Treatment of Colitis with a Commensal Gut Bacterium Engineered to Secrete Human TGF-β1 Under the Control of Dietary Xylan

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Background: While cytokine therapy and the use of immunosuppressive cytokines such as transforming growth factor-β (TGF-β) offer great potential for the treatment of inflammatory bowel disease (IBD), issues concerning formulation, stability in vivo, delivery to target tissues, and potential toxicity need to be addressed. In consideration of these problems we engineered the human commensal bacterium Bacteroides ovatus for the controlled in situ delivery of TGF-β1 and treatment of colitis.

Methods: Sequence encoding the human tgf-β1 gene was cloned downstream of the xylanase promoter in the xylan operon of B. ovatus by homologous recombination. Resulting recombinants (BO-TGF) were tested for TGF-β production in the presence and absence of polysaccharide xylan in vitro and in vivo, and used to treat experimental murine colitis. Clinical and pathological scores were used to assess the effectiveness of therapy. Colonic inflammatory markers including inflammatory cytokine expression were assessed by colorimetric assay and real-time polymerase chain reaction (PCR).

Results: BO-TGF secreted high levels of biologically active dimeric TGF-β in vitro and in vivo in a xylan-controlled manner. Administration of xylan in drinking water to BO-TGF-treated mice resulted in a significant clinical improvement of colitis, accelerating healing of damaged colonic epithelium, reducing inflammatory cell infiltration, reducing expression of proinflammatory cytokines, and promoting production of mucin-rich goblet cells in colonic crypts. These beneficial effects are comparable and in most cases superior to that achieved by conventional steroid therapy.

Conclusions: This novel drug delivery system has potential for the targeted and controlled delivery of TGF-β1 and other immunotherapeutic agents for the long-term management of various bowel disorders.

Key Words: colitis, TGF-β, drug delivery, gut bacteria

Inflammatory bowel disease (IBD), including Crohn’s disease and ulcerative colitis, is a significant public health problem, with an increasing incidence in Western societies. Human IBD is characterized by inflammation in the large and/or small intestine associated with aberrant mucosal immune responses to constituents of the intestinal microbiota characterized by imbalances in effector versus regulatory immune cell activity. Cytokines play a central role in regulating immune cell activity and maintaining intestinal immune homeostasis with alterations in the production of pro- (e.g., tumor necrosis factor alpha [TNF-α]) versus anti-inflammatory cytokines (e.g., interleukin [IL]-10 and transforming growth factor beta [TGF-β]) being linked to the development of IBD in both experimental models and patients. An immunosuppressive cytokine that is central to maintaining intestinal homeostasis is TGF-β1.

TGF-β1 is an important intestinal epithelial cell growth factor, coordinating the rapid cell turnover and helping maintain normal intestinal homeostasis to commensal luminal enteric bacteria. Inadequate TGF-β signaling in T cells has been implicated in the pathogenesis of various diseases, including IBD, with TGF-β1-deficient mice developing early onset and fatal intestinal inflammation. In addition, production of TGF-β1 is associated with protection from the development of colitis, which is related to its involvement in wound healing and epithelial reconstitution, its modulatory effects on T cell and dendritic cell function, and the development of T regulatory (TReg) cells able to

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suppress intestinal inflammation.\textsuperscript{17} These studies highlight the potential use of TGF-\(\beta\) for the treatment of IBD. However, its action is hampered by its rapid clearance and degradation by the liver, kidney, and spleen,\textsuperscript{18} requiring multiple doses to sustain therapeutic systemic levels. Furthermore, normal homeostatic actions of TGF-\(\beta\) in uncompromised cells may be altered by high dosage, leading to unwanted and unexpected complications.\textsuperscript{19} Systemic administration of TGF-\(\beta\) may not therefore be the optimal route of delivery. The finding that intranasal delivery of plasmid DNA encoding TGF-\(\beta_1\) is effective in preventing and ameliorating experimental colitis\textsuperscript{20} suggests mucosal delivery is a more effective route of administering TGF-\(\beta_1\). For IBD, oral delivery is the preferred route of administering TGF-\(\beta_1\), although it must be delivered in large enough amounts or in a form that would ensure sufficient biologically active protein survived the acid- and protease-rich environment of the upper gastrointestinal (GI) and reached the target tissues of the lower GI tract. Alternative means of delivery need to be considered therefore, of which mucosal delivery via live microorganisms shows promise.

