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Helicobacter bilis-Induced Inflammatory Bowel Disease in *scid* Mice with Defined Flora

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Helicobacter bilis **has been isolated from aged inbred mice with multifocal chronic hepatitis and from *scid* mice with diarrhea, proliferative typhlitis, and colitis. To determine the pathogenic potential of *H. bilis*, we inoculated 4-week-old female Tac:Icr:Ha(ICR)-*scid*fDF mice by intraperitoneal injection of $\sim 10^8$ CFU of *H. bilis* in phosphate-buffered saline (PBS) ($n = 15$) or PBS alone ($n = 10$) and necropsied them at 7 weeks postinfection. Sham-inoculated mice had no significant gross or histopathological findings. In contrast, all 15 experimentally inoculated mice (confirmed to be *H. bilis*-colonized by culture and PCR of cecal contents) exhibited varying degrees of inflammatory bowel disease (IBD). Proliferative typhlocolitis was characterized by focal to segmental areas of crypt hyperplasia and a predominantly histiocytic inflammatory cell infiltrate. Labeling indices for 5-bromo-2′-deoxyuridine incorporation were increased approximately 2.5-fold in the ceca and colons of *H. bilis*-inoculated mice. This is the first study to demonstrate experimentally that infection with *H. bilis* causes IBD in *scid* mice with defined flora. This result both confirms a pathogenic role for *H. bilis* in mice and provides a new model relating a specific microbial agent and IBD.**

The type strain of the *Helicobacter* genus, *H. pylori*, colonizes the gastric mucosa of humans and has been shown to cause peptic ulcer disease (7, 28). *H. pylori* is linked epidemiologically to the development of gastric adenocarcinomas (6). Gastric *Helicobacter* species, which also colonize the gastric mucosa and cause varying degrees of gastric pathology, have been identified in animal species. These species include *H. mustelae* in ferrets (20) and *H. felis* and *H. heilmannii* in cats and dogs (17, 25, 26).

Recently, there has been considerable interest in intestinal *Helicobacter* spp. of mice because of the association with hepatitis and inflammatory bowel disease (IBD) (5, 19, 22, 44–46). These organisms are particularly relevant for two reasons. First, they may be important models implicating bacterial infections in carcinogenesis. *Helicobacter hepaticus*, an intestinal helicobacter which has been shown to cause persistent hepatitis in A/JCr mice, has been linked with hepatitis and hepatic tumors in several strains of mice and has been proposed as a model of *Helicobacter*-induced tumorigenesis (13, 19, 22, 44–46). Second, many of these intestinal helicobacter species are widespread in commercial and academic rodent colonies and may significantly confound interpretations of some research studies (12, 37). For example, some transgenic rodent strains which have been proposed as spontaneous models of IBD are infected with rodent intestinal helicobacters (12), which are known to potentiate the development of IBD (5). *H. hepaticus* was first identified in a long-term toxicology study in which the control group was found to have an extremely high incidence of hepatic tumors (16, 46). Thus, it is important to determine the pathogenic potential of these novel helicobacter species, both to determine their usefulness as models and to assess the degree of interference they may cause in research with infected mice.

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Helicobacter bilis was recently identified as a novel *Helicobacter* species colonizing the bile, livers, and intestines of aged inbred mice (21). The organism is associated with hepatic lesions but has not been experimentally demonstrated to cause hepatic disease. Anecdotally, a mixed infection of *H. bilis* and the recently characterized *Helicobacter rodentium* (38) has been associated with severe necrotizing proliferative typhlocolitis and diarrhea in a colony of immunodeficient mice (39). The purpose of this study was to determine the pathogenic potential of *H. bilis* in immunocompromised mice with defined flora to fulfill Koch's postulates under controlled conditions.

MATERIALS AND METHODS

Animals. Twenty-five isolator-reared 3-week-old female Tac:Icr:Ha(ICR)-*scid*fDF mice were obtained from Taconic Farms (Germantown, N.Y.). The mice were obtained with a defined-flora status, meaning that they were colonized only with a proprietary cocktail (altered Schaedler's flora) of eight anaerobic bacteria. In contrast to gnotobiotic (germfree) mice, mice colonized with altered Schaedler's flora have normal gut anatomy and physiology. The mice were maintained in autoclaved microisolator caging for the duration of the experiment. In order to maintain their defined-flora status, all husbandry materials (bedding, food, water bottles, etc.) were autoclaved before use; all animal manipulations took place in a hood. The mice were given autoclaved pelleted rodent chow (Agway 3200) and sterile water ad libitum. Mice were housed five per cage. All manipulations performed on the mice were approved by the Institutional Animal Care and Use Committee of the Massachusetts Institute of Technology.

