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Helicobacter hepaticus Infection Triggers Inflammatory Bowel Disease in T Cell Receptor αβ Mutant Mice

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Abstract | The T cell receptor alpha chain-deficient (TCR α-/-) and TCR beta chain-deficient (TCR β-/-) mice develop chronic intestinal inflammation that resembles inflammatory bowel disease by 3 to 4 months of age. The objective of the study reported here was to determine the role of infection with the bacterial pathogen *Helicobacter hepaticus* in the pathogenesis of disease in TCR αβ mutant mice. The *H. hepaticus*-infected TCR αβ mutant mice were rederived by use of embryo transfer to produce *Helicobacter*-free animals. *Helicobacter*-free TCR α-/-, TCR β-/-, and TCR α-/-×β-/- mice were inoculated with *H. hepaticus*. Experimentally infected mice and uninfected control mice were examined for intestinal lesions at 3, 6, and 9 months after inoculation. The TCR αβ mutant mice inoculated with *H. hepaticus* developed intestinal epithelial cell hyperplasia and mucosal inflammation. By 6 months after inoculation, infected animals had moderate cecal and colonic lesions. *Helicobacter*-free TCR α-/- mice, but not TCR β-/- or TCR α-/-×β-/- mice, also developed *H. hepaticus*-independent colitis by 9 months after inoculation. Infection with *H. hepaticus* is sufficient to cause chronic proliferative intestinal inflammation in TCR αβ mutant mice. However, *H. hepaticus* infection is not necessary for intestinal disease in TCR α-/- mice.

Inflammatory bowel disease (IBD) comprises a group of idiopathic disorders characterized by chronic inflammation of the large bowel (ulcerative colitis) or any part of the gastrointestinal tract (Crohn's disease). A number of IBD models have been described in mice with altered immune function, including interleukin-2 deficient (IL-$2^{-/-}$), IL-$10^{-/-}$, or T cell receptor alpha chain-deficient (TCR $\alpha^{-/-}$) or TCR beta chain-deficient (TCR $\beta^{-/-}$) mice (1–3). TCR $\alpha^{-/-}$ or TCR $\beta^{-/-}$ mice develop colitis by 3 to 4 months of age, and exhibit chronic diarrhea, wasting, anorectal prolapse, and intestinal lesions characterized by thickening of the colon, cecum, and rectum. Substantial mortality occurs between 6 months and 1 year of age. Because TCR αβ mutant mice were initially housed in a "pathogen free" environment where sentinel mice were consistently seronegative and/or culture negative for known pathogenic bacteria, parasites, and viruses, it was assumed that infectious agents did not contribute to disease pathogenesis (3). Subsequently, a newly identified pathogen, *Helicobacter hepaticus*, was found to enzootically infect multiple lines of genetically altered mice exhibiting rectal prolapse, including TCR $\alpha^{-/-}$ and TCR $\beta^{-/-}$ mice (4), which suggested a role for infection with *H. hepaticus* in intestinal disease.

Because the germ-free state protects IL-$2^{-/-}$, IL-$10^{-/-}$, and TCR $\alpha^{-/-}$ mice from IBD (1, 5, 6), it has been postulated that disease pathogenesis involves an abnormal and uncontrolled immune response to normal gut constituents, particularly resident microbiota. However, ex-germ-free TCR $\alpha^{-/-}$ mice colonized with a limited microbiota consisting of *Lactobacillus plantarum*, *Streptococcus faecalis*, *S. faecium* and *Escherichia coli*, also are protected from disease (6). These results indicate that TCR $\alpha^{-/-}$ mice do not develop IBD spontaneously, and are consistent with the idea that the pathogenesis of intestinal disease is not a consequence of luminal microbiota per se, but may result from infection with a specific pathogen or group of pathogens.

Helicobacter hepaticus is a gram-negative, urease-producing murine pathogen, which was first isolated from the livers of mice with chronic-active hepatitis (7). This organism is related to *H. pylori*, which is known to cause chronic gastric inflammation in humans and is linked to development of peptic ulcer disease and gastric cancer (8, 9). *Helicobacter hepaticus* has been found to infect many strains of immunocompetent mice in commercial and academic mouse colonies (10). The primary site of colonization for *H. hepaticus* appears to be the cecum and colon. In strains of mice susceptible to hepatitis, *H. hepaticus* can be isolated from the liver as well as from the distal portion of the bowel of infected animals (7). *Helicobacter hepaticus*-associated hepatitis is linked to the development of liver cancer in male A/JCr and B6C3F1 mice (11, 12). There is a high prevalence of *H. hepaticus* in strains of mice predisposed to IBD, and natural infection with the organism is associated with IBD in *scid* and nude mice (13, 14). Furthermore, experimentally induced infection with *H. hepaticus* is sufficient for the expression of IBD in defined flora *scid* mice reconstituted with $CD4^+$ $CD45RB^{high}$ T cells (15). More recently, RAG-$2^{-/-}$ mice exposed to *H. hepaticus*, and IL-$10^{-/-}$ mice experimentally infected with *H. hepaticus* have been documented to develop IBD (16, 17). Infection with a novel urease-negative *Helicobacter* species, provisionally designated "*H. typhlonicus*," in IL-$10^{-/-}$ and *scid* mice also has been shown to be associated with the development of colitis and typhlitis (18, 19). These data indicate that infection with *Helicobacter* species, especially *H. hepaticus*, can cause IBD in susceptible lines of genetically altered mice.

The purpose of the study reported here was to test the hypothesis that infection with a single bacterial pathogen is suffi-

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cient to induce IBD in TCR αβ mutant mice. We now report that experimentally induced infection with *H. hepaticus* causes IBD in TCR αβ mutant mice.

