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Rapid Onset of Ulcerative Typhlocolitis in B6.129P2-IL10^{tm1Cgn} (IL-10$^{-/-}$) Mice Infected with *Helicobacter trogontum* Is Associated with Decreased Colonization by Altered Schaedler's Flora$^{\nabla}$

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Infection with *Helicobacter trogontum*, a urease-positive helicobacter isolated from subclinically infected rats, was evaluated in B6.129P2-IL10^{tm1Cgn} (interleukin-10$^{-/-}$ [IL-10$^{-/-}$]) and C57BL/6 (B6) mice. In a first experiment, IL-10$^{-/-}$ mice naturally infected with *Helicobacter rodentium* had subclinical typhlocolitis but developed severe diarrhea and loss of body condition with erosive to ulcerative typhlocolitis within 1 to 3 weeks of experimental infection with *H. trogontum*. A second experiment demonstrated that helicobacter-free IL-10$^{-/-}$ mice dosed with *H. trogontum* also developed severe clinical signs and typhlocolitis within 2 to 4 weeks, whereas B6 mice colonized with *H. trogontum* were resistant to disease. In a third experiment, using helicobacter-free IL-10$^{-/-}$ mice, dosing with *H. trogontum* resulted in acute morbidity and typhlocolitis within 8 days. Acute typhlocolitis was accompanied by signs of sepsis supported by degenerative hemograms and recovery of *Escherichia coli* and *Proteus* spp. from the livers of infected mice. Quantitative PCR data revealed that *H. rodentium* and *H. trogontum* may compete for colonization of the lower bowel, as *H. trogontum* established higher colonization levels in the absence of *H. rodentium* ($P < 0.003$). *H. trogontum*-induced typhlocolitis was also associated with a significant decrease in the levels of colonization by five of eight anaerobes that comprise altered Schaedler's flora ($P < 0.002$). These results demonstrate for the first time that *H. rodentium* infection in IL-10$^{-/-}$ mice causes subclinical typhlocolitis and that infection with *H. trogontum* (with or without *H. rodentium*) induces a rapid-onset, erosive to ulcerative typhlocolitis which impacts the normal anaerobic flora of the colon and increases the risk of sepsis.

A variety of inbred mutant and genetically engineered strains of mice with immune function abnormalities develop spontaneous enterocolitis, which can be promoted by natural or experimental helicobacter infection (36). A notable example is the spontaneous typhlocolitis that progresses to neoplastic sequelae over 6 months in B6.129P2-IL-10^{tm1Cgn} (interleukin-10$^{-/-}$ [IL-10$^{-/-}$]) mice with undefined intestinal flora (4). The time course for lesions of typhlocolitis, epithelial hyperplasia, and dysplasia in IL-10$^{-/-}$ mice can be accelerated by experimental infection with *Helicobacter hepaticus* (24), *Helicobacter bilis* (8), or *Helicobacter typhlonius* (13). Notably, IL-10$^{-/-}$ mice develop Th1-mediated typhlocolitis whose severity depends on the genetic susceptibility of the background strain (4) and do not develop disease when maintained germfree (3). Thus, the proximal cause for the chronic typhlocolitis that develops under conventional husbandry conditions is presumed to be enteric flora, including natural or experimental infection with bacteria such as *Helicobacter* spp. (13) and enterococci (3).

During the past decade, the isolation and characterization of novel murine helicobacters have been an active area of interest (49). Enterohepatic helicobacter infections of mice can cause not only subclinical, intercurrent disease that may confound research but also overt disease, such as typhlocolitis in immunodeficient and immune-dysregulated mice, which has been studied as a model of human inflammatory bowel disease (IBD). *Helicobacter trogontum* is a urease-positive helicobacter first isolated from the colons of subclinically infected Wistar and Holtzman rats (28) and is closely related to *H. hepaticus* and members of "*Helicobacter* (*Flexispira*) *rappini*" taxa 1, 4, and 5 by 16S rRNA sequence analysis (10, 19). A taxon 1 isolate has been implicated in human gastroenteritis (2, 37), and taxon 4 contains an uncharacterized isolate from sheep (19). A taxon 5 isolate was associated with natural abortion in sheep (21) and experimental abortion in guinea pigs (7). *H. trogontum* also has morphology and growth characteristics similar to those of, and clusters phylogenetically with, a novel intestinal helicobacter associated with ulcerative colitis in cotton-top tamarins (39) that is in taxon 10 (10). Other than in vitro studies to demonstrate expression of urease and failure to express cytolethal distending toxin (22), the potential to model human disease with *H. trogontum* has been evaluated only in germfree mice that developed subclinical inflammatory lesions (31, 30, 32).

In an initial study of IL-10$^{-/-}$ mice on a C57BL/6 (B6) background that were naturally infected with multiple enterohepatic *Helicobacter* spp., experimental challenge with *H. trogontum* caused debilitating diarrhea associated with severe acute typhlocolitis (S. Danon, personal communication). An additional experiment using IL-10$^{-/-}$ mice from a separate colony naturally infected with *Helicobacter rodentium* replicated these observations; *H. trogontum* infection rapidly caused

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debilitating typhlocolitis. These experiments were expanded to determine if infection with *H. trogontum* alone would reproducibly cause severe typhlocolitis in helicobacter-free B6.129P2-IL-10^{tm1Cgn} (IL-10$^{-/-}$) mice similar to that observed in the IL-10$^{-/-}$ mice naturally infected with multiple enterohepatic helicobacters. Additionally, because both humans (45) and mouse models (5, 17) affected by IBD have been shown to develop differences in their colonic flora relative to control populations (45), we were interested in comparing the population dynamics of *H. rodentium* and *H. trogontum* and in the potential impact of inflammation on normal flora. We used real-time quantitative PCR (qPCR) to estimate levels of colonization in the proximal colon by both helicobacters and altered Schaedler's flora (ASF), which consists of eight anaerobes selected to provide gnotobiotic mice with a standardized intestinal flora (9, 17, 38).

