Comparative Medicine
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Vol 54, No 5
October 2004
Pages 549-557

Pathogenicity of *Helicobacter rodentium* in A/JCr and SCID Mice

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Helicobacter rodentium **was first recognized as a potential pathogen when it was isolated, along with** ***Helicobacter bilis*****, from a colony of scid/Trp53 knockout mice with diarrhea. Clinical disease in these mice was more severe than that previously reported in mice infected with** ***H. bilis*** **alone, thus suggesting that** ***H. rodentium*** **contributed to the pathogenesis of enteritis. The purpose of the study reported here was to address two questions: is** ***H. rodentium*** **pathogenic in mice, and when co-infection with a pathogenic helicobacter occurs, does** ***H. rodentium*** **augment disease? To this end, A/JCr and C.B-17/IcrCrl-scidBr mice were inoculated with** ***H. rodentium*** **and/or** ***H. hepaticus*****. Twelve weeks after inoculation, mice were euthanized. The cecum and liver were evaluated microscopically for evidence of disease. Cecal interferon-inducible protein 10 (IP-10), macrophage inflammatory protein 1α (MIP-1α), interleukin 10 (IL-10), and interferon gamma (IFN-γ) mRNA values were measured as an indicator of mucosal immune response. Hepatic lesions were not identified in mice mono-infected with** ***H. rodentium*****; likewise, cecal lesion scores were not significantly different from those of uninfected controls. With the exception of an increased IL-10 mRNA value in SCID mice, mean immune-related gene expression in** ***H. rodentium*** **mono-infected and uninfected control mice was not significantly different. In contrast, all mice infected with** ***H. hepaticus*** **developed moderate to severe hepatitis, significant increase in cecal lesion scores, and increased immune-related gene expression. The C.B-17/IcrCrl-scidBr mice co-infected with** ***H. hepaticus*** **and** ***H. rodentium*** **had liquid cecal contents and low terminal body weight. Further, compared with mice infected with** ***H. hepaticus*** **alone, co-infection was associated with significant increases of IL-10, MIP-1α, and IP-10 mRNA values in C.B-17/IcrCrl-scidBr and IFN-γ and MIP-1α mRNA values in A/JCr mice. These results suggested that** ***H. rodentium*** **alone does not cause hepatitis or enteritis in A/JCr or C.B-17/IcrCrl-scidBr mice; however, co-infection with** ***H. hepaticus*** **and** ***H. rodentium*** **was associated with augmented cecal gene expression and clinical manifestation of disease in immunodeficient mice.**

Over the past 5 decades, great strides have been made in the identification and eradication of infections in laboratory rodents (28). As a result, most contemporary rodent colonies are relatively free of the viruses, parasites, bacteria, and fungi that cause clinical disease. However, some microbes, especially agents that cause insidious disease, remain in an enzootic state in many research colonies. Members of the genus *Helicobacter* are one such group of agents. In a recent study by Livingston and Riley (11), *Helicobacter* spp. were found in 32% of mouse fecal specimens submitted to the University of Missouri Research Animal Diagnostic Laboratory from November 1, 2001 to October 31, 2002. These data corroborate results of a 1997 survey of 72 major biomedical research institutions, conducted by Jacoby and Lindsey, where members of the genus *Helicobacter* were found to be among the most prevalent infective agents in mouse colonies (8). Although some members of this genus clearly cause disease in immunocompromised mice, most infections are subclinical and often go unnoticed. Because of the uncertainty about the potential of certain *Helicobacter* species to cause disease, their impact on research is poorly defined and often overlooked, even despite their widespread distribution in rodent colonies.

Since 1983 when B. J. Marshall and J. R. Warren first described curved bacilli, now known as *H. pylori*, in gastric mucosa biopsy specimens from patients with gastritis and peptic ulceration (13), at least 22 additional helicobacters have been identified and formally named. Among these, *H. hepaticus* (25), *H. muridarum* (10), *H. bilis* (5), *H. rodentium* (21), *H. typhlonius* (7), *Helicobacter* sp. flexispira ("*Flexispira rappini*") (20), and an unnamed urease-negative helicobacter recently identified by Shomer and co-workers (24) have been isolated from mice. Several of these isolates, including *H. hepaticus, H. bilis* (6, 23), *H. rodentium* (22), and the newly identified urease-negative *Helicobacter* sp. (24) have been associated with disease. However, *H. hepaticus* is the only species that consistently causes enteritis and chronic active hepatitis in immunodeficient as well as susceptible strains of immunocompetent mice (3, 9, 25-27). Conversely, *H. rodentium* has only been convincingly associated with disease in immunodeficient mice co-infected with *H. bilis* (22).

Like all members of this genus, *H. rodentium* is a spiral-shaped, fastidious, microaerophilic bacterium. It has bipolar, single, nonsheathed flagella, and was the first urease-negative *Helicobacter* sp. isolated from the intestine of mice (21). Because *H. rodentium* was initially cultured from a colony of subclinically infected mice, it was thought to be part of the normal intestinal flora (21). However, Shomer and co-workers subsequently described an outbreak of diarrhea in a colony of *Prkdc*^{scid}/*Tpr53*^{tm1tyj} (*scid*/*Trp53*-/-) mice co-infected with *H. rodentium*

Received: 12/15/03. Revision requested: 3/10/04. Accepted: 5/13/04.
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and *H. bilis* (22). In that report, *scid* mice with a null mutation of the *p53* tumor suppressor gene developed moderate to severe hemorrhagic diarrhea with histopathologic evidence of proliferative typhlitis, colitis, and proctitis. *Helicobacter bilis* and *H. rodentium* were isolated by use of microaerobic culture of feces and cecal contents from affected mice, and the dual infection was confirmed by use of polymerase chain reaction (PCR) analysis (22). Although experimentally induced infection with *H. bilis* alone had been documented to cause enteritis in immunodeficient mice (6, 23), the severity of clinical disease in this instance suggested that *H. rodentium* may be an important component of the pathogenic process.

