Sex-Specific Immune Responses Shape Proviral Landscapes in Individuals on Long-Term Suppressive ART

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Background

- Biological sex impacts host immune responses (ref. 1) which may influence selection and evolution of HIV-1 reservoir cells during long-term antiretroviral therapy (ART) (ref. 2-3).
- Sex-specific differences in HIV reservoir landscape, including proviral size and composition, and integration sites profiles, among long-term ART-treated (LT-ART) individuals was previously demonstrated *(Tan et al., Oral presentation, CROI 2024)* (Fig. 1).
- However, host immune responses contribute to the shaping of such proviral landscapes in individuals on LT-ART remain elusive.

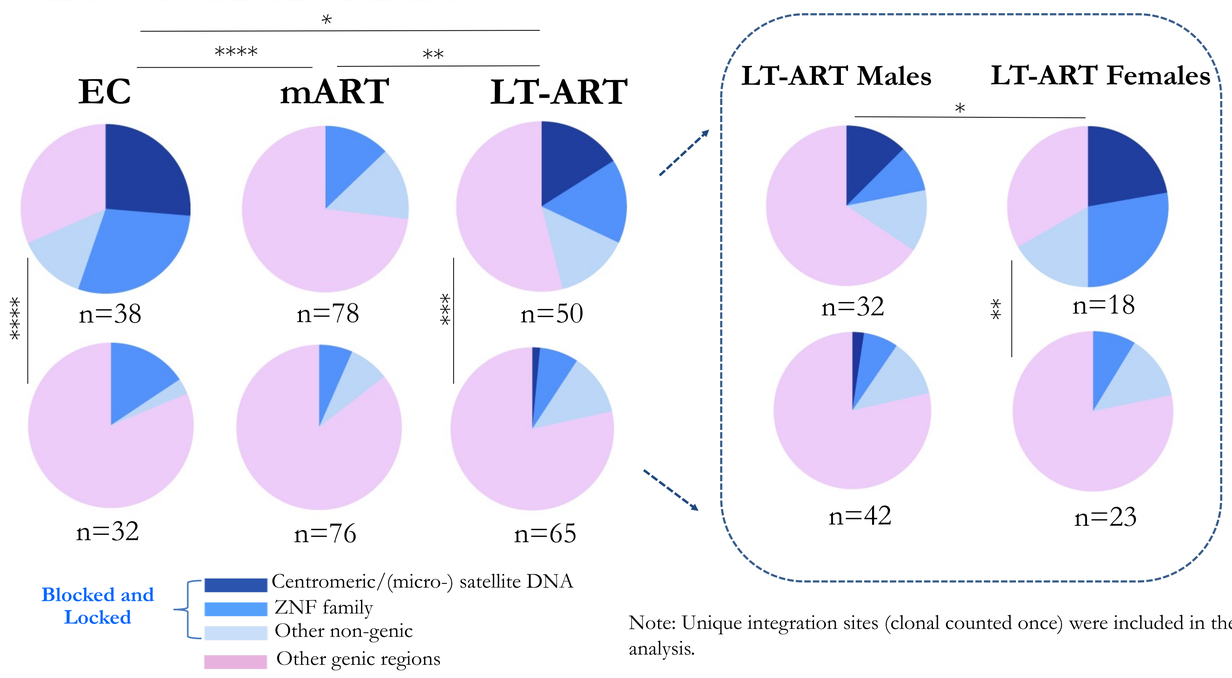


Fig 1: Enrichment of intact proviruses in heterochromatin regions in LT-ART individuals particularly in females. (Tan *et al., Oral presentation*, CROI 2024).

*EC: elite controllers; mART: individuals on moderate-term of ART (~8 years); and LT-ART: individuals on long-term ART (~20 years)

Methods

- We selected a total of 20 participants on LT-ART and categorized them into 4 study groups based on their HIV-1 integration site profiles: females and males with viral reservoirs dominated by genome-intact proviruses integrated in transcriptionally-repressed heterochromatin (HTC) sites (HTC^{high}) and those without evidence of selection for intact proviruses at HTC ($HTC^{low/neg}$).
- We conducted global immunophenotyping studies using multidimensional spectral flow cytometry with 27 colors for innate immune and 18 colors for T cell adaptive immune cells.

	HTC^{high} females	HTC^{high} males	$HTC^{low/-}$ females	$HTC^{low/-}$ males
Number of participants	5	5	4	6
% of intact proviruses in known heterochromatin (range)	77.8 – 100%	84.6 – 100%	0%	0 – 33.3%
Age	57 (51 – 66)	64 (53 – 73)	67 (64 – 76)	56 (54 – 68)
CD4 counts	908 (459 - 1204)	718 (501 - 1452)	837 (638 – 1053)	735 (636 – 1062)
Time since diagnosis (year)	21.5 (19 - 28)	27 (21 - 32)	21 (21 - 26)	21.5 (16 - 29)
Known duration of undetectable viremia (year)	17 (15 - 23)	20 (20 - 23)	21 (19 - 21)	19 (15 - 23)

Table 1: Demographic information of HTC^{high} and $HTC^{low/-}$ male and female study subgroups

Distinct Myeloid Immune Signatures in Females with HIV-1 Reservoir Enriched in Heterochromatin

- The proportions of CD64+PD-L1+ myeloid dendritic cells were higher in females, specifically in the HTC^{high} group (Fig 2B), and was associated with the proportions of intact proviruses in heterochromatin (Fig 2C).

