Varying Cecal Bacterial Loads Influences Colitis and Gastritis in HLA-B27 Transgenic Rats

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Background & Aims: Recent data support an important role of resident luminal bacteria in experimental colitis. We determined how altered cecal bacterial loads influence colitis and gastritis. *Methods:* A cecal self-filling blind loop (SFBL) was created or the cecum was excluded from the fecal stream in specific pathogen–free HLA-B27 transgenic (TG) rats with early colitis and in nontransgenic (nonTG) littermates; controls underwent sham operation (SHAM). Luminal bacterial concentrations were determined by culture and counting chamber. *Results:* TG rats with SFBL had more severe cecal inflammation and leukocytosis than TG SHAM controls. TG excluded rats with low cecal bacterial loads had no cecal inflammation and less colitis and gastritis than SHAM controls, despite having normal distal colonic and gastric bacterial concentrations. Metronidazole attenuated cecal inflammation and eliminated *Bacteroides* in SFBL TG rats. NonTG SFBL rats had mild cecal inflammation and no gastritis and colitis. The ratio of total anaerobic to aerobic bacteria was 1000-fold greater in SFBL than in SHAM rats, with a 10,000-fold increased ratio of *Bacteroides* spp. to aerobes. *Conclusions:* The luminal bacterial load and composition determines the activity of cecal inflammation in genetically susceptible hosts. Lowering cecal bacterial concentrations can diminish inflammation in remote organs.

Extensive data support an important role for normal luminal bacteria in inflammatory bowel disease, especially Crohn's disease and experimental enterocolitis.[1–3] Resident bacteria have been implicated in the pathogenesis of indomethacin-,[4] carrageenan-,[5] and dextran sulfate sodium–induced[6] intestinal inflammation, as well as spontaneous colitis in interleukin (IL)-$2^{-/-}$,[7] IL-$10^{-/-}$,[8] CD45RB^{high} reconstituted severe combined immunodeficient (SCID) mice,[9] and C3H/HeJBir mice.[10,11] In a recent study we showed the essential influence of resident bacteria on the spontaneous colitis and gastritis in HLA-B27/β2 microglobulin transgenic (TG) Fischer rats.[12] The onset of spontaneous colitis and gastritis in TG rats normally begins at 2 months of age with a 100% incidence of nonbloody diarrhea by 3–4 months.[13,14] In a sterile environment these animals do not develop colitis, gastritis, or arthritis.[12,15] However, when germfree rats were transferred into a specific pathogen–free (SPF) environment, all TG rats had histological and biochemical evidence of active colitis and gastritis after 4 weeks, although diarrhea was not evident at this time.[12]

Additional data show that altering bacterial populations influences the degree of intestinal and extraintestinal inflammation. Decreasing luminal bacterial concentrations, especially of anaerobic bacteria, can be beneficial in both clinical and experimental enterocolitis. Crohn's colitis and ileocolitis respond to several antibiotics including metronidazole,[16] which is selectively active against anaerobic bacteria. Metronidazole also attenuates chronic experimental intestinal inflammation in the indomethacin and carrageenan models[4,17] and in HLA-B27 TG rats.[18] Bowel rest, leading to long-term reduction of luminal bacteria, is used to treat severe Crohn's disease.[19–21] However, anaerobic bacterial overgrowth in bypassed jejunoileal segments for morbid obesity leads to systemic inflammation.[22] Experimental small intestinal anaerobic bacterial overgrowth in a jejunal self-filling blind loop (SFBL) can induce hepatobiliary inflammation and reactivate quiescent arthritis in genetically susceptible Lewis rats.[23,24] In both human and rat bacterial overgrowth models, broad-spectrum antibiotics or metronidazole treat systemic manifestations.[23,25–27] These results convincingly show that anaerobic bacteria play an

Abbreviations used in this paper: BBE, *Bacteroides* bile esculin; BHI, brain heart infusion; EX, excluded from the fecal stream; LPS, lipopolysaccharide; nonTG, nontransgenic; PARS, prereduced anaerobically sterilized; PG-PS, peptidoglycan-polysaccharide; SCID, severe combined immunodeficiency disease; SHAM, sham-operated; SFBL, self-filling blind loop; SPF, specific pathogen free; TCR, T-cell receptor; TG, transgenic; WBC, white blood cell (count).

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important role in induction of colitis and extraintestinal manifestations in genetically susceptible hosts. Our recent data support this hypothesis and show that all bacteria do not have equal abilities to induce gastrointestinal inflammation. Gnotobiotic B27 TG rats, colonized with 6 different obligate and facultative anaerobic bacteria including *Streptococcus faecium, Streptococcus avium, Peptostreptococcus productus, Escherichia coli, Eubacterium contortum,* and *Bacteroides vulgatus* developed much more active colitis and gastritis than littermates colonized with the same bacteria without *B. vulgatus.*[12]

This study shows how alterations in the autologous cecal bacterial composition and load influence the degree of gastrointestinal inflammation in HLA-B27 TG rats, without using antibiotics or selected heterologous bacteria. Attention was focused on *Bacteroides* spp. because our previous observations in gnotobiotic rats showed a dominant role for these species.[12]

