A Skewed NK Cell Repertoire Persists in People with HIV-1 Despite Long-Term ART

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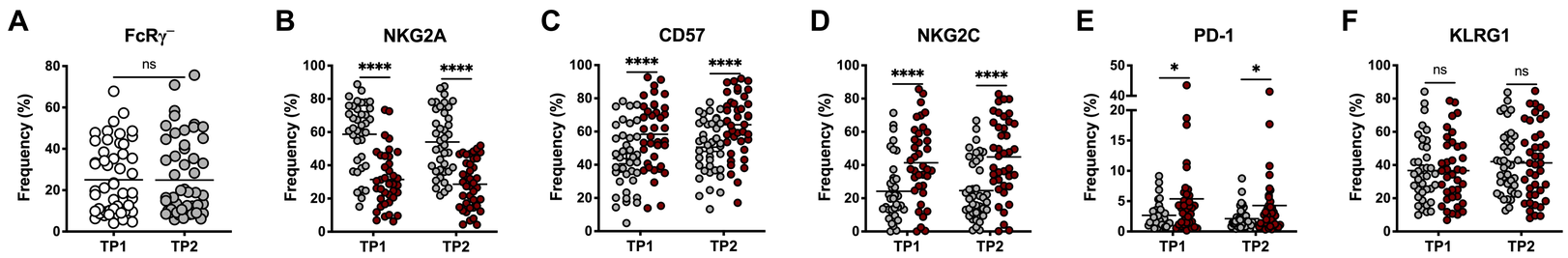
Background

- HIV-1 infection greatly alters the NK cell phenotypic and functional repertoire
- This is highlighted by the expansion of a rare population of FcRγ⁻ NK cells exhibiting characteristics of traditional immunologic memory in people with HIV (PWH)
- NK cell subset distribution and function are also sequentially deregulated with chronic HIV-1 viremia
- On average, 24 months of suppressive antiretroviral therapy (ART) are required before normalization of surface expression of CD56 and NKG2A and recovery in functionality
- PWH on effective ART continue to show signs of residual immune dysfunction, including persistence of NK cell activation
- Do chronic HIV-1 infection and long-term ART have enduring effects on conventional and memory-like NK cells?

Methods

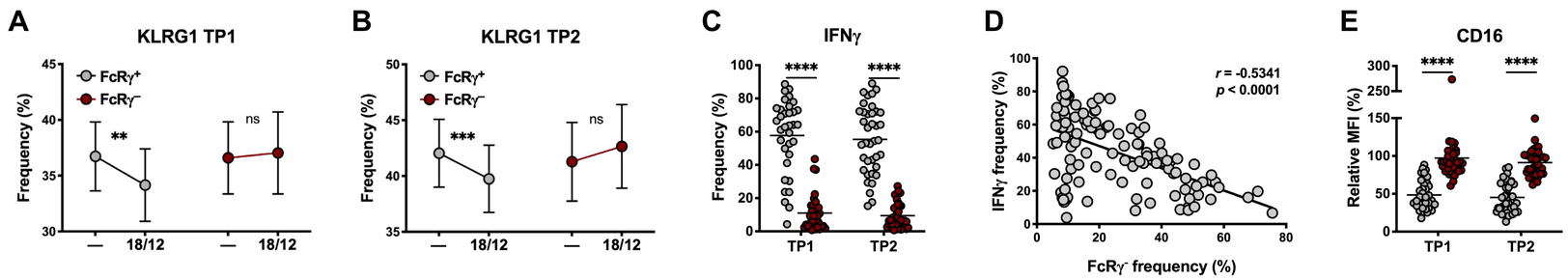
- Longitudinal analysis detailing conventional and memory-like NK cell characteristics in PWH (n=60) of the AIDS Clinical Trials Group (ACTG) cohort study A5321
- All participants initiated ART in ACTG trials during chronic infection and had well-documented consistent HIV-1 suppression
- Phenotypic and functional NK cell profiles, as well as frequencies of FcRγ⁻ NK cells, were determined by flow cytometry at 4 weeks, 1 year, and 4 years post-initiation of ART
- Single-cell multiomic analysis was performed using the BD Rhapsody™ Express Single-Cell Analysis System

Phenotypic differences of FcRγ⁻ and FcRγ⁺ NK cells do not diminish in response to ART



