Inflammatory bowel disease (IBD)-specific immune cell recruitment and response can be modulated with anti-TNF-α therapies in the human Colon Intestine-Chip

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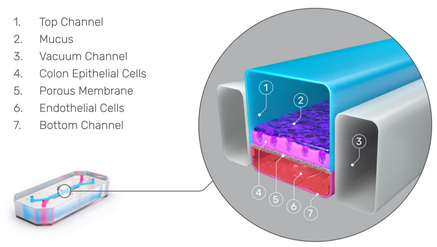
Colon Intestine-Chip Model for IBD

Inflammatory bowel disease (IBD) is a complex inflammatory disease, for which few effective therapies exist. The goal of our current work was to show that: i) such a complex, immune cell-driven pathogenesis could be captured on Emulate's human Colon Intestine-Chips; and ii) this could be used as a novel human-centric system to support IBD drug development including anti-TNF-α antibodies. We previously developed an advanced primary human vascularized Colon Intestine-Chip model and showed that it can recapitulate physiologic cell composition, morphology and barrier integrity [1].

The model described herein is advantageous in recapitulating *in vivo* inflammatory effector functions in that it supports immune cell trafficking under fluid flow conditions, uses primary cell co-culture, and provides a physiologically relevant peristaltic-like stretch.

1. Top Channel
2. Mucus
3. Vacuum Channel
4. Colon Epithelial Cells
5. Porous Membrane
6. Endothelial Cells
7. Bottom Channel



Recapitulating TNFα-Dependent Inflammatory Priming Stimulus on Colon Intestine-Chip

The IBD pathogenic response has been modeled though the natural disease progression pathway:

1) Inflammatory priming of endothelium with chemotactic gradient
2) Immune cell recruitment through vasculature into epithelium
3) Immune cell-dependent inflammation and release of pro-inflammatory cytokines

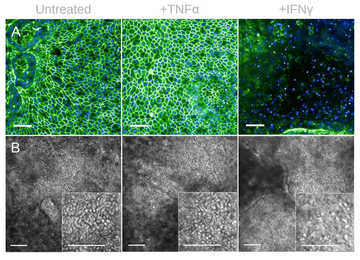


Figure 1. Epithelial Morphology Maintained with TNFα
A) Immunofluorescent tight junction protein (ZO-1) staining of Colon Intestine-Chips were treated basolaterally with TNFα cytokine at 50 ng/mL for 24 h to recapitulate inflammatory priming, or with IFNγ at 50 ng/mL for 72 h to replicate later stages of the immune response in IBD. Tight junctions are preserved in the TNFα cytokine group, whereas a marked loss in signal and structure was observed with IFNγ treatment. B) Phase contrast images of the epithelial monolayer showing morphological disruption with IFNγ treatment only. Scale bar = 100 µm.

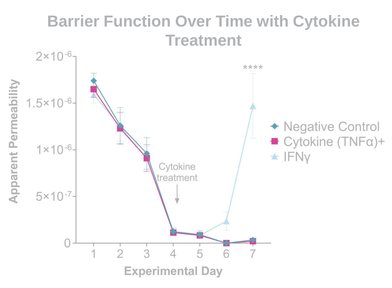


Figure 2. Epithelial Barrier Integrity Disrupted Only with Relevant Cytokines
Treatment of human intestinal microvascular endothelial cells (HIMECs) on Colon Intestine-Chip with TNFα (50 ng/mL) has no effect (p>0.99) on epithelial barrier permeability on Day 7, indicating immune cell-dependency. Cytokine IFNγ positive control (50 ng/mL) has marked (****p<0.0001) barrier disruption as seen in downstream IBD inflammatory pathways. All comparisons to Negative Control (untreated) group, significance determined by ordinary two-way ANOVA with Tukey's multiple comparison test. All groups n>4 chips, similar results demonstrated in n=5 experimental replicates.

