

Different Subsets of Enteric Bacteria Induce and Perpetuate Experimental Colitis in Rats and Mice

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Resident bacteria are incriminated in the pathogenesis of experimental colitis and inflammatory bowel diseases. We investigated the relative roles of various enteric bacteria populations in the induction and perpetuation of experimental colitis. HLA-B27 transgenic rats received antibiotics (ciprofloxacin, metronidazole, or vancomycin-imipenem) in drinking water or water alone in either prevention or treatment protocols. Mice were treated similarly with metronidazole or vancomycin-imipenem before or after receiving 5% dextran sodium sulfate (DSS). Germfree transgenic rats were colonized with specific-pathogen-free enteric bacteria grown overnight either in anaerobic or aerobic atmospheres. Nontransgenic rats colonized with anaerobic bacteria served as negative controls. Although preventive metronidazole significantly attenuated colitis in transgenic rats and DSS-treated mice, it had no therapeutic benefit once colitis was established. Ciprofloxacin also partially prevented but did not treat colitis in B27 transgenic rats. In both animal models vancomycin-imipenem most effectively prevented and treated colitis. Germfree transgenic rats reconstituted with enteric bacteria grown under anaerobic conditions had more aggressive colitis than those associated with aerobic bacteria. These results suggest that a subset of resident luminal bacteria induces colitis, but that a complex interaction of commensal aerobic and anaerobic bacteria provides the constant antigenic drive for chronic immune-mediated colonic inflammation.

Rapidly growing evidence supports the influence of normal enteric bacteria on the pathogenic process of intestinal inflammation and extraintestinal manifestations in experimental colitis and human inflammatory bowel diseases (IBD) (40–43). Both spontaneous and induced inflammation in multiple widely diverse rodent models have been associated with commensal luminal bacteria (1, 11–13, 16, 22, 31, 44, 45, 52, 54). The influence of resident bacteria on the induction and perpetuation of spontaneous colitis and gastritis has been thoroughly studied in HLA-B27/ β_2 -microglobulin transgenic (B27 TG) rats. Colitis, gastritis, and joint inflammation fail to develop in B27 TG rats raised under germfree (sterile) conditions (36, 49). Moreover, when transferred into a specific-pathogen-free (SPF) environment, B27 TG rats universally develop immune-mediated colitis and gastritis within 1 month of bacterial colonization (36).

However, not all luminal bacteria have equal abilities to cause inflammation. Antibiotics with narrow specificities, such as metronidazole, which is selectively active against anaerobic bacteria, are effective in Crohn's colitis and ileocolitis (47) and also attenuate chronic experimental intestinal inflammation induced by indomethacin or carageenan in rats and guinea pigs, respectively (32, 54). In addition, overgrowth of predominantly anaerobic bacteria in bypassed small intestinal segments can lead to systemic inflammation. A jejunal self-filling blind loop induces hepatobiliary inflammation resembling scler-

osing cholangitis and reactivates quiescent arthritis in genetically susceptible Lewis or Wistar rats (24, 25), and some patients undergoing surgical treatment for morbid obesity with creation of bypassed jejunoileal segments develop arthritis and hepatic and skin inflammation (8). In both examples, metronidazole or broad-spectrum antibiotics with anaerobic specificities can reverse these systemic manifestations (15, 23, 25). Creating a cecal self-filling blind loop in B27 TG rats alters the cecal bacterial composition by significantly increasing luminal concentrations of *Bacteroides* spp. and anaerobic bacteria relative to aerobic flora (37). This manipulation of cecal bacterial composition markedly enhances cecal inflammation in these rats, with submucosal inflammation and mucosal ulcers extending to the muscle layer (37). Furthermore, exclusion of the cecum from the fecal stream decreases the total bacterial load in the cecum and results in almost complete healing of cecal inflammation and gastritis, although the concentration of gastric bacteria does not change (37). The most convincing evidence for selective induction of experimental gastrointestinal inflammation with subsets of resident bacteria is provided by reconstitution studies in gnotobiotic B27 TG rats. Previously germfree B27 TG rats colonized with six different obligate and facultative intestinal anaerobic bacteria including *Bacteroides vulgatus* develop much more active colitis and gastritis than littermates colonized with the same selected bacteria without *B. vulgatus* (36). Of note, B27 TG rats colonized with a full complement of SPF bacteria had more inflammation than the B27 TG gnotobiotic rats colonized with the six bacterial species including *B. vulgatus*. The importance of *B. vulgatus* in induction of colitis in this model was confirmed by monoassociation studies, in which *B. vulgatus* but not *Escherichia coli* induced

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colitis equal to that induced by the group of six investigated bacterial species (38). Interestingly, TG rats monoassociated with *B. vulgatus* failed to develop gastritis, which was routinely present in gnotobiotic rats colonized with the six different bacterial strains (38) and in SPF B27 TG rats (36). However, Onderdonk et al. (33) reported that high concentrations of *E. coli* and *Enterococcus* species were associated with severe colitis in B27 TG rats. These results suggest that anaerobic bacteria, especially *B. vulgatus*, have a key role in initiating colitis in B27 TG rats and that other bacterial species, although unable to initiate colitis independently, have an important role in mediating inflammation in remote organs such as the stomach and modulating the intensity of colitis.

