UNIVERSITY OF CALIFORNIA SAN DIEGO

Evaluating Interleukin-10 Expressing Native *E. coli* in a Murine Model of Acute Colitis as a Potential Therapeutic

A Thesis submitted in partial satisfaction of the requirements
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in

Biology

by

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2024

The Thesis of Kevin Wei is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

University of California San Diego

2024

DEDICATION

This thesis is dedicated to my family, whose unwavering support and encouragement have been the bedrock of my academic journey in the United States since 2015. To my parents, for their endless sacrifices and for believing in my potential even when I was consumed by doubt. Your faith in me has been my guiding light. To my friends, for their relentless motivation and selfless sharing of their expertise, always propelling me towards success. Your camaraderie and support have been invaluable.

I also dedicate this work to my mentor, Nicole Siguenza, who bestowed upon me her mentorship and support when I needed it most. To my thesis advisor, Dr. Amir Zarrinpar, who recognized my potential and dedicated his utmost effort to foster my professional growth. Your guidance and wisdom have been a beacon throughout this challenging process. To my colleagues at lab, whose timely advice and assistance have been instrumental in navigating this journey, I express my deepest gratitude.

Lastly, this thesis is dedicated to all the patients and families affected by inflammatory bowel disease. It is my fervent hope that this research will contribute to a deeper understanding and more effective treatment of this debilitating condition, bringing hope and relief to those who suffer.

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ABSTRACT OF THE THESIS

Evaluating Interleukin-10 Expressing Native *E. coli* in a Murine Model of Acute Colitis as a Potential Therapeutic

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Inflammatory bowel disease (IBD) presents a significant healthcare challenge due to its rising global prevalence and the limitation of current treatments. This thesis investigated the feasibility of an engineered native bacteria (ENB) therapeutic for IBD which aimed to address the limitations of current treatments and offer an alternative for patients who do not respond to existing therapies. Building on the study by Russell *et al*. (2022) which identified a genetically

tractable, colonizable and functional native *E. coli* chassis, I tested the therapeutic effect of interleukin-10 (IL-10) expressing *E. coli* (EcAZ-2$^{IL\text{-}10+}$) in mice with dextran sulfate sodium (DSS)-induced acute colitis. Recombinant IL-10 molecules expressed by EcAZ-2$^{IL\text{-}10+}$ showed high bioactivity *in vitro*, reducing tumor necrosis factor-alpha (TNF-α) production by lipopolysaccharide (LPS) stimulated mouse macrophages. In addition, EcAZ-2$^{IL\text{-}10+}$ stably colonized the gut of C57Bl/6 mice after one single treatment via oral gavage, maintaining colonization after DSS administration. Intestinal delivery of bioactive IL-10 by EcAZ-2$^{IL\text{-}10+}$ did not show significant amelioration in colitis symptoms reflected by body weight change, colon measurements, and histology, but it was well tolerated by the mice and did not worsen disease severity. My thesis demonstrates that EcAZ-2$^{IL\text{-}10+}$ has the potential to be optimized for better therapeutic efficacy in managing IBD.

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic, debilitating, autoimmune disease that can greatly decrease quality of life. Ineffectively-treated patients can experience life-threatening complications, with the most fatal being cardiovascular disease and infections resulting from IBD flare-ups (Follin-Arbelet et al., 2022). Typical symptoms for a patient include abdominal pain, weight loss, diarrhea, or bloody stool (Mayo Clinic, n.d.). In the United States alone, there are an estimated 3.1 million patients (1.3% of the total US population) diagnosed with IBD (Dahlhamer et al., 2016). Although the highest incidence rate is generally reported in developed, industrialized parts of the world, such as Europe and North America, incidence of IBD is rapidly increasing in newly industrialized countries in Asia, Africa and South America (Ng et al., 2017). While the exact causes of IBD are unknown, several risk factors have been identified, including genetic predisposition, environmental influences, microbial imbalances, and immunological issues (Zhang, 2014). While IBD can manifest symptoms in different parts of the body, the pathology primarily occurs in the gut. Decreased epithelial barrier integrity in the gut allows commensal microbes to trigger an immune response, further damaging the gut barrier and leading to an even more uncontrolled immune reaction. (Maloy & Powrie, 2011). This provides an important clue that treatment for IBD should focus on modulating the inflammatory response of patients.

Current pharmaceutical interventions include anti-inflammatory drugs such as aminosalicylates (5-ASAs) or immunosuppressants like corticosteroids and anti-tumor necrosis factor (TNF) therapy (Imbrizi et al., 2023). However, each treatment option has drawbacks. 5-ASAs have limited efficacy in severe cases. Corticosteroids are effective in treating severe colitis, but long-term use can pose a high risk of detrimental side effects. Other

immunosuppressants help reduce systemic immune response to ameliorate inflammation, but also increase patients' susceptibility to infections or development of other autoimmune disorders. When patients fail to respond to these pharmaceutical treatments, surgical intervention becomes necessary. Not only is surgery invasive, but it also poses secondary infection risks and can create digestive issues without offering a long-term solution for IBD.

The intestine, as the body's largest interface with the external environment, must balance tolerance to food and microbiota-derived antigens while responding to pathogens. This is achieved through a highly developed immune system that should suppress inflammation under normal conditions. The immune system uses the production of cytokines to balance and regulate its reactions. For example, the intestine produces both pro-inflammatory cytokines like TNF-α, which protect against pathogens, and anti-inflammatory cytokines like IL-10, which prevent excessive inflammation. Maintaining this balance is essential for intestinal homeostasis (**Fig. 1A**) (Neurath, 2024). During an acute episode of colitis, the cytokine balance can break, and the colon epithelium can be overwhelmed with pro-inflammatory cytokines. IL-10, as its counterpart, becomes essential. Hence, there has been a growing interest in exploring IL-10 therapy.