One of the most commonly exploited bacteria for drug delivery is \textit{Lactococcus lactis}, a Gram-positive bacteria used extensively in the dairy industry and the first genetically modified organism to be used alive for the treatment of IBD.\textsuperscript{21} Strains of \textit{L. lactis} secreting the antiinflammatory cytokine IL-10 were shown to be effective in treating intestinal inflammation in mice.\textsuperscript{22–24} However, the use of genetically modified \textit{L. lactis} or other bacteria in current use have significant drawbacks, including lack of regulation of the production of therapeutic molecule production in vivo and biosafety and environmental contamination concerns. To overcome these drawbacks we utilized \textit{Bacteroides ovatus}, a major commensal anaerobic colonic Gram-negative bacterium in humans, as an improved genetically modified bacterium to deliver TGF-\(\beta\) under the control of the plant polysaccharide xylan. Xylan is a major component of plant material\textsuperscript{25} with the host relying on xylanolytic colonic bacteria such as \textit{B. ovatus}\textsuperscript{26} for its breakdown and release of short chain fatty acids that are an important energy source for intestinal epithelial cells.\textsuperscript{27} A potential advantage of this method of delivery is that the bacterium is administered less frequently. Furthermore, with this system the protein is delivered in a controlled manner\textsuperscript{28} and in smaller doses, reducing the likelihood of side effects. Here we tested the efficacy of \textit{B. ovatus}-delivered TGF-\(\beta_1\) in an experimental mouse model of acute colitis.

**MATERIALS AND METHODS**

**Animals**

Male C57BL/6 mice at 6–8 weeks of age were obtained (Harlan Laboratories, Bicester, UK). All procedures were carried out in accordance with the Animal Scientific Procedures Act, 1986. Mice were fed standard chow (5%–10% xylan content) and tap water ad libitum up until 24 hours prior to starting the experiment when the feed was replaced with xylan-reduced (~0.7%) chow (Special Diet Services, Witham, UK). Xylan was added to drinking water (30 mg/mL) when required. Bacteria (2 \(\times\) 10\(^8\) in 0.2 mL phosphate-buffered saline [PBS]) were administered by oral gavage.

**Generation of TGF-\(\beta_1\) Expressing Strains of \textit{B. ovatus}**

The region of the human \(tgf-\beta_1\) gene encoding the 112 amino acid mature protein was polymerase chain reaction (PCR)-amplified from a cDNA clone (MRC Geneservice, UK; GenBank Access. No. BE255274, IMAGE: 3356605). The \textit{Bacteroides fragilis} enterotoxin secretion signal sequence was cloned upstream of the \(tgf-\beta_1\) coding sequence. The \textit{B. ovatus} xylanase promoter (GenBank Access. No. EU334491) was then cloned upstream of the \(tgf-\beta\) and the construct introduced into chromosomal DNA of \textit{B. ovatus} (BOV975) by conjugation\textsuperscript{28,29} to generate the strain BO-TGF.

**Experimental Colitis**

Acute colitis was induced in mice by adding dextran sodium sulfate (DSS, 2.5% w/v, molecular weight, 35–45 kDa; MP Biomedicals, Irvine, CA) to drinking water for 5 days. In the treatment protocol DSS was changed on day 5 to either water alone or water supplemented with xylan until day 11, when the experiment was terminated. Five groups of mice (\(n = 8\) in each) were used, two receiving BO-TGF, one receiving BOV975, one receiving PBS alone on days 5, 7, and 9, and one group receiving dexamethasone (3 \(\mu\)g/g s.c.) once daily from days 5 to 10. Xylan was added to the drinking water of one of the groups receiving BO-TGF and to the BOV975 group. In the prevention protocol BO-TGF and xylan administration were started simultaneously with DSS treatment (2.5%, w/v), which was continued until day 5, when the experiment was terminated. Four groups of mice (\(n = 8\) in each) were used, two groups receiving BO-TGF, one receiving BO V975, and the other receiving PBS alone by oral gavage at days 0, 2, and 4. Xylan was added to the drinking water of one of the groups of animals receiving BO-TGF and to the BOV975 group. Normal healthy control mice received normal drinking water throughout the experiment. The course of disease activity was followed by monitoring body weight, fecal blood content, and stool consistency. Disease activity index (DAI) was calculated to assess colitis clinically as described previously.\textsuperscript{30} At autopsy, colonic length was measured and macroscopic inflammation graded according to percent colonic involvement as follows: 0, none; 1, 1%–25%; 2, 26%–50%; 3, 51%–75%; 4, >75%.
TGF-β1 Assays