Bacteria. The *H. bilis* type strain (ATCC 51630) was grown under microaerobic conditions in vented jars containing N_2, H_2, and CO_2 (80:10:10) at 37°C on Columbia blood agar plates (Remel Laboratories, Lenexa, Kans.) for 3 days. The bacteria were harvested, resuspended in phosphate-buffered saline (PBS), and visualized by Gram stain and phase microscopy for purity, morphology, and motility. The optical density at 600 nm was adjusted to 0.3, and 0.5 ml of this inoculum ($\sim 10^8$ CFU) was used for each intraperitoneal (i.p.) injection.

Bacterial isolation. Bacteria were cultured from fecal pellets (antemortem) or from ground cecal tissue (necropsy). At scheduled intervals, pooled samples of 3 to 5 fresh fecal pellets were obtained from each cage of mice. Fecal pellets were suspended in 1 ml of sterile PBS, and the resulting slurry was plated directly onto TVP (trimethoprim-vancomycin-polymyxin; Remel Laboratories) or Glaxo (vancomycin-polymyxin-bacitracin-amphotericin B-nalidixic acid [30]) plates for microaerobic isolation of *H. bilis*. Some of the slurry was passed through a 0.45-μm-pore-size filter. Filtered slurry was plated directly onto blood agar plates for microaerobic isolation of *H. bilis*. At necropsy, cecal tissue was obtained aseptically, ground in a tissue grinder, and plated on Glaxo media.

TABLE 1. Results for control and *H. bilis*-infected mouse ceca and colons

Indicator	Results for			
	Control mice		*H. bilis*-infected mice[a]	
	Cecum	Colon	Cecum	Colon
BrdU LI (%)[c]	8.5 ± 4.2 (n = 8[b])	4.3 ± 1.6 (n = 9[b])	21.7 ± 7.1 (n = 15)*	10.8 ± 6.7 (n = 15)***
Maximum crypt length (μm)[c]	170 ± 52 (n = 10)	140 ± 25 (n = 10)	284 ± 104 (n = 15)**	170 ± 44 (n = 15)****
Hyperplasia (no. of mice positive/no. tested)	0/10	0/10	14/15	11/15
Inflammation (no. of mice positive/no. tested)	0/10	0/10	12/15	6/15

[a] *, P = 0.0004 versus control cecum; **, P = 0.001 versus control cecum; ***, P = 0.002 versus control colon; ****, P = 0.037 versus control colon.
[b] Carnoy's-fixed tissue lost in processing.
[c] Data are expressed as means ± SDs.

The altered Schaedler's flora in the Tac:Icr:Ha(ICR)-*scid*fDF mice contained only one organism, a *Lactobacillus* species, that was not a fastidious anaerobe and could grow both aerobically and microaerobically. Like *H. bilis*, the *Lactobacillus* was not inhibited by the antibiotics in TVP agar, though it was excluded by the 0.45-μm-pore-size filter. *H. bilis* was most readily isolated from the Glaxo plates, which contained bacitracin and inhibited the growth of the *Lactobacillus* sp. *H. bilis* colonies were identified by their characteristic spreading morphology; *H. bilis* organisms were gram negative and had characteristic morphology and motility according to examination by phase microscopy (21). The organism was identified by strong catalase, urease, and oxidase reactions and by resistance to nalidixic acid and cephalothin. Although growth of *H. bilis* was generally evident within 5 days, plates were maintained for 3 weeks before a determination of no growth was made. At scheduled intervals, unfiltered fecal slurry was plated directly onto Columbia blood agar plates for aerobic monitoring of the defined flora.

PCR analysis. PCR evaluations of fecal samples and bacterial isolates were used to supplement culture techniques to identify *H. bilis*.

(i) DNA extraction. Bacterial DNA was extracted from fecal pellets, bacterial cultures, or mouse ceca as previously described (21, 37).

(ii) PCR amplification. The *H. bilis*-specific primers C62 (forward primer, AGAACTGCATTTGAAACTACTTT) and C12 (reverse primer, GTATTGCA TCTCTTTGTATGT) amplified a 640-bp product. Between 12 and 18 μl of DNA extract was added to a 100-μl (final volume) reaction tube containing Tth polymerase buffer (Boehringer Mannheim, Indianapolis, Ind.) supplemented with a solution containing 1 mM $MgCl_2$ to a final concentration of 2.75 mM, 0.5 μM (each) the two primers, 200 μM each deoxynucleotide, and 200 μg of bovine serum albumin per ml. Samples were heated at 94°C for 4 min, briefly centrifuged, and cooled to 60°C. Tth polymerase (3.2 U) (Pharmacia, Piscataway, N.J.) and 1.25 U of polymerase enhancer (Perfect Match; Stratagene, La Jolla, Calif.) were added, and then an overlay of 100 μl of mineral oil was added. Amplification took place in a thermal cycler under the following conditions: denaturation at 94°C for 1 min, annealing at 59°C for 2 min, and extension at 72°C for 2 min. A total of 35 cycles were performed, followed by a 4-min extension step. A 15-μl sample was electrophoresed though a 6% Visigel separation matrix (Stratagene) and was visualized by staining with ethidium bromide and viewing by UV illumination.

