The Role of Granzyme B on Inflammatory Cytokine Production by Human Gut Lamina Propria Mononuclear Cells

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BACKGROUND

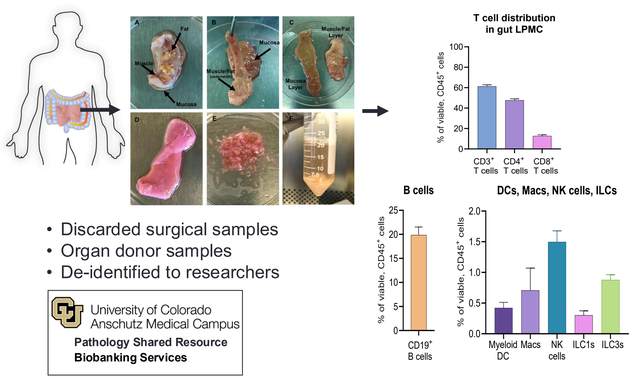
- Despite the success of antiviral treatment for HIV-1 infection, people living with HIV (PWH) have persistent gastrointestinal inflammation which contributes to continued comorbidity.[1]
- PWH exhibit both structural and immunological changes in their gut resulting in local inflammation and increased bacterial translocation.[1]
- Our lab has previously shown that in gut samples of PWH, higher frequencies of a serine protease called Granzyme B (GZB) were secreted by CD4 T cells. [2]
- This increased extracellular GZB was associated with greater abundance of potentially inflammatory bacteria.[2]
- GZB is classically utilized by CD8 T cells to induce apoptosis but has additional functions of interest including enhancement of production and activation of inflammatory cytokines.
- Here we assess the effect that *E. coli* has on the stimulation of inflammatory cytokines under conditions with and without inhibition of granzyme B.

RESEARCH QUESTION

How does GZB produced in response to enteric bacteria exposure drive gut associated inflammatory cytokine production in an in vitro gastrointestinal model?

LPMC MODEL

Mononuclear cells were harvested from procured gut samples for use in the *in vitro* human gut lamina propria mononuclear cell model. Cell population distributions are shown below.



- Discarded surgical samples
- Organ donor samples
- De-identified to researchers

University of Colorado Anschutz Medical Campus
Pathology Shared Resource
Biobanking Services

METHODS AND RESULTS

Figure 1: Stimulation of LPMC's with *E. coli* upregulated production of functional Granzyme B (GZB).

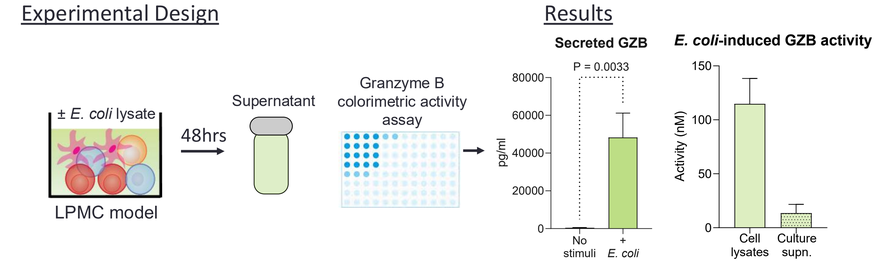


Figure 2: Stimulated LPMC's showed increased Secreted TNFα, IL-1α, IL-18, and IL-1β, and cell associated IL-1α