MATERIALS AND METHODS

Animals. Viral antibody- and parasite-free B6.129P2-IL-10^{tm1Cgn} (IL-10$^{-/-}$) mice naturally infected with *H. rodentium* were maintained at the Massachusetts Institute of Technology (Cambridge). Helicobacter-free IL-10$^{-/-}$ mice were rederived by embryo transfer from the *H. rodentium*-infected founder mice, and helicobacter-free C57BL/6 (B6) mice were purchased from Jackson Laboratories (Bar Harbor, ME). The helicobacter infection status of these separately housed colonies was confirmed by fecal PCR using genus- and species-specific primers (40, 41). Mice were provided pelleted rodent diet (ProLab 3000; Purina Mills, St. Louis, MO) and filtered water ad libitum and were maintained in AAALAC-accredited facilities in microisolator caging (Allentown Caging Equipment Inc., Allentown, NJ) under standard environmental conditions. All experiments were approved by the MIT Committee on Animal Care.

Natural infection with *H. rodentium*. Naturally acquired infection with *H. rodentium* had been confirmed in a colony of barrier-maintained IL-10$^{-/-}$ mice that had been obtained from a commercial vendor prior to the recognition of *H. rodentium* as a novel helicobacter of mice (41). This colony was free of all other known *Helicobacter* species as well as pathogenic murine viruses, *Salmonella* spp., *Citrobacter rodentium*, and parasites.

Experimental infection with *H. trogontum* (ATCC 700114). In a first experiment, 12 male and 13 female IL-10$^{-/-}$ mice from a colony naturally infected with *H. rodentium* were dosed at 6 to 8 weeks of age with 2×10^7 *H. trogontum* three times on alternate days by oral gavage or were sham-dosed as controls (11 males, 7 females). Mice were euthanized with carbon dioxide and necropsied at time points up to 6 weeks postinfection (PI) based on progressive evidence of bloody feces, dehydration, and weight loss in *H. trogontum*-infected mice. In a second experiment, five male and three female helicobacter-free IL-10$^{-/-}$ and five male and five female B6 mice were dosed at 6 to 8 weeks of age with 2×10^7 *H. trogontum* organisms twice over 5 days by oral gavage, and a similar number were sham-dosed as controls. Mice were necropsied 2 to 4 weeks PI based on the extent of morbidity. A third group of helicobacter-free male ($n = 10$) and female ($n = 10$) IL-10$^{-/-}$ mice were dosed at 6 weeks of age with 2×10^7 *H. trogontum* organisms twice over 5 days by oral gavage or were sham-dosed as controls (five males and six females). These mice were euthanized and necropsied 8 days PI due to rapid clinical deterioration.

Clinical assessment and necropsy. Throughout the experiments, mice were assessed for evidence of diarrhea, dehydration, and deteriorating body condition (47). Although there were some deaths due to acute disease, mice were euthanized with CO_2 and necropsied when they became moribund or when the PI interval of 6 weeks was reached.

Confirmation of helicobacter infection. To demonstrate that mice were helicobacter free or colonized with *H. rodentium* or *H. trogontum*, fecal or cecal samples were tested by PCR using previously described methods and primer sets specific for the genus or each *Helicobacter* species. (12, 41).

Quantitative PCR for *H. rodentium and H. trogontum*. After removal of gross cecal contents, approximately 0.5 cm of cecal tissue was sampled proximal to the cecal-colonic junction and stored at −20°C until DNA extraction. DNA was extracted using a Boehringer-Mannheim High Pure PCR template preparation kit (Roche Molecular Biochemicals, Indianapolis, IN) following the manufacturer's protocol for the isolation of nucleic acids from tissue. *H. rodentium* and *H. trogontum* organisms were quantified with species-specific primers in the samples, with host (mouse) DNA being measured using commercial 18S rRNA gene-based primers and probe (Applied Biosystems, Foster City, CA). The 16S rRNA gene-based primers for *H. rodentium* were previously reported (41), whereas the primers (forward, 5′-TATTGAGAGTAGTACTTATTGAA-3′; reverse, 5′-ATGCAAAGTTTTGATTTCAAGACCA-3′) for the *H. trogontum* genome were derived from the spacer region between the 16S and 23S rRNA genes of *H. trogontum*, which produce a 365-bp amplicon (J. Fox and Z. Shen, unpublished data). Real-time quantitative PCR was performed using the Applied Biosystems sequence detection system (model 7700). There was no cross-amplification between host DNA and *H. rodentium*- or *H. trogontum*-specific primers. For quantitation with Sybr green, duplicates of each sample contained the following in a 50-μl volume: 10 μl template DNA, 5 μl of 10× commercial buffer A, 7 μl of 25 mM $MgCl_2$, 1 μl each of 10 μM dATP, dCTP, dGTP, and dUTP, 0.5 μl uracil-*N*-glycosylase, 2.5 μl AmpliTaq Gold, and balance DNase-free double-distilled H_2O. Thermocycling was performed at 50°C for 2 min and 95°C for 10 min, followed by 40 repeats of 95°C for 15 s and 60°C for 60 s. To estimate copy numbers of *H. trogontum* and *H. rodentium* in samples, a standard curve was created using serial 10-fold dilutions (1×10^6 to 10^1) of copy number based on an estimate of the genome size of *H. trogontum* and *H. rodentium*. A mean mass value (1.71 Mb) was estimated by the average from two published *H. pylori* genomes (1, 46) and the *H. hepaticus* genome (44). The number of *H. trogontum* or *H. rodentium* genome copies was then normalized to μg of murine chromosomal DNA measured by qPCR using 18S rRNA gene-based primers and probe mixture (Applied Systems). Murine chromosomal DNA in each sample was compared to a standard curve based on 10^6, 10^5, 10^4, 10^3, and 10^2 pg of DNA obtained from *Helicobacter*-free mouse liver.

Quantitative PCR for ASF. ASF consists of eight anaerobes selected to provide gnotobiotic mice with a standardized intestinal flora (9). The eight organisms include ASF356 and ASF502, which are *Clostridium* spp., ASF360 and ASF361, which are *Lactobacillus* spp., ASF500, an unclassified gram-positive rod, ASF492, a *Eubacterium* sp., ASF457, recently named *Mucispirillum schaedleri* (35), and ASF519, a *Bacteroides* sp. Quantitative PCR was performed on proximal colon samples obtained from four male and four female helicobacter-free IL-10$^{-/-}$ mice and from eight male and seven female IL-10$^{-/-}$ mice 8 days after infection with *H. trogontum*. DNA extraction was modified to maximize isolation of nucleic acids from gram-positive as well as gram-negative bacteria, and assay reagents and conditions were used as previously described (17, 38).