Immunohistochemistry. (i) In vivo labeling with BrdU. A fresh solution of 5 mg of 5-bromo-2′-deoxyuridine (BrdU) per ml in PBS was made immediately prior to use. Mice were injected i.p. with 50 mg of BrdU per kg of body weight 1 h prior to sacrifice. At necropsy, sections of liver, distal colon, and cecum were fixed in Carnoy's fixative for assessment of proliferation. Tissues were processed within 24 h by standard methods, embedded in paraffin, and sectioned at a thickness of 5 μm.

(ii) Immunohistochemical detection of BrdU staining. Immunohistochemical detection of BrdU incorporation was performed on 5-μm sections and visualized by an avidin-biotin monoclonal antibody immunohistochemical technique as previously described (18). Briefly, sections were deparaffinized, hydrated by a series of xylene-to-70% ethanol baths, exposed to 1% hydrogen peroxide in methanol to inactivate endogenous peroxidase activity, and incubated in 1 M hydrochloric acid at 60°C for 8 min to denature the tissue DNA. The slides were incubated with 5% normal rabbit serum to block nonspecific binding of the primary BrdU antibody. Slides were then incubated with the following series of antibodies and reagents: monoclonal antibody to BrdU (Dako, Glostrup, Denmark), biotinylated antimouse immunoglobulin G (IgG), and peroxidase-conjugated streptavidin. Staining was visualized with diaminobenzidine in accordance with the directions in a commercially available kit (Kirkegaard & Perry Laboratories, Gaithersburg, Md.). Slides were washed between incubations in Tris-buffered saline. Control slides were included in each assay; these slides were incubated without the anti-BrdU antibody. Slides were counterstained with hematoxylin. BrdU was used to stain the nuclei of cells in the S phase of the cell cycle; these nuclei appeared to be brown on a blue background.

(iii) Quantitation of proliferation. Proliferation was expressed as labeling index (LI), the percentage of BrdU-stained cells divided by the total cells in the crypt. LI was calculated by counting each cell in each adequately aligned crypt in a given section of cecum or colon and noting whether the nucleus was stained with BrdU. Crypts were deemed adequately aligned if they were sectioned longitudinally in a single plane such that all cells in the crypt could be counted. For each mouse, all of the crypts in one section (a minimum of 10 well-oriented crypts) were counted; if there were fewer than 10 adequately aligned crypts in a tissue section, a second slide cut from the same tissue block was counted. The cells in each crypt were counted, and the location of each BrdU-stained cell was noted.

Immunofluorescence. Sections of ceca from *H. bilis*-infected mice were embedded in mounting media, snap frozen in liquid nitrogen, and sectioned at 7 μm. Slides were blocked for 30 min with Cas Block (Zymed, San Francisco, Calif.) and then incubated for 1 h with a 1:75 dilution of fluorescein isothiocyanate-conjugated anti-CD11b (Mac-1; Pharmacia). Slides were rinsed in PBS and mounted with aqueous mounting media (Dako, Carpinteria, Calif.) and viewed by fluorescent microscopy. Negative controls were incubated with streptavidin-fluorescein isothiocyanate (Zymed) in place of the primary antibody. Positive-staining cells were identified by characteristic apple green fluorescence.

Histology. Sections of liver and gastrointestinal tissues were fixed in neutral buffered 10% formalin and in Carnoy's fixative. Only Carnoy's-fixed tissues were used for immunohistochemistry. Formalin-fixed tissues were embedded in paraffin, sectioned at 5 μm, and stained with Warthin-Starry silver stain to visualize bacteria in tissues. Hematoxylin and eosin (H&E) stain was used to stain 5-μm sections of tissues fixed in both types of fixative for assessment of histopathology.

Statistics and analysis. Statistics reported in Table 1 and throughout the text are based on a two-tailed Student t test. The Student t test for equal variances or unequal variances was used, depending on the result of an F test of the data (used to determine whether variances were equal).