Materials and Methods

Animals: The TCR $\alpha^{-/-}$ and TCR $\beta^{-/-}$ mice on a C57BL/6 × 129-Ola background were a gift from Dr. Susumu Tonegawa. Control and experimentally infected mice were housed in two separate 80-ft^2 cubicles under negative pressure within an AAALAC-approved barrier facility under environmental conditions of 22°C, 40 to 70% humidity, 15 air changes/h, and a 12:12-hour light:dark cycle. Animals were housed in polycarbonate microisolator cages (7.5 × 11.5 × 5 in.) with filter tops. The bedding was composed of heat-treated hardwood chips (Sanichips, P. J. Murphy Inc., Montville, NJ). Mice were fed a pelleted diet (RMH3000, Purina Mills Inc., Richmond, IN) and water (produced by reverse-phase osmosis) ad libitum. Sentinel animals consistently tested seronegative for a standard panel of viral agents (4) and culture negative for respiratory pathogens, *Salmonella* species, and *Citrobacter rodentium*. In addition, sentinel animals were also consistently test negative for ectoparasites and endoparasites. All animal experiments were approved by the MIT Animal Care and Use Committee.

Genotyping: The DNA from tail tissue was isolated, using a GenomicPrep Cell and Tissue DNA Isolation kit (Amersham Pharmacia Biotech Inc., Piscataway, NJ) according to the recommendations of the manufacturer. Primers used to genotype the mice were complementary to sequences in the TCR α (5'-T G T T C A C C G A C T T T G A C T C C-3' and 5'-T G G C G T T G G T C T C T T T G A A G- 3'), TCR β (5'-T G A G A A A T G T G A C T C C A C C C-3' and 5'-C T G C T C A G G C A G T A G C A T A-3'), or the *pgk-neo* locus (5'-C T T G G G T G G A G A G G C T A T T C-3' and 5'-A G G T G A G A T G A C A G G A G A T C-3'), and were designed to specifically amplify either the wild-type or mutant allele, producing products of 200, 214, and 280 bp, respectively. Reactions consisted of 20 pmol of each primer, 200 μ*M* each dNTP, approximately 200 ng of template DNA, and 1 U of *Taq* DNA polymerase (Promega Corp., Madison, WI). Polymerase chain reaction analysis was performed at 94°C for 2 minutes, followed by 35 cycles of 94°C for 1 minute, 58°C for 1 minute, and 72°C for 1 minute, followed by a final extension of 7 minutes at 72°C. A tenth of each reaction product was separated by electrophoresis on a 2% agarose gel for analysis.

Embryo transfer rederivation: Embryo transfer was performed as described (20). Superovulation of donor females was accomplished by i.p. administration of 5 IU of pregnant mares' serum (Calbiochem, La Jolla, CA), followed 48 hours later with 5 IU of human chorionic gonadotropin (Organon, West Orange, NY), at which time the female mice were placed with fertile males. The next morning, females were checked for copulatory plugs. Three days later, mice with plugs were euthanized by CO_2 asphyxiation. Embryos were collected from the uterus and maintained at room temperature until implantation. Pseudopregnant Swiss Webster recipient female mice (Taconic, Germantown, NY) were anesthetized with 2.5% 2,2,2- tribromoethyl alcohol, tert-amyl alcohol (Aldrich, Milwaukee, WI; 0.015 ml/g of body weight, i.p.) for intrauterine implantation of the embryos. Recipient females were then allowed to carry the litters to term. The *Helicobacter* status of the animals was established on the basis of culture and PCR results.

Culture and PCR identification of H. hepaticus: Fecal pellets and mucosal scrapings from individual animals were processed for culture and isolation as described (10). The DNA was isolated from cultured bacteria and from cecal tissue as described (21). A modification of this method was used to isolate DNA from feces. Briefly, a single fecal pellet was suspended in 1.5 ml of phosphate-buffered saline (PBS) and was centrifuged at 700 ×*g* for 5 minutes. One hundred microliters of supernatant was diluted with an equal volume of PBS before being processed as described (21). A sensitive and specific PCR assay for determining the *Helicobacter* status of mice was performed as described, using *H. hepaticus*-specific primers B38 and B39 that produce a 417-bp product (10), or generic *all-Helicobacter* primers C97 and C98 that produce a 398-bp product (22).

Experimental design: *Helicobacter*-free TCR αβ mutant mice were randomized to remain as uninoculated controls or to be experimentally inoculated with *H. hepaticus* (Table 1). Between 2 and 3 months of age, mice in the experimentally inoculated group received approximately 10^8 colony-forming units of *H. hepaticus* (ATCC 51448) by oral gavage, 3 times, 48 hours apart in a volume of approximately 0.3 ml. Inocula were grown in a microaerobic environment at 37°C for 3 to 4 days on tryptic soy agar supplemented with 5% sheep blood, 20 μg of cefoperazone/ml, 10 μg of vancomycin/ml, and 2 μg of amphotericin B/ml, then were resuspended in tryptic soy broth. The inocula were assessed by use of phase-contrast microscopy to ensure adequate motility with few coccoid forms, and by use of Gram staining and aerobic and microaerobic culture to document absence of contaminating microorganisms. All animals were monitored daily for diarrhea, rectal prolapse, and poor body condition.

Necropsy, histopathologic examination, and immunohistochemical analysis: At 3, 6 or 9 months after inoculation, animals were euthanized by CO_2 asphyxiation. One hour prior to euthanasia, each mouse received a single injection of bromodeoxyuridine (BrdU; 50 mg/kg of body weight, i.p.) as described (15). Fecal pellets were collected for culture and PCR analysis. The cecum was divided into 3 sections to be processed for PCR analysis (apex), culture (middle), and histologic and immunohistochemical analyses (base with ileocecal junction). The base of the cecum, the ileocecal junction, and the entire colon and rectum were placed in Carnoy's fixative, processed in routine manner, and embedded in paraffin. Blocks were sectioned at 4-μm thickness and stained with hematoxylin and eosin. Slides were viewed by a veterinary pathologist (CAD) in blinded fashion and scored for goblet cell depletion, epithelial cell hyperplasia, cellular infiltration, and fibrosis. Cecal and colonic tissues were graded on a scale from 0 to 4 (none, minimal, mild, moderate, and severe, respectively) for hyperplasia and inflammation. Immunohistochemical analysis for BrdU was performed as described (15). Proliferation was assessed in the distal and proximal portions of the colon and the cecum. Labeling index values were determined by counting 10 well-oriented crypts from each of these regions, and were expressed as the percentage of labeled cells per total cells in the crypt.

Statistical analysis: Labeling index values were analyzed, using a paired *t*-test, and categorical lesion scores were analyzed, using the Mann-Whitney test, both at a significance level of $P < 0.05$.