Materials and Methods

Animals

A colony of HLA-B27 TG rats and nontransgenic (nonTG) littermates[13] originally obtained from Dr. Joel D. Taurog (Southwestern Medical School, Dallas, TX) was derived into a sterile environment, populated with SPF bacteria, then housed and maintained in an SPF environment.[12]

Experimental Design

TG rats (N = 21) raised in an SPF environment were divided into 3 groups at 2 months of age, when colitis first becomes evident. In one group a cecal SFBL was created as described later, in the second group the cecum was excluded from the fecal stream (EX), and the third group was sham operated (SHAM). NonTG littermates (n = 11) were SHAM or SFBL operated and served as negative controls. All rats were monitored weekly for clinical evidence of diarrhea and arthritis. One month after surgery, at the age of 3 months, all rats were killed by CO_2 asphyxiation. Anticoagulated cardiac blood was obtained for white blood cell (WBC) counts. The cecum and colon were inspected grossly in a blinded fashion for evidence of intestinal thickness and scored on a validated scale ranging from 0 to 4^+ as previously described.[12] The cecum, stomach, and right colon were fixed in 10% buffered formalin for histological evaluations. Tissues from the transverse colon were snap-frozen in isopentane for protein assays.

In a separate experiment, we treated TG SFBL rats with metronidazole, 40 mg · kg^{-1} · day^{-1}, in drinking water. Treatment was begun at the age of 8 weeks, and surgery was performed at 10 weeks. Metronidazole-treated rats (n = 4) and water controls (n = 4) were killed at 14 weeks of age. Cecal tissue was removed for histology.

All studies were performed with the approval of the University of North Carolina Animal Care and Use Committee.

Creation of a Cecal SFBL

Cecal SFBLs were created in TG rats (n = 8) and nonTG littermates (n = 6) at 2 months of age. Anesthesia was induced using 40 mg/kg intramuscular ketamine and 0.75 mg/kg acepromazine. After a midline incision, the cecum was ligated at the cecal-colonic junction using a 4.0 silk suture (Figure 1). Continuity of the fecal stream was accomplished by a side-to-side anastomosis between the terminal ileum and the transverse colon, using 6.0 Vicryl sutures (Ethicon Inc., Sommerville, NJ). SHAM operation (TG, N = 5; nonTG, N = 5) included a laparotomy and external manipulation of the cecum, but no ligation or anastomosis.

Exclusion of the Cecum

Surgery was performed on TG rats (n = 8) at 2 months of age. After anesthesia and midline incision the cecum was separated from the right colon and terminal ileum (Figure 1). The section of the terminal ileum connected to the cecum was ligated using a 4.0 silk suture. A colostomy was performed using the colonic end of the cecum. The transected right colon was ligated and an end-to-side anastomosis with the terminal ileum was created using 6.0 Vicryl sutures.

Determination of Bacterial Concentrations

The cecum, colon, jejunum, ileum, and stomach of TG and nonTG rats (SFBL, EX, and SHAM) were removed and

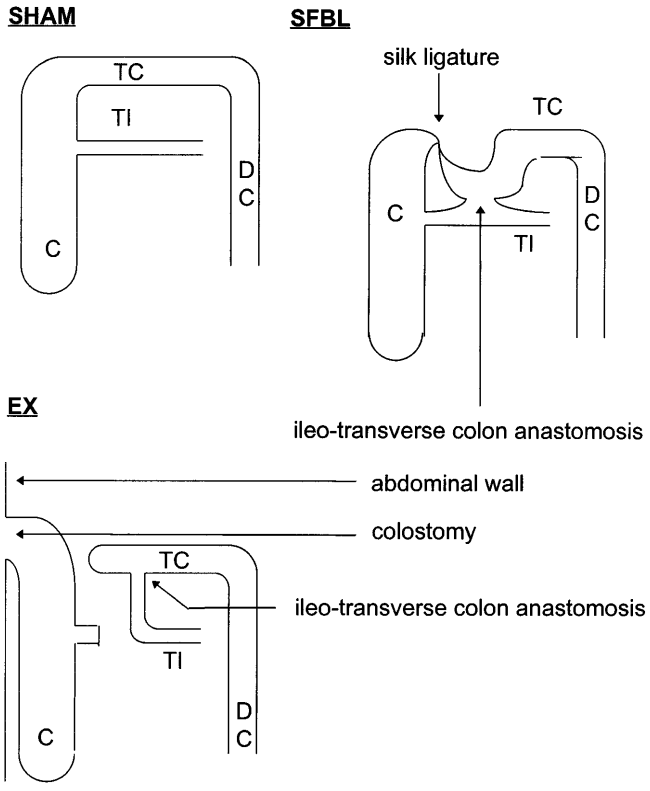

Figure 1. Surgical procedures to create an SFBL and exclusion of the cecum with a colostomy (EX). Surgical procedures were performed on 2-month-old TG Fischer rats and nonTG littermates. C, cecum; TI, terminal ileum; TC, transverse colon; DC, descending colon.

