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Original Research

Pathogenicity of *Helicobacter ganmani* in Mice Susceptible and Resistant to Infection with *H. hepaticus*

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***Helicobacter* spp. are some of the most prevalent bacterial contaminants of laboratory mice. Although abundant data regarding the diseases associated with *H. hepaticus* infection are available, little is known about the pathogenicity of *H. ganmani*, which was first isolated in 2001 from the intestines of laboratory mice. The objective of this study was to evaluate the host response to *H. ganmani* colonization in *H. hepaticus* disease-resistant C57BL/6 and disease-susceptible A/J and IL10-deficient mice. Mice were inoculated with *H. ganmani*, *H. hepaticus*, or *Brucella* broth. Cecal lesion scores, cecal gene expression, and *Helicobacter* load were measured at 4 and 90 d after inoculation. At both time points, mice inoculated with *H. ganmani* had similar or significantly more copies of cecum-associated *Helicobacter* DNA than did mice inoculated with *H. hepaticus*. When compared with those of sham-inoculated control mice, cecal lesion scores at 4 and 90 d after inoculation were not significantly greater in *H. ganmani*-inoculated A/J, C57BL/6, or IL10-deficient mice. Analysis of cecal gene expression demonstrated that *H. ganmani* infection failed to cause significant elevations of IFNγ in A/J, C57BL/6, or IL10-deficient mice. However, in IL10-deficient mice, *H. ganmani* infection was associated with a significant increase in the expression of the proinflammatory cytokine IL12/23p40. Although *H. ganmani* infection in this study failed to induce the typhlitis that is the hallmark of *H. hepaticus* infection, infection with *H. ganmani* was associated with alterations in inflammatory cytokines in IL10-deficient mice.**

Abbreviations: B6, C57BL/6NCr; HPRT, hypoxanthine guanine phosphoribosyl transferase; IL10 KO, B6.129P2-*IL10*^{tm1Cgn}/J.

Since the discovery of the link between *Helicobacter pylori* and chronic gastritis in 1982,[17] *Helicobacter* spp. in humans and animals have become a field of extensive study. Due to improved detection methods, there has been a rapid expansion in our understanding and ability to detect native *Helicobacter* spp. in mouse models. Several reports investigating their prevalence in mice housed in research institutions have found *Helicobacter* spp. to be some of the most common bacterial contaminants of laboratory rodents.[2,3,12,16,23] *Helicobacter hepaticus* is perhaps the most notorious of the murine helicobacters, by virtue of the early realization of its pathogenicity in adult mice.[8,24] The hallmarks of infection by *H. hepaticus* are typhlitis, colitis, and hepatitis.[10] In addition, *H. hepaticus* is commonly used as a microbial trigger in susceptible mouse strains used as models of inflammatory bowel disease.[5,9,19,21,28] In 2001, less than 10 y after *H. hepaticus* was discovered, *H. ganmani* was isolated from the intestines of laboratory mice.[26] During its initial characterization, 16S rDNA sequence analysis placed *H. ganmani* phylogenetically closest to *H. rodentium*, a urease-negative helicobacter that had been previously isolated from mouse intestines.[26]

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Despite the reported endemic presence of *H. ganmani* in many research colonies,[2,3,12] only a few reports to date have attempted to address *H. ganmani*'s potential pathogenicity.[22,30] One report describes an outbreak of inflammatory bowel-like disease associated with *H. ganmani* infection in an otherwise *Helicobacter*-free conventional colony of IL10-deficient mice.[22] The findings from another report describe the effect of natural colonization of IL10-deficient mice with *H. ganmani*, *H. hepaticus*, or both.[30] In that study, 8- to 20-wk-old mice monoinfected with *H. ganmani* had significantly lower lesion scores than did mice monoinfected with *H. hepaticus*, suggesting that infection with *H. ganmani* alone was not sufficient to cause severe typhlocolitis.[30] However, by 34 wk of age, clinical typhlocolitis (diarrhea) and grossly enlarged ceca were observed at necropsy in 2 of the 6 mice monoinfected with *H. ganmani*.[30]

Although these reports of naturally occurring infections have provided a glimpse into *H. ganmani*'s potential to produce intestinal disease in immunodeficient mice, a controlled study in immunocompetent and immunodeficient mice had not been conducted previously. The objectives of the current study were to evaluate the effect of *H. ganmani* infection on intestinal disease and to characterize alterations of inflammatory gene expression associated with infection. To this end, we selected A/J and IL10-deficient mice for this study because of their known susceptibility to *H. hepaticus*-induced typhlocolitis.[9,13,14,19,21,28] In contrast, although

C57BL/6 mice show an initial spike in inflammatory cytokines after *H. hepaticus* infection, they do not typically develop chronic disease.[19] We did not expect C57BL/6 mice to develop *H. hepaticus*-induced disease, but we deemed it prudent to characterize the possible effects—through unknown mechanisms—of *H. ganmani* on this common strain.

Previous studies characterizing cecal gene expression during *H. hepaticus* induced typhlocolitis demonstrated that IFNγ and IL12/23p40 (IL12/23) are key proinflammatory cytokines that drive typhlitis.[19] Expression of these cytokines was increased in *H. hepaticus*-inoculated A/J mice but not in *H. hepaticus*-inoculated C57BL/6 mice.[19] In addition, treatment with neutralizing monoclonal antibodies against these cytokines significantly decreased cecal lesion severity, implicating the roles of IFNγ and IL12/23 in modulating the pathogenesis of typhlitis.[19] We hypothesized that characterizing the effect of *H. ganmani* infection on expression of IFNγ and IL12/23 would uncover aspects of the host response that are not readily apparent by histologic evaluation of cecal tissue alone.

