# INTESTINAL INTERFERON-LAMBDA RECEPTOR 1 EXPRESSION AND RESPONSES ARE SIGNIFICANTLY DECREASED IN PEDIATRIC INFLAMMATORY BOWEL DISEASE PATIENTS


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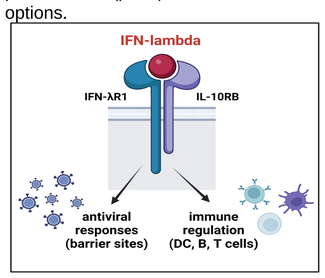
## Background

### Inflammatory Bowel Disease and IFN-λ Signalling

- Inflammatory bowel diseases (IBD), Crohn's disease (CD) and ulcerative colitis (UC), are chronic and severely debilitating intestinal diseases.
- IBD affects ~300,000 Canadians with incidence rates rising, especially in children.
- Improving our understanding of how intestinal inflammatory processes are regulated in pediatric IBD (pIBD) and the connection to the microbiome, could reveal novel therapeutic options.




- Interferon-lambdas (IFN-λs) are important innate cytokines that inhibit viruses at mucosal barriers and modulate immune responses.
- Commensal gut microbes induce IFN-λs to promote baseline antiviral gene expression.[2]
  - ✓ IFN-λ utilizes a unique heterodimeric receptor **IFN-λR1/IL-10RB.**[3]
  - ✓ The receptor is highly expressed on intestinal epithelial cells and subsets of immune cells.[2,3]

### Role of IFN-λ in Inflammation

- Mouse colitis models demonstrate Ifnlr1 deficiency leads to exacerbated inflammation, while Ifn-λ treatment decreases inflammation and promotes gut barrier integrity in wildtype mice.[4-6]
- With little known about IFN-λR biology in **human pIBD**, we hoped to fill the **gap in knowledge** if IFN-λR1 expression and IFN-λ responses were altered in pIBD patients.

## Hypothesis

We hypothesize that children with IBD have lower levels of IFN-λ receptor and downstream activity, which decreases the promotion of healing and anti-inflammatory pathways by IFN-λs naturally induced in the gut.

## Materials and Methods

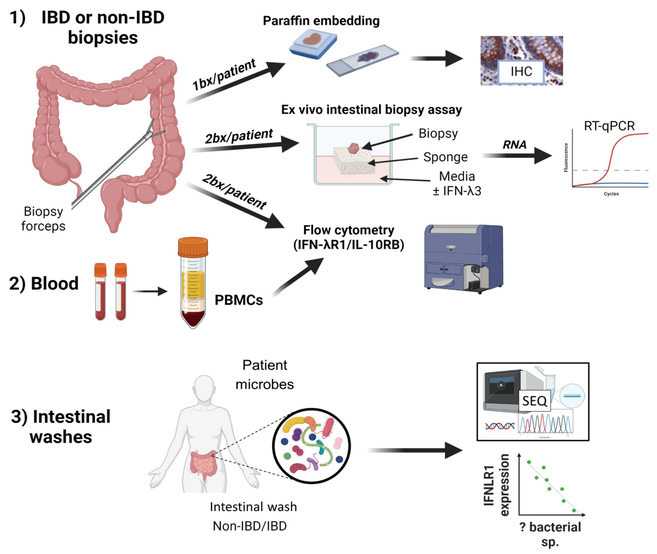



**Figure 1: Overview of methods used in this study.**

**Patient information:** Patients (ages 6-16) were enrolled into this study and samples (biopsies, blood and intestinal washes) were collected at routine colonoscopies. Patients had been diagnosed with IBD (CD or UC) or found not to have IBD (non-IBD) during their procedure. Consent was obtained from all patients' parents/guardians.

- **Sex-disaggregated data is being collected and a full analysis of sex-based differences will be completed upon full recruitment of our patient cohort.**

**Sample processing:** Intestinal biopsies from uninflamed regions of the ascending colon were processed after collection and set-up for 1) culturing ex vivo in specific media +/- IFN-λ3 for 24hrs → RT-qPCR or 2) formalin fixation and embedding in paraffin for immunohistochemistry (IHC). DNA from whole microbiota colonic intestinal mucosal washes were sent to Novogene for shotgun metagenomics analysis. Blood was processed by density gradient centrifugation (Lymphoprep) and peripheral blood mononuclear cells (PBMCs) were cryopreserved until thawing for flow cytometry analysis.