immediately placed into an anaerobic isolator. One milliliter of cecal contents was taken, weighed, and serially diluted in phosphate-buffered saline or prereduced thioglycolate broth. From every 10-fold dilution, 100 μL were plated on modified Bryant's medium 10 (MM10) agar or prereduced anaerobically sterilized (PRAS) agar plates in an anaerobic 5% CO_2, 10% H_2, and 85% N_2 atmosphere. Aerobic culture was performed using enriched brain heart infusion (BHI) agar or blood agar plates. Colonies were counted after 2 (aerobic) and 6 (anaerobic) days of incubation at 37°C. *Bacteroides* spp. were selectively grown on *Bacteroides* Bile Esculin (BBE) Agar under anaerobic conditions. Morphology was determined by Gram stain. Total bacterial concentrations were determined on serial dilutions with phase-contrast microscopy using a Petroff–Hauser counting chamber. Identification of presumed *Bacteroides* spp. was performed according to standard procedures.[28] Results were normalized for stool dry weights.

Histology

Tissues were prepared as previously described.[12] A validated histological inflammatory score ranging from 0 to 4^+ was used for blinded evaluation of colonic and gastric inflammation.[12]

Rat IL-1β ELISA

We measured IL-β protein concentrations in the cecal tissue by enzyme-linked immunosorbent assay (ELISA) as previously described,[12] using antibodies that were provided by Dr. S. Poole, National Institute of Biological Standards and Controls, Hertfordshire, England.

Statistical Analysis

Statistics were performed using the analysis of variance and the Student *t* test for comparison between the groups and the χ^2 test for nominal data. Significance was considered to be $P < 0.05$. All data are presented as mean ± SEM.

Results

Clinical Features

One hundred percent of the TG SFBL rats had diarrhea 3–4 weeks after surgery compared with 0% of the SHAM and EX groups ($P < 0.0005$). There were no differences in weight gain in the various groups. One TG rat with SFBL developed active arthritis, but no arthritis was observed in the SHAM and EX groups. NonTG rats had no evidence of diarrhea or arthritis.

Cecal Inflammation

The blinded gross gut score of the cecum (Figure 2) was significantly increased in the TG SFBL compared with TG SHAM rats (3.6 ± 0.1 vs. 1.6 ± 0.2; $P <$ 0.0001). The predominant visible abnormality was thickening of the large intestine, which was more evident in the cecum than in the colon. There were no macroscopic

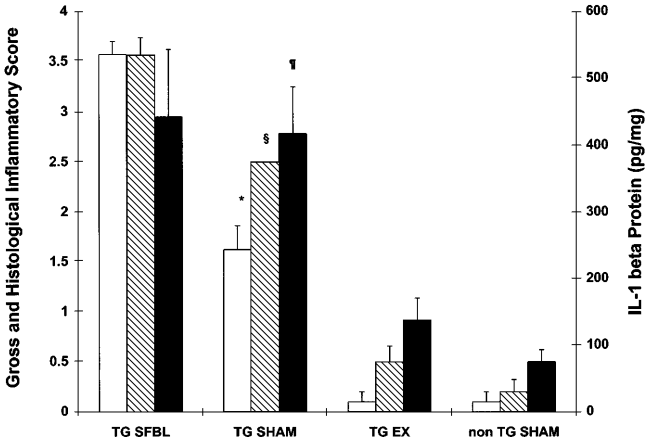

Figure 2. Quantitation of cecal inflammation in HLA-B27/β2 microglobulin TG rats exposed to variable bacterial loads. Blinded gross (□) and histological (▨) evaluations showed significantly increased inflammation in TG rats with SFBL (N = 8) and significantly decreased inflammation in TG EX rats (N = 8) compared with TG SHAM controls (N = 5). There was no difference between TG EX and nonTG SHAM (N = 5). IL-1β protein concentrations (■) in the cecal tissue confirmed decreased inflammation in TG EX vs. TG SHAM, but showed no difference between TG SFBL and TG SHAM. *$P < 0.0001$ vs. all other groups. §$P < 0.002$ vs. TG SFBL and $P < 0.0001$ vs. TG EX and nonTG SHAM. ¶$P < 0.007$ vs. TG EX and nonTG SHAM.

signs of inflammation in the TG EX and nonTG SHAM groups (0.1 ± 0.1 for both; $P < 0.0001$ vs. TG SHAM).

Histological inflammation in the lamina propria of the cecum of TG SHAM rats (Figure 3*A*) was characterized by diffuse infiltration of predominantly mononuclear cells and scattered eosinophils but few neutrophils, thickening of the mucosa with crypt hyperplasia, few scattered crypt abscesses, and rare superficial ulcerations. Inflammation was confined to the mucosa. Mucosal inflammation was increased in TG SFBL rats and extended to the submucosa compared with TG SHAM controls (Figure 3*C* and *D*). Seventy-five percent of TG SFBL rats had submucosal inflammation vs. 0% of TG SHAM rats ($P < 0.01$). More aggressive cecal inflammation in SFBL TG rats was confirmed by blinded histological inflammatory scores (3.6 ± 0.2 vs. 2.5 ± 0; $P <$ 0.005; Figure 2). There was almost no evidence of cecal inflammation in TG EX rats (Figure 3*B*), as confirmed by the lack of difference in histological scores of TG EX and nonTG SHAM rats (0.5 ± 0.2 vs. 0.2 ± 0.1; both $P <$ 0.0001 vs. TG SHAM; Figure 2).