To date, our understanding of the potential for *H. ganmani* to cause intestinal disease has been limited to reports that focused on the evaluation of histologic disease in naturally infected IL10-deficient mice. Despite the reported endemic presence of *H. ganmani* in many research colonies,[2,3,12] there are no published reports of disease associated with *H. ganmani* infection in immunocompetent mice. In addition, *H. ganmani* shares close sequence homology with *H. rodentium*, which has been found to be nonpathogenic in monoinfected immunodeficient and immunocompetent mice.[20] Therefore, we hypothesized that experimental infection with *H. ganmani* would not produce disease in *H. hepaticus*-susceptible or -resistant mice.

Materials and Methods

Animals. The current study was conducted in accordance with the guidelines set forth by the 8th edition of the *Guide for the Care and Use of Laboratory Animals*[11] and was approved by the University of Missouri–Columbia Animal Care and Use Committee.

Female A/JCr and C57BL/6NCr (B6) mice were obtained from the Frederick Cancer Research and Development Center (Frederick, MD) at 3 wk of age. Female offspring from inhouse breeding colonies of B6.129P2-*IL10*^{tm1Cgn}/J (IL10 KO) mice, originally obtained from the Jackson Laboratory (Bar Harbor, ME), were used in this study also. All mice were 4 wk of age when they began experimental manipulations related to this study. Female mice were chosen for this study in light of previous reports that demonstrated increased susceptibility to *H. hepaticus* in female mice.[15]

Female IL10 KO mice used in this study were born and maintained within inhouse colonies documented via sentinel testing to be free of ecto- and endoparasites and pathogenic and opportunistic enteric and respiratory bacteria. Sentinel testing also confirmed the absence of antibodies against the following viral agents: mouse hepatitis virus, mice minute virus, mouse parvovirus, Sendai virus, *Mycoplasma pulmonis*, Theiler murine encephalomyelitis virus, epizootic diarrhea of infant mice virus, pneumonia virus of mice, reovirus type 3, lymphocytic choriomeningitis virus, mouse adenoviruses, ectromelia virus, and polyoma virus. Female A/JCr and B6 mice were obtained from colonies documented by the supplier to be free of the listed agents. In addition, prior to the start of this study, each experimental mouse was tested by using a genus-specific fecal PCR assay and confirmed to be free of *Helicobacter* spp.

All mice were maintained under SPF conditions, and all materials (water, feed, cages, bedding) were autoclaved or irradiated prior to entry into the animal room. All mouse manipulations were performed in a class II biologic safety cabinet. Mice were socially housed according to treatment group in standard shoebox-type microisolation cages on individually ventilated racks (Thoren Caging Systems, Hazelton, PA) with paperchip bedding (Shepherd Specialty Paper, Milford, NJ) and nesting pads (Nestlets, Ancare, Bellmore, NY). A commercial rodent diet (LabDiet 5008, LabDiets, St Louis, MO) and water were provided ad libitum. The room was maintained on a 14:10-h light:dark cycle (lights on, 0600). Animals were maintained under these conditions from birth or at the time of arrival from the supplier until the end of the study.

Experimental design and tissue collection. A total of 60 female A/JCr mice, 60 female B6 mice, and 60 female IL10 KO mice were used for this study. Within each strain, 20 mice were each assigned to 1 of 3 treatment groups: sham-inoculated, *H. ganmani*-inoculated, and *H. hepaticus*-inoculated. All mice were 4 wk of age at the time of inoculation. Of the 20 mice in each of these treatment groups, 10 mice were euthanized at 4 d after inoculation, with the remaining 10 mice euthanized at 90 d, for collection of cecal tissue and cecal contents.

At either 4 or 90 d after inoculation, mice were euthanized by an inhaled overdose of CO_2 followed by cervical dislocation. The cecum was removed from each mouse, and cecal contents were frozen and stored at –20 °C for DNA extraction for confirmation of infection and bacterial quantification. The cecum was opened along the mesenteric border, laid flat, cut into 2 equal longitudinal sections, and rinsed in PBS to remove remaining fecal contamination. One cecal section was immediately snap-frozen and stored at –80 °C for gene expression analysis; the remaining cecal strip was placed in 10% neutral buffered formalin for histolologic processing and evaluation.

Bacterial cultivation and inoculation. *H. hepaticus* (MU94) and naturally isolated *H. ganmani* (MU1) were grown in 250-mL Erlenmeyer flasks or on 5% sheep blood agar plates (Becton Dickinson, Franklin Lakes, NJ) containing *Brucella* broth (Becton Dickinson) supplemented with 5% fetal bovine serum (Sigma–Aldrich, St Louis, MO) and incubated for 48 h at 37 °C in a microaerobic chamber with 90% N_2, 5% H_2, and 5% CO_2.

At 4 wk of age, experimental mice were orally gavaged with either 10^8 *H. hepaticus* or *H. ganmani* organisms suspended in 0.5 mL *Brucella* broth. Sham-inoculated mice were inoculated with 0.5 mL sterile *Brucella* broth.

Scoring of cecal lesions. To confirm differential disease susceptibility between mouse strains and to determine the timing of disease onset, cecal tissue sections were collected at 4 and 90 d after inoculation from sham-inoculated, *H. hepaticus*-inoculated, and *H. ganmani*-inoculated mice. Cecal tissues were formalin-fixed and embedded in paraffin, cut in 5-µm sections, and processed for hematoxylin and eosin staining. All scoring was performed by a researcher who was blinded to the treatment of each mouse.