BO-TGF was cultured in RGM-hemin\(^{31}\) supplemented with 0.1% glucose. Xylan (0.05% w/v) was added for variable periods before culture supernatants were assayed in triplicate for TGF-β\(_1\) by enzyme-linked immunosorbent assay (ELISA) (BD Biosciences, Oxford, UK). For assaying TGF-β production in vivo, C57BL/6 mice were administered wildtype \(B. \text{ovatus}\) (BO V975) or BO-TGF by oral gavage. Xylan was added to drinking water to induce TGF-β\(_1\) production and the colonic contents were removed 24 hours later, weighed, and mixed thoroughly with 500 μL of PBS containing 1 μg/mL protease inhibitor cocktail and 1 mM phenylmethanesulfonyl fluoride (Sigma-Aldrich, Poole, UK). The suspension was centrifuged and the supernatant assayed by ELISA. TGF-β\(_1\) biological activity was assayed according to its ability to inhibit IL-5-induced proliferation of the erythroleukemia cell line, TF-1. Briefly, the BO-TGF culture supernatant or TGF-β\(_1\) standards were serial diluted and added to TF-1 cells cultured in RPMI 1640 with 5% fetal bovine serum (FBS), 50 μg/mL streptomycin, 50 U/mL penicillin, and 5 ng/mL recombinant human IL-5 (NIBSC, Potters Bar, UK). Cells were incubated for 48 hours at 37°C, at which point proliferation was assessed by \(^{3}H\)thymidine incorporation and scintillation counting (1450 Wallac Microbeta Trilux). A dose–response curve of cpm versus dilution of standard or unknown was plotted, and the amount of TGF-β\(_1\) in the samples was determined from standard curve linear regression analysis of cpm versus log standard concentration. In neutralization experiments, purified chicken anti-TGF-β\(_1\) (1:200 dilution) was included with samples for 1 hour at 37°C before testing. For immunoblotting, BO-TGF conditioned media was precipitated with acetone, resuspended in 500 μL nonreducing loading buffer, then separated on a 16% tricine sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and transferred onto PVDF membrane (Millipore, Bedford, MA). Membranes were sequentially incubated with biotinylated anti-TGF-β\(_1\) antibody (BD Biosciences), streptavidin-horseradish peroxidase (HRP), and chemiluminescent substrate and visualized on X-ray film.

Histology

The colon was sampled at 1, 2, and 3 cm from the anal verge. Tissue samples were fixed in 10% formalin and paraffin-embedded and sections were stained with hematoxylin and eosin (H&E). The sections were graded as to severity and extent of crypt damage, severity of acute and chronic inflammatory cell infiltrate, and mucin depletion. The grading index for severity of crypt damage was as follows: 0, none; 1, crypt epithelial flattening ± necrotic debris in crypt lumen; 2, crypt epithelial injury in <50% mucosal thickness with basal crypt preserved; 3, crypt epithelial injury in >50% of mucosal thickness or crypt epithelium completely destroyed, but superficial epithelium; 4, grade 3 + superficial epithelium destroyed; 5, ulceration involving muscularis mucosa or deeper. The extent of crypt damage was scored as follows: 0, none; 1, focal; 2, multifocal (≥2 areas); 3, diffuse (≥50% circumference). The grading index for severity of acute and chronic inflammation was: 0, none; 1, mild; 2, moderate; 3, severe. The grading index for mucin depletion was: 0, none; 1, mild/moderate; 2, severe. The total colitis score was the sum of the five subscores. The final score for the colon of individual mice was the average score of three samples plus the macroscopic inflammation grade. To exclude bias histological scores were determined in a blinded manner twice, by a colorectal pathologist and by another trained clinical investigator. Final scores represent the average of both scores.

