Bacteria-Induced Granzyme B Contributes to HIV-1-Mediated Gut CD4 T Cell Depletion *Ex Vivo*

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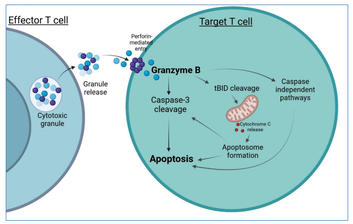
Background

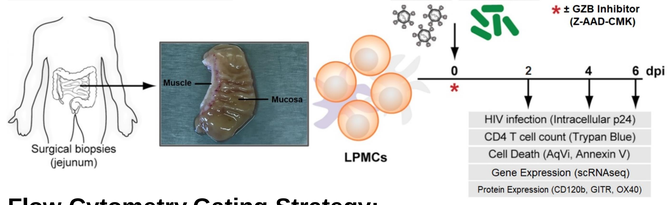
- The gastrointestinal tract is a major site of early and massive CD4 T cell depletion following HIV-1 infection.
- However, the mechanisms driving the death of gut CD4 T cells during HIV remain poorly understood.
- Among persons with HIV-1 (PWH), a **disrupted epithelial barrier** results in the **translocation** of bacteria from the gut lumen into the underlying lamina propria and systemic circulation.
- We previously reported that *ex vivo* exposure of human gut lamina propria mononuclear cells (LPMC) to enteric bacteria:
  - increased HIV-1-mediated CD4 T cell death, shifting the death mechanism from pyroptosis **to apoptosis**[1],
  - upregulated **granzyme B** (GZB) in CD4 T cells[2,3].
- GZB is an enzyme typically associated with CD8 and NK cell cytotoxicity mediated via apoptosis.



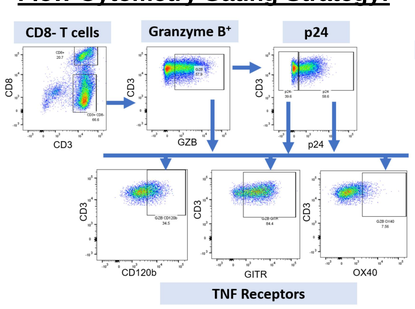
- A higher frequency of GZB+ LP CD4 T cells was **observed in colon tissue of PWH** versus HIV-1 negative controls, despite having lower numbers of colon CD4 T cells[3].
- In this current study, we utilized our *in vitro* Lamina Propria Aggregate Culture (LPAC) model[4] to determine if GZB plays a critical role in HIV-1-mediated gut CD4 T cell death.

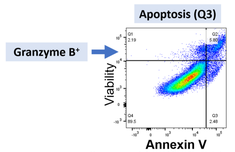
Methods

***In vitro* LPAC Model**



Flow Cytometry Gating Strategy:

