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Research Article

Microencapsulated Interleukin-10 Therapeutics for Inflammatory Bowel Disease

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Abstract

Background and Aims: Crohn's disease and ulcerative colitis are chronic disorders of the gastrointestinal tract, often grouped together under the term Inflammatory Bowel Disease (IBD). Symptoms of diarrhea, nausea, abdominal pain, and weight loss cause significant morbidity and reduced quality of life for over 1.4 million Americans. Despite the use of therapies including prednisone, immunomodulators, antibiotics, and biologics, the majority of sufferers fail to see lasting remissions and experience little impact on their eventual need for surgical interventions, revealing an urgent need for a deeper understanding of pathogenesis and development of novel, targeted therapies. Interleukin-10, a potent anti-inflammatory cytokine, has been evaluated as a potential therapeutic for IBD since shortly after its discovery. Despite its effectiveness in rodent models, its efficacy in human clinical trials proved lack luster due, in large part, to its short *in vivo* half-life and toxicity associated with high systemic doses.

Methods: Phase Inversion encapsulation, a non-mechanical encapsulation approach that retains full protein bioactivity, dramatically improves shelf-life and affords an extended release of protein, was utilized to encapsulate IL-10, largely overcoming the cytokine's therapeutic shortcomings. Studies employing encapsulated IL-10 in rodent models of IBD were used to demonstrate a novel therapeutic application of this sustained-release technology aimed at IBD.

Results: We show that oral treatment with encapsulated IL-10 ameliorated weight loss and improved endoscopy scores in the murine model of DSS induced colitis.

Conclusion: These observations support a potential role for Phase Inversion encapsulated IL-10 in the prevention and resolution of colitis flares.

Keywords: *Crohn's Disease; Ulcerative Colitis; IBD; IL-10*

Introduction

Crohn's disease (CD) and ulcerative colitis (UC) are chronic disorders of the gastrointestinal (GI) tract. Symptoms of diarrhea, nausea, abdominal pain, and weight loss cause significant morbidity and reduce quality of life for over 1.4 million Americans [1]. While symptoms are similar, significant differences exist between these diseases. For example, UC is limited to the colon while CD can occur anywhere in the digestive tract. CD patients generally have healthy areas in between lesions, but in UC, there are no healthy areas in between inflamed tissue. Fistulas complicate Crohn's disease in up to 33% of cases [2], but much less frequently in UC [3]. Commonalities in symptoms

and intestine-centric inflammation lead to their grouping together under the term inflammatory bowel disease (IBD). Untreated, IBD is associated with an increased risk of colorectal cancer [4], progression of disease [5] and fatal outcomes [6]. Conventional therapies include prednisone, immunomodulators and antibiotics [7]. Over the last 20 years, the so-called biological response modifiers or 'biologics', macromolecules that target pro-inflammatory lymphocytes or the cytokines they produce [8], have revolutionized IBD treatment. For example, infliximab, a chimeric anti-human TNF-α antibody, earned FDA approval in 1998 due to its high response rate, significant mucosal and fistula healing, and long-term remissions in CD and UC [9,10]. Emerging biologics include anti-p40, anti-p19, anti-IL-12, anti-IL-17 and anti-alpha 4 integrin antibodies [8,11]. Unfortunately, remissions are all too often short-lived and associated with significant side-effects [4]. An estimated 30% of patients will not respond to biologics at all, and of those who initially respond, approximately 40% will cease to respond within a year. Finally, observational studies suggest that biologics have only modest impact on surgical intervention rates [12]. Despite therapy, 80% and 45% of CD and UC patients, respectively, will have required surgery after 20 years of disease [7]. These observations reveal the urgent need for a deeper understanding of pathogenesis and development of novel, targeted therapies.

Decades ago, observations in rodent models and IBD patients implicated inappropriate gut immune responses in the development of IBD. The immune suppressive cytokine interleukin 10 (IL-10) was first discovered in 1989, and since then, intense investigation has revealed potent anti-inflammatory and immune regulatory activities [13]. IL-10 is an 18 kDa cytokine produced by subsets of T- and B-cells, dendritic cells, macrophages, monocytes, and mast cells [14,15]. Along with TGFβ, IL-10 represents a major anti-inflammatory cytokine. It suppresses Th1/Th2 T-cell activity and production of IFN-γ, IL-2, IL-4 and IL-5, as well as antigen presenting cell (APC) activity (dendritic cells, macrophages and monocytes) and the production of IL-12, IL-1β and TNF-α [14,15]. Other immune activities include the suppression of NK-cell production of IFN-γ, pro-inflammatory chemokines, COX-2 and NO [15]. Within a few years of its discovery, the potential of IL-10 as an IBD therapeutic was evaluated in several rodent models.