In immunocompetent A/JCr and B6 mice, intestinal disease was assessed by using a previously described scoring system[21] that was adapted from an earlier system.[18] Accordingly, ceca were evaluated for intensity of inflammation (score of 0, no inflammation; 1, mild; 2, moderate; and 3, severe), longitudinal extent

of inflammation (1, one or 2 small foci; 2, patchy foci; and 3, diffuse foci), and vertical extent of inflammation (1, basal mucosal inflammation; 2, full-thickness mucosal inflammation; and 3, transmural inflammation). In addition, ceca were evaluated for hyperplasia, defined as the presence of basophilic crypt epithelial cells in at least 2/3 of the gland or at least doubling of the height of the mucosal epithelium. The distribution of hyperplasia was scored as 1 for focal hyperplasia and 2 for diffuse hyperplasia. Because the lowest inflammation score is 3 according to this system, compared with a minimum of 1 for hyperplasia, 2 was subtracted from the total inflammation score to normalize the results when inflammation was present. After this correction, the total score (sum of inflammation plus hyperplasia) was calculated for each mouse. Accordingly, scores could range from 0 (no typhlitis) to 9 (severe typhlitis).

Cecal tissues in IL10 KO mice were evaluated by using a scoring system developed in our lab (ML Hart) specifically for scoring typhlitis in these animals. Because these mice typically develop more severe typhlitis than wildtype mice due to the deletion of the antiinflammatory cytokine IL10, this scoring system allows for detailed assessment of epithelial hyperplasia and inflammation. In this system, the severity of epithelial hyperplasia (0, none; 1, mild; 2, moderate; and 3, severe with crypt branching or herniation or both) and inflammatory changes (0, no inflammation; 1, mild inflammation limited to the mucosa; 2, moderate inflammation limited to the mucosa and submucosa; 3, severe inflammation with obliteration of normal architecture, erosions, or crypts abscesses; and 4, severe inflammation with ulceration) are characterized. In addition, a separate score is assigned regarding the longitudinal extent of epithelial hyperplasia and inflammation (0, no significant changes; 1, one or 2 foci occupying less than 10% of the mucosa; 2, multifocal lesions occupying 10% to 60% of the mucosa; 3, diffuse lesions occupying more than 60% of the mucosa). The final score for each IL10 KO mouse was calculated as follows: (hyperplasia score × extent of hyperplasia score) + (inflammation score × extent of inflammation score). With this system, scores could range from 0 (no typhlitis) to 24 (severe typhlitis).

DNA extraction from cecal contents. Sterile PBS (1 mL) was added to frozen cecal contents. Samples then were homogenized thoroughly and centrifuged at 200 × *g* for 5 min. After centrifugation, 200 µL of the fecal supernatant was processed for DNA extraction (DNeasy Tissue Kit, Qiagen, Valencia, CA). After extraction, DNA purity and quantity was assessed spectrophotometrically (Nanodrop 1000 Spectrophotometer, Nanodrop, Wilmington, DE).

Confirmation of infection and quantification of *Helicobacter* DNA from cecal contents. DNA samples isolated from cecal contents were submitted to a diagnostic lab (IDEXX BioResearch, Columbia, MO) to confirm infection status by using species-specific primers for *H. hepaticus* and *H. ganmani*.

By using quantitative RT–PCR analysis and previously described genus-specific *Helicobacter* primers, DNA extracted from cecal contents was processed to determine the relative number of *Helicobacter* DNA copies.[1,25] A standard curve was generated from serial dilutions of DNA isolated from a pure culture of *H. hepaticus*. Estimates of the number of *Helicobacter* genome copies in the standards were based on a genome size of 1.8 Mb and a molecular mass of 1.09×10^9 Da.[27] Each sample was assayed in duplicate in 20-µL reactions that each contained 0.5 µL of each primer, 0.4 µL 3 mM $MgCl_2$, 10.0 µL QuantiTect SYBR Green PCR Master Mix (Qiagen), 4.6 µL nuclease-free water, and 4.0 µL sample DNA. Reactions were performed in a real-time rotary thermocycler (Rotor-Gene Q; Qiagen) using the following parameters: incubation at 95 °C for 15 min, 15 s of denaturation at 94 °C (40 cycles), 20 s of annealing at 58 °C, and 30 s of extension at 72 °C. Fluorescence was monitored at the end of each extension phase (Rotor-Gene 1.7.94 software, Qiagen). Technical replicates were averaged, and normalization of each biologic replicate was performed by dividing the number of detected copies of *Helicobacter* 16S rRNA by the amount of template DNA used in the PCR reaction (16S rRNA copies per nanogram DNA).

Cecal RNA extraction and reverse transcription. Frozen ceca were thawed in a solution of phenol and guanidine isothiocyanate (TRIzol Reagent, Invitrogen, Carlsbad, CA). Ceca then were homogenized (Tissuelyser, Qiagen) for 4 min at 30 Hz. Total RNA was isolated according to the manufacturer's protocol (TriZol Reagent; Invitrogen) and extracted RNA was dissolved in 40 µL water treated with diethyl pyrocarbonate (Sigma–Aldrich). RNA purity and quantity were assessed by absorbance at 260 and 280 nm (Nanodrop). Extracted RNA (5 µg each sample) was reverse-transcribed according to the manufacturer's protocol (Superscript, Invitrogen) by using M-MLV reverse transcriptase and oligo(dT) primers. The resulting cDNA was diluted in water treated with diethyl pyrocarbonate to produce a final concentration of 20 ng/µL.