The purposes of the study reported here were to examine the pathogenic potential of *H. rodentium* as a single infecting agent in immunocompetent and immunodeficient mice and to determine whether *H. rodentium* alters the severity of disease caused by a known pathogenic helicobacter such as *H. hepaticus*. We observed that *H. rodentium* mono-infection in C.B-17/IcrCrl-scidBr (SCID) and A/JCr mice was not associated with disease. Further, although the severity of enteric and hepatic lesions in mice co-infected with *H. rodentium* and *H. hepaticus* was not significantly different from that in mice infected with *H. hepaticus* alone, co-infection was associated with a significant increase in expression of the immune-regulatory gene interleukin 10 (IL-10) and the proinflammatory genes interferon gamma (IFN-γ), IFN-γ-inducible protein of 10 kDa (IP-10), and macrophage inflammatory protein 1α (MIP-1α).

Materials and Methods

Animals. The following study was conducted in accordance with guidelines set forth by the *Guide for the Care and Use of Laboratory Animals* and approved by the University of Missouri–Columbia Animal Care and Use Committee. Four-week-old female A/JCr (Frederick Cancer Research and Development Center, Frederick, Md.) and C.B-17/IcrCrl-scidBr (Charles River Laboratories, Wilmington, Mass.) mice used in this study were obtained from colonies reported to be free from adventitious viruses, ecto- and endoparasites, and pathogenic and opportunistic enteric and respiratory tract bacteria, including those of the genus *Helicobacter*. Mice were housed in polycarbonate static microisolation cages (Thoren, Hazelton, Pa.) with autoclaved pelleted bedding (Paperchip, Canbrands International, Ltd., Moncton, New Brunswick, Canada), and were allowed ad libitum access to Irradiated Picolab 20, 5053 Rodent Chow (Purina Mills, Richland, Ind.) and autoclaved tap water. The animals were acclimated to a room environment with 10 to 15 air changes hourly, room temperature of 21 ± 2°C, relative humidity between 30 and 70%, and a 12/12-h light/dark cycle for 7 days before research began. Cages, water, and bedding were autoclaved prior to use, and cage changes were performed in strict compliance with procedures designed to maintain specific-pathogen-free status. At the conclusion of this study, sera from five A/JCr sham-inoculated control and *Helicobacter*-infected mice were submitted to the RADIL for serologic evaluation. These mice were documented to be free of antibodies directed against mouse hepatitis virus, mice minute virus, mouse parvovirus, Sendai virus, *Mycoplasma pulmonis*, Theiler's 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.

Bacteria and cultivation. *Helicobacter hepaticus* (strain MU-94) (12) was obtained from an endemically infected mouse colony using a previously described culture technique. *Helicobacter rodentium* (strain MIT-1707) (22) was a gift from J. G. Fox (Cambridge, Mass.). For inoculation, *H. hepaticus* and *H. rodentium* cultures were grown in *Brucella* broth (Becton Dickinson, Franklin Lakes, N.J.) supplemented with 5% fetal bovine serum (Sigma-Aldrich Co., St. Louis, Mo.). Cultures in a 250-ml Erlenmeyer flask were agitated by use of a stir bar and were incubated for 24 h at 37°C in a microaerobic environment with $90\%N_2$-$5\%H_2$-$5\%CO_2$.

Experimental design. A total of 50 SCID and 40 A/JCr mice were separated into four groups: 1, uninfected control (SCID n = 5, A/JCr n = 10); 2, *H. rodentium* infected (SCID n = 15, A/JCr n = 10); 3, *H. hepaticus* infected (SCID n = 15, A/JCr n = 10); and 4, *H. rodentium / hepaticus* coinfected (SCID n = 15, A/JCr n = 10). Mice of groups 1 and 3 were initially gavaged with 0.5 ml of sterile *Brucella* broth, and mice of groups 2 and 4 were gavaged with 10^8 *H. rodentium* colony-forming units (CFU) suspended in 0.5 ml of *Brucella* broth.

Two weeks after the first inoculation, mice of groups 1 and 2 were gavaged with sterile *Brucella* broth and those of groups 3 and 4 were inoculated with 10^8 CFU of *H. hepaticus*. Fecal samples from all mice were submitted to the University of Missouri Research Animal Diagnostic Laboratory for detection of *Helicobacter* infection at postinoculation week 4 and when mice were euthanized for sample collection. *Helicobacter hepaticus* and *H. rodentium* infection was determined using a species-specific PCR assay designed to amplify non-conserved regions of the 16S rRNA gene. Experimentally infected and control mice were euthanized by an inhaled overdose of carbon dioxide at postinoculation month 3. The cecum and biopsy specimens of the right and left medial lobes of the liver were collected at necropsy. The cecum was equally divided into two longitudinal sections. One cecal section was snap frozen and stored at –80°C for gene expression analysis, and the remaining cecal strip and liver biopsy specimen were placed in neutral-buffered 10% formalin for histologic evaluation.

Histologic examination and intestinal lesion scoring. Formalin-fixed cecum and liver specimens were trimmed, embedded in paraffin, cut in 5-μm-thick sections, and processed for staining with hematoxylin and eosin. To objectively assess the severity of intestinal disease in A/JCr mice, we adapted the scoring system described by Mohammadi and coworkers for analysis of chronic gastritis in *H. felis*-infected mice (14). Briefly, lesions were scored for intensity of inflammation (0 = none, 1 = mild, 2 = moderate, and 3 = severe), longitudinal extent (1 = one or two small foci, 2 = patchy, and 3 = diffuse), and vertical extent of inflammation (1 = basal mucosal inflammation, 2 = full-thickness mucosal inflammation, and 3 = transmural inflammation).

