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Eradication of *Helicobacter* spp. by Using Medicated Diet in Mice Deficient in Functional Natural Killer Cells and Complement Factor D

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A commercial 4-drug diet has shown promise in eradicating *Helicobacter* spp. from rodents; however, its effectiveness in immunocompromised mice is unknown. This study evaluated the efficacy of this treatment in eradicating *Helicobacter* spp. from mice deficient in functional natural killer cells (*Cd1*$^{-/-}$) or complement factor D (*Df* $^{-/-}$). *Cd1*$^{-/-}$ mice naturally infected with *H. hepaticus* with or without *H. rodentium* were fed either control or medicated diet for 8 wk followed by 4 wk on control diet. Fecal samples were PCR-evaluated for *Helicobacter* spp. before mice began treatment and then every 2 wk thereafter for 12 wk. The same experimental design was repeated for eighteen 9- to 21-wk-old *Df* $^{-/-}$ mice naturally infected with *H. bilis* with or without *H. rodentium.* All *Df* $^{-/-}$ mice and 8- to 21-wk-old *Cd1*$^{-/-}$ mice ceased shedding *Helicobacter* spp. after 2 wk of treatment and remained negative throughout the study. In contrast, the *Cd1*$^{-/-}$ mice that were 24 wk or older shed *Helicobacter* spp. for the first 8 wk but tested negative at 10 and 12 wk. All treated animals had enlarged ceca and gained less weight than control untreated mice, and 6 of 7 treated *Cd1*$^{-/-}$ male mice developed mild portal fibrosis. These findings show that within 2 wk of treatment, the 4-drug diet eradicated *H. hepaticus* and *H. rodentium* from young *Cd1*$^{-/-}$ mice and *H. bilis* and *H. rodentium* from *Df* $^{-/-}$ mice, but eradication of established infections in *Cd1*$^{-/-}$ mice required 8 wk of treatment.

Helicobacter spp. infections are widespread within academic institutions, whereas most rodent vendors have successfully eliminated the bacteria from their stock.[25] Infections of *Helicobacter hepaticus* alone or in combination with other *Helicobacter* spp. are the most frequently diagnosed infections,[35] and species prevalence varies both by geographic location and by colony within individual institutions.[14,25,35]

The research implications of intercurrent infection with various *Helicobacter* spp. have recently been reviewed.[6] For example, *H. hepaticus* was responsible for hepatitis and hepatic tumors in control mice on long-term carcinogenesis studies in A/JCr, SCID/NCr, and C3H/HeNCr[40] and B6C3F1 mice,[15] and *H. bilis* infection caused hepatitis in outbred SW mice in a long-term oral supplementation study looking for organ-specific histologic lesions.[11] In addition, *Helicobacter* spp. have been implicated in the alteration of immunologic parameters, such as inhibition of oral tolerance.[21] Mice with immune deficiencies often develop severe pathology: *scid/Trp53*$^{-/-}$ mice developed typhlocolitis and proctitis when infected with *H. bilis* and *H. typhlonius*,[42] and *IL10*$^{-/-}$ mice developed reproductive problems when infected with *H. typhlonius* or *H. rodentium*.[31] These and other examples demonstrate a need to eliminate *Helicobacter* spp. from infected mouse colonies, particularly those that are immunocompromised.

To date the most successful methods of *Helicobacter* eradication have been labor-intensive. Methods that have proven effective include embryo transfer,[8,30,38] cross-fostering,[2,5,33,36,41] treatment of individual mice with antibiotics,[9,10,26] and cross-fostering in combination with a medicated diet.[18] In contrast to these methods, successful dietary treatment has the potential to be very useful for eradicating multiple *Helicobacter* spp. in large mouse colonies without the need for surgery or individual manipulation, particularly from colonies of genetically manipulated mice that are not available commercially and are expensive or difficult to rederive by existing methods. However until recently, attempts to eliminate *Helicobacter* spp. by using dietary treatment alone have been largely unsuccessful. Eradication of *Helicobacter* spp. was not achieved in *scid/Trp53* knockout mice[32] or TCR × Rag, HNT/TCR BALB/c, and TNF transgenic mice[18] by using a diet containing amoxicillin, metronidazole, and bismuth or in B6.129P2-IL10^{tm1Cgn}/J mice[31] by using a dietary treatment with amoxicillin, clarithromycin, metronidazole, and omeprazole. Successful eradication of *Helicobacter* spp. by using this same 4-drug combination diet has been reported in rats[17] and mice with a musculoskeletal deficiency but no known immune deficiency,[19] although the infection status of individual mice in that study was not determined before treatment. Preliminary information from our institution suggests that this 4-drug diet was effective in eradicating *Helicobacter* spp. in mice deficient in functional natural killer cells. Therefore, the current prospective controlled study was undertaken to evaluate the effectiveness of the 4-drug medicated diet in eradicating *H. hepaticus*, *H. bilis*, and *H. rodentium* from 2 naturally infected strains of immunocompromised mice.