Colitis induced by dextran sodium sulfate (DSS) in BALB/c mice was first described in 1990 by Okayasu et al. (30) and is now an established induced model of acute and chronic colitis. Its association with enteric bacteria is well described (28, 30). Okayasu et al. reported significant increases in the population of members of the bacterial species *Bacteroides*, *Enterobacter*, and *Clostridium* but decreases in *Eubacterium* spp. and *Enterococcus* spp. after acute DSS colitis (30). *Bacteroides* spp. were identified as predominantly *Bacteroides distasonis*. Protective effects of metronidazole and the combination of metronidazole and ciprofloxacin in acute DSS colitis were demonstrated by Ohkusa et al. (29) and Hans et al. (19). However, Axelsson et al. (6) have reported that acute DSS colitis can be induced even in a germfree environment and is not significantly different than intestinal inflammation in rodents maintained in a conventional environment, suggesting a direct toxic effect of DSS. In contrast, other investigators found significantly attenuated colitis in germfree mice treated with DSS (50). Several groups have demonstrated that colitis occurs in SCID mice treated for short periods with DSS (5, 14), suggesting that T lymphocytes, B cells, and NK cells are not essential in the acute phase of DSS colitis. Of interest, sulfasalazine treated colitis in germfree DSS mice, even in the absence of bacteria, which are essential to split sulfasalazine into active 5 amino salicylic acid (5-ASA) (4).

To help determine which broad subsets of the complex resident luminal bacterial population preferentially initiate and perpetuate colitis in two distinctly different rodent models, we administered several antibiotics with selective antimicrobial activities to B27 TG rats and BALB/c mice with DSS-induced colitis before and after the onset of intestinal inflammation. In addition, we colonized germfree B27 TG rats with commensal cecal bacteria grown under aerobic or anaerobic conditions. B27 TG rats were selected because these rats develop well-characterized chronic T-lymphocyte-mediated inflammation when exposed to resident nonpathogenic enteric bacteria. The DSS model was chosen to determine which commensal bacteria mediate acute induced intestinal inflammation.

MATERIALS AND METHODS

Animals. Colonies of B27 TG rats and nontransgenic (NT) littermates on a Fischer F344 background (18), originally obtained from Joel D. Taurog (Southwestern Medical School, Dallas, Tex.), were housed and maintained in an SPF environment and under germfree conditions (36). SPF female BALB/c mice were obtained from Charles River, Sulzfeld, Germany, and housed under standard conditions.

Experimental design. B27 TG rats raised in an SPF environment were treated with either antibiotics in drinking water or water alone (as controls) in preven-

tion and treatment protocols. NT rats housed under identical conditions served as negative controls. Prevention (TG rats given water, $n = 8$; TG rats given ciprofloxacin, $n = 8$; TG rats given metronidazole, $n = 9$; TG rats given vancomycin-imipenem, $n = 9$; NT rats given water, $n = 9$) started at 4 weeks of age, soon after weaning and prior to the onset of colitis, which develops between 2 and 3 months of age in SPF conditions at our institution (36). Treatment (TG rats given water, $n = 5$; TG rats given ciprofloxacin, $n = 8$; TG rats given metronidazole, $n = 8$; TG rats given vancomycin-imipenem, $n = 8$; NT rats given water, $n = 9$) was begun at 3 months of age, when SPF B27 TG rats have developed colitis by gross, histologic, and immunologic parameters (36). All rats were killed at 4 months of age, after 1 month of antibiotic treatment and 3 months of prevention. At necropsy cecal tissues were taken for histology and determination of interleukin-1 β (IL-1 β) protein, which has been validated as an indicator of tissue inflammation (36–38). The antibiotics used in this experiment were selected for their activities against various enteric bacterial populations. Metronidazole (40 mg/kg of body weight/day) is selectively active against anaerobic bacteria, including gram-negative bacteria such as *Bacteroides* spp., whereas ciprofloxacin (50 mg/kg/day) is most effective against enteric aerobic gram-negative organisms, with an extended spectrum to anaerobic gram-positive bacteria. Imipenem (50 mg/kg/day), is a very-broad-spectrum antimicrobial drug with efficacy against almost all gram-positive and gram-negative bacteria, including anaerobes, except methicillin-resistant staphylococci, enterococci, and some *Pseudomonas* spp. Vancomycin (50 mg/kg/day) was added to imipenem to cover staphylococci and enterococci.

Acute colitis in BALB/c mice was established by adding 5% DSS (ICN Bio-medicals Inc., Aurora, Ohio) to drinking water for 7 days (20, 30). DSS-treated mice were also treated with either metronidazole (40 mg/kg/day) or vancomycin-imipenem (50 mg/kg/day) in drinking water or with water alone as a positive control. Water consumption was monitored on a daily basis. We were unable to administer ciprofloxacin, since mice refused to drink water containing this agent. Prevention started 3 days before DSS administration, and treatment began 2 days after the onset of DSS feeding. All mice ($n = 5$ for each group) were killed on day 7. At necropsy cecal tissues were taken for histology.

B27 TG rats and NT littermates raised under germfree conditions (36) were divided in two groups at the age of 2 months. Both groups were transferred into separate isolators and colonized with different bacterial populations by oral and anal gavage of 1 ml of culture medium containing the bacteria and placing 0.5 ml on food pellets as previously described (36). One group of B27 TG rats ($n = 6$) received fecal bacteria from SPF TG rats; the bacteria were incubated overnight in a thioglycolate broth in an anaerobic 5% CO₂-10% H₂-85% N₂ atmosphere. The second group of B27 TG rats ($n = 9$) received fecal bacteria from the same rat which were grown overnight in brain heart infusion broth under aerobic conditions. NT rats ($n = 9$) with fecal bacteria grown under anaerobic conditions served as negative controls. Rats were killed 1 month after bacterial colonization.

Gross inflammatory scores. At necropsy the degree of thickening of the mid-cecum of rats was blindly scored using a previously validated scale from 0 to 4+ scale (36).

Histology. Tissues were prepared as previously described (36). A validated histologic inflammatory score ranging from 0 to 4+ was used for blinded evaluation of cecal inflammation in the B27 TG rats (36). This score was adapted to mice with DSS-induced colitis.

