Anti-inflammatory properties of Lactobacillus gasseri expressing manganese superoxide dismutase using the interleukin 10-deficient mouse model of colitis

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Carroll IM, Andrus JM, Bruno-Bárcena JM, Klaenhammer TR, Hassan HM, Threadgill DS. Anti-inflammatory properties of Lactobacillus gasseri expressing manganese superoxide dismutase using the interleukin 10-deficient mouse model of colitis. Am J Physiol Gastrointest Liver Physiol 293: G729–G738, 2007. First published July 19, 2007; doi:10.1152/ajpgi.00132.2007.—Emerging evidence has implicated reactive oxygen species (ROS) in the pathogenesis of inflammatory bowel disease (IBD). Although intestinal epithelial cells produce the ROS-neutralizing enzyme superoxide dismutase (SOD), the protein and activity levels of copper/zinc (Cu/Zn) and manganese (Mn) SOD are perturbed in inflamed tissues of IBD patients. Thus we investigated the ability of MnSOD from (Cu/Zn) and manganese (Mn) SOD are perturbed in inflamed tissues epithelial cells produce the ROS-neutralizing enzyme superoxide dismutase (SOD), the protein and activity levels of copper/zinc (Cu/Zn) and manganese (Mn) SOD are perturbed in inflamed tissues of IBD patients. Thus we investigated the ability of MnSOD from Streptococcus thermophilus to reduce colitis symptoms in interleukin (IL) 10-deficient mice using Lactobacillus gasseri as a delivery vehicle. Cohorts of 13–15 IL-10-deficient mice were left untreated or supplemented with native L. gasseri or L. gasseri expressing MnSOD for 4 wk. Colonic tissue was collected and inflammation was histologically scored. The presence of innate immune cells was investigated by immunohistochemistry and the host antioxidant response was determined by quantitative PCR. It was demonstrated that L. gasseri was stably maintained in mice for at least 3 days. L. gasseri producing MnSOD significantly reduced inflammation in IL-10-deficient mice compared with untreated controls (P < 0.05), whereas the anti-inflammatory effects of both native and MnSOD producing L. gasseri were more pronounced in males. The anti-inflammatory effects of L. gasseri were associated with a reduction in the infiltration of neutrophils and macrophages. Transcripts of antioxidant genes were equivalent in colonic tissues obtained from control and probiotic-treated IL-10-deficient mice. This study demonstrates that L. gasseri producing MnSOD has significant anti-inflammatory activity that reduces the severity of colitis in the IL-10-deficient mouse.

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Lactobacilli; oxidative stress; inflammatory bowel disease

UCOLERATIVE COLITIS (UC) and Crohn’s disease (CD), collectively known as inflammatory bowel disease (IBD), are disorders with unknown causes and are characterized by recurrent intestinal inflammation. IBD is believed to result from one or a combination of factors including genetic-based host susceptibility, enteric microflora, and a dysfunctional immune response (33). In addition to these factors, emerging evidence has suggested a role for oxidative stress in the initiation and progression of IBD since increased levels of oxidized proteins, lipids, and nucleic acids are found in the inflamed mucosa of IBD patients (10, 16).

It is well established that inflammation in the gut is associated with the recruitment of activated phagocytic leukocytes and the production of inflammatory mediators such as proteases, cytokines, arachidonic acid metabolites, and reactive oxygen species (ROS) (34). Recent studies have demonstrated that ROS, such as the superoxide anion, are not merely by-products of inflammation but are central to its pathogenesis (16, 21, 30). The normal intestinal mucosa is equipped with a network of antioxidant enzymes that neutralize ROS in a two-step pathway. First, superoxide dismutases (SODs) convert the primary superoxide anion (O2·−) into the more stable metabolite, hydrogen peroxide (H2O2). Second, H2O2 is converted to water by catalase or glutathione peroxidase. The activities of these enzymes are usually balanced to maintain a low and continual steady-state level of ROS. However, the levels of SOD in IBD patients are frequently depleted (14, 17), highlighting the potential for elevated levels of this enzyme to function as a therapeutic for IBD.

