34, 39, 40], but, since immunity can be achieved in IL-4-- but not in IL-12-deficient mice [4, 5], it is agreed that Th1 responses are necessary for protection. IFN-γ is associated with gastritis [3, 24, 41], but evidence suggests that Th1 responses, and not IFN-γ responses, are required for protection [6]; the mechanisms of Th1-mediated protection remain elusive. In contrast to this, IL-10 appears to both inhibit protective immunity [42] and prolong *H. pylori* infections in mice [43]. Although the ruvC mutant elicited a polarized Th1 response and was cleared, there was little evidence of gastric inflammation [12]. This finding is puzzling, since clearance of *H. pylori* in mice is thought to occur through gastritis [3, 24]. The *H. pylori* SS1 wt strain has a nonfunctional cag pathogenicity island (PaI) [44], which could reduce its proinflammatory potential in vivo (although there is no direct link between cag PaI and gastritis in mice [45]). Immunity and inflammation are strongly interlinked, and Th cytokines have profound effects on activation of macrophages. In the presence

Figure 4. Dot plots depicting immunofluorescent detection of CD4, interferon (IFN)-γ, interleukin (IL)-10, and IL-4 among antigen-stimulated splenic lymphocytes from mice, after flow-cytometric analysis. The data shown are from 1 mouse administered a control inoculum of brain-heart infusion (BHI) broth (A–C), 1 infected for 14 days with the wild-type (wt) strain (D–F), and 1 infected with the ruvC mutant (G–I). Fluorescence from staining with anti–CD4–R-phycoerythrin–Texas Red (ECD) is plotted on the X-axes, whereas fluorescence from staining with anti–IFN-γ–fluorescein isothiocyanate (FITC) (A, D, and G), anti–IL-10–allophycocyanin (APC) (B, E, and H), and anti–IL-4–phycoerythrin (PE) (C, F, and I) is plotted on the Y-axes. The frequency of cytokine-positive CD4-positive events per 10^6 cells is displayed in the top right quadrant of each panel. The results are typical of those obtained for each group.
of IFN-γ, macrophages are triggered to become “angry” bactericidal effectors [46], which would efficiently clear colonizing bacteria. The CD1 mouse strain appears to be preprogrammed for Th2 responses, and, in the presence of IL-4, macrophages become alternately activated and the expression of bactericidal factors is partially inhibited [47]. Increased expression of IL-10 can also inhibit activation and inflammation of macrophages [42, 46]. The ruvC mutant is significantly more sensitive to oxidative stress than is the wt strain [12]. Therefore, high-level gastritis may not be necessary for clearance of this mutant strain. Future studies will compare the gastric immune and inflammatory responses.

Our results demonstrate that polarization of the host response governs susceptibility and resistance to *H. pylori* infection. Th1-dominated responses appear to be effective in eradicating *H. pylori*, whereas Th2 responses provide incomplete protection. The polarizing signals that commit differentiation of naive T cells to Th1 or Th2 subsets are complex and are influenced by several factors, including microbial signals [48, 49]. Exploitation of a host’s capacity to produce immunosuppressive cytokines provides a means for pathogens to evade immunity [50]. *H. pylori* stimulates many inflammatory mediators [51, 52] but is able to down-regulate T cell activity [53, 54] and presentation of antigens [55]. Several studies of *H. pylori*–mediated immunomodulation by known and unknown mechanisms have been published recently [53, 56–58]. We, therefore, investigated the expression of potentially immunomodulatory virulence determinants, which could have been influenced by the absence of ruvC activity [52, 53, 59–62], but found no differences between the wt strain and the ruvC mutant. Future proteomic analyses will investigate differences between the strains and identify bacterial immunomodulatory factors. Before performing these extensive studies, we will test the vaccine efficacy of our *H. pylori* mutants, in particular whether they induce protection against unrelated strains. Since the number of mouse-colonizing *H. pylori* strains is limited, we will use the Mongolian gerbil model for these future experiments (reviewed in [63]).

As well as defining the parameters of the immune response correlating with persistence and clearance of *H. pylori*, our model has the potential to identify the bacterial factors that drive them. Such factors could be candidates for therapeutic intervention and could allow the rational design of vaccines against *H. pylori* and, possibly, other pathogens that are able to circumvent the host immune response.

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