Inflamed Intestine-Chip: Recreating the Mucosal Microenvironment to Understand the Pathogenesis of Ulcerative Colitis

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Abstract

Ulcerative colitis (UC), a common form of inflammatory bowel disease (IBD), is a chronic, idiopathic intestinal disorder affecting close to a million patients in the United States. The pathogenesis of UC involves immune dysregulation in response to commensal microbes in genetically susceptible individuals. Recently, the severity of inflammation has been correlated with increased IL-9 production and elevated populations of mucosal T_H9 T-cells. Unfortunately, our understanding of IL-9's contribution to the pathogenesis of UC has been hampered by contradictory findings between animal and human studies. To overcome these challenges, we are developing more accurate *in vitro* models of the intestine using Organs-on-Chips technology that place living human cells in micro-engineered environments. Our Intestine-Chip recapitulates key aspects of the intestinal milieu including mechanical forces, extracellular matrix, tissue-tissue interfaces, immune cells, and blood components.

Methods

We have developed an Intestine-Chip model which includes intestinal epithelial cells, vascular endothelial cells, and primary derived, resident immune cells isolated from the lamina propria (LPDC) of healthy and ulcerative colitis (UC) patients including inflamed and non-inflamed regions of UC tissue.

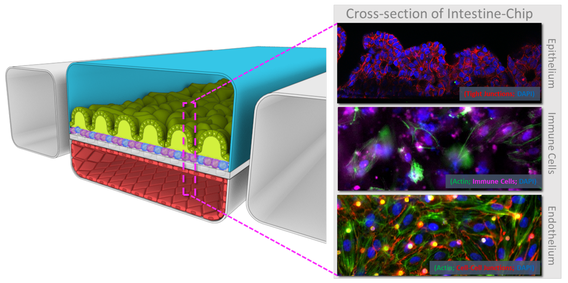


Fig 1. Schematic of the Intestine-Chip

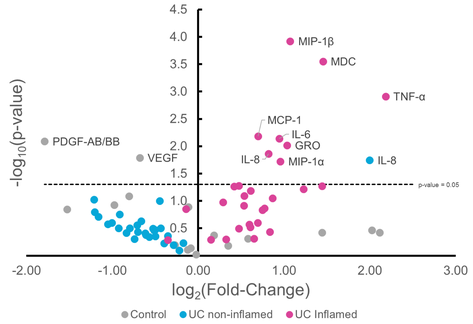
The Intestine-Chip is approximately the size of a AA battery and is composed of two independent fluidic compartments and, in this iteration, three cell types. Each channel sustains on the order of 100,000 cells.

Donor	Diagnosis	State
1	Control	Non-Inflamed
2	Ulcerative Colitis	Non-Inflamed
		Inflamed

Fig 2. Donor-Derived Lamina Propria

CD45⁺ resident immune cells were isolated from donor colon resections. Immune cells were isolated from a healthy control donor and from non-inflamed and inflamed regions of a donor diagnosed with UC.

Results



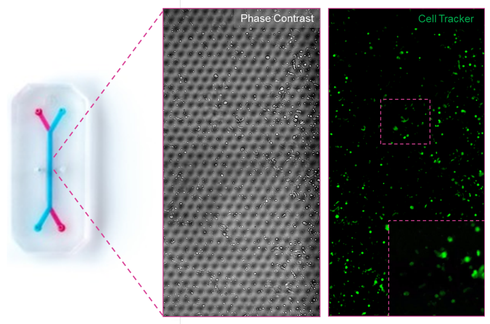


Fig 3A,B. Cytokine Responses

Donor-derived CD45⁺ immune cells were cultured on plates and challenged with a TLR2 agonist to generate a cytokine response. The resulting cytokine levels were measured by Luminex multi-analyte assay. LPDCs derived from inflamed regions of an UC patient have a stronger response to bacterial challenge than LPDCs isolated from non-inflamed regions of the same tissue as indicated by secreted cytokine levels. B) Representative phase and fluorescent images of tagged LPDCs showing typical density and distribution of cells on the Intestine-Chip in the context of the semi-porous membrane separating independent fluidic channels.