Real-time PCR

RNA was extracted from samples of colonic tissue removed from the distal third of the colon using the SV Total RNA Isolation System (Promega, Southamptom, UK). One microgram of RNA was reverse- transcribed with M-MuLV reverse transcriptase (New England Biolabs, Hitchin, UK) and resulting cDNA analyzed for TNF-α, IL-1β, cyclooxygenase-2 (COX-2), and intestinal trefoil factor (ITF) by real-time quantitative PCR using an iCycler (Bio-Rad, Hercules, CA) with SYBER Green Jump Start Taq ready mix (Sigma-Aldrich, St. Louis, MO) and the following reaction conditions: 95°C for 3 minutes followed by 40 cycles of 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds. A melting curve was obtained to verify the presence of a single amplicon. Primers used were: TNF-α Forward: ACGG CATGGATCTCAAGAAC, Reverse: GTGGGTGAGG CACGTAGT; IL-1β Forward: CAGGCAGCGATATCA CTCA, Reverse: TGTCCTCATCCTGGAAAGGT; COX-2 Forward: TGCAAAATTGAGCCCTCTC, Reverse: CCCC AAAAGATAGCATCTGGA and β-actin Forward: CTTCTT CTTGGTATGGAATCCTG, Reverse: GTAATCTCCTTCT GGATCCTGTC. β-Actin was used as reference for normalization. Quantification of mRNA was performed using the standard curve method with the starting quantity of the gene normalized to the amount of β-actin product for each condition to determine gene expression relative to the β-actin gene.

Statistical Analysis

Data are expressed as means ± standard error of the mean (SEM). Statistical significance was determined using Student’s t-test for comparison within groups or single factor analysis of variance (ANOVA) for comparison between groups. Nonparametric data were assessed using Mann–Whitney U-test for comparison within groups or Kruskal–
Wallis test for comparison between groups. An associated P-value of <0.05 was considered significant.

RESULTS

Utilization of Xylan to Control Human TGF-β1 Production by B. ovatus

A strain of B. ovatus capable of secreting human TGF-β1 in a xylan-inducible manner was generated (BO-TGF). Xylan-induced TGF-β1 production was verified by ELISA. Secreted TGF-β1 protein (>100 pg/mL) was detected within 4 hours of exposure to xylan in cultures of BO-TGF, the level of which increased up to >300 pg/mL after 24 hours (Fig. 1a). By contrast, levels of TGF-β1 in cultures containing no exogenous xylan were less than 3 pg/mL, 100-fold lower than that produced in the presence of xylan (P = 0.037). TGF-β1 was detected in the colon of C57BL/6 mice after receiving a single dose of BO-TGF maintained on a xylan-containing diet. The levels present were significantly higher (11–14 ng/gm colonic content) than in mice administered wildtype BOV975 maintained on a xylan-containing diet (P = 0.018) and in nontreated mice (P = 0.012) (Fig. 1b). TGF-β1 was detected in the colonic contents of mice administered BO-TGF and maintained on

FIGURE 1. Generation of recombinant strains of B. ovatus producing TGF-β1 in response to xylan. (a) Levels of TGF-β1 in culture supernatants of B. ovatus wildtype (BOV975) and BO-TGF cultured in the absence (BOTGF-X) or presence of 0.05% (w/v) xylan for 4 hours (BOTGF+4X) or 24 hours (BOTGF+24X) were determined by ELISA. The data represents the mean (±SEM) values from triplicate values obtained from three independent experiments, *p < 0.05. (b) Amounts of TGF-β1 in the colonic washout of C57BL/6 mice were determined by harvesting the colonic contents 24 hours after oral administration of 2 × 10⁸ cfu BO V975 or BO-TGF and assaying by ELISA. All mice were maintained on a xylan-reduced diet with normal drinking water (−X) or water supplemented with xylan (+X). Nontreated (Control) mice served as an additional control group. Data points represent the mean (±SEM) values from three independent experiments, *P < 0.05 comparing BO-TGF+X to other groups. (c) The structure of BO-TGF produced TGF-β1 was determined by immunoblotting of BO-TGF + xylan conditioned media using biotinylated anti-TGF-β1 antibody to detect TGF-β1. (d) The biological activity of BO-TGF produced TGF-β1 was determined by bioassay and inhibition of IL-5 induced proliferation of TF-1 cells. Cells were incubated with serial dilutions of supernatant from cultures of BOV975 or BO-TGF grown in the presence of 0.05% xylan with or without anti-TGF antibody (Ab) measured proliferation by [³H] thymidine uptake. Data represent the mean (±SEM) values from two experiments.
a xylan-reduced diet (4 ng/gm, \( P = 0.034 \)) (Fig. 1b), although at significantly lower levels than that seen in mice given BO-TGF and a xylan-containing diet. This low level of TGF-\( \beta \) production by BO-TGF in the absence of exogenous xylan was most likely due to the presence of residual xylan in the xylan-reduced chow. It was not possible to maintain mice on a xylan-free diet (unpubl. obs.). These findings demonstrate the ability of BO-TGF to produce TGF-\( \beta_1 \) in vivo in a xylan controlled manner.

