Advanced Modeling of Inflammatory Immune Cell Recruitment and Response on Human Colon Intestine-Chip for IBD Therapeutic Development

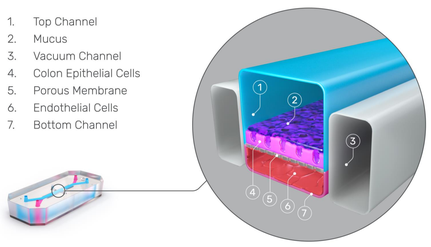
Marianne Kanellias[1], Dennis Ramos[1], Kairav Maniar[1], Andrew W Woodham[1], Adriana Cespedes[1], John Sauld[1], Carolina Lucchesi[1], Gauri Kulkarni[1], Athanasia Apostolou[1], Ville J. Kujala[1], Lorna Ewart[1] & Christopher V. Carman[1]

[1] Emulate, Inc., USA

Colon Intestine-Chip Model for IBD

Immune cell recruitment into tissues is an essential step in inflammatory responses. This occurs in a highly tissue- and stimulus-specific manner, which presents a significant challenge to modeling disease and testing therapeutics *ex vivo*. We previously developed an advanced primary human vascularized Colon Intestine-Chip model and showed that it recapitulates physiologic cell composition, morphology and barrier integrity. The goal of this work was to test the ability of this system to model inflammatory bowel disease (IBD)-like immune cell responses *ex vivo*.

The described model 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.



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 (e.g., IFNγ)

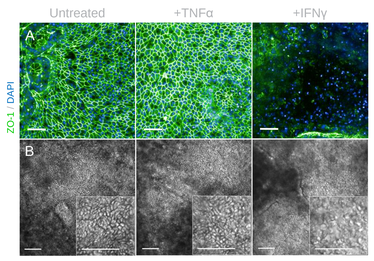


Figure 1. Epithelial Morphology Maintained with TNFα
A) Immunofluorescent tight junction (ZO-1) staining of Colon Intestine-Chips were treated basolaterally with TNFα cytokine for 24 hrs to recapitulate inflammatory priming, or with IFNγ for 72 hrs to replicate later stages of the immune response in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

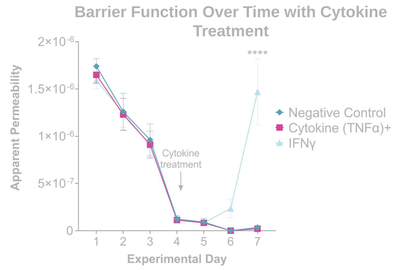


Figure 2. Epithelial Barrier Integrity Disrupted Only with Relevant Cytokines
Treatment of human intestinal microvascular endothelial cells (HIMECs) on Colon Intestine-Chip with TNFα has no effect (p>0.99) on epithelial barrier permeability on Day 7, indicating immune cell-dependency. Cytokine IFNγ positive control 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.

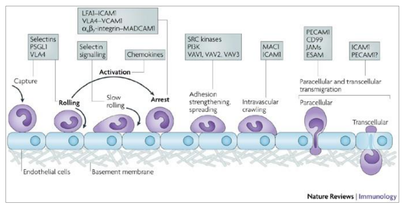


Figure 3. Leukocyte Adhesion Cascade Under Shear Flow
Shear forces are crucial for allowing stimulus and selection-driven arrest of rolling immune cells on the endothelium [1].

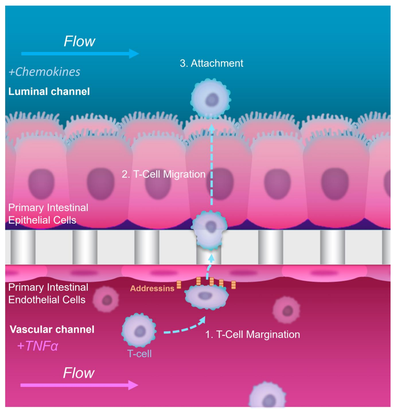


Figure 4. Modelling Inflammatory Immune Cell Recruitment on the Colon Intestine-Chip

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 (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 uL/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 migrate upwards through the membrane pores along the chemoattractant gradient. After PBMC are allowed to migrate to intestinal epithelial structures for 72 hours, release of inflammatory cytokines are measured through top-channel effluent collection and multiplex analysis.