Materials and Methods

Animals. Twenty 8- to 12-wk-old male and female B6.129-*Cd1*^{tm1Gru} mice (*Cd1* $^{-/-}$) were bred inhouse and naturally infected with *H. hepaticus* with or without *H. rodentium* and 12 *Cd1* $^{-/-}$ mice (age, 24 wk or more) were acquired from an inhouse colony naturally infected with *H. hepaticus* with or without *H. rodentium*. *Cd1* $^{-/-}$ treatment groups consisted of 5 male and 5 female mice (young groups) and 3 male and 3 female

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mice (old groups). Eighteen 9- to 21-wk-old male and female C.129-*Cfd*^{tm1Yxu} (*Df* $^{-/-}$) mice were bred inhouse and naturally infected with *H. bilis* with or without *H. rodentium*. *Df*$^{-/-}$ mice were assigned randomly to a control group (4 male, 5 female) and a treatment group (5 male, 4 female). Male and female mice were assigned separately to control or treatment groups so that there were approximately equal numbers of male and female mice in each group. Mice were housed individually for the duration of the study to prevent cross-infection.

Housing. Mice were housed in an AAALAC-accredited facility in compliance with the *Guide for the Care and Use of Laboratory Animals*,[16] and procedures were approved by the Johns Hopkins Institutional Animal Care and Use Committee. Mice were housed in individually ventilated cages (Allentown Caging Equipment, Allentown, NJ) on autoclaved corncob bedding (Bed-O'Cobs, The Andersons, Maumee, OH) and received reverse-osmosis–treated water by means of an in-cage automated watering system (Rees Scientific, Trenton, NJ). Cages were changed on a 2-wk cycle by using chlorine-dioxide–based disinfectant (MB10 tabs, 100-ppm solution, Quip Laboratories, Wilmington, DE) in filtered-air change stations (Lab Products, Seaford, DE) with precautions to avoid cross-contamination among cages, including changing gloves between cages. All mice were acclimated to the control diet prior to beginning of the study.

Health surveillance. The *Cd1* $^{-/-}$ and *Df* $^{-/-}$ mice as tested were free of a wide range of viral and parasitic pathogens by soiled bedding sentinel surveillance. Pathogens excluded were Sendai virus, pneumonia virus of mice, mouse hepatitis virus, mouse minute virus, mouse parvovirus 1 and 2, Theiler mouse encephalomyelitis virus, reovirus, epizootic diarrhea of infant mice, lymphocytic choriomeningitis virus, ectromelia virus, murine adenovirus, murine cytomegalovirus, *Mycoplasma pulmonis*, fur mites, and pinworms.

Diet and feeding protocol. Control diet consisted of a 5-g, irradiated and nutritionally complete grain-based, bacon-flavored tablet (product no. S05072, BioServ, Frenchtown, NJ). Medicated diet was the same diet with the addition of 3.0 mg amoxicillin, 0.5 mg clarithromycin, 1.0 mg metronidazole, and 0.02 mg omeprazole to each 5-g tablet (Rodent *Helicobacter* MD Four-Drug Combo, product no. S05723, BioServ).

Prior to beginning the study, each mouse was weighed and acclimated to the control diet for 2 wk because the diet used in the animal facility was from a different vendor than the study diet. All study mice were individually confirmed positive for one or more *Helicobacter* spp. by PCR analysis of fecal samples. Two experimental groups were established for each mouse strain: one group received control diet (control), and the other received medicated diet (treatment). The treatment protocol consisted of 8 wk ad libitum medicated diet followed by 4 wk of control diet. Control animals were fed control diet for 12 wk.