Liver culture. To screen for translocation of bacteria from the gut in the third experiment, liver samples from four helicobacter-free and four *H. trogontum*-infected IL-10$^{-/-}$ mice were aseptically collected at necropsy 8 days PI. A 0.5-cm^3 sample from the left liver lobe of each mouse was homogenized, plated on Trypticase soy agar (TSA) and MacConkey's medium (Remel, Inc., Lenexa, KA), and incubated under aerobic conditions at 37°C. For potential recovery of *H. trogontum*, tissue was inoculated onto TSA and incubated under microaerobic conditions (80:10:10 N_2-H_2-CO_2) at 37°C.

Complete blood counts. Whole blood was collected into EDTA at necropsy for evaluation of the hemogram from helicobacter-free and *H. trogontum*-infected IL-10$^{-/-}$ mice at 8 days PI. Most parameters were measured with a commercial hematology analyzer (CDC Technologies, Oxford, CT), while the differential was determined manually by a certified medical technologist blinded to the experimental status of the samples.

Histology. Formalin-fixed tissues were routinely processed, embedded in paraffin, sectioned at 4 μm, stained with hematoxylin and eosin, and evaluated by a board-certified veterinary pathologist blinded to the sample identity. Inflammation, edema, epithelial defects, hyperplasia, and dysplasia of the cecal-colonic junction were scored on an ascending scale (0 [for normal] to 4) of severity and invasiveness of the lesion (Table 1). Scores for inflammation, edema, epithelial defects, hyperplasia, and dysplasia were totaled to generate a lesion index for comparison between groups of mice.

Statistics. Statistical comparisons of controls to helicobacter-infected mice for morbidity and lesion severity were performed using a software package (GraphPad Software, Inc., San Diego, CA.). Chi-square analysis and the nonparametric Mann-Whitney test were used for categorical data involving morbidity and lesion scores. Quantitative PCR data for levels of colonization by helicobacter and ASF species were first log transformed and then compared by Student's *t* test. Results were considered significant when P was <0.05.

RESULTS

***H. rodentium* infection was associated with subclinical typhlocolitis in IL-10$^{-/-}$ mice.** In the first experiment, IL-10$^{-/-}$ mice naturally infected with *H. rodentium* were clinically nor-

TABLE 1. Lower bowel lesion scoring criteria

Score	Inflammation	Edema	Epithelial defects	Hyperplasia	Dysplasia
0	None	None	None	Normal crypt length	None
1	Small multifocal lamina proprial and/or transepithelial leukocyte accumulations	Mild segmental expansion of submucosa	Decreased goblet cells, occasional dilated glands, mild surface "tattering"	~1.5× normal crypt length	Mild dysplasia characterized by epithelial cell pleomorphism, plump and attenuated forms, aberrant crypt foci, gland malformation with mild splitting, branching, and infolding, rare cystic dilation
2	Coalescing mucosal inflammation with or without early submucosal extension	Moderate diffuse submucosal with or without mucosal lamina propria expansion	Focally extensive surface epithelial tattering, many dilated glands with attenuated lining and luminal cell debris	~2×; with or without mitotic figures one-third way up to surface	Moderate dysplasia characterized by pleomorphism, early cellular and nuclear atypia, mild piling and infolding, occasional cystic dilation, bulging towards muscularis mucosae and projection into lumen, loss of normal glandular, mucous, or goblet cells, also known as atypical hyperplasia
3	Coalescing mucosal inflammation with prominent multifocal submucosal extension with or without follicle formation	Severe edema of mucosa and submucosa	Erosions	~3×; with or without mitotic figures half-way up to surface	Gastrointestinal intraepithelial neoplasia (6), severe dysplasia confined to mucosa, features as above but greater severity, frequent and sometimes bizarre mitoses
4	Severe diffuse inflammation of mucosa, submucosa, and deeper layers	Transmural; very severe	Ulceration	4×+; with or without mitotic figures more than half-way up to surface	Invasive carcinoma: Submucosal invasion, or any demonstrated invasion into blood or lymphatic vessels, regional nodes, or other metastasis.

mal until necropsied at 10 to 12 weeks of age. *H. rodentium* infection was consistently associated with mild enlargement of mesenteric lymph nodes. Typhlocolitis associated with *H. rodentium* infection was mild to moderate (Table 2). Total lesion indices were significant in both male and female IL-10$^{-/-}$ mice infected with *H. rodentium* compared to helicobacter-free IL-10$^{-/-}$ mice used in the second and third experiments ($P < 0.005$ for males, $P < 0.01$ for females) (Table 2; Fig. 1 and 2). Reflected in the individual parameter scores for inflammation, epithelial defects, hyperplasia, and dysplasia as well as in the total lesion indices (Table 2), there was a trend for male IL-10$^{-/-}$ mice infected with *H. rodentium* to have moderate disease, compared to mild disease in female mice. In contrast to helicobacter-free IL-10$^{-/-}$ mice, IL-10$^{-/-}$ mice infected with *H. rodentium* had multifocal to coalescing areas of mucosal inflammation in the lamina propria of the cecal-colonic junction along with mild to moderate edema of the submucosa. Goblet cell numbers were decreased, and crypts were elongated, with features of moderate dysplasia.