IL-10 has shown therapeutic promise in preclinical and early clinical trials for IBD. However, previous IL-10 treatment attempts, such as subcutaneous administration of the recombinant IL-10 protein in humans, are well tolerated at low doses but are clinically ineffective (Schreiber et al., 2000 & Buruiana et al., 2010), possibly due to insufficient concentration of IL-10 reaching the site of inflammation in the intestines. Attempts at increasing systemic dosage of IL-10 leads to more adverse events, such as anemia (Tilg, 2002). Therefore, a more precise and targeted IL-10 administration method is needed, for instance, oral

administration to deliver IL-10 directly to the gastrointestinal tract. However, direct oral administration of IL-10 can be ineffective due to the protein's short half-life and risk of denaturation in stomach acid. Alternatively, engineered probiotics can help protect sensitive therapeutic compounds, allowing for their oral delivery.

Delivering IL-10 molecules *in situ* using engineered probiotic *Lactococcus lactis* (*L. lactis*) has shown promise in murine models, achieving a 50% reduction in inflammation in dextran sulfate sodium (DSS)-induced chronic colitis (Steidler et al., 2000). This approach has also been proven safe in human trials (Braat et al., 2006). However, it has not been further developed into a viable pharmaceutical product; one of the key pitfalls of using probiotics is that, while probiotic strains and other lab bacterial strains are used for their known safety profiles and increased ease of genetic manipulation, they do not show consistent nor stable engraftment in a host with an intact microbiome (Mimee et al., 2016). We, therefore, hypothesize that the *L. lactis* chassis cannot effectively survive passage through the human digestive system and consequently did not produce a notable therapeutic effect. Additionally, due to the chronic nature of IBD, frequent re-administration would be costly and burdensome for patients. Thus, there remains a need for a better bacterial chassis that can stably colonize the intestines to achieve long-term treatment.

To address this issue, we have optimized the use of a native bacterial chassis – an undomesticated *E. coli* strain isolated from the murine gut (EcAZ-1) – that can be engineered to express transgenes of interest. EcAZ-1 was first engineered to express antibiotic-resistance genes and green fluorescent protein (GFP) for ease of isolation and tracking during *in vivo* experiments (EcAZ-2). In our published study, we showed that EcAZ-2-based live bacterial therapeutics can cause functional changes in the host. For example, when the ENB overexpressed a bile salt

hydrolase gene, it improved insulin sensitivity in mouse models of type 2 diabetes. Even more impressive is that EcAZ-2 can perpetually colonize conventionally-raised (CR), wild-type (WT) mice with an intact microbiome. Unlike probiotic strains which are typically cleared from an intact gut microbiome upon cessation of treatment, especially in humans, the ability of EcAZ-2 to engraft in the murine gut microbiome is promising for translation to future human trials. Thus, the Zarrinpar lab has engineered native bacteria EcAZ-2 to express IL-10 (EcAZ-2$^{\mathbf{IL\text{-}10+}}$), which is able to colonize in CR mice without loss of function (Russell et al., 2022).

To evaluate the effectiveness of EcAZ-2$^{\mathbf{IL\text{-}10+}}$ in preventing colitis, I chose the DSS-induced mouse model of acute colitis. DSS damages colonic epithelial cells, mimicking the breach of the gut barrier in IBD (Eichele et al., 2017). Acute induction of colitis simulates an IBD flare-up. Compared to other murine IBD models, such as adoptive T cell transfer or IL-10 receptor gene knock-out, the DSS-induced colitis model offers the advantage of easily controlling the onset date, disease severity, and duration of colitis by adjusting the timing and concentration of DSS administration. This flexibility makes DSS a widely-used model for studying IBD.

In this thesis, I hypothesized that EcAZ-2$^{\text{IL-10+}}$ could express bioactive IL-10, colonize the murine gut, and lessen the disease severity of DSS-induced colitis in CR-WT mice. The positive translation impact of this research lies in the development of a live native bacterial therapeutic for delivering IL-10 locally to the gut, offering an alternative and promising avenue for IBD treatment.

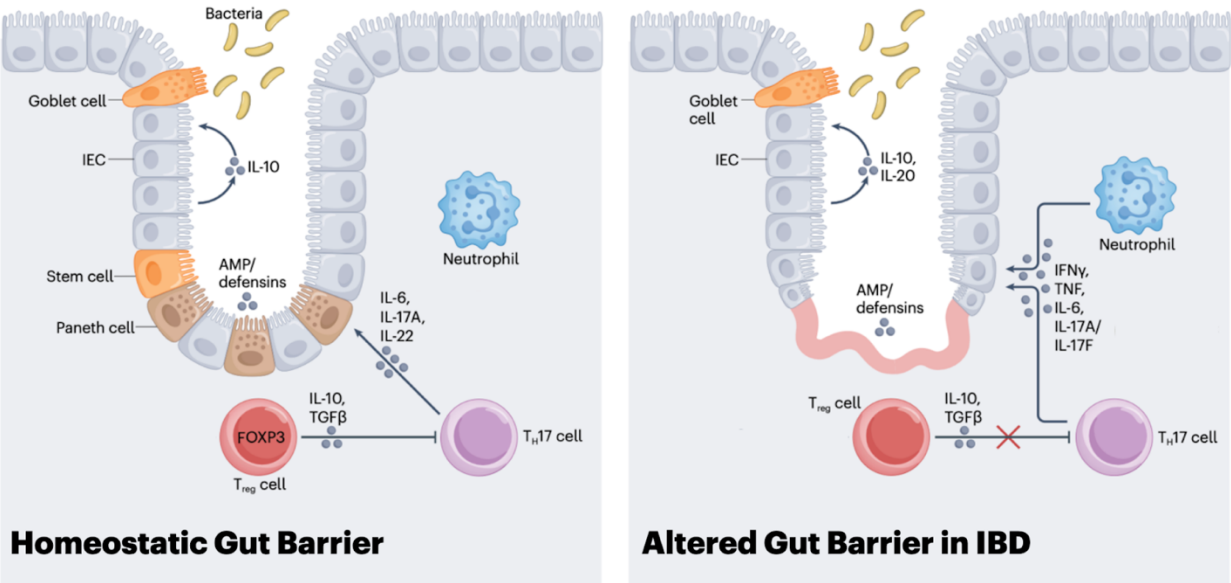


Figure 1A: Schematics of IL-10 immune pathway of ameliorating colitis. (Adapted from *Neurath*)

Chapter 1 ECAZ-2^{IL-10+} CAN EXPRESS BIOLOGICALLY ACTIVE IL-10

In our published study, the addition of the *il10* gene did not affect the *in vitro* growth rate of EcAZ-2 (**Fig. 2A**). Additionally, using an enzyme-linked immunosorbent assay (ELISA), we verified the production of the recombinant IL-10 (rIL-10) protein from EcAZ-2^{IL-10+} (**Fig. 2B**) (Russell et al., 2022). Note that rIL-10 was only detected in the cell lysate and not in the supernatant, indicating that rIL-10 is present intracellularly. However, since we engineered a prokaryotic organism to express a eukaryotic protein, there is potential lack of bioactivity. Factors such as protease activity in the cytoplasm, lack of post-translational modification, and potentially improper protein folding could affect the bioactivity of the rIL-10 protein, making it non-functional. Hence, it is essential to verify the biological activity of rIL-10 before giving it as a treatment to the mouse model (Mergulhão et al., 2007).