Analysis of cecal expression of IFNγ and IL12/23. Previous studies characterizing cecal gene expression during *Helicobacter*-induced typhlocolitis demonstrated that IFNγ and IL12/23 are key proinflammatory signaling molecules through which the clinical manifestation of typhlitis occurs.[19]

A standard curve was created by using known concentrations of a pCR4-TOPO (Invitrogen) plasmid containing the transcripts of interest, IFNγ and IL12/23, and the housekeeping gene hypoxanthine guanine phosphoribosyl transferase (HPRT). Reactions were performed in a real-time rotary thermocycler (Rotor-Gene Q, Qiagen). Primer sequences and cycling parameters used were described previously.[19] Each sample was assayed in duplicate for IFNγ, IL12/23, and HPRT expression. Technical replicates from each mouse were averaged and normalized to HPRT expression (mean copies of cytokine of interest per 10,000 HPRT copies) to allow for comparison between mice.

Statistical analysis. All data were analyzed by using the statistical software SigmaPlot (SPSS, Chicago, IL). Normally distributed data were analyzed with Student *t* tests (2 groups) or one-way ANOVA with posthoc analysis according to the Student–Newman–Keuls method (3 groups). Data that was not normally distributed were analyzed with one-way ANOVA on ranks (Kruskal–Wallis) with posthoc analysis according to the Student–Newman–Keuls method. A *P* value of less than or equal to 0.05 was considered significant. Exact *P* values are reported for all parametric analyses. However, the statistical software did not provide exact *P* values for posthoc analysis performed after nonparametric tests, which were used to analyze cecal inflammation. Thus, these *P* values have been reported as either greater than or less than 0.05.

Results

Confirmation and quantification of *Helicobacter* colonization. Qualitative PCR analysis of DNA isolated from cecal contents

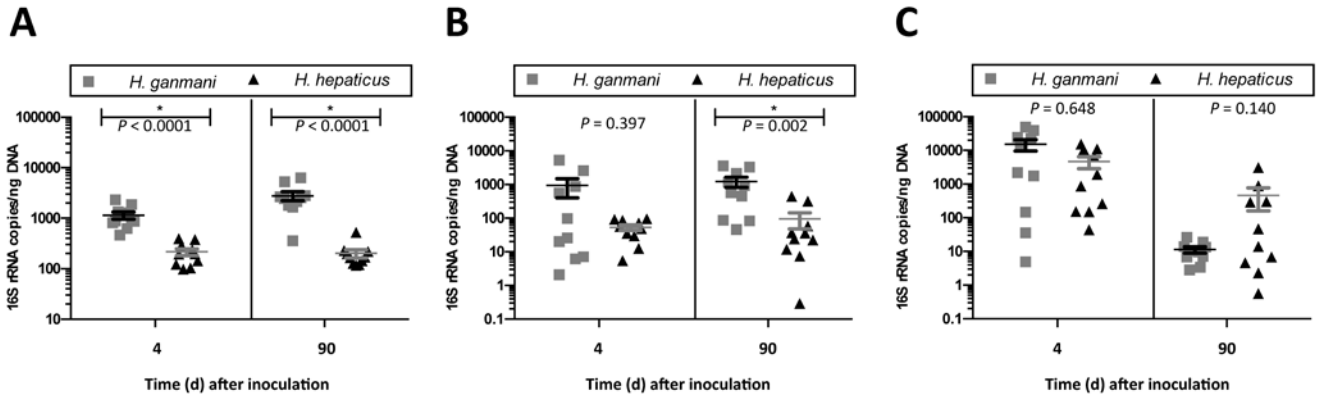

Figure 1. Number (mean ± SEM) of cecum-associated *Helicobacter* DNA copies per ng of DNA in *H. hepaticus* and *H. ganmani*-inoculated mice (n = 10 per group) at 4 and 90 d after inoculation in (A) A/JCr, (B) C57BL/6NCr, and (C) B6.129P2-*IL10*^{tm1Cgn}/J mice. *, Values significantly ($P < 0.05$) different according to Student t test.