Sample collection. Fresh fecal samples were collected aseptically from each mouse by restraining each animal and collecting freshly voided samples. To avoid cross contamination, gloves were changed between animals. Samples were collected and animals weighed at the start of the study and every 2 wk for the 12-wk study duration. Mice were euthanized by carbon dioxide asphyxiation.

Gross pathology and histopathology. A complete necropsy for gross pathology and tissue collection was performed on all study animals. Liver (a representative section of the left lateral and median lobes), stomach, small and large intestine samples were collected for histopathology. Weights of the liver and cecum were collected; ceca were weighed on 21 of the 32 *Cd1*$^{-/-}$ mice. The small and large intestines were prepared by using the Swiss roll technique. Tissues were fixed in 10% buffered formalin and embedded in paraffin blocks, and slides were stained with hematoxylin and eosin (5-µm tissue sections). Histopathologic analysis was performed on *Cd1* $^{-/-}$ mice but not *Df*$^{-/-}$ mice. The following histologic parameters were assessed and graded: hepatic and intestinal inflammation (0, no inflammation; 1, mild multifocal neutrophilic infiltrates; 2, moderate multifocal to diffuse neutrophilic infiltrates), portal fibrosis (0, no portal fibrosis; 1, portal fibrosis present), hepatic necrosis (0, none; 1, mild multifocal necrosis), and hepatocyte vacuolation (0, none; 1, mild multifocal hepatocyte vacuolation; 2, moderate multifocal to diffuse hepatocyte vacuolation).

***Helicobacter* testing.** Fresh fecal samples were frozen at –20 °C prior to testing and analyzed inhouse for *Helicobacter* spp. by PCR as previously described.[3] Briefly, genus-specific primers (5′ TAT GAC GGG TAT CCG GC 3′ and 5′ ATT CCA CCT ACC TCT CCC A 3′) from regions of the 16S rRNA gene were synthesized by Integrated DNA Technologies (Coralville, IA). PCR amplification was done by using a Chromo4 real-time detector (Bio-Rad Laboratories, Hercules, CA) and the Quanti-Tect SYBRGreen PCR kit (SYBRGreen, Valencia, CA). Primers were used at 0.20 µM and $MgCl_2$ at 2.5 mM. DNA was extracted from approximately 20 mg fecal material (equivalent to 50 to 600 ng/mL feces as tested by spectrophotometer) by using the QIAamp DNA Stool Mini Kit (Qiagen, Ocala, FL). Sample DNA was heat-denatured at 95 °C for 10 min and then amplified during 40 cycles at 95 °C for 15 s, 58 °C for 30 s, and 72 °C for 30 s. A standard curve was generated based on serial dilutions of DNA spiked into buffer. At the completion of cycling, a melting curve analysis was performed on samples with late amplification to check for products other than *Helicobacter* spp. Controls included DNA from a negative fecal sample and *Helicobacter* spp. DNA spiked into a negative fecal sample. To control for false negatives caused by PCR inhibitors in the feces, each sample was divided and also tested by using primers for an arbitrarily chosen mouse gene during the same PCR run. In addition to inhouse testing, paired samples were collected at the beginning and end of the study and submitted to an outside laboratory (Research Animal Diagnostic Laboratory, Columbia, MO) for speciation and to corroborate inhouse test results.

Statistical analysis. Statistical analyses were performed by using GraphPad Prism (Graphpad, San Diego, CA). Liver weights, cecal weights, and weight gain were compared by using the unpaired Student *t* test. Histologic scores were analyzed by using the Mann–Whitney test for nonparametric data. Distribution of *H. rodentium* in each animal at the start of the study was analyzed by Fischer exact test. Significance was established at a *P* level of less than 0.05. *Helicobacter* fecal PCR results were not compared statistically because only 100% eradication was considered relevant.

Results

***Helicobacter* tests.** Before treatment, all B6.129-*Cd1*^{tm1Gru} mice tested positive for *H. hepaticus*, and 28 (17 mice 8 to 12 wk old, 11 of those 24 wk and older) of the 32 mice (87.5%) were concurrently infected with *H. rodentium*. All *Df* $^{-/-}$ mice tested positive for *H. bilis*, and 12 of the 18 mice (66.7%) were concurrently infected with *H. rodentium*. The proportion of mice infected with *H. rodentium* did not differ by treatment group ($P = 1.0$) or sex ($P = 0.13$) in either strain.

