HIV-SPECIFIC CD8⁺ T CELL RESPONSES ASSOCIATE WITH SMALLER HIV RESERVOIR SIZE IN INFANTS AFTER ART

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MHRP U.S. MILITARY HIV RESEARCH PROGRAM #863

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BACKGROUND:

The infant immune system is characterized by reduced HIV-specific immune responses, resulting in an inability to control HIV viremia. Early administration of antiretroviral therapy (ART) in HIV infected infants is associated with a rapid decay of HIV DNA and RNA in PBMCs and smaller HIV reservoir size. In order to develop new interventions for HIV remission in infants, it is important to better understand how the developing immune system and HIV-specific T cell responses affect the size of the viral reservoir after long-term ART. In this study, we investigated the levels of total and HIV-specific T cell activation and their relation to HIV viral reservoir size in HIV infected Thai infants.

METHODS

- Forty-one HIV-infected Thai infants enrolled in the HIVNAT209 study who started ART within the first 6 months of age were included: 8 virally non-suppressed at baseline, either pre-ART (n=4) or on ART (median 23 days, range 4-45) and 33 successfully treated infants (**Table 1**). Seven HIV exposed uninfected (HEU) Thai infants enrolled in RV519 were also included. Comparisons were made with HIV infected Thai adults from RV254 who were acutely infected in 4G Stage 1 (n=14, NAT+/4th Gen EIA-/3rd Gen EIA-), 4G Stage 2 (n=18, NAT+/4th Gen EIA+/3rd Gen EIA-), and 4G Stage 3 (n=21, NAT+/4th Gen EIA+/3rd Gen EIA+), or who started treatment in 4G Stage 3 and had been successfully treated for a median of 2.8 years (range 1.9-5.9 yrs).
- Activation of total CD45RA⁻ CD8⁺ and CD4⁺ T cells was analyzed by measuring expression of Ki-67 alone, Ki-67 and bcl-2 (Ki-67⁺bcl-2^{lo}), or CD38 and HLA-DR (CD38⁺HLA-DR⁺) by flow cytometry.
- Total and integrated HIV DNA levels in CD4⁺ T cells were quantified by ultrasensitive PCR.
- HIV-specific CD8⁺ T cells were identified by flow cytometry using a pool of tetramers to identify HLA-A*11:01 cells specific for a Nef antigen and HLA-C*01:02 cells specific for a Gag or Env antigens.
- HIV-specific production of IFNγ, TNFα, and IL-2 was measured by flow cytometry after an 8-hour stimulation of expanded CD8 T cell lines activated by autologous B-EBV cells pulsed overnight with 74 dominant CRF01_AE HIV peptides.

Table 1. Age and ART duration at time of sampling

	n	Age (mos) Median (range)	ART duration (mos) Median (range)
HIVNAT209 V1	8	3.3 (0.9-5.3)	0.1 (0-1.5)
HIVNAT209 V3	16	15.4 (12.5-20.0)	12.6 (10.8-15.8)
HIVNAT209 V5	10	28.4 (24.9-31.8)	25.4 (20.7-26.4)
HIVNAT209 V7	7	40.9 (38.4-42.9)	37.3 (35.5-38.1)
HIV Exposed Uninfected	7	13.2 (12.2-18.5)	NA

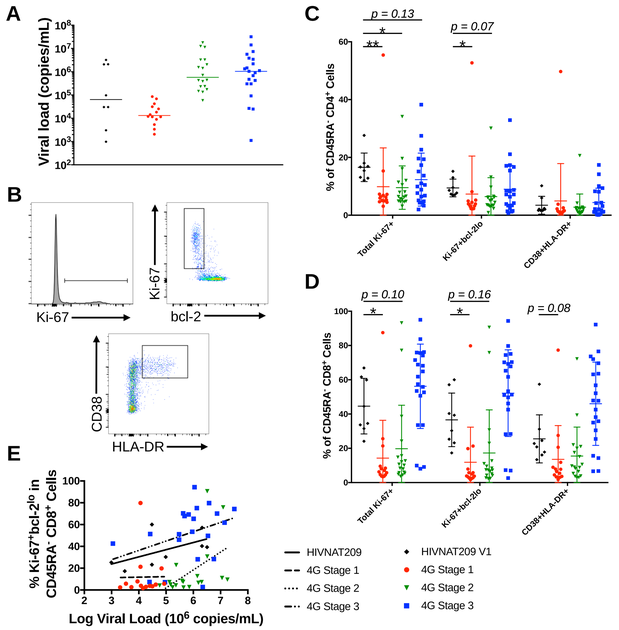


Figure 1. Activation of T cells in infants and adults at acute HIV infection (AHI). A. Plasma viral loads at pre-ART AHI in infants and adults diagnosed during 4G Stages 1, 2, and 3. Percentages of Ki-67⁺, Ki-67⁺bcl-2^{lo}, and CD38⁺HLA-DR⁺ **(B)** activated CD45RA⁻ CD4⁺ T cells **(C)** and CD45RA⁻ CD8⁺ T cells **(D)** were analyzed by flow cytometry. *p<0.05, **p<0.01 by Kruskal-Wallis test with post-test comparison to HIVNAT209 V1. E. Correlation between the viral load and frequency of Ki-67⁺bcl-2^{lo} cells among CD45RA⁻ CD8⁺ T cells. Linear regression was used to compare the elevation of the best-fit lines.