***H. trogontum* infection was associated with rapid onset of ulcerative typhlocolitis in IL-10$^{-/-}$ mice.** In contrast to *H. rodentium* infection, clinical morbidity was severe in mice coinfected with *H. rodentium* and *H. trogontum*, particularly in male IL-10$^{-/-}$ mice ($P < 0.002$). Coinfected IL-10$^{-/-}$ male mice rapidly developed dehydration and weight loss and were either found dead of acute disease or euthanized when moribund at 1 or 3 weeks after infection with *H. trogontum* ($n = 12$). The extent of typhlocolitis and hyperplasia of the epithelium in males that developed acute morbidity by 1 week PI was similar to that in males that were necropsied at 3 weeks PI ($P = 0.25$ and 0.14, respectively), although dysplasia was more severe at 3 weeks PI ($P = 0.05$). Coinfected male IL-10$^{-/-}$ mice consistently developed gross thickening of the cecal-colonic junction, and cecal and colon contents were often grossly hemorrhagic. Mesenteric lymph nodes were markedly enlarged in the cecal-colonic mesentery. The cecal-colonic junctions of male IL-10$^{-/-}$ mice coinfected with *H. rodentium* and *H. trogontum* were extensively infiltrated with mononuclear cells that distorted crypt morphology. Crypt abscesses, depletion of goblet cells, and elongation of crypts to 2 to 3 times normal length with mild to moderate dysplasia were noted (Fig. 2C). Comparison of total lesion indices (Table 2) indicates that *H. trogontum* significantly exacerbated typhlocolitis in male IL-10$^{-/-}$ mice compared to natural infection with *H. rodentium* ($P < 0.004$).

In contrast to males, only 4 of 13 (31%) female IL-10$^{-/-}$

TABLE 2. Lesion indices observed at the cecal-colonic junction

Expt	Infection status	Mouse	*n*	Severity score for[a]:						Lesion index[a]
				Time point	Inflammation	Edema	Epithelial defect	Hyperplasia	Dysplasia	
1	*H. rodentium*	Male IL-10$^{-/-}$	11	3-6 wk	2 (0–3)	1 (0–1.5)	0.5 (0–2.5)	1 (0–3)	0.5 (0–2)	6 (0–10)
		Female IL-10$^{-/-}$	7	4-6 wk	1 (0–2.5)	1 (0–1.5)	0 (0–1.5)	0.5 (0–2.5)	0 (0–1.5)	2 (0–9)
	H. rodentium and *H. trogontum*	Male IL-10$^{-/-}$	12	1-3 wk	2.5 (0–2.5)	1.75 (0.5–3)	2.5 (0–3)	1.5 (0–2.5)	1.25 (0–2)	9.75 (0.5–11.5)
		Female IL-10$^{-/-}$	13	2-6 wk	0 (0–2)	0 (0–3)	0 (0–2.5)	0 (0–2)	0 (0–1.5)	0 (0–10.5)
2	Uninfected controls	Male B6	5	4 wk	0 (0)	0 (0–0.5)	0 (0)	0 (0)	0 (0)	0 (0–0.5)
		Female B6	4	4 wk	0 (0)	0 (0–0.5)	0 (0)	0 (0)	0 (0)	0 (0–0.5)
	H. trogontum	Male B6	4	4 wk	0 (0–0.5)	0 (0–0.5)	0.5 (0–1)	0 (0)	0 (0)	0 (0–1)
		Female B6	5	4 wk	0 (0–0.5)	0.5 (0–1)	0 (0)	0 (0)	0 (0)	0.5 (0–1.5)
	Uninfected controls	Male IL-10$^{-/-}$	5	4 wk	0 (0)	0 (0–0.5)	0 (0)	0 (0)	0 (0)	0 (0–0.5)
		Female IL-10$^{-/-}$	5	4 wk	0 (0–1)	0 (0–2.5)	0 (0–0.5)	0 (0)	0 (0)	0 (0–4)
	H. trogontum	Male IL-10$^{-/-}$	5	2-3 wk	2.5 (2.5–3)	2 (1.5–2)	2.5 (2–3)	1 (0.5–2)	1 (0.5–1.5)	8.5 (7.5–11)
		Female IL-10$^{-/-}$	3	3-4 wk	2 (1–2.5)	2 (1.5–2.5)	1.5 (0–2)	0.5 (0–1.5)	0.5 (0–1)	7 (2.5–9)
3	Uninfected controls	Male IL-10$^{-/-}$	5	8 days	0 (0–0.5)	0 (0–0.5)	0 (0)	0 (0)	0 (0)	0 (0–0.5)
		Female IL-10$^{-/-}$	6	8 days	0 (0)	0 (0–0.5)	0 (0)	0 (0)	0 (0)	0 (0–0.5)
	H. trogontum	Male IL-10$^{-/-}$	10	8 days	2.5 (0–3)	2.25 (0–3.5)	2.5 (0–3.5)	1.5 (0–2)	0.5 (0–1)	9.75 (0–13)
		Female IL-10$^{-/-}$	10	8 days	3 (1–3.5)	2 (0–2.5)	3 (0–3.5)	1.75 (0–2)	1 (0–1)	10 (1–12.5)

[a] Values are medians and ranges.

mice became clinically debilitated from concurrent *H. rodentium* and *H. trogontum* infection. Two female mice were euthanized for deteriorating clinical condition at 3 weeks, one female mouse was euthanized at 5 weeks, and one female mouse was euthanized at 6 weeks PI. Clinically normal female IL-10$^{-/-}$ mice infected with both *H. rodentium* and *H. trogontum* were necropsied for comparison of lesions to those of male mice and had gross and histologic changes that were mild in the majority of mice (Table 2; Fig. 1). Notably, experimental challenge with *H. trogontum* in females naturally infected with *H. rodentium* did not significantly exacerbate inflammation, hyperplasia, or dysplasia attributable to *H. rodentium* infection alone ($P = 0.09$).

