The microbial-derived short chain fatty acid butyrate differentially inhibits gut T helper cell subset proliferation.

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Hallmarks of HIV-1 infection in the gut

- High concentration of activated T cells in the lamina propria (LP).

- Increased levels of HIV-1 infection and subsequent CD4 T cell depletion (Th17, Th22).

- HIV-1 associated dysbiosis of the mucosal microbiome.
  - Lower abundance of bacteria families that produce regulatory short chain fatty acids such as butyrate.
Butyrate and gut homeostasis

- Butyrate is one of the metabolic products of the digestion of dietary fiber by anaerobic bacteria.

- Butyrate concentrations:
  - lumen 20mM,
  - portal circulation 30µM
  - systemic circulation 4µM.

- Butyrate has been shown to have immune modulating effects on innate and adaptive immune cells and acts as a major nutrient source for colonocytes.
  - Lack of studies on human gut immune cells

- Various mechanisms
  - HDAC inhibitor (HDACi),
  - G-coupled Protein Receptor (GPR) signaling
  - PPARγ signaling (Alex 2013)
Butyrate and HIV-1 infection

- Total butyrate producing bacteria (BPB) species decreased in relative abundance in the colonic mucosa of chronically infected and untreated individuals.

- Levels of a representative BPB inversely associated with clinical markers of inflammation and microbial translocation.

- The addition of butyrate to in vitro HIV-infected lamina propria mononuclear cells exposed to enteric bacteria resulted in:
  - decreased CD4 and CD8 T cell activation
  - decreased HIV-1 infection levels
  - decreased production of IL-17 and IFNγ
Objective

To better understand the mechanisms by which butyrate impacts gut LP CD4 T helper cell activation, proliferation and HIV-1 infection levels.
**Assay Design**

**ELISA:**
- Secreted IL-17, IFNγ

**Multi-color flow cytometry:**
- CD4 T cell activation and proliferation
- Th17, Th22, Th1 proliferation
- Th17, Th22, Th1 infection

**CFSE-labeled LPMC**

- ±T cell activating beads
- ± butyrate

+/- HIV-1 (TF CH40)

4 days
Butyrate reduces LP CD4 T cell activation and proliferation in a dose dependent manner

- Similar responses with bacteria and in purified LP CD4 T cells.
- Significant toxicity at higher doses (>4mM).

Values: Mean, N=3
*P<0.05
Butyrate reduces LP CD4 T cell cytokine production in a dose dependent manner

Values: Mean, N=3
*P<0.05

• Similar responses in purified LP CD4 T cells.
Does butyrate differentially effect LP T helper cell subset proliferation and HIV-1 infection levels?
Th17 cells are more sensitive to the effect of butyrate

**Identification of Th subsets**

- **4 day culture**
- **4 hour mitogenic stimulation** (PMA/ionomycin)

**Proliferation of Th subsets**

IC50 Values
- Th17 = 0.147 mM
- Th1 = 0.229 mM
- Th22 = 0.258 mM

Values: Mean, N=6
*P<0.05
Lower doses of butyrate increase the frequency of HIV-1 infected T helper cells in the setting of activation.
Conclusions

• In the setting of lower concentrations of butyrate:
  – Decreased Th17 proliferation relative to other T helper subsets
  – Increased T helper subset HIV-1 infection, with Th17 productive infection levels peaking at lower concentrations of butyrate

• In the setting of higher concentrations of butyrate:
  – Decreased T helper cell proliferation
  – Decreased T helper subset HIV-1 infection levels
Clinical Importance and Future Directions

• Our *in vitro* modeling suggest that lower amounts of butyrate in the gut lumen may exacerbate infection and depletion of certain T helper subsets
  – increasing amounts of available butyrate may decrease total T helper cell activation, infection and depletion.

• Future Studies:
  – Mechanistic studies into HDACi, GPR and PPARγ signaling
  – Gene expression study on isolated T helper cell populations
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