Three isofoms of SOD are found in humans: copper/zinc (Cu/Zn)-SOD, manganese (Mn)-SOD, and extracellular (EC)-SOD. The abundance and activity of all three isofoms of SOD in paired inflamed and noninflamed tissues from CD and UC patients compared with samples from normal control mucosa were recently investigated (13, 14). The level of Cu/ZnSOD protein was mildly reduced in the lamina propria of inflamed tissue whereas MnSOD protein was increased, although this was not accompanied by an increase in activity. Since Cu/ZnSOD is a key antioxidant and is reduced in inflamed colonic tissues, the potential therapeutic effects of this enzyme as a treatment for IBD have been investigated. Interestingly, both protective (15, 34, 35) and antagonistic (12) effects have been reported for Cu/ZnSOD. The variability of efficacy of Cu/ZnSOD in these studies may be due to the different experimental models used and the mode of delivery of the enzyme. Similar studies have not been reported for MnSOD.

Another important characteristic of IBD is an aggressive reaction to enteric microflora, an aspect that is reiterated in all current mouse models of colitis. Although specific members of the gut flora can be more colitogenic than others (33), it is not...
known which members are most relevant in human patients. Because the microflora plays an important role in IBD, numerous studies have focused on how to utilize commensal bacteria or “probiotics” as a treatment strategy for IBD. Probiotics are live microorganisms that when administered in adequate amounts confer a health benefit on the host (FAO 2001, www.fao.org). It is believed that increasing the numbers of specific organisms within the gut microbiota can have a beneficial effect either directly by regulating the host immune system (23, 28, 41, 44) or indirectly by altering the composition of the gut flora (7, 8). The beneficial effects of the Lactobacilli, members of the lactic acid bacteria, have received particular attention. These organisms are present at relatively low levels in the human colon, but recent studies have demonstrated their ability to modulate inflammation in the IL-10-deficient mouse model of colitis using Lactobacillus reuteri (18), a combination of L. reuteri and L. paracasei (27), L. plantarum (25), and L. salivarius (19, 23) and to help prevent enteric infections by using L. casei (29). The use of microorganisms as vehicles to deliver biologically active molecules for the treatment of gastrointestinal diseases has also been demonstrated (39). Consequently, the present study was designed to investigate the therapeutic effects of L. gasseri producing the MnSOD from Streptococcus thermophilus in the IL-10-deficient mouse model of colitis.

MATERIALS AND METHODS

**Bacterial strains.** The probiotics used in these studies were L. gasseri NC1500 and L. gasseri NC1501 (3, 4). L. gasseri NC1500 harbors the empty plasmid pTRK563 whereas L. gasseri NC1501 harbors the plasmid pSodA. pSodA was constructed by cloning the superoxide dismutase gene, sodA, from S. thermophilus into pTRK563. It has been demonstrated that L. gasseri expressing MnSOD offers protection to this organism from the superoxide radical, hydrogen peroxide, and the hydroxyl radical, in vitro (3, 4). Both strains of L. gasseri were grown aerobically in Lactobacillus broth according to de Man, Rogosa, and Sharpe (MRS broth) containing erythromycin at a final concentration of 2 μg/ml at 37°C. The L. gasseri strain used in this study was chosen due to its human origin (3) and its effective ability to colonize the mouse gastrointestinal (GI) tract.

**IL-10-deficient model.** The wild-type C57BL/6J mice and its congenic interleukin 10-deficient (IL-10−/−) mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Mice were bred and maintained under specific pathogen-free conditions (SPF) in ventilated racks with HEPA-filtered air at constant temperature and humidity on a 12:12-h light-dark cycle in American Association for Accreditation of Laboratory Animal Care-approved facilities at the University of North Carolina at Chapel Hill. Mouse cages were fitted with wire bottoms at the initiation of experiments to prevent coprophagy. Mice were fed Purina 5058 rodent chow and water ad libitum under SPF conditions. All studies were performed under Institutional Animal Care and Use Committee-approved protocols.