**TGF-\( \beta_1 \) Produced by BO-TGF Is Dimeric and Biologically Active**

Only dimeric TGF-\( \beta \) has biological activity.\(^{32}\) Immunoblotting analysis of xylan-induced BO-TGF culture supernatants identified a 25-kDa protein (Fig. 1c) corresponding to the dimeric form of TGF-\( \beta_1 \) produced by mammalian cells.\(^{33}\) Biological activity of BO-TGF-produced TGF-\( \beta_1 \) was verified using a bioassay and demonstrating suppression of IL-5-induced proliferation of TF-1 cells (Fig. 1d). By contrast, no biological activity was detected in supernatants from cultures of BOV975. Addition of anti-TGF-\( \beta \) antibody to BO-TGF + xylan supernatants neutralized the inhibition of proliferation seen with BO-TGF supernatants confirmed that the growth inhibition activity in these culture supernatants was attributable to TGF-\( \beta_1 \) (Fig. 1d). The level of biologically active TGF-\( \beta_1 \) detected in BO-TGF supernatants ranged from 50–110 pg/mL.

**Treatment of Acute Colitis by BO-TGF**

DSS was given to adult C57BL/6 mice for 5 days. Animals receiving DSS alone lost almost a quarter (22%) of their body weight after 5 days. Similar levels of weight loss were seen in animals receiving BO-TGF alone (22%) and BO-V975 and xylan (24%). By contrast, weight loss was less dramatic in BO-TGF-treated animals given xylan (14%–16%). BO-TGF and xylan treatment significantly improved stool consistency (\( P = 0.035 \)) and reduced rectal bleeding (\( P = 0.029 \)) compared to the non-treated DSS control group (Fig. 2a). The DAI scores were significantly lower in mice treated with BO-TGF and xylan (5.5 ± 0.7) compared to animals receiving DSS alone (8.2 ± 0.5, \( P = 0.012 \)), BOV975 plus xylan (7.6 ± 0.4, \( P = 0.025 \)), and BO-TGF without xylan (8.5 ± 0.3, \( P = 0.005 \)). The improvement in the disease activity was better than that achieved by steroid treatment (7.3 ± 0.6), although the difference seen was not significant (\( P = 0.096 \)) (Fig. 2b).

The colon of animals treated with BO-TGF and xylan (7.6 cm ± 0.19) were significantly longer than those in animals treated with BO-TGF alone (6.3 cm ± 0.16; 17% shorter, \( P = 0.002 \)), BOV975 and xylan (6.7 cm ± 0.2, 12% shorter, \( P = 0.013 \)), nontreated DSS control (6.5 ± 0.16, 14% shorter, \( P = 0.004 \)), or in animals treated with steroids (6.8 ± 0.2, 10% shorter, \( P = 0.045 \)) (Fig. 3a,b). Colons of animals administered DSS were edematous and inflamed compared to healthy colons. Colons from animals treated with BO-TGF and xylan had less pronounced edema and the proportion of inflamed colon appeared less than nonxylan-treated DSS controls (Fig. 3b). The pathology score of BO-TGF and xylan-treated animals was significantly better than nontreated animals (\( P = 0.041 \)) and animals treated with BO-TGF alone (\( P = 0.028 \)) (Fig. 3c). In particular, inflammation was reduced by 38% with BO-TGF plus xylan treatment compared to nontreated mice (\( P = 0.002 \)), BOV975, and xylan-treated mice (\( P = 0.042 \)).
Collectively, these data show that treatment with BO-TGF and xylan significantly reduced inflammation and intestinal crypt damage.