We verified the bioactivity of rIL-10 by testing its inhibitory effect on LPS-induced TNF-α production in J774A.1 mouse macrophage cells (**Fig. 2C**). TNF-α, a pro-inflammatory cytokine, decreases with increased IL-10 signaling. We used an ELISA to ensure similar LPS levels in both EcAZ-2 and EcAZ-2^{IL-10+} samples which we then used to stimulate the macrophage cells. The positive control group was treated with 100 ng/ml of commercially available recombinant IL-10 (c-rIL-10) (which should significantly suppress TNF-α production), while the negative control group received heat-denatured c-rIL-10 post-LPS stimulation (and thus leaves LPS-induced TNF-α production unaffected). The treatment group received EcAZ-2^{IL-10+} lysate, and the IL-10 concentration-matched comparison group was treated with 440 pg/ml c-rIL-10 post-LPS stimulation (**Fig. 2B**). We expected the TNF-α levels to be lowest in the positive control, highest in the negative control, with the treatment and comparison groups falling in between. If the rIL-10 produced by EcAZ-2^{IL-10+} has complete bioactivity, we expect

the resultant TNF-α levels to be similar to that of the comparison group. Each treatment group was run in biological triplicates.

In the mouse TNF-α assay, the cells that received heat-denatured c-rIL-10 post-LPS stimulation (negative control) exhibited TNF-α levels of 401.6 ± 12.18 pg/ml (**Fig. 2D**). The group that received 100 ng/ml c-rIL-10 (positive control) showed a significantly lower TNF-α level of 52.38 ± 4.68 pg/ml, representing an 86.96% reduction in pro-inflammatory cytokine expression (**Fig. 2D**). The cell line treated with EcAZ-2$^{IL\text{-}10+}$ lysate (treatment group) demonstrated a 36.70% decrease in TNF-α levels (254.2 ± 4.47 pg/ml) compared to the negative control (**Fig. 2D**), indicating a significant anti-inflammatory effect. In addition, the TNF-α level observed in treatment group showed a similar TNF-α reduction to the comparison group (EcAZ-2 lysate + 440 pg/ml c-rIL-10), confirming high biological activity of the rIL-10 produced by EcAZ-2$^{IL\text{-}10+}$. These results confirm the *in vitro* bioactivity of IL-10 produced by EcAZ-2$^{IL\text{-}10+}$, paving the way for future *in vivo* experiments.

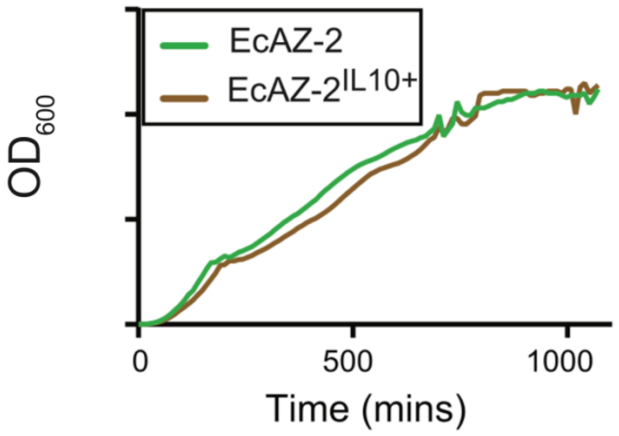


Figure 2A: *In vitro* growth curve of EcAZ-$2^{IL\text{-}10+}$ compared with EcAZ-2. The line represents an average of three measurements per strain. (Adapted from *Russell et al.*)

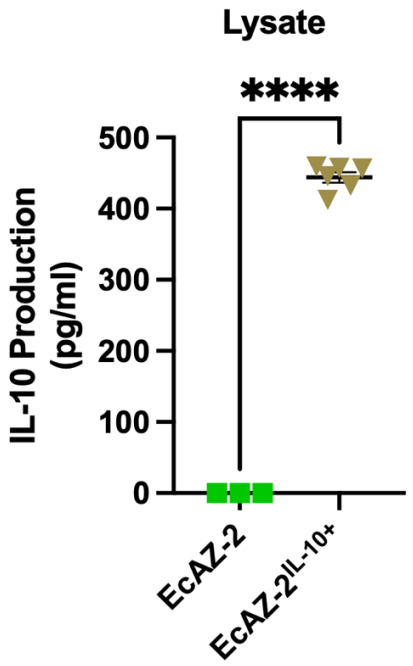


Figure 2B: IL-10 levels detected with ELISA from EcAZ-$2^{IL\text{-}10+}$ cell lysates (n = 6).