In addition, lesions were scored for hyperplasia. Hyperplasia is defined as the presence of basophilic staining "crypt" epithelial cells in at least the lower two-thirds of the gland or at least doubling of the height of the mucosal epithelium. Focal hyperplasia was given a score of 1, and diffuse hyperplasia was given a score of 2. The scores for intensity of inflammation, longitudinal and vertical extents of inflammation, and hyperplasia were added together to give a total score for each animal. Because the minimal inflammation score using this system is 3 (mild, focal, basal), inflammation and hyperplasia were not on comparable scales. To

Table 1. Sense and anti-sense primer pairs used for PCR amplification[†]

Gene name	Sense (5'-3')	Anti-sense (5'-3')	Fragment size (bp)
HPRT	GTA ATG ATC AGT CAA CGG GGG ACC CA	GCA AGC TTG CAA CCT TAA CCA	165
IL-10	CGG GAA GAC AAT AAC TG	CAT TTC CGA TAA GGC TTG G	186
IFN-γ	TGG AGG AAC TGG CAA AAG GAT GGT	TTG GGA CAA TCT CTT CCC CAC	336
MIP-1α	GCT CAA CAT CAT GAA GGT CTC C	TGC CGG TTT CTC TTA GTC AGG	222
IP-10	CCT ATC CTG CCC ACG TGT TGA G	CAT CCT GCA GGA GGA GTA GCA G	301

HPRT = hypoxanthine-guanine phosphoribosyltransferase; IL-10 = interleukin 10; IFN-γ = gamma interferon; MIP-1α = macrophage inflammatory protein-1α; IP-10 = IFN-γ-inducible protein of 10 kDa.

rectify this bias, lesion score > 2 was adjusted by subtracting 2 from the total score to give a total adjusted score. The total adjusted score was used to compare lesions in uninfected, *H. hepaticus*-, *H. rodentium*-, and dually infected mice. In contrast to helicobacter-induced enteritis in immunocompetent A/JCr mice, typhlitis in SCID mice is predominantly proliferative. The scoring system used by Franklin and co-workers for analysis of enteric lesions in SCID mice experimentally infected with *H. bilis* (6) or *H. typhlonius* (7) was used to objectively evaluate proliferative typhlitis in *Helicobacter*-infected SCID mice.

Extraction of RNA from cecal tissue. Frozen ceca were thawed in a solution of phenol and guanidine isothiocyanate (TRIzol Reagent, Invitrogen Corporation, Carlsbad, Calif.). The ceca were homogenized using a polypropylene pestle that was driven by a Pellet Pestle Motor (Kimble/Kontes, Vineland, N.J.). Total RNA was isolated according to the manufacturer's protocol (TRIzol Reagent, Invitrogen Corporation), and the extracted RNA was dissolved in 100 µl of water treated with diethyl pyrocarbonate (DEPC, Sigma-Aldrich Co.). The quantity of RNA was assessed by measuring the absorbance at 260 nm.

Real-time reverse transcriptase (RT)-PCR analysis. Semi-quantitative RT-PCR analysis was used to examine the transcriptional activation of four immune genes (IP-10, MIP-1α, IL-10, and IFN-γ) that were known from previous cDNA expression array analysis studies to be differentially expressed in cecal tissue from *H. hepaticus*-infected female A/JCr mice (15).

For reverse transcription, 5 µg of total RNA was reverse transcribed by use of Moloney murine leukemia virus reverse transcriptase (MMLVRT) and oligo (dT) primers according to the manufacturer's protocol (Superscript, Invitrogen Corporation). The cDNA was diluted with water to a final concentration of 20 ng/µl.

The primer sequences for IFN-γ (16), IL-10 (1), IP-10 (2), and hypoxanthine-guanine phosphoribosyltransferase (HPRT) (16) have been reported. The primer sequences for MIP-1α were designed from published mRNA sequences using OMIGA software (Accelrys Inc., San Diego, Calif.). Sequences for all primer pairs are shown in Table 1. Standard samples were generated from linearized plasmids containing cloned amplicons (Zero Blunt Topo PCR-cloning kit, Invitrogen Corporation). Samples were quantified by comparing fluorescence of experimental samples with that of plasmid standards.

Messenger RNA for selected genes was quantified using real-time RT-PCR (LightCycler, Roche Diagnostic Corporation, Indianapolis, Ind.). The reactions and melting curves were performed in a 20-µl volume in glass capillaries that contained 0.5 µ*M* each primer, 3 m*M* $MgCl_2$, QuantiTect SYBR Green PCR Master Mix containing dNTP mix, HotStart *Taq* DNA polymerase, reaction buffer, and SYBR green I (QIAGEN, Valencia, Calif.), and cDNA. To quantify the number of copies of specific cDNA, a standard curve was run using known concentrations (10^1 to 10^5 copies) of linearized pCR-Blunt II-TOPO (Invitrogen Corporation) plasmid containing the amplicon. The reactions were incubated at 95°C for 15 min to activate the polymerase. Forty cycles consisting of a 15 sec of denaturing at 94°C, 20 sec of annealing at 55°C (MIP-1α), 57°C (IL-10) or 60°C (IFN-γ and IP-10), and 30 sec of extension at 72°C were used to amplify the genes of interest. The ramp rate was 3°C/s for annealing and 20°C/s for all other steps. For MIP-1α, IP-10, IL-10, and HPRT, fluorescence was measured at the end of each extension step. To exclude primer-dimer artifacts, the fluorescence from IFN-γ was measured at 83°C. Following amplification, melting curves were generated to verify PCR product identity. The HPRT values were used to normalize *Helicobacter*-infected and sham-infected samples for comparison.

Statistical analysis. Tissue sections were evaluated without knowledge of mouse infection status. A median lesion score for each group of animals was determined, and these scores were used for statistical comparisons. Because these data are ordinal data, they were statistically compared using the nonparametric one-way analysis of variance (ANOVA) on ranks, Kruskal-Wallis.

The significance of differences between mean expression amounts of selected genes, measured by use of real-time RT-PCR analysis, was determined using a one-way ANOVA, Student-Newman-Keuls. The statistical software package, SigmaStat (SPSS, Inc., Chicago, Ill.) was used for all statistical analyses.

Results

Histopathologic findings and lesion scores. All mice that were experimentally infected with either *H. hepaticus* or *H. rodentium* alone or with both bacteria were colonized by postinoculation week 4 and remained persistently infected throughout the study. Sham-inoculated control mice remained free of infection for the duration of the study. All mice were euthanized and necropsied at postinoculation month 3.

There were no gross or histologic lesions in the liver (Fig. 1A) or cecum (Fig. 3A) of *H. rodentium*-infected or uninfected A/JCr mice. In contrast, all *H. hepaticus*-infected mice had moderate, multifocal, non-suppurative, necrotizing hepatitis (Fig. 1B), and cecal lesion scores were significantly ($P < 0.05$) increased compared with uninfected control and *H. rodentium*-infected A/JCr mice (Fig. 2A). The median cecal lesion score of mice co-infected with both *H. hepaticus* and *H. rodentium* was not significantly different than that of mice mono-infected with *H. hepaticus* (Fig. 2A). Cecal lesions were similar to those previously described (3, 4, 29) and were characterized by patchy to diffuse mononuclear cell infiltrates in the lamina propria, with mild to moderate mucosal epithelial hyperplasia (Fig. 3B).