After treatment, all 20 young (age, 8 to 12 wk) *Cd1* $^{-/-}$ and all *Df* $^{-/-}$ mice in the treatment group tested negative for *Helicobacter* spp. at the 2-wk time point and subsequently remained

negative through the end of the study. Old (age, 24 wk or more) *Cd1* $^{-/-}$ mice in the treatment group continued to shed *Helicobacter* spp. (10 of 12 continuously, 2 of 12 intermittently) during the 8 wk of medicated diet but subsequently tested negative at the 10- and 12-wk time points when being fed control diet. All controls remained positive for *Helicobacter* spp. throughout the 12 wk of study (Table 1).

Long-term follow-up of nonexperimental *Cd1* $^{-/-}$ mice from the same source colony as those for the mice in our study supported the efficacy of the presented treatment protocol. We followed 58 mice (5 wk of age, $n = 34$; 7 wk, $n = 3$; 9 wk, $n = 16$; 12 wk, $n = 2$; 16 wk, $n = 3$) housed as breeding trios or single-sex groups that were treated by following the same procedures. These mice were confirmed as negative for *Helicobacter* spp. by PCR of pooled fecal samples 5 mo after treatment cessation (data not shown).

Weight. At the start of the study, there were no significant differences in weight between treated and control males or treated and control females. All the *Cd1* $^{-/-}$ and *Df* $^{-/-}$ mice in the treatment groups and 15 of the 16 *Cd1* $^{-/-}$mice in the control group accepted the new diet and either maintained or gained body weight through the study period. The remaining mouse (in the young *Cd1* $^{-/-}$ control group) lost weight at the 2-wk time point but regained it subsequently. At necropsy, *Cd1*$^{-/-}$ control male mice had gained significantly ($P < 0.0001$) more weight than had treated males (8.12 ± 0.68 g compared with 2.44 ± 0.41 g; Figure 1), and *Cd1*$^{-/-}$ control male mice gained more weight than did *Cd1*$^{-/-}$ control female mice (8.14 ± 0.68 g compared with 2.36 ± 0.74 g, $P < 0.0001$).

Gross pathology. Mice were necropsied in random order over a 3-d period. One animal from the old *Cd1* $^{-/-}$ treatment group was found dead, and tissues were too autolyzed to analyze histopathologically; however, we obtained PCR data. Gross necropsy findings were unremarkable except that all treated mice from both strains had enlarged ceca, and all *Cd1* $^{-/-}$ treated mice also had diffusely distended small and large intestines, compared with those of controls. In contrast, only one treated female among the *Df* $^{-/-}$ mice had a distended distal small intestine; the others were grossly normal. Cecal and liver weights were evaluated statistically because of differences in size observed at necropsy. *Cd1* $^{-/-}$ and *Df* $^{-/-}$ mice were analyzed separately. The young and old *Cd1* $^{-/-}$ groups were combined for statistical analysis, as were the *Df* $^{-/-}$ male and female mice. The following categories of mice were compared: all treated mice versus all control mice; treated males versus control males; treated females versus control females; control males versus control females; and treated males versus treated females. All comparisons were unremarkable except that ceca were significantly ($P < 0.001$) larger in treated mice than in controls (*Cd1*$^{-/-}$: 2.25 ± 0.21 g compared with 0.32 ± 0.02 g; *Df* $^{-/-}$:1.12 ± 0.11 g compared with 0.63 ± 0.12 g; Figure 2), and liver weight in control *Cd1*$^{-/-}$ male mice (1.88 ± 0.14 g) was significantly greater than that in treated *Cd1*$^{-/-}$ male mice (1.38 ± 0.12 g, $P = 0.0235$) or control *Cd1*$^{-/-}$ female mice (1.02 ± 0.03 g, $P < 0.0001$).