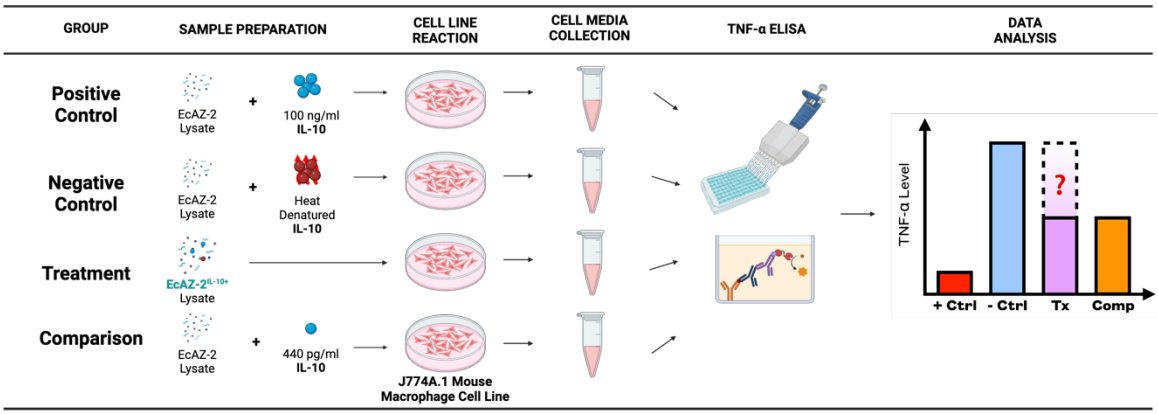
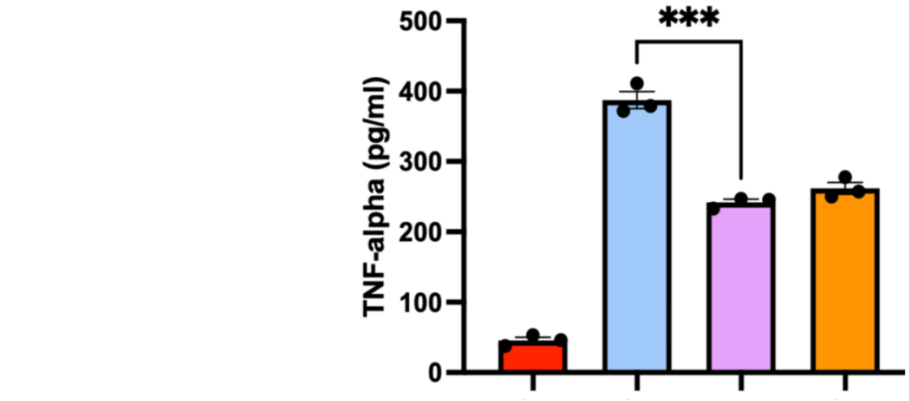


Figure 2C: Schematics of different experimental groups in the verification of bioactivity experiment.



EcAZ-2 Lysate (LPS)	+	+	-	+
100 ng/ml Commercial IL-10	+	-	-	-
Denatured Commercial IL-10	-	+	-	-
EcAZ-2^{IL-10+} Lysate	-	-	+	-
440 pg/ml Commercial IL-10	-	-	-	+

Figure 2D: TNF-α cell assay shows comparable *in vitro* anti-inflammatory effect between commercially available recombinant IL-10 and the lysate of EcAZ-2^{IL-10+} (n=3).

Data are analyzed with unpaired parametric t-test with Welch's correction (B and D).

Chapter 2 ECAZ-2$^{IL\text{-}10+}$ SHOWS STABLE COLONIZATION BEFORE AND AFTER DSS TREATMENT

To assess the therapeutic effect of EcAZ-2$^{IL\text{-}10+}$ in ameliorating colitis, we used conventionally raised, six-week-old wild-type C57BL/6 male mice from Jackson Laboratory (Bar Harbor, ME). This strain has previously shown stable colonization by our engineered native *E. coli* (Russell et al., 2022). We selected young male mice because DSS-induced colitis develops more gradually in younger animals due to their lower food and water intake. Additionally, males exhibit a more aggressive progression of DSS-induced colitis, making them suitable for this disease model (Chassaing et al., 2015). As illustrated in the experimental timeline (**Fig. 3A**), mice from different groups received a single oral gavage of either PBS (vehicle control), EcAZ-2 (bacterial chassis control), or EcAZ-2$^{IL\text{-}10+}$ (treatment). One week after the initial gavage, acute colitis was induced by providing water with 2% DSS for 7 days, while the healthy control group received normal water without the supplement (**Fig. 3B**).

Since DSS significantly disrupts the gut microbiome (Liu et al., 2023), we could not assume stable colonization of EcAZ-2$^{IL\text{-}10+}$ throughout the experiment. To monitor bacterial engraftment, we collected stool samples before and after DSS administration. The stool samples were processed and plated on Luria Broth agar plates containing strain-selective antibiotics. After overnight incubation, we checked colonies to verify colonization levels before and after DSS treatment. The data showed no ENB colonization in the PBS group and stable bacteriaL colonization in EcAZ-2 and EcAZ-2$^{IL\text{-}10+}$ groups before and after DSS administration, with no cross-contamination. Although one mouse from each bacterial colitis group failed to produce sufficient stool for analysis due to DSS pathology, the remaining four mice demonstrated stable colonization comparable to pre-DSS levels. This indicates that the DSS model of acute murine

colitis does not significantly affect the colonization of EcAZ-2 and EcAZ-2$^{IL\text{-}10+}$ which provided validity to interpretation of later results (**Fig. 3C**).

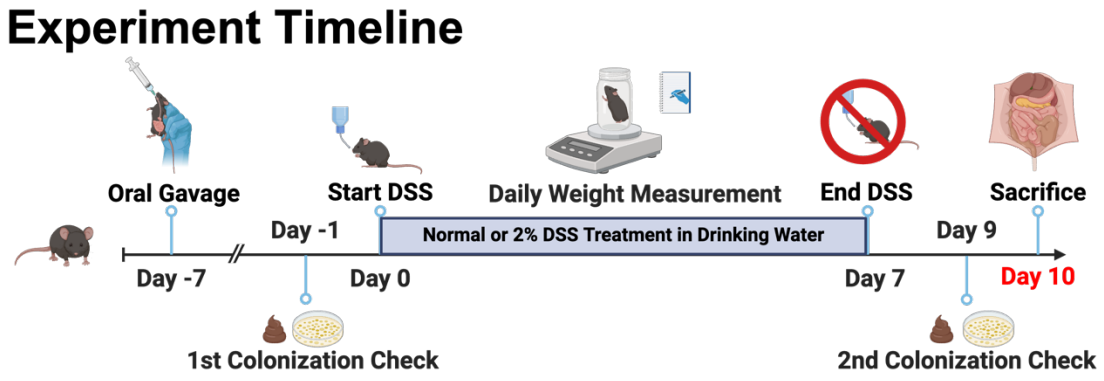


Figure 3A: Schematic of *in vivo* acute DSS-induced colitis experimental timeline.