**Detection of L. gasseri.** Colonization/persistence of each of the L. gasseri strains (NC1500 and NC1501) in the gut of the wild-type mice was tested by orally administering 1 × 10⁹ colony-forming units (CFU) of each strain in 20 μl of sterile PBS to C57BL/6J mice (n = 5 mice for each strain) every day for 5 days. Fecal pellets were collected from before, during, and up to 7 days after the probiotic administration. Each fecal pellet was homogenized in 1 ml of sterile PBS. The homogenates were serially diluted and plated onto MRS agar containing 2 μg/ml erythromycin. The daily average CFU of either probiotic strain in mouse feces were determined.

**Probiotic therapy.** C57BL/6J-IL-10−/− mice begin developing inflammation around 4 wk of age when housed in the University of North Carolina animal facilities. Although the C57BL/6J-IL-10−/− mice are regarded as colitis resistant (1), the presence of antagonistic gut flora like Helicobacter hepaticus (H. hepaticus) can dramatically accelerate colitis. Random sampling of feces from of our IL-10−/− mice demonstrated the presence of H. hepaticus and Enterococcus faecalis (potentially colitogenic) in this mouse colony. Consequently, L. gasseri administration was carried out on 3-wk-old mice before the onset of inflammation and continued for 4 wk. Fresh cultures of L. gasseri NC1500 and NC1501 were grown in MRS broth containing 2 μg/ml erythromycin. The bacterial cells were pelleted and washed twice with PBS. The concentration of each L. gasseri strain was adjusted to 1 × 10⁹ CFU/ml based on OD₆₀₀ readings that had previously been correlated with CFU numbers (data not shown). Next, 1 ml of each suspension was pelleted by centrifugation and then resuspended in 20 μl of PBS. Mice were administered sterile PBS (control group; 8 male and 7 female), L. gasseri NC1500 (5 male and 8 female) or L. gasseri (MnSOD) NC1501 (7 male and 7 female).
Each mouse was supplemented with 20 μl PBS (control) or 1 × 10⁹ CFU of either *L. gasseri* strain orally in 20 μl PBS every day for 5 days and then every third day until the completion of the study. After 4 wk of treatment the colon from each CO₂-euthanized mouse was removed, flushed, weighed, and splayed open longitudinally before processing for histological analysis.

The composition of the gut microflora can vary between mice from different litters (5), much the same way it varies between unrelated humans (45). Therefore, a control (PBS-treated) mouse from each litter was used to control for interlitter heterogeneity.

**Histopathology.** Cecal and colonic tissues from wild-type and *IL-10⁻/⁻* mice were fixed in 10% neutral buffered formalin and embedded in paraffin before being cut into 7-μm sections and stained with hematoxylin and eosin (H&E) for light microscopic examination. Evidence of colitis was based on the presence of lymphocyte infiltration, goblet cell depletion, crypt architectural distortion, and ulceration. The degree of inflammation in each tissue section was graded on a scale from 0 to 4 based on the following criteria: 0, no evidence of inflammation; 1, low level of lymphocyte infiltration, minimal epithelial hyperplasia; 2, generalized infiltration of lymphocyte cells