Promising observations in these models incentivized clinical trials of IL-10 in human IBD patients. This progression was reviewed by Leach., *et al*. in 1999. Exogenous IL-10 administration was shown to ameliorate or prevent colitis in IBD models, including the $CD4+CD45RB^{high}$ adoptive cell transfer model in IL-10 knockout mice. The spontaneous development of IBD in mice lacking IL-10 showed the importance of the cytokine in mucosal immune regulation [16]. IL-10 knockout mice develop anemia, lose weight, and die prematurely because of chronic enterocolitis [16-18]. High levels of IFN-γ and TNF-α are detected in the intestinal explants of these mice, indicating abnormal Th1 T-cell and monocyte/macrophage activity like that seen in human IBD patients [18,19]. Parenteral administration of recombinant IL-10 partially prevented disease development but did not ameliorate established disease in these mice [18,19].

Following these observations, the safety and efficacy of parenteral administration of IL-10 as an IBD treatment was evaluated in two multinational multicenter studies [20,21]. In a 24-week double-blind placebo-controlled study in 95 patients with moderately active, steroid-refractory CD, IL-10 was administered subcutaneously (SC) at 1, 5, 10 and 20 μg/kg daily [20]. After a 28-day treatment regimen there was a modest response in the group receiving the 5 μg/kg dose (23.5% of patients in the treatment group vs 0% in the placebo group). In a second study involving 329 treatment-refractory patients with active CD, patients were administered 1, 4, 8 or 20 μg/kg daily [21]. Although no significant differences were observed in clinical remission between treatment and placebo groups, some clinical improvement was achieved in the 8 μg/kg group compared to placebo (46% response vs. 27%, respectively). These modest results, combined with significant side effects associated with higher-dose IL-10 [22,23], dampened enthusiasm for its systemic use in the treatment of IBD [24]. The short *in vivo* half-life (1.0 - 2.5 hr) and the side effects associated with high doses following systemic delivery, including anemia and thrombocytopenia, limited the efficacy of systemic IL-10 therapy [14]. It was suggested that development of new delivery technologies for the targeted delivery of IL-10 to the GI tract might significantly improve the efficacy in IBD patients [24]. One option for targeted delivery is encapsulation within protective microspheres.

Phase Inversion (PIN®) encapsulation is an innovation utilizing a non-mechanical encapsulation approach 8 that retains full protein bioactivity, dramatically improves shelf-life and expands storage options. PIN is capable of producing particles within a size range of 0.1-

10 µm, ideally suited to oral delivery, as such particles can cross the GI barrier either through the Peyer's Patch and/or the absorptive epithelium [25-27] exhibit minimal immunogenicity/toxicity, and display favorable degradation/clearance profiles [13,14]. We have successfully encapsulated different cytokines into PIN particles and demonstrated the *in vivo* therapeutic utility of such local sustained-release formulations in murine models of cancer [15-18], infectious disease [19] and immune mediated inflammatory disease [20]. Having established the applicability of PIN encapsulation technology across a wide array of cytokines and indications, we developed proprietary scaled-up manufacturing methods for producing large batches of PIN-encapsulated cytokine particles with optimized physicochemical properties, suitable for non-clinical and clinical studies. The proprietary polymers and excipients used in our formulation are either FDA-approved or were deemed non-novel by the FDA. Importantly, we have already shown that orally administered FITC-BSA-loaded PIN microparticles are efficiently taken up and retained in the intestinal lamina propria and the mesenteric lymph nodes for at least 24 hours after administration.

The studies reported here represent a novel therapeutic application of this sustained-release PIN microsphere technology aimed at IBD. We show that oral treatment with microsphere-encapsulated IL-10 ameliorated weight loss and improved endoscopy scores in the murine model of DSS induced colitis. These observations support a potential role for PIN encapsulated IL-10 in the prevention and resolution of colitis flares.

Materials and Methods

Preparation and characterization of Il-10 loaded microspheres

Recombinant mouse IL-10 (mIL-10) purchased from Peprotech, Rocky Hill, NJ, was encapsulated into poly-lactic acid (PLA) microspheres (0.6 mg cytokine per gram of particles) using PIN as described previously [28].

In vitro drug release

mIL-10 loaded microspheres were release tested using an *in vitro* release assay described previously [29]. Briefly, 10 mg of PIN particles were suspended in 1.0 mL release buffer and transferred to the wells of a 96-well plate in triplicate. The plate was incubated at 37°C in 5% CO_2, and the supernatant was replaced daily and stored at -20°C for analysis in mIL-10 ELISA (R and D Systems, Quantikine mouse IL-10 ELISA kit, Cat # M1000B and bioassay (see below).

Mouse IL-10 bioactivity

mIL-10 bioactivity was determined by a MC/9 cell-based mIL-10 assay. MC/9 cells were plated at 20,000 cells/well in media with mIL-4. mIL-10 standards or release samples were added and the plate was incubated at 37°C, 5% CO_2 for 72 hours. Promega CellTiter 96 Aqueous One Solution Reagent was added to each well and plates were read at 490 nm after 4 hours. Total levels of mIL-10 activity were calculated from the standard curve and the dilution factor used in the assay. Data are expressed as ng/ml.