IL-1β protein concentrations in the cecal tissue (Figure 2) of TG EX were significantly reduced compared with TG SHAM (138.0 ± 32.9 vs. 417.9 ± 69.5 ng/g tissue; $P < 0.01$). However, there were no differences evident between TG SHAM and SFBL (442.9 ± 100.5) or between TG EX and nonTG SHAM (75.3 ± 17.8) groups.

NonTG SFBL rats had mild-to-moderate grossly de-

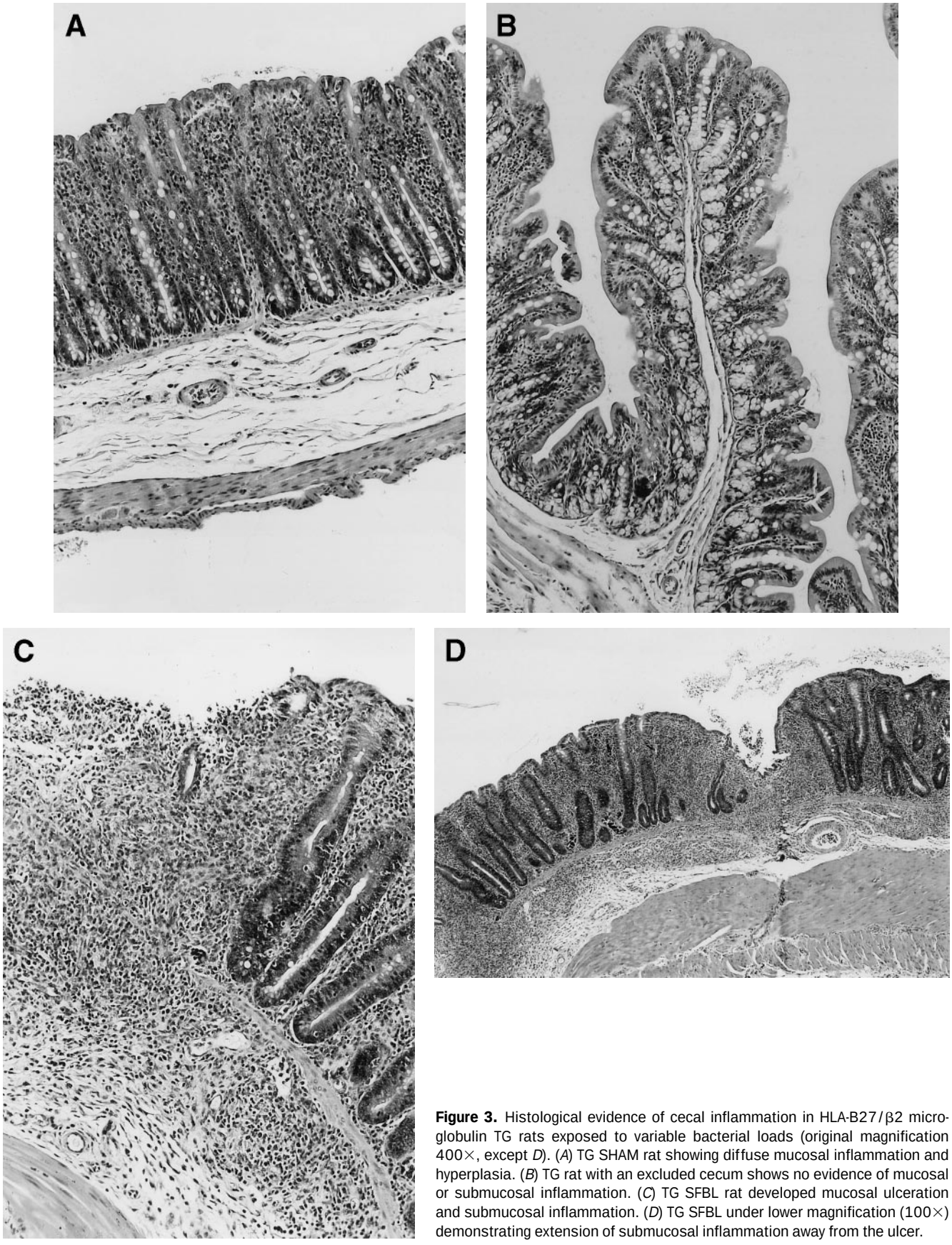

Figure 3. Histological evidence of cecal inflammation in HLA-B27/β2 microglobulin TG rats exposed to variable bacterial loads (original magnification 400×, except *D*). (*A*) TG SHAM rat showing diffuse mucosal inflammation and hyperplasia. (*B*) TG rat with an excluded cecum shows no evidence of mucosal or submucosal inflammation. (*C*) TG SFBL rat developed mucosal ulceration and submucosal inflammation. (*D*) TG SFBL under lower magnification (100×) demonstrating extension of submucosal inflammation away from the ulcer.