Results

Rederivation of TCR αβ mutant mice: To test the hypoth-

Table 1. Design of *Helicobacter hepaticus* inoculation studies in T cell receptor (TCR) αβ mutant mice

	3 Months*	6 Months	9 Months
Uninoculated control mice†			
TCR $\alpha^{-/-}$	ND	ND	6
TCR $\beta^{-/-}$	2	5	5
TCR $\alpha^{-/-}\times\beta^{-/-}$	2	10	ND
H. hepaticus-infected mice†			
TCR $\alpha^{-/-}$	ND	ND	7
TCR $\beta^{-/-}$	2	7	5
TCR $\alpha^{-/-}\times\beta^{-/-}$	5	8	ND

*Number of animals euthanized at each time point.
†Prior to the beginning of the study, all mice were test negative for infection with *Helicobacter* species on the basis of fecal polymerase chain reaction analysis. Mice were tested for infection with *H. hepaticus* 1 week after inoculation and at the time of necropsy.
ND = Not done.

esis that infection with *H. hepaticus* is sufficient to cause IBD in TCR αβ mutant mice, we rederived *Helicobacter*-free animals. After embryo transfer rederivation into *Helicobacter*-free Swiss Webster recipient mice, all offspring (10 animals) were tested for *Helicobacter* infection. These mice were then interbred, and half of their progeny (25 animals) were similarly tested. *Helicobacter* species were not detected by use of PCR analysis or culture, confirming that the embryo transfer procedure was successful. None of the rederived mice developed clinical signs of IBD through 6 months of age. At 1 year of age, four of >100 mice (all TCR $\alpha^{-/-}$) developed rectal prolapse. These animals were also free of *Helicobacter* infection, as judged on the basis of PCR analysis and culture results. To generate animals for the experimentally induced infection studies, the *Helicobacter*-free TCR αβ mutant mice were interbred.

Experimentally induced infection of TCR αβ mutant mice with *H. hepaticus*: A total of 34 mice were experimentally inoculated with *H. hepaticus*. Thirty age-matched uninoculated animals served as controls (Table 1). All uninoculated control mice remained free of *Helicobacter* infection throughout the duration of the experiment. Experimentally inoculated animals became infected within one week of oral gavage, and remained infected throughout the duration of the experiment, as determined by fecal PCR amplification. At necropsy, culture and PCR amplification of fecal and cecal DNA samples gave concordant results for each of the uninoculated control and the *H. hepaticus*-infected animals. During the course of the experiment, none of the animals exhibited ≥ 20% loss of body weight and mortality was not noted.

Microscopic lesions: *H. hepaticus* infection was consistently and significantly associated with moderate to marked proliferative typhlitis in all TCR αβ mutant mice tested at each time point (Figure 1). *H. hepaticus* infection was significantly associated with intense proliferative colitis in TCR $\beta^{-/-}$ mice at 6 months after inoculation (Figure 2) and 9 months after inoculation (Figure 3), and in TCR $\alpha^{-/-} \times \beta^{-/-}$ mice at 6 months after inoculation (Figure 4). The TCR $\alpha^{-/-}$ mice infected with *H. hepaticus* also consistently developed marked proliferative colitis; however, *H. hepaticus*-independent colitis, which was often of marked intensity, was also seen at 9 months after inoculation (Figure 5).

Three months after inoculation: *H. hepaticus* infection in TCR $\beta^{-/-}$ and TCR $\alpha^{-/-} \times \beta^{-/-}$ mice was significantly associated with moderate to marked proliferative typhlitis (data not shown). In the absence of *H. hepaticus* infection, inflammation and hyperplasia were typically limited. At this time point, *H. hepaticus*-associated lesions were polarized to the cecum. However, median scores for colonic hyperplasia also were increased in

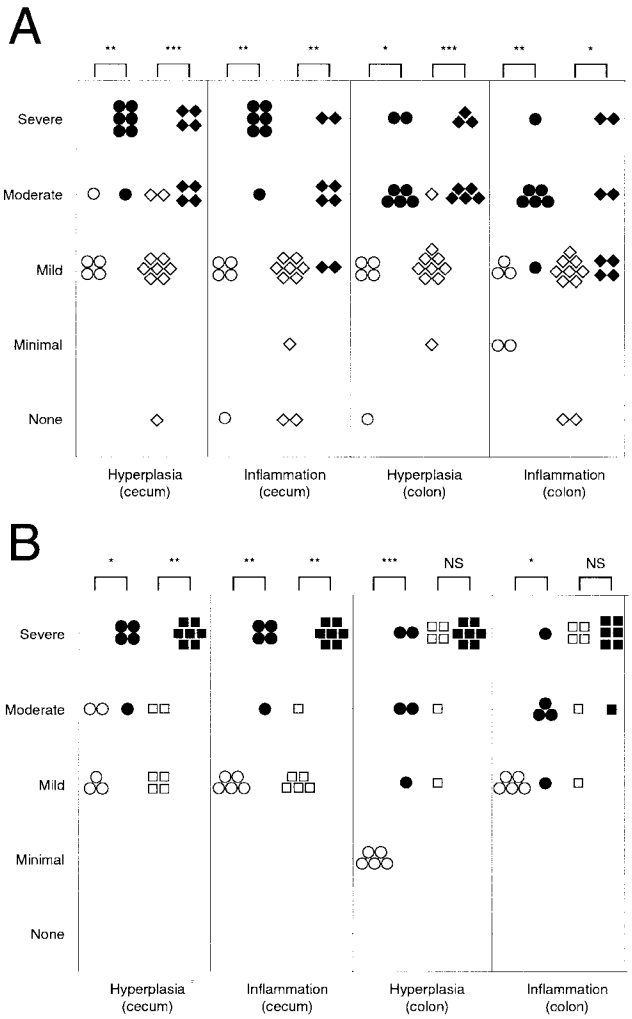

Figure 1. Lesion scores for uninoculated control and *Helicobacter hepaticus*-infected T cell receptor (TCR) αβ mutant mice. **(A)** control ❍ and infected ● TCR $\beta^{-/-}$ mice, and control ◇ and infected ◆ TCR $\alpha^{-/-}\times\beta^{-/-}$ mice 6 months after inoculation. **(B)** control ❍ and infected ● TCR $\beta^{-/-}$mice, and control □ and infected ■ TCR $\alpha^{-/-}$ mice 9 months after inoculation. The *P* values are expressed as < 0.05 (*), < 0.01 (**), < 0.001 (***), or not significant (NS).