Experimental design. Weanling mice were cultured aerobically and microaerobically upon arrival to ensure that they had no *Helicobacter* spp. and no contaminants to their defined flora. They were randomly assigned to control or *H. bilis* inoculation groups. After 1 week, the 4-week-old mice were inoculated i.p. with 0.5 ml of *H. bilis*-containing PBS (15 mice) or sterile PBS (10 sham-dosed control mice). Fresh fecal samples (a pooled sample of five pellets from each cage) were obtained at 1-week intervals and were used for culture (weeks 1 and 2 postinoculation) or saved at −70°C for PCR. At 7 weeks postinoculation, mice were necropsied. Samples were taken for histopathology and assessment of proliferation as described above. Cecal tissue samples from each mouse were used for *H. bilis* culture. Samples were saved at −70°C for PCR.

RESULTS

Colonization by *H. bilis*. The pooled fecal samples from the infected cages were PCR positive for *H. bilis* at 1 week postinoculation and culture positive at 2 weeks postinoculation. Positive cultures were identified by characteristic spreading colony morphology; strong oxidase, urease, and catalase reactions; gram-negative staining; characteristic morphology and motility by phase-contrast microscopy; and resistance to nalidixic acid and cephalothin (21). All 15 *H. bilis*-inoculated animals were culture positive at necropsy. The sham-dosed animals remained negative for *H. bilis* by PCR and culture for the duration of the study and were negative for *H. bilis* at necropsy. Aerobic culture of feces confirmed that all mice in both groups maintained their defined-flora status for the duration of the study.

Clinical and gross findings. Three of the *H. bilis*-infected mice developed mild diarrhea, which did not appear to affect

their growth. There was no significant difference between the weights of control (31.0 ± 4.1 g) (mean ± standard deviation [SD]) and *H. bilis*-infected (29.0 ± 4.3 g) mice. At necropsy, one control mouse was found to have an unusually long cecum (3 cm) which was neither thickened nor dilated. Three of the *H. bilis*-infected mice had no formed feces in their distal colons at necropsy. One of the *H. bilis*-infected mice had a mild corneal scar.

Histopathology. No lesions were found in the gastrointestinal sections of any of the control mice. The unusually long cecum that was found in one control mouse had no histological abnormalities. In contrast, all of the *H. bilis*-infected mice had histological evidence of IBD, including one or more of the following types of lesions: proliferative typhlitis, proliferative colitis, cecal hyperplasia, and colonic hyperplasia. Mild to moderate proliferative typhlitis was the most prominent feature. Cecal hyperplasia, seen in all but one of the *H. bilis*-inoculated mice, was characterized by focal areas of increased crypt length (approximately twofold longer than normal crypts), with increased mucosal epithelial cell hyperchromicity and crypt cell density (Fig. 1). Cecal hyperplasia was most apparent in the ileo-cecal junction area. Mild to moderate multifocal typhlitis was observed in 80% of the *H. bilis*-inoculated mice (Table 1). Cecal inflammation was characterized by submucosal edema and mucosal cellular infiltration consisting primarily of mononuclear cells with neutrophils and occasional eosinophils. The multifocal inflammatory infiltrate surrounded cecal crypts and was accompanied by epithelial hyperplasia. Crypt abscesses, suppurative exudates, and inflammatory infiltration through the mucosa and the hyperplastic epithelium were observed in the most severely affected mice (Fig. 1). Mononuclear cell inflammation in the infected mice typically comprised cells morphologically characterized as macrophages, based on the observation of moderate to abundant eosinophilic cytoplasm with indistinct borders and large oval or indented nuclei with vesicular chromatin. The inflammatory infiltrate also contained a small proportion of small mononuclear cells and granulocytes. In comparison, infrequent small, discrete aggregates of macrophages and small mononuclear cells resembling lymphocytes, characterized by round, dense basophilic nuclei and scant cytoplasm, were observed in control mice. The lymphoid cells were not characterized; however, the lack of associated epithelial changes suggests that these aggregates may represent poorly formed gut-associated lymphoid tissue.

Colonic hyperplasia and colitis were also observed (Fig. 2). Focal to segmental areas of mild to moderate colonic hyperplasia (crypt lengths were increased approximately 1.5-fold) were observed in the distal colons of 75% of the *H. bilis*-inoculated mice (Fig. 2 and Table 1). Mild to moderate colitis was observed in approximately half of the *H. bilis*-inoculated mice; the colonic inflammatory infiltrate consisted predominantly of mononuclear cells that were similar in appearance to those described for the cecal infiltrates (Fig. 2). Sections of liver, stomach, ileum, jejunum, and duodenum were histologically normal. Warthin-Starry–stained sections (not shown) revealed organisms consistent with *H. bilis* in the mucosal crypts of the cecum. No organisms were seen in the liver.