Stability studies

Particles were aliquoted and stored at -20°C and aliquots were removed at selected time points. For each time point, an overnight release assay was performed as above. mIL-10 concentration and bioactivity values in supernatants were then performed as above and compared with those observed for the same samples prior to storage (un-stored). Final values for activity were expressed as % bioactivity of unstored particles.

DSS-induced colitis

mIL-10 microspheres vs intraperitoneally-administered soluble mIL-10 +/- blank spheres

Female BALB/C mice (8 per group) were purchased from Jackson Laboratories, (Bar Harbor, ME). The mice were kept under standard laboratory conditions with free access to food and water. They were allowed to adapt one week before starting the study. The care and

use of laboratory animals was in accordance with a University at Buffalo IACUC-approved animal use protocol. Colitis was induced in 40 female BALB/C mice (6 - 8 weeks old) with 3.5% dextran sodium sulfate (40 kDa) in drinking water for days 0 - 5. Mice were weighed and observed daily for disease score as in previous studies (Table 1). At disease onset, mice were randomized into 4 groups of 8 animals per group based on weight loss and disease score. Groups of mice were treated with either IL-10 microspheres (1 µg of IL-10 in 2 mg of particles in 100 µL of PBS; given by gavage), with soluble IL-10 (1 µg; given i.p.), or with blank microspheres (2 mg in 100 µL of PBS; given by gavage) 3 times per week. Another group of DSS mice remained untreated as a negative control. Mice were monitored for body weight and disease score daily during therapy. Mice were sacrificed on day 10; SAA levels, and colon weight to length ratio determined as in our previous studies.

Disease Score	0	1	2	3
Posture	Normal	Occasional hunching	Mostly hunched	Hunched at all times
Activity	Explores environment	Reduced activity	Lethargic/mostly stationary	Immobile
Coat Condition	Well-groomed coat	Reduced grooming	Matted/not clean	Ruffled, severe hair loss
Fecal Character	Normal stool; small, firm, and dry	Melena (tarry or dark)	Watery	Hematochezia (red and bloody)

Table 1: *Disease scoring criteria.*

Ideal dosing frequency determination

For dose optimization studies involving endoscopy, colitis was induced in C57BL/6 male mice (6 - 8 weeks old) with 2.5% dextran sodium sulfate (40 kDa) in drinking water for the 6 days. Treatments with either saline or IL-10-loaded spheres began on day 3. Mice were treated every other day for the next 7 days. On day 6, DSS was removed from water. On day nine colitis was evaluated by endoscopy. Mice were sacrificed on day 12 and colons removed for analysis as in our previous studies. Animals used in the DSS colitis protocol consisted of C57/Bl6 mice ordered from Jackson Laboratories. All aspects of the study were carried out under protocols approved by the Institutional Animal Care and Use Committee at the University of Louisville in accordance with an IACUC-approved animal use protocol.

Prior to colonoscopy, mice were anesthetized using intraperitoneal injection of ketamine/xylazine (100/10 mg/kg). The colonoscopy apparatus (Karl Storz, Tuttlingen, Germany) consisted of a miniature endoscope (scope 1.9 mm diameter), a xenon light source, a triple chip camera, and an air pump to achieve regulated inflation of the mouse colon. After mice were adequately sedated, the colonoscope was lubricated with sterile water then inserted under direct visualization into the rectum. The colonoscope was advanced after obtaining a view of the colon lumen until the bowel was obstructed by stool or the scope could not be advanced any further without impacting the wall of the colon. The colonoscopic procedure was viewed on a color monitor and digitally recorded for later viewing and archiving. Endoscopic scoring was performed in blinded fashion. A score was assigned immediately after the colonoscopy. The following scoring criteria were used to measure endoscopic disease severity by the endoscopist.

Colitis Score	0	1	2	3
Colon Thickening	Transparent	Blunting sharpness	Vague colors	Opaque
Vessel Changes	Normal	Small aneurysms	Loss of hierarchy	No vessels visible
Fibrin	Rare	White coats	Many clumps	Entire colon coated
Granularity	Normal	Patchy	More involved	Entire mucosa
Stool Consistency	Spicules	Barely deformable	Deep deformation	Coats mucosa

Table 2: *Endoscopic index of severity scoring criteria.*

Colitis scores are determined by endoscopy with each criterion being assigned a value between 0 - 3, with 3 being the most severe. Total severity scores are calculated by adding all five sub-scores, for a possible maximum score of 15 [30]. After colonoscopy was completed,

mice were placed in clean cages, which were then placed on a warming pad until movement was detected. Once mice began purposeful movements, cages were replaced in the housing racks.

Statistical analysis

Significance ($p < 0.05$) between experimental and control groups was determined using Student's two tailed t-test analysis. In experiments with multiple groups, homogeneity of inter-group variance was analyzed by ANOVA.