H&E-stained sections of colon revealed severe crypt epithelium destruction, inflammatory cell infiltration, submucosal edema, and severe goblet cell depletion in DSS-administered animals that received no treatment and in animals treated with BOV975 plus xylan and BO-TGF alone (Fig. 4). However, in mice treated with steroid or BO-TGF and xylan, crypt regeneration and restoration of colonic mucosa were frequently observed and the inflammatory cellular infiltrate was limited and had a more normal appearance (Fig. 4).

**FIGURE 3.** Pathological effect of BO-TGF and xylan treatment of DSS-induced colitis. (a) Colonic length was measured from ileocecal junction to the anal verge at necropsy (day 11). Data are expressed as means ±SEM (n = 8), *P < 0.05 comparing BO-TGF + X to other treatment groups. (b) Representative photos of the colon appearance at day 11 after treatment of DSS colitis with BO-TGF with (+X) or without (−X) xylan. (c) Inflammation severity score was based on severity of acute and chronic inflammatory cells infiltrate of the colonic mucosa from eight animals in each group, **P < 0.05 comparing BO-TGF + X to DSS Ctrl, BOV975 + X and BO-TGF-X groups. (d) Pathology score was based on cumulative scores of the depth and extent of crypt injury, acute and chronic inflammatory infiltrate, goblet cell depletion, and percent of colonic involvement (as detailed in Materials and Methods) from eight animals in each group. *P < 0.05 comparing BO-TGF + X to DSS Ctrl, BOV975 + X and BO-TGF-X groups. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

and BO-TGF-alone treated mice (P = 0.044) (Fig. 3d). Collectively, these data show that treatment with BO-TGF and xylan significantly reduced inflammation and intestinal crypt damage.

**Modulation of Proinflammatory Cytokine Expression by BO-TGF and Xylan Treatment**

Acute DSS-colitis is characterized by an increased production of macrophage-derived cytokines such as IL-1β and TNF-α. Expression of TNF-α (Fig. 5a) and IL-1β (Fig. 5b) in colonic mucosa was reduced in mice treated with BO-TGF and xylan compared to nontreated mice and mice treated with BO-TGF alone (P < 0.05). Furthermore, expression of TNF-α mRNA was lower in BO-TGF and xylan-treated animals compared to animals treated with BOV975 and xylan (P = 0.004), although BOV975 and xylan treatment did have a modest beneficial effect on reducing TNF-α expression compared to nontreated colitic mice. Likewise, the amount of COX-2 mRNA was significantly reduced by
BO-TGF and xylan treatment compared to nontreated mice ($P = 0.05$) and mice treated with BO-TGF alone ($P = 0.001$) (Fig. 5c). The amount of mRNA encoding the epithelial growth factor, intestinal trefoil factor (ITF), was significantly increased by BO-TGF and xylan treatment compared to nontreated mice ($P = 0.011$) and mice treated with BO-TGF alone ($P = 0.013$) (Fig. 5d).

Prevention of Acute Colitis by BO-TGF

The ability of xylan-regulated TGF-$\beta_1$ production by BO-TGF to affect the onset and development of colitis was assessed by administering BO-TGF and xylan at the inception of DSS exposure. This dosing regimen produced a significant and beneficial effect on weight loss on days 2, 3, and 4 (2.4%) compared to nontreated animals (6.6%) and animals receiving BO-TGF alone (7.2%) or BOV975 and xylan (6.6%) ($P < 0.05$) (Fig. 6a).

Colons of BO-TGF and xylan-treated mice were significantly longer than nontreated mice (6.8 cm ± 0.16 versus 6.2 cm ± 0.1, $P < 0.01$) and in mice treated with BO-TGF alone (6.4 cm ± 0.07, $P < 0.01$). Consistent with BO-TGF and xylan treatment limiting colitis development, histopathology showed that this treatment regimen reduced epithelial damage, inflammatory infiltrate, and goblet cell depletion scores by >25% compared to mice treated with BO-TGF alone ($P = 0.015$). Pathology scores were also improved compared to mice treated with BO-TGF alone (>22%) ($P = 0.032$) and BOV975 with xylan-treated mice (15%) but was not statistically significant (Fig. 6b). Furthermore, amounts of TNF-$\alpha$ and IL-1$\beta$ mRNA were significantly reduced by BO-TGF and xylan treatment as compared to nontreated mice ($P = 0.022$ and 0.005, respectively) and mice treated with BO-TGF alone ($P = 0.001$ and 0.03, respectively) (Fig. 6c).