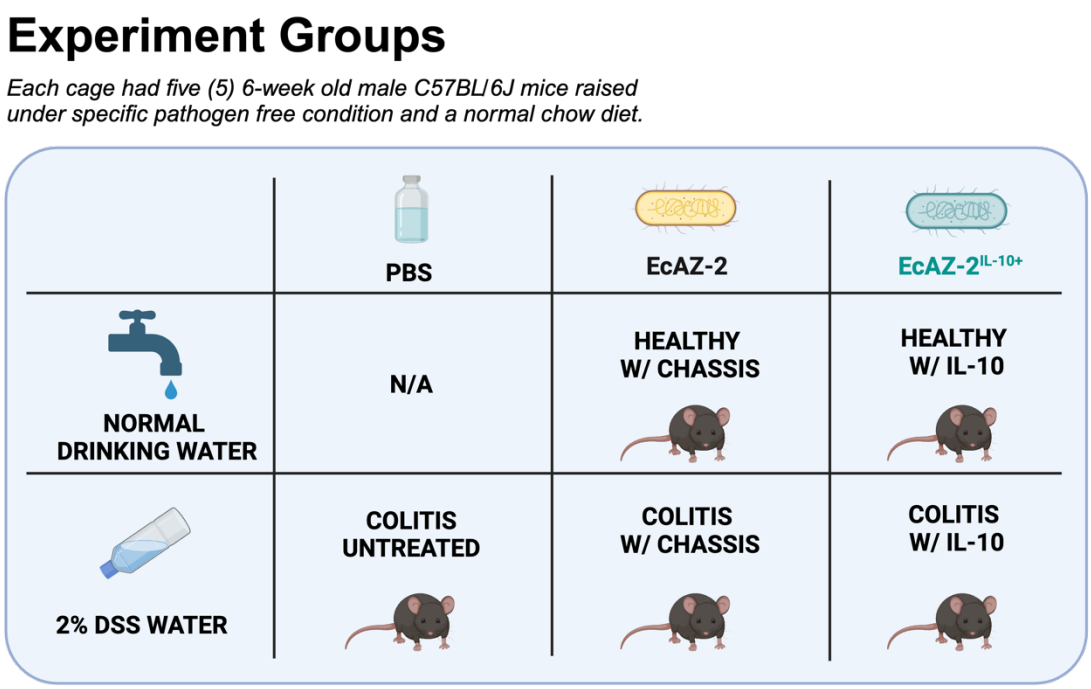


Figure 3B: Schematics of the experiment groups.

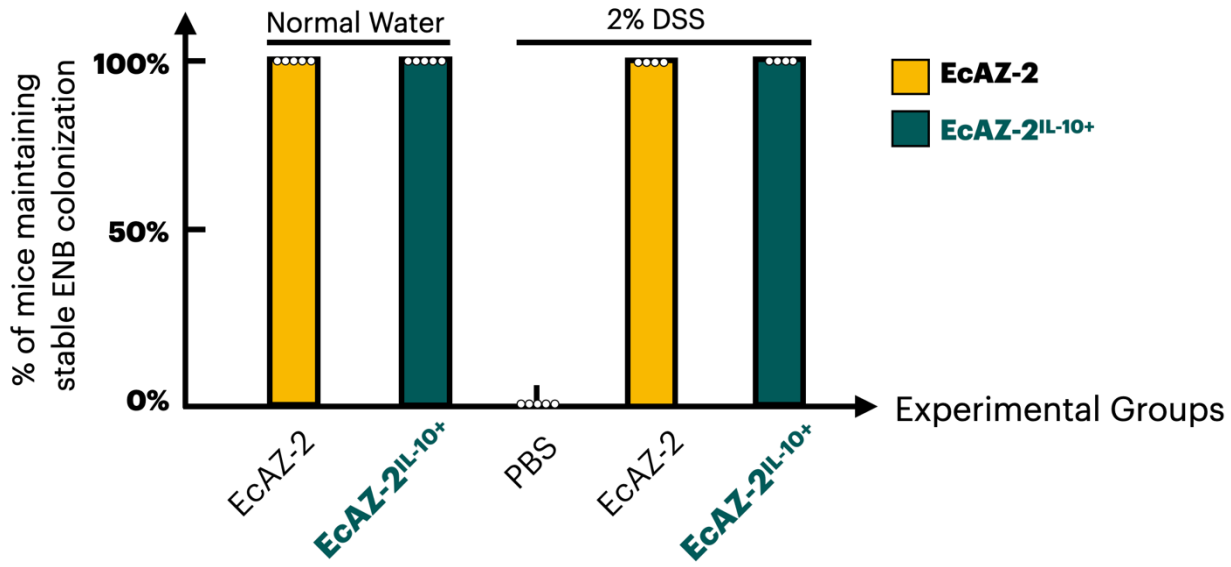


Figure 3C: Qualitative colonization chart showcasing different strains of bacteria tested (n=4 or 5) before and after DSS treatment after gavage of 10^{10} CFUs of EcAZ-2 or EcAZ-$2^{IL\text{-}10+}$ in non-antibiotic-treated CR-WT mice housed in an SPF facility (4-5 mice/condition).

Chapter 3 INTESTINAL DELIVERY OF IL-10 BY ECAZ-2^{IL-10+} DID NOT LESSEN THE SEVERITY OF MURINE COLITIS PHENOTYPE INDUCED BY DSS

To evaluate the severity of colitis under different conditions, we used three metrics to analyze the therapeutic efficacy of EcAZ-2^{IL-10+}: percentage change in body weight, colon measurements, and colon histology analysis.

Body Weight Change: Body weight serves as a proxy for overall health, with mice experiencing more severe colitis typically showing more weight reduction during DSS administration. Weight monitoring started from the first day of DSS administration and continued daily until the day of sacrifice. From day 6 onwards, groups treated with DSS exhibited a significantly lower weight compared to healthy controls, validating the disease model (**Fig. 4A**). None of the groups experienced more than a 20% weight loss, which complies with the Animal Care Program (ACP) ethical regulations. According to these guidelines, mice that lose more than 20% of their initial body weight are to be discontinued from the study and euthanized. There were no significant differences in weight change between healthy groups receiving either EcAZ-2 or EcAZ-2^{IL-10+}, indicating that the IL-10 expressing bacteria were safe (**Fig. 4B**). Among the DSS-treated groups, all displayed significant weight loss, but there were no significant differences between those receiving different oral gavage contents. This suggests that the IL-10 produced by EcAZ-2^{IL-10+} did not ameliorate colitis-induced weight decrease (**Fig. 4C**).