Results

PIN encapsulation and characterization of mIL-10: We have previously demonstrated that different cytokines can be encapsulated efficiently into and released in a sustained fashion in bioactive form from PIN particles both *in vitro* and *in vivo* [29,31-34]. IL-10 can also be encapsulated and released from PIN particles for extended periods. Recombinant murine IL-10 was encapsulated into PIN particles and the short-term release profile of the cytokine from four separate small (3g) bench-top lots (092916, 040517, 011818 and 012318) was determined using an *in vitro* release assay (Figure 1). While lot to lot differences were observed, the 3-day release pattern was strikingly similar and suggests that physiologically-relevant quantities of mIL-10 are released from PIN particles for at least 3 days. The released IL-10 was fully bioactive, as demonstrated using the MC/9 mIL-10 bioassay (Figure 2). In this assay, the concentration of IL-10 in the 24-hour release sample was first determined by ELISA, then bioactivity was calculated from the standard curve and the dilution factor used in the assay. In all three lots tested, the bioactivity observed was approximately equal to the concentration of IL-10 as determined by ELISA, indicating no loss of bioactivity upon encapsulation.

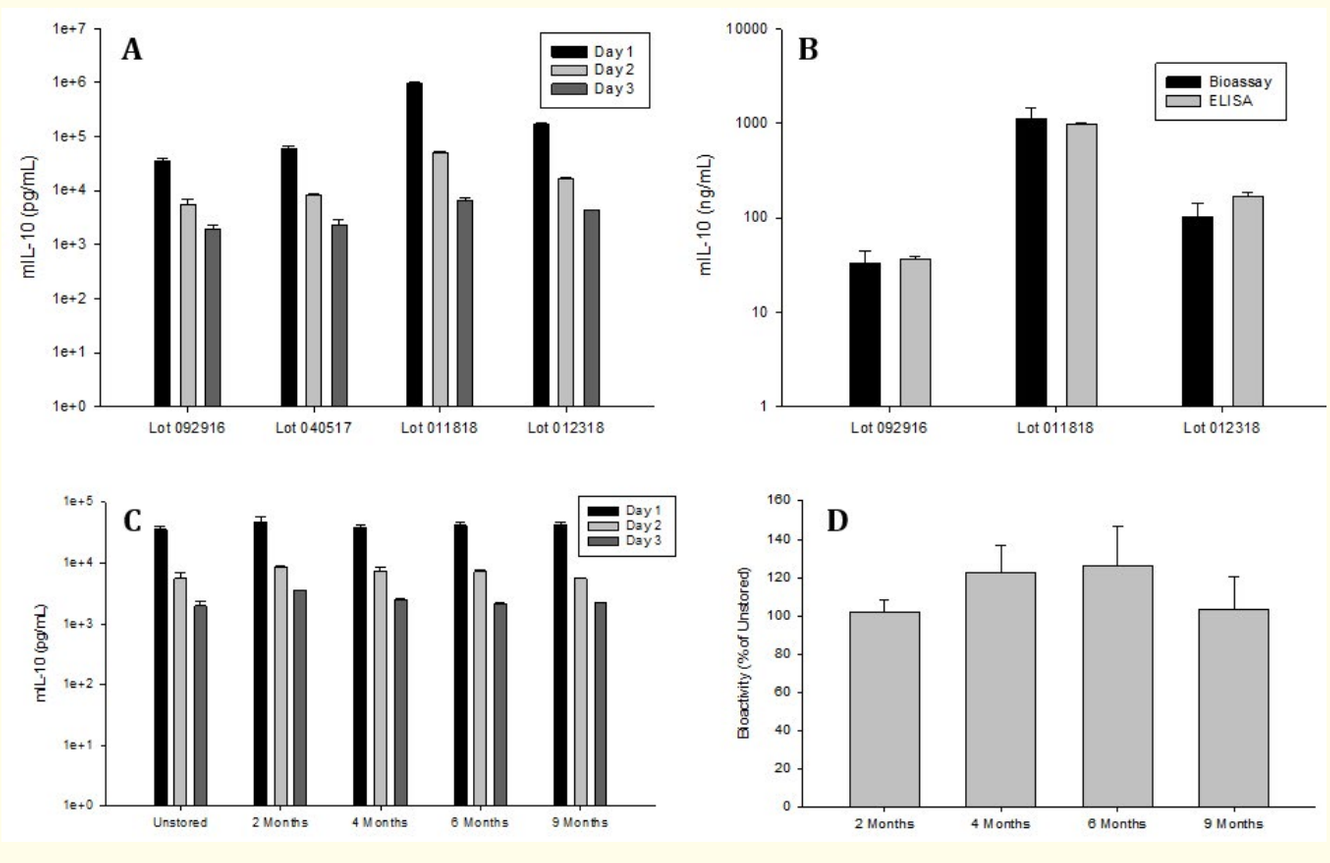

Figure 1: *Release profile, bioactivity, and 9-month stability of mIL-10 microspheres.*

Stability of PIN-encapsulated mIL-10: A selected lot of mIL-10 loaded microspheres was stored at -20°C for 0 through 9 months. At each time point, 10 mg/mL suspensions were prepared in standard release buffer and supernatants were collected at 24 hours. The resulting release samples were tested for mIL-10 levels in an ELISA (Figure 3). All release samples contained higher mIL-10 levels than initial concentrations (Zero store). The three-day release patterns of stored samples were not significantly different than unstored.