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**Fig. 3.** A: macroscopic findings of colons extracted from IL-10-deficient mice challenged with either PBS (top), *L. gasseri* NC1500 (middle), or *L. gasseri* NC1501 (MnSOD, bottom). B: inflammatory scores of hematoxylin and eosin (H&E)-stained colonic tissue after *L. gasseri* or PBS challenge (*P* < 0.05, nonparametric 1-way ANOVA). C: H&E staining of normal colonic tissue from a wild-type C57BL/6 mouse. D: inflamed colonic tissue from an IL-10-deficient control (PBS) mouse. E: colonic tissue from an IL-10-deficient mouse treated with wild-type *L. gasseri*. F: colonic tissue from an IL-10-deficient mouse treated with wild-type *L. gasseri* expressing SodA (NC1501). All histological images are at ×20 magnification. G: proximal and distal inflammatory scores of H&E-stained colonic tissue after probiotic or PBS challenge.
in the lamina propria, obvious epithelial hyperplasia, mild goblet cell depletion; 3, pronounced lamina propria cell infiltration, marked epithelial hyperplasia, marked decrease in goblet cells, architectural distortion; 4, all the criteria from grade 3 plus the presence of crypt abscesses and mucosal ulceration. Inflammatory scores were recorded for a generalized state of the entire colon as well as for the proximal and distal colon. All samples were masked until grading of inflammation was complete.

**Immunohistochemistry.** To quantify inflammatory cells, myeloperoxidase (MPO) and cyclooxygenase-2 (COX-2) were detected by immunohistochemistry (IHC) in paraffin-embedded tissues as previously described (11). Briefly, slides with sectioned colonic tissues were deparaffinized in xylene and graded alcohols. After a 5-min microwave exposure in 10 mM citrate buffer, the slides were immersed in buffer containing 3% H2O2 to inhibit endogenous peroxidase activity. Blocking was performed for 1 h with normal goat serum and then the slides were incubated with a primary antibody overnight at 4°C. Rabbit anti-MPO (DakoCytomation, Carpinteria, CA) was used to detect neutrophils (11), and rabbit anti-COX-2 (Cayman Laboratories, Ann Arbor, MI) was used to detect activated macrophages (36). After brief washes the slides were incubated with biotinylated goat anti-rabbit secondary antibody for 1 h then with avidin-horseradish peroxidase (Vector Laboratories, Burlingame, CA). After additional washes, the slides were developed with 3,3’-diaminobenzidine (Biomedia). Slides were then counterstained with hematoxylin, dehydrated, and mounted with a coverslip. Normal epithelial tissue and test tissues lacking a primary antibody incubation step were used as controls for both MPO and COX-2 staining.

**Quantitative PCR.** Total colonic RNA was extracted from IL-10−/− mice administered either PBS (control), L. gasseri NC1500, or L. gasseri (MnSOD) NC1501 (n = 3, for each group) with Trizol (Invitrogen, Carlsbad, CA) according to the manufacturer’s protocol. Briefly, each colon was homogenized in 1 ml Trizol solution using a Retsch MM300 bead beating apparatus (Retsch, Newton, PA). Chloroform (220 μl) was added to the homogenate, mixed, and then centrifuged at 12,000 g for 15 min at 4°C. RNA was precipitated from the aqueous layer by adding an equal volume of 2-propanol and incubating the mixture at room temperature for 10 min. The mixture was then centrifuged at 12,000 g for 10 min at 4°C. The RNA pellet was washed with 1 ml of 70% ethanol and resuspended in 100 μl of nuclease-free water. The concentration of each RNA preparation was determined by measuring optical density at 260 nm.

The High Capacity cDNA Archive Kit (Applied BioSystems, Foster City, CA) was used to reverse transcribe 500 ng of total colonic RNA into first-strand complementary DNA (cDNA) with 250 U of reverse-transcriptase, 1× random primers, and 1 mM dNTPs in a 100 μl reaction mixture at 37°C for 120 min. Quantitative PCR was carried out with a real-time MX3000P thermocycler (Stratagene, La Jolla, CA) using a fluorescently labeled (FAM) oligonucleotide probe (Applied BioSystems, Taqman Gene Expression Assays). The genes investigated were Sod2 (Mm00449726_m1), Sod3 (Mm00448831_m1), Cat (Mm00437992_m1), and Gpx1 (Mm00656767_g1). The PCR reaction mixture contained 10 ng cDNA, 1× primer/probe mix, and 0.5 U of AmpliTaq Gold PCR master mix in a final volume of 15 μl. An initial denaturing step of 10 min at 95°C was followed by 40 cycles of amplification at 95°C for 15 s and 60°C for 1 min. PCR products were quantified by using cDNA standards of known concentration for each gene investigated. Each gene analysis was normalized with Gapdh, yielding a relative transcript level. Gapdh has been previously used to normalize oxidative stress related genes in different organs in the mouse (2, 20, 42).