BrdU immunohistochemistry. BrdU staining was used to obtain semiquantitative estimates of cell proliferation in cecal and colonic crypts. The LI in the ceca and colons of *H. bilis*-infected mice was significantly increased compared to that of control mice (Table 1). Mice with BrdU LIs lower than the mean plus 1 SD of the value for the controls were considered not to be proliferative; by this standard, 14 of 15 *H. bilis*-infected mice were considered to exhibit cecal proliferation,

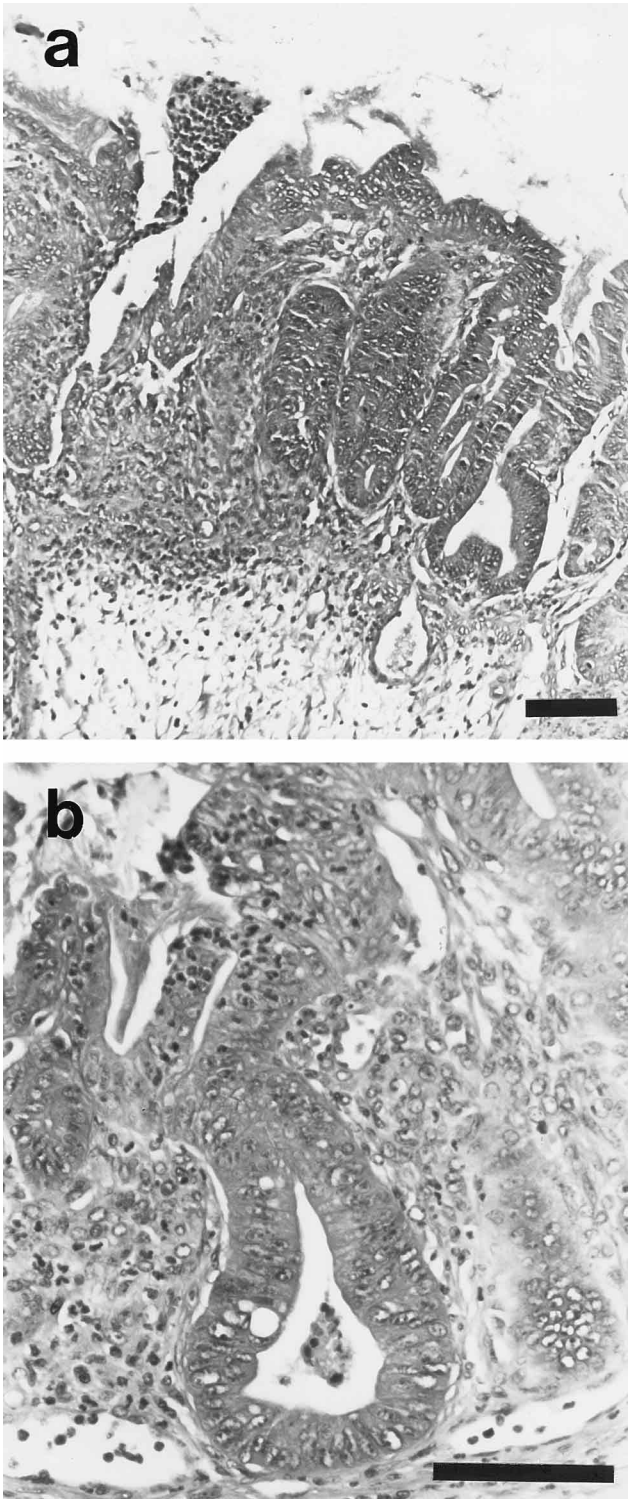

FIG. 1. Histopathology of *H. bilis*-infected mouse ceca. H&E-stained sections. (a) Cecal hyperplasia is evident as increased crypt length and cell crowding and hyperchromicity of the crypt epithelial cells. An inflammatory infiltrate consisting mainly of mononuclear cells and neutrophils is present in the lamina propria and submucosa. An area of cecal epithelial erosion with a suppurative exudate is present. Bar = 200 μm. (b) Sloughed cellular debris is present in a dilated cecal crypt. A mixed population of mononuclear cells and neutrophils has infiltrated the lamina propria and the hyperplastic epithelium. Bar = 100 μm. (Control mouse tissue is shown in Fig. 3a.)

