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Synthetic Biotic Producing AHR Metabolites for the Treatment of Inflammatory Bowel Disease

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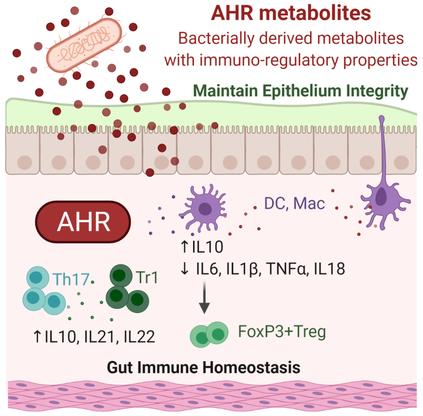
Introduction

- Inflammatory bowel diseases (IBD) are chronic intestinal inflammatory conditions attributed to an intricate interplay of genetic and environmental factors.
- Mammalian aryl hydrocarbon receptor (AHR) is a ligand-dependent transcription factor with barrier-protective and immunomodulatory roles in cells within the intestinal microenvironment that can bind structurally diverse ligands, including indole acetic acid (IAA).



EcN engineered pathway for IAA production

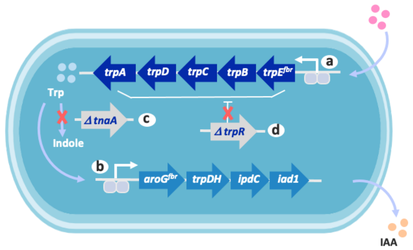


Fig 1. EcN-IAA strain contains two plasmids: **(a)** plasmid encoding the enzymatic pathway for tryptophan (Trp) production, *trpE^{fbr}BCDA* **(b)** plasmid encoding the enzymatic pathway which converts Trp to indole-3-acetic acid (IAA), *trpDH-ipdC-iad1C*. A feedback inhibition resistant DAHP synthase, encoded by *aroG^{fbr}*. Regulation of Trp and IAA production is carried out by P_{frrS} anaerobic-inducible promoter. The *E. coli* Nissle (EcN) chassis contains two key chromosomal deletions: **(c)** *tnaA*, encoding tryptophanase, which converts Trp to indole, and **(d)** *trpR*, encoding Trp operon repressor, which inhibits production of Trp pathway enzymes.

Engineered EcN produces and secretes bioactive IAA ligand

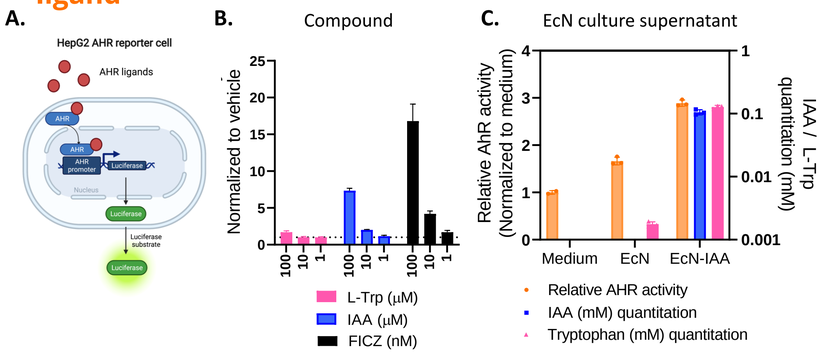


Fig 2. A) AHR luciferase reporter cells were stimulated with AHR ligands for 48 hrs and luciferase activity readout. **B**) The bioactive range of IAA to activate AHR is between 10-100 μM and the intermediate tryptophan product is not able to activate AHR efficiently, FICZ included as a positive control. **C)** EcN cultured medium was used in AHR reporter assay and levels of IAA and L-Trp measured with mass spectrometer.

IAA maintains integrity of intestinal epithelium barrier

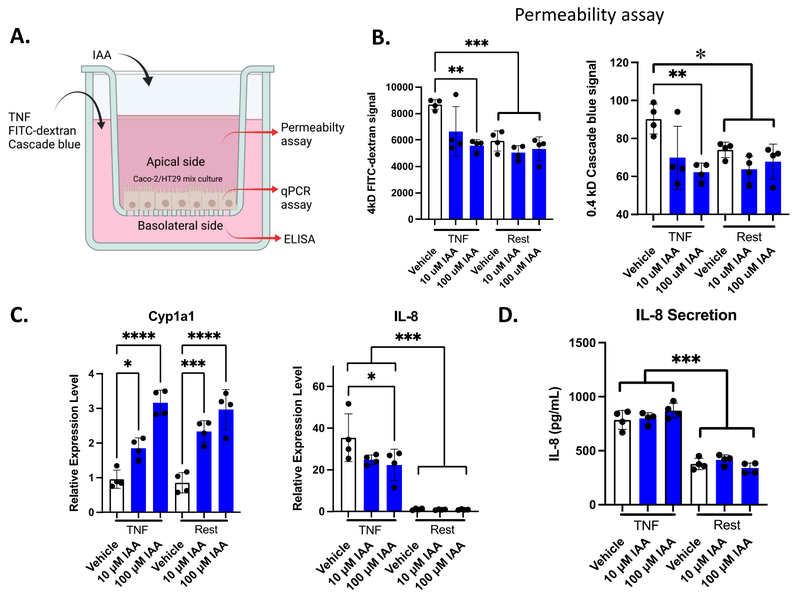


Fig. 3 A) Caco-2/HT-29 epithelial cell transwell assay used to evaluate the effect of IAA on intestinal barrier (apical side medium), gene expression (cell) and cytokine secretion (basolateral medium). **B**) IAA treatment reduces inflammation-induced leakage in intestinal barrier. 4kD FITC-dextran and 0.4kD cascade blue were used. **C)** IAA induces AHR downstream gene, Cyp1a1, expression and reduces inflammatory IL8 expression. **D**) IAA treatment in intestinal epithelial cells has no effect in basolateral IL8 secretion. (ANOVA multiple comparison, *, p<0.05; **, p<0.01; ***, p<0.001; ****, p<0.0001)

