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IN VIVO CHARACTERIZATION OF SER-301, A RATIONALLY-DESIGNED INVESTIGATIONAL MICROBIOME THERAPEUTIC FOR PATIENTS WITH ACTIVE MILD-TO-MODERATE ULCERATIVE COLITIS

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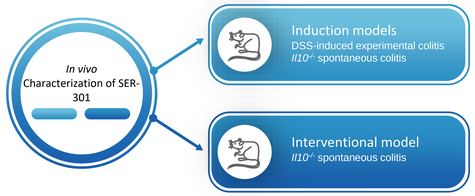
INTRODUCTION

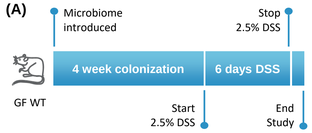
- Ulcerative colitis (UC) is a relapsing-remitting chronic inflammatory disorder affecting the mucosal surface of the colon (Danese and Fiocchi 2011). The role of the microbiome in the development, progression, and treatment of UC has been a subject of considerable interest.
- SER-301 is an oral, rationally-designed investigational microbiome therapeutic composed of cultivated spores and vegetative bacterial strains intended to treat UC by modifying the gastrointestinal microbiome to reduce intestinal inflammation and repair epithelial barrier damage that are central to UC pathogenesis.
- We used mouse models of colitis to evaluate the ability of SER-301 to promote immune homeostasis in the colon and counterbalance inflammatory conditions to prevent the development of colitis.
- In multiple preclinical models, SER-301 demonstrated the ability to modulate gut immune cell populations towards a noninflammatory phenotype, promoting the development of regulatory T cells and decreasing the frequency of inflammatory Th1 and Th17 effector T cells



SER-301 MODULATES COLONIC CD4+ T CELLS IN THE DSS-INDUCED COLITIS MODEL TOWARDS A NONINFLAMMATORY PHENOTYPE

In the dextran sodium sulfate (DSS)-induced model of colitis, germ-free (GF) wild-type C57BL/6 mice were colonized with either SER-301 or a composition containing strains isolated from UC patients with proinflammatory properties ("INFL+"). SER-301 colonization modulated colonic CD4+ T cell populations towards a noninflammatory phenotype resulting in a significantly lower frequency of pro-inflammatory Th1 and Th17 effector T cells, and increased peripheral regulatory T cells, compared to mice colonized with INFL+.

Figure 1



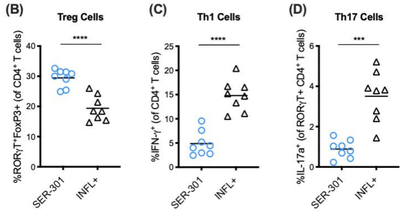


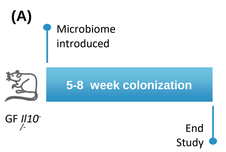
Figure 1: SER-301 colonization led to a noninflammatory effect in the GF DSS-induced mouse model of colitis. A) GF wild-type C57BL/6 mice were colonized with SER-301 (n=8) or INFL+ (n=8) for 4 weeks and subsequently subjected to 2.5% DSS for 6 days. On Day 7, lymphocytes were isolated from the colonic lamina propria for flow cytometric analysis. **B)** SER-301 colonization resulted in an increased frequency of Foxp3+ RORγt+ CD4+ T cells ("Treg"), which represent a stable peripheral Treg population known to have a highly anti-inflammatory phenotype (Kim et al. 2016), relative to INFL+. **C-D)** Additionally, the frequency of pro-inflammatory Th1 and Th17 effector T cells was lower in SER-301 colonized mice compared to mice colonized with INFL+ after DSS administration. Lines represent the mean and points represent individual mice.