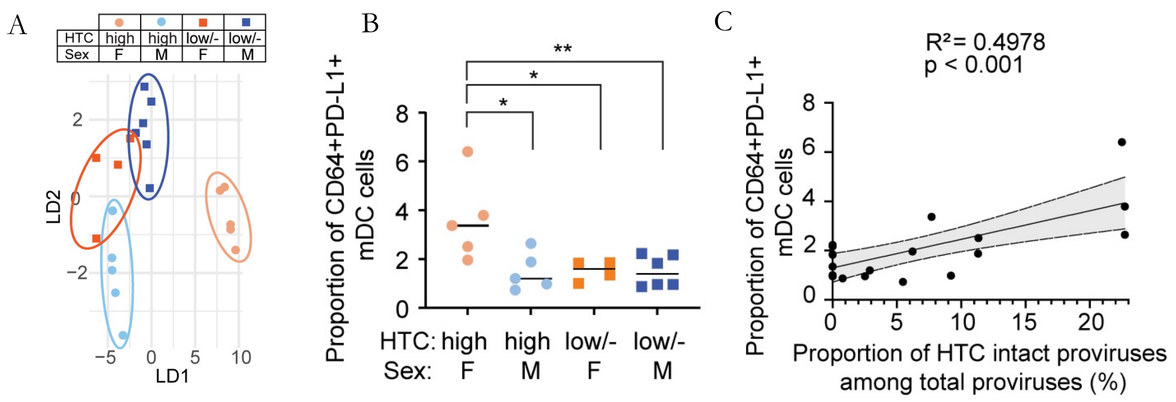


Fig 2. A: Linear discriminant analysis of total innate immune cells in indicated study subgroups (A). **B-C:** Proportion of CD64+PD-L1+ myeloid dendritic cells (mDCs) in indicated study subgroups (B) and their associations with the proportions of intact proviruses in heterochromatin regions among total proviruses (C).

Distinct NK Immune Signatures in Females with HIV-1 Reservoir Enriched in Heterochromatin

- NK cells in the HTC^{high} group were characterized by a more limited ensemble expression of inhibitory KIR receptors and TIGIT compared to other groups, particularly relative to the males from the $HTC^{low/-}$ group (Fig. 3A-B), and this was associated with the proportions of intact proviruses in heterochromatin (Fig. 3C).