A second experiment was then performed to determine if the gender differences were reproducible and to determine the effects of infection with *H. trogontum* in helicobacter-free IL-10$^{-/-}$ and wild-type B6 mice. One of 10 B6 mice was not colonized with *H. trogontum* and thus was not included in the analysis. Four male and five female helicobacter-free B6 mice experimentally colonized with *H. trogontum* remained clinically normal, with no gross abnormalities noted at necropsy. Histology assessment revealed that *H. trogontum* caused only mild typhlocolitis in B6 mice by 4 weeks PI, with no development of epithelial hyperplasia or dysplasia (Table 2; Fig. 2D). The body condition of helicobacter-free male and female IL-10$^{-/-}$ mice subsequently infected with *H. trogontum* progressively declined, necessitating euthanasia by 2 to 4 weeks PI. Similar to the first study, *H. trogontum*-infected IL-10$^{-/-}$ mice were clinically dehydrated and had enlarged mesenteric lymph nodes and grossly thickened ceca and colons. Lower bowel contents

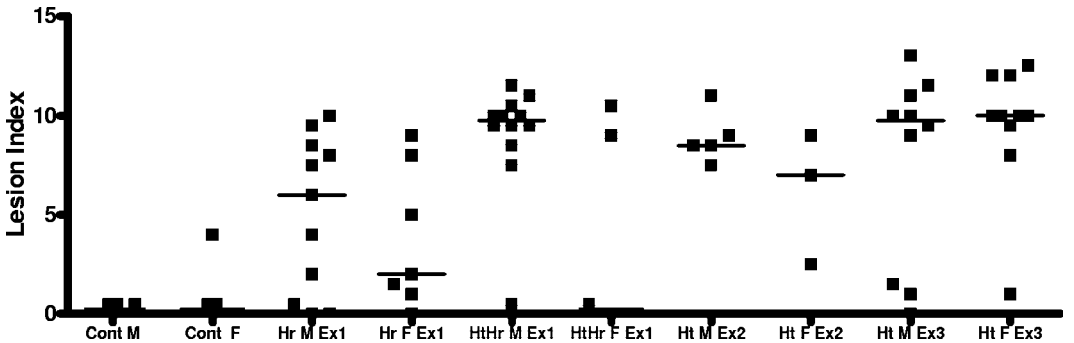

FIG. 1. Total lesion index for individual IL-10$^{-/-}$ mice evaluated in the three experiments (Ex). Cont M and Cont F, helicobacter-free male and female IL-10$^{-/-}$ controls; Hr, *H. rodentium*; Ht, *H. trogontum*; HtHr, coinfection. Lines represent median values. See Table 2 for the numbers of mice evaluated.

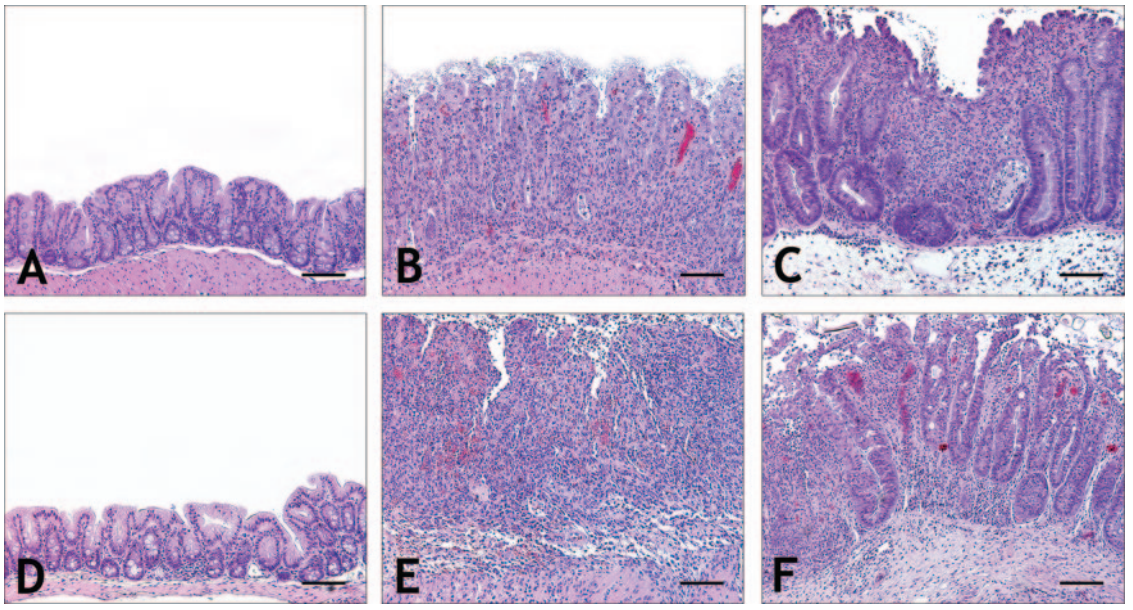

FIG. 2. (A) Normal cecum from helicobacter-free IL-10$^{-/-}$ male mouse. (B) Moderate typhlitis from *H. rodentium* infection in an IL-10$^{-/-}$ male mouse. Note the moderate hyperplasia, loss of goblet cells, and inflammatory infiltrate. (C) Severe, diffuse typhlitis with focal erosion, glandular dysplasia and hyperplasia, loss of goblet cells, and crypt abscesses from *H. rodentium* and *H. trogontum* infection in an IL-10$^{-/-}$ male mouse. (D) Minimal typhlitis from *H. trogontum* infection in a male B6 mouse. (E) Severe erosive typhlitis with near-total crypt atrophy from *H. trogontum* infection in an IL-10$^{-/-}$ male mouse. (F) Lower magnification of the field adjacent to the lesion in panel E, showing severe inflammation, hyperplasia and dysplasia. Bar = 160 μm (A to E) and 250 μm (F).

were fluid and sometimes hemorrhagic. Notably, there were no gender-associated differences in the extent of disease attributable to *H. trogontum* ($P = 0.25$), although there was a trend for females to resist clinical effects somewhat longer (3 to 4 weeks PI) than male IL-10$^{-/-}$ mice (2 to 3 weeks PI) (Table 2). Histologically, inflammation, edema, epithelial defects, hyperplasia, and dysplasia were moderate to severe in both male and female IL-10$^{-/-}$ mice, with similar total lesion indices ($P = 0.07$).