**Statistical analysis.** Experimental groups were analyzed for significance of differences between the means of treatment groups and control groups by ANOVA with Tukey’s multiple comparison test or an unpaired t-test using GraphPad (v4.0a; Prism, San Diego, CA).

**RESULTS**

**Gastrointestinal transit.** The presence of L. gasseri NC1500 and L. gasseri NC1501 could be detected within the feces of wild-type C57BL/6J mice for up to 4 days after cessation of treatment following 5 consecutive days of feeding 1 × 109 CFU (Fig. 1). Indeed, the 16S rDNA gene was sequenced from random colonies obtained on the erythromycin-containing MRS plates to confirm the selectivity of this method for the administered L. gasseri strains. A fluctuation was observed in

![Graph](http://ajpgi.physiology.org/)

Fig. 4. Inflammatory scores of H&E-stained colonic tissue after L. gasseri or PBS treatment in male (A), *P < 0.05, **P < 0.01, nonparametric 1-way ANOVA) or female (B) mice. C: sex bias displayed by probiotic or control treatments. Average inflammatory scores for male and female IL-10-deficient mice are plotted on the x-axis and y-axis, respectively.
the CFU levels of each organism during the first 4 days of probiotic administration. However, CFU levels of the two L. gasseri cultures stabilized on day 5 (last day of probiotic feeding) through day 9, showing little variation between the viable counts of either strain in feces. It was concluded that after 5 days of supplementation both L. gasseri NC1500 and L. gasseri NC1501 (MnSOD) persisted within the mouse GI tract for the same length of time and at approximately the same viable cell numbers. Additionally, no obvious differences were observed in bacterial fecal densities or persistence in the GI tract between male and female mice in this study. A feeding regimen was constructed based on the knowledge that bacterial numbers significantly decline 3 days after the cessation of bacterial supplementation (Fig. 1). Thus each test mouse received $1 \times 10^9$ CFU of the respective L. gasseri strain every third day after an initial 5-day administration ensuring that either bacterium would be present in the mouse gut for the duration of the experiments (Fig. 2). The presence of either L. gasseri strain was detected in the feces of randomly sampled test mice throughout the 4 wk of bacterial supplementation (data not shown).

L. gasseri and MnSOD biotherapy. Following 4 wk of treatment, the dissected colons from L. gasseri-treated and control mice displayed distinct phenotypic differences. The colons from PBS-treated control mice were distinctly thicker than the colons dissected from littermates fed either L. gasseri strain (Fig. 3A). This observation was quantified when the wet weight of the majority of colons in each group was measured, revealing that the wet weights of the colons from L. gasseri-fed mice were lower than those from corresponding control mice. Although the colons from mice fed with either strain displayed a marked decrease in weight, these reductions were not statistically significant [colonic weights: control = $0.5546 \pm 0.0518$ g, $n = 5$ males and 7 females; L. gasseri NC1500 = $0.4540 \pm 0.0195$ g, $n = 4$ males and 7 females; L. gasseri NC1501 (MnSOD) = $0.4504 \pm 0.03172$ g, $n = 7$ males and 5 females].