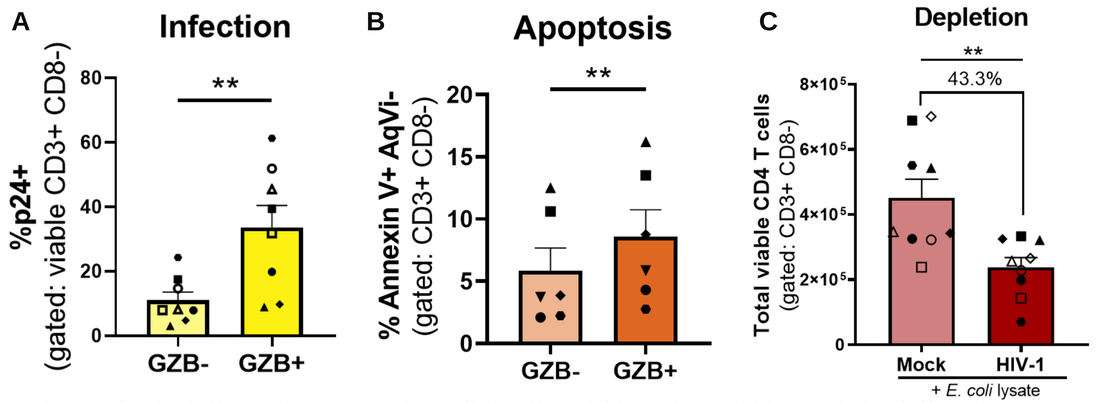




LP CD4 T cells were identified within viable cells as CD3+ CD8- T cells due to the down-regulation of CD4 by HIV-1[5]. Apoptotic CD4 T cells were identified as CD3+CD8- T cells without an initial viability gate. Gates for GZB, CD120b, GITR and OX40 expression were established using isotype controls; intracellular p24 expression was established using mock-infected cultures

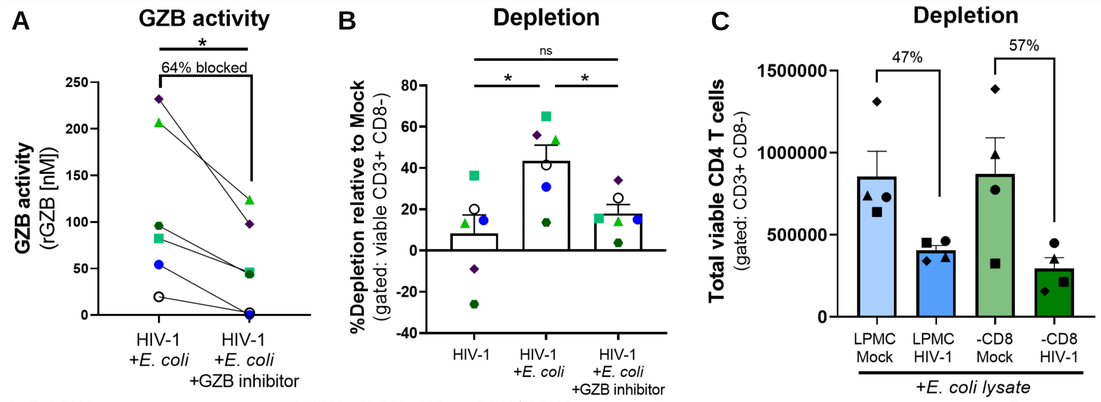
Enteric bacteria-mediated Granzyme B induction in human gut CD4 T cells *in vitro* is linked to HIV-1-associated CD4 T cell death.

GZB-expressing CD4 T cells are preferentially infected and a higher fraction undergo apoptotic death versus CD4 T cells lacking GZB



LPMCs were spinoculated with Transmitter/Founder (TF) HIV-1 (CH040; 200ng p24/10^6 LPMC) or mock infected and cultured with or without commensal *Escherichia coli* lysate (10μg/ml) for 4 days. **A.** Percentages of productively infected (p24+) GZB+ or GZB- CD3+CD8- T cells (N=8) **P<0.01; Bars indicate mean ± SEM. **B.** Percentages of apoptotic (Annexin V+ Viability dye-) GZB+ or GZB- $CD3^+CD8^-$ T cells. (N=6) **C.** HIV-specific LP CD4 T cell depletion was enumerated based on the number of viable LPMC collected 4dpi (determined by trypan blue exclusion) multiplied by the percentage of CD3+CD8- T cells in viable LPMC determined using flow cytometry. The percent depletion relative to mock is then calculated. (N=9).

Inhibition of GZB during HIV-1 infection decreases CD4 T cell death



A, B. LPMCs were spinoculated with TF HIV-1 (CH040; 200ng p24/10^6 LPMC) or mock infected and cultured with or without commensal *E. coli* lysate (10μg/ml) in the presence/absence of the GZB inhibitor Z-AAD-CMK (250μM) for 4 days. **A** Intracellular GZB activity was determined in cell lysates by colorimetric assay: cleavage of a GZB substrate (Ac-IEPD-pNA) by active GZB releases p-nitroanilide (pNA) and levels of pNA are detected at absorbance 405nm. N=6; *P<0.05. **B.** In the presence of *E. coli*, HIV-1 mediated CD4 T cell depletion was, on average, 43%; in the presence of GZB inhibitor, LP CD4 T cell depletion decreased to 18%. N=6; *P<0.05, NS: not significant. **C**. *In vitro* CD8 T cells do not significantly contribute to CD4 T cell death. Total LPMCs or LPMCs depleted of CD8 cells (-CD8) using magnetic beads were cultured with *E. coli* lysate (10μg/ml) for 4 days (N=4). For total LPMC CD4 T cell depletion was, on average, 47%; in the absence of CD8 T cells, LP CD4 T cell depletion still occurred at 57%.