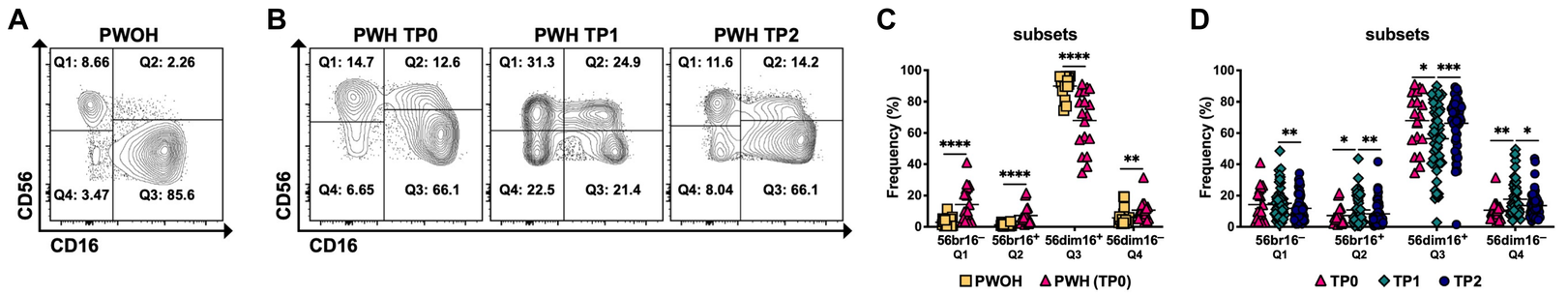
Evaluation by flow cytometry of PBMCs from PWH of the ACTG A5321 study with an FcRγ⁻ NK cell frequency >10% (n = 44) at 1 year (TP1) and 4 years (TP2) post-ART initiation. **(A)** The proportion of FcRγ⁻ NK cells remained stable over the course of treatment with ART. **(B-F)** Direct comparisons were drawn between FcRγ⁻ and FcRγ⁺ NK cells within each timepoint. The FcRγ⁻ population was enriched for cells with lower levels of NKG2A (B) but higher levels of CD57 (C), NKG2C (D), and PD-1 (E). FcRγ⁻ and FcRγ⁺ NK cells expressed comparable levels of KLRG1 (F). Statistical significance was determined by the paired Student's t-test (****p < 0.0001; *p < 0.05).

Persistence of reduced IL-18 responsiveness in the FcRγ⁻ population



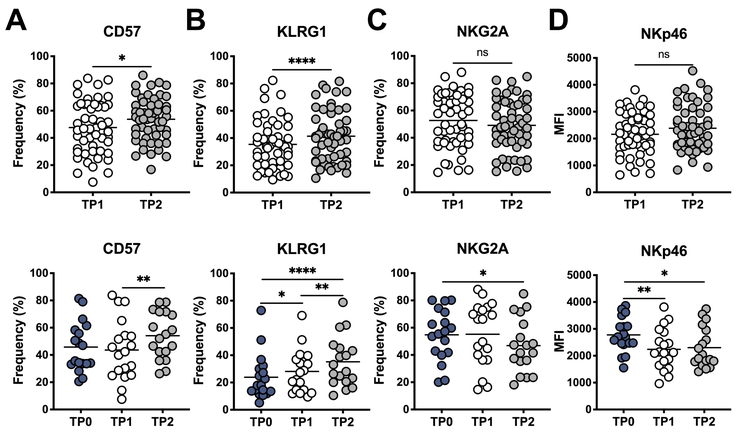
Evaluation by flow cytometry of PBMCs from PWH of the ACTG A5321 study with an FcRγ⁻ NK cell frequency >10% (n = 44) at 1 year (TP1) and 4 years (TP2) post-ART initiation. Cultures were exposed to media alone or IL-18+IL-12 for 24h. **(A, B)** Comparisons of KLRG1 surface levels on NK cells, at baseline (—) and following a 24h exposure to IL-18+IL-12 (18/12). Although conventional NK cells downregulated KLRG1 following IL-18+IL-12 stimulation, its expression increased modestly within the FcRγ⁻ subset. **(C)** The lack of responsiveness of the FcRγ⁻ population to innate stimuli was magnified by their dismal production of IFNγ following a 24h culture with IL-18+IL12. **(D)** The proportion of FcRγ⁻ NK cells inversely correlated with the frequency of NK cells positive for IFNγ by intracellular staining. **(E)** Unlike the FcRγ⁺ subset, cytokine co-stimulated FcRγ⁻ NK cells maintained CD16 expression relative to unstimulated controls at both timepoints. Statistical significance was determined using the paired Student's t-test (A-C, E) or Pearson correlation (D) (****p < 0.0001; ***p < 0.001; **p < 0.01).