Similar to the findings in A/JCr mice, gross or histologic lesions were not discovered in the liver (Fig. 1C) or cecum (Fig. 3C) of *H. rodentium*-infected or uninfected control SCID mice. *Helicobacter*

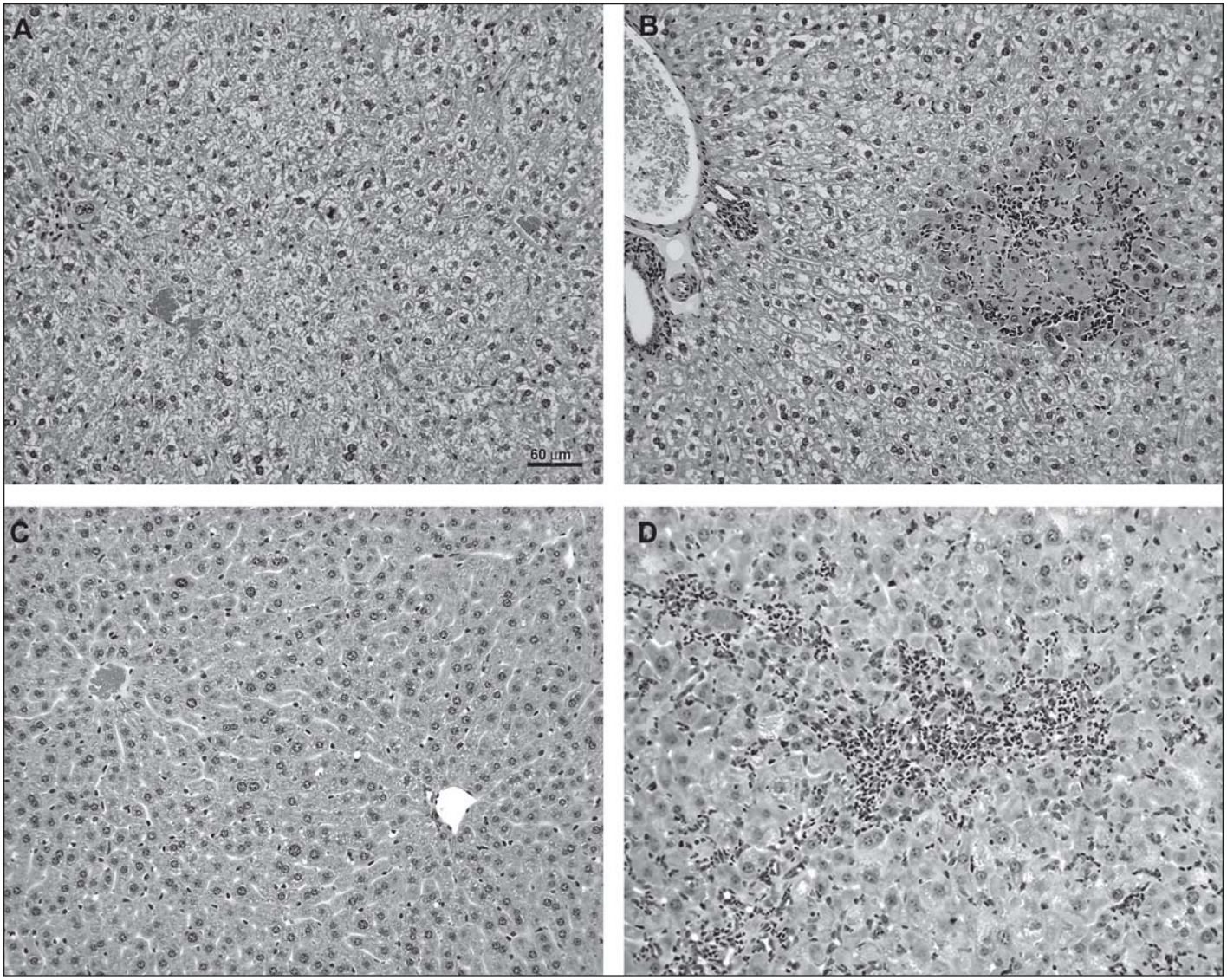

Figure 1. Photomicrographs of liver sections from A/JCr (A, B) and C.B-17/IcrCrl-scidBr (C, D) mice infected with *Helicobacter rodentium* (A and C) alone or in combination with *H. hepaticus* (B and D). Lesions in *H. hepaticus*-infected A/JCr (B) mice are characterized by multifocal necrosis, with mild to moderate mononuclear cell inflammation. Lesions in *H. hepaticus*-infected C.B-17/IcrCrl-scidBr mice are characterized by patchy to coalescing necrosis associated with moderate mixed inflammatory cell infiltration. Lesions were not identified in *H. rodentium*-infected mice. H&E stain; bar = 60 µm.