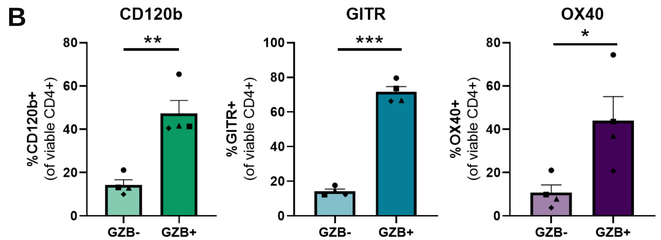
Enteric *E. coli* expanded GZB+ CD4 T cells preferentially express several TNFSFRs

A

Gene ID	Gene Name	Fold difference*
IFNG	IFNγ	7.15
GZMA	GZA	2.36
IL2RA	IL-2 Receptor	2.23
MKI67	KI-67	1.94
NKG7	NK granule 7	1.57
TNFRSF18	GITR	2.60
TNFRSF4	OX40	1.94
TNFRSF1B	CD120b (TNFR2)	1.65

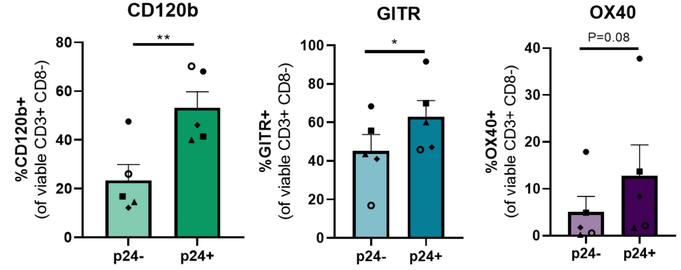
A. Unbiased single-cell RNA sequencing was performed on highly purified LP CD4 T cells isolated from total LPMC following exposure to commensal *E. coli* lysate (N=6). Differentially expressed genes were determined in GZMB+ versus GMZB- CD4 T cells and are expressed as fold difference.

B



B. Higher protein expression of TNFSFRs on *E. coli*-expanded GZB+ versus GZB- LP CD4 T cells was confirmed by flow cytometry. N=4; Bars represent mean ± SEM; *P<0.05, **P<0.01, ***P<0.001.

A greater fraction of surviving GZB+ p24+ CD4 T cells express TNFRs versus p24- CD4 T cells



LPMCs were spinoculated with TF HIV-1 (CH040; 200ng p24/10^6 LPMC) or mock infected and cultured with commensal *E. coli* lysate (10μg/ml) for 4 days. Percentages of **A.** CD120b, **B.** OX40 or **C.** GITR expressing cells were determined in p24+ GZB+ CD3+ CD8- T cells using flow cytometry. N=5, * P<0.05, ** P<0.01.

Conclusions

- Our findings suggest that bacteria-mediated GZB induction in CD4 T cells may be a mechanism contributing to massive CD4 T cell death in the gut during acute HIV-1 infection.
- Higher expression of anti-apoptotic TNFRs in GZB+ CD4 T cells that survive *in vitro* HIV-1 infection raise the possibility that surviving GZB+ CD4 T cells in the gut may serve as a significant HIV-1 reservoir.

Acknowledgements

University of Colorado Anschutz Medical Campus
Pathology Shared Resource
The Wilson and Santiago Collaboratory.

National Institute of Allergy and Infectious Diseases — **R01 AI145428** (Wilson & Santiago) **T32 AI052066** (Mickens)

National Center for Advancing Translational Sciences — **T32 TR004367** (Mickens)

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