An abnormal NK cell subset distribution in HIV-1 infection



(A, B) Representative FACS plots, illustrating the expected distribution at baseline of CD56^{dim} and CD56^{bright} NK cells (A) and unusually large CD56^{bright}CD16^{+} and CD56^{dim}CD16^{-} populations at the three on-ART timepoints (B). **(C, D)** Relative to PWOH, PWH of the ACTG A5321 study experienced an aberrant NK cell subset distribution, which was particularly pronounced at TP1 and began to normalize by TP2; n = 20 PWOH, n = 20 PWH at TP0, n = 60 PWH at TP1 and TP2. Statistical significance was determined by the unpaired Student's t-test (C) or mixed-effects analysis, with correction for multiple comparisons by Tukey's HSD post-hoc test (D) (****p < 0.0001; ***p < 0.001; **p < 0.01; *p < 0.05).

NK cells become more differentiated over the course of ART



Evaluation by flow cytometry of PBMCs from PWH of the ACTG A5321 study at ~4 weeks on-ART (TP0, n = 20), 1 year on-ART (TP1, n = 60), and 4 years on-ART (TP2, n = 60). **(A, B)** NK cells gained expression of CD57 and KLRG1, both of which are markers of terminal differentiation, with the highest levels observed at TP2. **(C, D)** Concomitant with the acquisition of this differentiated phenotype was a reduction in the expression of NKG2A and NKp46. Statistical significance was determined by the paired Student's t-test or mixed-effects analysis, with correction for multiple comparisons by Tukey's HSD post-hoc test (****p < 0.0001; **p < 0.01; *p < 0.05).

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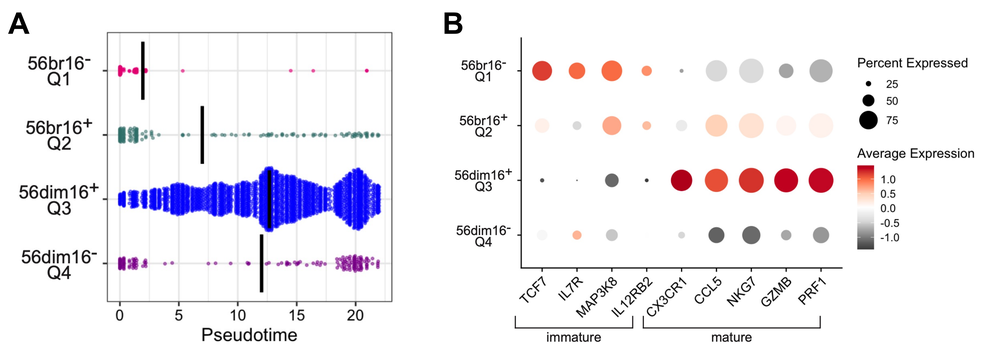
Multiomic analysis points to interruption of NK-poiesis



scRNA-seq analysis across NK cell subsets at the on-ART timepoint of 4 years (TP2; n = 4 PWH). NK cells were grouped by surface CD56 and CD16 protein expression in silico via AbSeq staining. **(A)** Mean pseudotime values from the same participant, indicated by the vertical lines, increased from the CD56^{bright}CD16^{-} to CD56^{dim}CD16^{+} subsets. **(B)** Expression of genes associated with NK cell development changed significantly throughout the trajectory for all 4 participants and were further analyzed at the subset level.

Conclusions

- NK cells progress along a spectrum of differentiation in PWH on long-term ART
- Conventional but not FcRγ⁻ NK cells downregulate KLRG1 upon IL-18+IL-12 exposure
- PWH display atypical NK cell subsets, representing intermediate stages of NK-poiesis
- NK cell irregularities persist in PWH despite long-term ART, underscoring the need to better understand the causative mechanisms that prevent full restoration of immune health in PWH

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