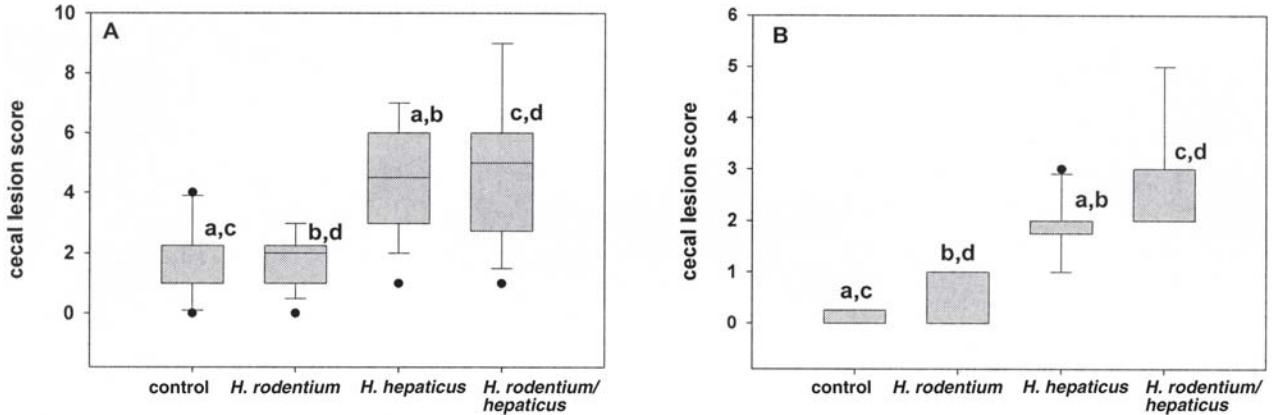

Figure 2. Median cecal lesion scores of sham-inoculated control, *H. rodentium*-, *H. hepaticus*-, and *H. rodentium*/*hepaticus*-infected A/JCr (A) and C.B-17/IcrCrl-scidBr (B) mice. Inflammation and hyperplasia were evaluated microscopically and were scored as described in the text. The center line within the box gives the median lesion score. The top and bottom of the box represents the 25 and 75 percentiles, respectively. The top and bottom of the whisker give the 10th and 90th percentiles, respectively. Outliers are indicated by dots. Statistically significant differences ($P < 0.05$) are denoted by like letters (a, b, c, and d), One-way analysis of variance (ANOVA), Kruskal-Wallis.

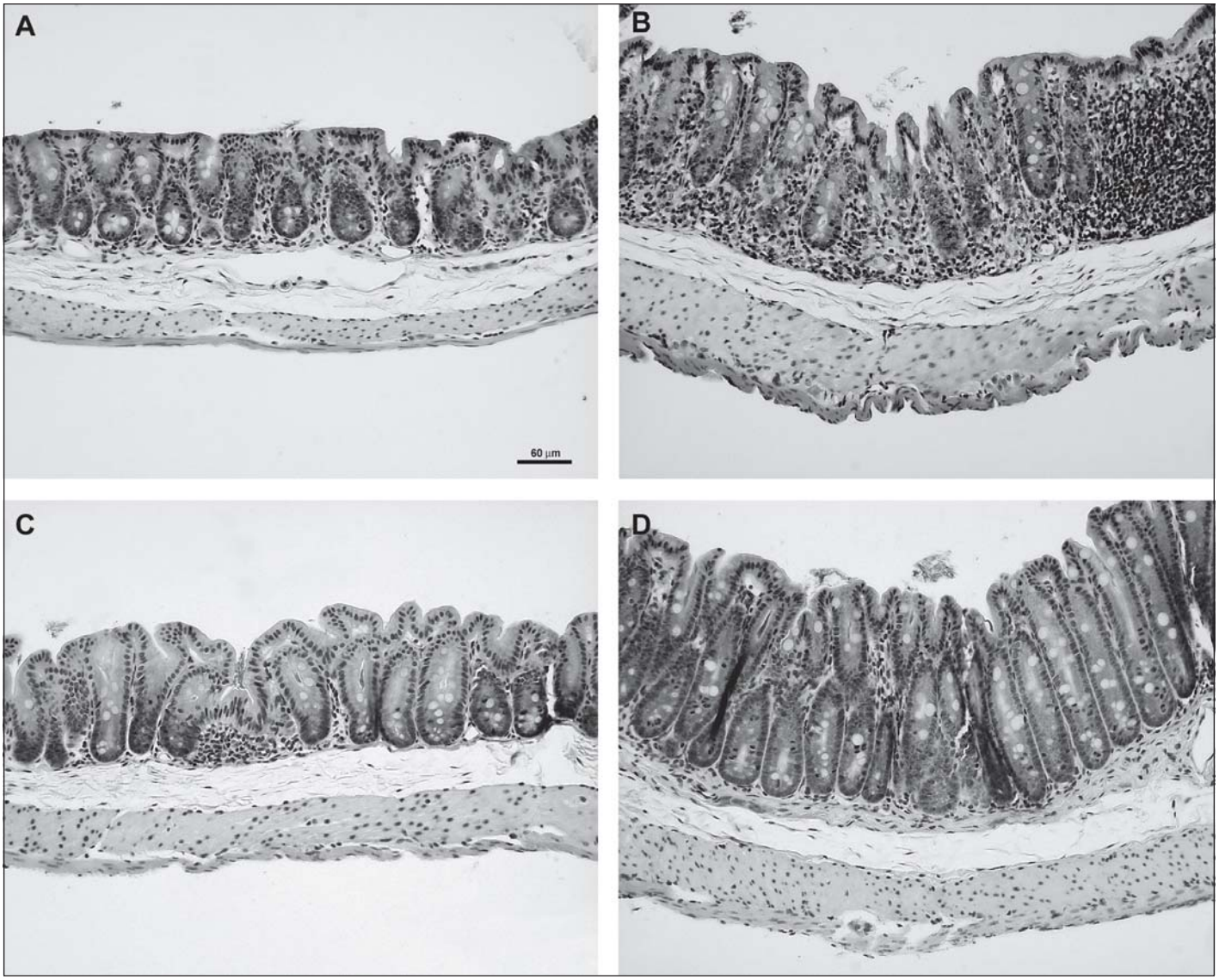

Figure 3. Photomicrographs of sections of cecum from A/JCr (A and B) and C.B-17/IcrCrl-scidBr (C and D) mice infected with *H. rodentium* (A and C) alone or in combination with *H. hepaticus* (B and D). Histologic lesions were not identified in cecal sections from sham-inoculated control (not shown) and *H. rodentium*-infected (A and C) mice. Typhlitis in *H. hepaticus/rodentium* co-infected A/JCr (B) mice is characterized by moderate mononuclear cell infiltrates and mild mucosal hyperplasia. Typhlitis in co-infected C.B-17/IcrCrl-scidBr (D) mice is characterized predominantly by mucosal hyperplasia, with mild to moderate mixed inflammatory cell infiltration. H&E stain; bar = 60 µm.

hepaticus-infected mice had moderate to severe, patchy to coalescing, necrotizing hepatitis (Fig. 1D). Lesions in the cecum were characterized by patchy to diffuse mucosal hyperplasia with infiltration of mixed inflammatory cells into the lamina propria (Fig. 3D). Cecal lesion scores of *H. hepaticus*-infected SCID mice were significantly ($P < 0.05$) increased compared with those of uninfected control and *H. rodentium*-infected SCID mice (Fig. 2B). The median cecal lesion score of co-infected mice was not significantly different than that of mice mono-infected with *H. hepaticus*. However, all SCID mice in the co-infected group had liquid cecal contents, and the terminal mean body weight of these mice was significantly ($P < 0.05$) less than that of the uninfected control, *H. rodentium*-, and *H. hepaticus* mono-infected groups (Fig. 4).