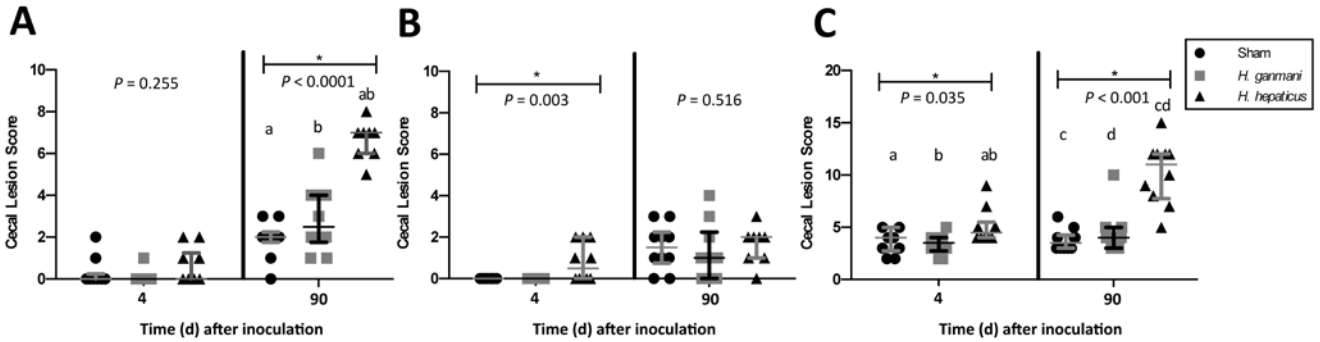

Figure 2. Median cecal lesion scores (bar, interquartile range) of (A) A/JCr, (B) C57BL/6NCr, and (C) B6.129P2-*IL10*^{tm1Cgn}/J mice inoculated with *Brucella* broth (sham), *H. ganmani*, or *H. hepaticus*. Mice (n = 10 per group) were euthanized at 4 and 90 d after inoculation. The cecum from each mouse was collected and evaluated microscopically. Inflammation and epithelial hyperplasia were evaluated by using different scoring systems for immunocompetent (A/JCr and B6) and immunocompromised (IL10 KO) mice. *, Significant ($P < 0.05$; one-way ANOVA on ranks [Kruskal–Wallis]) difference between group medians; like letters indicate posthoc differences in pairwise comparisons according to Student–Newman–Keuls tests.

confirmed that all *H. ganmani*- and *H. hepaticus*-inoculated mice were positive for the *Helicobacter* species with which they were experimentally infected (data not shown). Sham-inoculated control mice were confirmed to be negative for DNA from all *Helicobacter* species at both 4 d and 90 d after inoculation (data not shown).

Helicobacter DNA in the cecal contents of *H. ganmani*- or *H. hepaticus*-inoculated mice was quantified by using quantitative PCR analysis to determine whether colonization differed significantly between *H. hepaticus* and *H. ganmani*-inoculated mice, thereby potentially accounting for differences in disease severity. Overall, when compared with mice inoculated with *H. hepaticus*, mice inoculated with *H. ganmani* had similar numbers or significantly more copies of cecal associated *Helicobacter* DNA (Figure 1 A through C). Specifically, A/JCr mice inoculated with *H. ganmani* had more *Helicobacter* DNA copies at 4 and 90 d after inoculation ($P < 0.0001$ for both comparisons) than did mice inoculated with *H. hepaticus* (Figure 1 A). In B6 mice, levels of *Helicobacter* DNA at 4 d after inoculation did not differ ($P = 0.397$) between *H. ganmani*-inoculated mice and animals inoculated with *H. hepaticus*. However, at 90 d after inoculation, *H. ganmani*-inoculated B6 mice had greater ($P = 0.002$) levels of *Helicobacter* DNA than did *H. hepaticus*-inoculated mice (Figure 1 B). In IL10 KO mice, *Helicobacter* DNA copy numbers did not differ between *H. ganmani*- and *H. hepaticus*-inoculated animals at either 4 d ($P = 0.648$) or 90 d ($P = 0.140$) after inoculation (Figure 1 C).

Cecal lesion scores. At 4 d after inoculation, median lesion scores differed significantly between treatment groups in B6 mice ($P = 0.003$) and IL10 KO mice ($P = 0.035$) but not in A/JCr mice ($P = 0.255$; Figure 2 A through C). Posthoc analysis of cecal lesion scores found no significant difference between median lesion scores of sham-inoculated and *H. ganmani*-inoculated IL10 KO mice (Figure 2 C). In contrast, *H. hepaticus*-inoculated IL10 KO mice had greater median lesion scores than did their sham-inoculated and *H. ganmani*-inoculated counterparts ($P < 0.05$ for both comparisons). Posthoc analysis of data from B6 mice did not reveal significant differences after pairwise comparisons of median lesion scores of sham-inoculated, *H. hepaticus*-inoculated, and *H. ganmani*-inoculated animals (Figure 2 B).

At 90 d after inoculation, median cecal lesion scores between treatment groups were significantly different in A/JCr mice ($P < 0.0001$) and IL10 KO mice ($P < 0.001$) but not in B6 mice ($P = 0.516$; Figure 2 A through C and Figure 3). Posthoc analysis showed that the median lesion scores of A/JCr and IL10 KO mice infected with *H. ganmani* were similar to those of their sham-inoculated counterparts ($P > 0.05$ for all comparisons; Figure 2 A and C). Conversely, *H. hepaticus*-inoculated A/JCr and IL10 KO mice had significantly greater lesion scores when compared with sham-

