Characterization of a novel human primary intestinal epithelial monolayer platform in response to a cytokine challenge

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Introduction

The ability to grow and expand primary human epithelial cells *in vitro* has been a significant advancement in the field of gastrointestinal biology. The ALTIS RepliGut Planar system uses a proprietary biomimetic scaffold on a porous membrane to form a human intestinal epithelial monolayer (Figure 1). These stem cell driven monolayers form a polarized functional barrier after differentiation as seen by transepithelial electrical resistance (TEER) and the localization of tight junction proteins/apical brush border (Figure 3). Additionally, the cells are heterogenous with the expression of epithelial differentiation markers as seen by immunostaining (Figure 2).

Epithelial responses to cytokines, such as TNFα, have been well characterized in murine models. Here, we test human primary intestinal epithelial cells from multiple human donors in the RepliGut Planar system to see if they can recapitulate a similar response and to capture the variability that may occur in humans from a genetically diverse background.

Formation of RepliGut Planar human IEC monolayers

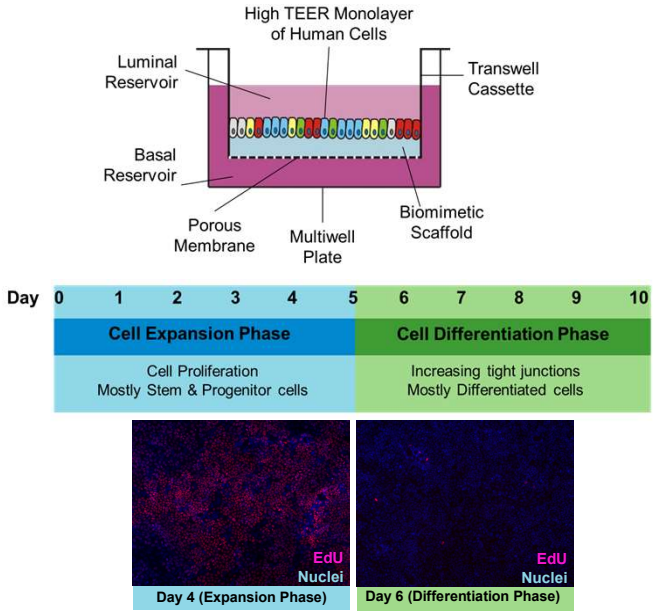

Figure 1. RepliGut Planar is a platform that allows for the culture of a contiguous monolayer of primary human intestinal epithelial cells. The transwell format allows for easy access to apical and basolateral compartments for individual compound addition or supernatant analysis. The 10-day culture timeline allows for investigation and analysis of proliferative or differentiated cell populations. During the Cell Expansion Phase, RepliGut monolayers contain a majority of proliferative (EdU$^+$) cells (in red), but lack proliferative cells during the Cell Differentiation Phase, after a 24-hour EdU pulse.

Monolayers can be differentiated to various IEC types

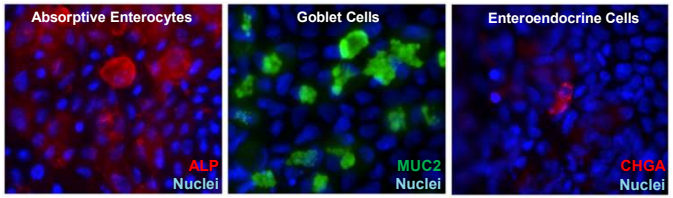

Figure 2. Day 10 monolayers contain differentiated IECs, including absorptive enterocytes (ALP: Alkaline Phosphatase), goblet cells (MUC2: Mucin 2), and enteroendocrine cells (CHGA: Chromagranin A) by immunofluorescence staining.

RepliGut monolayers form polarized functional barrier

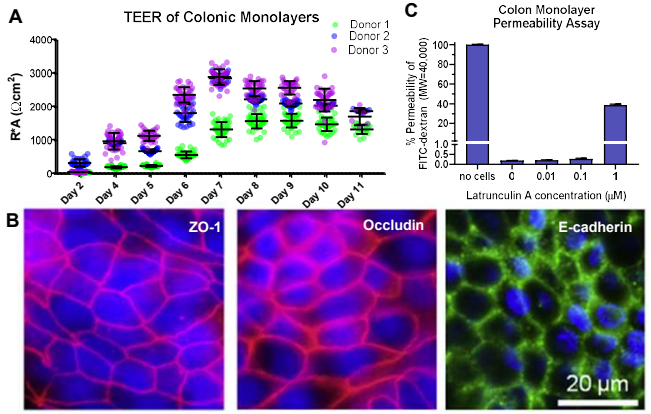

Figure 3. A) Confluent monolayers have increasing transepithelial electrical resistance (TEER) after differentiation. Daily TEER measurement of colonic monolayers from 3 donors through day 11 revealed peak resistance of 2251 ± 71.84 Ω x cm^2 (mean ± SEM) on day 7. B) At Day 10, RepliGut Planar monolayers stain positive for the tight junction proteins ZO-1, E-cadherin, and Occludin. C) Differentiated RepliGut monolayers are impermeable to FITC-dextran. Monolayer permeability can be modulated by Latrunculin A, an actin polymerization inhibitor.