Table 1. Bacterial Concentrations From Various Regions of TG and NonTG Rat Gastrointestinal Tract

	Stomach	Jejunum	Ileum	Cecum	Colon
Total count (organism/mL)	$0.5–1.6 \times 10^{12}$	$3–8 \times 10^{11}$	$2–3 \times 10^{11}$	$3–4 \times 10^{12}$	3×10^{12}
Anaerobic culture (CFU/mL)	$4–6 \times 10^{9}$	$3–6 \times 10^{8}$	$3–5 \times 10^{9}$	$0.2–2 \times 10^{10}$	$0.5–1 \times 10^{10}$
Aerobic culture (CFU/mL)	$0.4–2.5 \times 10^{9}$	$1–5 \times 10^{7}$	$3–8 \times 10^{8}$	$0.2–2.7 \times 10^{9}$	$0.4–3.8 \times 10^{9}$
Bacteroides spp. (CFU/mL)	$0–1 \times 10^{5}$	$2–4 \times 10^{2}$	$0.1–1 \times 10^{5}$	$0.4–2 \times 10^{8}$	$1–3 \times 10^{8}$

NOTE. Results are expressed in organisms/mL luminal contents for the total count and in CFU/mL for the cultures. After serial dilutions of the luminal contents, the total number of microscopically visible bacteria, colonies grown on MM10 agar under anaerobic conditions, colonies grown on BHI enriched agar under aerobic conditions, and colonies grown on BBE agar under anaerobic conditions, which permits selective growth of *Bacteroides* spp., were counted. Results are expressed as the range of 3 experiments.

tectable cecal thickening (gross score, 1.3 ± 0.4) but had only very mild inflammation by histological criteria (0.6 ± 0.1, nonsignificant vs. TG EX and $P < 0.02$ vs. nonTG SHAM). By histology this thickening was caused by muscular hypertrophy and mild submucosal edema. IL-1β protein concentrations were not elevated in the nonTG SFBL group (83.9 ± 22.2), confirming the lack of active inflammation.

These results show a consistent increase in cecal inflammation in TG rats with a SFBL and a virtual absence of inflammation in the excluded cecum by gross, histological, and biochemical parameters.

TG SFBL rats treated with metronidazole had significantly less cecal inflammation than water controls, as shown by blinded histology score (2.4 ± 0.1 vs. 3.5 ± 0.4; $P < 0.05$).

Colitis

There was no gross evidence of inflammation in the colon in TG EX and nonTG SHAM rats (0.0 ± 0.0 for both; $P < 0.005$ vs. TG SHAM) by blinded examination. TG SFBL rats had slightly more colonic inflammation than TG SHAM (2.0 ± 0.2 vs. 1.1 ± 0.3; $P < 0.05$). Histological evidence of colitis was also reduced in the TG EX compared with TG SHAM (1.1 ± 0.2 vs. 1.8 ± 0.1; $P < 0.05$), but was significantly higher than nonTG SHAM controls (0.1 ± 0.1; $P < 0.01$). There was no significant difference between histological inflammatory scores in the TG SFBL (2.2 ± 0.2) and TG SHAM groups. Grossly and histologically there was no colitis in the nonTG SFBL group (0.2 ± 0.1 and 0.3 ± 0.2), respectively. These results indicate that alterations in cecal bacterial loads can affect inflammation in the distal colon.

Gastritis

Antral gastritis, with mucosal thickening and mononuclear cell infiltration predominantly in the basal two thirds of the crypts, was a consistent histological feature in TG SHAM and SFBL rats (histological score, 2.6 ± 0.1 vs. 2.5 ± 0.1; NS). Surprisingly, gastritis was significantly decreased in the TG EX group (0.4 ± 0.2; $P < 0.0005$) and totally absent in some of these animals. There was no gastritis in nonTG SFBL animals (0.1 ± 0.1).

Hematologic Features

TG SFBL rats had higher peripheral WBC counts (21.6 ± 1.1 vs. 15.7 ± 2.1; $P < 0.02$) than TG SHAM, whereas TG EX had almost normal WBC counts (8.8 ± 1.0; $P < 0.01$ vs. TG SHAM). NonTG SFBL rats had slightly elevated WBC counts (12.0 ± 1.3; $P < 0.03$ vs. TG EX and nonTG SHAM; NS vs. TG SHAM). There was no difference in hemoglobin and hematocrit values between the groups (data not shown).

Microbial Results

In SHAM rats (Table 1) the total, microscopically visible number of bacteria per milliliter luminal contents in the stomach, jejunum, and ileum was 1 log less than in the cecum and colon. Aerobically cultivable bacteria had the lowest concentrations in the jejunum and highest concentrations in the cecum and colon. Our results show a luxurious presence of bacteria in the rat stomach. Bacteria in the stomach and jejunum were predominantly gram-positive rods and coccobacilli; in addition, spore-forming rods were present in the ileum. The microscopic picture of the cecal bacteria was quite complex, yet similar to the colon, dominated by gram-positive bacilli and spore-forming rods with less frequent gram-negative bacilli. Using selective culture media, concentrations of presumed *Bacteroides* spp. were 3–6 logs higher in the cecum and colon compared with the jejunum and ileum. One of 3 rats had no detectable ($<10^2$/mL) *Bacteroides* spp. in the stomach.