Histological evaluation. Similar to total colonic weights, the average histological scores for IL-10$^{-/-}$ mice supplemented with either strain of L. gasseri showed a decrease in the degree of inflammation compared with control mice; however, only L. gasseri NC1501 (MnSOD) displayed a statistically significant difference (Fig. 3B; $P < 0.05$). Compared with wild-type mice (Fig. 3C), IL-10$^{-/-}$ from the control group displayed strong inflammatory damage evident in the form of crypt architectural distortion, pronounced cell infiltration in the lamina propria, goblet cell destruction, hyperplasia, and the presence of crypt abscesses (Fig. 3D). Although some tissues from mice fed L. gasseri NC1500 showed a reduction in inflammation, the majority of tissues from this group exhibited characteristics of severe inflammatory damage (Fig. 3E). In contrast, the tissues from mice fed L. gasseri expressing MnSOD, NC1501, displayed a substantial reduction in inflammation characteristics.
Colons from \textit{L. gasseri} 1501 (MnSOD)-treated mice exhibited infiltrating cells within the lamina propria, but crypt architecture was normal with intact goblet cells with a greatly reduced occurrence of hyperplasia.

The level of inflammation in colonic tissues was also recorded for the proximal and distal ends of each colon, revealing that the degree of inflammation was more pronounced at the distal end (Fig. 3G). Although not statistically significant, there was a slight reduction in inflammatory scores for the proximal colon for both probiotics. \textit{L. gasseri} NC1501, however, produced a significant reduction in the inflammatory score for the distal colon ($P < 0.05$).

Sex effects. To determine whether males and females respond equivalently, the inflammatory scores for whole colons was evaluated with respect to sex. The female \textit{IL-10}^{-/-} control group displayed a markedly lower inflammatory score than the male controls (Fig. 4A vs. B). Interestingly, \textit{L. gasseri} NC1500 treatment was associated with an increase in the inflammatory score in female colons but a significant reduction of inflammation in males ($P < 0.05$). The antagonistic and protective effects of \textit{L. gasseri} NC1500 in female and male mice, respectively, were also observed in both the proximal and distal regions of the colon (data not shown). The SodA-producing \textit{L. gasseri} (NC1501) caused a reduction of inflammation in both male and female mice, but only males reached statistical significance (Fig. 4B; $P < 0.01$). A comparison of the male and female inflammatory scores from each treatment group revealed that the inflammation was in general more severe in male IL-10-deficient mice (Fig. 4C).

**Innate immune response.** Colonic tissue sections from the majority of mice in each experimental group (control, $n = 7$; \textit{L. gasseri}, $n = 13$; \textit{L. gasseri} MnSOD, $n = 13$) were analyzed by IHC to evaluate the innate immune response. Since neutrophil infiltration in the lamina propria is a characteristic of the inflammation score, staining for myeloperoxidase (MPO), a marker for neutrophils (11), was used to support the H&E-based score. Compared with wild-type mice (Fig. 5A), colonic tissues from control mice contained a large number of MPO-positive cells within the lamina propria (Fig. 5B). The number of neutrophils present in colonic tissues was correlated with the severity of inflammation and the histological score (Fig. 5, B–D).

Colonic tissue sections from the same mice used for MPO staining were also stained for COX-2, a marker for macrophages (36) by IHC. Many infiltrating cells in the lamina propria stained positive for COX-2 in inflamed colonic tissue (Fig. 6). These COX-2-positive cells occurred in patches throughout the entire colon, and consistent with MPO staining the number of these positive cell patches was reduced in less...
inflamed tissues (Fig. 6), supporting the H&E inflammation scores. The COX-2-positive cells were not as abundant as MPO-positive cells and occurred mainly where cellular infiltrate in the lamina propria was dense or near breaches in the epithelial layer (Fig. 6B).

