A high-throughput gut-immune co-culture model for identifying anti-inflammatory therapies

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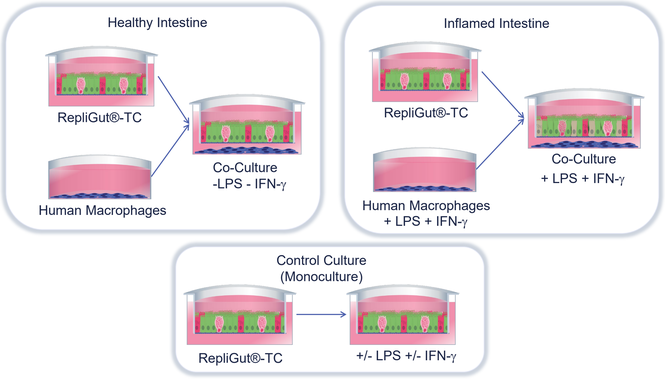
Purpose and Objectives

Current treatments for inflammatory bowel disease (IBD) are limited in their long-term effectiveness, as many patients become non-responsive to anti-inflammatory drugs after several months of treatment, underscoring the urgent need for new therapeutic options. Animal models are poor tools for screening anti-inflammatory leads due to their low throughput, interspecies variability in immune response, and differences in gut physiology.

Objectives

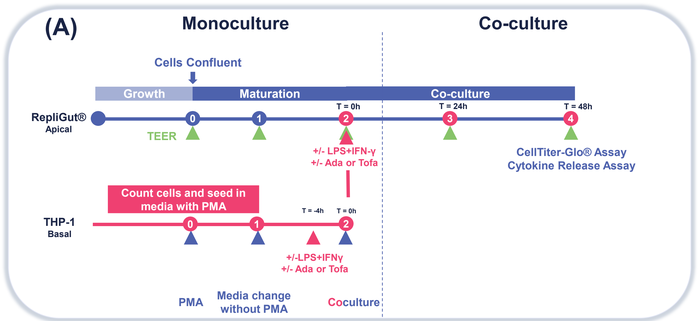
- Develop a human-relevant and high-throughput indirect gut-immune co-culture system that accurately mimics the human IBD microenvironment
- Demonstrate co-culture model responsiveness to endotoxin and prevention of damage with anti-inflammatory treatments

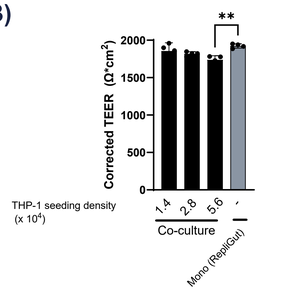
Methods



- RepliGut® Planar – Transverse Colon was established on Transwell® membrane inserts in 96-well format with standard methods
- THP-1 monocytes were directed to the macrophage lineage using PMA on bottom surface of standard 96-well plate.
- The two culture systems were brought together in the presence or absence of macrophage activation stimuli (IFN-γ and LPS) to create comparable “healthy” and “inflamed” intestine environments.

Experimental Overview



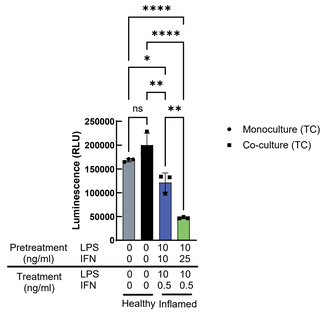


n=4. **p<0.01, One-way ANOVA with Tukey’s multiple comparison test

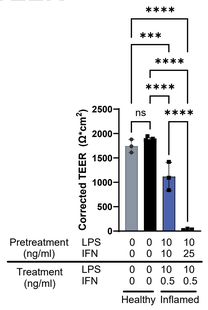
- **(A)** Schematic description of gut-immune co-culture and treatment and assay timelines.
- **(B)** RepliGut® mean TEER values remained steady at 48h of co-culture with increasing THP-1 cell counts.