Rat IL-1 β enzyme-linked immunosorbent assay. We measured IL-1 β protein concentrations in homogenized cecal tissues by enzyme-linked immunosorbent assay as previously described (37), using antibodies that were provided by S. Poole, National Institute of Biological Standards and Controls, Hertfordshire, United Kingdom. We previously have documented upregulation of IL-1 and other proinflammatory cytokines produced by activated macrophages in SPF B27 TG rats (36) and correlated tissue IL-1 β with histology scores and tissue myeloperoxidase (36–38).

Determination of luminal bacterial concentrations. The ceca of euthanized non-B27 TG rats were removed, and 1 ml of cecal contents was taken immediately, weighed, and serially diluted in prerduced thioglycolate broth. From every dilution 100 μ l was plated on prerduced anaerobically sterilized agar plates in an anaerobic 5% CO₂-10% H₂-85% N₂ atmosphere. Aerobic culture was performed using blood agar plates. Colonies were counted after 2 (aerobic culture) and 6 (anaerobic culture) days of incubation at 37°C. *Bacteroides* spp. were selectively grown on *Bacteroides* Bile Esculin agar under anaerobic conditions. Morphology was checked by Gram staining. Total bacterial concentrations were determined on serial dilutions with phase-contrast microscopy using a Neubauer counting chamber. Identification of presumed *Bacteroides* spp. was performed by standard procedures (46). Results were normalized for stool dry weights.

Statistical analysis. All data are expressed as the mean \pm standard error of the mean (SEM). After testing for equal distribution, analysis of variance was used

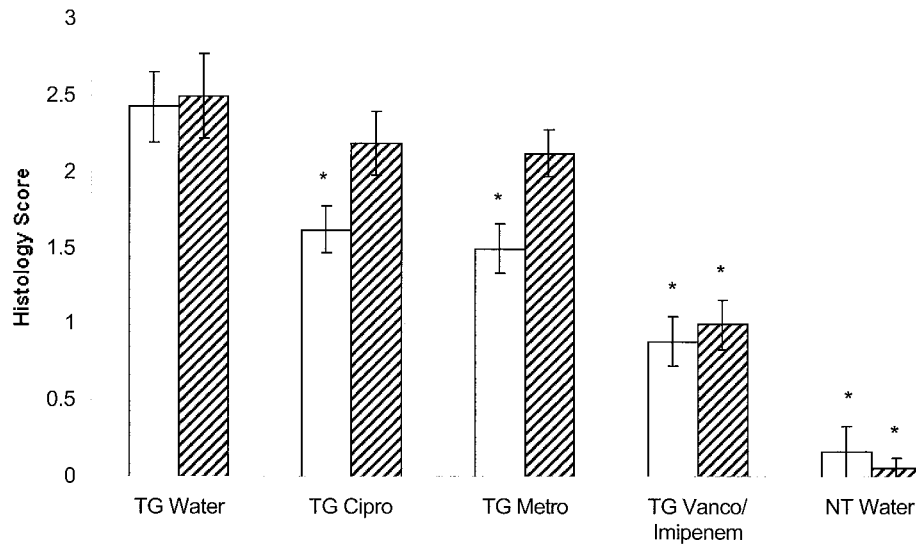


FIG. 1. Blinded histologic scores of the ceca from B27 TG treated with different antibiotics starting before (4 weeks of age, prevention protocol) (□) and after (3 months of age, treatment protocol) (▨) the onset of colitis. All rats were killed at 4 months of age. $n = 8$ or 9 rats in each group. *, $P < 0.05$ versus TG water controls. Cipro, ciprofloxacin; Metro, metronidazole; Vanco, vancomycin. Error bars indicate SEMs.

to test for differences between the groups. Gross and histologic inflammatory scores, tissue IL-1 β levels, and fecal bacterial concentrations with equal distribution were compared to the control group (TG rats given water) using multiple-comparison procedures (Bonferroni t test). Gross and histologic inflammatory scores which did not pass the equal distribution test were analyzed using the nonparametric Kruskal-Wallis one-way analysis of variance on ranks and Dunn's method for the multiple-comparison procedures. All tests were performed using SigmaStat software from SSPS Inc. A P value of < 0.05 was considered statistically significant.

RESULTS

Antibiotics. To determine which broad classes of luminal bacteria are responsible for initiation of colitis, 4-week-old SPF B27 TG rats were given antibiotics with selective activities prior to onset of colitis (preventive protocol). The same antibiotics were administered to 3-month-old B27 TG rats after onset of colitis in a therapeutic protocol. The degree of colitis was measured by blinded gross and histologic scores as well as tissue IL-1 β concentration, which is a validated marker of macrophage activation and inflammation in this model (36–38). Although preventive metronidazole significantly attenuated colitis in B27 TG rats grossly (0.5 ± 0.2 , versus 1.6 ± 0.3 for B27 TG rats given water; $P < 0.05$) and histologically ($P < 0.001$) (Fig. 1), there was no benefit in treating with metronidazole once colitis was established (gross gut score, 1.6 ± 0.3 for metronidazole versus 1.9 ± 0.6 water [not significant {NS}]) (Fig. 1 and 2A and B). These results were confirmed by cecal IL-1 β (Fig. 3). Similarly, mice were treated with antibiotics 3 days before (prevention) or 2 days after (treatment) onset of DSS administration. Metronidazole reduced the severity of DSS-induced colitis when given prophylactically to mice ($P = 0.01$) (Fig. 4), but there was no statistically significant therapeutic effect. The effect of ciprofloxacin on histologic scores was similar to that of metronidazole ($P < 0.05$ in prevention, NS in treatment) (Fig. 1), but it did not significantly reduce the gross scores (prevention, 0.4 ± 0.1 [NS]; treatment, 1.2 ± 0.3 [NS]) or tissue IL-1 β concentrations (Fig. 3) with either protocol.