Colon Measurements: During sacrifice, colons were harvested, cleaned of stool, washed in PBS, and measured for length and weight. Severe colitis is typically characterized by shorter colon lengths and higher weight per length ratios due to immune cell infiltration (**Fig. 4D**). As expected, all DSS-treated groups showed a significantly higher colon length-to-weight ratio

compared to healthy groups, with no significant differences observed among the colitis groups, indicating limited therapeutic effect of EcAZ-$2^{IL\text{-}10+}$ (**Fig. 4E**).

Histology Analysis: Colon sections were stained with hematoxylin and eosin (H&E) and scored by a blinded pathologist using a defined scoring system (0-3: 0 healthy, 1 mild, 2 moderate, 3 severe). Colon tissue exhibiting severe colitis featured ulceration and enlarged lymphoid tissue (**Fig. 4F**). Consistent with body weight and colon measurement results, all DSS-treated groups were rated as severe with a score of 3, while all healthy groups were rated as healthy with a score of 0 (**Fig. 4G**).

Overall, all three metrics consistently indicated that while the intestinal delivery of EcAZ-$2^{IL\text{-}10+}$ was safe, it did not exhibit a significant therapeutic effect in rescuing acute colitis.

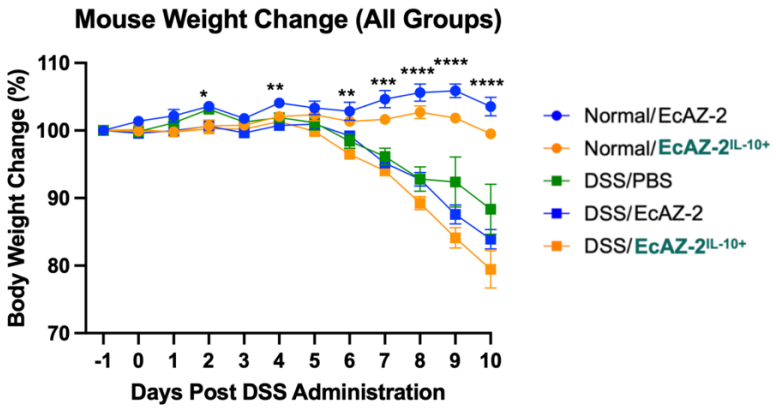


Figure 4A: Daily body weight changes as a percentage of the initial weight for all treatment groups (n=5).

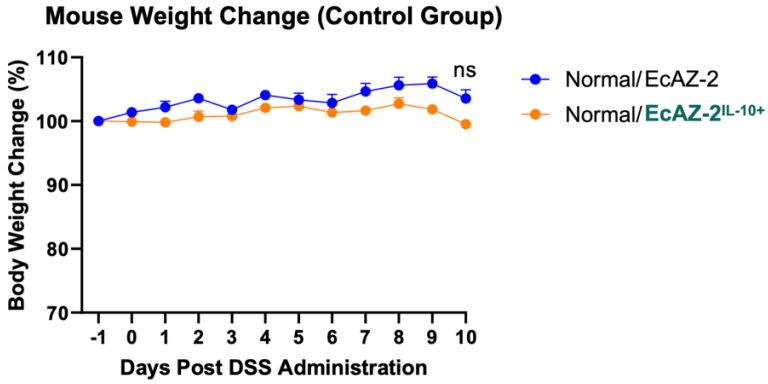


Figure 4B: Daily body weight changes as a percentage of the initial weight in normal water groups (n=5).

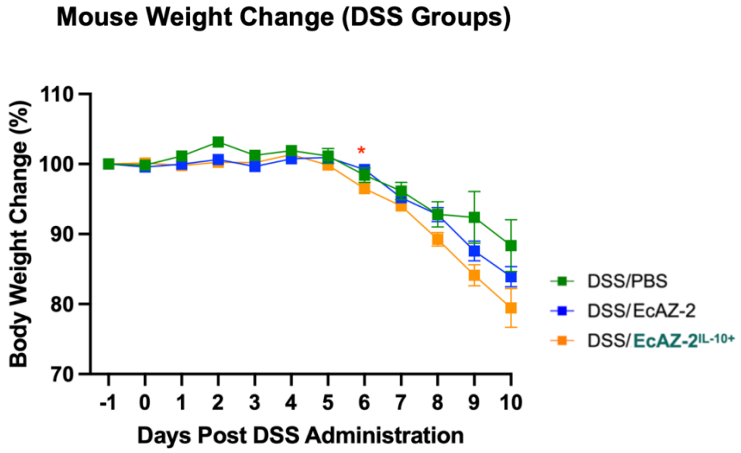


Figure 4C: Daily body weight changes as a percentage of the initial weights among DSS groups (n=5).

Figure 4D: Sample comparison of the colon shortening between healthy and colitis mice.

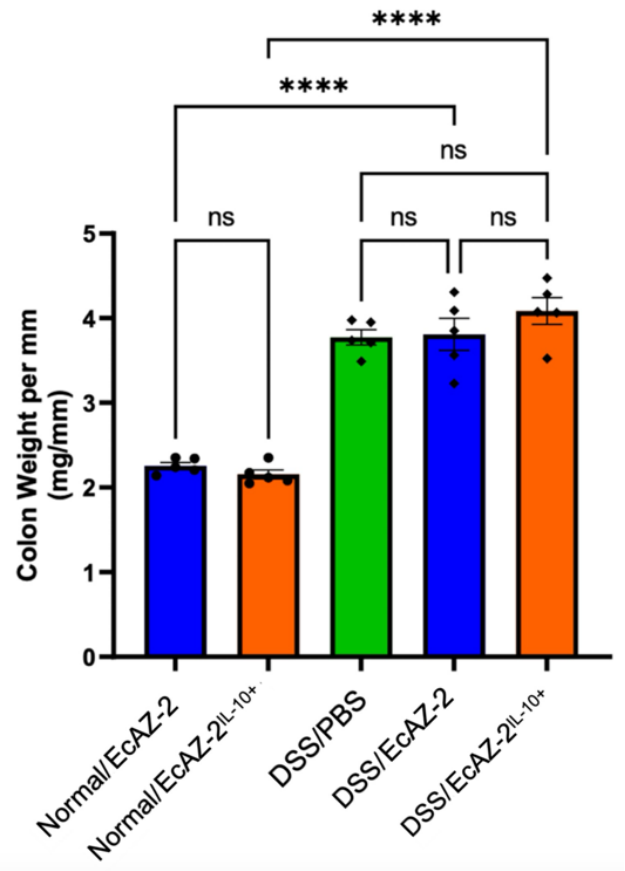


Figure 4E: Normalized comparison of colon weight-to-length ratios (n=5).