Mucosal IFN-γ, IL-10, MIP-1α, and IP-10 gene expression. As an indicator of host immune response to *Helicobacter* infection, cecal IFN-γ, IL-10, MIP-1α, and IP-10 mRNA values were measured by use of semi-quantitative real-time PCR assays. Significant differences were not found in cecal IP-10, MIP-1α, IL-10, and IFN-γ mRNA values between uninfected control and *H. rodentium*-infected A/JCr (Fig. 5A-D) mice. Similarly, in *H. rodentium*-infected SCID mice, IFN-γ, IP-10, and MIP-1α mRNA expression was not significantly different from that in uninfected controls; however, IL-10 expression was significantly ($P < 0.05$) increased (Fig. 6A-D). *Helicobacter hepaticus*-infected A/JCr (Fig. 5A-D) and SCID (Fig. 6A-D) mice had significantly ($P < 0.05$) increased cecal IFN-γ, IL-10, MIP-1α, and IP-10 mRNA expression compared with that of *H. rodentium* mono-infected and uninfected control mice. Moreover, co-infection with *H. rodentium* and *H. hepaticus* was associated with significant ($P < 0.05$) increases of IL-10, MIP-1α, and IP-10 mRNA values in SCID (Fig. 6A-D) and IFN-γ and MIP-1α mRNA values in A/JCr (Fig. 5A-D) mice, compared those in with mice infected with *H. hepaticus* alone.

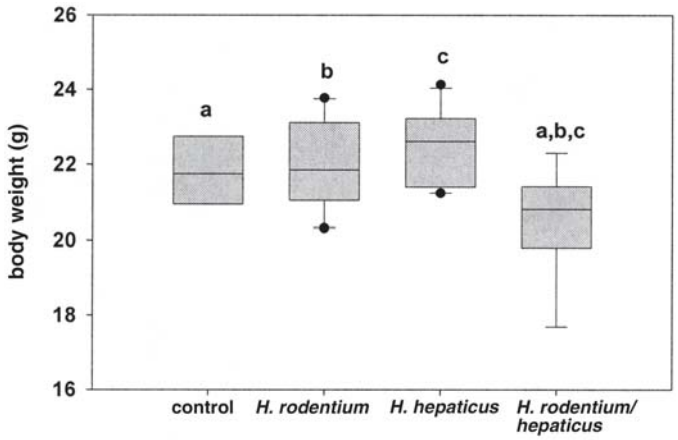

Figure 4. Mean terminal body weights of sham-inoculated control, *H. rodentium*-, *H. hepaticus*-, and *H. rodentium*/*hepaticus*-infected C.B-17/IcrCrl-scidBr mice. The mean terminal body weight of the *H. hepaticus*/*rodentium* co-infection group is significantly different from that for all other groups ($P < 0.05$), denoted by like letters (a, b, c). One-way ANOVA, Student-Newman-Keuls was used for statistical analysis.

Discussion

Helicobacter rodentium infection is prevalent in contemporary rodent colonies. Of the fecal specimens tested at the University of Missouri Research Animal Diagnostic Laboratory from November 2001 through October 2002, approximately 20% were positive for *H. hepaticus*, 17% were positive for *H. typhlonius,* 10% were positive for *H. rodentium*, 5% were positive for *H. bilis*, and 1% was positive for uncharacterized *Helicobacter* sp. (11). Although, as these data suggest, *H. rodentium* is the third most common helicobacter infection in rodent colonies, little is known about its pathogenic potential. In the study reported here, we assessed the pathogenicity of *H. rodentium* in immunocompetent and immunocompromised mice as a single infecting agent and in combination with *H. hepaticus*.

Helicobacter rodentium was initially isolated from the cecum of clinically normal mice (21) and, therefore, was thought to be a member of the normal intestinal flora. However, when Shomer and co-workers reported an outbreak of diarrhea in a colony of *scid*/*Trp53*-/- mice, co-infected with *H. rodentium* and *H. bilis*, the "normal flora" designation for this bacterium was called into