H. hepaticus-infected mice, typically in association with mild inflammation. Lesions in the cecum, although often of marked intensity, tended to be restricted to discrete foci of hyperplastic mucosal epithelium, with local infiltration consisting predominantly of lymphocytes and macrophages.

Six months after inoculation: In TCR $\beta^{-/-}$ and TCR $\alpha^{-/-}\times\beta^{-/-}$ mice, *H. hepaticus*-associated IBD lesions were more extensive, and lesion foci were more extensive and tended to coalesce. Colonic lesion scores were significantly increased in TCR $\beta^{-/-}$ and TCR $\alpha^{-/-} \times \beta^{-/-}$ mice (Figure 1). However, lesions were typically discrete and multifocal. In several TCR $\beta^{-/-}$ mice, *H. hepaticus*-associated colitis involved the distal portion of the colon, manifesting as an intense proctitis. Although TCR $\beta^{-/-}$ and TCR $\alpha^{-/-}\times\beta^{-/-}$ mice developed moderate to severe IBD in response to *H. hepaticus* infection, cecal inflammation scores were significantly higher as a group in TCR $\beta^{-/-}$ mice ($P = 0.0247$).

Nine months after inoculation: *H. hepaticus* infection was significantly associated with extensive, moderate to marked

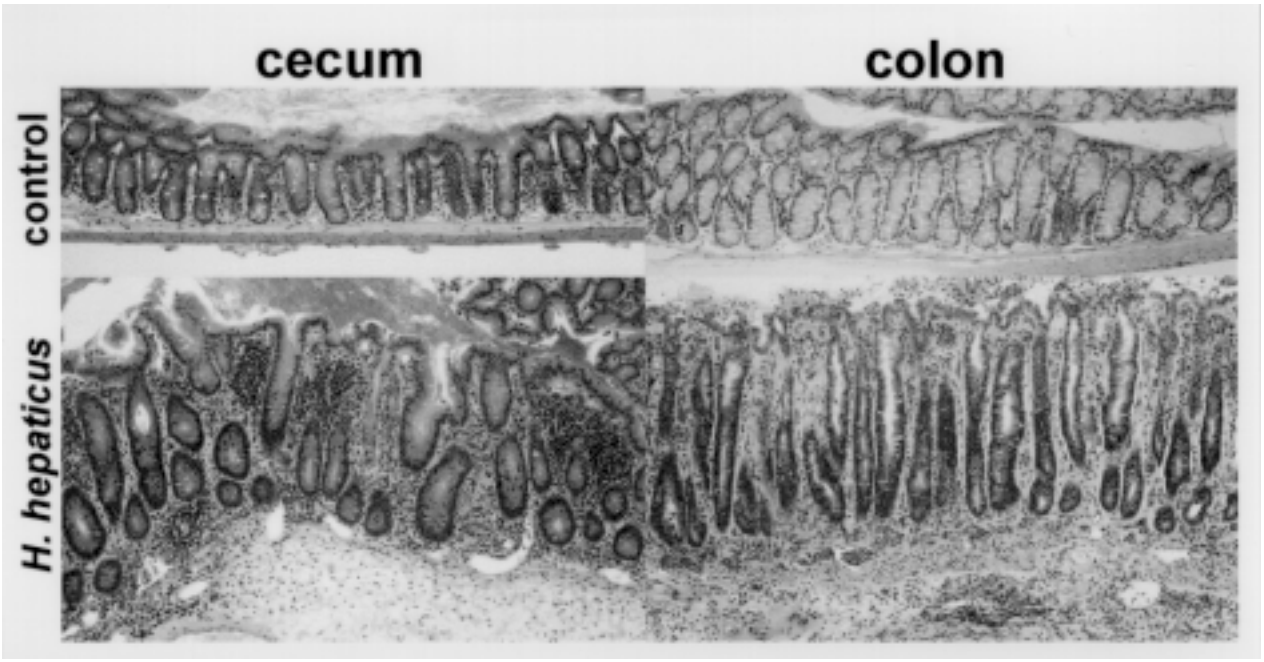

Figure 2. Histopathologic changes in TCR $\beta^{-/-}$ mice at 6 months after inoculation. The TCR $\beta^{-/-}$ mice infected with *H. hepaticus* developed moderate to marked mucosal hyperplasia and inflammation in the cecum and colon. Erosive changes are present on the surface epithelium of the colon. In contrast, the cecum and colon from uninoculated control mice contained only limited inflammatory changes; those shown are within normal limits. H&E stain; 55× magnification.