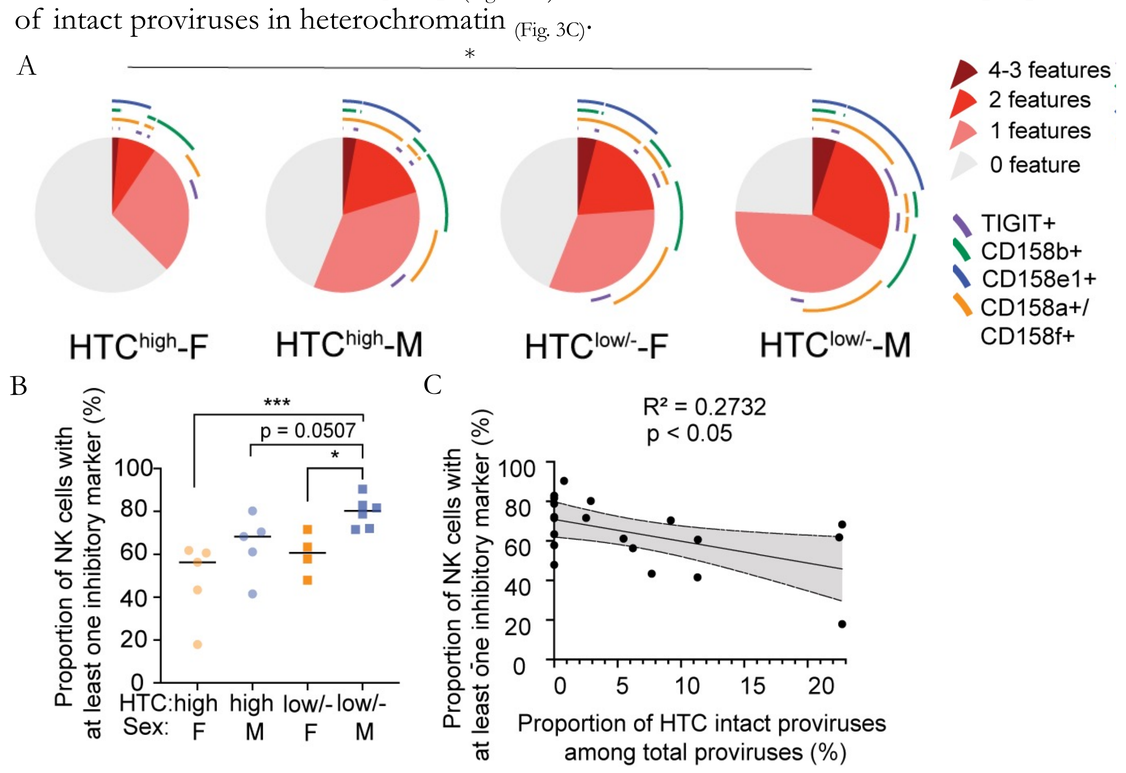


Fig 3. A: SPICE diagrams highlighting proportions of NK cells with 0, 1 or ≥2 inhibitory features. Pie chart colors reflect the proportions of cells expressing the indicated numbers of markers; arcs reflect the proportion of cells expressing a given marker. **B-C:** Proportions of NK cells with at least one inhibiting feature (B) and their associations with the proportions of intact proviruses in heterochromatin regions among total proviruses (C).

T cell Adaptive Immune Signatures in People Living with HIV on Long-term Suppressive ART

- HTC^{high} male subgroup was characterized by more polyfunctional HIV-1-Gag specific CD8+ T cells, compared to other groups, particularly relative to the females from the $HTC^{low/-}$ group (Fig. 4A-B), although no association with the proportions of intact proviruses in heterochromatin was noted (Fig 4C).

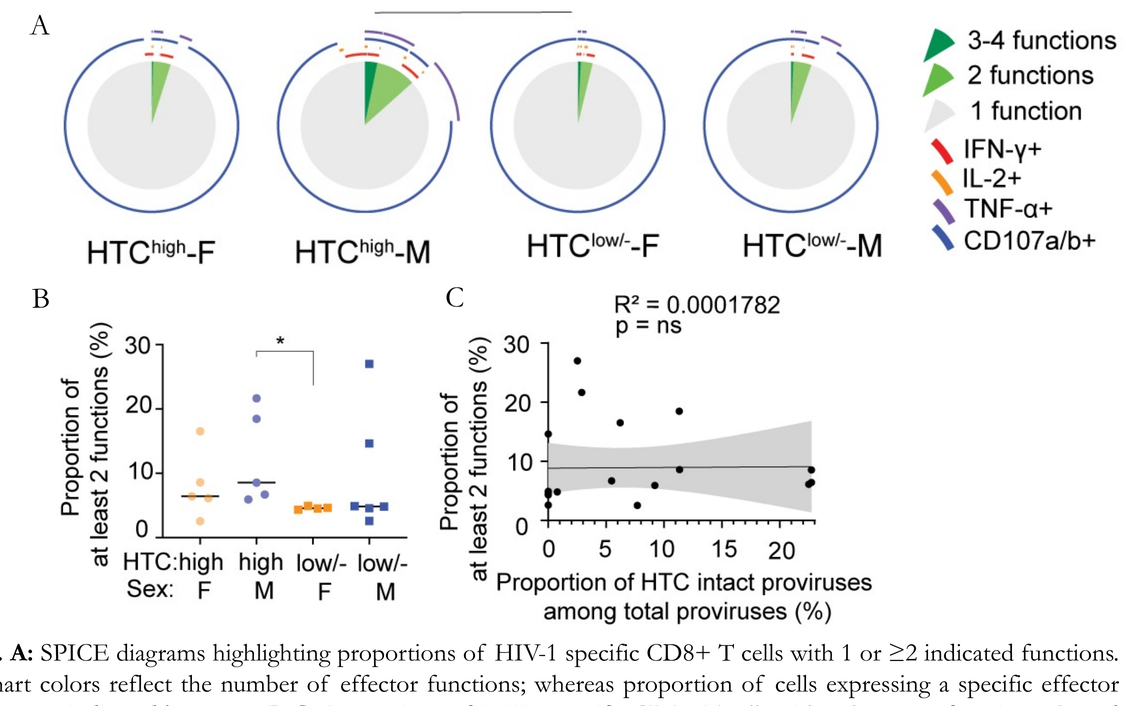


Fig 4. A: SPICE diagrams highlighting proportions of HIV-1 specific CD8+ T cells with 1 or ≥2 indicated functions. Pie chart colors reflect the number of effector functions; whereas proportion of cells expressing a specific effector function are indicated by an arc. **B-C:** Proportions of HIV-1 specific CD8+ T cells with at least two functions (B) and their associations with the proportions of intact proviruses in heterochromatin regions among total proviruses (C).

Conclusion

- Innate immune responses may drive efficient immune selection of intact proviruses in heterochromatin locations and suggests that females may be better candidates to explore innate-immunity-dependent strategies for targeting HIV-1 reservoir cells.

References

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