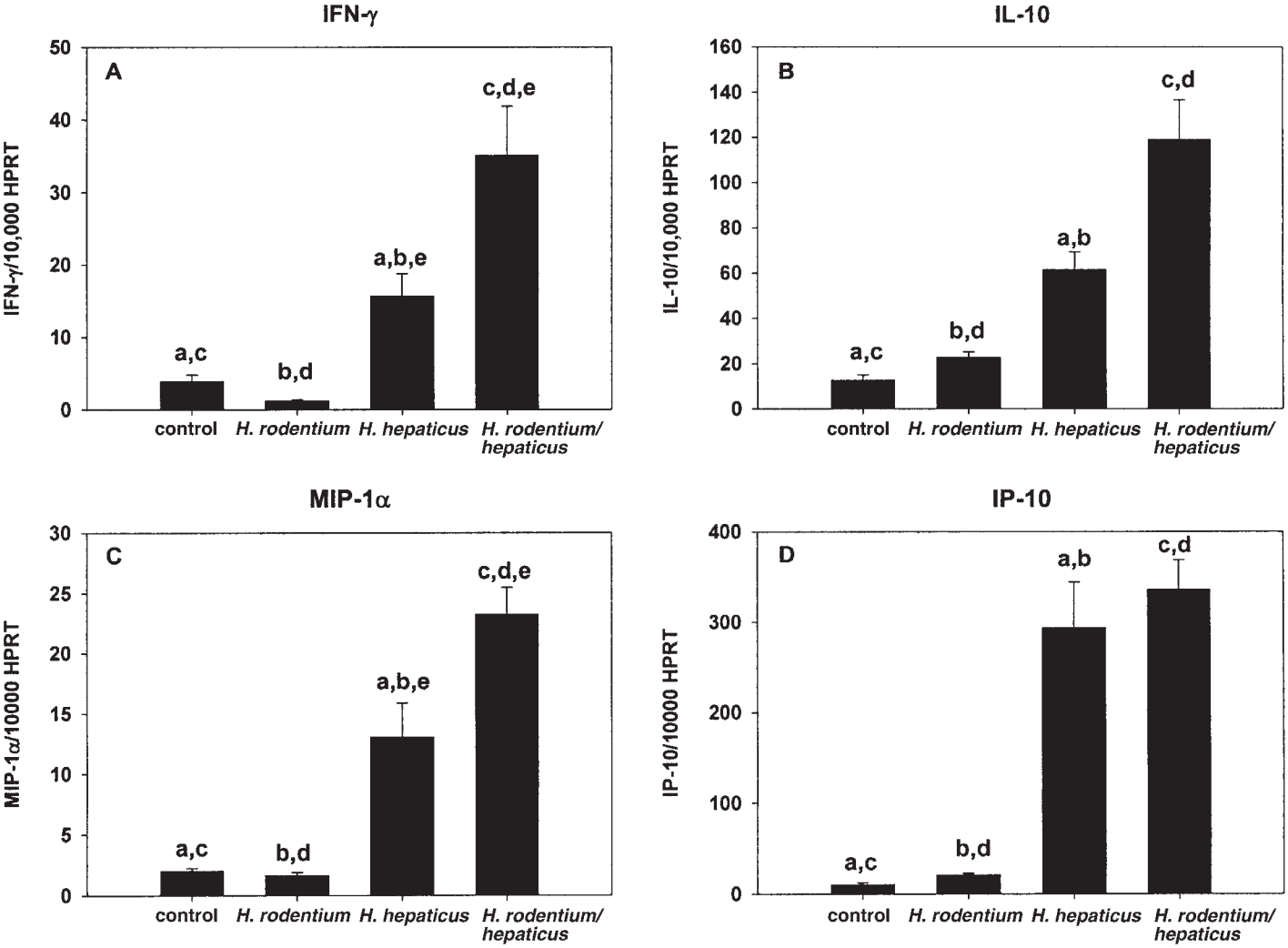

Figure 5. Mean number of interferon gamma (IFN-γ; A), interleukin 10 (IL-10; B), macrophage inflammatory protein 1α (MIP-1α; C), and IFN-inducible protein of 10 kDa (IP-10; D) mRNA molecules relative to the number of hypoxanthine-guanine phosphoribosyltransferase (HPRT) mRNA molecules in the cecum of sham-inoculated control, *H. rodentium*-, *H. hepaticus*-, and *H. rodentium*/*hepaticus*-infected A/JCr mice. These data represent the mean ± SEM of 10 mice/group. Statistically significant differences ($P < 0.05$) are labeled with like letters (a, b, c, d, e). A one-way ANOVA, Student-Newman-Keuls, was used for statistical analysis.