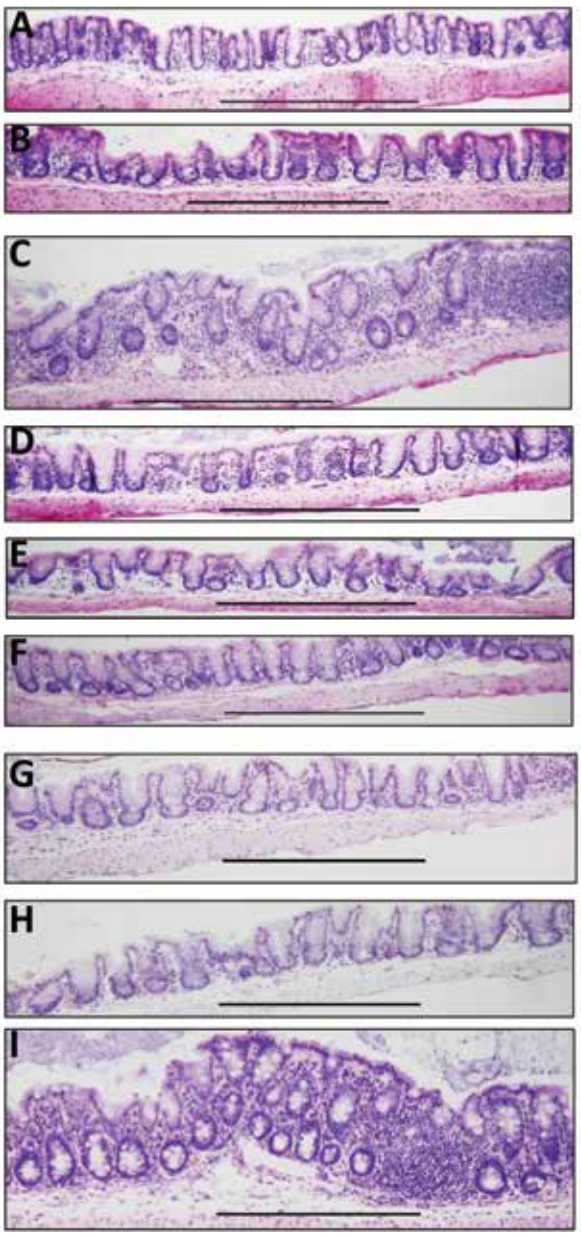

Figure 3. Representative photomicrographs of cecal sections taken from (A through C) A/JCr, (D through F) C57BL/6NCr, and (G through I) B6.129P2-*IL10*^{tm1Cgn}/J mice at 90 d after inoculation. Mice of each strain were inoculated with either (A, D, and G) *Brucella* broth, (B, E, and H) *H. ganmani*, or (C, F, and I) *H. hepaticus*. Hematoxylin and eosin stain; bar, 500 µm.

inoculated and *H. ganmani*–inoculated mice ($P < 0.05$ for all comparisons).

Cecal expression of IFNγ and IL12/23. Cecal tissue collected at necropsy at 4 and 90 d after inoculation was analyzed by RT–PCR for mRNA expression of IFNγ and IL12/23. These cytokines were selected in light of current understanding regarding genes upregulated during infection by *H. hepaticus* and those involved in the ontogeny of mucosal inflammation.[13,14,19,21]

Mean IFNγ expression levels at 4 d after inoculation differed significantly among treatment groups in A/JCr mice ($P < 0.001$) and B6 mice ($P < 0.001$) but not in IL10 KO mice ($P = 0.336$) (Figure 4 A through C). Posthoc analysis revealed that *H. ganmani*-inoculated A/JCr mice had significantly ($P = 0.011$) lower IFNγ expression than did sham-inoculated A/JCr mice (Figure 4 A). In B6 mice, IFNγ expression did not differ ($P = 0.795$) between *H. ganmani*-inoculated and sham-inoculated mice (Figure 4 B). In contrast, IFNγ expression at 4 d after inoculation was significantly increased in *H. hepaticus*-inoculated A/JCr and B6 mice compared with sham- and *H. ganmani*-inoculated counterparts ($P < 0.001$ for all comparisons).

Mean IFNγ expression levels at 90 d after inoculation were significantly different among treatment groups in A/JCr ($P < 0.001$) and IL10 KO ($P < 0.001$) mice but not in B6 mice ($P = 0.102$; Figure 4 A through C). Posthoc analysis of mean IFNγ levels at 90 d after inoculation revealed that IFNγ expression in *H. ganmani*-inoculated A/JCr and IL10 KO mice did not differ from that in sham-inoculated mice (A/JCr, $P = 0.962$; IL10 KO, $P = 0.277$; Figure 4 A and C). Conversely, IFN-γ expression was significantly greater in *H. hepaticus*-inoculated A/JCr and IL10 KO mice compared with their sham-inoculated and *H. ganmani*-inoculated counterparts ($P < 0.001$ for all comparisons).

Mean IL12/23 expression levels at 4 d after inoculation were significantly different among treatment groups in A/JCr ($P < 0.001$), B6 ($P = 0.004$), and IL10 KO ($P < 0.001$) mice (Figure 4 D through F). Posthoc analysis revealed that compared with those in sham-inoculated mice, mean IL12/23 expression levels were not significantly elevated in *H. ganmani*-inoculated A/JCr ($P = 0.530$), B6 ($P = 0.184$), or IL10 KO ($P = 0.284$) mice. In contrast, IL12/23 expression was increased in *H. hepaticus*-inoculated A/JCr, B6, and IL10 KO animals compared with sham-inoculated (A/JCr, $P = 0.001$; B6, $P = 0.038$; IL10 KO, $P < 0.001$) and *H. ganmani*-inoculated (A/JCr, $P = 0.001$; B6, $P = 0.003$; IL10 KO, $P = 0.002$) mice.

Mean IL12/23 expression levels at 90 d after inoculation differed significantly among treatment groups in A/JCr ($P = 0.005$) and IL10 KO ($P < 0.001$) mice but not B6 ($P = 0.142$) mice (Figure 4 D through F). Posthoc analysis showed that the expression of IL12/23 was similar ($P = 0.965$) in *H. ganmani*-inoculated and sham-inoculated A/JCr mice (Figure 4 D). Conversely, *H. hepaticus*-inoculated A/JCr mice had significantly greater expression of IL12/23 than did sham-inoculated ($P = 0.01$) and *H. ganmani*-inoculated ($P = 0.004$) animals. In *H. ganmani*-inoculated IL10 KO mice, IL12/23 expression was significantly ($P = 0.015$) increased compared with that of sham-inoculated mice. Mean IL12/23 expression was significantly increased in *H. hepaticus*-inoculated IL10 KO mice compared with *H. ganmani*-inoculated mice ($P = 0.018$) and sham-inoculated mice ($P < 0.001$; Figure 4 F).