In both animal models broad-spectrum antibiotic therapy with combined vancomycin-imipenem was effective in both prevention (gross score, 0.2 ± 0.1 [$P < 0.05$]; histologic scores, $P < 0.0001$ [Fig. 1 and 4]; IL-1 β , $P < 0.01$ [Fig. 3]) and treatment of established colitis (gross score, 0.3 ± 0.1 [$P < 0.01$]; histologic scores, $P < 0.0001$ [Fig. 1, 2A and C, and 4]; IL-1 β , $P < 0.05$ [Fig. 3]). However, even vancomycin-imipenem did not completely abrogate histologic inflammation in the B27 TG rats (histologic score, $P < 0.005$ versus NT control rats given water [Fig. 1]), although there was no difference in IL-1 β concentrations relative to NT control rats given water (Fig. 3). In contrast, this broad-spectrum antibiotic combination almost completely blocked DSS-induced colitis (Fig. 4).

These results indicate that selective antibiotics can attenuate the onset of colitis in two disparate models, but only broad-spectrum treatment can reverse established disease. These data suggest that both aerobic and anaerobic bacteria can initiate inflammation but that a more complex group of bacteria is required to perpetuate disease.

Microbial assessments. We then measured total cecal concentrations of bacteria in antibiotic-treated versus water-treated control rats as well as the relative concentrations of anaerobic and aerobic subsets and *Bacteroides* species, which have been implicated in the pathogenesis of colitis in B27 TG rats (36, 38). Total cecal bacterial concentrations in the rats were significantly reduced after 4 weeks of antibiotic treatment in all therapeutic groups relative to the water-treated controls (control, $2.7 \times 10^{10} \pm 1.0 \times 10^{10}$ CFU/g of stool; vancomycin-imipenem, $2.0 \times 10^8 \pm 0.8 \times 10^8$ CFU/g of stool [$P < 0.025$ versus control]; metronidazole, $1.9 \times 10^9 \pm 0.6 \times 10^9$ CFU/g of stool [$P < 0.035$ versus control]; ciprofloxacin, $3.9 \times 10^9 \pm 0.5 \times 10^9$ CFU/g of stool [$P < 0.045$ versus control]) (Fig. 5) but were lowest in the vancomycin-imipenem-treated animals ($P < 0.02$ versus metronidazole and $P < 0.0002$ versus ciprofloxacin) (Fig. 5), which had at least 1-log-unit-fewer organisms than all other groups. Sequential evaluation of cecal bacterial

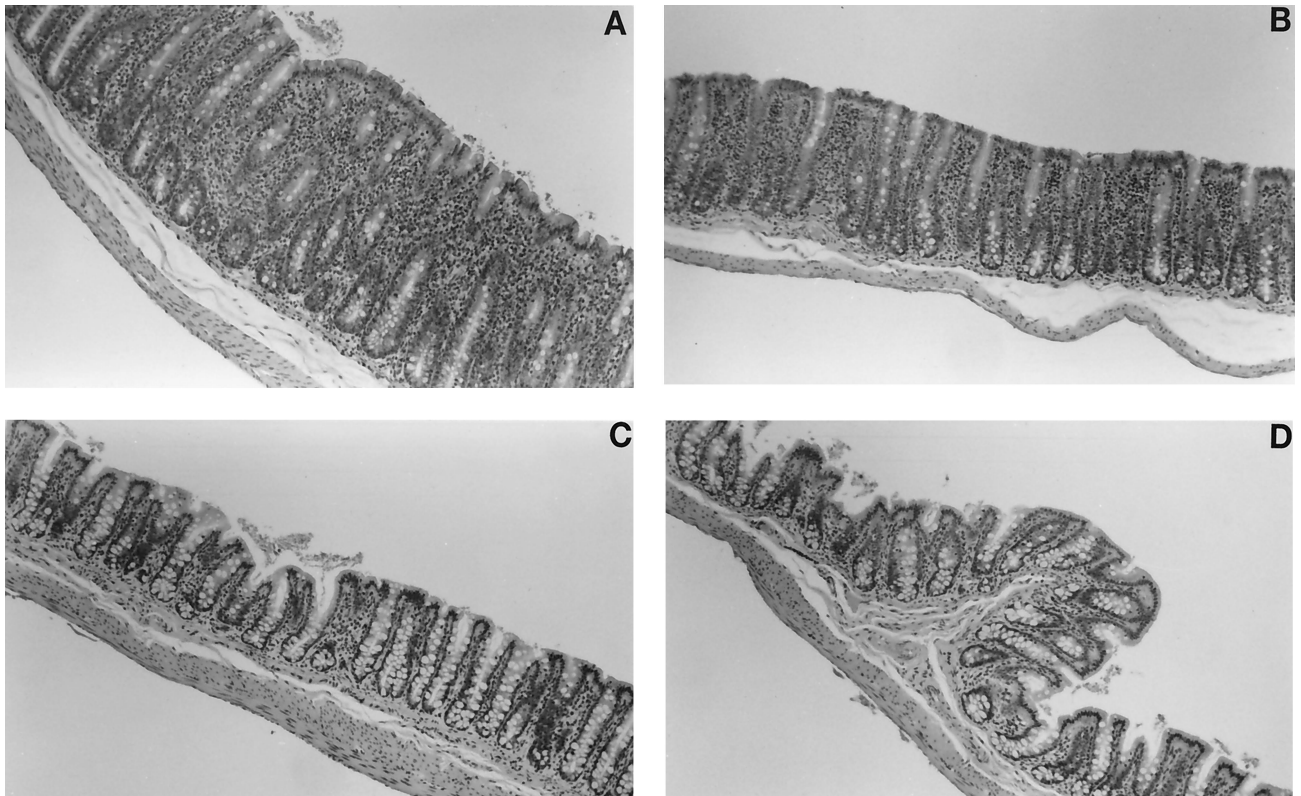


FIG. 2. (A) Photomicrograph of cecal inflammation in a 4-month-old SPF B27/ β_2 -microglobulin TG rat treated with water alone. (B) A TG rat treated with metronidazole after the onset of colitis (3 months of age) showed only slight reduction of cecal inflammation. (C) The cecum of a TG rat treated with vancomycin-imipenem after the onset of colitis (3 months of age) revealed substantially less cecal inflammation. (D) Normal cecum of a 4-month-old NT control rat treated with water alone.