In a third experiment, 10 male and 10 female IL-10$^{-/-}$ mice infected with *H. trogontum* rapidly developed diarrhea with associated dehydration and weight loss that necessitated euthanasia at 8 days PI. Five uninfected control male and six control female IL-10$^{-/-}$ mice remained clinically normal. Within just 8 days, weight loss in *H. trogontum*-infected mice was severe, with an average loss of 32% in infected females and an average loss of 24% in infected males. Gross findings at necropsy in infected mice were consistent with observations from the first two experiments: dehydration, weight loss, enlarged mesenteric lymph nodes, and thickened cecal-colonic junction and distal colon, often with dark, liquid intestinal contents. Total lesion indices, incorporating inflammation, edema, epithelial defects, hyperplasia, and dysplasia at the cecal-colonic junction, were similar ($P = 0.17$) in male and female IL-10$^{-/-}$ mice infected with *H. trogontum* (Table 2; Fig. 1, 2E, and 2F). Coalescing, diffuse inflammation of the mucosa, submucosa, and deeper layers was observed in 9 of 10 female and 7 of 10 male IL-10$^{-/-}$ mice infected with *H. trogontum* and was accompanied by erosive to ulcerative epithelial lesions in seven mice of each gender (Fig. 2E). Crypt necrosis, crypt abscesses, erosion and ulceration of epithelium, submucosal edema, and extension of transmural inflammation progressed to serositis in some mice. Although lesions were most severe at the cecal-colonic junction in the *H. trogontum*-infected IL-10$^{-/-}$ mice, segmental areas of the middle and distal colon were similarly affected with moderate to severe inflammation, submucosal edema, epithelial defects, and mild to moderate hyperplasia and dysplasia of the epithelium (data not shown).

***H. trogontum* infection caused acute morbidity associated with leukocytosis and translocation of intestinal bacteria to the liver.** In the *H. trogontum*-infected IL-10$^{-/-}$ mice that became clinically ill within 8 days, a leukocytosis with a degenerative left shift in the hemogram suggested sepsis (data not shown), supporting the deteriorating clinical status of these mice. A subset of four liver samples from controls and four samples from *H. trogontum*-infected mice were collected aseptically at necropsy and cultured to determine if necrotic changes in the intestinal epithelia allowed translocation of intestinal bacteria. Although *H. trogontum* was not recovered by culture of liver samples, *Escherichia coli* and *Proteus* spp. were recovered by aerobic culture from the livers of three of the four selected *H. trogontum*-infected IL-10$^{-/-}$ mice, whereas liver samples from four selected control IL-10$^{-/-}$ mice were sterile. Mild portal hepatitis (not shown) was observed in the livers from the *H. trogontum*-infected mice but not in the control, sterile livers.

Natural infection with *H. rodentium* inhibited colonization of *H. trogontum* in the cecum. Conventional PCR was used to confirm the infection status of controls infected with *H. rodentium* and the helicobacter-free status of control mice used in the additional two studies. Real-time quantitative PCR was used for estimating the levels of colonization by *H. rodentium* and *H. trogontum* in the ceca of experimentally infected mice. In the first experiment, which evaluated male and female IL-

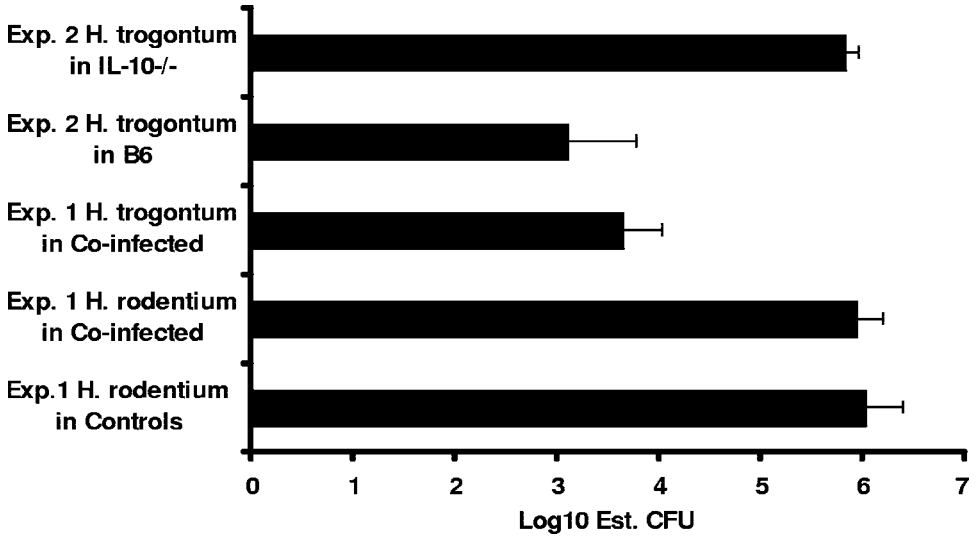

FIG. 3. Data from quantitative PCR indicated that *H. rodentium* levels in the cecum were approximately 2 logs higher than *H. trogontum* ($P < 0.001$) in coinfected mice and were not lowered by concurrent infection with *H. trogontum* ($P = 0.43$). B6 mice were colonized with *H. trogontum* at levels approximately 3 logs lower than IL-10$^{-/-}$ mice ($P < 0.004$). *H. trogontum* levels were significantly higher in monoinfected IL-10$^{-/-}$ mice (experiment 2) than in coinfected IL-10$^{-/-}$ mice (experiment 1) ($P < 0.003$). Data are medians and standard errors.

10$^{-/-}$ mice naturally infected with *H. rodentium* and then experimentally challenged with *H. trogontum*, levels of colonization by *H. rodentium* and *H. trogontum* were similar in both genders of mice ($P = 0.14$ and higher; data by gender not shown). Levels of *H. rodentium* were approximately 2 logs higher than those of *H. trogontum* ($P < 0.001$) in coinfected mice (Fig. 3) and were not lowered by subsequent coinfection with *H. trogontum* ($P = 0.43$). In the second experiment, B6 mice were colonized with *H. trogontum* at levels approximately 3 logs lower than those in IL-10$^{-/-}$ mice ($P < 0.004$) (Fig. 3). Notably, in the absence of *H. rodentium*, *H. trogontum* levels were significantly higher in the monoinfected IL-10$^{-/-}$ mice in the second experiment than in coinfected IL-10$^{-/-}$ mice in the first experiment ($P < 0.003$).

