A20 May Contribute to Recovery of Intestinal Epithelial Function in Treated HIV Disease

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Introduction

- Untreated HIV disease is characterized by progressive intestinal CD4 loss and Th17 depletion.
- Gut epithelial barrier dysfunction has been described in untreated HIV disease. Specifically there is:
  - Increased epithelial permeability
  - Decreased tight junction expression
- Antiretroviral therapy (ART) reverses these changes to variable degrees.
- Mediators of intestinal epithelial cell (IEC) dysfunction and restoration are not known.
- Epithelial cells have not been specifically investigated in HIV infected individuals.

Materials & Methods

Tissue Samplling & Cell Isolation. Rectosigmoid biopsies were obtained under a protocol approved by the Committee on Human Research. Intestinal epithelial cells were isolated by EDTA treatment and lymphocytes were isolated using collagenase.

Measurement of Cytokine Production. Cytokine production by CD4 T cells was determined by *ex vivo* stimulation and intracellular flow cytometry. Global cytokine levels were measured by qPCR on RNA extracted from whole biopsies.

Transcriptome Analysis. RNA was extracted from epithelial isolates and the transcriptome was assessed by RNAseq. The dataset was analyzed by DESeq2 method.

Organoid Cultures. Small intestines were harvested from mice homozygous for floxed A20 allele, with estrogen-responsive Cre-recombinase expressed under the villin promoter (A20 F/F villin-ERCre). Murine gut crypts were obtained by EDTA wash and embedded into matrigel. Once established, organoids were treated with 4-hydroxytamoxifen (4OHT) to induce A20 deletion for 24 hours prior to stimulation with recombinant cytokine for 12 hours. Cultures were harvested and assayed for relevant genes by qPCR.

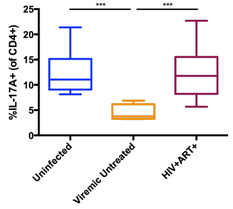
Cohort Characteristics

Subgroup	Number	Age	Gender	CD4 T Cell Count (Cells/ml)
Uninfected	9	**45** (24-57)	100% Male	**581** (378-1178)
Viremic Untreated	6	**45** (29-52)	100% Male	**659** (318-600)
HIV+ ART+	19	**53** (29-73)	95% Male	**603** (220-1207)

Results

IL-17A producing CD4 T cells are depleted during viremia but restored with therapy

Figure 1: Gut lymphocytes were stimulated *ex vivo* with PMA and ionomycin for five hours, then stained intracellularly for cytokines by flow cytometry.



Epithelial expression signature is consistent with A20(TNFAIP3)-mediated inhibition of NFkB activity during ART

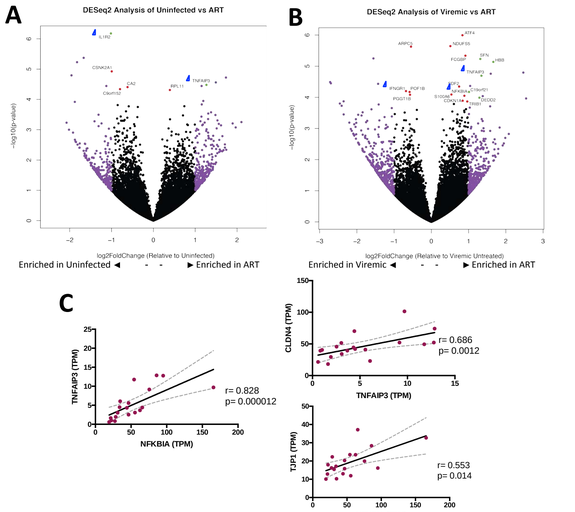


Figure 2: Differential expression analysis of RNAseq on IECs from ART-treated individuals relative to (A) uninfected and (B) viremic untreated individuals using DESeq2 workflow. TNFAIP3 is the gene name for A20. (C) Positive correlation of TNFAIP3 and NFKBIA with tight junction genes was done by Spearman's rank.

A20 deletion in murine organoids leads to increased inflammatory gene expression after IL-17 stimulation

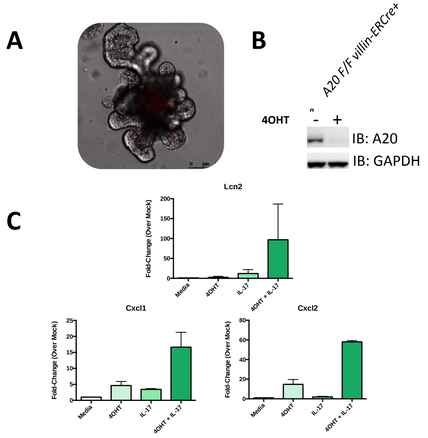


Figure 3: Murine model with conditional epithelial-restricted A20 deletion. (A) Confocal image of an intestinal organoid culture. (B) Western blot demonstrating effective deletion of A20 after 4OHT. (C) Expression of inflammatory genes in organoids after stimulation with IL-17A as measured by qPCR. Plots show two independent experiments.

HIV viremia is characterized by elevated global IFNγ and enhanced Type-I IFN responses

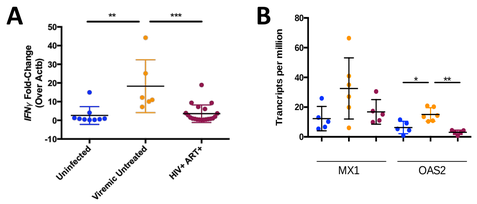


Figure 4: (A) Whole biopsy mRNA expression of IFNγ by qPCR. (B) Expression of interferon-stimulated genes in epithelial isolates assessed by RNAseq. P-value significance was determined using Mann Whitney test.

IFNγ decreases expression of tight junction and mucin genes, further exacerbated by A20 absence

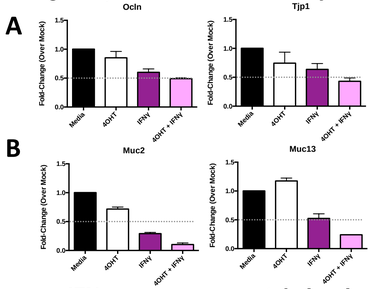


Figure 5: qPCR for (A) tight junction and (B) mucin genes in organoid cultures after IFNγ stimulation. Plots show two independent experiments.

IFNα suppresses protein levels of A20 in organoid cultures

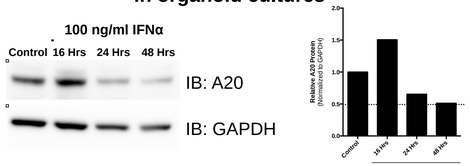
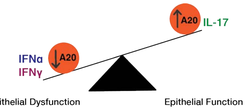


Figure 6: (A) Western blot for A20 after treatment with IFNα. (B) Quantification of protein bands using Bio-Rad Image Lab (N=1).

Conclusions & Implications



- Treatment of HIV disease reduces Type-I IFN activity and IFNγ presence, potentially removing suppression of A20 and limiting IFNγ-mediated damage respectively.
- IL-17 resurgence after treatment could induce A20 expression and directly promote barrier function.
- The A20 pathway may be a potential therapeutic target to promote intestinal health in HIV infected individuals on ART.

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