The bioactivity of the murine IL-10 released from stored PIN microspheres mimicked the ELISA results in that the bioactivity of samples stored at -20°C were not significantly different than unstored (Figure 4). These results show mIL-10 loaded PIN microspheres can be stored for at least 9 months at -20°C with no significant reduction in the biological activity of encapsulated IL-10.

Therapeutic potential of oral encapsulated IL-10 in treating acute IBD flairs: We first compared the ability of mIL-10 loaded microspheres (lot 092916) and soluble mIL-10 + blank microspheres to ameliorate acute disease using the murine DSS model of colitis. Mice were treated orally with a constant dose of encapsulated mIL-10 loaded PIN microspheres (2 mg microspheres; 0.6 mg cytokine per gram of microspheres) at disease onset. Selected disease markers were then monitored. The differences between control (blank microspheres) and the IL-10 treatment groups in terms of % body weight change (Figure 5, top panel), were significant between days 6-9, suggesting benefit of IL-10 whether given orally in the form of encapsulated cytokine or soluble as an IP injection. However, colon weight to length (Figure 5, right panel) were improved in the encapsulated IL-10 treated group, but appeared exacerbated in the soluble IP IL-10 group. No differences in other parameters, including disease score or Serum Amyloid A (SAA) levels at the end of the study were noted. This was likely due to the late onset nature of the study and natural resolution in all groups once the DSS was removed from the drinking water. This suggested a potential for mIL-10 loaded microspheres in attenuating IBD flairs.

Therapeutic potential of oral encapsulated IL-10 in IBD flair resolution: In the next study, we tested the ability of the same encapsulated mIL-10 to speed disease resolution. In this study, treatment was delayed until day 6, twenty-four hours after DSS was removed. Encapsulated mIL-10 was compared to soluble material given three times per week, as well as sulfasalazine (50 mg/kg) given daily for six days. Mice receiving encapsulated IL-10 were spared the more dramatic body weight loss (~7% vs ~11% respectively) seen in all other groups (Figure 6), although significant reduction in disease score was observed only in the sulfasalazine treated group. All groups had improved colon weight to length ratios compared to mice receiving no treatment. Together, the results of these two studies suggest the potential of encapsulated IL-10 to reduce severity of IBD flairs and speed their resolution.

Optimized Treatment of established IBD disease with mIL-10 loaded microsphere:. To test the ability of a higher dose (5 mg) of oral mIL-10 loaded microspheres (again, loaded at 0.6 mg cytokine per gram of particles) to ameliorate ongoing IBD, a larger study with various dose schedules was undertaken. mIL-10 loaded (lot 012318) or blank microspheres were administered orally 1, 2 or 3 times per week, starting from day 3. Mice were monitored daily for weight loss and for colitis scores by endoscopy 24 hours before the end of the study.

Treatment three times per week significantly (between days 8 and 12) ameliorated weight loss as in our previous studies, however treatment once or six times per week was not as effective (Figure 7). Treatment two or three times per week, but not six times per week, improved colon weight to length ratios. Interestingly, all treatment schedules significantly reduced colitis score at endoscopy. These results suggest a therapeutic dosing window may be necessary to achieve the optimum positive effects. Once again, no differences in other parameters, including disease score or SAA levels at the end of the study were noted (data not shown).