Producing Fluidics-Based IBD Model on Colon Intestine-Chip

In contrast to standard *ex vivo* models including leukocyte-endothelial cell co-culture systems and transwell migration assays, the Colon Intestine-Chip is subjected to fluid flow and shear forces, which contribute to physiologic selectivity of leukocyte attachment *in vivo* (Fig. 3). Moreover, the Colon Intestine-Chip includes primary cell co-culture with a flexible membrane that can accurately recreate the intestinal microenvironment and peristalsis that is lacking in other *in vitro* flow based systems.

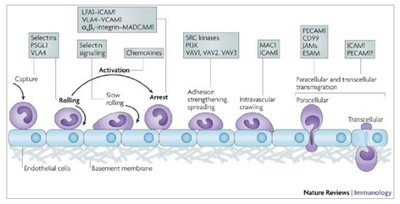


Figure 3. Leukocyte Adhesion Cascade Under Shear Flow
Shear forces are crucial for allowing stimulus and selection-driven arrest of rolling immune cells on the endothelium [2].

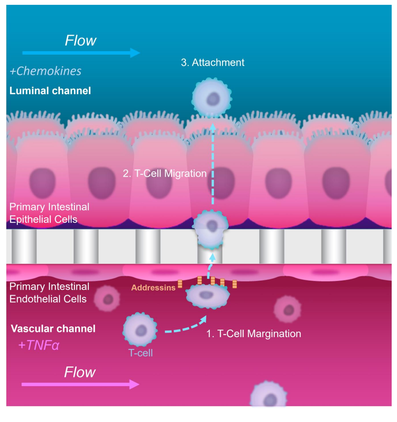


Figure 4. Modelling Inflammatory Immune Cell Recruitment on the Colon Intestine-Chip

Primary cells are cultured on the Chip-S1® Stretchable Chips: human intestinal microvascular endothelial cells (HIMEC) in the bottom vascular channel, and human colonic organoids in the top epithelial channel. When a mature co-culture has been reached under flow (30 µL/hr) approximately 3 days post-seeding, inflammatory priming is stimulated by administering cytokine TNFα at 50 ng/mL into the vascular channel for 24 hours.

At 24 hours post-cytokine treatment, peripheral blood mononuclear cells (PBMCs) are administered into the vascular channel for 4 hours under high flow (1000 µL/h) at physiologic cell density (2x10^6 cells/mL). The top epithelial channel is meanwhile continuously perfused with exogenous IBD-relevant chemokines (CXCL2, CXCL10, CXCL12, CCL19, CCL20, CCL25, CCL28, GPR15L) at 100 ng/mL each to provide a chemotactic gradient.

During administration, the PBMCs interface with the activated endothelium upregulating addressins including MAdCAM-1 and ICAM-1 and migrated upwards through the membrane pores along the chemoattractant gradient. After PBMC were allowed to migrate to intestinal epithelial structures for 72 hours, release of inflammatory cytokines were measured through top-channel effluent collection and multiplex analysis.

PBMCs Demonstrate Inflammation-Specific Attachment

We captured our model's selectivity and stimulus-dependent attachment and migration of PBMCs using confocal, 3D imaging analysis. Further downstream effector analyses were performed by measuring cytokine release and barrier permeability of the epithelium as a result of recruited PBMC. Finally, this work also presents donor-to-donor variability in attachment from three PBMC donors (Figure 6A).

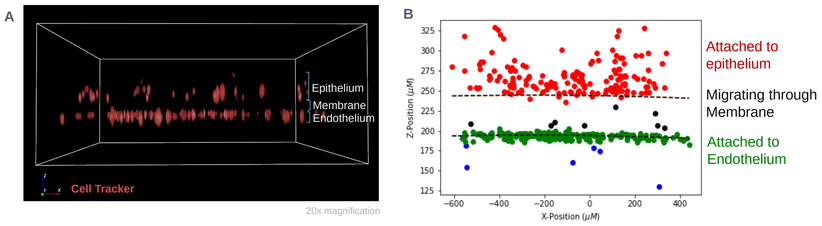


Figure 5. Opera Phenix z-stack Imaging and PBMC Location Classification
A) Full-tile (n>24 fields of view at 20x mag.) confocal images are acquired of the Colon Intestine-Chip 72 hours after PBMC administration into the endothelial channel. B) PBMCs (labeled with Cell Tracker) are then automatically classified by z-location: attached to endothelium (green), transmigrating through membrane (black), and attached to the epithelium (red).