In TG and NT rats the concentrations of total visible bacteria in the cecal contents were equal between the SFBL and SHAM groups (Table 2). However, the number of bacteria recovered under anaerobic growth conditions (obligate and facultative anaerobes) was significantly increased in the SFBL group, whereas bacteria recovered under aerobic growth conditions (aerobes and facultative anaerobes) in the SFBL group were significantly decreased compared with the SHAM group. Thus, the ratio

Table 2. Cecal Bacterial Concentrations

	NonTG (N = 6)			TG (N = 1–2)		
	SFBL	SHAM	EX	SFBL	SHAM	EX
Total	$2–6 \times 10^{12}$	$3–6 \times 10^{12}$	$2–4 \times 10^{10}$ [a]	$2–3 \times 10^{12}$	$2–3 \times 10^{12}$	2×10^{10}
Anaerobic	$2 (\pm 0.6) \times 10^{10}$ [a]	$4 (\pm 1) \times 10^{9}$	$2 (\pm 1) \times 10^{6}$	$2–4 \times 10^{10}$	$3–4 \times 10^{9}$	5×10^{8}
Aerobic	$7 (\pm 3) \times 10^{6}$ [a]	$9 (\pm 4) \times 10^{8}$	$2 (\pm 0.4) \times 10^{6}$	$2–6 \times 10^{7}$	$2–3 \times 10^{9}$	4×10^{7}
Bacteroides spp.	$9 (\pm 4) \times 10^{9}$ [a]	$2 (\pm 0.8) \times 10^{7}$	$4 (\pm 0.4) \times 10^{5}$	2×10^{9}	4×10^{7}	ND
Ratio anaerobic/aerobic	2846 ± 2186[a]	5 ± 2.5	1 ± 0.3	333–2000	1–2	13
Ratio *Bacteroides* spp./aerobic	1703 ± 815[a]	0.02 ± 0.007	0.2 ± 0.1	33–100	0.01–0.02	ND
Cecal weight including contents	ND	ND	ND	14.8 g	4 g	1.5 g

NOTE. Results are expressed in organisms/mL luminal contents for the total count and in CFU/mL for the cultures using the techniques described in Table 1. After serial dilutions in phosphate-buffered saline or prereduced thioglycolate, cecal contents were cultured anaerobically on MM10, PRAS, and BBE agar plates and aerobically on enriched BHI and blood agar plates. Results of transgenic and microscopic counts of nonTG rats are expressed as the range of 1–2 experiments. Culture results of nonTG rats (n = 6) are expressed as mean values.
ND, not done.
[a] $P < 0.05$ vs. nonTG SHAM.

of anaerobically cultured bacteria to aerobically grown organisms in the SFBL rats was almost 3 logs higher than that of the SHAM group. Similarly, the concentrations of isolates on the *Bacteroides* selective media were significantly higher in the SFBL rats compared with the SHAM group, and the ratio of cecal *Bacteroides* spp. to aerobically grown bacteria was almost 5 logs higher in the SFBL group (Table 2). The dominant *Bacteroides* spp. were *B. fragilis* and *B. vulgatus*, present in approximately equal frequencies, with no difference in the relative frequency of these strains in SFBL vs. SHAM rats. Rats from the TG EX group had 2–3 log decreased total and viable cecal bacterial concentrations and 2 log fewer *Bacteroides* spp. in the cecum compared with SHAM. In TG SFBL rats treated with metronidazole no *Bacteroides* spp. were detectable in cecal contents. Although total bacterial concentrations per milliliter cecal contents were similar between the SFBL and SHAM groups, the enlarged ceca of SFBL rats were considerably heavier (Table 2) because of increased luminal contents and the thickened cecal walls, leading to much higher bacterial loads in the SFBL ceca.

The total concentrations of bacteria in the stomach and colon were not different between the groups (SHAM, SFBL, EX; data not shown).

Discussion

These data emphasize the important role of luminal resident bacteria in the pathogenesis of spontaneous colitis in HLA-B27 transgenic rats by showing that the degree of local cecal inflammation depends on the luminal bacterial load and composition in this genetically susceptible host. In the excluded cecum both the bacterial concentration and total bacterial load are decreased, with a corresponding decrease in inflammation. However, although the overall bacterial concentration is normal in the cecal blind loop, the total load of bacteria is increased, because of the higher volume of cecal contents, as manifested by cecal weight, and is associated with more aggressive mucosal and submucosal inflammation. However, cecal inflammation is not exclusively dependent on the bacterial load, because disease fails to develop in the blind loop of nonTG rats, showing a corequirement of host genetic susceptibility. These findings are consistent with previous observations in several different models. Lichtman et al. induced hepatobiliary inflammation and arthritis in genetically susceptible Lewis rats by creating small intestinal bacterial overgrowth with a jejunal SFBL[23,24] and reported pouchitis in Lewis rats with an ileal pouch–rectal anastomosis.[29] Each of these models were related to bacterial overgrowth of predominantly anaerobic bacteria, including *Bacteroides* spp., and responded to metronidazole treatment, which eliminated *Bacteroides* spp.[23,25,29] Similarly, proximal diversion of the fecal stream by ileostomy induces remission in Crohn's disease, reduces steroid requirements, and prevents postoperative disease recurrence, but symptoms recur soon after reestablishing intestinal continuity[20,21,30] or infusion of luminal contents.[31]