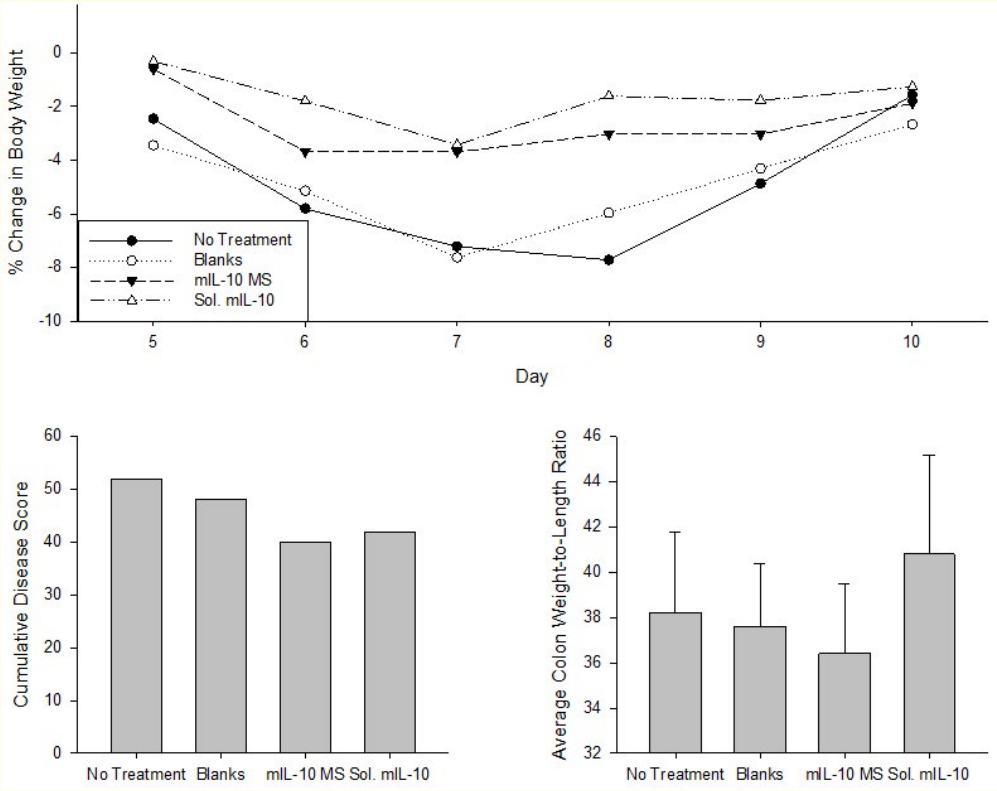

Figure 2

Discussion

We have shown that murine IL-10 can be encapsulated in PLA based microspheres with acceptable batch to batch consistency, a slow-release pattern (over days) and stability similar to other cytokines we have encapsulated. Oral treatments, three times per week, with encapsulated mIL-10 to mice beginning at the onset of symptoms, or 24 hours after removal of DSS, reduces weight loss and colon inflammation associated with DSS-induced colitis. These observations suggest that treatment with encapsulated human IL-10 could reduce severity of IBD flares and speed resolution in human sufferers. We have also shown that treatment with encapsulated murine IL-10 before disease onset could lessen the severity of ongoing IBD. Thus, encapsulated human IL-10 may also be a useful maintenance therapy to attenuate IBD.

Based on the same expectation that local delivery of IL-10 to the gut would reduce inflammation, several other approaches aiming at local delivery of IL-10 to the GI tract have been tested in murine models [35-37]. Daily oral administration of bacteria genetically engineered to secrete murine IL-10 prevented disease development in two different models of murine IBD [35]. Safety concerns associated with release of genetically-modified organisms into the environment, development of anti-bacterial immunity and potential

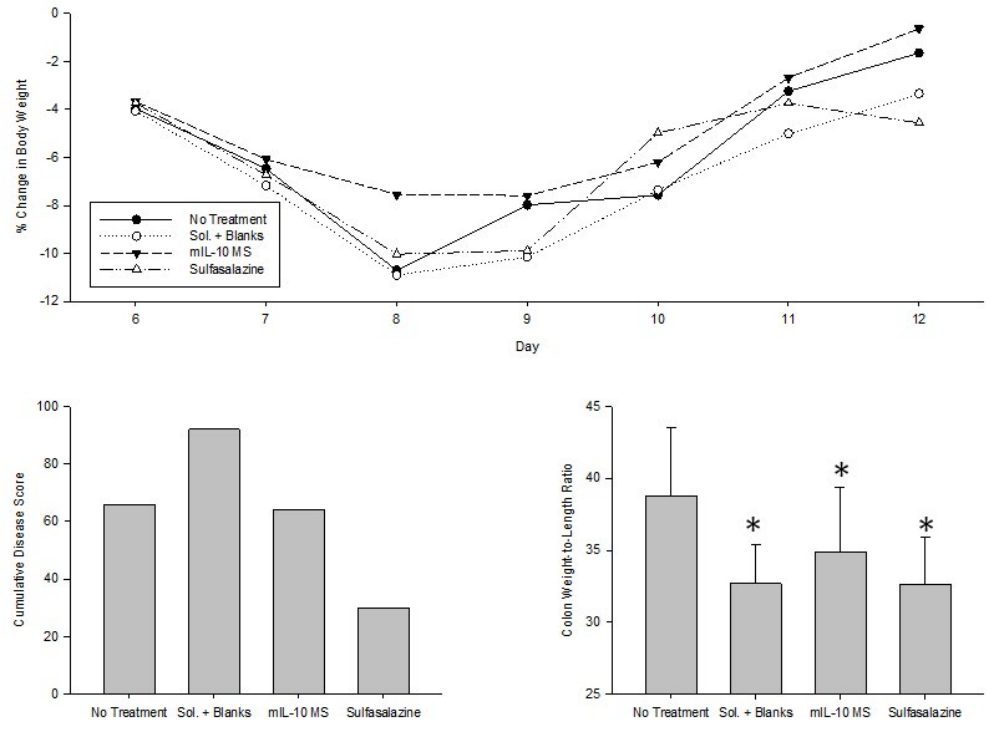

Figure 3: *mIL-10 loaded microspheres (mIL-10 MS) and soluble IL-10 (Peprotech, Inc, Rocky Hill, NJ) + blank microspheres are compared in their impact on disease resolution.*