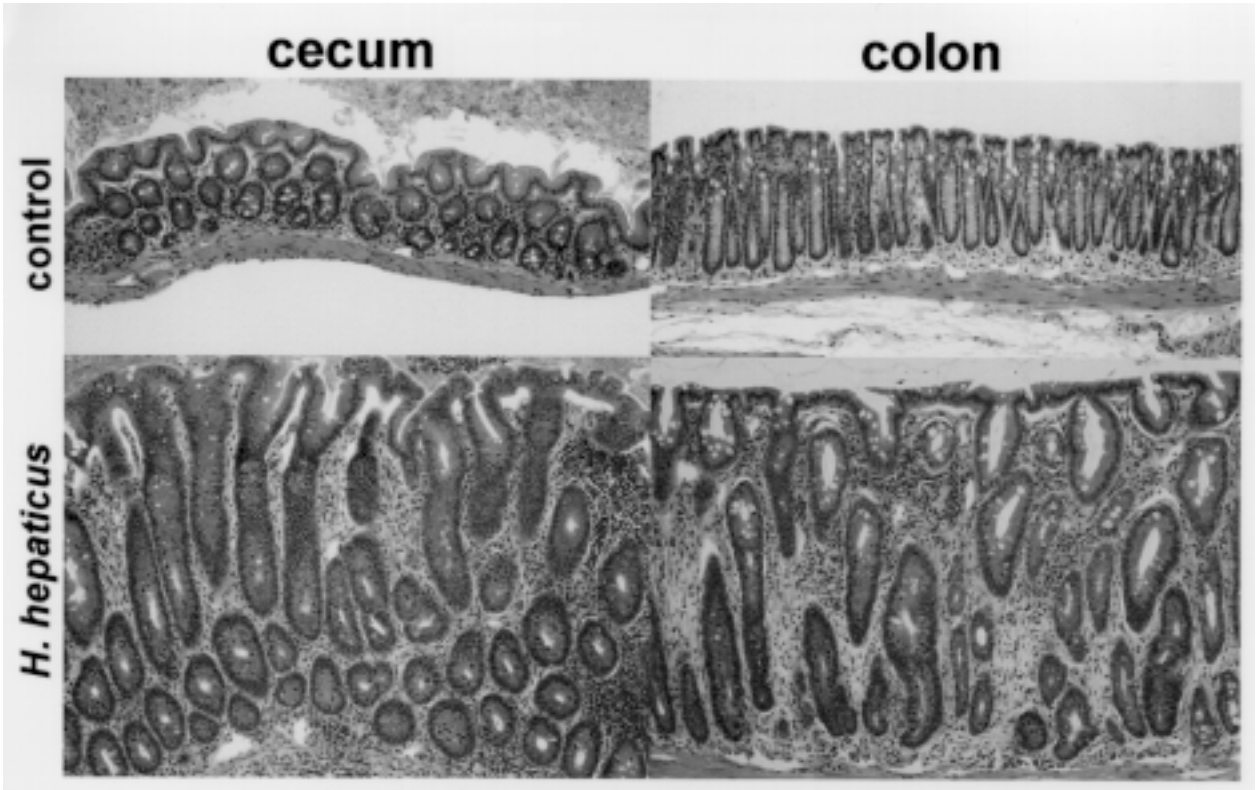

Figure 3. Histopathologic changes in TCR $\beta^{-/-}$ mice at 9 months after inoculation. Cecal and colonic lesions have increased in intensity in TCR $\beta^{-/-}$ mice infected with *H. hepaticus*. The lesions are characterized by elongated, irregular crypts and chronic lymphohistocytic inflammation. As observed at the earlier time point, control mice developed only limited inflammatory changes. H&E stain; 55× magnification.

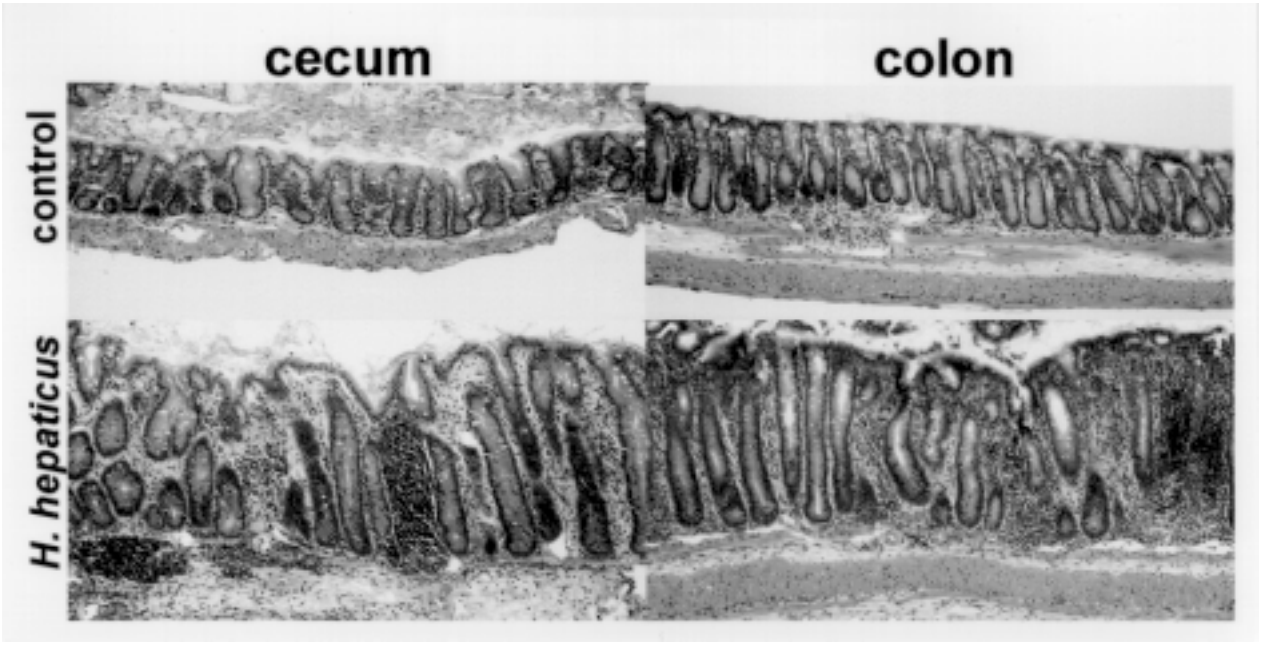

Figure 4. Histopathologic changes in TCR $\alpha^{-/-}\times\beta^{-/-}$ mice at 6 months after inoculation. The TCR $\alpha^{-/-}\times\beta^{-/-}$ mice infected with *H. hepaticus* developed a similar pattern of lesions as that in infected TCR $\beta^{-/-}$ mice. The histologic lesions of proliferative typhlocolitis were slightly less intense in comparison, though typically were still moderate to marked in intensity. Similar to the TCR $\beta^{-/-}$ mice, the control TCR $\alpha^{-/-}\times\beta^{-/-}$ mice did not develop notable inflammatory changes. H&E stain; 55× magnification.

IBD in the cecum and colon of TCR $\beta^{-/-}$ mice, and similarly in the cecum of TCR $\alpha^{-/-}$ mice. In the absence of infection, inflammatory changes were limited in the TCR $\beta^{-/-}$ mice. In contrast, control TCR $\alpha^{-/-}$ mice typically had limited changes in the cecum, but often manifested moderate to marked proliferative colitis, independent of *H. hepaticus* infection. Associated with this manifestation of colitis, colonic inflammation scores for control and infected TCR $\alpha^{-/-}$ mice were significantly greater than those of TCR $\beta^{-/-}$ mice, which did not develop *H. hepaticus*-independent colitis ($P = 0.0332$).

