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Enterotoxigenic *Bacteroides fragilis:* A Potential Instigator of Colitis

Shervin Rabizadeh, MD, MBA[*], **Ki-Jong Rhee, PhD**[†], **Shaoguang Wu, MD**[†], **David Huso, DVM, PhD**[‡], **Christine M. Gan, PhD**[§,|], **Jonathan E. Golub, PhD, MPH**[†], **XinQun Wu, MD**[†], **Ming Zhang, MS**[†], and **Cynthia L. Sears, MD**[†,§,|]

[*] Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, Maryland [†] Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland [‡] Department of Comparative Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland [§] Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, Maryland [|] Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Baltimore, Maryland

Abstract

Background—Inflammatory bowel disease (IBD) is proposed to result from a dysregulated mucosal immune response to the colonic flora in genetically susceptible individuals. Enterotoxigenic *Bacteroides fragilis* (ETBF), a molecular subclass of the common human commensal, *B. fragilis*, has been associated with IBD. This study investigated whether ETBF colonization of mice initiated colitis or modified the clinical course of a colitis agonist, dextran sodium sulfate (DSS).

Methods—Four- and 6-week-old C57BL/6 mice were inoculated with buffer, nontoxigenic *B. fragilis* (NTBF) strain 9343(pFD340), or ETBF strain 86-5443-2-2 via orogastric tube. A subset of mice received 2% DSS several days pre- or post-inoculation of bacteria. Clinical status was assessed throughout the experiment and severity of colonic inflammation was scored after sacrifice.

Results—All mice, including those receiving DSS, were clinically well prior to bacterial inoculation. NTBF and ETBF colonization was similar. Regardless of mouse age or timing of DSS administration, mice who received ETBF+DSS experienced worse colitis reflected by less weight gain, enhanced gross disease, and greater inflammation in their colons ($P < 0.05$), especially in the cecum. In particular, younger mice had more extensive disease. Mice inoculated only with ETBF also exhibited colitis with more severe inflammation when compared to all other groups ($P < 0.05$) except the ETBF+DSS group.

Conclusions—ETBF, a colonic commensal, alone stimulates colitis and significantly enhances colonic inflammation in DSS-treated mice. This study suggests that acquisition of ETBF colonization may be a potential factor in initiation and/or exacerbation of colitis.

Keywords

enterotoxigenic *Bacteroides fragilis* (ETBF); colitis; dextran sodium sulfate (DSS)

Reprints: Shervin Rabizadeh, MD, MBA, 600 North Wolfe St., Brady 320, Baltimore, MD 21287 (srabiza1@jhmi.edu).

Inflammatory bowel disease (IBD), which includes Crohn's disease and ulcerative colitis, is a disorder characterized by chronic inflammation of the gastrointestinal tract. The etiology of IBD is unknown; however, it is postulated to result from a dysregulated mucosal immune response to the colonic flora in genetically susceptible individuals.[1] IBD patients exhibit excess epithelial permeability that enhances direct contact between the colonic flora and the immune system possibly permitting disease initiation.[2–4] In fact, Crohn's disease is partially responsive to antibiotic treatment (e.g., metronidazole, ciprofloxacin, or rifaximin), suggesting that enteric bacteria are important in disease pathogenesis.

Bacteroides fragilis are normal colonic commensals in the majority of adults.[5,6] Colonization rates in children have been less well studied.[7] Although humans are typically asymptomatically colonized, *B. fragilis* is identified as the leading anaerobe in bloodstream infections and intraabdominal abscesses.[8–10] A molecular subset of *B. fragilis*, termed enterotoxigenic *B. fragilis* (ETBF), secrete a 20-kDa proinflammatory zinc-dependent metalloprotease toxin, designated the *B. fragilis* toxin (BFT). ETBF are associated with diarrheal illnesses in animals, children, and adults.[11–13] In considerably more limited data, ETBF have been linked to IBD where these bacteria have been identified in mucosal washings of IBD patients and associated with clinically active IBD.[14,15] However, in all human studies evaluating ETBF colonization to date, a sizeable number of individuals (≈4%–30%) appear to be colonized, apparently asymptomatically, with ETBF.[16] Whether long-term colonization with ETBF is truly without consequence to the host is unclear, particularly given a recent report associating ETBF colonization with colorectal carcinoma.[17]

In this study we asked whether ETBF colonization of mice modified the clinical course of a known colitis agonist, dextran sodium sulfate (DSS). DSS induces predominantly colonic inflammation, the anatomic site of ETBF colonization, through direct epithelial cytotoxicity as well as immune-mediated interactions resulting in an IBD-like disorder.[18] We hypothesized that ETBF colonization may exacerbate colitis due to DSS. Our results suggest that colonization with ETBF is a potential risk factor in the initiation and exacerbation of colonic inflammation.