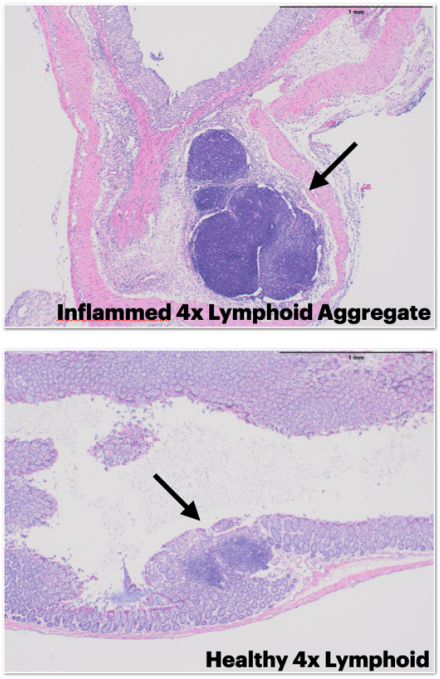


Figure 4F: Representative colon histology sections stained with hematoxylin and eosin between healthy and colitis mice.

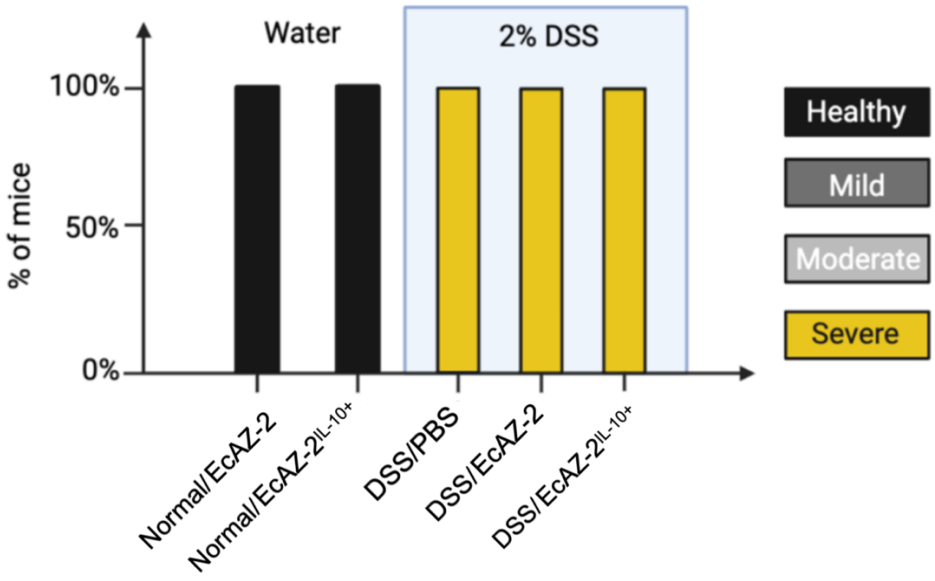


Figure 4G: Histological scoring of colitis severity: 0 = healthy, 1 = mild (neutrophilic cryptitis), 2 = moderate (neutrophilic crypt abscess), 3 = severe (ulceration present).

Data reflect 5 mice per group, and are presented as mean ± SEM (A, B, C, and E). Data were analyzed with a two-way ANOVA with Tukey's post hoc test.

DISCUSSION

My thesis aimed to address the pressing need for more effective therapeutic interventions in IBD by exploring the potential of IL-10-expressing native *E. coli* as a novel treatment strategy. Unlike treatments such as systemic administration of recombinant IL-10 or attempted local delivery using mal-adapted probiotics, our approach leveraged the natural colonization ability of native *E. coli* to deliver IL-10 directly to the gut lumen. A major milestone achieved is the verification that the engineered bacteria produce bioactive rIL-10 and continue to colonize the gut after DSS administration. Compared to commercially available IL-10, the rIL-10 produced by EcAZ-$2^{IL\text{-}10+}$ showed a comparable anti-inflammatory effect *in vitro* by reducing TNF-α production in mouse macrophages after LPS stimulation. *In vivo*, using the DSS-induced mouse model of acute colitis to assess the therapeutic potential of IL-10-expressing *E. coli* in IBD, we did not observe significant improvements within the 10-day experimental timeframe for parameters such as weight loss, colon weight to length ratio, and histological scores. However, the safety and potential for long-term treatment using the EcAZ-2 chassis warrant further pursuit.

I hypothesize that the lack of therapeutic effect of EcAZ-$2^{IL\text{-}10+}$ is due to inadequate IL-10 delivery levels. Literature indicates that the IL-10-secreting *L. lactis* used by Steidler et al. (2000) generated 3 ug/mL of IL-10 concentration in the supernatant – significantly higher than the 440 pg/ml measured in EcAZ-$2^{IL\text{-}10+}$ lysate. Additionally, our bacterial chassis does not secrete the therapeutic protein into the gut lumen, but instead releases it through cell lysis during regular cell turnover. To improve therapeutic efficacy of EcAZ-$2^{IL\text{-}10+}$, a synchronized bacterial lysis approach to release more IL-10 (Din et al., 2016) can be explored. However, lysis of the majority of the EcAZ-$2^{IL\text{-}10+}$ population might disrupt stable colonization as *E. coli* already has low abundance in the gut (Tenaillon et al., 2010). Moreover, programming a suicide mechanism

could lower the overall species fitness and ultimately jeopardize its perpetual colonization feature. Alternatively, developing a secretion mechanism could lead to increased luminal IL-10 levels and promote *in vivo* bioactivity. Although the rIL-10 in the lysate of manually -lysed EcAZ-$2^{IL\text{-}10+}$ is biologically active, natural cell death in the gut may encourage rIL-10 proteolysis in the cytoplasm, resulting in a lower dosage of bioactive IL-10 released. As pointed out by Choi & Lee (2004), secretory production of heterologous proteins in *E. coli* offers several benefits over cytoplasmic production, such as the prevention of inclusion body formation, proper disulfide bond formation, and an authentic N-terminal amino acid sequence after signal peptide cleavage, without the extension of methionine. Secretion could help achieve a higher dosage of bioactive IL-10 in the inflamed tissue.