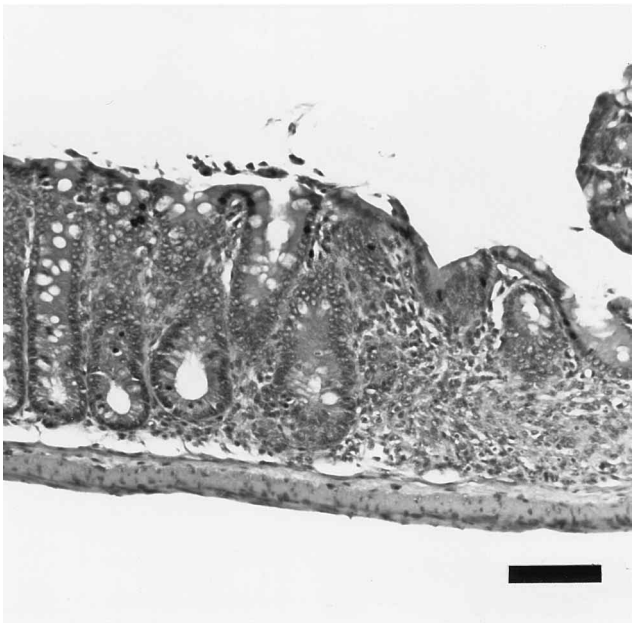

FIG. 2. Histopathology of *H. bilis*-infected mouse colon. H&E-stained section. Colonic hyperplasia is evident as increased crypt length and cell crowding and hyperchromicity of the crypt epithelial cells. An inflammatory infiltrate consisting mainly of mononuclear cells and neutrophils is present in the lamina propria and submucosa. Bar = 100 μm. (Control mouse tissue is displayed in Fig. 3c.)

and 11 of 15 *H. bilis*-infected mice were considered to exhibit colonic proliferation. BrdU staining revealed a 2.5-fold increase in the LI of the ceca and colons of *H. bilis*-infected mice compared to that of control mice (Table 1 and Fig. 3).

Mac-1 immunofluorescence. The ceca of selected *H. bilis*-infected mice were examined in order to augment the morphological characterization of the inflammatory infiltrate. Mac-1, which specifically binds to CD11b and stains macrophages and NK cells, stained cells corresponding to the histiocytic inflammatory infiltrate (not shown); however, we cannot rule out the possibility that some of the fluorescent cells were NK cells.

DISCUSSION

The results of this study show that experimental infection with the type strain of *H. bilis* can cause proliferative typhlocolitis in outbred *scid* mice with defined flora. Although *H. bilis* has been reported to occur in association with liver lesions in aged inbred mice (21), this study is the first to report *H. bilis* causing proliferative typhlitis and diarrhea in mice. *H. bilis* is found widely in conventional mouse colonies, which may be of real clinical concern and may interfere significantly with research. We also suggest that *H. bilis* infection of *scid* mice might be a good model for the investigation of bacterium-related IBD.

Although *H. bilis* has previously been isolated from aged mice with hepatic lesions, the *H. bilis*-infected *scid* mice in this study developed lesions limited to mild to moderate proliferative typhlitis and colitis. Several factors, including strain and sex, have been shown to modulate the severity of helicobacter-induced disease (22). We have previously reported an outbreak of severe diarrhea and proliferative necrotizing ulcerative typhlitis associated with a dual infection of *H. bilis* and *H. rodentium* in inbred (CB.17) *scid* mice (39). We used outbred ICR *scid* mice, which may be more resistant to disease than inbred CB.17 *scid* mice. Also, we used only female mice to avoid problems with aggressive behavior in group-housed males; however, male mice of some strains are reported to have more severe manifestations of *H. hepaticus*-induced disease (19, 22). In addition, more severe lesions or hepatic lesions similar to those occurring in *H. hepaticus*-infected mice (19) may require longer infection times to develop. Finally, bacterium-specific factors may be important determinants of the severity of disease. For instance, strains of *H. pylori* that elaborate vacuolating cytotoxin have been reported to be more pathogenic than strains without the cytotoxin (11, 42). We report here on the type strain of *H. bilis*; it remains to be determined whether there are strains that are more or less pathogenic.

