Phenotype of Gamma-delta T Cells in Acute HIV-1 Infection Predict Neutralization Breadth

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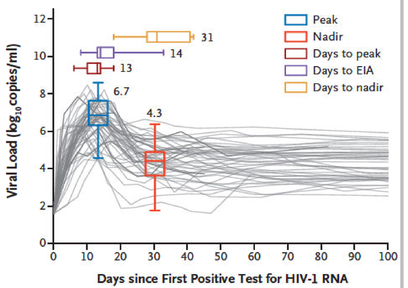
Background

The Natural HIV Infection RV217 Cohort: A Prospective Study of Acute HIV Infection (AHI) in Participants from East Africa and Thailand



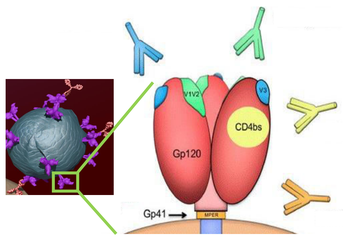
2,276 volunteers at high-risk for HIV-1 acquisition
- Participants tested 2X weekly
- 112 AHI identified

Viral loads the first 100 days post HIV-1 positive test[1]



Essential Study Time Points Captured
- Pre-HIV-1+ Test
- Peak viral load
- Viral set point
- Chronic infection

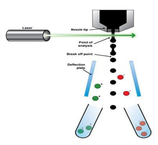
Broadly neutralizing antibodies (bNAbs)
- The HIV envelope (ENV) has sites of vulnerability targeted by bNAbs.
- Only 1% of people living with HIV naturally develop bNAbs.[2]
- No vaccine has induced the production of bNAbs thus far (including the most successful HIV vaccine with 31% efficacy developed by MHRP).



bNAbs for a better HIV vaccine
- MHRP previously identified RV217 participants with neutralization breadth.[3]
- Broad neutralizers neutralized >70% of a 34-multi-clade virus panel. Non-broad neutralizers were unable to neutralize 35% of the same panel.
- Innate immune cells could impact bNAb development.
- This study investigated the role of innate immune cells in neutralization breadth development in the RV217 cohort.

Methods

1. RV217 Participants Chosen Based on the Neutralization of 34 Pseudoviruses[3]
   - 11 broad and 11 non-broad neutralizers
     - Four time points chosen per participant
       1. Median -185 days pre first HIV+ Test
       2. Median 18 days post first HIV+ Test
       3. Median 42 days post first HIV+ Test
       4. Median 980 days post first HIV+ Test
2. Phenotype determined by flow cytometry
   - Longitudinal and non-broad vs. broad neutralizer analysis
3. Gene expression analyzed by RNA sequencing of sorted cells



Results

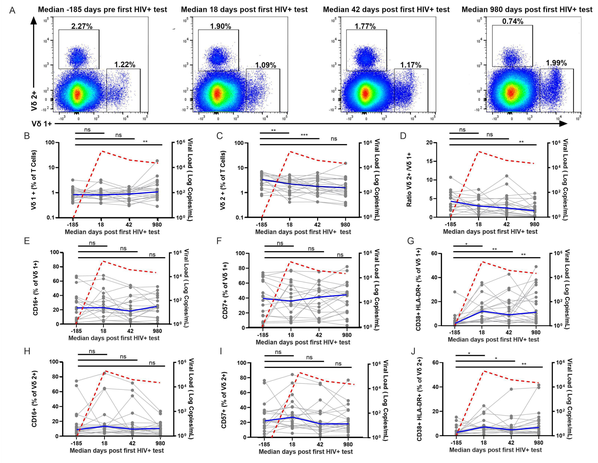


Figure 1. Dynamics of Vδ1 and Vδ2 T cells activation during AHI. Representative flow plots **(A)** of Vδ1 and Vδ2+ T cell frequencies at median -185 pre HIV+ test and 18, 42, and 980 days post HIV+ test showing a decrease in the frequency Vδ2+ T cells and an increase in Vδ1+ T cells. Longitudinal analysis of Vδ1 and Vδ2+ T cells frequency and ratio (**B-D**), effector molecules (CD16 and CD57) and activation markers (CD38+ HLA-DR+) expression (**E-J**). Each participant is indicated by a grey line and the blue line indicate the median. Dashed red line is indicative of median viral load. *P < 0.05, **P < 0.01, ***P < 0.001.