PBMCs Demonstrate Inflammation-Specific Attachment

Our model demonstrated 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.

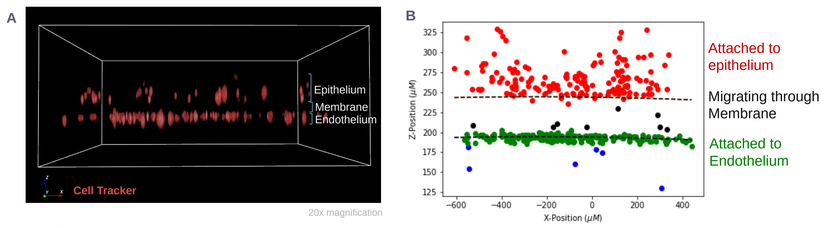


Figure 5. Opera Phenix z-stack Imaging and PBMC Location Classification
A) Full-tile (n>24 fields of view at 20x mag.) confocal images are acquired of the Colon Intestine-Chip 72 hours after PBMC administration into the endothelial channel. B) PBMCs (labeled with Cell Tracker) are then automatically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

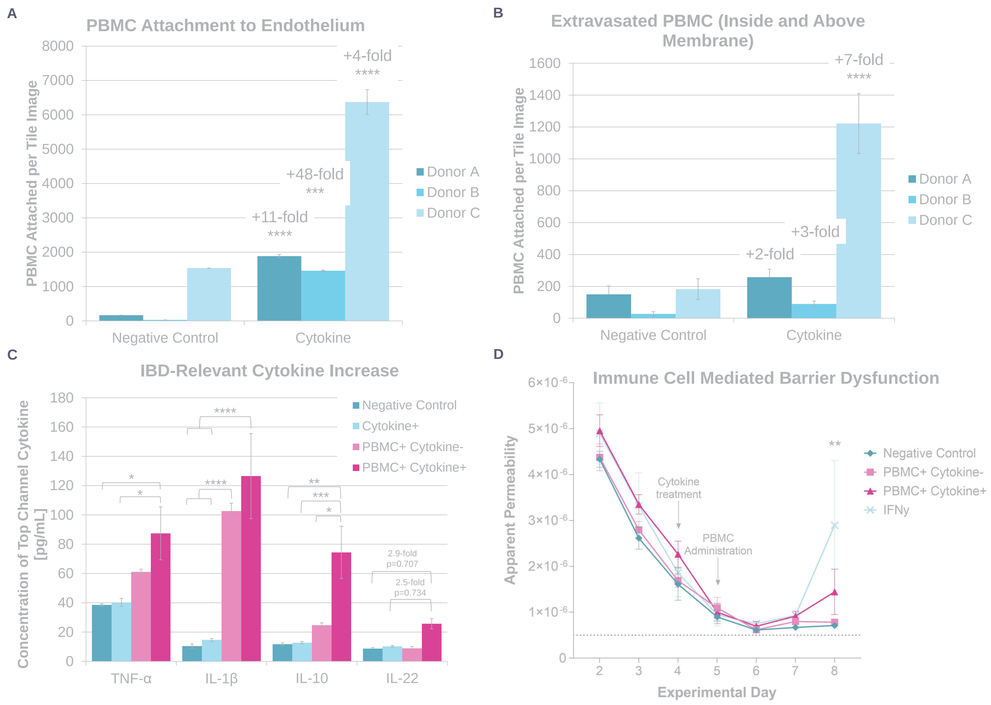


Figure 6. Specificity of PBMC Attachment and Cytokine Release with Priming Stimulus
PBMC bolus was introduced into Colon Intestine-Chips as described in Figure 4. A) PBMC attachment was quantified through 3D confocal analysis of Cell Tracker-stained PBMC for three PBMC donors. Cytokine treated groups 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 alpha-4 Integrin Inhibition (AJM300) and Corticosteroid Stimulation (Dexamethasone)