Experimental infection with *H. trogontum* lowered colonization of ASF. In the third experiment, samples from the proximal colon were obtained at necropsy to evaluate potential population shifts in resident ASF as the result of *H. trogontum* infection. Samples from 15 IL-10$^{-/-}$ mice infected with *H. trogontum* for 8 days were evaluated and showed significantly lower levels of colonization by five of the eight ASF species in the proximal colon than in eight helicobacter-free control IL-10$^{-/-}$ mice (Fig. 4). ASF356 (*Clostridium* sp.), ASF457 (*M. schaedleri*), ASF492 (*Eubacterium* sp.), ASF500 (unclassified gram-positive rod), and ASF502 (*Clostridium* sp.) all colonized at lower levels in *H. trogontum*-infected mice ($P < 0.002$). Levels of colonization by the other three ASF species (ASF360 and ASF361 [both *Lactobacillus* spp.] and ASF519 [*Bacteroides* sp.]) in the proximal colon were not im-

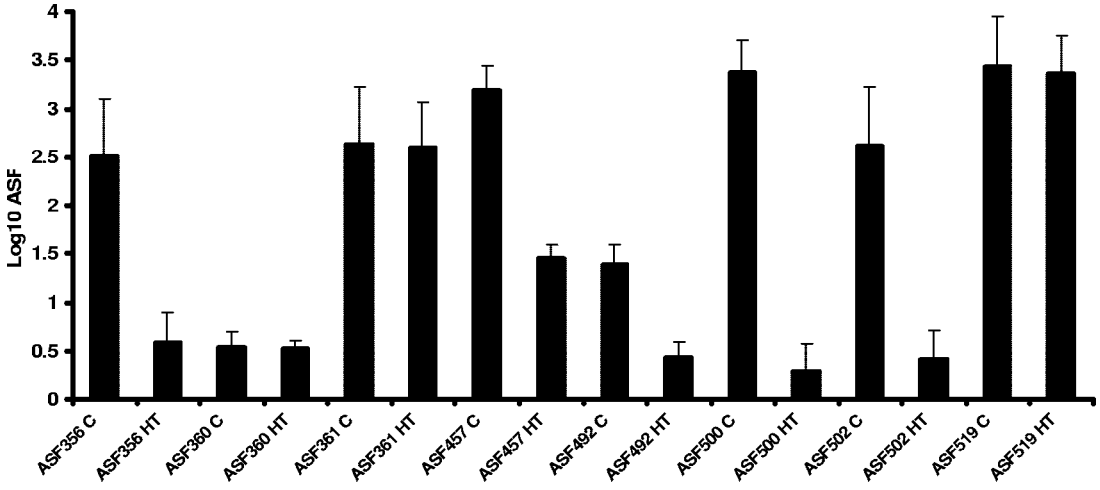

FIG. 4. IL-10$^{-/-}$ mice infected with *H. trogontum* for 8 days had significantly lower levels of colonization by five of the eight ASF species in the proximal colon than helicobacter-free control IL-10$^{-/-}$ mice ($P < 0.002$). Levels of colonization by ASF360, ASF361, and ASF519 were not impacted by *H. trogontum* infection ($P = 0.43$). Data are medians and standard errors.

pacted by *H. trogontum* infection ($P = 0.43$), nor were any of the changes in colonization by the eight species of ASF impacted by gender in the *H. trogontum*-infected mice ($P = 0.2$).

DISCUSSION

Infection with *H. trogontum* caused severe morbidity and typhlocolitis in IL-10$^{-/-}$ mice that were naturally infected with *H. rodentium* or were initially helicobacter free. Additionally, lesions attributable to *H. rodentium* infection in IL-10$^{-/-}$ mice have not been reported, but *H. rodentium* infection was associated with mild to moderate typhlocolitis in this study. Results from the first experiment suggested that male IL-10$^{-/-}$ mice may be predisposed to more severe disease from *H. trogontum* infection than females, but data from the two follow-up studies indicated that both genders were susceptible, albeit with a possible time course delay in female mice. Development of acute typhlocolitis associated with weight loss and diarrhea from *H. trogontum* infection was also associated with declines in ASF colonization, suggesting that *H. trogontum* infection impacts the levels of colonization by normal, potentially beneficial flora. Erosive to ulcerative epithelial changes from the acute inflammation may have increased mucosal permeability and permitted translocation of *E. coli* and *Proteus* spp. to the liver, suggesting that sepsis contributed to clinical deterioration. Compared to more chronic lesion development associated with other enterohepatic helicobacter infections in IL-10$^{-/-}$ mice (24, 13), *H. trogontum*-induced typhlocolitis is an acute model of bacterial inflammatory bowel disease.

Lack of disease attributable to *H. trogontum* infection in B6 mice and the acute typhlocolitis, epithelial hyperplasia, and dysplasia induced by *H. trogontum* in the IL-10$^{-/-}$ mice are consistent with previously reported models using *H. hepaticus* (24), *H. bilis* (8), and *H. typhlonius* (13); however, the clinical course of *H. trogontum* infection in IL-10$^{-/-}$ mice was faster and the lesions more severe. *H. trogontum* did not colonize B6 mice to the same level as IL-10$^{-/-}$ mice for unknown reasons, but lack of typhlocolitis in the B6 mice is likely more closely related to normal homeostatic IL-10 responses than to lower colonization. IL-10 is a Th2-associated cytokine with potent anti-inflammatory properties and has been shown to be critical to T-regulatory-cell function in a mouse model of *H. hepaticus*-mediated colon cancer (11). The acute nature of severe inflammation caused by *H. trogontum* infection in IL-10$^{-/-}$ mice provides a rapid-onset model for dysregulated intestinal epithelial cell responses to a bacterial pathogen.