Overall, the presence of MPO- and COX-2-positive cells in the lamina propria of tissue sections tightly mirrored the histological grade applied to each section. For example, when each stained section was grouped by histological grade the number of MPO- and COX-2-positive cells progressively increased in tandem with the inflammatory histological scores applied. Although the number of neutrophils and macrophages were significantly decreased in the L. gasseri and L. gasseri MnSOD groups, there was no significant difference observed between the two probiotic-treated groups with respect to these inflammatory cells (Patches of MPO positive cells in tissue sections: control, 15 ± 4.925; L. gasseri, 5.5338 ± 1.403, P < 0.05; L. gasseri MnSOD, 5.231 ± 1.49, P < 0.05. Patches of COX-2 positive cells in tissue sections: control, 10.75 ± 1.66; L. gasseri, 3.231 ± 1.105, P < 0.001; L. gasseri MnSOD, 2.846 ± 1.78, P < 0.001). The sex effects seen with histological grading were not fully reiterated by MPO and COX-2 scoring, with the exception that the reduction in MPO and COX-2 staining reached statistical significance in males but not females.

Antioxidant gene expression. In response to inflammatory stimuli, several antioxidant genes involved in the neutralization of the superoxide radical [Sod2, MnSOD, and Sod3, (EC) Cu/ZnSOD] and hydrogen peroxide (Cat, catalase and Gpx1, glutathione peroxidase) are expressed. Quantitative PCR analysis showed that the expression of the antioxidant genes associated with superoxide radicals (Sod2 and Sod3) increased in colons of untreated IL-10-deficient mice (Fig. 7, A–B), whereas the genes involved with detoxifying hydrogen peroxide decreased in expression (Fig. 7, C–D). The transcript levels of the antioxidant genes in the colons from mice administered either L. gasseri NC1500 and L. gasseri NC1501 (MnSOD) were similar to those of the control mice.

DISCUSSION

The implication of microbial antigens in the pathogenesis of IBD has led to the concept of using natural intestinal organisms to alter the composition of the gut flora or to deliver therapeutic molecules to target areas in the intestinal tract. The use of probiotics has been extensively investigated in animal models and clinical trials (7, 18, 19, 23, 27–29, 32, 41), including L. gasseri, which has been tested for therapeutic effects in a rat model of colitis (24). In addition, the use of genetically modified bacteria as delivery vehicles for biologically active molecules for immunization (37, 38) and to treat IBD (37, 43) has been demonstrated. In this study, we show that unmodified L. gasseri had a non-statistically significant effect on colitis in the IL-10-deficient mouse model overall (i.e., with both males and females included) but did significantly reduce the inflammatory damage in male IL-10-deficient mice. In contrast, modified L. gasseri showed significant effects on colitis reduction both overall and in male mice, thus supporting the preferred use of the modified strain.

It has been suggested that an effective probiotic strain must transiently persist and reach high numbers at the target area of the gut (25). Previous studies have demonstrated that when administered orally L. salivarius spp. salivarius and L. plantarum can persist in mouse feces for up to 2 and 11 days, respectively.
respectively, after cessation of probiotic administration (22, 25). We found that both L. gasseri strains can persist for up to 4 days in the mouse GI tract. Since the engineered strain of L. gasseri does not display a colonization advantage in wild-type C57BL/6j mice, the therapeutic benefits arising from the use of this organism may not be due to a prolonged retention time or higher viable numbers within the gut. However, the oxidative environment in IL-10-deficient mice differs from wild-type C57BL/6j mice; thus the retention time of L. gasseri producing MnSOD in the inflamed GI tract may also differ. On the basis of random bacterial sampling throughout the experimental period, there was no significant retention time difference (data not shown) for either strain in the inflamed GI tract, suggesting this is not a major factor in the treatment outcomes.