Current therapeutics for IBD either target lymphocyte adhesion (via α4β7 integrin) 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 demonstrate the ability of the Colon Intestine-Chip to show reduction in total PBMC adhesion and downstream epithelial inflammation using the small-molecule α4 integrin inhibitor AJM300 in Phase 3 clinical trials [2] against current gold standard corticosteroid dexamethasone [3].

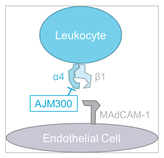


Figure 7. Mechanism of AJM300
AJM300 is a small molecule antagonist of the lymphocyte α4 subunit. AJM300 showed a statistically significant response rate compared to control in clinical studies [2].

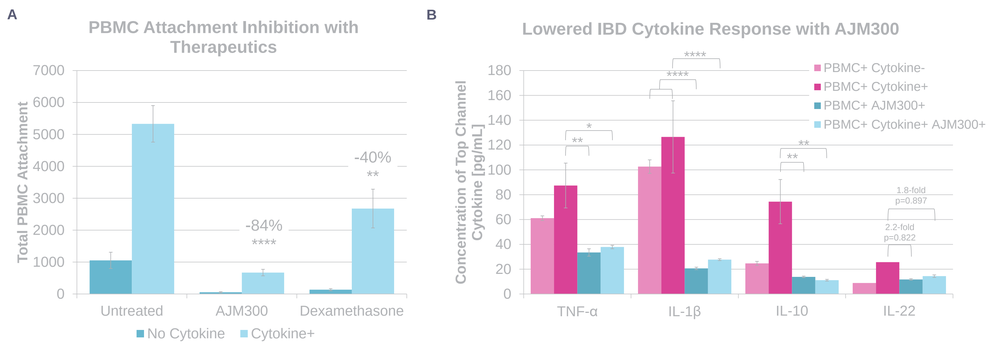


Figure 8. Reduction in Inflammatory Reaction Demonstrated in Intestine-Chip IBD Model
A) Total PBMC attachment on chip was reduced significantly with AJM300 treatment (****p<0.0001) and Dexamethasone (**p<0.01), which notably also decreased the baseline PBMC attachment without cytokine. Data shown as mean ± SEM, n>12 samples from at least n=3 pooled experiments and PBMC donors; * p<0.05 determined by one-way ANOVA with Tukey's multiple comparison test. B) Cytokine multiplex analysis of effluent from epithelial channel demonstrates the effectiveness of AJM300 in reducing cytokine release by at least 1.8-fold from cytokine-treated control. * p<0.05 determined by two-way ANOVA with Tukey's multiple comparison test, n=4 chips.

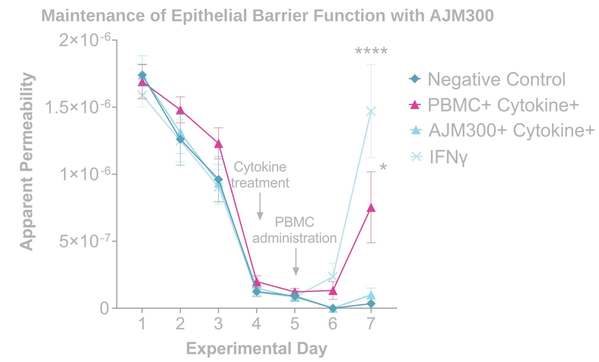


Figure 9. Healthy Barrier Function Maintained with AJM300 Treatment
Barrier function of cytokine-inflamed samples treated with AJM300 maintained levels similar (p>0.1) to untreated control at Day 7, compared to positive IFNγ disruption. IFNγ and PBMC+Cytokine+ groups were found to have significantly higher disruption than Negative control on Day 7 (****p<0.0001 and *p<0.05, respectively, ordinary Two-way ANOVA with Tukey's multiple comparison test). All groups n>4 samples. Similar significant results have been demonstrated in n=3 experimental replicates.