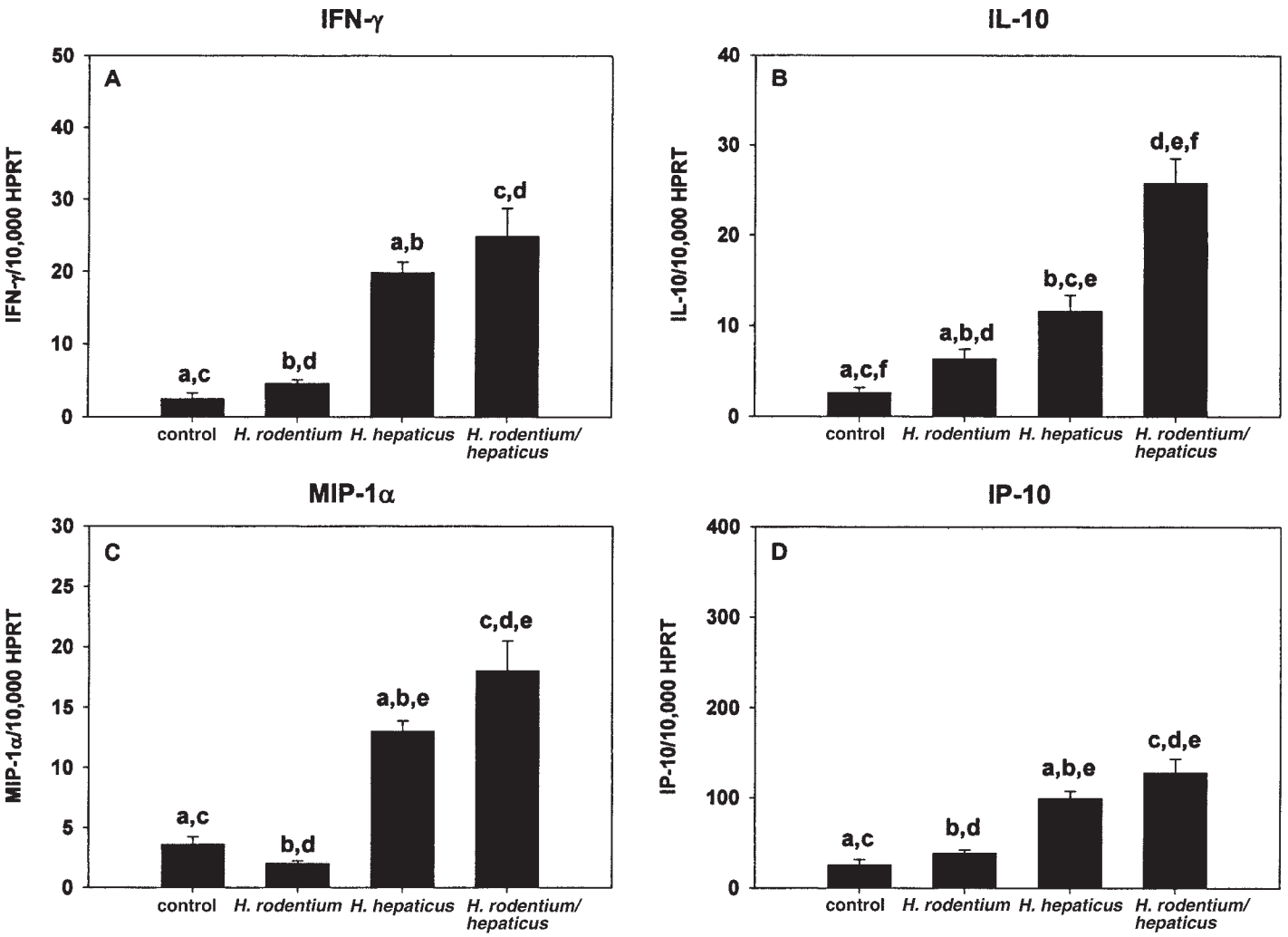

Figure 6. The mean number of IFN-γ (A), IL-10 (B), MIP-1α (C), and IP-10 (D) mRNA molecules relative to the number of HPRT mRNA molecules in the ceca of sham-inoculated control, *H. rodentium*-, *H. hepaticus*-, and *H. rodentium / hepaticus*-infected C.B-17/IcrCrl-scidBr mice. Data represent the mean ± SEM of 5 mice in the control group and 15 mice in the helicobacter-infected group. Statistically significant differences ($P < 0.05$) are labeled with like letters (a, b, c, d, e, f). A one-way ANOVA, Student-Newman-Keuls, was used for statistical analysis.

question (22). Although *H. bilis* is known to be pathogenic in some immunodeficient mice (6, 23), the severity of hemorrhagic diarrhea and proliferative typhlocolitis in mice from the index clinical case suggested that *H. rodentium* was complicit in the disease. Providing further evidence to support this theory, Shomer and co-workers induced mild typhlocolitis in A/JCr mice experimentally infected with *H. rodentium*; however, the experimental details of that study were not provided (22). By contrast, in our *H. rodentium* mono-infected mice, hepatic and cecal lesions were not found. Furthermore, the severity of proliferative typhlitis and cholangiohepatitis lesions observed in mice co-infected with *H. rodentium* and *H. hepaticus* were not significantly different than that in mice infected with *H. hepaticus* alone. Curiously, although the severity of histologic disease was not measurably different among these groups, we consistently observed liquid cecal contents and low terminal body weight exclusively in SCID mice from the co-infected group.

We previously characterized the gene expression profile in the cecum of A/JCr mice with *H. hepaticus*-induced typhlitis, using cDNA array and real-time RT-PCR (15). In that study, we identified a subset of immune-related genes, including IL-10, IFN-γ, the CXC chemokine IP-10, and the CC chemokine MIP-1α, that were up-regulated prior to the onset of histologically detectable disease and remained up-regulated during the height of pathologic changes (15). Since gene dysregulation caused by helicobacter infection can occur in the absence of clinical and histologic disease, measuring the expression level of these key genes can be particularly useful in assessing host response to subclinical infection. To this end, using semi-quantitative real-time RT-PCR, we measured cecal IFN-γ, IL-10, MIP-1α, and IP-10 mRNA values in A/JCr and SCID mice infected with either *H. hepaticus*, *H. rodentium*, or both bacteria. Results of our current study indicate that, with the exception of a slight, albeit statistically significant increase in cecal IL-10 expression in SCID mice, *H. rodentium* mono-infection did not induce the cecal gene expression alterations observed in *H. hepaticus*-infected mice. Surprisingly, co-infection with *H. hepaticus* was associated with significant increase of IL-10, MIP-1α, and IP-10 mRNA values in SCID mice and MIP-1α and IFN-γ mRNA values in A/JCr mice compared with mRNA values in mice infected with *H. hepaticus* alone. The biologic significance of this augmented gene expression found in the co-infected mice is unclear. However, in light of

the low terminal body weight and liquid cecal contents observed in the co-infected SCID mice, these changes may represent a potentiated host immune response.

The pathogenic mechanisms leading to these changes are unknown, but conceivably could be due to a higher helicobacter load or interactions between the *Helicobacter* species. Mice in the co-infection groups were initially inoculated with 10^8 CFU of *H. rodentium* followed by a second inoculation of 10^8 CFU of *H. hepaticus* 2 weeks later. By contrast, mice in the mono-infected groups were given only a single inoculum dose of 10^8 CFU. It is conceivable that *H. hepaticus* and *H. rodentium* share antigenic epitopes, and co-infected mice respond more robustly simply because of a larger helicobacter inoculation dose and subsequent colonization. It is equally plausible that the co-infected mice are responding to a synergism between *H. rodentium* and *H. hepaticus*. Evidence to support this theory comes from studies of inflammatory bowel disease using HLA-B27/beta 2 (β_2)-microglobulin transgenic rats. These studies suggest that certain bacteria, although unable to initiate colitis independently, play an important role in augmenting inflammation (17-19).

In conclusion, results of this study indicate that *H. rodentium*, as a single infecting agent, does not induce hepatitis or typhlitis in immunocompetent A/JCr mice or immunocompromised SCID mice. Furthermore, there is no significant difference in the severity of histologic lesions in mice co-infected with *H. rodentium* and *H. hepaticus* compared with that in mice infected with *H. hepaticus* alone. These results alone suggest that *H. rodentium* is nonpathogenic in mice. However, this interpretation is confounded by the findings of augmented cecal proinflammatory gene expression in co-infected A/JCr and SCID mice, liquid cecal material, and low mean terminal body weight observed solely in co-infected SCID mice, and increased IL-10 expression in *H. rodentium* mono-infected SCID mice. The implication of this study for rodent facility managers and veterinarians is that *H. rodentium* may be an acceptable contaminant in most conventional mouse colonies. However, since co-infection by *H. rodentium* may potentiate disease caused by more pathogenic members of this genus such as *H. hepaticus* or *H. bilis*, veterinarians should not overlook the potential for complicity of this bacterium in a clinical disease outbreak.

Acknowledgment

We thank Beth Livingston for expert technical advice and Kim Mullinax, Laurie Roesel, and Lisa Zell for assistance with animal necropsy and sample preparation. This work was supported by funds from National Institutes of Health training grant NIH-T32-RR07004, University of Missouri College of Veterinary Medicine Committee on Research grant, and Research Animal Diagnostic Laboratory.

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