MATERIALS AND METHODS

Bacterial Strains

The wild-type ETBF strain used in this study was 86-5443-2-2 (piglet isolate; source Dr. L.L. Myers), while the nontoxigenic *B. fragilis* (NTBF) strain was NCTC 9343 (source American Type Culture Collection, Rockville, MD). *Bacteroides fragilis* strains were propagated anaerobically on BHI medium containing 37 g of brain heart infusion base (Difco Laboratories, Detroit, MI) per liter along with 5 g of yeast extract (Difco), 0.1 mg of vitamin K per liter, 0.5 mg of hemin per liter, 50 mg of L-cysteine, and 6 μg of clindamycin per liter (all from Sigma, St. Louis, MO).

Mice

Male C57BL/6 mice were purchased from Jackson Laboratories (Bar Harbor, ME) at ≈3 weeks of age or were bred at Johns Hopkins using C57BL/6 breeder pairs from the National Cancer Institute. Mice were maintained under specific pathogen-free conditions and studied according to protocols approved by the Johns Hopkins University Animal Care and Use Committee in accordance with the Association for the Assessment and Accreditation of Laboratory Animal Care International. For most experiments, similar to other mouse models of enteric infection,[19] mice were pretreated for 5 days with 5 g/L of streptomycin (Sigma) and 100 mg/L of clindamycin (Pharmacia, Kalamazoo, MI) in their drinking water;

antibiotics at the same dose were continued until the day of sacrifice. The goal of this protocol was to enhance colonization with *B. fragilis* and to permit ready isolation and quantification of inoculated bacteria. ETBF strain 86-5443-2-2 is inherently resistant to clindamycin, whereas NTBF strain 9343 was engineered to be clindamycin-resistant through transformation with plasmid FD340 [yielding NCTC9343(pFD340)]; *B. fragilis* are naturally resistant to aminoglycosides. Subgroups of mice were given 2% DSS (MW: 30,000–50,000; MP Biomedicals, Solon, OH) in their drinking water as described in the Results.

Freshly plated *B. fragilis* strains were grown in BHI broth for 24 hours, resuspended in BHI broth and grown for another 24 hours, washed twice with 0.1N sodium bicarbonate, resuspended in buffer, and adjusted to an optical density corresponding to $\approx 10^9$ colony-forming units (CFU)/mL (as quantitated by serial dilution on BHI plates) prior to inoculation. Inoculation was performed via orogastric tube (≈0.2 mL; 10^8 CFU/mouse) under isoflurane anesthesia; sodium bicarbonate (≈0.2 mL) was used for sham inoculation of control mice. Mouse clinical status (weight, activity, appearance) was assessed at 1- to 3-day intervals and on the day of euthanasia. Mice were euthanized using carbon dioxide euthanasia at timepoints as discussed in the Results. Gross findings were recorded and cecum and colon were harvested for histology. A subgroup of mice had spleen weights measured at the time of sacrifice.

Fecal Analysis

Stool was obtained from each mouse prior to bacterial inoculation and periodically post-inoculation including the day of sacrifice. Serial dilutions were cultured on BHI plates with clindamycin to quantitate strain colonization. The presence of *B. fragilis* and ETBF was confirmed using polymerase chain reaction (PCR). *Bacteroides* species-specific primer pairs were developed to amplify the 16S rRNA intergenic spacer region (ISR) as previously described.[20] One of these forward primers, 1392A (5′-GTACACACCGCCCGT) was combined with a reverse primer of our own design, Bfra-Rev20 (5′-AATTTAGAACCAATGAACG) to specifically amplify a 190-bp region of the *B. fragilis* ISR. A primer pair was also designed to generate amplicons of the *B. fragilis* enterotoxin (BFT) produced by ETBF, yielding a 109-bp fragment [BFT-F1 (5′-GAAAGTCAGACACGTGCAG-TACC) and BFT-R1 (5′-CCTGCATCTGGGCACTAAC)]. PCR reactions were carried out in 25 μL reactions containing 1.25 U platinum *Taq* DNA polymerase (Invitrogen, La Jolla, CA), 1× PCR buffer, 1 mM of each dNTP, 6% v/v DMSO, and 1 μM of each primer. The following cycling conditions were employed: for the *B. fragilis* and BFT-specific primers, 95°C for 4 minutes; 30 cycles of 94°C for 30 seconds; 52°C for 30 seconds; 72°C for 30 seconds; 72°C for 7 minutes; cooled to 4°C. PCR products were resolved on 2% agarose gels.

Histology

Cecum and colon were preserved in 10% buffered formalin prior to histologic examination by hematoxylin and eosin staining of 5-μm sections. To facilitate examination of the colon, tissue samples were “Swiss rolled” prior to embedding and sectioning.[21] Cecal and colon sections were graded for inflammation using a scale of 0 (no inflammation), +1 (mild increase in inflammatory cells; no mucosal changes), +2 (moderate increase in inflammatory cells; mild scattered proliferation +/− focal loss of crypt architecture), +3 (severe increase in inflammatory cells; diffuse or nearly diffuse proliferation, focally extensive loss of crypt architecture), +4 (complete or nearly complete mucosal destruction).