concentrations revealed an initial 1,000-fold decrease of intestinal flora after 3 weeks of therapy with vancomycin-imipenem (5.4×10^7 CFU/g) with a subsequent increase in cecal concentrations to 1.3×10^9 CFU/g after 6 weeks, which was still 50-fold less than those in water-treated controls. The interval

increase in bacterial concentration between 3 and 6 weeks of antibiotic administration was minimal in the metronidazole-treated group and no increase was seen in the ciprofloxacin-treated group, although the initial decrease was not nearly as marked as with combination therapy (data not shown). At the

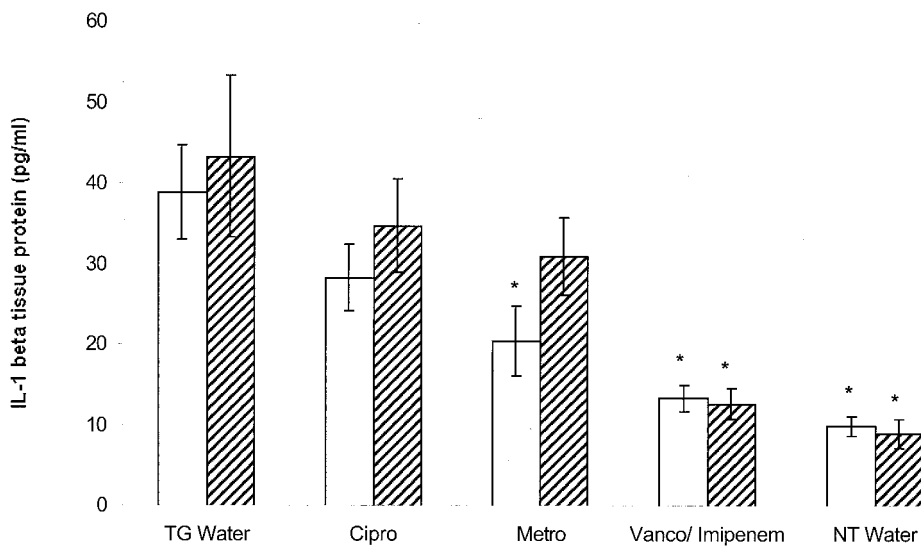


FIG. 3. Tissue IL-1 β protein concentrations of the ceca from B27 TG rats treated with different antibiotics starting before (4 weeks of age) (\square) and after (3 months of age) (▨) the onset of colitis. *, $P < 0.05$ versus TG water. Cipro, ciprofloxacin; Metro, metronidazole; Vanco, vancomycin. Error bars indicate SEMs.

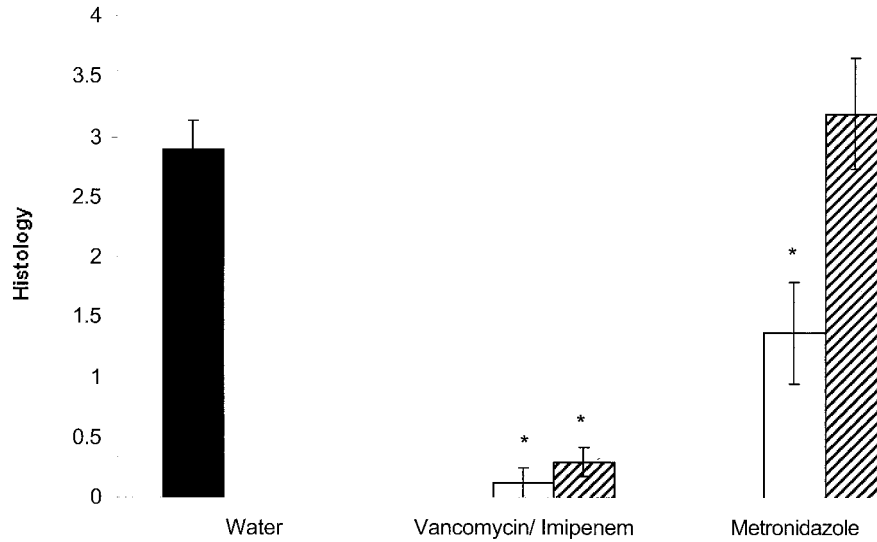


FIG. 4. Blinded histologic scores of the ceca from BALB/c mice with DSS-induced colitis treated with different antibiotics starting 3 days before or 2 days after the onset of DSS administration. The 5% DSS was continued for a total of 7 days. *, $P < 0.05$ versus water. □, Prevention; ▨, treatment; ■, water control. Error bars indicate SEMs.

end of 8 weeks of antibiotic treatment cecal *Bacteroides* spp. were absent in the metronidazole-and vancomycin-imipenem-treated groups (with one exception in the vancomycin-imipenem-treated group) but were detected in all animals of the ciprofloxacin-treated and control groups. The ratio of anaerobically to aerobically grown bacteria (reflecting the concentration of obligate anaerobic bacteria) compared with that in the control group after three weeks of antibiotic therapy was 0.5 in the vancomycin-imipenem-treated group, 0.1 in the metronidazole-treated group, and 1.5 in the ciprofloxacin-treated group and did not change over the treatment period (Fig. 6),

suggesting a strong decrease of obligate anaerobic bacteria in the metronidazole-treated group, a decrease of both aerobic and anaerobic bacteria in the vancomycin-imipenem-treated group (with slightly more effect on anaerobes than aerobes), and a selective decrease of aerobic bacteria in the ciprofloxacin-treated group. These results suggest that the decrease in luminal bacterial concentrations correlates with the therapeutic efficacy of antibiotics, that both aerobic and anaerobic bacterial populations are implicated in disease, and that while *Bacteroides* spp. may be important in the pathogenesis of disease, clearly other bacterial strains have contributory roles.