problems with patient compliance (ingestion of live bacteria) limit this approach. In another study, rectal enema with IL-10-engineered adenoviral vectors successfully suppressed disease progression in adult IL-10 knockout mice demonstrating the potential of local gene therapy strategies for the treatment of IBD [36]. However, the long-term utility of this strategy is limited by anti-adenoviral immune responses. Finally, delivery of recombinant IL-10 to the colon by rectally administered IL-10-encapsulated gelatin microspheres was shown to prevent disease development in young IL-10 knockout mice [37]. While this approach circumvents drawbacks associated with the use of live bacteria or viral vectors (safety, the ability to control dosage and anti-bacterial/viral immune responses), efficacy required repeated rectal administration, thus raising potential patient compliance issues. Given the copious and robust published data with IL-10 in animal models of IBD, we were content with the abbreviated animal studies presented here indicating efficacy of our encapsulated IL-10, reasoning that additional supporting pre-clinical data already existed to justify IND enabling development of encapsulated human IL-10, and associated toxicology and clinical studies. Those efforts are in progress.

The observations reported here suggest PIN encapsulated IL-10, given orally 3 times per week at the time of an IBD flair, may speed resolution. While all treatment schedules improved colitis as judged by endoscopy score, more frequent dosing (6x per week), at least in terms of weight loss and colon W/L ratios, appeared less effective. This is in keeping with the well-known pleiotropic nature of IL-10,

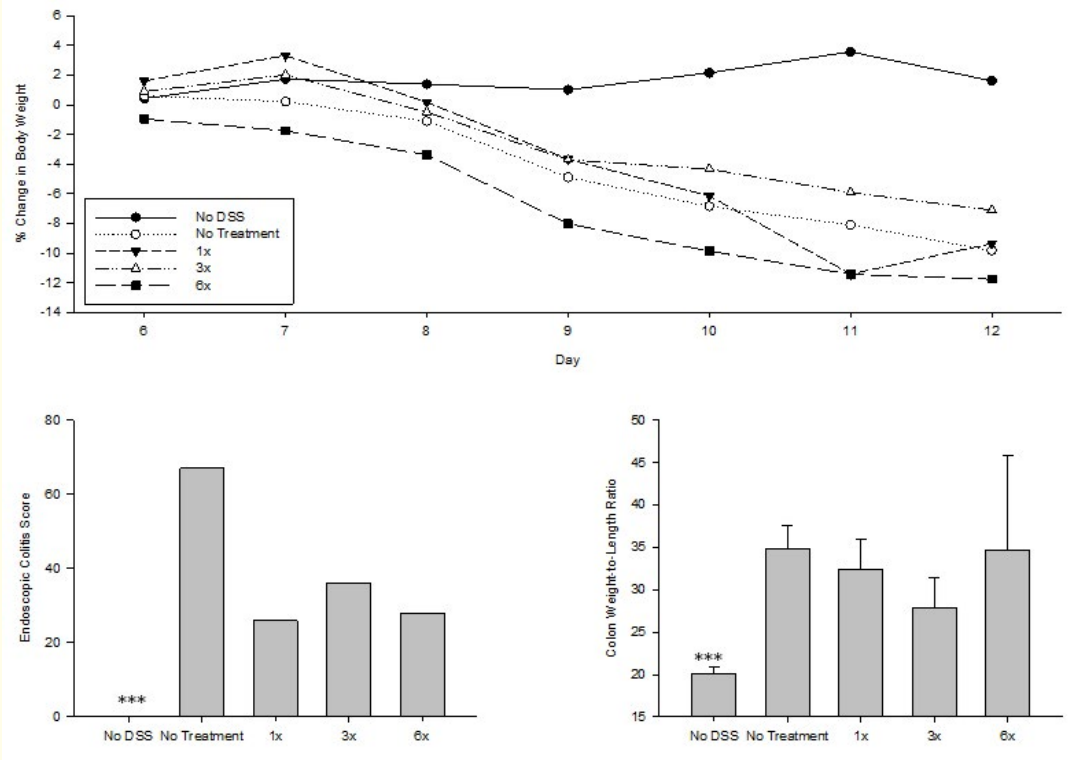

Figure 4: *mIL-10 loaded microspheres are optimized for treatment of established IBD disease.*

which may help explain the discrepancy between preclinical observations and controlled IL-10 clinical trials. Optimal tissue levels of IL-10 levels may attenuate inflammatory diseases, while higher or lower levels might be ineffectual, or worse, even detrimental. For example, immunostimulatory effects of IL-10 on B cells and activated CD8+ T cells at certain levels may overwhelm or even reverse the anti-inflammatory effects of IL-10 [38]. Endogenous IL-10 levels appear to fluctuate in the inductive phase of IBD and systemic administration may not produce local levels that can positively affect the cellular and molecular mediators involved in perpetuating gut inflammation. Low IL-10 levels appeared to increase disease severity in CD patients compared to high levels [39,40]. This perhaps relates to mechanistic differences between CD and UC.