Discussion

Although *H. ganmani* infection appears to be common in rodent colonies, reports of clinical disease to date have been isolated to studies of natural infection in immunodeficient mouse strains.[22,30] The goal of the current study was to evaluate the effect of *H. ganmani* infection on cecal inflammation and to identify alterations in IFNγ and IL12/23 gene expression associated with infection.

Our first objective was to confirm that *H. ganmani* effectively colonizes the mouse cecum. Failure of colonization could account for a lack of histologic lesions or of changes in cytokine gene expression. However, our data show that at each time point within

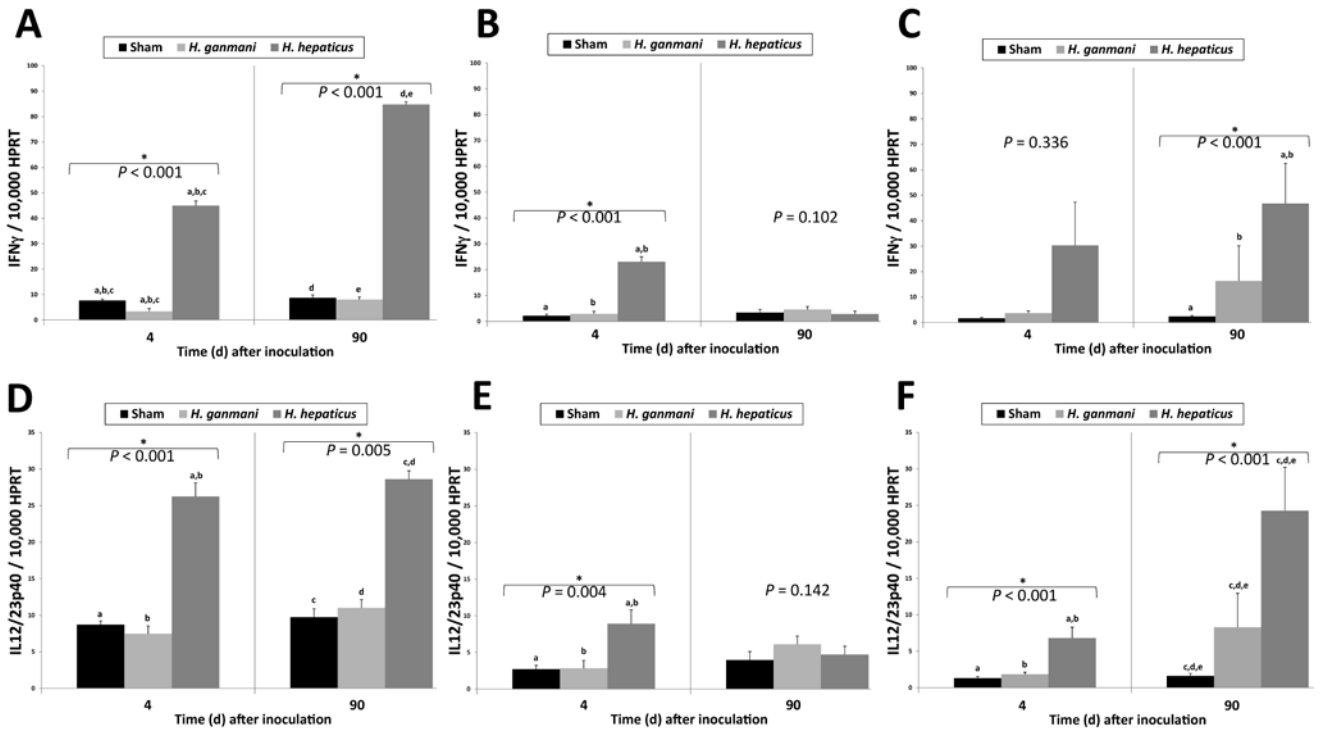

Figure 4. Number (mean ± SEM) of (A through C) IFNγ and (D through F) IL12/23p40 mRNA molecules relative to the number of hypoxanthine guanine phosphoribosyl transferase (HPRT) mRNA molecules in cecal tissue. Ceca were collected 4 and 90 d after inoculation with either *Brucella* broth (sham), *H. ganmani*, or *H. hepaticus* from (A and D) A/JCr, (B and E) C57BL/6NCr, and (C and F) B6.129P2-*IL10*^{tm1Cgn}/J mice. *, Significant ($P < 0.05$; one-way ANOVA) difference between group means; like letters indicate posthoc differences in pairwise comparisons according to Student–Newman–Keuls tests.

each strain, cecal levels of *H. ganmani* were similar, if not significantly greater, than those of *H. hepaticus.* These findings suggest that the lack of clinical disease and typhlitis found in *H. ganmani*-inoculated mice was not a result of decreased or absent bacterial colonization.