SER-301 PROMOTES REGULATORY IMMUNE RESPONSES AND DOES NOT INDUCE COLITIS IN THE *Il10*$^{-/-}$ SPONTANEOUS COLITIS MODEL

Experimental Design

SER-301 was evaluated in a spontaneous colitis model using GF *Il10*$^{-/-}$ C57BL/6 mice. Mice were colonized with either SER-301 or a human fecal microbial transplant prepared from IBD patients ("IBD FMT") and monitored for 5-8 weeks (Experiment 2a). The severity of intestinal inflammation was assessed by fecal lipocalin, histopathology (cecum, colon, rectum) and flow cytometric characterization of colonic immune cell populations. The same evaluation was repeated in a second experiment (Experiment 2b), colonizing mice with SER-301 or the inflammatory composition INFL+. In both experiments, SER-301 colonization induced a noninflammatory immune response in the colon and did not induce colitis, while colonization with IBD FMT or INFL+ significantly increased levels of lipocalin and frequency of proinflammatory T cell populations, indicative of intestinal inflammation and colitis.

Figure 2



Histology and Lipocalin Results

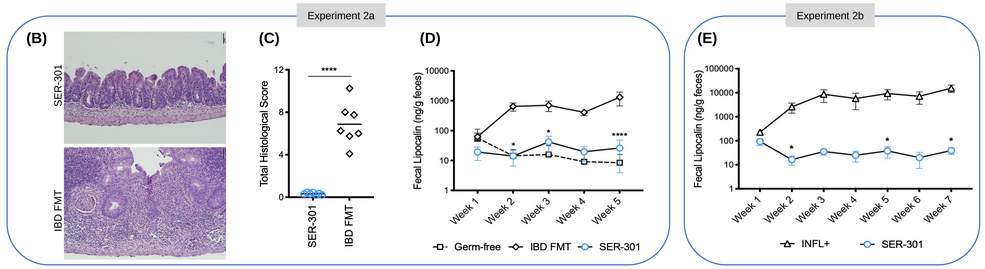


Figure 2: SER-301 colonization does not induce intestinal inflammation or disease histopathology in the *Il10*$^{-/-}$ model. A) Experimental design. GF *Il10*$^{-/-}$ C57BL/6 mice were colonized with SER-301 (n=7) and pooled IBD FMT (experiment 2a), or another composition containing strains isolated from UC patients with proinflammatory properties ("INFL+") (experiment 2b) for 5-8 weeks in two separate studies. **B)** Representative histological images of cecum, H&E stain. **C)** Total histological score (0-16) of the cecum, proximal and distal colon, and rectum for alterations and signs of inflammation, quantitated in a blinded fashion. **D-E)** Fecal lipocalin-2 was measured weekly in feces by ELISA in experiment 2a (**D**) and experiment 2b (**E**). Statistical significance determined by paired t-test across timecourse, represented as a p-value of p<0.05*, p<0.01**, p<0.001***, p<0.0001****