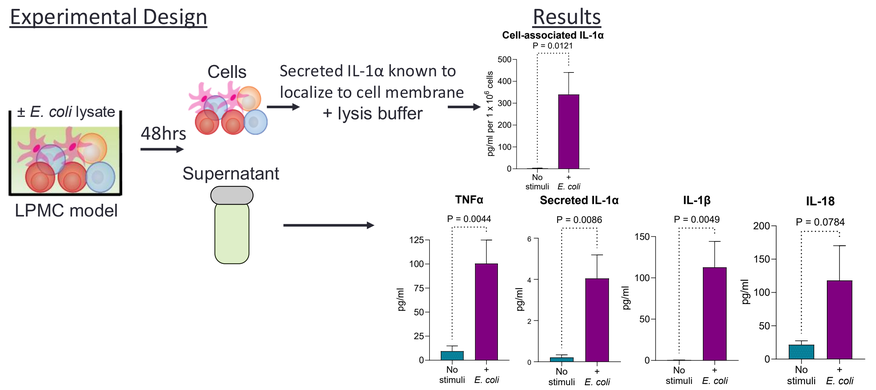
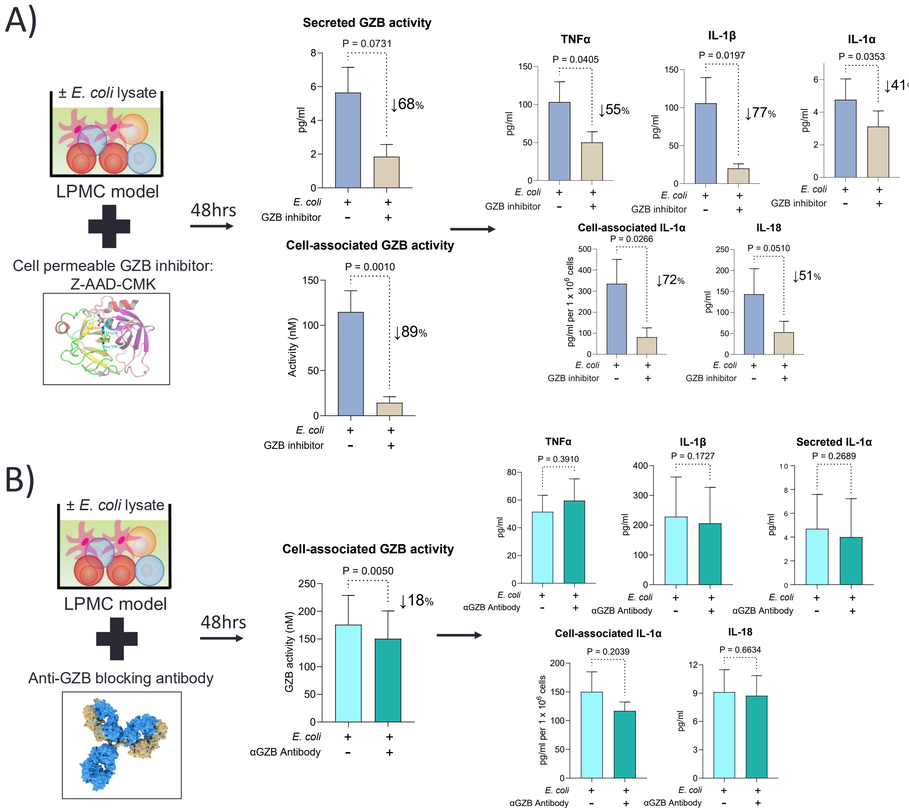


Figure 3: (A) Cell permeable GZB inhibitor saw significant drops in secreted cytokines. **(B)** Anti-GZB blocking antibody showed no significant cytokine attenuation. (methods are similar Figures 1 and 2)



SUMMARY AND FUTURE DIRECTIONS

- Simultaneously blocking both extracellular and intracellular GZB activity in response to E. coli stimulation of LPMCs in-vitro resulted in decreased production of inflammatory cytokines while inhibition of exclusively extracellular GZB did not attenuate production
- We propose that intracellular GZB, either produced or taken up by immune cell populations is the primary driver for enhancing microbe-induced inflammatory cytokine production within gut LPMCs
- Future Directions: Treatments targeting intracellular or cell-associated Granzyme B function could be used in the future to better prevent and control inflammatory gut conditions

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- References:
  1. Dillon S, et. al. The gut microbiome and HIV-1 pathogenesis: a two-way street. AIDS. 2016.
  2. Dillon S, et. al. Granzyme B+ CD4 T cells accumulate in the colon during chronic HIV-1 infection. Gut Microbes. 2022.