Despite harboring bacterial colonization similar to or greater than that in *H. hepaticus*-inoculated mice, *H. ganmani*-inoculated mice had no increases in median cecal lesion scores when compared with those of sham controls. This finding was true for all strains in our study, including those with known susceptibility to *H. hepaticus*-induced disease. In the current study, *H. hepaticus*-susceptible A/JCr and IL10 KO mice infected with *H. hepaticus* responded to infection as previously reported,[9,13,14,19,21,28] developing severe typhlitis, unlike control and *H. ganmani*-inoculated mice. Our *H. hepaticus*-resistant B6 mice showed an initial acute response when infected with *H. hepaticus*, and this response did not persist chronically, consistent with previous reports.[19] These findings show that the lack of noteworthy typhlitis in *H. ganmani*-inoculated mice was not due to failure of this particular cohort of mice to respond to *Helicobacter* infection, because those with a previously reported susceptibility to *H. hepaticus* responded as expected.

Analysis of IFNγ and IL12/23 gene expression revealed that *H. ganmani* infection was not associated with alterations in proinflammatory gene expression in immunocompetent mice. *H. hepaticus*-susceptible A/JCr mice and -resistant B6 mice inoculated with *H. ganmani* did not show noteworthy alterations in gene expression when compared with that of sham-inoculated mice at both the acute and chronic time points. As previously reported, we observed that *H. hepaticus*-infected A/JCr mice manifested the expected upregulation of IL12/23 and IFN-γ at both the acute and chronic time points, whereas infected B6 mice exhibited an increase in expression at day 4, with a subsequent return to baseline by day 90 after inoculation.[19]

Whereas median cecal lesion scores in *H. ganmani*-inoculated IL10 KO mice were not significantly greater than those of sham controls, analysis of cytokine gene expression revealed that *H. ganmani* infection significantly increased expression of the IL12/23 gene in IL10 KO mice at 90 d after inoculation. Although IL12/23 levels in *H. ganmani*-inoculated IL10 KO mice were significantly lower than those in mice inoculated with *H. hepaticus*, subclinical disease cannot be ruled out, even though median cecal lesion scores for these mice were not significantly greater than those of sham mice. These findings demonstrate the potential for *H. ganmani* to alter the expression of proinflammatory cytokines in the absence of clinical or histologic disease in female IL10 KO mice.

A careful look at cecal lesion scores 90 d after inoculation shows that in A/JCr and IL10 KO mice inoculated with *H. ganmani*, a single mouse in each group had a score that was well beyond the median seen in their respective cohorts. This observation suggests that within a given cohort, *H. ganmani* may have the potential to cause morbidity in a select number of animals. These findings are in agreement with a previous report of IL10 KO mice naturally colonized with *H. ganmani*,[30] in which 8- to 20-wk-old IL10 KO mice monoinfected with *H. ganmani* had significantly lower lesion scores than did mice monoinfected with *H. hepaticus*.[30] However, in an older cohort of mice monoinfected with either *H. ganmani* or

H. hepaticus, 2 of 6 mice from both groups presented with diarrhea prior to necropsy at 36 wk of age. At necropsy, these mice were noted to have grossly enlarged colonic and rectal tissue.[30]

Although our study has provided insight regarding the possible pathogenicity of this endemic helicobacter in commonly used mouse strains, many additional variables likely modulate the pathogenicity of *H. ganmani*, such as concurrent infection with another *Helicobacter* species. In one study, mice infected with both *H. rodentium* and *H. hepaticus* exhibited more severe intestinal disease than did mice infected with *H. hepaticus* alone.[20] A similar study found that mice concurrently infected with *H. rodentium* and *H. typhlonius*, a murine helicobacter that shares 97.64% 16S rDNA homology with *H. hepaticus,* had more severe typhlocolitis and a higher incidence of intestinal neoplasia than did mice infected with *H. typhlonius* alone.[6] The cited study also evaluated the effect of age of infection on disease pathogenesis and found marked differences in the course of intestinal disease and development of neoplasia that were dependent on age at the time of infection.[6]

Experimental or breeding stresses, sex-associated effects, and other host factors should also be considered as potential factors that may affect disease progression in *H. ganmani*-infected mice. Although spontaneous disease has been reported to occur in IL10 KO mice infected with *H. ganmani*, this event occurred in a colony of breeding animals.[22] In our current study, *H. ganmani*-inoculated IL10 KO mice did not develop clinical disease, but we inoculated young, naïve, nonbreeding female mice. Consequently, reproductive status may play a role in disease progression. In addition, although we used female mice in our study in light of evidence[15] suggesting their increased susceptibility to *H. hepaticus* infection, this sex-associated effect may not be true for *H. ganmani*. Histologic lesion scores in male mice infected with *H. hepaticus* or *H. ganmani* were significantly higher than those of their infected female cohorts in another study,[30] suggesting that sex may interact with other host or environmental factors to modulate disease progression. Finally, recent studies investigating the role of the intestinal microbiome suggest that the composition of the host's microbiota greatly influences disease phenotype, especially in models of inflammatory bowel disease.[4,7,29]

In conclusion, previous and current findings show that although *H. ganmani* can colonize and maintain a population within the mouse strains studied, the organism did not cause severe typhlitis or alterations in proinflammatory gene expression in immunocompetent A/JCr or B6 mice. However, infection was associated with increased expression of the proinflammatory cytokine IL12/23 in IL10 KO mice. Given that our report is the first to document experimental infection of mice with *H. ganmani*, additional studies are needed to identity how disease progression is modulated in light of differences in the host microbiome, coinfection with other *Helicobacter* spp., host genetics, or as a result of husbandry or experimental procedures. Because of the potential of *H. ganmani* to serve as a confounding variable that could affect research reproducibility, we recommend that, until additional characterization, *H. ganmani* should be regarded as a potential pathogen, especially in research facilities housing immunodeficient mice or mice used in gastrointestinal and immunologic studies.

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