Statistics

Data are presented as means ± standard error of the mean (SEM) unless otherwise stated. Comparison of means was done by unpaired Student's *t*-test. A *P*-value ≤0.05 was considered a significant difference.

RESULTS

DSS Colitis Is Exacerbated by ETBF in Young Mice (Fig. 1A)

We initially tested whether the colonic commensal, ETBF, modified the course of DSS-induced colitis by treating 4-week-old C57BL/6 mice with 5 days of 2% DSS followed by inoculation with NTBF, ETBF, or buffer only (sham inoculation). All mice, whether receiving DSS or not, were clinically well, with positive weight gain for the 5 days prior to bacterial inoculation (data not shown). There were no significant differences in weight change between groups prior to bacterial inoculation. NTBF and ETBF colonization, confirmed by PCR analysis (data not shown), was not modified by DSS administration and colonization on the day of sacrifice was similar ($4.40 \times 10^9 \pm 1.40 \times 10^9$ CFUs/gm stool and $2.25 \times 10^{10} \pm 9.34 \times 10^9$ CFUs/gm stool, respectively; $P = 0.09$). On the day of sacrifice (day 9, Fig. 1A), the mean weight gain since the day of inoculation in the DSS+ETBF group was significantly less than all other groups ($P = 0.02$ versus ETBF; $P < 0.001$ versus all other groups) (Fig. 2A). Similarly, mice colonized with ETBF, but not treated with DSS, gained less weight than the other groups; a result that was significant when ETBF group was compared to the sham ($P = 0.02$) or DSS+sham ($P = 0.04$) groups but only approached significance compared to the NTBF ($P = 0.08$) or DSS+NTBF ($P = 0.08$) groups. All mice appeared clinically well except the DSS+ETBF group. DSS+ETBF mice developed rectal bleeding (2/10), recto-anal ulceration (2/10) (Fig. 3), lethargy (5/10), or death (2/10) by day 2 post-inoculation. At necropsy, it was noted that ETBF mice, especially those in the DSS +ETBF group, generally had contracted, bloody ceca as opposed to the NTBF and buffer-inoculated mice. Histologic examination of the colon revealed focal inflammation along the colonic axis in all the DSS-treated mice as well as the mice only colonized with ETBF (Table 1, Fig. 4). As previously reported,[18] inflammation was predominantly enhanced in the distal colon of mice treated only with DSS. NTBF colonization did not modify this pattern of inflammation. In contrast, mice inoculated only with ETBF developed predominantly cecal/proximal colonic inflammation that was markedly exacerbated by concomitant DSS treatment (Fig. 4). Average cecum and colon inflammation scores were significantly higher in the DSS+ETBF group compared to all other groups ($P < 0.05$) (Table 1). Average cecum inflammation scores of the ETBF group were significantly higher than all other groups ($P < 0.01$) except the DSS+ETBF group (Table 1).

ETBF-associated Colitis Is Blunted in Older Mice (Fig. 1A)

Our preliminary observations suggested that ETBF-associated colitis in mice may be modified by the age of the mice. To assess whether age was a factor in the extent of colitis induced in mice inoculated with ETBF with or without pretreatment with DSS, groups of 6-week-old C57BL/6 mice were treated with 5 days of 2% DSS and inoculated with NTBF, ETBF, or buffer alone and compared to groups of mice inoculated with NTBF, ETBF, or buffer in the absence of DSS pretreatment. Similar to the 4-week-old C57BL6 mice, all 6-week-old C57BL/6 mice, whether treated with DSS or not, were clinically well, with positive weight gain prior to bacterial inoculation. Unlike the younger C57BL/6 mice, after bacterial inoculation there was no significant difference in weight change among the various groups (all $P > 0.05$) (Fig. 2B) and, clinically, all mice appeared well, with no evidence of rectal bleeding, recto-anal ulceration, or death. NTBF and ETBF colonization, confirmed by culture of stool samples and PCR, was similar on the day of sacrifice ($2.10 \times 10^{10} \pm 1.30 \times 10^{10}$ CFUs/gm stool and $3.84 \times 10^9 \pm 1.00 \times 10^9$ CFUs/gm stool, respectively; $P = 0.18$) in

all subgroups of mice. Nevertheless, the average cecum inflammation scores were significantly higher in the DSS+ETBF group compared to all other groups ($P \leq 0.05$) (Table 1, *P*Fig. 4). There was also enhanced cecitis in the ETBF-only mice compared to all other groups (< 0.02) with the exception of the DSS+ETBF mice. The colon inflammation scores were also significantly higher in the DSS+ETBF group compared to all other groups ($P \leq 0.05$) except the DSS+sham group ($P = 0.08$). This latter group had higher colonic inflammation scores compared to the sham-only and ETBF-only group ($P \leq 0.05$); otherwise, no other significant differences were noted (Table 1). These results suggest that, although older mouse age blunts the clinical expression of colitis, ETBF colonization still worsens colitis in mice with subclinical DSS colitis.