Flow Cytometry Results

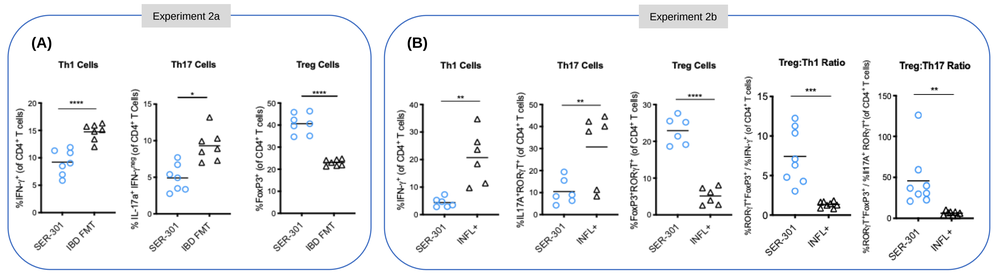


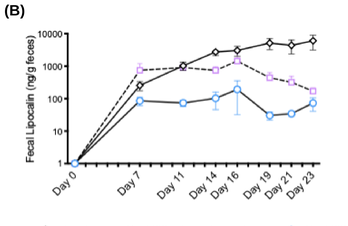
Figure 3: SER-301 treatment modulated colonic immune cell populations towards a noninflammatory phenotype. Measurement of lamina propria lymphocytes via flow cytometry from spontaneous *Il10*$^{-/-}$ colitis model. **A)** Results from experiment 2a including IFNγ+ IL17- or IFNγ+ CD4+ ("Th1"), IL17+ or IL17+ IFNγ- CD4+ ("Th17"), and Foxp3+ CD4+ ("Treg"), T cells. **B)** Results from experiment 2b, including Th1, Th17, Foxp3+ RORγt+ CD4+ Peripheral Tregs and the ratio of the frequency of percent Treg cells to percent Th1 or Th17 cells was calculated. Lines represent the mean and points represent individual mice. Data were analyzed using one-way or two-way ANOVA with a post-hoc Fisher's LSD test. Significance was determined as a p-value of p<0.05*, p<0.01**, p<0.001***, p<0.0001****.

INTERVENTIONAL TREATMENT WITH SER-301 ATTENUATES INTESTINAL INFLAMMATION IN THE *Il10*$^{-/-}$ SPONTANEOUS COLITIS MODEL

Experimental Design

Interventional dosing of SER-301 was also evaluated in the GF *Il10*$^{-/-}$ C57BL/6 mouse model. Mice were first colonized with the INFL+ composition to induce intestinal inflammation. Once a sustained increase in fecal lipocalin was observed indicating colitis development, mice were randomized into a group that remained untreated or repeatedly dosed with SER-301. A third group was only colonized with SER-301 as a comparator. The severity of intestinal inflammation was assessed by fecal lipocalin and flow cytometric characterization of colonic immune cell populations. SER-301 intervention led to a decrease in lipocalin levels compared to untreated INFL+ mice and modulated colonic CD4+ T cell populations towards a more noninflammatory phenotype, suggesting SER-301 treatment attenuates intestinal inflammation and colitis development.

Figure 4



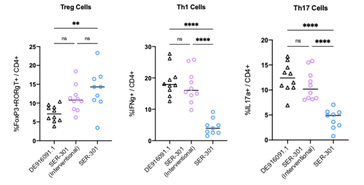


Figure 4: SER-301 interventional treatment attenuates established intestinal inflammation in the *Il10*$^{-/-}$ model. A) Experimental design. GF *Il10*$^{-/-}$ C57BL/6 mice were colonized with INFL+ (n=20) for 1-2 weeks and then left untreated or dosed 3x a week with SER-301. **B)** Fecal lipocalin-2 was measured weekly in feces by ELISA. Statistical significance determined by paired t-test across timecourse for lipocalin and , represented as a p-value of p<0.05*, p<0.01**, p<0.001***, p<0.0001**** **C)** Measurement of lamina propria lymphocytes via flow cytometry. Results include IFNγ+ IL17- or IFNγ+ CD4+ ("Th1"), IL17+ or IL17+ IFNγ- CD4+ ("Th17"), and Foxp3+ CD4+ ("Treg"), T cells. Lines represent the mean and points represent individual mice. Data were analyzed using one-way or two-way ANOVA with a post-hoc Fisher's LSD test.

CONCLUSIONS

- Preclinical assessments of SER-301 in two prophylactic mouse models of colitis show that SER-301 treatment promotes a noninflammatory immune profile in the colon and does not induce the development of colitis in germ-free *Il10*$^{-/-}$ C57BL/6 mice.
- Additionally, interventional dosing of SER-301 in the *Il10*$^{-/-}$ mouse model showed that SER-301 is capable of reversing established intestinal inflammation and the development of colitis.
- SER-301 design and in vitro properties will be presented in the Microbiome and Host Response in IBD session at DDW 2021.
- A Phase 1b study evaluating SER-301 for the treatment of active mild-to-moderate UC is currently enrolling (ACTRN12620000963921).