## Results

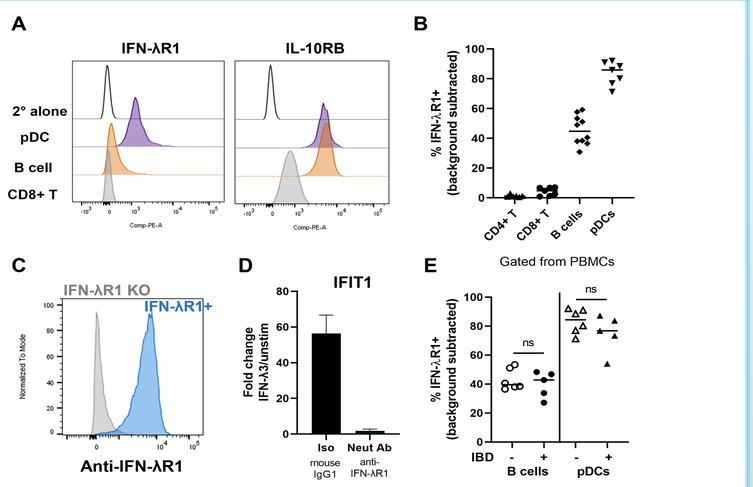



**Figure 2: Measurement of human IFN-λ receptor (IFN-λR1/IL-10RB) levels by flow cytometry.** A-B) IFN-λR1 and IL-10RB staining on the surface of various immune cells within blood. IFN-λR1%+ is shown after subtracting secondary antibody alone (background <0.5%). IL-10RB %+ was >97% on each cell type investigated. Each symbol is a different individual. C) Stable cell lines were used to confirm the new antibody specificity where IFNLR1 is knocked out by CRISPR/Cas9 (gray) and then IFNLR1 expression is added back with an expression plasmid (blue). D) The same flow cytometry antibody binds IFN-λR1 to block IFN-λ3 signaling/downstream IFN-stimulated gene (IFIT) induction as measured by RT-qPCR. E) IFN-λR1 levels comparing B cells and pDCs stained from PBMCs from non-IBD and IBD individuals.

### Highlight

We detected lower IFN-λR1 expression in pIBD gut tissue. Targeting IFN-λR1 and promoting optimal IFN-λ responses could be a novel future therapeutic option for pIBD patients.

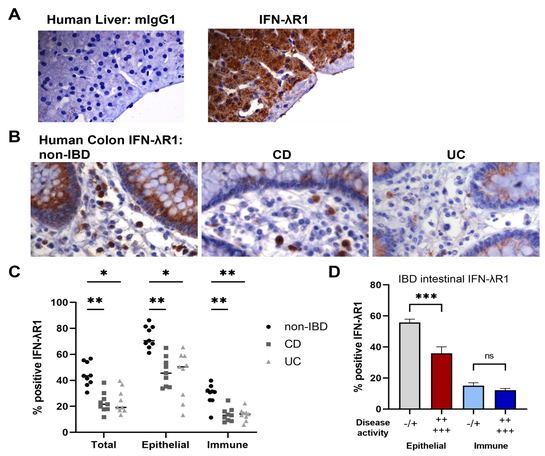



**Figure 3 IFN-λR1 protein levels on intestinal epithelial and immune cells are significantly decreased in pIBD.** A) IFN-λR1 IHC staining of positive control human liver compared to mouse IgG1 isotype. B-C) IFN-λR1 IHC staining of intestinal biopsies from pediatric non-IBD, CD or UC patients with blinded counts of IFN-λR1+ cells within 5 separate fields of view shown as percentage. D) IBD patient data shown relative to remission/mild (-/+) or moderate/severe (++/+++) disease activity. CD = Crohn disease, UC = ulcerative colitis. *, P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001. Each dot is an individual participant.