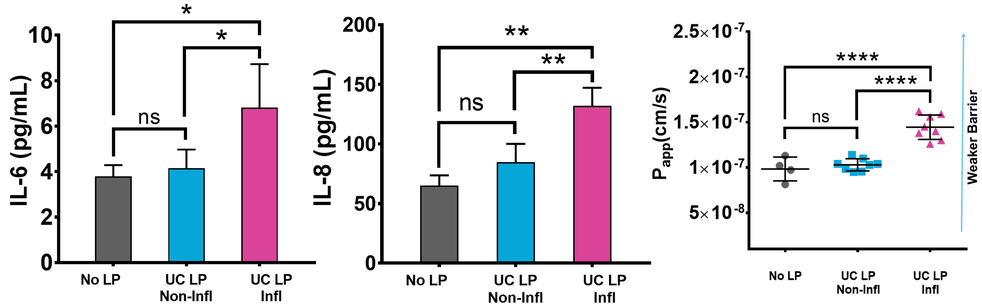


Fig 4A-C. Epithelial Response On-Chip

Intestine-Chips cultured in the presence of LPDCs from inflamed UC tissue have a higher baseline production of pro-inflammatory cytokines (A,B) and weaker epithelial barrier function (C) than chips cultured with non-inflamed LPDC or without LPDCs.

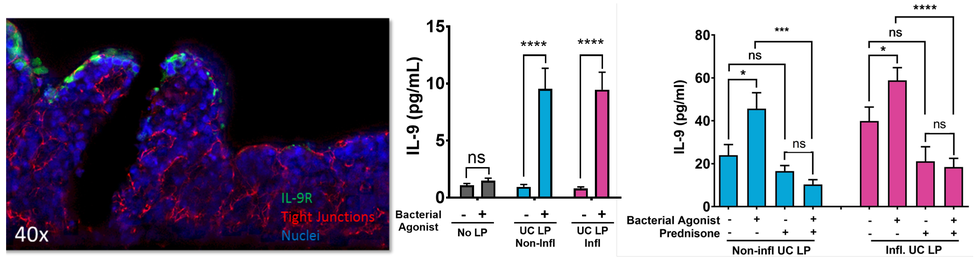
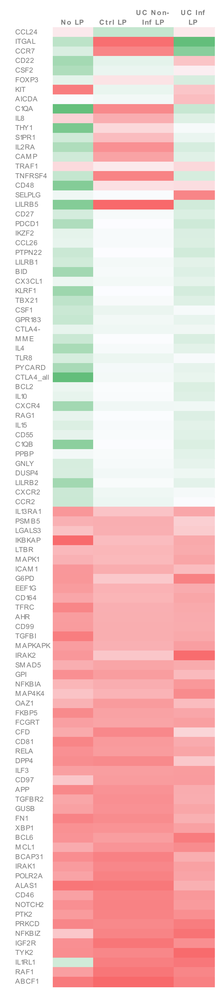


Fig 5. IL-9 Mediated Damage

The severity of UC is correlated with elevated levels of mucosal IL-9 and populations of Th9 CD4⁺ effector cells. IL-9 has a direct effect on epithelial barrier function by weakening cell-cell junction complexes via activation of the IL-9R receptor. The Intestine-Chip epithelium expresses the IL-9R receptor and IL-9 is expressed as a function of LPDCs in response to bacterial challenge. IL-9 mediated inflammation is mitigated during treatment of the Intestine-Chip with the gluococorticoid prednisone, a standard-of-care treatment.



Gene Expression

Gene expression analysis showing the top 100 up- and down-regulated genes from the Intestine-Chip's Caco-2 epithelium after TLR2 activation relative to untreated Chips and comparing the response as a function of LPDCs from different donors and inflamed versus non-inflamed regions.

Qualitatively, gene expression of Caco-2 from Intestine-Chips with LPDCs from control and UC non-inflamed tissues have a similar expression pattern and expression levels.

Gene expression of Caco-2 from Intestine-Chips with UC inflamed LPDCs show a different pattern of regulation possibly reflecting pathogenic mechanisms of UC disease recapitulated on the Intestine-Chip.

These results indicate that patient derived LPDCs retain their *in vivo* phenotype and continue to promote disease pathogenesis *ex vivo* in Intestine-Chips.

Summary

We described an Intestine-Chip model that incorporates donor-derived, resident intestinal immune cells that recapitulate key aspects of inflammatory bowel disease including dysregulated cytokine responses to bacterial challenge and loss of barrier function.

Mucosal dysregulation in inflammatory bowel disease (IBD) is characterized by localized ulceration. What are the location-specific disease mechanisms that define healthy and diseased tissue? Future studies will focus on location specific LPDC activation and cell population and with Intestine-Chip inflammation.

Conclusions

Organ-Chips technology provides a unique window into organ-level function by allowing precise control over tissue microenvironments including elements such as dynamic flow, mechanoactuation, and tissue-tissue interfaces. The Intestine-Chip provides a new platform for 1) mechanistic investigations of the pathogenic cross-talk between the epithelium and the mucosal immune system 2) to facilitate more predictive screening of therapeutic candidates in a patient-specific context. The Caco-2 based Intestine-Chip represents a robust platform to explore the pathophysiological and pathophysiological relationships between critical cell-types of the intestinal mucosa.

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