TNFα leads to loss of barrier, chemokine production

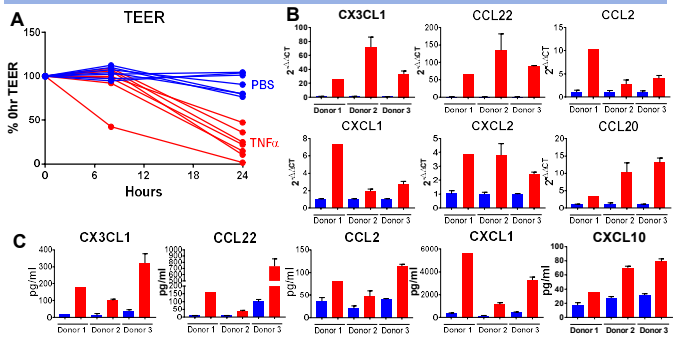

Figure 4. A) Monolayers established from 3 different donors treated with 10ng/ml TNFα results in loss of epithelial resistance by 24 hours. Each line represents a single transwell. B) Gene expression analysis shows upregulation of chemokines and cytokines after TNFα treatment of monolayers. C) Luminex analysis shows increased chemokine secretion by the epithelial monolayers after TNFα treatment. Similar responses are observed across the 3 donors, however variability in the magnitude of the responses is observed.

Tofacitinib protects IECs from IFNγ responses

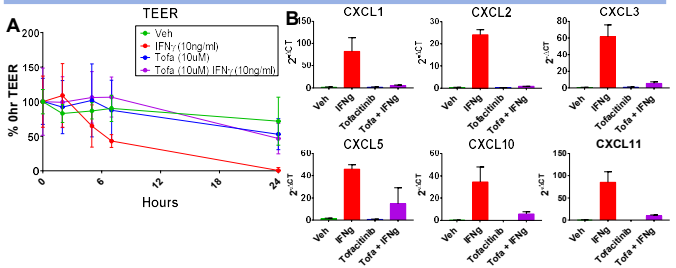

Figure 5. A) TEER time-course measurements after treatment with 10ng/ml IFNγ shows a complete loss of resistance by 24 hours. Treatment with 10μM Tofacitinib protects against the IFNγ-induced loss of barrier, resulting in similar TEER as vehicle or Tofacitinib alone. B) IFNγ leads to the upregulation of several chemokines, including CXCL1, CXCL2, CXCL3, CXCL5, CXCL10, and CXCL11. Tofacitinib reduces the IFNγ-induced upregulation of these genes.

Conclusion

Human intestinal cells in the Altis RepliGut Planar system form differentiated primary human IEC monolayers *in vitro*. These stem cell-driven monolayers display various markers of intestinal epithelial cell, including absorptive enterocytes, goblet cells, and enteroendocrine cells. Additionally, the differentiated monolayers form a polarized, functional barrier, as assessed by increase in TEER, expression of tight junction markers, and FITC-Dextran permeability assays.

RepliGut Planar IEC monolayers from 3 different donors were treated with TNFα to determine if they could recapitulate the expected TNFα responses, and capture the variability that may occur in humans from a genetically diverse background. After 10ng/ml TNFα treatment, we observed a loss of barrier by TEER, and an increase in pro-inflammatory chemokines/cytokines by mRNA and protein. While all three donors demonstrated similar gene expression and cytokine secretion responses to TNFα, the variability in the extent of response likely reflect intrinsic genetic differences in the donors. As a proof-of-concept of this system to test potential anti-inflammatory compounds, jejunum monolayers were pretreated with tofacitinib, a JAK inhibitor with proven efficacy for ulcerative colitis. When challenged with IFNγ, cells treated with the inhibitor were protected from IFNγ-induced responses, including enhanced barrier function and reduced pro-inflammatory chemokines/cytokines. These preliminary results highlight the utility of the RepliGut Planar monolayers in looking at biologically relevant IEC responses while incorporating the genetic variability across multiple donors.