DISCUSSION

In this study we constructed a recombinant strain of $B.\ ovatus$ for mucosal delivery of human TGF-$\beta_1$ under the control of the dietary plant polysaccharide xylan. Xylan regulation of TGF-$\beta_1$ production is a novel and important safety feature of this drug delivery system. In addition, the anaerobic nature of $B.\ ovatus$ provides an additional important environmental safety feature that is lacking in other recombinant mucosal delivery bacteria currently in use. This TGF-$\beta$ delivery system was evaluated for its prophylactic and therapeutic effect in an established mouse model of acute...
colitis. Xylan supplementation to BO-TGF-treated mice resulted in significant improvement of acute colitis; reducing weight loss, diarrhea, and rectal bleeding, inflammation and neutrophil infiltration, and expression of proinflammatory cytokines, and promoting healing of damaged colonic crypt epithelium and upregulating trefoil factor. These beneficial effects were comparable and in some cases superior to that achieved by steroid treatment. BO-TGF and xylan treatment also had a prophylactic effect, limiting the development of intestinal inflammation both clinically and histologically. These beneficial effects were comparable and in some cases superior to that achieved by steroid treatment. BO-TGF and xylan treatment also had a prophylactic effect, limiting the development of intestinal inflammation both clinically and histologically. The use of genetically engineered B. ovatus for the controlled mucosal delivery of antiinflammatory proteins has potential clinical applications for the treatment of various intestinal disorders, including IBD.

TGF-β is an important epithelial cell growth factor and is a potent inducer of intestinal epithelium repair after mucosal injury. However, administration of recombinant TGF-β intraperitoneally is ineffective at treating experimental colitis. An ideal treatment, therefore, would restore TGF-β signaling locally in affected epithelial cells necessitating the development of localized and specific delivery systems. As we have shown here, the temporal and spatial control of TGF-β delivery is achieved through the xylan-controlled delivery of TGF-β by B. ovatus, leading to enhanced epithelial cell repair and reduced inflammatory infiltrate. How BO-TGF produced TGF-β is taken up and acts in the inflamed intestine was not established in this study. However, the ability of Bacteroides to adhere to the surface of epithelial cells and the expression of functional TGF-β receptors by differentiated epithelial cells in the intestine suggests that BO-TGF-derived TGF-β is directly acquired by receptor-bearing epithelial cells.
regulating their growth and phenotype.\textsuperscript{5} The therapeutic effect of increasing TGF-\(\beta_1\) levels in the intestine via BO-TGF and xylan treatment appears to be at odds with the high levels of TGF-\(\beta_1\) seen in active Crohn’s disease and ulcerative colitis patients.\textsuperscript{37,38} This can be explained by an inability of mucosal cells in these patients to fully respond to TGF-\(\beta_1\) due to defective TGF-receptor mediated signaling and overexpression of the inhibitory protein of the Smad family, Smad7, that inhibits TGF-\(\beta\)-receptor signaling.\textsuperscript{4,8,38} Consideration of TGF-\(\beta\) responsiveness and expression of Smad7 in IBD patients would be a factor in determining the potential use and benefit of BO-TGF and xylan treatment in IBD patients.

A potential limitation of TGF-\(\beta\) therapy is its profibrogenic activity\textsuperscript{39} and scar formation after wound healing. This scarring is reduced after TGF-\(\beta_1\) neutralization or after the exogenous addition of TGF-\(\beta_3\) to cutaneous wounds.\textsuperscript{40} TGF-\(\beta_3\) could serve as the antiinflammatory nonfibrogenic therapeutic substitute for TGF-\(\beta_1\). TGF-\(\beta_1\), however, is 100-fold more potent than TGF-\(\beta_3\),\textsuperscript{41} hence its preferred use in this study. The amount of TGF-\(\beta\) produced by BO-TGF detected by bioassay was less than that detected by ELISA. This discrepancy may be explained by differences in the sensitivity of the assays and/or the short half-life of the biologically active dimeric form of TGF-\(\beta_1\) in tissue culture, although immunoblotting of xylan induced BO-TGF cultures clearly shows that the bacteria exclusively secrete the dimeric form of TGF-\(\beta_1\).