Colonization with ETBF Predisposes Mice to More Severe Colitis (Fig. 1B)

To model the reported clinical scenario of apparent asymptomatic ETBF colonization, we next colonized C57BL/6 mice with ETBF and then treated with DSS as a stimulus known to trigger colitis. For this experiment, 4-week-old C57BL/6 mice were first inoculated with NTBF, ETBF, or buffer only. Ten days post-inoculation a subgroup of the mice were treated with 2% DSS in their drinking water for 1 week. All mice were clinically well with normal activity 10 days post-inoculation and there were no significant differences in the weight change among the experimental groups at the time of DSS treatment initiation (data not shown). Similar to our previous experiments, NTBF and ETBF colonization, confirmed by stool culture and PCR, was similar in all mouse subgroups at the time of sacrifice ($3.47 \times 10^{10} \pm 1.53 \times 10^{10}$ CFUs/gm stool and $2.42 \times 10^{10} \pm 7.35 \times 10^{9}$ CFUs/gm stool, respectively; $P = 0.50$). There was significantly less weight gain by day 5 postinitiation of DSS in the ETBF+DSS group when compared to all other mouse groups ($P < 0.01$) (Fig. 2C). However, this difference was only significant when the ETBF+DSS group was compared to the sham, NTBF, and ETBF groups ($P < 0.05$) on the day of sacrifice, 7 days postinitiation of DSS. On the day of sacrifice there was evidence of rectal blood in 4/8 ETBF+DSS mice, 1/7 ETBF mice, 1/7 NTBF+DSS mice, and 1/4 sham+DSS mice and 1 of the ETBF+DSS mice exhibited recto-anal ulceration. At necropsy, the ETBF-inoculated mice had contracted, bloody ceca compared to the NTBF and buffer-inoculated mice. A trend was noted for higher spleen weights in both ETBF groups as compared to NTBF groups and mice treated with buffer only (Table 2). Histopathology revealed that ETBF +DSS mice had significantly higher cecum ($p \leq 0.05$) and colonic ($p \leq 0.01$) inflammation and proliferation scores than all other groups (Table 1, Fig. 4). Together, these results suggest that ETBF colonization alone induces asymptomatic inflammation of the cecum/ proximal colon and that colonization with ETBF predisposes mice to more significant colitis when exposed to an inflammatory stimulus. The splenomegaly suggests that ETBF induce systemic inflammation even though the mice remained clinically asymptomatic.

To examine effects in the absence of antibiotics, a group of mice without any antibiotic treatment in their drinking water was inoculated with NTBF, ETBF, or buffer prior to treatment with 2% DSS beginning 10 days postbacterial inoculation. Stool colonization, as determined by culture and PCR, was again similar at the time of sacrifice in the NTBF-and ETBF-inoculated mice (2.88×10^{10} to 3.99×10^{11} CFUs/gm stool and 1.45×10^{10} to 4.14×10^{10} CFUs/gm stool, respectively) and the mice were clinically well prior to initiation of DSS. The ETBF+DSS group had less weight gain (data not shown) and more rectal bleeding and recto-anal ulceration (3/3 mice as compared to none in all other groups [$N = 2–3$/group] except for 1/2 sham+DSS mice). Similar to mice who received antibiotics, the ETBF+DSS group had enhanced cecal and colonic inflammation/proliferation scores compared to all other groups (Table 1, Fig. 4).

DISCUSSION

Our results indicate that ETBF have the potential to initiate and exacerbate colitis in C57BL/6 mice. These mice are inbred but lack defined defects in host immunity as are usually present in other current murine models of IBD focused on examining the role of the colonic flora in disease.[22,23] Identification of colitis in C57BL/6 mice colonized with ETBF suggest that these organisms are potent proinflammatory bacteria. IBD is thought to result from a dysregulated mucosal immune response to the colonic flora in genetically susceptible individuals.[1] Furthermore, patients with bowel inflammation, but not controls, have been observed to have high concentrations of mucosal bacteria (exceeding 10^9/mL) and a dense, adherent mucosal biofilm mass composed predominantly of *B. fragilis* group organisms.[14,15] The *B. fragilis* group consists of at least 10 distinct species of organisms[20] and subspeciation of the adherent *B. fragilis* group organisms in the mucosal biofilm mass has not yet been reported.

Our results further suggest that ETBF may initiate both a mucosal inflammatory response as well as a systemic response based on our observations of splenomegaly in some mice. It has previously been proposed that NTBF are critical to induction of systemic T-cell development.[24] Further studies will be required to delineate if ETBF, similar to NTBF, influence systemic T-cell development, potentially adversely, and if this contributes to colitis risk. We also note that ETBF-induced colonic inflammation is predominantly proximal (cecitis) in contrast to the distal localization of DSS-induced inflammation. Despite this difference in the site of predominant disease localization, ETBF do appear to affect the entire colon and promote DSS-induced colonic inflammation as well as cause rectoanal ulceration in some mice, suggesting that ETBF induced subtle perturbations of mucosal immunity throughout the colon.