The ability to cause proliferation and inflammation in gastrointestinal mucosae may be common to several helicobacter species. In addition to the examples of proliferative bowel disease cited above, proliferation of gastric crypts occurs in infections of cats and humans with *H. pylori* (4, 14), mice with *H. felis* (18), and ferrets with *H. mustelae* (15). One factor previously proposed to play a role in the proliferation and inflammation is urease, which is produced by the gastric helicobacter species and by all *H. hepaticus* and *H. bilis* strains. Urease may cause tissue injury by releasing ammonia (1). *H. bilis* has recently been reported to contain the gene encoding VacA (3), the vacuolating cytotoxin produced by some strains of *H. pylori*, and the *cag* gene (3), associated with a pathogenicity island found in some strains of *H. pylori* (8), *H. mustelae* (2), and *H. hepaticus* (29). It is not known whether *H. bilis* produces the granulating cytotoxin found in *H. hepaticus* and some strains of *H. pylori* (41). Further work to identify determinants of pathogenicity should include experimental infection studies to determine the pathogenic potential of urease-negative murine intestinal helicobacters, including *H. rodentium* (38), which was implicated previously in a clinical outbreak of severe typhlitis and colitis at our institution (39).

It has been hypothesized that some human IBD is triggered by bacteria and that IBD is a poorly modulated response to bacterial insult (9, 10, 33). There is even evidence that intestinal helicobacters may play a role in human IBD; *Helicobacter cinaedi* (an intestinal helicobacter of hamsters) (23) and *Helicobacter fennelliae* were first identified as agents causing IBD in immunocompromised humans (43). In this case, the bacterium *H. bilis*, which is part of the normal flora of many mice (21), produced mild to moderate IBD in outbred *scid* mice. One major advantage of this defined-flora *scid* mouse model is that it allows the study of the effects of a specific agent, e.g., *H. bilis*, in isolation from the effects of other pathogens. Powrie et al. reported that while reconstitution of CB.17 *scid* mice with a subset of $CD4^+$ T cells expressing high levels of CD45RB ($CD45RB^{high}$) alone causes IBD, reconstitution with $CD45RB^{low}$ T cells or with a mixed population of both $CD45RB^{high}$ and $CD45RB^{low}$ T cells does not (24, 34, 35). In contrast, Rudolphi et al. reported that reconstituting CB.17 mice with unfractionated $CD4^+$ T cells (a population containing both $CD45RB^{high}$ and $CD45RB^{low}$ cells) does cause IBD (36). The helicobacter status and microflora of the mice used in these two studies were not known, so the different results in these two studies could be due to differing microbial flora. Future work to understand the pathogenesis of bacterium-related IBD could include reconstituting *H. bilis*-infected *scid* mice with $CD45RB^{high}$ cells to see if the IBD is exacerbated or reconstituting *H. bilis*-infected *scid* mice with $CD45RB^{low}$ or mixed cells to see if the response is immunomodulated.

As noted in these previous studies, a major advantage of *scid* mouse models is that specific parts of the immune response may be studied in isolation. *scid* mice are deficient in B and T cells, and the repertoire of inflammatory cells is thus likely

