2`-Fucosyllactose Directly Modulates Macrophages in An Induced Pluripotent Stem Cell Model of Crohn's Disease

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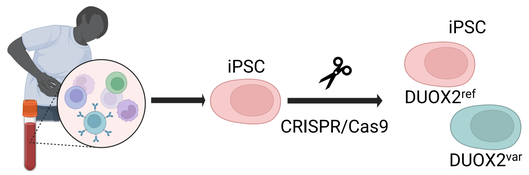
Background

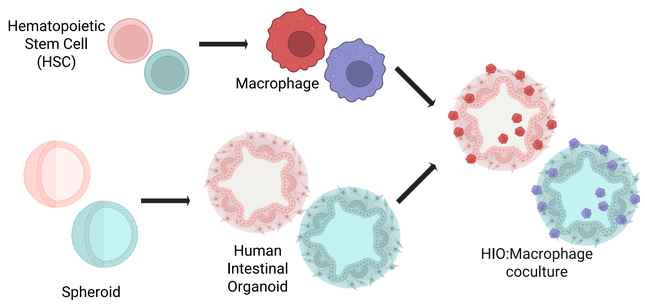
- Crohn's disease (CD) patients present with inflammatory behavior, many develop stricturing complications
- Rare loss-of-function mutations of the dual oxidase 2 (DUOX2) gene have been identified as risk factors for inflammatory bowel disease and linked to maintenance of mucosal homeostasis
- The human milk oligosaccharide 2`-Fucosyllactose (2`-FL) was found to be a direct modulator of immune cells and a beneficial prebiotic
- An ongoing clinical trial in CD patients hopes to enlist 2`-FL as an adjunct to anti-TNF treatment
- Here we show a model to study macrophage and tissue fibrosis mechanisms *in-vitro* by creating induced pluripotent stem cells (iPSC) from pediatric CD patients and differentiating them into an organoid system

Methods

- iPSCs were derived from peripheral blood mononuclear cells of a CD patient (DUOX2 reference)
- A loss-of-function mutation was introduced to the DUOX2 gene using CRISPER/Cas9 (DUOX2 variant)



- Hematopoietic progenitor cells were separately differentiated from each line, then they were terminally differentiated into macrophages
- Macrophages were exposed to lipopolysaccharide (LPS) as an inflammatory activator, cytokine release was analyzed using Luminex assay
- Human intestinal organoids (HIO) were separately differentiated from each iPSC line and then exposed to 2`-FL
- HIOs were cocultured with isogenic LPS-activated macrophages with or without 2`-FL pre-treatment
- Collagen content of the cocultures was measured using Sirius Red staining with polarized light microscopy (PLM)



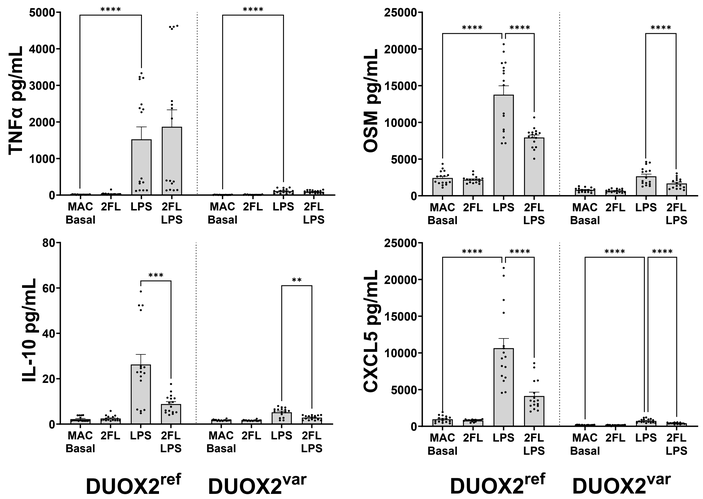
Results

1. iPSC-derived macrophages showed morphology and cell surface markers similar to primary macrophages (data not shown)

2. LPS induced a gene expression pattern comparable to that seen in inflamed CD ileum (Jurickova et al. *J Crohns Colitis* 2024)