Establishing Inflamed Conditions

(A) CellTiter-Glo®

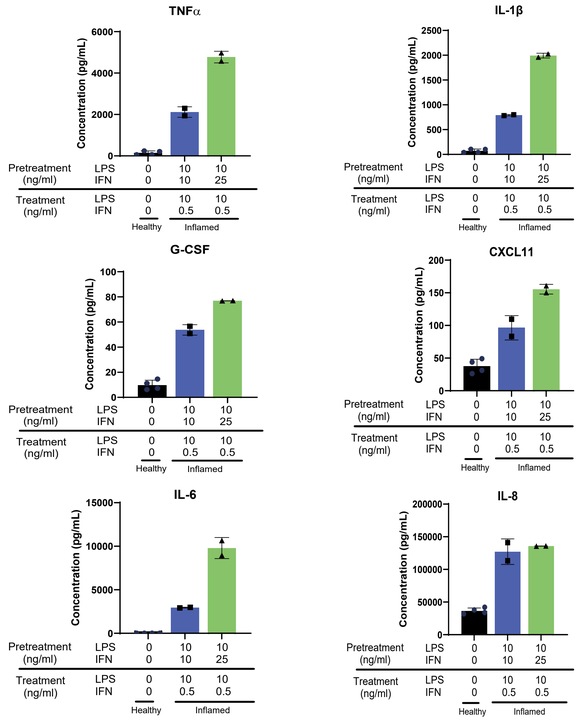


(B) TEER



n=3 technical replicates. ****p<0.0001, Two-way ANOVA with Tukey’s multiple comparison test

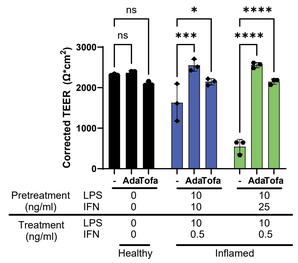
(C) Cytokine Analysis



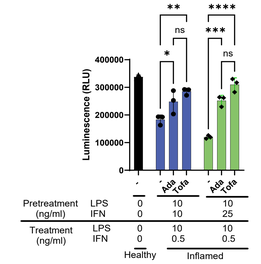
- RepliGut®-TC + THP1 co-cultures maintained high TEER and viability similar to RepliGut®-TC monoculture in the absence of IFN-y and LPS.
- Pretreatment of THP-1 and co-treatment of RepliGut®-TC + THP-1 with IFN-γ and LPS caused a dose dependent decrease in **(A) Cell Viability** and **(B) TEER** after 48h of co-culture
- **(C)** Dose dependent increases in released **cytokines** with increasing pre-treatment of cultures by IFN-γ

Preventing Inflammation

(A) TEER



(B) Cell Titer Glo®



n=3 technical replicates. ****p<0.0001, Two-way ANOVA with Tukey’s multiple comparison test.

- **TEER (A)** and **CellTiter-Glo (B)** assays on inflamed tissues treated with Adalimumab (Ada,TNF-α inhibitor) and Tofacitinib (Tofa, Jak-Stat inhibitor)
- Ada and Tofa treatments fully prevented TEER reduction and cell viability loss

Conclusions

- Human intestine-immune co-culture system established by co-culturing RepliGut® TC with THP-1-derived macrophages.
- Activation of macrophages to inflamed state using IFN-γ and LPS damaged RepliGut®-TC monolayer in the co-culture system and caused increased inflammatory cytokine release.
- Induction of inflammation in the RepliGut®-TC + THP-1 co-culture provides a tunable environment for comparing treatment efficacies in healthy and inflamed models.

References

- Pike, Colleen M., et al. “Characterization and Optimization of Variability in a Human Colonic Epithelium Culture Model”. ALTEX - Alternatives to Animal Experimentation, vol. 41, no. 3, July 2024, pp. 425-38, doi:10.14573/altex.2309221.
- Kämpfer AAM, Urbán P, Gioria S, Kanase N, Stone V, Kinsner-Ovaskainen A. Development of an in vitro co-culture model to mimic the human intestine in healthy and diseased state. Toxicol In Vitro. 2017 Dec;45(Pt 1):31-43. doi: 10.1016/j.tiv.2017.08.011. Epub 2017 Aug 12. PMID: 28807632; PMCID: PMC5744654.

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