Of interest, mice inoculated with ETBF at the weaning transition were more susceptible to colitis as well as promotion of DSS-induced disease. This could, in part, be due to differences in initiation of bacterial tolerance at different ages and/or a result of differing colonic flora in younger mice. Prior data indicate that rapid changes occur in mouse fecal flora and immune responses at weaning.[25] ETBF-associated diarrheal disease occurs in children over the age of 1. In contrast, in studies conducted to date, children under the age of 1 are colonized apparently asymptomatically with ETBF.[11–13,16] Together, these results suggest a critical shift in the mucosal response to ETBF associated with age and likely other factors including diet or other environmental exposures.

Several potential factors need to be considered in interpretation of this study. Administration of DSS to induce subclinical colitis, while convenient, does not yield a model that perfectly mimics the pathogenesis of IBD, since DSS causes a chemical colitis through epithelial injury.[26] Nevertheless, DSS has been commonly used to study the pathogenesis of colitis[18,26–31] and was useful to allow us to model the clinical scenarios of ETBF colonization preceding or following the emergence of colitis in the host. Another potential study limitation was the use of antibiotics to enhance bacterial colonization. Use of antibiotics in the drinking water of the mice disrupts the native mouse flora influencing bacterial interactions and possibly enhancing disease post-inoculation with NTBF or ETBF. Our experiment performed without antibiotics demonstrated results consistent with experiments involving mice treated with antibiotics, further validating our results. Importantly, resistance to antibiotics is relatively common in *B. fragilis*[32] and antibiotics are commonly used drugs. Thus, our results with and without antibiotics may have relevance to the pathogenesis of human colitis. Lastly, despite using an inbred mouse strain, we observed mouse-to-mouse variability in disease induction. This may relate to potential inconsistencies in bacterial inoculation. Mice may not receive the full inoculum secondary to technical

difficul-ties or gastric contents delaying transit time (mice were not fasted prior to bacterial inoculations). Although our stool cultures showed similar colonization of mice with their respective bacteria at the times analyzed, the impact of bacterial number on the initial mucosal changes and disease progression in mice that, despite similar ages, vary in weight (for example) are, as yet, unknown. Further, whether mucosal colonization with ETBF differs from NTBF is unknown.

In summary, ETBF alone cause and significantly worsen colitis in DSS-treated mice. This appears to be influenced by age, as younger mice have more profound inflammation. Because ETBF are colonic commensals in some individuals,[16] this study has potential implications for IBD patients. Our results suggest the hypothesis that ETBF, at least in part, through secretion of its proinflammatory toxin (BFT), are capable of initiating an inflammatory mucosal immune response that contributes to initiation and/or exacerbation of IBD in the susceptible host. Potential future studies should focus on determining the presence of ETBF at the time of diagnosis of IBD, especially in children, as well as evaluation for ETBF in the stools of patients with disease flairs. Studies to evaluate the mucosal and systemic immune responses to ETBF may contribute to our understanding of the pathogenesis of IBD.

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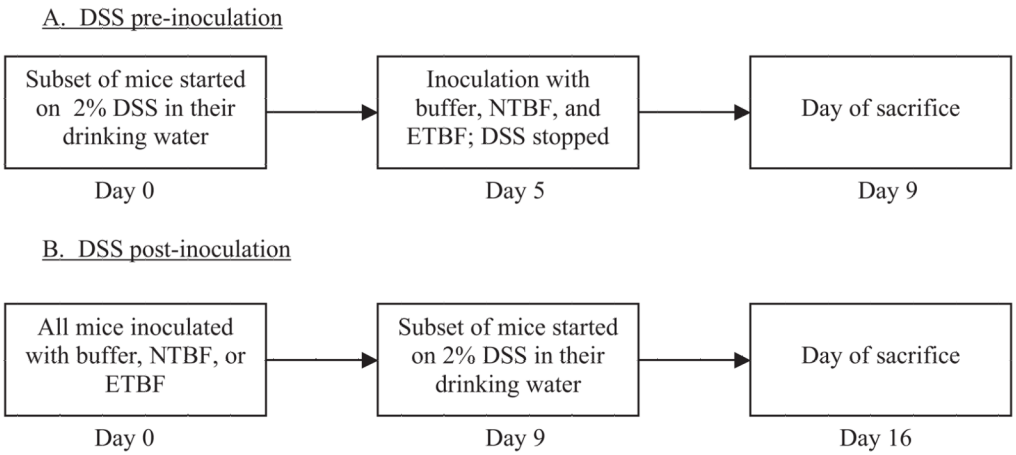

FIGURE 1.