The ratio of anaerobic to aerobic bacteria and the concentration of *Bacteroides* spp. seem to be additional determinants of inflammation. Although the luminal concentration of cultivable anaerobic bacteria in SFBL rats was only slightly increased by 1 log, the number of aerobically grown bacteria was decreased by 2 logs. Therefore, the ratio of anaerobic (which includes obligate and facultative anaerobes) to facultative anaerobic bacteria was up to 1000-fold higher in the SFBL vs. SHAM rats. The ratio of *Bacteroides* spp. to facultative anaerobic bacteria was increased to an even greater degree (10,000–100,000-fold higher in the SFBL vs. SHAM group). Moreover, concentrations of bacteria grown in *Bacteroides*

selective media in the cecum and colon of the SHAM group were 5–6 logs higher than in the proximal small intestine. Although the selected BBE agar probably does not recover all *Bacteroides* and we were able to recover 1% or less of organisms observed by phase-contrast microscopy, an equal percentage of organisms should be recovered in all groups. Total bacterial concentrations were 2 logs lower in the colon of the EX group compared with SHAM rats. Anaerobic bacteria, especially *Bacteroides* spp., have been incriminated in the pathogenesis of Crohn's colitis and experimental colitis by earlier studies. Fecal concentrations and serum antibodies to anaerobic bacteria, such as *Bacteroides*, *Eubacteria*, and *Peptostreptococcus*, are increased in Crohn's disease.[2,32–35] Furthermore, patients with Crohn's disease have increased mucosal antibodies against luminal bacteria, including *Bacteroides* spp.[36] Metronidazole, which is selectively active against anaerobic bacteria and which can chronically suppress *Bacteroides* spp.,[37] is effective in Crohn's colitis and ileocolitis,[16] in spontaneous colitis in B27 TG rats,[18] and in indomethacin-induced chronic intestinal inflammation[4] and prevents hepatobiliary injury and arthritis associated with small bowel bacterial overgrowth in the jejunal SFBL model.[23,25] In the jejunal SFBL model, metronidazole and tetracycline had very little effect on total bacterial concentrations but eliminated *Bacteroides* spp., whereas gentamycin and polymyxin B had no effect on *Bacteroides* concentrations and exhibited no protective benefit on inflammation.[25] Similarly, we show that metronidazole attenuates local inflammation in the cecal SFBL model and eliminates detectable *Bacteroides* spp. *B. vulgatus* has been incriminated in the pathogenesis of HLA-B27 TG rats[12] and carrageenan-induced colitis in guinea pigs[5] by reconstitution studies. In addition, Cong et al.[11] documented $CD4^+$ T cell responses to *B. vulgatus* in C3H/HeJ Bir mice. Furthermore, Garcia-La Fuente et al.[38] recovered *B. fragilis* and *B. uniformis* from inflamed colonic walls after trinitrobenzene sulfonic acid treatment in rats and showed that anaerobes played a key role in transmural inflammation in this model. In the present study, we found *B. vulgatus* and *B. fragilis* to be the dominant *Bacteroides* spp. Although *Bacteroides* spp. appeared to be involved in cecal inflammation, other anaerobes and even aerobes contribute to the inflammatory response, because metronidazole eliminated detectible *Bacteroides* spp. but did not completely eliminate cecal inflammation. However, anaerobic bacteria seem to play only a minor role in ulcerative colitis[2] and antibiotics are not effective with the exception of ciprofloxacin.[39] Although spontaneous colitis in HLA-B27 TG rats more closely resembles ulcerative colitis than Crohn's disease, the presence of focal mucosal ulcerations and transmural inflammation in the presence of increased bacterial loads following creation of an SFBL suggests a Crohn's-like pattern. Furthermore, the TH_1 profile of cytokines in HLA-B27 TG rats[12] more closely resembles Crohn's disease than ulcerative colitis.[40]