Histopathology. Histopathologic examination of the liver and gastrointestinal tract was performed on *Cd1* $^{-/-}$ mice. Categories for statistical comparison were the same those described for gross pathology. Histopathology changes were never more than moderate in all tissues examined, and the young and old *Cd1* $^{-/-}$ groups were pooled for statistical analysis. Comparisons were unremarkable except that control mice had a significantly ($P = 0.0378$) higher incidence of inflammatory foci within the hepatic parenchyma than did treatment mice, and control female mice had a higher ($P = 0.0379$) frequency of hepatic inflammation than did treated female mice. Hepatitis was never more than mild and usually was portal to periportal. In addition, treated male mice had a significantly ($P = 0.014$) higher prevalence of portal fibrosis than did control male mice (Figure 3). Several sections of liver from *Helicobacter*-positive mice with hepatitis were stained with Warthin–Starry silver stain and examined carefully for spiral organisms but none were found. (data not shown) There were no significant changes in the histopathology of the gastrointestinal tract in any group.

Discussion

To our knowledge, the current report is the first to describe successful eradication of *H. hepaticus*, *H. bilis*, and *H. rodentium* in immunocompromised mice by using a medicated diet containing amoxicillin, clarithromycin, metronidazole, and omeprazole. This diet had previously been used successfully to eradicate *Helicobacter* spp. from immunocompetent rats[17] and from mice with musculoskeletal deficiencies but no known immune deficiencies.[19] Mice with both documented and unsuspected immune deficiencies are quite common in mouse research colonies and when infected with mouse bacterial pathogens, existing mouse models with immune deficiencies typically develop pathology that is more severe or persistent than that of their immunocompetent counterparts.[1,4,12,20,23,24,29,32,39] These infections are often difficult to treat and eradicate by using antibiotics alone.[12,26,32] Therefore any potential antibiotic treatment for *Helicobacter* spp. should be evaluated for its ability to eradicate infection in immunocompromised mice.

The B6.129-*Cd1*^{tm1Gru} mice (*Cd1*$^{-/-}$) used in our study were deficient in functional natural killer cells because they lack the antigen-presenting molecule CD1d.[37] Although a complete understanding of the importance of natural killer cells in *Helicobacter* spp. immunity has yet to be reached,[22] these cells play an important role during the clearance of bacterial infections, host defense against parasitic disease, modulation of host immunity through fungal infection, and amelioration of viral infections.[34] C.129-*Cfd*^{tm1Yxu} (*Df* $^{-/-}$) mice lack factor D, a molecule that is crucial for complement activation through the alternative pathway.[43] Therefore both the *Cd1* $^{-/-}$ and the *Df* $^{-/-}$ mice used in this study have deficiencies in their innate immune systems that could increase their susceptibility to *Helicobacter* spp. infection and impede efforts to eradicate the bacteria through medication. Nevertheless, *Helicobacter* shedding in the feces ceased after 2 wk of treatment in both the young *Cd1*$^{-/-}$ mice and in all the *Df* $^{-/-}$ mice. This result contrasts with that from the old *Cd1* $^{-/-}$ mice, which continued to test positive for the first 8 wk of the study. Shorter infection period, younger age, different genotype, and different *Helicobacter* spp. (*H. bilis* and *H. rodentium* in the *Df*$^{-/-}$ mice compared with *H. hepaticus* and *H. rodentium* in the *Cd1*$^{-/-}$) could have played a role in the rate of response to treatment.

The eradication of *Helicobacter* spp. infections is challenging in older mice.[26] Our results suggest that treatment for 8 wk or longer may be necessary to eradicate *Helicobacter* spp. in old mice with long-established infections. Indeed the manufacturer recommends treatment for 8 wk.[13] The older *Cd1* $^{-/-}$ mice in our study tested positive through week 8 but negative at weeks 10 and 12 when treatment had been discontinued, raising the question whether the later negative tests represented only temporary cessation of shedding rather than eradication. However, had that been the case, we would have expected the infection to rebound in weeks 10 and 12 after treatment discontinuation. A likely explanation for the observed results is that the PCR

Table 1. Progression of *Helicobacter* status of mice (no. positive per time point) during the study

	Time point (wk)						
	0	2	4	6	8	10	12
Treatment group							
8- to 12-wk-old *Cd1*$^{-/-}$ (n = 10)	10	0	0	0	0	0	0
>24-wk-old *Cd1*$^{-/-}$ (n = 6)	6	5	6	4	6	0	0
9-21-wk-old *Df* $^{-/-}$ (n = 9)	9	0	0	0	0	0	0
Control group							
8- to 12-wk-old *Cd1*$^{-/-}$ (n = 10)	10	10	10	10	10	10	10
> 24-wk-old *Cd1*$^{-/-}$ (n = 6)	6	6	6	6	6	6	6
9-21-wk-old *Df* $^{-/-}$ (n = 9)	9	9	9	9	9	9	9

