GLADSTONE INSTITUTES

HIV Reservoir Cells From ART-Suppressed PWH Exhibit Distinct but Heterogeneous Glycomic Signatures

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BACKGROUND

Glycans play essential roles in cell differentiation, homeostasis, and immunity:

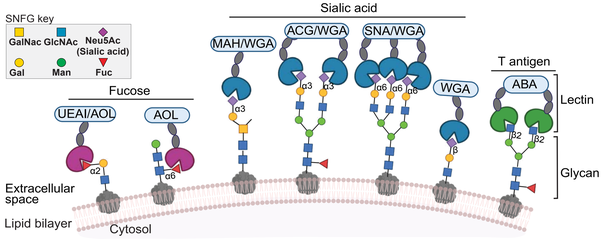
- Sialylation enables cells to evade immune surveillance[1].
- Fucosylation is essential for T cell activation and clonal expansion[2].

However, whether glycans are differentially expressed on HIV reservoir cells remains unknown.

Here, we implemented CyTOF-Lec[3], together with CyTOF/PP-SLIDE[4], to define glycan and protein features of reservoir cells from people with HIV on ART (ART-PWH).

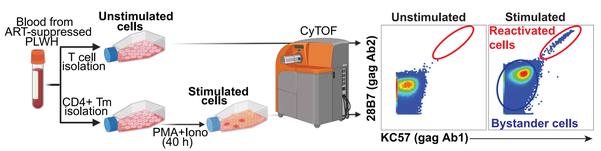
METHODS

Figure 1: Specificity profiles of glycan-recognizing lectins utilized in this study



Our CyTOF-Lec panel comprised of antibodies against 33 proteins including HIV Gag. In addition, the panel included 7 Lectins with distinct glycan specificities. Fucose-binding lectins comprised UEAI (*Ulex europaeus* agglutinin-I) which binds to α1-2 branched fucose, and AOL (*Aspergillus oryzae* lectin) which binds to total/core fucose. Sialic acid-binding lectins comprised MAH (*Maackia amuerensis*-II) which binds to α2-3 sialylated O-glycans, ACG (*Agrocybe cylindracea* galectin) which binds to α2-3 sialylated glycans, SNA (*Sambucus nigra* agglutinin) which binds to α2-6 sialylated glycans, and WGA (wheat germ agglutinin) which recognizes total sialylated and terminal GlcNAcα glycans. The lectin ABA (*Agaricus bisporus* agglutinin) was used to profile T-antigen core 1 O-glycans (Galβ1-3GalNAcα1) and GlcNAc-terminated glycans.

Figure 2: Experimental design for profiling of HIV reservoir cells by CyTOF-Lec/PP-SLIDE



CD4+ T memory (Tm) cells were isolated from peripheral blood of ART-suppressed PWH enrolled in SCOPE, and stimulated with PMA + Ionomycin for 40 hours in the presence of ART to identify reactivated cells, and then phenotyped by CyTOF-Lec. Unstimulated CD4+ Tm cells were phenotyped in parallel. Reactivated HIV reservoir cells were identified by co-expression of two HIV gag antibodies (KC57 & 28B7 clones). Dot plots show the unstimulated (left) and stimulated (right) conditions, with reactivated and bystander cells indicated with the red and blue circles, respectively. We compared the total CD4+ Tm cells to the "kNN latent cells" which were identified by the PP-SLIDE pipeline and harbor the predicted phenotypes of HIV reservoir cells prior to ex vivo reactivation (see Figure 3).

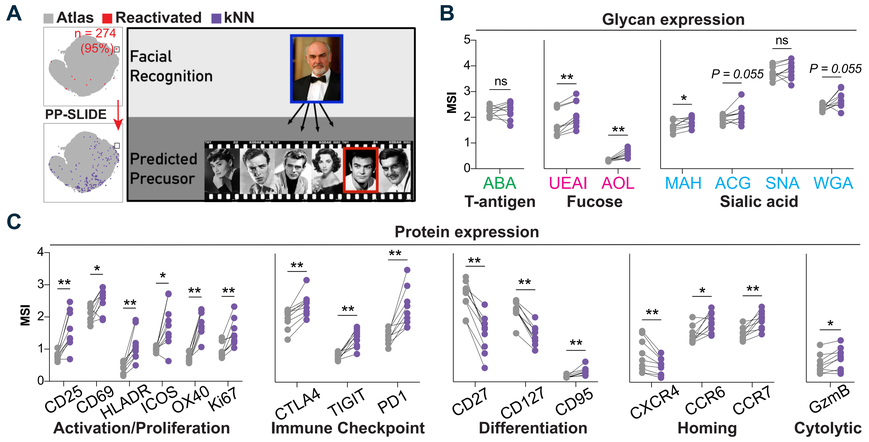
Fucosylation and to a lesser extent sialylation profiles distinguish inducible HIV reservoir cells from their uninfected counterparts. If validated, these profiles can serve as biomarkers for inducible reservoir cells, and also can reveal on mechanisms of HIV persistence given the regulatory roles of glycosylation on cellular homeostasis.

PLAIN LANGUAGE SUMMARY:

HIV reservoir cells have different sugars on their surface as compared to uninfected cells, which can serve not only as biomarkers for these cells, but can inform on how they are able to persist long-term in people with HIV.