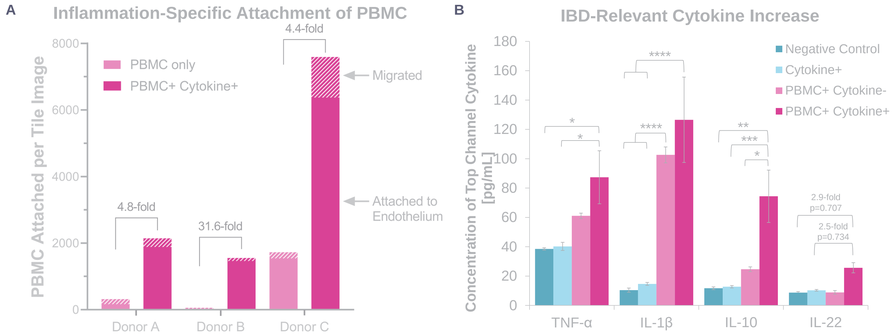


Figure 6. Specificity of PBMC Attachment and Cytokine Release with Priming Stimulus
PBMC bolus was introduced into Colon Intestine-Chips as described in Figure 4. A) PBMC attachment was quantified through 3D confocal analysis of Cell Tracker-stained PBMC for three PBMC donors. Cytokine treated groups showed a significant (>4-fold) increase in PBMC attachment across donors. B) Migrated PBMC transmigrating through the membrane pores and into the epithelial channel increased with cytokine treatment, due to chemotactic gradient in top channel. Unpaired t test was performed separately for each donor compared to Negative Control (*p<0.05); n>24 fields of view per sample, n>4 samples per group. C) Effluent samples from epithelial outflow was measured using multiplex analysis. Treatment with PBMC ± Cytokine (TNFα + chemokines) led to at least a 2-fold increase in cytokine response for IBD-relevant cytokines. D) Cytokine-dependent PBMC effector function was measured through permeability of the epithelial barrier after administration (Day 5) and was shown to increase in permeability with PBMC+ Cytokine+ treatment (p=0.1318) and significantly with IFNγ (**p<0.01) in comparison to Negative Control. Significance determined by Two-way ANOVA with Tukey's multiple comparison test, n>4. All data shown as mean ± SEM.

IBD Model Accurately Recapitulates Biological Mechanisms of TNF-α Inhibitors

Current therapeutics for IBD either target lymphocyte adhesion or reduce cell signaling associated with inflammatory pathways (e.g., cytokine release or MAdCAM-1). Current *ex vivo* screening models typically involve static cultures; and flow-based systems do not recapitulate 3D cell-cell interactions with primary cells. Here, we demonstrated the ability of the Colon Intestine-Chip to show reduction in total PBMC adhesion and downstream epithelial inflammation using biologic α-TNFα inhibitors certolizumab and adalimumab against current gold standard corticosteroid dexamethasone [3]. We also demonstrated migration of gut-trophic immune cell subtypes and a clinically-relevant reduction of cytokine production at 72h.

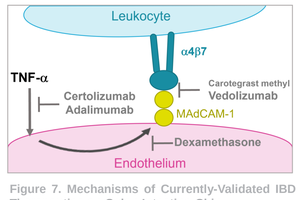


Figure 7. Mechanisms of Currently-Validated IBD Therapeutics on Colon Intestine-Chip
The IBD Colon Intestine-Chip model has been validated with clinical compounds of various mechanisms of action:

1) Corticosteroids: Dexamethasone
2) α4-integrin blockers: AJM300 and vedolizumab (Entyvio)
3) TNFα inhibitors: certolizumab pegol (Cimzia) and adalimumab (Humira)