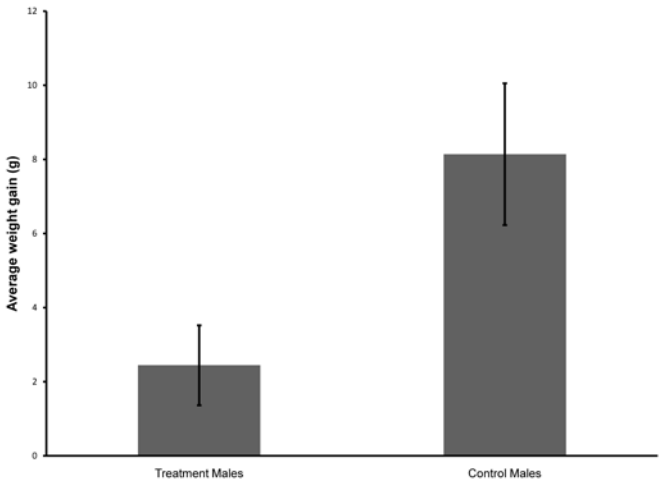

Figure 1. Control *Cd1*$^{-/-}$ male mice gained significantly (P < 0.0001) more weight than did treated *Cd1*$^{-/-}$ male mice. Data are given as mean ± SEM.

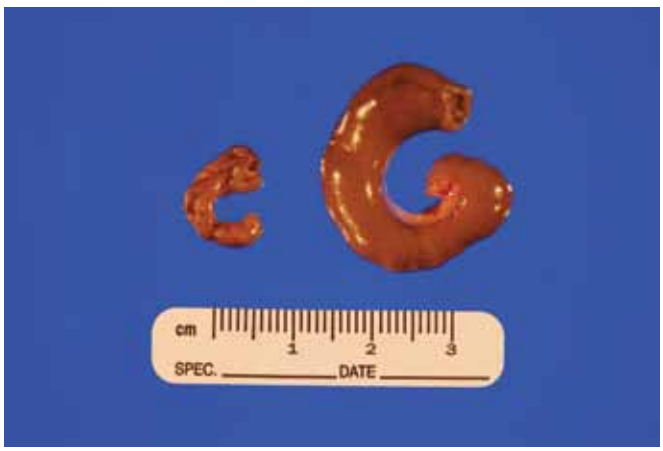

Figure 2. Treated mice (right) had significantly (P < 0.0001) larger ceca than did control mice.

indiscriminately amplified template from nonviable organisms during the later positive tests. In support of this theory, we reviewed the mean PCR cycle thresholds for the old *Cd1* $^{-/-}$ mice: these measures were 26.50, 31.71, 34.68, 34.74, and 35.13 in weeks 0 through 8. Although the SYBRGreen assay is not quantitative, the cycle threshold values clearly show that copy number decreased at each successive test and indeed was very close to the detection threshold for the last positive test. Further support of the efficacy of this treatment protocol comes from our long-term follow-up of 58 nonexperimental *Cd1* $^{-/-}$ mice, all of which were PCR-negative for *Helicobacter* spp. 5 mo after treatment cessation.

One hypothesis for the slow treatment response we observed in old mice was sequestration of bacteria in chronic inflammatory tissue with limited drug penetration; however, the relatively normal histopathology results did not support this hypothesis. In another study,[7] chronic inflammatory bowel disease did not prevent cessation of *H. rodentium* and *H. typhlonius* shedding after 7 to 10 wk of treatment. However, in that study mice were maintained on the medicated diet until tissue collection, and whether the infection would have reemerged after discontinuation of the medicated feed is unknown. In addition, IL10-deficient mice produce an exaggerated, prolonged inflammatory response rather than an impaired immune response, making it difficult to compare results.

Another potential mechanism for slow drug response was poor absorption resulting in prolongation of time to reach therapeutic drug concentrations in the liver; however, drug concentrations were not evaluated in our study. In addition, poor treatment efficacy has been attributed to aged diet;[10] however, the diet used in the present study was fed shortly (within 2 to 3 mo) after manufacture and was stored appropriately.