3. DUOX2 Genotype and 2`-FL Attenuate Macrophage Inflammatory Response

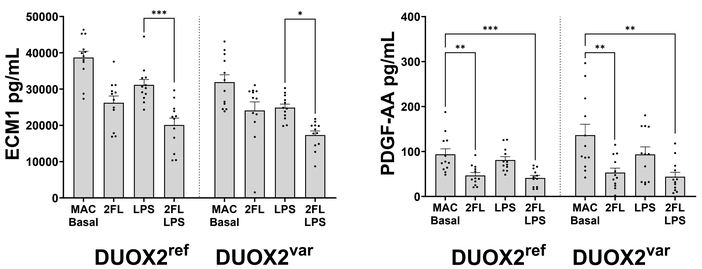
- LPS induced TNF-α, OSM, IL-10, and CXCL5, in both lines
- Absolute values were lower for DUOX2^{var}
- Pre-treatment with 2`-FL attenuated OSM, IL-10, and CCXL5



(A) Cytokine secretion quantified using a custom Luminex assay

4. 2`-Fucosyllactose Attenuates Profibrotic Response

- Mesenchymal factors ECM1 and PDGF-AA decreased with 2`FL, while LPS did not result in their increase



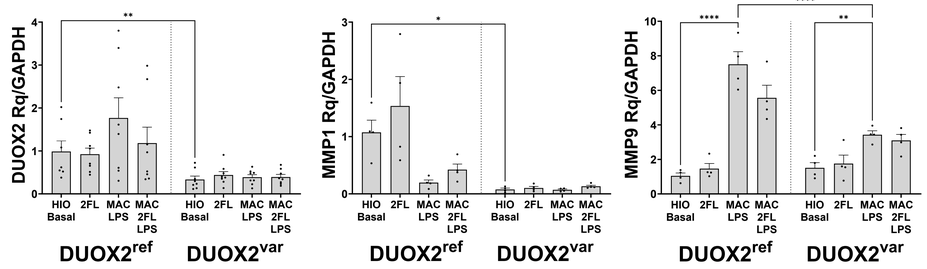
(B) Cytokine secretion quantified using a custom Luminex assay

5. Coculturing activated macrophages with isogenic HIOs resulted in an increase in CD14 and CD68 cells, as well as an increase in cells expressing myofibroblast (ACTA2) and fibroblast (VIM) markers (data not shown)

Results

6. Stimulation of Fibrogenic Markers In HIO:Macrophage Cocultures

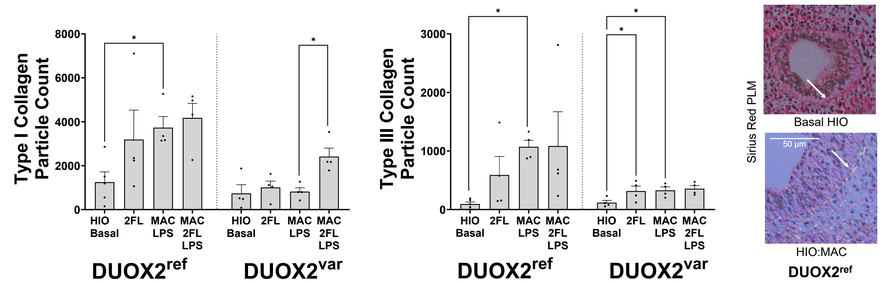
- DUOX2^{ref} HIOs had higher basal DUOX2 gene expression (left)
- Basal levels of MMP1 were higher in the DUOX2^{ref} line (middle)
- MMP9 basal levels did not vary between the lines (right)
- HIO:Mac:LPS coculture had increased MMP9 expression, significantly more in DUOX2^{ref}



(C) Gene expression quantified using TaqMan low-density array real-time PCR

7. Collagen Formation Differs Depending On DUOX2 Genotype and Exposure

- Type I and III collagen increased in the DUOX2^{ref} coculture in response to LPS independently of 2`-FL
- DUOX2^{var} type I collagen increased only with the addition of 2`-FL to HIO:Mac coculture
- Type III increased in the DUOX2^{var} line with 2`-FL addition as well as HIO:Mac coculture



(D) HIO collagen content demonstrated using Sirius red staining with PLM (white arrows), quantified in ImageJ

Conclusions

- We showed direct effects of a small molecule on macrophage activation patterns, key mediators were significantly attenuated, with trends that exhibit downstream effects on fibrosis
- Multiple factors in the pathogenesis of fibrosis were activated with a standard inflammatory activator, a pattern which we were able to quantify
- HIO:Macrophage coculture resulted in formation of tissue architecture alongside immune mediators
- By introducing a risk variant mutation, we demonstrated differential effects of inflammatory stimuli, as well as modulation using an external regulating molecule
- We plan to further investigate the mechanisms underlying these responses with RNA sequencing, in hopes to continue to uncover mechanisms by which the inflamed ileum can be shifted towards healing rather than fibrosis