Inoculation protocols and timeline. (A) DSS preinoculation. This protocol used 4- and 6-week-old mice. All mice had antibiotics in their drinking water from day 0 through day 9. In the text, mice from these experiments will be classified as sham (buffer), NTBF, or ETBF if they did not receive DSS and DSS+sham, DSS+NTBF, or DSS+ETBF if they received DSS. (B) DSS post-inoculation. This protocol used 4-week-old mice. All but 1 subset of mice (see text) received antibiotics in their drinking water from day −5 through day 16. In the text, these mice will be classified as sham (buffer), NTBF, or ETBF if they did not receive DSS and sham+DSS, NTBF+DSS, or ETBF+DSS if they received DSS.

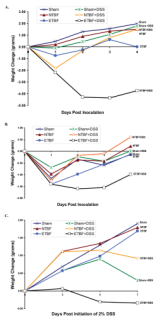

FIGURE 2.

ETBF colonization increases weight loss in 4- and 6-week-old C57BL/6 mice with DSS-induced colitis. Four- and 6-week-old C57BL/6 mice were inoculated with buffer, NTBF, or ETBF after a subset had received 5 days of 2% DSS in their drinking water (A, B). In a separate experiment, 4-week-old C57BL/6 were inoculated with buffer, NTBF, and ETBF 10 days prior to a subset receiving 1 week of 2% DSS in their drinking water (C). The mice were weighed daily to once every third day. The graphs depict weight change for each group after normalizing the weights to the day of inoculation (A, B; note the difference in the scale of the weight change axis between A and B) or initiation of DSS (C). The ETBF+DSS mice had significantly less weight gain from day 2 through 4 ($P < 0.02$) in the first experiment (A) and by day 5 ($P < 0.01$) in the third experiment (C); however, this was not significantly different ($P > 0.05$) at any timepoint in the second experiment (B). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

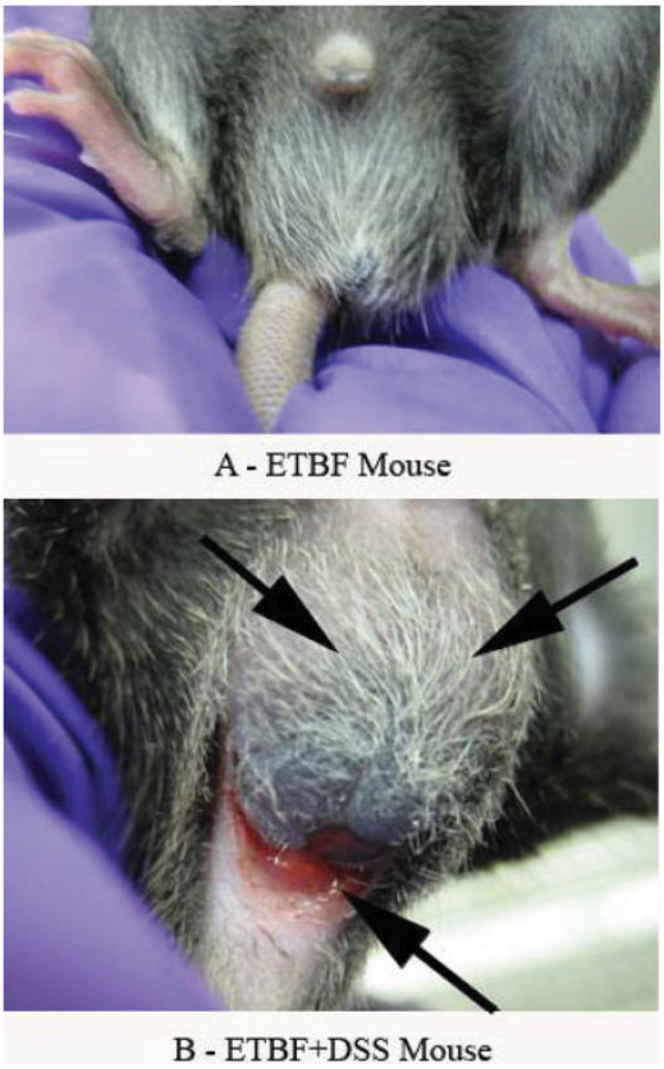

FIGURE 3.

ETBF induce recto-anal ulceration in 4-week-old C57BL/6 mice with DSS-induced colitis. Four-week-old C57BL/6 mice were inoculated with buffer, NTBF, or ETBF after a subset had received 5 days of 2% DSS in their drinking water. Mouse clinical status (weight, activity, appearance) was assessed at 1- to 3-day intervals and on the day of euthanasia. Photographs were taken at the time of sacrifice at which time, in all experiments, ETBF mice (as well as other mouse groups, see text) had no gross evidence of disease (A) except for the ETBF+DSS mice who had evidence of recto-anal breakdown, ulceration, and necrosis (B; arrows). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