Gastritis is a common feature in several colitis models[7,12,41] and is found in up to 60% of Crohn's disease patients after careful investigation.[42] Surprisingly, gastritis in TG rats seems to be more dependent on cecal than gastric bacterial loads. EX and SHAM rats had equal bacterial concentrations in the stomach, yet gastritis was significantly reduced in the EX group and completely absent in some of these animals. We hypothesize that exposure to anaerobic bacteria, such as *Bacteroides* spp., increases mucosal uptake of bacterial components in the cecum, which contains large lymphoid aggregates.[43] This uptake of bacteria and bacterial antigens stimulates mucosal lymphocytes, which then circulate and home to different mucosal sites, such as the colon and stomach, where in the rat the total bacterial load is higher than in the jejunum and ileum. This hypothesis is supported by observations that enterotoxins secreted by several *B. fragilis* spp. increases epithelial permeability and bacterial internalization by HT-29 enterocytes,[44] small bowel bacterial overgrowth enhances mucosal uptake of the bacterial cell wall polymer peptidoglycan-polysaccharide (PG-PS),[26] and the homing of mucosal lymphocytes in SCID mice is dependent on enteric bacteria.[9] Furthermore, this concept is consistent with suppression of colitis in T cell receptor (TCR)-α deficient mice by resection of the cecal tip ("appendix"), which contains a large lymphoid aggregate.[43] Although our results agree in principle with those of Mizoguchi et al.[43] in which early "appendectomy" prevented the onset of colitis in TCR-α knockout mice for up to 6 months, the present study includes several important new advances in knowledge. (1) The influence of the cecum on inflammation in the stomach, an even more remote organ than the colon, further supports the concept of local T-cell induction in the cecum followed by lymphocyte trafficking to remote sites. (2) Microbial assessments strongly implicate normal cecal luminal bacteria in the pathogenesis of local inflammation in the cecum and in remote organs such as the stomach, because the excluded cecum had 2 logs less of cecal bacteria but equal gastric bacteria compared with SHAM controls. In contrast, cecal overgrowth of anaerobic bacteria dramatically aggravates the local inflammation but does not influence gastritis. (3) Appendectomy in the TCR-α knockout mice was performed at 3 weeks of age (before the onset of colitis) and prevented colitis for 6 months,[43] whereas the exclusion of the cecum in B27 TG rats was performed at 2 months of age (after the onset

of gastrointestinal inflammation) and therefore treated established colitis and gastritis. In contrast, appendectomy performed after 5 weeks of age did not prevent colitis in TCR-α mice.[43] Of interest, luminal bacterial stimulation also appears to influence colitis in TCR-α–deficient mice as evidenced by development of an oligoclonal immune response to cecal bacteria[45] and absence of colitis in a sterile environment.[46]

An alternative, but less likely, explanation of the remote inflammation is that cytokines and soluble inflammatory mediators secreted by the inflamed cecal mucosa cause antral mucosal injury. Therefore, in the absence of cecal inflammation, systemic cytokine concentrations could be lower than controls. The lack of enhanced gastritis with significantly more aggressive cecal inflammation with cecal bacterial overgrowth in SFBL TG rats makes this explanation less likely.

Systemic manifestations such as arthritis and leukocytosis, which were more pronounced in the SFBL group, are probably caused by increased mucosal absorption of proinflammatory bacterial products such as lipopolysaccharide (LPS) and PG-PS, or translocation of viable bacteria.[2] In the jejunal SFBL model small intestinal bacterial overgrowth causes hepatobiliary injury,[24] reactivates quiescent arthritis in genetically susceptible Lewis rats,[23] and leads to bacterial translocation into mesenteric lymph nodes[24] and systemic absorption of LPS[47] and PG-PS.[24,26] Furthermore, systemic endotoxemia occurs in trinitrobenzene sulfonic acid– and acetic acid–induced colitis,[48,49] and correlates with disease activity in ulcerative colitis and Crohn's disease.[50] It was somewhat surprising that arthritis occurred only in 1 of 8 TG rats with a cecal SFBL. There might be two explanations: (1) arthritis and hepatobiliary injury in the jejunal SFBL model was induced in genetically susceptible Lewis rats,[23] whereas our TG rats are on the resistant Fischer background[24]; and (2) arthritis in B27 TG Fischer rats in our SPF environment is mild, inconsistent, and follows an undulating pattern with a very late onset.[12] Our rats were killed at 3 months of age corresponding with the earliest onset of arthritis in this model.[14] A mild degree of leukocytosis is expected in B27 TG rats.[12,13] The increased leukocytosis in the TG SFBL group is consistent with our previous observations that TG rats with a jejunal SFBL had higher WBC counts than TG rats without surgery.[51]

This study emphasizes the direct influence of the cecal anaerobic bacterial load, especially *Bacteroides* spp., and the ratio of anaerobic to aerobic bacteria on local inflammation in the cecum, because the degree of inflammation correlates with levels of isolates on *Bacteroides*-selective medium and increased anaerobic/aerobic and *Bacteroides*/aerobic ratios and cecal inflammation was attenuated by metronidazole, which dramatically decreased cecal *Bacteroides* concentrations. Moreover, it shows that the cecal bacterial load influences remote inflammation in the distal colon, stomach, and bone marrow through as yet undefined mechanisms. These results have important therapeutic implications for human inflammatory bowel diseases that may extend past the established approaches of bowel rest and antibiotic therapy. Future studies should focus on more specific antimicrobial therapeutic protocols targeting anaerobic bacteria and *Bacteroides* spp., on blockade of membrane receptors for PG-PS, LPS, and chemotactic peptides from anaerobes, on enhancing luminal growth of protective bacteria competing for anaerobic niches, and on direct immunologic approaches targeting *Bacteroides* antigens.

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