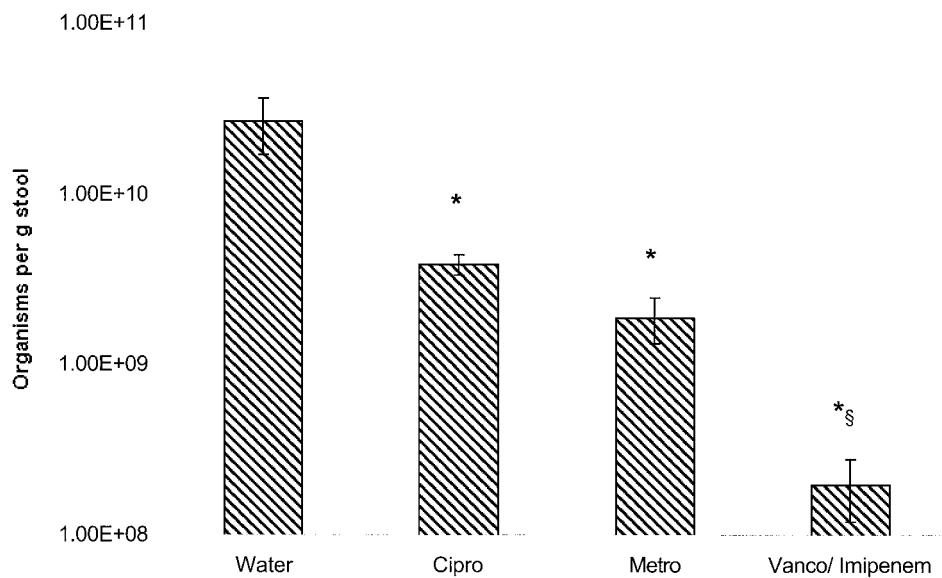


FIG. 5. Total bacterial concentrations in the cecal contents of NT rats treated with different antibiotics for 4 weeks, measured in a counting chamber. *, $P < 0.05$ versus control; §, $P < 0.05$ versus ciprofloxacin (Cipro) and metronidazole (Metro). Vanco, vancomycin. Error bars indicate SEMs.

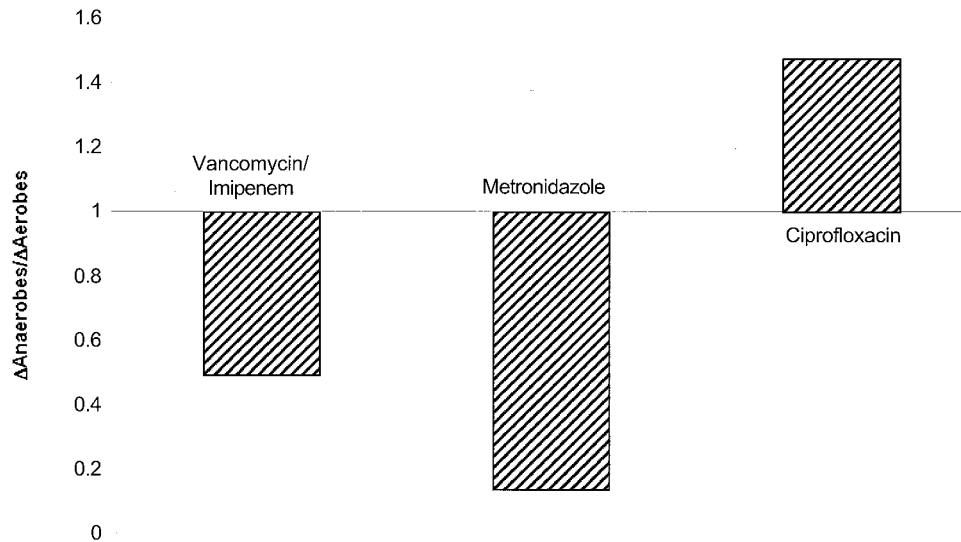


FIG. 6. Alteration of the ratio of anaerobic bacteria to aerobic bacteria by antibiotics compared to the ratio of anaerobic bacteria to aerobic bacteria in the water control group ($[\text{anaerobes/aerobes with antibiotics}]/[\text{anaerobes/aerobes for water control}]$). Antibiotic treatment resulted in an altered bacterial composition after 6 weeks. Compared with the water controls, vancomycin-imipenem reduced more anaerobes than aerobes as demonstrated by the decreased ratio of anaerobes to aerobes to 0.5 of that of the water control group. This alteration of bacterial composition was even exceeded by metronidazole, where the ratio of anaerobes to aerobes dropped to 1/10 of that of the water control group. In contrast ciprofloxacin reduced predominantly aerobes as shown by the increase of the anaerobic/aerobic ratio to 1.5-fold that of the water control group.