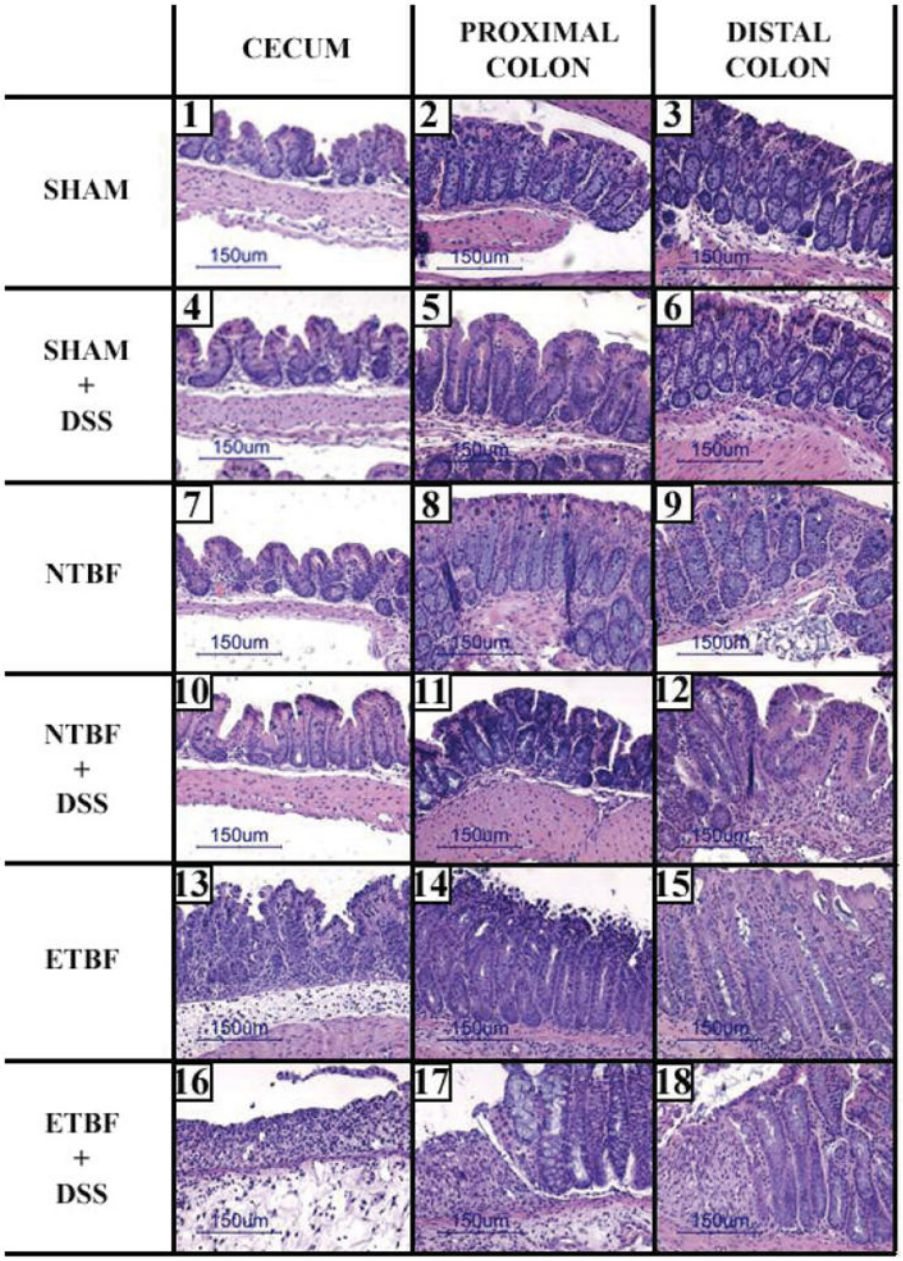

FIGURE 4.

Cecum and proximal and distal colon histopathology of 4- and 6-week-old C57BL/6 mice inoculated with [NTBF, ETBF, or sham] in the presence or absence of 2% DSS. Representative histopathology is shown. Cecum is from 4-week-old C57BL/6 mice inoculated with buffer, NTBF, or ETBF after a subset had received 5 days of 2% DSS in their drinking water. Colon is from 4-week-old C57BL/6 inoculated with buffer, NTBF, or ETBF 10 days prior to select mice receiving 1 week of 2% DSS in their drinking water. Anoikis (detaching of cells from the epithelial surface) (insert 13 and 14), proliferation (insert 14, 15, 17, right, and 18, right), and ulceration/mucosal destruction (insert 16, 17, left, and 18, left) were seen more often in the ETBF group and ETBF+DSS group. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE 1

Enhanced Inflammation of Cecum and Colon in 4-Week and 6-Week-Old C57BL/6 Mice Inoculated with ETBF in the Presence of 2% DSS