The L. gasseri host strain used in this study is the neotype strain of this species and is of human origin (3). Indeed, the transit of an L. gasseri strain in the human intestinal tract has been demonstrated (26). It is possible that the retention time in the intestinal tract of the mouse of the L. gasseri strains used in this study may vary between other strains and animal hosts. Regardless, the transient presence of either strain of L. gasseri in the mouse gastrointestinal tract implies that this organism cannot overcome competitive exclusion by the autochthonous flora. Thus it is unlikely these particular strains of L. gasseri reduce inflammation by permanently altering the composition of the gut flora, a mechanism that has been previously proposed for probiotics (6). It is more likely that L. gasseri introduced into the gut in relatively large numbers temporarily dominates a particular niche in the host gut flora, altering its composition. Thus, because lactic acid bacteria are relatively unreactive organisms (40), the host is exposed to a less antigenic microbiota. This mechanism might also reduce exposure of the host immune system to highly antagonistic organisms such as Enterococcus faecalis (9) and Helicobacter hepaticus (31).

The use of endogenous SOD as a therapeutic molecule for IBD has been investigated previously (12, 15, 34, 35). Attempts to replenish Cu/ZnSOD levels either by subcutaneous delivery or through transgenesis have demonstrated some protective effect (15, 35). Unlike the present study, these investigations did not demonstrate that SOD can reduce inflammatory damage at the histological level. An explanation for the partial success of Cu/ZnSOD therapy may be that in inflamed tissues of IBD patients the activity and protein levels of MnSOD are more significantly altered than Cu/ZnSOD (13). Thus replenishing MnSOD levels in inflamed tissues would appear a better strategy. We describe herein a novel approach using bacterial delivery of a bacterial MnSOD to curtail inflammation in the IL-10-deficient mouse model of colitis.

Interestingly, we found that both the parental and MnSOD producing strain of L. gasseri exhibit a sex bias in the IL-10-deficient mouse model of colitis. Consistent with our results, female mice are less responsive to bacterially induced inflammation in a mouse model for liver cancer (31). Unexpectedly, when the composition of gut flora in female mice was altered by introducing the wild-type form of L. gasseri in large numbers, the resultant inflammation increased. This suggests that although the female IL-10-deficient mice used in this study do not in general develop severe colitis, they are capable of developing an aggressive form of colitis when the right stimulus is applied.

The additional beneficial effects exerted by the MnSOD producing strain of L. gasseri relative to the native strain can potentially occur via one of two mechanisms. The MnSOD produced in L. gasseri may act directly to relieve oxidative stress in the gut or indirectly by enhancing the natural beneficial properties of L. gasseri. The direct mechanism implies that SOD produced by the engineered L. gasseri reduces the levels of superoxide radicals in the gut imposed by specific members of the gut flora (9, 31) and the immune system. Consistent with this mechanism, we found that colonic tissue from mice administered MnSOD producing L. gasseri show a noticeable infiltrate of immune cells but retention of normal goblet cell numbers and crypt architecture. The increased transcriptional levels of host antioxidant genes in inflamed colons from IL-10-deficient mice implies that the superoxide radicals are the primary source of oxidative stress in this model. This supports the importance of a molecule that can disarm oxygen free radicals as therapy for colitis. Since the response in control or probiotic-treated groups is equivalent regardless of inflammatory status, there may be a threshold level of endogenous antioxidant activity supporting the observed benefit of additional MnSOD.

An alternative mechanism by which MnSOD may function is indirectly by enhancing the probiotic activity of L. gasseri. We have previously demonstrated that the MnSOD producing strain of L. gasseri has increased survival rates in vitro in the presence of both superoxide radicals and hydrogen peroxide (3, 4). Given that oxidative stress is abundant in the mammalian gut, particularly in inflamed tissues, the elevated level of MnSOD may overcome the oxidative insult from the immune system, allowing for the engineered form of L. gasseri to establish a more intimate contact with the colonic epithelium. Because the native strain of L. gasseri is more sensitive than its MnSOD-producing strain to superoxide radicals and hydrogen peroxide, the native L. gasseri may be unable to overcome the host defenses to exert greater beneficial effects (3).

In summary, the present study outlines the use of L. gasseri treatment of inflammation in the colon of IL-10-deficient mice. The use of MnSOD to enhance the therapeutic properties of L. gasseri for colitis was also demonstrated.

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