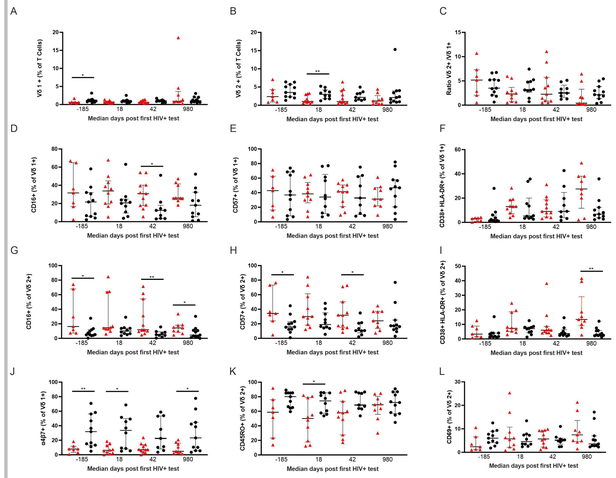


Figure 4. Differences in phenotype of Vδ1 and Vδ2+ T Cell between broad and non-broad neutralizers. Comparison between the frequency (**A-C**) and phenotype (**D-L**) of Vδ1 and Vδ2+ T cells between broad and non-broad neutralizers pre- and post-HIV acquisition. Broad neutralizer had a more effector like profile as evidence by increased CD16 levels on both Vδ1 and Vδ2+ T cells as well as elevated CD57 expression on Vδ2+ T cells. Non broad neutralizers had elevated levels of α4β7 on Vδ1 T cells and CD45RO on Vδ2+ T cells. Each data set is presented as the mean ± SEM. Broad and non-broad neutralizers were compared using the Mann-Whitney test. *P < 0.05 **P < 0.01

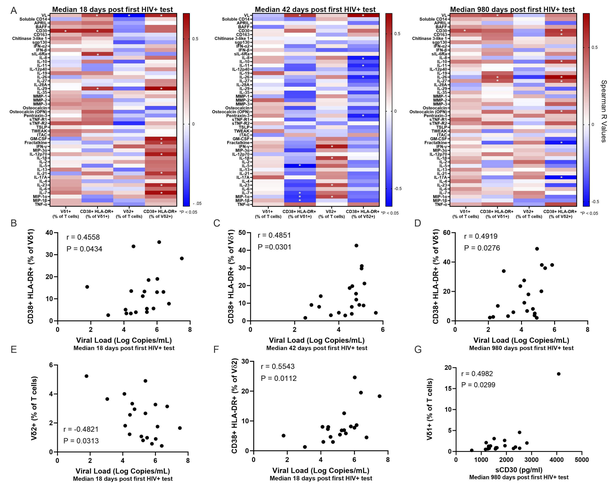


Figure 2. Association between the levels of soluble markers and Vδ1 and Vδ2+ T cell frequency and activation. Heat map showing the Spearman R values for associations between soluble markers and Vδ1 and Vδ2+ T cells frequency and activation at median 18, 42, and 980 days post HIV+ test (**A**). Association between viral and Vδ1 T cell activation (**B-D**), Vδ2 T cell frequency (**E**), and activation (**F**). Association between sCD30 levels and Vδ1 T cell frequency (**G**). *P < 0.05.