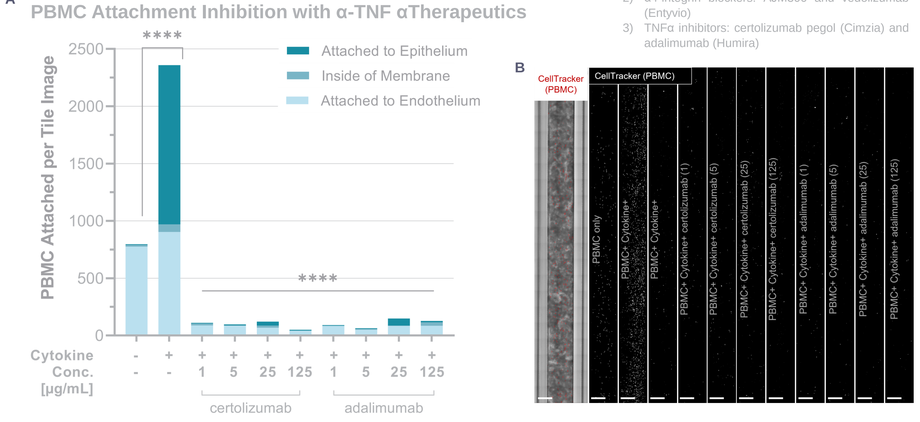


Figure 8. Reduction in Inflammatory Reaction Demonstrated in Intestine-Chip IBD Model
A) Total PBMC attachment on chip was reduced significantly with certolizumab and adalimumab treatment (****p<0.0001), which notably decreased baseline PBMC attachment without cytokine. Certolizumab and adalimumab treatment also demonstrated a reduction in the PBMC migrated to the epithelium (dark teal) compared to PBMC+Cytokine+ group. Data shown as mean; *p<0.05 determined by one-way ANOVA with Tukey's multiple comparison test, n=4 chips. B) Representative stitched tile immunofluorescent images of fixed Colon Intestine-chips 72-hours post-administration of PBMC, labeled with Cell Tracker dye. Brightfield merged image shown on left. Tile images composed of 42 images at 20X magnification; scale bar = 500 µm.

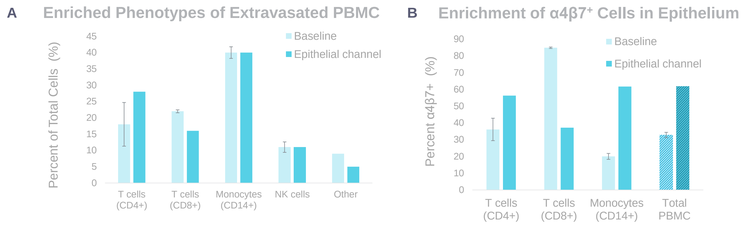


Figure 9. Immunophenotyping of Extravasated Immune Cells from the Epithelial Channel
Cells were digested enzymatically from the epithelial channel of the Colon Intestine-chip, and immune cells were phenotyped via flow cytometry. A) Comparison of baseline PBMC phenotype (%) before administration to chip versus extravasated immune cells. B) Enrichment of α4β7 integrin observed in extravasated cells compared to baseline (pre-administration). For data with n=3 technical triplicates, all data shown as mean ± SEM, otherwise n=1 chip with mean shown.