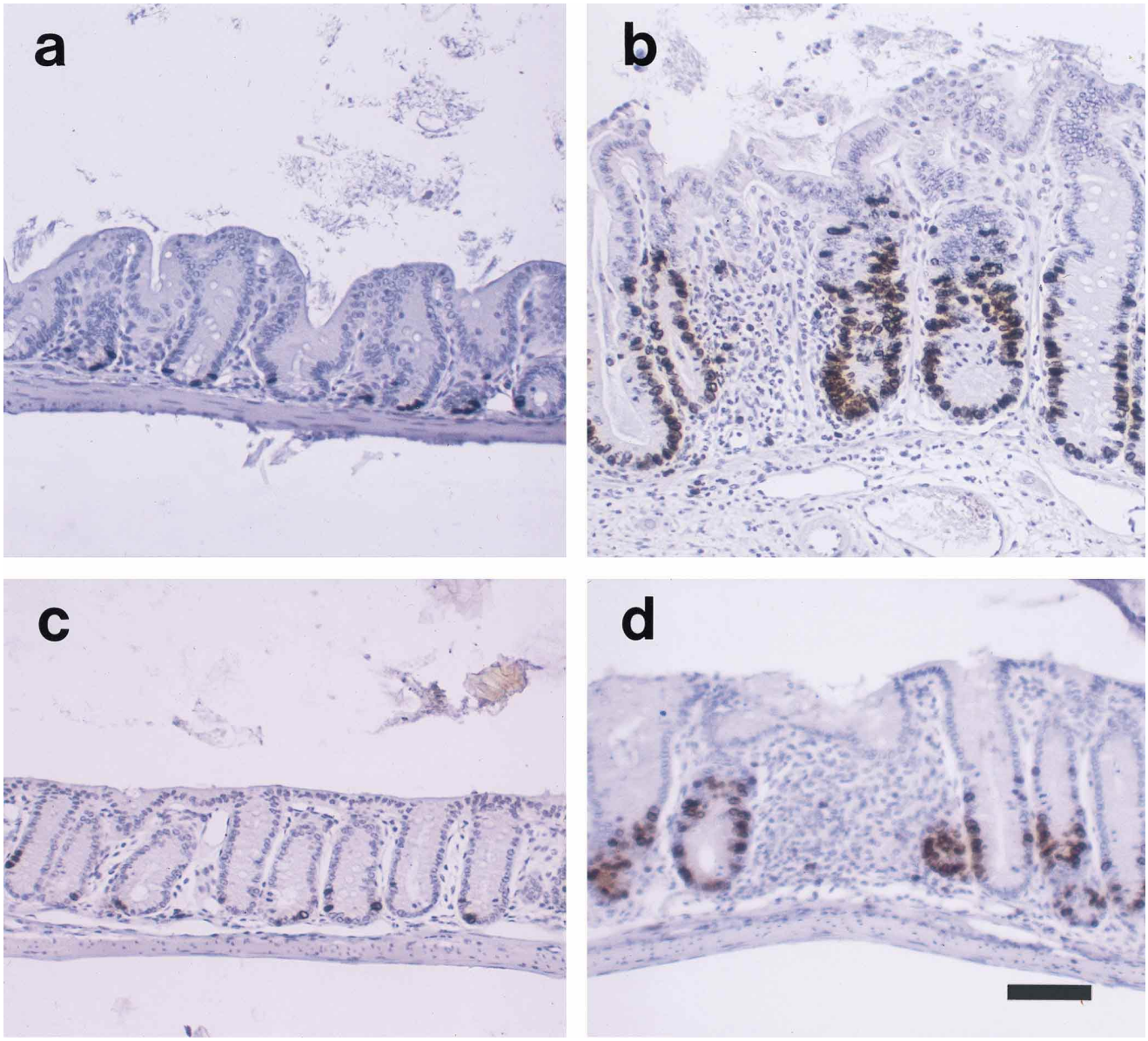

FIG. 3. BrdU immunohistochemistry of control and *H. bilis*-infected mouse ceca and distal colons. BrdU-stained sections of (a) control cecum; (b) *H. bilis*-infected cecum; (c) control colon; and (d) *H. bilis*-infected colon. BrdU labels the nuclei in the S phase of cell division. Labeled cell nuclei appear to be brown. Proliferation is evident in panels b and d as an increased number of BrdU-stained cells per crypt and a higher LI. Bar = 100 μm.

limited to macrophages, NK cells, and granulocytes. Our observation of inflammatory infiltrates, largely comprising macrophages in the *H. bilis*-infected mice described in this report, is consistent with this assumption. Macrophages are also prominent in the early development of IBD in *scid* mice reconstituted with CD45RB^{high} T cells (24, 34, 35). Macrophages have been speculated to play a role in *H. pylori*-related disease in humans; inducible nitric oxide synthase (iNOS) is increased in the macrophages of humans infected with *H. pylori* and may play an important role in the production of carcinogenic nitrosamine compounds in the stomachs of *H. pylori*-infected humans (27). Expression of iNOS is also increased in the colonic epithelium in humans with IBD (40). Thus, the *scid* mouse model allows the study of the role of iNOS and macrophages in bacterium-mediated inflammation of the gastrointestinal tract.

It should be noted that the roles of other mononuclear inflammatory cell populations in the *scid* model merit further evaluation in future studies. NK cells as well as T and B cell activity arising from residual immunocompetent activity ("leakiness") may modulate the course of IBD activity. With regard to the latter phenomenon of residual immune activity in *scid* mice, while many CB.17 *scid* mice develop detectable serum Ig levels ("leak"), <1% of ICR *scid* mice have any detectable level of serum Ig (32). C3H *scid* mice are also less leaky than CB17 mice; less than 15% have detectable serum Ig, and none have B or T cells detectable by flow cytometry, in contrast to CB.17 *scid* mice, in whom B and T cells are occasionally detected (31). Thus, we presume that the ICR *scid* mice used in this study, which are even less leaky than the C3H *scid* mice, would have no detectable B or T cells.

Intestinal helicobacters may play an important role as fac-

tors or cofactors in the development of IBD in many species. It is particularly important to consider *H. bilis* and other murine helicobacters as potential pathogens. Some "spontaneous" genetic mouse models of IBD may now be attributable to helicobacter infection (12), and some long-term toxicology studies have been compromised by the helicobacter infection of control mice (19, 44, 46). In addition, there is some evidence that intestinal helicobacters such as *H. cinaedi* and *H. fennelliae* may indeed play a primary role in the development of human IBD (43). Thus, the *H. bilis*-infected *scid* mouse with defined flora may be a valuable model for bacterium-mediated IBD.

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