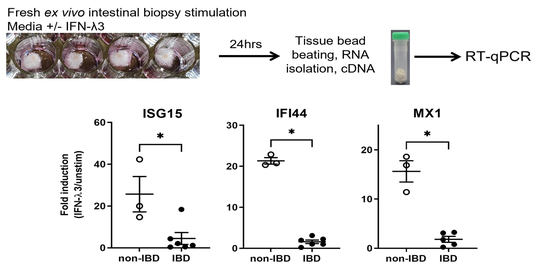



**Figure 4: IFN-λ3-mediated antiviral gene induction is significantly dampened in pIBD patient intestinal biopsies compared to non-IBD controls.** Protocols are now optimized for 24hr 3D intestinal biopsy ex vivo culture +/- IFN-λ3 before tissue homogenization and RNA isolation for downstream RT-qPCR. Media/secretions are saved for future protein validation work. IFN-stimulated gene expression (ISG15, IFI44 and MX1) is shown comparing unstimulated (unstim) and IFN-λ3 (100 ng/ml) treated ascending colon biopsies (from uninflamed regions). Each dot is a different participant. Lines represent mean +/- SEM.

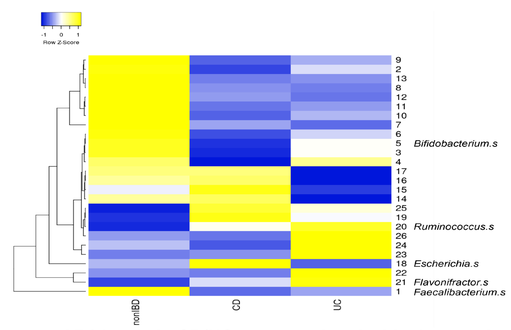



**Figure 5: Lower IFN-λR1 levels in CD/UC are associated with changes in colonic microbial abundance.** Whole intestinal microbiota were isolated from intestinal washes collected during colonoscopy from pediatric CD or UC (n=7 each) patients, and pediatric non-IBD controls (n=7). Microbial abundance was identified by shotgun metagenomics. Z score indicates the relative abundance between groups.

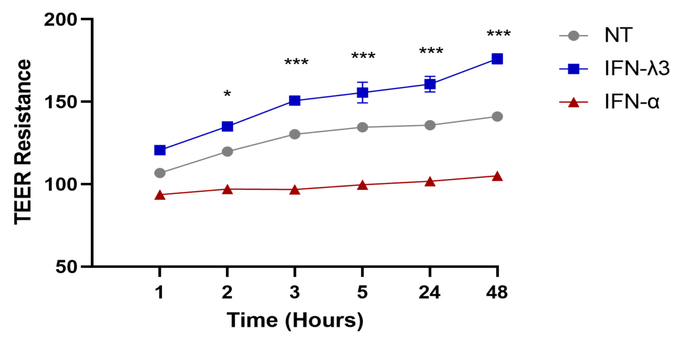



**Figure 6: IFN-λ3, but not type I IFN, IFN-α, promotes barrier function in an intestinal epithelial cell line.** HT-29 human colonic epithelial cells were cultured in transwells to create a monolayer barrier and trans-epithelial electrical resistance (TEER) measurements were taken, as indicated (hours), to examine epithelial barrier integrity in response to IFN-λ3 and IFN-α2, compared to no treatment (NT) controls. *, P<0.05, ***, P<0.001 comparing IFN-λ3 to NT or IFN-α; n=3 independent experiments.

## Conclusions

- IFN-λR1 protein levels are lower in intestinal tissue from children with IBD even at non-inflamed tissue sites.
- Patients with severe disease display lower IFN-λR1 levels on epithelial cells compared to those with mild disease or in remission.
- IFN-λ3-mediated ISG induction is significantly decreased in pIBD intestinal biopsy cells compared to non-IBD patient controls
- Specific microbes and/or their products may contribute to changes in IFN-λR1 levels
- IFN-λ3 promotes tight junctions and barrier in intestinal epithelial cell lines.

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