H. hepaticus-associated IBD lesions in the cecum and colon of TCR $\alpha^{-/-}$ mice and TCR $\beta^{-/-}$ mice at 9 months after inoculation typically were characterized by marked hyperplasia of the mucosal crypts and extensive, sometimes effacing mucosal inflammatory infiltrates. Mucosal hyperplasia was manifested by prominent lengthening of the crypts, basophilia of the epithelium, loss of goblet cell differentiation, and occasional multifocal hyperplastic invasion through the muscularis mucosae by the crypt epithelium. Inflammatory infiltrates were typically comprised of lymphocytes and macrophages. Neutrophilic infiltration was associated with foci of mucosal erosion or frank ulceration, the latter being observed infrequently. Inflammation occasionally penetrated the wall, and occasionally, granulomas were present in the mesenteric attachment and serosa.

Epithelial cell proliferation: To independently assess epithelial cell hyperproliferation, immunohistochemical analysis for BrdU incorporation was performed. By 6 months after inoculation, *H. hepaticus* infection was significantly associated with an approximate twofold increase in epithelial cell labeling in the cecum and the colon (Figure 6). Similarly, a twofold increase in the length of the crypts also was associated with *H. hepaticus* infection at this time point (Figure 6). In addition to the absolute increase in labeling index, mapping the position of labeled cells in each crypt revealed significant expansion of the proliferative compartment (Figure 7).

Discussion

Helicobacter species were first recognized in laboratory rodents, where they were considered part of the resident intestinal microbiota (23). Indeed, infection with *H. hepaticus* in immunocompetent rodents is typically subclinical. However, in addition to causing liver lesions, *H. hepaticus* infection also causes lesions in the gastrointestinal tract. Experimentally infected A/JCr male mice develop mucosal hyperplasia and inflammation in the cecum that can be seen 12 months after inoculation (24). A particular recombinant inbred strain, AXB2, in addition to hepatitis, also develops proctitis after experimentally induced infection with *H. hepaticus* (25). Cecal and colonic lesions also are seen in exgerm-free Swiss Webster female mice experimentally infected with *H. hepaticus*, typically by 18 months after inoculation, although not every animal develops colitis (26). Lesions in immunodeficient mice develop earlier and are often more severe. *Scid* mice naturally infected with *H. hepaticus* have mucosal hyperplasia and inflammation in the cecum by 4 to 6 months of age, and can develop proliferative colitis with substantial morbidity by the time they are 9 to 10 months old (13). Natural infection with *H. hepaticus* in athymic nude mice is associated with typhlitis, colitis, and rectal prolapse (14). Although clinical disease is not seen in immunocompetent mice with *H. hepaticus* infection, this organism is considered a murine pathogen because of its consistent association with gastrointestinal tract lesions.

We have now documented that *H. hepaticus* infection causes IBD in TCR αβ mutant mice. At 6 months after inoculation, *H. hepaticus* infection was significantly associated with mucosal hyperplasia and inflammation in the cecum and the colon of TCR $\beta^{-/-}$ mice. TCR $\alpha^{-/-}\times\beta^{-/-}$ mice were similarly affected by *H. hepaticus*