	4-Week-Old Mice Given 2% DSS Prior to Bacteria Inoculation		6-Week-Old Mice Given 2% DSS Prior to Bacteria Inoculation		4-Week-Old Mice Inoculated with Bacteria Prior to 2% DSS			
					Mice with Antibiotics		Mice without Antibiotics	
	Cecal Inflammation ± SEM	Colon Inflammation ± SEM	Cecal Inflammation ± SEM	Colon Inflammation ± SEM	Cecal Inflammation ± SEM	Colon Inflammation ± SEM	Cecal Inflammation ± SEM	Colon Inflammation ± SEM
Sham	0.4 ± 0.25 (*n* = 5)	0.6 ± 0.25 (*n* = 5)	0.5 ± 0.29 (*n* = 5)	0.4 ± 0.19 (*n* = 5)	1.4 ± 0.13 (*n* = 4)	0.9 ± 0.23 (*n* = 4)	0.3 (0.5, 0.0) (*n* = 2)	0.5 (1.0, 0.0) (*n* = 2)
Sham+DSS	0.6 ± 0.25 (*n* = 5)	1.2 ± 0.20 (*n* = 5)	0.7 ± 0.25 (*n* = 5)	1.3 ± 0.20 (*n* = 5)	2.3 ± 0.14 (*n* = 4)	2.1 ± 0.24 (*n* = 4)	1.8 (1.5, 2.0) (*n* = 2)	2.3 (2.5, 2.0) (*n* = 2)
NTBF	0.8 ± 0.20 (*n* = 5)	0.2 ± 0.20 (*n* = 5)	0.6 ± 0.19 (*n* = 5)	1.0 ± 0.22 (*n* = 5)	1.0 ± 0.29 (*n* = 7)	0.7 ± 0.17 (*n* = 7)	1.3 ± 0.17 (*n* = 3)	0.7 ± 0.17 (*n* = 3)
NTBF+DSS	0.8 ± 0.37 (*n* = 5)	0.8 ± 0.20 (*n* = 5)	1.0 ± 0.20 (*n* = 5)	0.9 ± 0.24 (*n* = 5)	2.3 ± 0.14 (*n* = 7)	2.4 ± 0.08 (*n* = 7)	1.3 ± 0.33 (*n* = 3)	1.7 ± 0.33 (*n* = 3)
ETBF[a]	2.2 ± 0.20 (*n* = 5)	1.2 ± 0.20 (*n* = 5)	1.7 ± 0.12 (*n* = 5)	0.5 ± 0.27 (*n* = 5)	2.8 ± 0.33 (*n* = 7)	2.7 ± 0.33[b] (*n* = 7)	2.1 (2.0, 2.1) (*n* = 2)	1.8 (1.5, 2.0) (*n* = 2)
ETBF+DSS[c]	3.3 ± 0.25 (*n* = 8)	2.2 ± 0.30 (*n* = 8)	2.6 ± 0.29 (*n* = 10)	1.8 ± 0.13 (*n* = 10)	3.8 ± 0.14 (*n* = 8)	3.8 ± 0.14 (*n* = 8)	3.5 ± 0.29 (*n* = 3)	3.7 ± 0.17 (*n* = 3)

4-week and 6-week-old C57BL/6 mice were inoculated with buffer, NTBF, or ETBF after a subset had received 5 days of 2% DSS in their drinking water. In a separate experiment, 4-week-old C57BL/6 were inoculated with buffer, NTBF, or ETBF 10 days prior to select mice receiving 1 week of 2% DSS in their drinking water. Cecal and colon sections were graded for inflammation as described in Materials and Methods.

[a] ETBF cecum scores were significantly worse than all other groups except ETBF+DSS for the experiments in which DSS was given first ($P \leq 0.05$) and when compared to the NTBF and sham cecums of mice given DSS after inoculation with bacteria ($P \leq 0.05$).

[b] ETBF colon scores were significantly worse than all NTBF and sham colon scores in mice inoculated with bacteria prior to initiation of 2% DSS ($P \leq 0.01$)

[c] ETBF+DSS cecum and colon scores were significantly worse than all other groups for each experiment ($P \leq 0.05$).

TABLE 2

Larger Spleens in 4-Week-Old Mice Colonized with ETBF for 10 Days Prior to Receiving 2% DSS for 1 Week

	Sham[a]	Sham+DSS[a,b]	NTBF[a]	NTBF+DSS[a]	ETBF[a,c]	ETBF+DSS[a,d]
Spleen Wt (mg) ± SEM	73.1 ± 4.4 (*n* = 4)	100.1 ± 10.8 (*n* = 4)	66.2 ± 3.4 (*n* = 3)	60.1 ± 5.5 (*n* = 3)	117.2 ± 7.2 (*n* = 3)	136.1 ± 17.6 (*n* = 4)

4-week-old C57BL/6 were inoculated with buffer, NTBF, and ETBF 10 days prior to a subset of mice receiving 1 week of 2% DSS in their drinking water. Spleens were weighed at the time of sacrifice.

[a] All other comparisons between groups were not significant.

[b] Sham+DSS has significantly large spleens vs. sham, NTBF, and NTBF+DSS ($P \leq 0.05$).

[c] ETBF had significantly larger spleens vs. sham, NTBF, and NTBF+DSS ($P \leq 0.05$).

[d] ETBF+DSS had significantly larger spleens vs. sham, NTBF, and NTBF+DSS ($P \leq 0.01$).