These data complicate discussions surrounding the failure of IL-10 in IBD clinical trials, particularly the lack of differences in histology scores and inconsistent efficacy across various outcomes within experiments. Yet they argue for why an oral encapsulated product might succeed in specific IBD indications. Marlow and colleagues proposed several reasons for clinical IL-10 failures in IBD. The first and perhaps most important reason was that systemic administration resulted in insufficient gut tissue levels of IL-10. We measured gut tissue levels of IL-10 following oral administration of encapsulated IL-10, as presented in our recent work, pending publication. We showed significantly increased IL-10 levels in various gut tissues (e.g. small intestines, mesenteric lymph nodes, and colon, but not spleen) vs low or no systemic levels over at least 4 days. Whether or not these tissue levels might be sufficient to treat IBD remains to be determined in the clinic. Nevertheless, the results reported here suggest both a prophylactic and therapeutic potential for encapsulated IL-10.

Differential prophylactic vs therapeutic efficacies for IL-10 in IBD remain controversial. Early reports in mouse models indicated that IL-10 administration was only successful when administered prophylactically, prior to colitis induction, and was unable to treat established inflammation [41,42]. Herfarth and colleagues reported that subcutaneous IL-10 treatments inhibited inflammatory responses in the Lewis rat model of peptidoglycan-polysaccharide (PG-APS) induced colitis. Treatments were started 12 hours before or at several intervals after PG-APS injection into the intestine. IL-10 treatments starting before PG-APS injection significantly attenuated gut inflammation, effects that were enhanced when the IL-10 was given in combination with sub-therapeutic doses of dexamethasone. Nearly no benefit was conferred when treatment started at disease onset. Almost immediately following this, a report by Barbara and colleagues accomplished IL-10 delivery to the gut by gene transfer via intraperitoneal injection of non-replicating human type 5 adenovirus bearing the IL-10 gene. Virus was given either 24 hours before or one hour after intra-rectal administration of dinitrobenzene sulphonic acid in rats. Treatment prior to colitis significantly improved disease and reduced myeloperoxidase activity and leukotriene B4 concentrations in the colon. In contrast, treatment after the onset of colitis had no beneficial effect.

More recent work, including the results reported here, strongly support a therapeutic potential for IL-10 in IBD. Zheng and colleagues used acetylcholine (ACh), a major neurotransmitter, to induce increased IL-10 synthesis in gut monocytic myeloid-derived suppressor cells. ACh was given via enema directly to colon tissue two days after DSS was introduced in the drinking water to induce colitis. ACh benefits included reduced weight loss, disease score index and histological score. Significant tissue reductions in pro inflammatory signals, including TNF-α, IFN-γ, IL-1β, IL-6, and IL-17 were also observed. These effects were dependent on increased IL-10. These results not only implicated a potentially significant neuro-immune axis in IBD, but also suggest a therapeutic potential for IL-10 in IBD [43].

Observations from Fey and colleagues also suggest a therapeutic potential for IL-10 in IBD. The group employed a genetic fusion of exotoxin cholix and human IL-10. The resulting chimera, termed AMT-101, was used to ferry IL-10 across intestinal epithelial cells via a transcytosis pathway, delivering an IL-10 payload to the underlying lamina propria. In those studies, mice were dosed orally once daily for 10 days starting from the induction of DSS-induced colitis. While not strictly therapeutic in the same sense as the studies reported here, AMT-101 significantly reduced DSS-induced weight loss during the in-life portion of the study, but interestingly, did not statically improve colon weight/length ratios. Improvements in gut tissue erosion, edema, mucosal thickness, gland loss, hyperplasia, and mucosal thickness were also observed. It is also interesting to note that prophylactic AMT use was indeed more effective in attenuating disease in this DSS-induced model than in a commonly used hapten Oxa-based murine model of intestinal inflammation.

Conclusion

In conclusion, we have presented data in the DSS induced colitis model suggesting both a prophylactic and therapeutic potential for encapsulated IL-10 in the treatment of colitis. Whether this translates to a clinical benefit for IBD patients, either alone or in combination with other therapies, remains to be demonstrated in the clinic.

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Conflicts of Interest

Dominick Auci holds shares in Therapyx, Inc. The authors report that there are no other conflicts of interest.

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