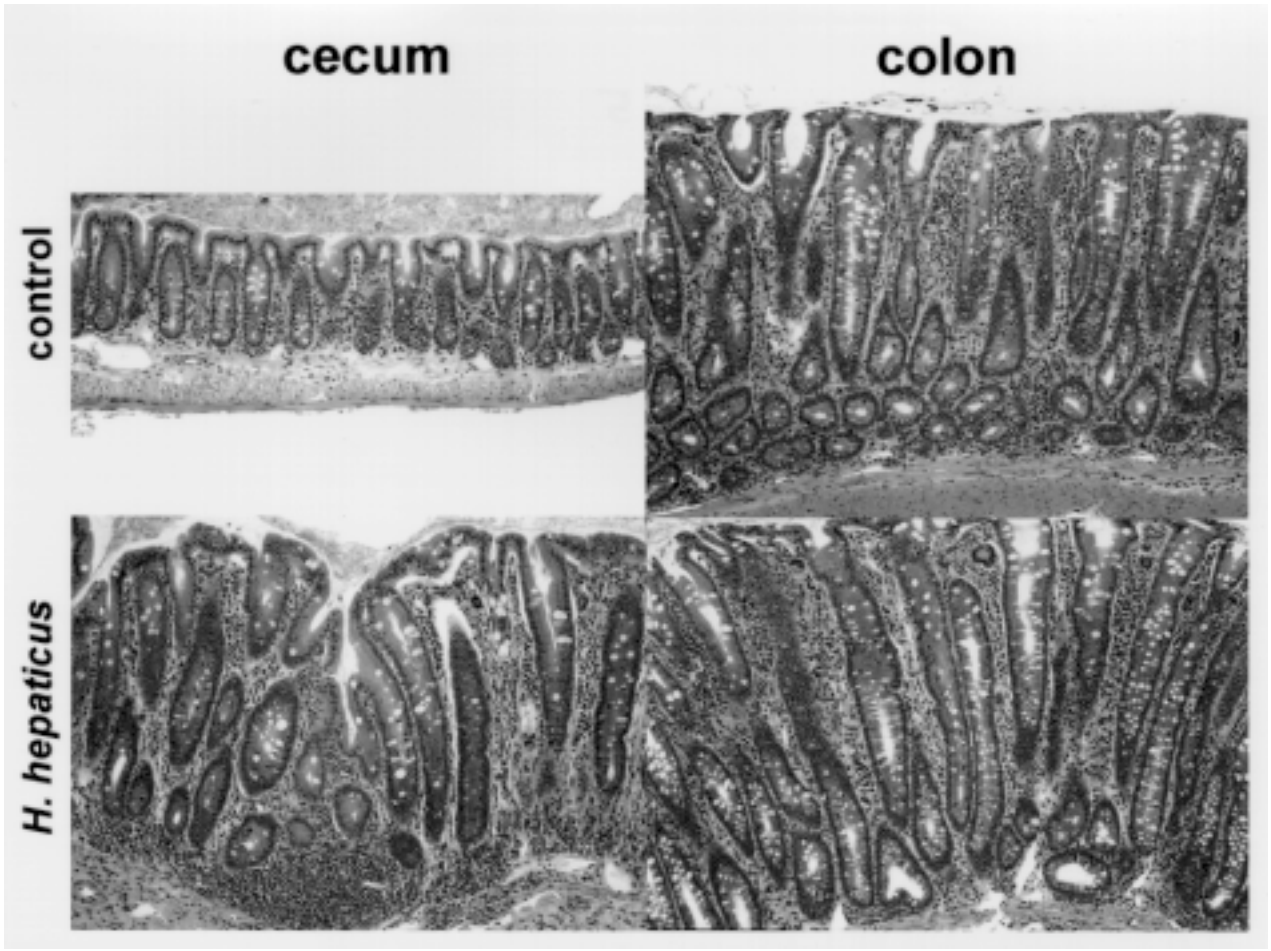

Figure 5. Histopathologic changes in TCR $\alpha^{-/-}$ mice at 9 months after inoculation. The TCR $\alpha^{-/-}$ mice infected with *H. hepaticus* consistently had marked proliferative typhlocolitis. However, unlike the other TCR αβ mutant mice, control TCR $\alpha^{-/-}$ mice developed moderate to marked inflammation in the colon. Cecal hyperplasia and inflammation were slightly increased in control mice, but these changes were significantly less intense than the *H. hepaticus*-associated cecal lesions. H&E stain; 55× magnification.

infection, and TCR $\beta^{-/-}$ and TCR $\alpha^{-/-}\times\beta^{-/-}$ mice had colonic and cecal epithelial hyperproliferation and expansion of the proliferative compartment that was significantly associated with *H. hepaticus* infection at this time point. Increased epithelial cell proliferation and expansion of the proliferative compartment are reported to be biomarkers of cancer risk among patients suffering from chronic ulcerative colitis (27). Nine months after inoculation, the lesions had progressed and there were differences between TCR $\alpha^{-/-}$ mice and TCR $\beta^{-/-}$ mice in the response to *H. hepaticus* infection. *Helicobacter hepaticus* infection remained significantly associated with IBD in TCR $\beta^{-/-}$ mice. The TCR $\alpha^{-/-}$ mice also exhibited mucosal hyperplasia and inflammation in the cecum that was significantly associated with *H. hepaticus* infection. Infected TCR $\alpha^{-/-}$ mice had proliferative colitis at this time point, but *H. hepaticus*-independent colitis was observed in the uninoculated control animals as well. Rearrangement of the TCR β chain precedes TCR α chain rearrangement, and with regard to thymocyte development, TCR $\alpha^{-/-}\times\beta^{-/-}$ mice have a phenotype that is similar to that of TCR $\beta^{-/-}$ mice (28). This also appears to be the case for *H. hepaticus*-associated IBD. Superficially, TCR $\alpha^{-/-}\times\beta^{-/-}$ mice and TCR $\beta^{-/-}$ mice may more closely resemble *scid* and nude mice in their susceptibility to *H. hepaticus*-associated IBD, whereas the pathogenesis of IBD in TCR $\alpha^{-/-}$ mice may be somewhat distinct. This is consistent with the observation that a unique population of TCR $\alpha^{-}\beta^{+}$ T cells plays a role in the pathogenesis of IBD in TCR $\alpha^{-/-}$ mice (29, 30).

The role of *H. hepaticus* infection in the etiopathogenesis of IBD in mice with altered immune function remains controversial. We have previously documented that experimentally induced infection with *H. hepaticus* is sufficient for the expression of IBD in defined flora *scid* mice reconstituted with $CD4^{+}$ $CD45RB^{high}$ T cells (15). RAG-$2^{-/-}$ mice exposed to *H. hepaticus* and IL-$10^{-/-}$ mice experimentally infected with *H. hepaticus* also have been documented to develop IBD (16, 17). However, a separate report indicates that IBD can develop in barrier-maintained IL-$10^{-/-}$ mice in the absence of *H. hepaticus* infection (5). A novel urease-negative *Helicobacter* species, *H. typhlonicus*, has been documented to cause IBD in IL-$10^{-/-}$ mice and *scid* mice (18, 19), and *H. bilis* infection can cause IBD in *scid* mice (31, 32). Therefore, in the absence of *H. hepaticus* infection, other *Helicobacter* species may be sufficient to cause IBD in susceptible lines of genetically altered mice. Contributing to the controversy is the term specific pathogen free (SPF). SPF animals are free of a specified list of pathogens (33). Pathogens for this purpose are defined as infectious agents that can cause overt disease and/or alter biologic responses during experimentation. However, there is no