The potential for *H. trogontum* to cause disease in mice with complex intestinal flora was previously unrecognized. However, a series of reports detailed how *H. trogontum* monoassociation in germfree outbred NIH mice caused vacuolation of ileal epithelial cells, loss of microvilli, and pronounced desquamation of cecal enterocytes (31). *H. trogontum* colonized the stomachs, ceca, and colons of these monoassociated mice and caused moderate, diffuse inflammation within at least one segment of the gastrointestinal tract in individual mice (30). Although *H. trogontum* was not recoverable from the liver, focal infiltration of inflammatory cells in the liver was suggestive of *H. trogontum*-associated hepatitis in monoassociated outbred NIH mice evaluated at 6, 12, and 18 months PI (32). Although consistent with *H. hepaticus*-associated (16, 14) and *H. bilis*-associated (15) hepatobiliary disease of selected inbred and outbred mice, infection with *H. trogontum* in the IL-10$^{-/-}$ and B6 mice did not cause liver lesions except for mild portal hepatitis (data not shown) in helicobacter-infected IL-10$^{-/-}$ mice. Helicobacter-associated hepatobiliary disease would not be expected in B6 mice (20, 48), particularly when they are infected for a short time. We believe that the mild portal infiltrates observed in the mice euthanized at day 8 after *H. trogontum* infection reflected antigenic stimulation from the lower bowel via the portal circulation.

Reports on *H. rodentium* indicate it may have a role in potentiating disease but probably causes minimal host tissue response in normal mice. Both *H. rodentium* (25, 34, 18) and *H. trogontum* (28) have been detected in laboratory rats by PCR or culture, with no reports of clinical disease or histologic bowel lesions. Of the formally named enterohepatic helicobacters, only *H. bilis* (43, 26), *H. hepaticus* (24), and *H. typhlonius* (13) have been shown to cause typhlocolitis in immunodeficient or immune-dysregulated mice, such as IL-10$^{-/-}$ mice. *Prkdc*^{scid}/*Tpr53*^{tm1tyi} mice on a B6.129/Sv × C.B-17 background developed severe diarrhea and typhlocolitis from coinfection with *H. rodentium* and *H. bilis* (42), although *H. rodentium* monoinfection was shown to be nonpathogenic in A/JCr and C.B-17/IcrCrl-scidBr mice (33). *H. rodentium* and *H. hepaticus* coinfection in C.B-17/IcrCrl-scidBr mice has also been shown to augment cecal gene expression and produce more severe clinical disease than can be attributed solely to *H. hepaticus* (33). *H. rodentium* was also recently shown to promote cholesterol gallstone formation in C57BL mice coinfected with *H. hepaticus* and fed a lithogenic diet (27).

Infection with *H. trogontum* in IL-10$^{-/-}$ mice in the second experiment quickly achieved higher levels of *H. trogontum* colonization than in coinfected mice from the first experiment, even though the mice received one less experimental dose of *H. trogontum*. Relatively higher *H. trogontum* colonization in the absence of *H. rodentium* may have been responsible for the acute morbidity and development of erosive typhlocolitis noted in the second and third experiments. The initial appearance of a gender bias for more severe disease in coinfected male IL-10$^{-/-}$ mice of the first experiment may have been related to preexisting *H. rodentium* infection. At necropsy, male and female IL-10$^{-/-}$ mice in the first experiment were colonized with *H. trogontum* at similar levels (data not shown), but *H. rodentium* infection may have primed an immune response against experimental challenge with *H. trogontum* or otherwise initially inhibited *H. trogontum* colonization in some mice, thereby allowing more time before colonization levels rose and morbidity developed.

The livers from the *H. trogontum*-infected mice in the third experiment were culture positive for *E. coli* and *Proteus* spp., and mild portal hepatitis was observed histologically. Mononuclear cells accumulating around the portal vessels may have responded to antigenic stimulation from the inflamed lower bowel. Recovery of the intestinal bacteria by culture from the liver may indicate true sepsis or, at a minimum, enhanced mucosal permeability from inflammation. Less probably, agonal events in concert with inflammatory damage to the epithelium may have allowed translocation of bacteria to the portal circulation (29).

Additionally, as observed in this study, it is unknown how *H.*

trogontum acutely impacts the population balance of other resident flora. As a broad indicator of potential alterations in colon bacterial populations, many of which are unculturable, we used quantitative PCR to estimate levels of colonization by each of the eight ASF anaerobes that naturally colonize mice and have historically been used to colonize germfree mice to reestablish normal gut physiology. *H. trogontum* infection acutely lowered colonization density of five of the eight ASF species, which is consistent with observations that both murine enterohepatic *Helicobacter* spp. and the ASF anaerobes colonize mice in high numbers at the cecal-colonic junction and lower bowel (38). Others have also shown using terminal restriction fragment length polymorphisms that *H. hepaticus* infection decreased overall diversity of unculturable microbiota of the cecum in B6 mice within 14 days PI (23). Profiles of denaturing gradient gel electrophoresis of PCR products indicated that colon microbiota were altered by developing colitis in IL-10$^{-/-}$ mice (5); in particular, loss of lactobacilli and an increase in a *Bacteroides* sp. that has 99% identity by 16S rRNA gene sequence analysis to the *Bacteroides* organism ASF519, which in our study remained elevated despite *H. trogontum*-associated inflammation, were observed. *H. trogontum* may have inhibited ASF levels by direct competition for nutrients or other factors in the natural niche in the lower bowel, or, more likely, ASF inhibition was indirectly influenced by the inflammatory response elicited by *H. trogontum*. The latter possibility is supported by a previous report that *H. hepaticus* infection had limited impact on levels of colonization by ASF strains in outbred Swiss Webster mice that did not develop helicobacter-associated colitis (17). These results suggest that acute typhlocolitis impacted the normal resident intestinal flora and may have indirect consequences of inhibiting beneficial organisms or promoting overgrowth of opportunistic bacteria, such as *E. coli* and *Proteus* spp. cultured from the livers of three IL-10$^{-/-}$ mice.

Our results demonstrate that concurrent infection with *H. trogontum* and *H. rodentium* or infection with *H. trogontum* alone in IL-10$^{-/-}$ mice on the B6 background causes acute erosive typhlocolitis with the risk of sepsis. These models should prove useful in dissecting the pathogenesis of various clinical and pathological features noted in inflammatory bowel disease of humans. Further studies using germfree mice subsequently infected with one or more ASF species in combination with *H. trogontum* or other enterohepatic helicobacters are warranted to further establish the role of *Helicobacter* and ASF species and other resident flora in gut homeostasis and disease during pathogenic bacterial infections.

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