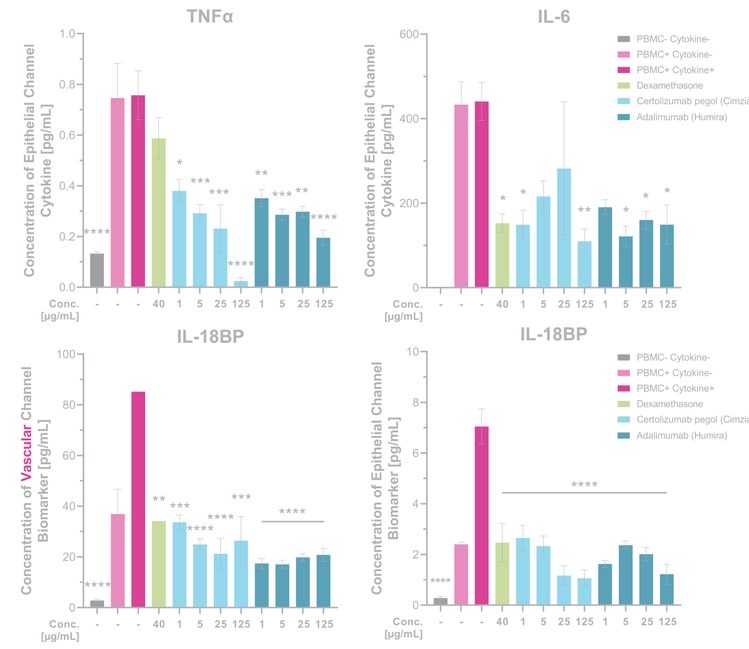


Figure 10. IBD-Relevant Cytokine Release Lowered with α-TNFα Treatment at 72h
At 72 hours post-administration of PBMC into the vascular channel, effluent outflow samples were collected from the vascular and epithelial channels and measured using multiplex analysis. Cytokines from epithelial channel demonstrated a significant reduction of TNFα (****p<0.0001), IL-6 (**p<0.01), and IFNγ (not shown) with α-TNFα treatment at 72h. Interestingly, IL-18 was observed to increase with α-TNFα treatment above PBMC+ Cytokine+ at 72h, but not at 48h (not shown) potentially relating to monocyte activity. IL-18-binding protein (IL-bp), a plasma biomarker for IBD, was found to be lowered in both the epithelial and vascular channel (****p<0.0001). Fecal biomarker calprotectin was also measured from the epithelial channel and was shown to be lowered >4-fold in α-TNFα treated conditions compared to PBMC+ Cytokine+ control which aligns with clinical predictions [4].
Significance determined by One-way ANOVA with Tukey's multiple comparison test, n>2 chips. Samples with flow abnormalities were removed from analysis. All data shown as mean ± SEM.

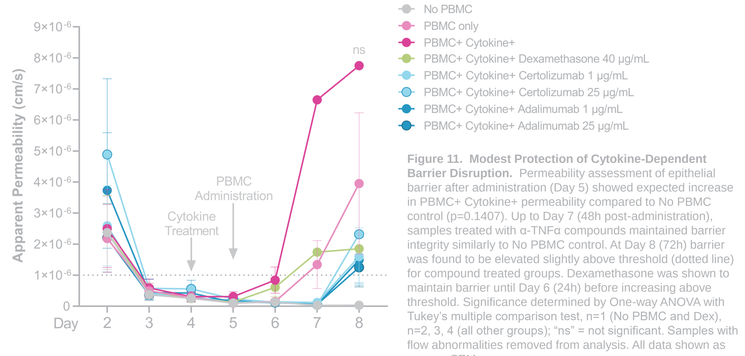


Figure 11. Modest Protection of Cytokine-Dependent Barrier Disruption. Permeability assessment of epithelial barrier after administration (Day 5) showed expected increase in PBMC+ Cytokine+ permeability compared to No PBMC control (p=0.1407). Up to Day 7 (48h post-administration), samples treated with α-TNFα compounds maintained barrier integrity similarly to No PBMC control. At Day 8 (72h) barrier was found to be elevated slightly above threshold (dotted line) for compound treated groups. Dexamethasone was shown to maintain barrier until Day 6 (24h) before increasing above threshold. Significance determined by One-way ANOVA with Tukey's multiple comparison test, n=1 (No PBMC and Dex), n=2, 3, 4 (all other groups); "ns" = not significant. Samples with flow abnormalities removed from analysis. All data shown as mean ± SEM.

Conclusion

Currently there exists a need for a comprehensive *ex vivo* model system that recapitulates inflammatory cell-cell interactions within the intestine under flow conditions. The Colon Intestine-Chip microfluidic platform creates a relevant inflammatory environment, using a cytokine treatment regime on primary cell material, that mimics the early stages of pro-inflammatory stimulus found in IBD. Our model captures inflammation-specific PBMC attachment and subsequent effector immune responses which can be targeted by clinical therapeutics including corticosteroids and TNF-α inhibitors. Overall, the human Colon Intestine-Chip can both model complex immune cell-driven IBD and validate effects of clinically relevant IBD drugs, which could provide predictive validity for pre-clinical development of new IBD drugs.

References

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