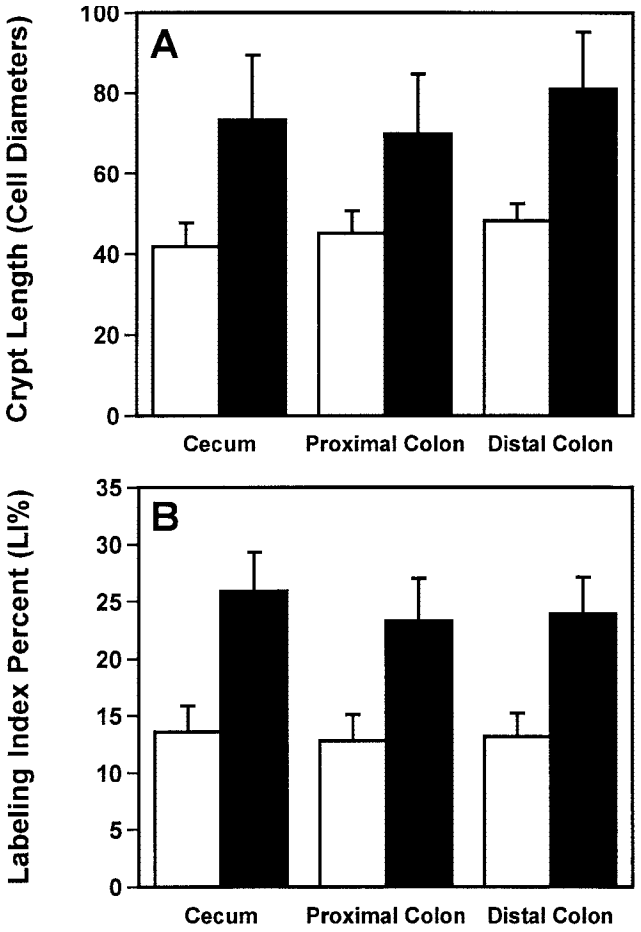

Figure 6. *Helicobacter hepaticus* infection is significantly associated with increased crypt length and increased labeling index in the cecum and colon of TCR αβ mutant mice 6 months after inoculation. **(A)** Crypt length in cell diameters, and **(B)** labeling index expressed as mean ± SD percentage of bromodeoxyuridine (BrdU)-positive cells per crypt, in uninoculated control mice □, and in *H. hepaticus* infected TCR $\beta^{-/-}$ and TCR $\alpha^{-/-} \times \beta^{-/-}$ mice ■ 6 months after inoculation.

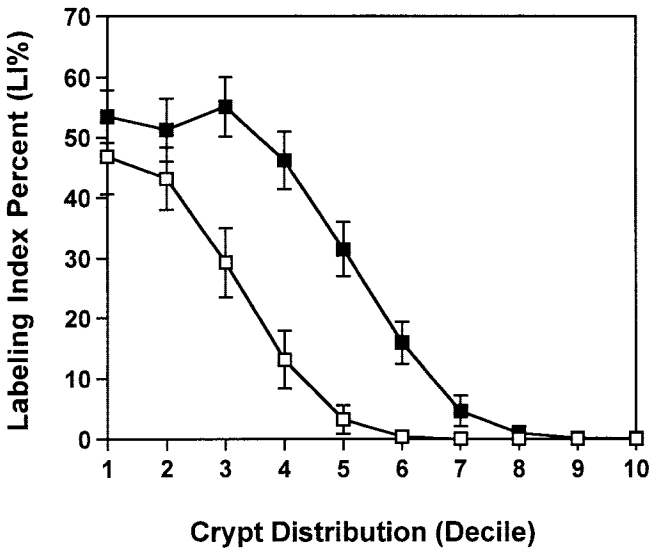

Figure 7. Longitudinal crypt distribution of proliferating cells in the cecum of *H. hepaticus*-infected and control TCR αβ mutant mice 6 months after inoculation. Labeling index expressed as mean ± SD percentage of BrdU-positive cells per crypt, by decile of total cell number, along the crypt from base (1) to apex (10) in uninoculated control mice □, and in *H. hepaticus*-infected TCR $\beta^{-/-}$ and TCR $\alpha \times \beta^{-/-}$ mice ■ 6 months after inoculation

universal agreement on which agents are pathogens, and designating a particular barrier facility SPF does not necessarily indicate that all potential pathogens have been excluded. It has also been reported by Gaskin et al. (34) that TCR $\alpha^{-/-}$ mice develop IBD when housed in an SPF barrier facility, but not when housed in a non-SPF conventional facility. However, the role of a specific agent or agents in the pathogenesis of disease was not investigated. Infection with a pathogen appears to be sufficient to trigger IBD in TCR αβ mutant mice. This has been documented by Sacco et al. with the protozoan *Cryptosporidium parvum* (35), and by our data with *H. hepaticus*. Nonetheless, the role of non-pathogenic resident microbiota in the progression of disease, and possibly in protection against the effects of pathogens (36), should not be ignored.

The mechanism by which *H. hepaticus* infection causes IBD in susceptible lines of genetically altered mice remains unclear. In addition to the presence of granulating cytotoxin activity, we recently identified a cytolethal distending toxin (CDT) in *H. hepaticus* (37, 38). The latter candidate virulence determinant may play a role in the pathogenesis of IBD, possibly by targeting lymphocytes and causing cell cycle arrest. To test this hypothesis, we are currently generating isogenic mutant strains of *H. hepaticus* that do not produce CDT. Furthermore, we are interested in the role of urease activity in the pathogenesis of *H. hepaticus*-induced disease. Urease is a virulence factor of *H. pylori* as documented by in vivo studies that indicate that urease-negative *H. pylori* mutants fail to colonize the gastric mucosa of gnotobiotic piglets and nude mice (39, 40). We have created a urease-negative isogenic mutant strain of *H. hepaticus* and are characterizing its capacity to infect and induce histologic lesions in a murine model.

Early typhlitis and later involvement of the colon mirror the normal sites of infection by *H. hepaticus* (23). *H. hepaticus*-independent colitis in TCR $\alpha^{-/-}$ mice may be associated with a distinct microbial agent or agents present in the intestinal microbiota of our mice. We did not attempt to define the intestinal microbiota of these animals. Since all of our *Helicobacter*-free TCR αβ mutant mice are housed in a common cubicle, we speculate that the putative microbial agent or agents are also present in our TCR $\beta^{-/-}$ mice and our TCR $\alpha^{-/-} \times \beta^{-/-}$ mice. These animals may be less susceptible to this particular microbial insult, since they lack the population of unique TCR $\alpha^{-}\beta^{+}$ T cells present in TCR $\alpha^{-/-}$ mice. Further studies will be needed to define the relationship between *H. hepaticus*-independent colitis and *H. hepaticus*-associated IBD in TCR $\alpha^{-/-}$ mice. The ability to trigger IBD in TCR αβ mutant mice with *H. hepaticus* infection should facilitate the use of this model to identify and characterize microbial virulence determinants and antigenic epitopes that are involved in the pathogenesis of disease. Use of the recently taxonomically classified Altered Schaedler Flora, which is used to colonize the intestine of mice with specific anaerobic bacteria, will also facilitate these efforts (41). Studies to establish the role of microbial pathogens in disease expression in mice that develop IBD are critically important to elucidating the etiopathogenesis of Crohn's disease and ulcerative colitis. The results of these studies may provide a strong impetus to investigate

the contribution of enterohepatic *Helicobacter* infection to IBD in humans.

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