Although our mice showed no clinical side effects of drug treatment, on gross examination treated *Cd1* $^{-/-}$ and *Df* $^{-/-}$ mice had significantly (P < 0.001) enlarged ceca compared with controls, presumably due to dysbiosis. Cecal enlargement and altered cecal flora as a result of antimicrobial therapy have previously been reported in mice after 14 d of medication.[27,28] In one study,[27] cecal size did not return to normal for as long as 14 d. In our current study, *Cd1*$^{-/-}$ mice were necropsied at 4 wk and *Df* $^{-/-}$ mice at 5 wk after treatment cessation. Ceca were still enlarged in *Df* $^{-/-}$ mice compared with controls, but *Cd1*$^{-/-}$ mice were more severely affected, with larger ceca and intestines than those of *Df* $^{-/-}$ mice. We did not investigate the cecal enlargement microbiologically, given that this finding was unexpected when treatment had ceased at least 1 mo before necropsy. Perhaps microbial overgrowth with organisms such as *Clostridium difficile* would persist and cause long-term complications, although our nonexperimental group bred successfully after dietary *Helicobacter* eradication (data not shown). Methods to hasten the return of normal flora, such as cohousing with untreated *Helicobacter*-negative animals should be considered. Control *Cd1*$^{-/-}$ male mice gained more weight and had heavier livers than did treated male mice, suggesting that dietary antibiotics may have caused a reduction in appetite. Body weight changes are more striking than they appear because the increased cecal size inflates the weight of the treated mice, which already were lighter than untreated mice. Female mice were not similarly affected however.

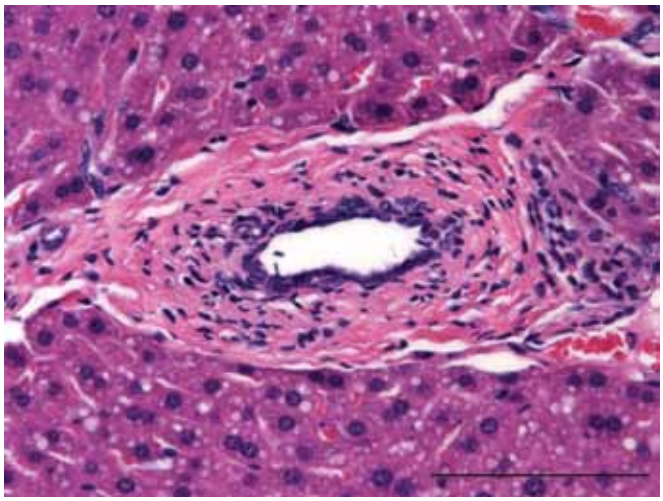

Figure 3. Representative sample of mild portal fibrosis in treated *Cd1*$^{-/-}$ mice. Hematoxylin and eosin stain; scale bar, 100 µm.

Compared with treated mice, control *Cd1*$^{-/-}$ mice had a higher incidence of hepatitis, suggesting that elimination of *Helicobacter* spp. with the medicated diet reduced the incidence of inflammation even when organisms are not readily discernible. In the present study, none of several sections of silver-stained liver from *Helicobacter*-positive mice with hepatitis demonstrated spiral organisms. This result was not unexpected given that *Helicobacter* organisms are often difficult to find even in severely affected specimens. One unexpected finding in our study was the increased incidence of portal fibrosis in treated compared with control male mice. This outcome deserves further study, because it may be a previously unreported side effect of the drugs used.

This study showed that dietary treatment with amoxicillin, clarithromycin, metronidazole, and omeprazole eradicated combinations of *H. hepaticus*, *H. bilis*, and *H. rodentium* from 2 immunocompromised mouse strains, including older *Cd1*$^{-/-}$ mice with long-established infections, although those mice required at least 8 wk treatment for eradication, and prolonged follow up (more than 4 wk) may be advisable to confirm eradication. Our results suggest that dietary treatment of young mice for use as founders of *Helicobacter*-free colonies would be successful. Also intriguing is the possibility that dietary treatment could be used to eradicate *Helicobacter* spp. from an entire existing colony of mice, including older and immunocompromised animals, similar to the current use of medicated diets for the eradication of intestinal parasites.

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