IAA modulates immune response toward epithelium tissue repair and immunosuppression

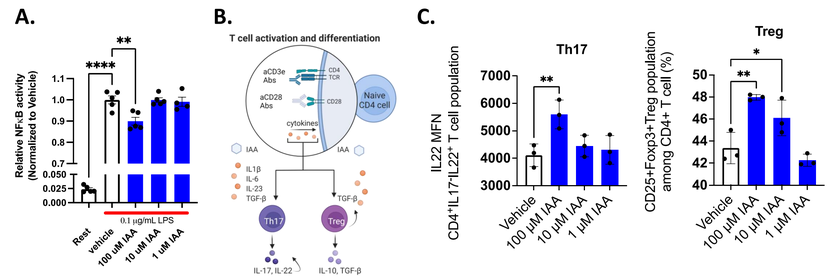
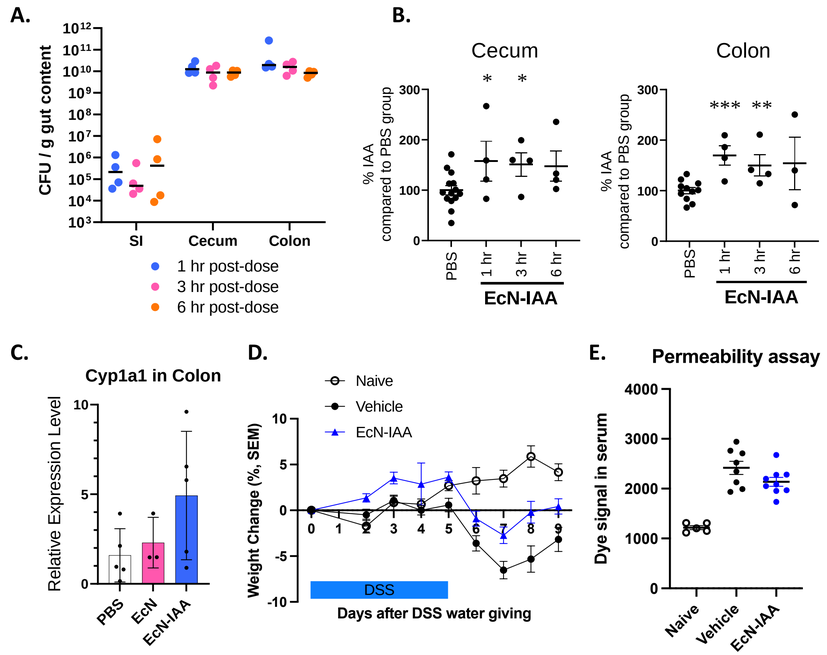


Fig. 4. A) NFκB luciferase reporter THP-1 cells were pretreated with IAA for 24 hrs followed with 24 hrs of LPS stimulation, and luciferase activity readout. **B**) Human naïve CD4+ T cells were stimulated with anti-CD3e, anti-CD28 antibody and cytokines to induce T cell differentiation. Th17 were induced with IL1β, IL6, IL23 and TGFβ; and Treg were induced with TGFβ. **C**) Additional IAA treatment promotes IL22 expression in Th17 cells and increases FOXP3$^+$Treg population. (ANOVA multiple comparison is applied, *, p<0.05; **, p<0.01; ***, p<0.001; ****, p<0.0001)

Engineered EcN-IAA therapy can ameliorate IBD in DSS mouse model



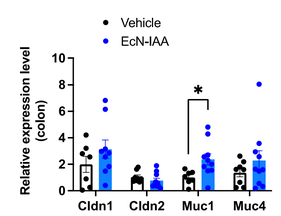


Fig. 5. A) Naïve mice orally dosed with 1E+10. EcN-IAA transits and resides in cecum and colon for at least 6 hrs post-dose. Gut contents were weighed, homogenized and plated for colony formation assay. **B**) EcN-IAA produces and significantly increases IAA concentration in cecum and colon. Gut contents were weighed and homogenized in 80% methanol and LCMS was used to quantify IAA in gut content. **C**) EcN-IAA produces bioactive IAA and activates AHR to drive downstream Cyp1a1 expression. **D-F**) EcN-IAA can reverse diseases progress in DSS induced colitis model by maintaining intestinal barrier. **D)** EcN-IAA was orally dosed before DSS giving and have it colonized by providing selection antibiotic in drinking water for whole experiment. **E)** CW800 dye was given orally the day before blood harvest. Serum was isolated for signal detection **F)** Expression of epithelium functional genes in colon. Colon is harvest at day 9, as shown in Fig. 5D. (ANOVA multiple comparison or t test is applied, *, p<0.05; **, p<0.01; ***, p<0.001)

Conclusions

- Our data demonstrate that IAA can efficiently activate AHR signaling, maintain intestinal barrier and promote immunomodulation *in vitro.*
- Engineered EcN secretes bioactive IAA to activate AHR in mice and lead to reversed disease progression in DSS IBD model.
- Synthetic Biotics activating AHR transcriptional pathway may provide a therapeutic option for reducing inflammation and enhancing mucosal healing in IBD.