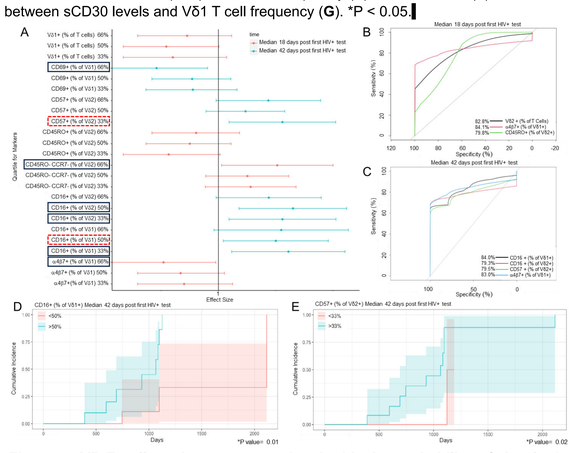


Figure 5. Vδ T cell markers are associated with the probability of developing neutralization breadth. Odds ratios (OR, solid symbols) and 95% CI (error bars) are indicated on the forest plot **(A)** with the vertical line at to indicate an OR of 1. The right side of this line represents markers that are associated with increased probability of developing neutralization breadth. Markers with error bars that overlap with the vertical line are not significant. Markers found to be significant are outlined with a black box. Markers CD16 and CD57 (red, dashed box) are significant markers noted as markers of interest in this study. AUROC curves **(B)** indicate the prediction of neutralization breadth accuracy for the frequency of Vδ2+ T cells (82.8%), α4β7+ Vδ1 T cells (84%), and CD45RO+ Vδ2 T cells (79.8%) at peak viral load. AUROC curves **(C)** indicate the prediction of neutralization breadth accuracy for the frequency of CD16+ Vδ1 T cells (84%), CD16+ Vδ2 T cells (79.3%), CD57+ Vδ2 T cells (79.3%), and α4β7+ Vδ1 T cells (83%) at set point viral load. Cumulative incidence curves showing that CD16+ Vδ1 T cells expression level above the median **(D)** and above the first tertile for CD57+ Vδ2 T cells **(E)** 1 month post initial viremia can significantly separate the participants that will achieve neutralization breadth during the course of the study to those that did not. P values were determined by the LogRank test.

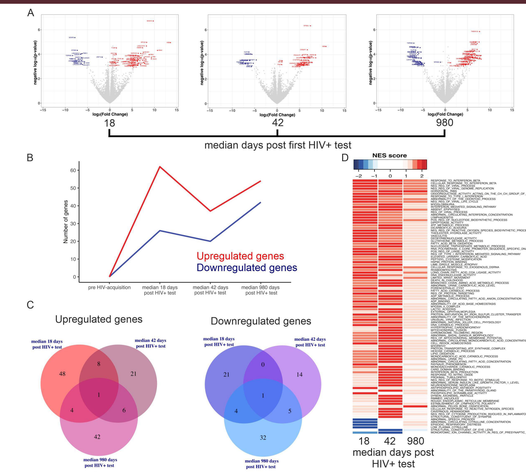


Figure 3. IFN-responsive and anti-viral pathways are induced in γδ T cells post HIV acquisition. RNA-Seq was performed on sorted total γδ T cells from PBMC of longitudinal samples from pre and post-acquisition time points. Volcano plots depict upregulated (red) or downregulated (blue) genes compared to pre-acquisition at three post-acquisition time points (**A**). Highlighted genes have a -$\log_{10}$(p-value) ≥ 3 and a $\log_2$(Fold Change) of 1 or −1 (corresponding to p ≤ 0.001, and fold change of 2 or 1/2, in a generalized linear model). The temporal dynamics of the upregulated and downregulated genes are shown longitudinally in post-HIV-1 acquisition (**B**). Shared and unshared differently expressed genes compared to pre-acquisition between all three post-acquisition time points are highlighted as a Venn diagram (**C**). Gene expression patterns were subjected to Gene Set Enrichment Analysis (GSEA), and upregulated and downregulated pathways in post-acquisition time points compared to pre-acquisition are displayed as a Normalized Enrichment Score (NES) heat map (**D**).

Conclusions

- γδ T cells are activated during AHI and express IFN-responsive and anti-viral genes.
- Viral load is a main driver of γδ T cells activation and loss of δ2 T cells during AHI.
- sCD30 was the only factor associated with the expansion of δ1 T cells during chronic HIV.
- Participants that developed neutralization breadth had higher expression of CD16 and CD57, two markers associated with effector functions.
- The levels of these markers on γδ T cells during AHI could predict which participants would develop neutralization breadth.
- Further work is needed to determine the mechanism used by γδ T cells to promote neutralization breadth.
- This work could help identify new pathways for HIV-1 vaccine development.

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Material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author, and are not to be construed as official, or as reflecting true views of the Department of the Army or the Department of Defense. The investigators have adhered to the policies for protection of human subjects as prescribed in AR 70–25.