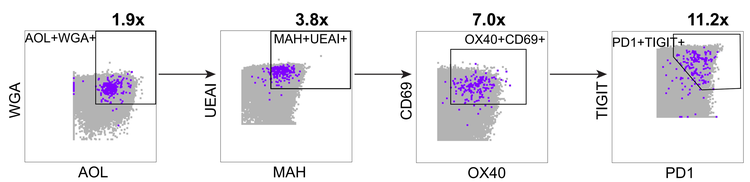
RESULTS

Figure 3: CyTOF-Lec/PP-SLIDE reveals the glycoproteomic features of the inducible HIV reservoir from ART-suppressed PWH



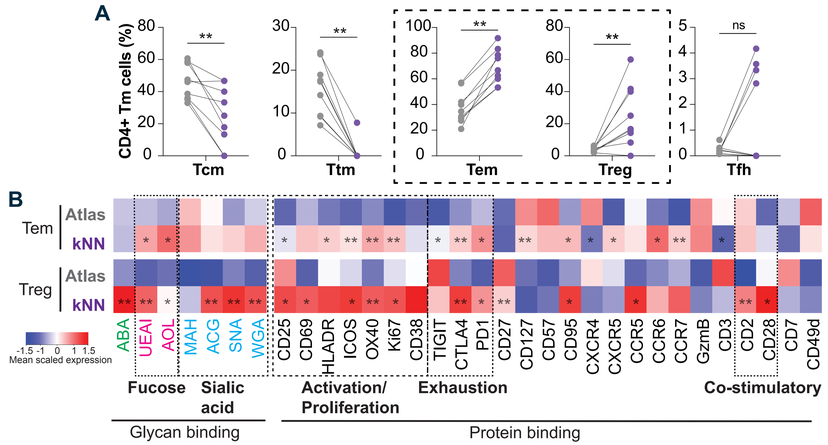
(A) Predicted precursor single-cell linkage using distance estimation (PP-SLIDE) analysis was used to predict the original phenotypes (kNN) of reactivated HIV reservoir cells prior to *ex vivo* stimulation. This "molecular facial recognition" method entails mapping the high-parameter information from reactivated cells onto an "Atlas" of unstimulated CD4+ Tm cells generated from the corresponding participant and identifying the most similar cell using a k-nearest neighbor (kNN) approach. The identified "Predicted Precursor" cells correspond to cells in the Atlas identified by kNN, and hence are referred to as kNN cells. The approach is analogous to matching a contemporary photo to an "atlas" of old photos to find the most similar one, and relies on the notion that features of the original unstimulated HIV reservoir cell will be retained even after HIV reactivation (B). **Inducible HIV reservoir cells** (**kNN cells, purple**) exhibit elevated fucosylation (AOL+UEAI+) and sialylation (MAH+), relative to **total CD4+ Tm cells** (**grey**). (C) Inducible HIV reservoir cells differentially express markers of T cell activation/proliferation, exhaustion, differentiation, homing, and cytolytic activity. Data are from blood samples of n=9 ART-suppressed PWH. *p<0.05, **p<0.01, ns (not significant) by Wilcoxon test.

Figure 5: Design of glycan/protein antigen sorting panel to enrich for HIV reservoir cells



Shown is a sequential gating strategy depicting inducible reservoir cells (identified by kNN) in purple, and total CD4+ Tm cells in grey. By sorting based on binding by four lectins (AOL+WGA+MAH+UEAI) and expression levels of four protein antigens (OX40+CD69+PD1+TIGIT+), we can theoretically enrich inducible reservoir cells by 11.2x as determined by CyTOF/PP-SLIDE. Numbers at the top correspond to the theoretical enrichment at each stage of the sequential gating strategy. This panel will be implemented on cells from ART-suppressed PWH, and the sorted population will be quantitated for HIV reservoir by measuring intact/total HIV DNA and HIV cell-associated RNA.

Figure 4: Inducible HIV reservoir cells are enriched for Tem and Treg phenotypes and exhibit a unique fucosylation and sialylation signature



(A) Inducible HIV reservoir cells (**kNN cells, purple**) are enriched in effector memory (Tem) and regulatory (Treg) phenotypes, but are depleted in central memory (Tcm) and transitional memory (Ttm) phenotypes, relative to total CD4+ Tm cells (**grey**). (B) Compared to Atlas cells, kNN-Tem cells exhibit high binding to UEAI and AOL, while kNN-Treg cells exhibit high binding to ABA, ACG, SNA, and WGA. Both reservoir subsets preferentially expressed activation/proliferation markers CD25, ICOS, OX40 and Ki67, and exhaustion markers PD1 and CTLA4, but only kNN-Treg ones had elevated CD2 and CD28. Data are from blood samples of n=9 ART-suppressed PWH. *p<0.05, **p<0.01, ns (not significant) by Wilcoxon test.

CONCLUSIONS

CyTOF-Lec technique paired with PP-SLIDE enables characterization of the glycomic and proteomic features of HIV reservoir cells from ART-suppressed PWH

HIV reservoir cells are enriched within exhausted Tem and Treg with unique glycan features:

- Fucosylation is broadly elevated across both reservoir subsets, consistent with its role in promoting T cell activation and clonal expansion, mechanisms that may support HIV persistence.
- By contrast, sialylation is preferentially upregulated in Treg reservoir cells, which may facilitate immune evasion of these cells.

REFERENCES

(1) Daly J, O'Dwyer ME et al., (2019). Blood, 134(1):138. (2) Liang W, Li W, et al., (2018). Front. Immunol. 9:78. (3) Ma T, Roan NR, et al., (2022). eLife, 11:e78870. (4) Neidlemman J, Roan NR, et al., (2020). eLife, 9:e60933.

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