In summary, while the intestinal delivery of IL-10 by EcAZ-$2^{IL\text{-}10+}$ did not show a significant rescue effect in acute colitis, our findings contribute to the understanding of using engineered bacteria for therapeutic purposes, particularly in the context of IBD. The study underscores the importance of optimizing therapeutic production and delivery mechanisms. It also highlights the potential of native bacterial chassis for sustained colonization and therapeutic protein delivery, despite the need for further refinement. Addressing the current challenges and advancing our understanding of IL-10-based therapies will be crucial steps towards developing effective and durable treatments for patients suffering from IBD.

MATERIALS & METHODS

In vitro Assays for Characterization of IL-10 Biological Activity: To verify the bioactivity of recombinant IL-10, we tested its inhibitory activity on the lipopolysaccharide (LPS)-induced production of TNF-α by macrophages.

Samples were prepared by first growing EcAZ-2$^{IL\text{-}10+}$ overnight in Super Optimal Broth supplemented with 12.5 ug/mL of kanamycin and 10 ug/mL of chloramphenicol. The next day, the cells were pelleted by centrifugation at 4,000 RCF at 4ºC for 10 minutes, and the resulting pellet was resuspended in 1 ml of 1x PBS. Samples were then lysed via 6 rounds of sonication (20 second of sonication followed by a 20 second pause). IL-10 protein levels in the cell lysates were measured by ELISA (Catalog 430601, BioLegend), following manufacturer instructions. Bicinchoninic acid (BCA) protein quantification assay was done on bacterial samples to help determine concentration and prevalence with respect to all bacterial protein production (Catalog G1002, CoreBio).

J774A.1 cells (a mouse macrophage cell line) were seeded at a density of 5×10^4 cells per well in 24-well tissue culture plates and incubated for 2 hours at 37°C, 5% CO_2. The cells were then stimulated with LPS, a common component of the cell wall in gram-negative bacteria such as E. coli. Note that, through performing an LPS quantification ELISA, we determined that EcAZ-2 and EcAZ-2$^{IL\text{-}10+}$ lysates had similar levels of LPS if they originally had similar bacterial density as measured by OD600, ensuring controlled stimulation across experiment groups.

For the positive control and comparison groups, commercially available recombinant IL-10 (c-rIL-10) was preincubated alone at 37°C. These IL-10 samples were then added to the incubated J774A.1 cells at concentrations of 100 ng/ml for the positive control and 440 pg/ml for the comparison group. For the negative control group, c-rIL-10 was denatured at 95°C for 30

minutes before being added along with LPS-containing native E. coli (EcAZ-2) to the incubated J774A.1 cells. For the treatment group, EcAZ-$2^{IL\text{-}10+}$ lysate was added at a comparable density to EcAZ-2 in the other groups, with the IL-10 concentration matching that of the comparison group.

After 4 hours of incubation at 37°C, 5% CO_2, the amount of TNF-α released in the medium was measured in triplicate by ELISA (Catalog 430901, BioLegend) according to the manufacturer's instructions.

Colonization Assessment: 2-4 stool pellets were collected from each mouse and weighed. 1 mL of sterile deionized water was added to each sample along with one sterile chrome bead (Neta Scientific, Hainesport, NJ). Samples were homogenized for 2 min. at 3500 rpm in Mini-Beadbeater-24 (BioSpec, Bartlesville, OK). For control samples, homogenized stool was plated on Luria Broth plates containing kanamycin or chloramphenicol to check for bacterial contamination. For EcAZ-2 colonized mice, samples were plated on chloramphenicol to check for unintended bacterial contamination and diluted and plated on kanamycin to check for colonization. The EcAZ-$2^{IL\text{-}10+}$ stool was diluted and plated on kanamycin and chloramphenicol.

Animal Experiments: All animal experiments were conducted in accordance with the guidelines of the IACUC of the University of California, San Diego. C57BL/6 mice (Jackson Laboratories) were housed in a specific pathogen free facility (irradiated chow and autoclaved bedding). Mice were housed 5 per cage and fed a normal-chow diet (Diet 7912, Teklad Diets, Madion, WI). After an acclimation period, 6-week-old male mice were gavaged with 200 uL of either PBS or ~10^{10} CFU of the corresponding bacterial strain. One week after oral gavage, mice received either normal autoclaved drinking water or 2% dextran sulfate sodium (DSS) in the autoclaved drinking water for 7 days. Weight loss was monitored daily, and colon tissues were collected at day 10.

Histology Analysis: Paraformaldehyde-fixed colon tissue was transferred to 70% ethanol before processing by routine paraffin embedding, sectioning and hematoxylin and eosin staining by Moores Cancer Center (La Jolla, CA). A pathologist (J.H.), blinded to experimental parameters, determined colitis scores. Each of the following four histologic parameters were scored as healthy (0), mild: neutrophilic cryptitis (1), moderate: neutrophilic crypt abscess (2), or severe: ulceration present (3). Images were captured at 4x magnification with a Nikon Eclipse NI-U and NSI-Element Basic Research software (Nikon).

Computer Software: Figures were generated using BioRender and PRISM GraphPad (v.10.2.3).

Statistical Analysis: Statistical analyses were performed using PRISM GraphPad (v.10.2.3). Details of statistical analysis and sample size are provided in the figure legends. No samples were excluded from any experiments performed in this study unless in the case of technical failure. Experimenters, with the exception of the pathologist, were not blinded to experimental conditions.

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