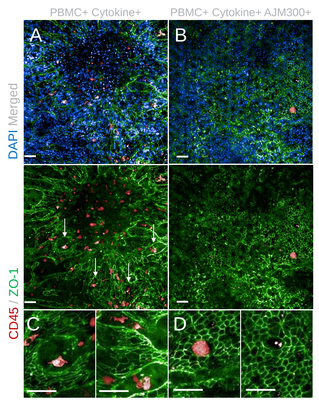


Figure 10. PBMC Attachment and Disruption of Tight Junctions Reduced with AJM300
Fixed chips treated with PBMC + Cytokine ± AJM300 were stained with Rat anti-CD45 primary overnight, then Donkey anti-Rat DyLight™ 650 secondary with conjugated Mouse anti-ZO-1 Alexa Fluor™ 488 for 3 hours. Samples were counterstained with DAPI and were imaged at 20x magnification. Qualitative images show disruption of epithelial tight junction network (ZO-1) at sites of PBMC attachment in PBMC+Cytokine+ condition (A). In contrast, samples treated with AJM300 (B) show less overall disruption of tight junctions. Two representative image focus are provided in (C) and (D) for demonstration. Scale bars = 50 µm.

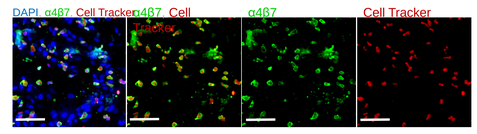


Figure 11. Co-localization of Gut-tropic α4β7 Marker on PBMC Attached to Cytokine-Treated Primary Intestinal Endothelium
Primary intestinal endothelial monolayers were treated with cytokine (TNFα) and then dosed with a bolus of PBMCs (labeled with CellTracker™). Paraformaldehyde fixed chips were then stained using human anti-α4β7 primary and goat anti-human Alexa Fluor™ 488 secondary antibodies with DAPI counterstain. IF images above were taken at 20X magnification show co-localization of the α4β7 surface marker on attached PBMC with MAdCAM-1 (merged signal = yellow), demonstrating adhesion via the α4β7-MAdCAM-1 adhesion pathway. Scale bars = 50 µm.

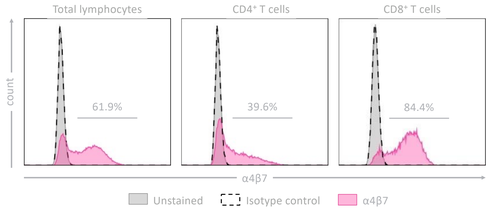


Figure 12. Expression of Gut-Tropic α4β7 Marker on PBMC subsets.
Freshly thawed PBMCs (Donor C) were analyzed for α4β7 with flow cytometry. Cells were stained with fluorophore-conjugated anti-CD4, anti-CD8, and anti-α4β7 antibodies or isotype control. Lymphocytes were identified based on FSC and SSC profiles. The x-axis is in log scale. Shown are representative histograms with the percent of α4β7$^+$ cells indicated, n=3.

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 demonstrated increased PBMC attachment and extravasation as well as capturing effector immune responses including cytokine release and epithelial barrier disruption in inflamed conditions. Furthermore, the Colon Intestine-Chip confirmed the efficacy of targeted therapeutic interventions that inhibit inflammatory response. Overall, this work presents the application of the Colon Intestine-Chip for modelling IBD-like conditions and for efficacy screening for IBD therapeutics.

References

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