The reduction in expression of proinflammatory cytokines (TNF-\(\alpha\) and IL-1\(\beta\)) in BO-TGF and xylan-treated animals could be a direct antiinflammatory effect of TGF-\(\beta\) on immune cells. For example, by inhibiting the differentiation of CD4\(^+\) into Th1 or Th2\textsuperscript{52,43} and promoting T\(_{\text{Reg}}\) generation.\textsuperscript{16} The antiinflammatory effect of xylan-induced BO-TGF could also be indirect by improving epithelial barrier function secondary to the wound-healing effect\textsuperscript{13} and

\begin{figure}[h]
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\caption{Prophylactic effect of BO-TGF and xylan on the development of DSS-induced colitis. At day 0 DSS was added to normal drinking water or to water containing xylan and continued for 5 days. At days 0, 2, and 4 groups of mice (\(n = 8\)) were gavaged with 2 \times 10^6 cfu of wildtype B. ovatus (BOV975) or TGF-\(\beta\) producing B. ovatus (BO-TGF). Control groups comprised animals that received no bacteria (DSS Ctrl) and normal healthy animals (Normal). The experiment was terminated at day 6. (a) Body weight was determined daily between day 0 and day 5. *\(P < 0.05\) comparing BO-TGF+X group with other groups. (b) Pathology score was based on cumulative scores for depth and extent of crypt injury, acute and chronic inflammatory infiltrate, goblet cell depletion, and percent of colonic involvement from eight animals in each group. Data are expressed as means ± SEM (\(n = 8\)), *\(P < 0.05\). (c) The amount of TNF-\(\alpha\) and IL-1\(\beta\) mRNA in the colonic mucosa at day 5 was determined by real-time PCR and by standardizing values against \(\beta\)-actin. Data are expressed as means (±SEM) obtained from five animals in each group, *\(P < 0.05\) comparing BO-TGF+X to DSS Ctrl, BOV975+X, and BO-TGF-X groups.}
\end{figure}
consequent reduced exposure of underlying cells to enteric antigens. The finding that treatment with the parental wild-type BOV975 strain of *B. ovatus* in combination with xylan had a modest positive effect on proinflammatory cytokine production and disease severity could be related to the probiotic effects of xylan and its ability to modulate the colonic microbiota. Our data also showed that expression of COX-2 was reduced in mice with colitis and treated with BOTGF and xylan. COX expression can be induced by a wide variety of stimuli including inflammatory mediators and growth factors. It has been shown that COX-2-mediated prostaglandin production contributes to protection from colonic injury, increased crypt cell survival, and inhibition of proinflammatory cytokine production by activated macrophages. However, consistent with our results, other antiinflammatory therapies such as gamma-oryzanol, hrhFGF, and curcumin have significantly reduced COX-2 expression in DSS-induced colitis. Our finding of reduced COX-2 expression may therefore be attributed to reduced inflammation in the colonic tissues of mice in response to BO-TGF and xylan treatment. Furthermore, expression of goblet cell-derived ITF, which is a potent inducer of epithelial cell restitution, was upregulated by BO-TGF and xylan treatment, which may have indirectly reduced inflammation by improving barrier function. Another mode of TGF-β action may be induction of differentiation of intestinal epithelial cells to goblet cells with the increased ITF mRNA expression in colonic mucosa of mice treated with BOTGF being a consequence of the recovery of goblet cell function.

In summary, we engineered a prominent member of the colonic microbiota for the controlled delivery of a therapeutic growth factor to treat and prevent acute colitis. The results are promising in offering a safe, localized, regulated, and tissue-specific bacterial drug delivery system. This approach may contribute to the next generation of symbiotic therapy for IBD as an additive or alternative to current strategies. This drug delivery system may also have applications in the treatment of other bowel disorders.

REFERENCES


