Ragon Institute of MGH, MIT and Harvard

BOTSWANA HARVARD AIDS INSTITUTE PARTNERSHIP FOR HIV RESEARCH AND EDUCATION

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HIV-1 RESERVOIR CELL EVOLUTION IN EARLY-TREATED CHILDREN IN BOTSWANA

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BACKGROUND

Despite remarkable advances in prevention of vertical HIV-1 transmission and antiretroviral drug development, pediatric HIV-1 infection remains a frequent and difficult-to-treat disease. Early initiation of antiretroviral therapy (ART) in neonates infected with HIV-1 may limit the frequency and stability of HIV-1 reservoir cells, possibly improving response to interventions aimed at viral eradication and cure.

METHODS

37 children from the Early Infant Treatment cohort in Botswana, who started ART at a median of 2 days from birth, were included in this study. HIV-1 near full-length genome sequencing of individual proviral species were used to characterize the proviral reservoir landscape. Integration sites associated with each proviral sequence were obtained using Matched Integration site and Proviral Sequencing (MIP-Seq). Immune responses were measured using flow cytometry.

RESULTS

I. Proviral DNA levels decreased by 5-10 fold after 84-96 weeks of early and continuous treatment; with more significantly pronounced decrease for intact compared with defective HIV-1 proviruses

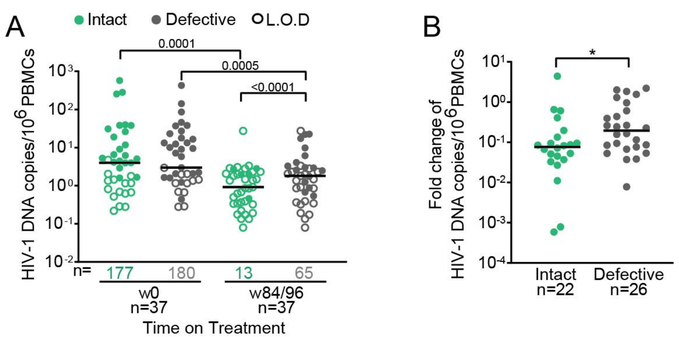

Figure 1: Decrease in viral reservoir in early-treated HIV-1-infected infants. (A): Frequency of intact and defective proviruses in early-treated HIV-1-infected infants at week 0 after birth and week 84/96. Limit of defection (L. O. D.) was calculated as 0.5 copies per maximum number of cells tested without target identification. (B): Fold change in proportion of intact and defective proviruses between baseline (week 0) and week 84/96 in early-treated infants. Data from all infants with detectable proviruses at baseline were included.

II. In two study participants, intact proviruses at week 84 were frequently integrated in heterochromatin regions that represent atypical sites for proviral integration during primary infection.

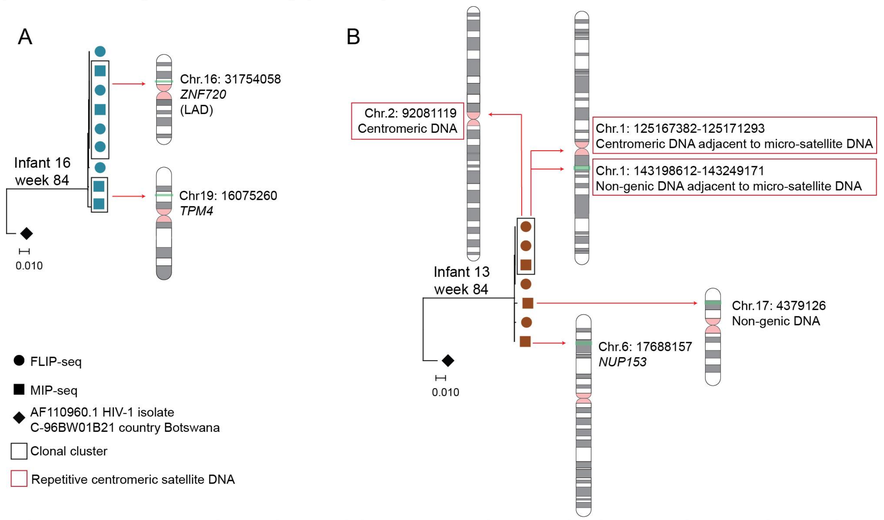

Figure 2: Integration sites of intact proviruses in heterochromatin regions from early-treated infants. (A-B): Linear maximum-likelihood phylogenetic trees for genome-intact proviral sequences from two early-treated infants after 84 weeks of antiretroviral therapy. Chromosomal coordinates and relative positioning of integration sites are depicted; genes that contain integration sites are listed. Clonal genome-intact proviral sequences, defined by identical proviral sequences and identical corresponding integration sites, are highlighted in black boxes. LAD, lamina-associated domain.