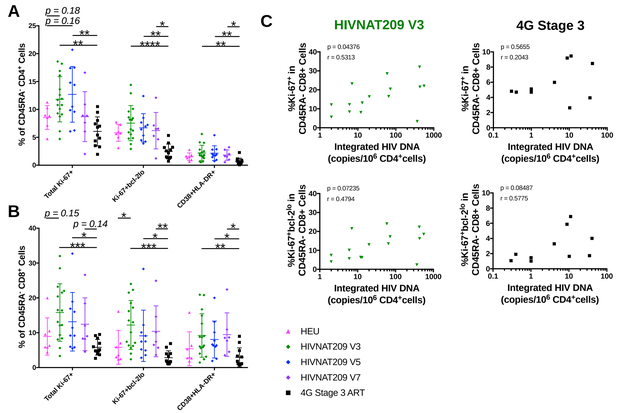


Figure 2. T cell activation in infants and adults after ART. Percentages of activated CD45RA⁻ CD4⁺ T cells **(A)** and CD45RA⁻ CD8⁺ T cells **(B)** were analyzed by flow cytometry at visits 3, 5, and 7 after ART initiation in infants, and compared to those in HEU infants and adults who initiated ART during 4G Stage 3. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 by Kruskal-Wallis test with post-test comparison to HEU or adults. **C.** Correlations between the frequency of total Ki-67⁺ cells and Ki-67⁺bcl-2^{lo} cells with levels of integrated HIV DNA were analyzed by a Spearman correlation.

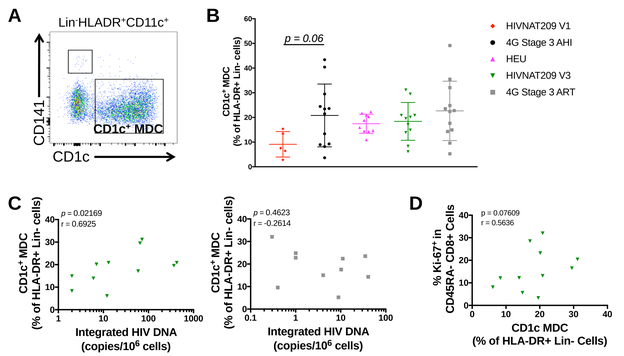


Figure 3. CD1c⁺ myeloid dendritic cell frequency correlation with HIV reservoir and CD8 T cell activation after one year of ART in infants. A-B. Frequencies of CD1c⁺ myeloid dendritic cells (MDCs) (HLA-DR⁺Lin⁻CD11c⁺CD141⁻CD1c⁺) were measured by flow cytometry in HIV⁺ infants at baseline (V1) and after 1 year of ART (V3), HEU infants, and adults before ART initiation in AHI stage 3 or after ART. Differences in frequency were measured between infants and adults at AHI using a Mann-Whitney test. **C.** Correlation of CD1c⁺ MDC frequency with integrated HIV DNA levels was analyzed. **D.** Correlation between CD1c⁺ MDC frequency and Ki-67⁺ CD45RA⁻ CD8⁺ T cells at V3 in infants. Correlations were analyzed by Spearman correlation.

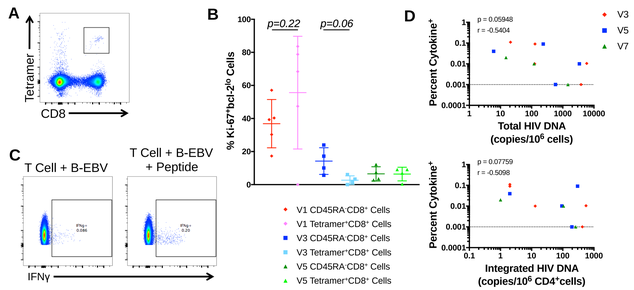


Figure 4. HIV-specific CD8⁺ T cell activation and function after ART. HIV-specific T cells were defined as CD8⁺ tetramer⁺ cells by flow cytometry **(A)** in order to analyze the frequency of Ki-67⁺bcl-2^{lo} activated cells in HIV-specific cells compared to total CD45RA⁻ CD8⁺ cells from the same participants **(B)**. Differences in T cell activation between tetramer⁺ cells and total CD45RA⁻ CD8⁺ cells were analyzed with a Mann-Whitney test. **C-D**. HIV-specific CD8⁺ T cell function was measured as the difference in frequency of cells producing cytokines after 8 hours of stimulation with autologous B-EBV cells pulsed with HIV peptide versus with B-EBV cells alone. Correlations between the frequency of cytokine producing cells and integrated HIV DNA levels were measured with Spearman correlations.

CONCLUSIONS

- Infants had T cell activation levels similar to adults in 4G Stage 3 during AHI.
- After one year of ART, HIV DNA levels correlated with CD8⁺ T cell activation levels and CD1c⁺ MDC frequency in infants. CD8⁺ T cell activation correlated with increased levels of CD1c⁺ MDC frequency.
- HIV-specific CD8⁺ T cells had lower activation levels after one year of ART than total CD45RA⁻CD8⁺ T cells. Smaller HIV reservoir size was negatively correlated with the frequency of HIV-specific CD8⁺ T cells producing IFNγ, TNFα, or IL-2 after ART in infants.
- These data suggest that after one year of ART, total immune activation in infants drives a larger HIV viral reservoir, while a greater HIV-specific CD8⁺ T cell response associates with a lower viral reservoir size.

Acknowledgements: The volunteers in HIVNAT209, RV519, and RV254/SEARC010; the staff at SEARCH Thailand and the Thai Red Cross AIDS Research Center. This research was funded by the following sources: 5R01AI114236-04 REVISED; 9/13/2017, 1R01AI108433 and a cooperative agreement (W81XWH-07-2-0067) between the Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc., and the U.S. Department of Defense (DoD). Thai Pharmaceutical Organization, Merck, ViiV Healthcare and Gilead.

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