

A Model System for Studying Inflammatory Pathways and Small Molecular Inhibitors in Inflammatory Bowel Disease Co-Culturing Human Intestinal Organoids and Macrophages
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Introduction: Inflammatory Bowel Disease (IBD) is a chronic inflammatory condition of the gastrointestinal tract. Symptoms are managed with broad immune suppression such as biologics blocking TNF- α , however a portion of patients do not respond to therapeutic efforts, and progress to end-organ disease stricturing and fibrosis requiring surgical resection. Patient-derived human intestinal organoids (HIOs) have emerged as a promising model to further study this disease process and investigate more specific candidate therapeutic agents. In this study, HIOs were co-cultured with macrophages to emulate the inflammatory intestinal environment that can lead to stricturing and fibrosis. By better understanding the inflammatory pathways at play between epithelial cells, fibroblasts, and macrophages that lead to fibrosis, potential routes for more precise therapeutics may be revealed.

Methods: Wild-type pediatric human blood samples were collected and induced into pluripotent stem cell (iPSC) cultures. iPSCs were differentiated into human intestinal organoids and macrophage-like cell populations separately and then co-cultured. Co-culture cell populations were exposed to lipopolysaccharide (LPS), a molecule commonly used to induce inflammatory response, eicosatetraenoic acid (ETYA), a PPAR receptor agonist and arachidonic acid analog, or a combination of the two small molecules for 3 days. Flow cytometry, cytospin, and immunohistologic staining were performed to characterize HIO and macrophage colonies. Co-culture populations were then harvested, and protein, DNA, and RNA were isolated for downstream analyses and quantification. Analysis focused on quantifying measures of inflammation and fibrosis, and whether introduction of ETYA abated any of these inflammatory changes.

Results: ETYA treatment of LPS-primed HIO:macrophage co-culture suppressed fibroblast activation genes ACTA2 and Vimentin. However, no significant changes were noted in COL1A1 protein production across all treatment groups. ETYA treatment was found to significantly increase expression of the epithelial cell NADPH oxidase gene DUOX2. While LPS was found to induce IL-1 β protein production in macrophages alone, there was no significant increase in IL-1 β production in co-culture experiments, and ETYA had no significant effect on IL-1 β production.

Conclusion: In conclusion, ETYA treatment appeared to have an effect predominantly on fibroblast and epithelial cell gene expression when HIO:macrophage co-cultures were exposed to LPS and ETYA. DUOX2 is highly involved in maintaining intestinal epithelial barrier function, and DUOX2 mutations in pediatric patients associated with a more severe inflammatory phenotype, and decreased response to anti-TNF- α therapy. Therefore, the result of increased DUOX2 gene expression with ETYA treatment is very notable. Future studies should also incorporate Crohn's Disease genetic variant iPSC HIOs and macrophages to understand how mutations in critical epithelial barrier maintenance genes affect inflammatory pathways leading to fibrosis. ETYA had limited effect on macrophage inflammatory cytokine production. Future work should aim to better characterize the inflammatory pathways unregulated in macrophages of this co-culture system, and the effect of ETYA on these pathways.

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