III. At birth, the frequency of intact proviruses was inversely associated with IL-8-secreting CD4 T cells (Th8), which represent a dominant cell subset in neonates and displayed higher levels of cell-intrinsic resistance to HIV-1 infection.

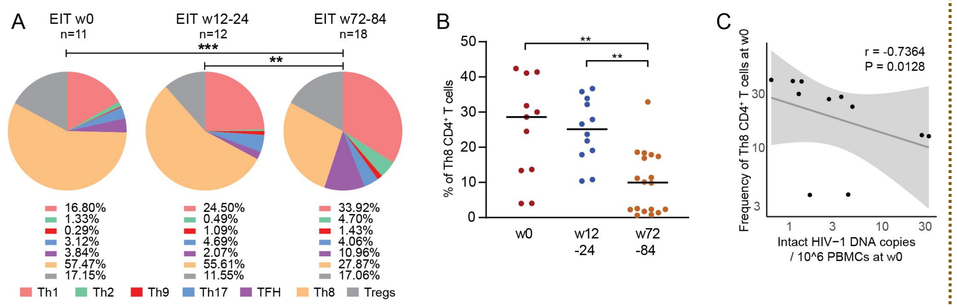

Figure 3: Th8 CD4 T cells were the dominant subset at birth, which inversely associated with HIV-1 reservoir size in early-treated infants. (A): Pie charts reflecting average proportions of monofunctional CD4 T cells expressing the indicated signature markers/cytokines, without concomitant expression of alternative cytokines. Longitudinal data from early-treated infants at indicated timepoints are shown. (B): Longitudinal changes in the proportion of Th8 cells in early-treated infants at indicated timepoints. **p<0.01; Kruskal Wallis test with post-hoc Dunn's test. (C): Association between the proportion of Th8 cells at week 0 and corresponding frequency of intact proviruses at week 0. Spearman correlation coefficient is shown.

IV. The decline of intact proviruses was inversely associated with an expansion of CD57+ NK cells, characterized by enhanced cytotoxic activities. Conversely, proportions of NK cells expressing the inhibitory receptor NKG2A decreased over time and correlated positively with intact provirus frequency.

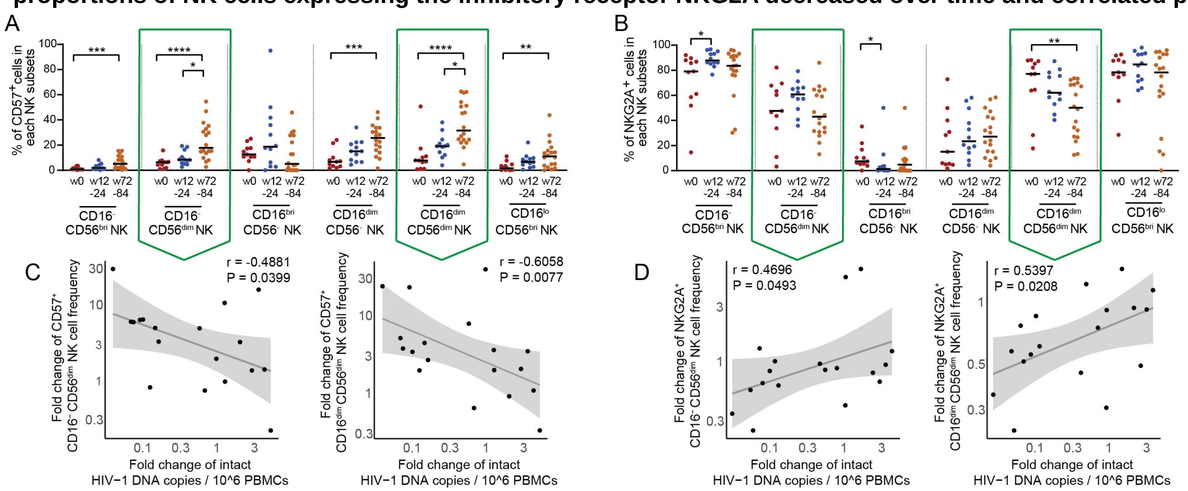

Figure 4: Longitudinal evolution of NK cell responses correlates with trajectory of intact HIV-1 proviruses. (A-B): Longitudinal evolution of CD57-expressing (A) and NKG2A-expressing (B) NK cell subsets in early-treated infants. Data from week 0, week 12/24 and week 72/84 are shown. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001; Kruskal Wallis test with post-hoc Dunn's test. (C-D): Correlation between proportional changes of indicated NK cell subsets (between week 0 and week 72/84) and corresponding changes in intact HIV-1 proviruses. Spearman correlation coefficient is indicated.

CONCLUSIONS

Together, these results suggest that HIV-1 reservoir cell seeding and evolution in early-treated children is markedly influenced by innate immune responses.