Reconstitution with enteric bacteria cultured under aerobic versus anaerobic conditions. We then populated germfree B27 TG rats with aerobically and anaerobically cultivated enteric bacteria to determine the relative abilities of these subsets to induce experimental colitis. Rats were maintained in Trexler isolators with sterile food and water for 1 month to prevent colonization with other organisms. Both groups of rats had similar concentrations of fecal bacteria (10^{12} CFU/g of stool), which were comparable to levels in SPF rats. B27 TG germfree

rats colonized with a culture of SPF fecal flora incubated for 24 h in an anaerobic atmosphere developed more aggressive cecal inflammation than littermates associated with aerobically cultivated bacteria from the same source (histology scores, 2.3 ± 0.2 for anaerobic atmosphere versus 1.4 ± 0.1 for aerobic atmosphere [$P < 0.01$]) (Fig. 7). However, B27 TG rats colonized with the aerobic flora had significantly more inflammation than NT littermates colonized with anaerobes, which had no evidence of colitis (0.3 ± 0.1 [$P < 0.0001$]). These experi-

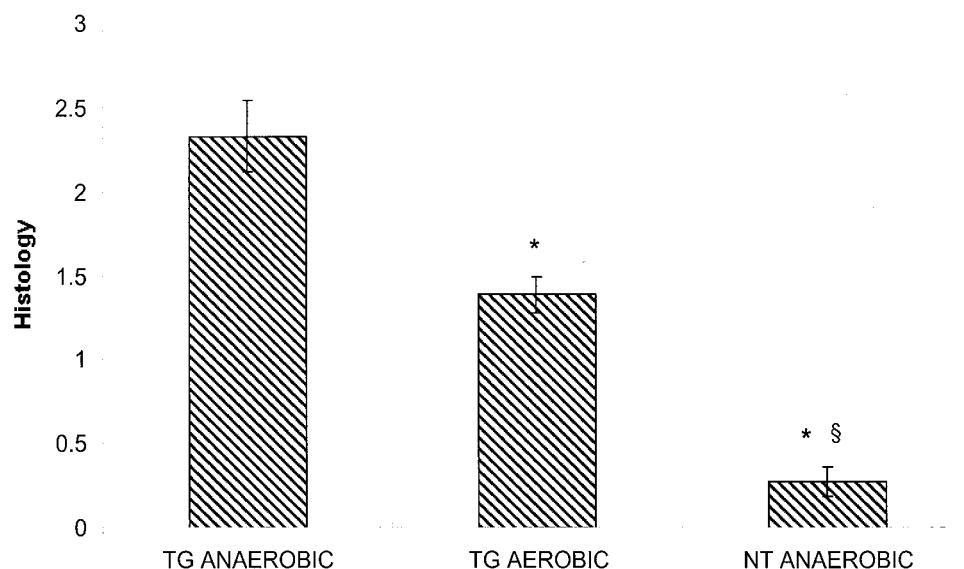


FIG. 7. Blinded histologic scores of the ceca from germfree B27 TG rats colonized for 1 month with cultures of SPF cecal bacteria incubated overnight under anaerobic or aerobic conditions. $n = 6$ to 9 rats/group. *, $P < 0.05$ versus anaerobic; §, $P < 0.05$ versus aerobic. Error bars indicate SEMs.

ments indicate that both aerobic and anaerobic nonpathogenic commensal bacteria can induce colitis in genetically susceptible rats, but the anaerobic population is more aggressive in this model, despite equal fecal concentrations of the two bacterial subsets.

DISCUSSION

Growing evidence in the past five years has supported the crucial role of enteric bacteria in the pathogenesis of chronic intestinal inflammation (41, 42). However, rodent models indicate that not all bacteria have equal capabilities of inducing gastrointestinal inflammation. Our present data further illustrate the complexities of bacterial composition in the pathogenesis of experimental colitis in two different rodent models. In the present study we used the DSS model as a prototype of an acute inducible colitis and the B27 TG rat model as an example of spontaneous, T_{H1} chronic immune-mediated gastrointestinal inflammation in a genetically engineered host (36, 48).

One major finding of the present study is the lack of therapeutic effects for antibiotics with narrow specificities. Metronidazole was unable to treat inflammation once colitis was established, although it was effective in both animal models in a prevention protocol. On the other hand, the broad-spectrum combination of vancomycin-imipenem was able to more effectively prevent colitis as well as treat established disease. These results are consistent with our previous observation that mono-association of gnotobiotic B27 TG rats with *B. vulgatus* induced colitis which was less aggressive than that present in SPF B27 TG rats (36, 38). Together these antibiotic and gnotobiotic studies suggest a predominant role for *Bacteroides* spp. in initiating colitis in this model, with a synergistic effect of an unknown number of other enteric bacteria in perpetuating and mediating disease (37, 38). However, the observation that the broad-spectrum combination of vancomycin-imipenem was also significantly more effective than metronidazole in preventing colitis suggests initial proinflammatory capabilities from bacterial species other than *Bacteroides*, since *Bacteroides* spp. were undetectable in both metronidazole and vancomycin-imipenem treatment groups. The ability of certain aerobic bacteria to initiate inflammation is further supported by the induction of mild colitis by colonization of germfree B27 TG rats with aerobic bacteria and a modest protective effect of ciprofloxacin in the present study. Videla et al. and Mourelle et al. also showed that the vancomycin-imipenem combination was superior to single antibiotics, including metronidazole, in experimental colitis induced by trinitrobenzene sulfonic acid (53) and could prevent fibrosis in these rats with induced colonic ulcers (27). Likewise, Madsen et al. (26) reported that the combination of metronidazole and neomycin prevented and treated colitis in IL-10-deficient mice and was superior to ciprofloxacin, which prevented the onset of colonic inflammation but only partially reversed established colitis, while Hans et al. (19) demonstrated that intraperitoneal metronidazole plus ciprofloxacin could improve acute but not chronic DSS-induced colitis. Clinical trials further support the ability of metronidazole to prevent Crohn's disease with modest therapeutic effects as a single agent but improved efficacy in combination with other antibiotics. Rutgeerts et al. (39) demonstrated that met-

ronidazole given after curative ileal resection for 3 months significantly decreased postoperative recurrence of Crohn's disease for up to 1 year. In a therapeutic protocol monotherapy with metronidazole significantly decreased the Crohn's disease activity index in patients with colitis and ileocolitis but had no effect on patients with isolated ileal disease and did not lead to a significant induction of remission (Crohn's disease activity index, <150) (47). More recently, Prantera et al. (35) reported that metronidazole in combination with ciprofloxacin was effective in the treatment of moderately active Crohn's disease.

The preventive effect of ciprofloxacin in B27 TG rats, although supported only by blinded histologic score and not by IL-1 β tissue concentration, was somewhat surprising in light of our previous observations that *B. vulgatus* has a preferential ability to induce colitis in this model (36, 38). Although others report some effect of ciprofloxacin on *Bacteroides* spp. (34), in our hands ciprofloxacin given in drinking water reduced total intestinal bacterial concentrations less than 1 log unit and had no effect on *Bacteroides* spp. Unfortunately we were unable to test these findings in DSS-treated mice, since these mice refused to drink water containing ciprofloxacin. Similarly, Madsen et al. (26) showed only a modest decrease (1 log unit) in mucosally adherent-invasive *Bacteroides* spp. following 8 weeks of oral ciprofloxacin treatment in IL-10 $^{-/-}$ mice, in contrast to clearance of all detectable *Bacteroides* with neomycin-metronidazole administration. The lack of any effect of preventive ciprofloxacin on the gross gut score and tissue IL-1 β level in our study and the preliminary results of Braat et al. (7), where IL-10 $^{-/-}$ mice treated either preventively or therapeutically with the same dose of ciprofloxacin received no benefit, and of Madsen et al. (26), where ciprofloxacin had only a modest therapeutic benefit in established colitis in IL-10 $^{-/-}$ mice, are in contrast to our observed histologic findings. Similarly, reports of the clinical effect of ciprofloxacin in IBD are somewhat inconsistent. Turunen et al. described a concomitant role of this antibiotic in preventing relapse of ulcerative colitis (51) but no effect on Crohn's disease using a similar protocol. However, recent reports by Colombel et al. (9) and preliminary data by Arnold et al. (3) demonstrated beneficial therapeutic effects of ciprofloxacin in mild to moderate active Crohn's disease. Gionchetti et al. have demonstrated efficacy of ciprofloxacin in combination with a broad-spectrum nonabsorbable antibiotic, rifaximin, in refractory pouchitis (17). These results suggest that aerobic gram-negative bacteria contribute to intestinal inflammation, which is supported by our observations of modest induction of experimental colitis in B27 TG rats obtained by aerobic cultures of cecal contents and by data from Onderdonk and colleagues, who revealed a dramatic increase of *E. coli* and *Enterococcus* spp. in cecal contents of HLA-B27 transgenic rats with severe chronic colitis (33).

The mechanism of action of metronidazole has been questioned in light of the possible direct immunomodulating capabilities of this antibiotic in indomethacin-induced small bowel inflammation (2, 10). In support of an antimicrobial role of metronidazole in our experiments, we demonstrated a prolonged suppression of *Bacteroides* spp. with chronic administration of metronidazole and with imipenem-vancomycin, which were the agents which most effectively prevented colitis in the B27 TG rats. Germfree B27 TG rats colonized with SPF fecal bacterial flora grown overnight under anaerobic condi-

tions developed active colitis, whereas population with aerobic bacterial cultures devoid of all obligate anaerobic species, including *Bacteroides*, produced only mild inflammation. While these experiments do not exclude a possible synergistic immunomodulatory effect of metronidazole, they indicate the importance of obligate anaerobic bacteria in the pathogenesis of colitis in this model.

Microbial assessment following antibiotic therapy revealed interesting findings. Although vancomycin-imipenem predictably suppressed bacterial counts after 3 weeks of administration, this was a transient effect with a 2-log-unit rebound in total bacterial counts between 3 and 6 weeks, consistent with the proliferation of resistant bacteria which partially repopulated the colon despite continued antibiotic administration. However, after 6 weeks of vancomycin-imipenem administration, total luminal bacterial counts remained 1 log unit less than control levels. This delayed increase in bacterial concentrations was less evident with metronidazole treatment and was absent with ciprofloxacin therapy. The discrepancy between the prolonged therapeutic effect of vancomycin-imipenem administration and increasing bacterial concentrations suggests that this combination of antibiotics may have a lasting effect on colitis by altering some components of the bacterial milieu in a sustained fashion. This concept is supported by lack of detection of *Bacteroides* spp. even after 8 weeks of therapy with vancomycin-imipenem despite a rebound in total bacterial counts. Therapeutic effects of protracted administration of vancomycin-imipenem may be prolonged because of the long-term elimination of *Bacteroides* spp. and possibly other resident bacterial species, which may be sufficient to prevent recurrence after induction of remission. Metronidazole, which also permanently suppressed *Bacteroides* spp., was able to prevent onset of colitis in B27 TG rats and to prevent relapse of Crohn's disease after a surgically induced remission (39). Clinical studies have shown that chronic metronidazole therapy can eliminate fecal *Bacteroides* for at least 6 months (21).

The present study convincingly reveals an important but complex effect of luminal bacteria in the acute phase as well as the chronic phase of experimental colitis. The present results in conjunction with our previous observations strongly support the following hypotheses (i) Normal luminal bacteria are required for development of chronic immune-mediated intestinal inflammation. (ii) Commensal enteric bacterial species have unequal proinflammatory capabilities, with some being more aggressive than others. (iii) Various endogenous bacteria have different roles in the inflammatory process. Some, including *Bacteroides* spp. and other, yet-to-be identified species partially suppressed by ciprofloxacin and more completely eliminated by vancomycin-imipenem, preferentially initiate inflammation, while another, perhaps larger spectrum of intestinal bacteria perpetuate disease. (iv) An initial reduction of the total bacterial load with a broad-spectrum antibiotic combination alters the bacterial composition with a lasting effect on intestinal inflammation, although the total luminal concentration recovers rapidly.

These findings have important clinical therapeutic implications and lay the foundation for clinical trials of broad-spectrum antibiotics or antibiotic combinations in human IBD. It may be possible to treat active Crohn's disease with a broad-

spectrum antibiotic combination until remission is achieved and then switch to prophylactic therapy with metronidazole.

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