# NK cell responses during acute HIV-1 infection are associated with slow disease progression in subtype A

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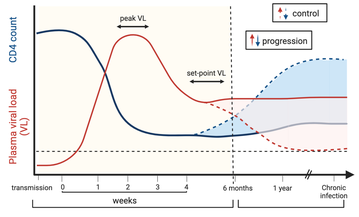
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## BACKGROUND

### Acute HIV-1 infection

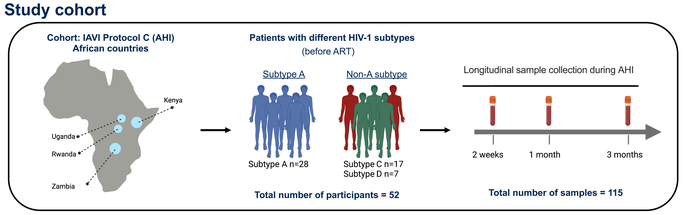



- HIV-1 subtypes influence disease progression [1,2].
- Acute HIV-1 infection (AHI) is the first stage of infection, and immune responses during AHI are critical to determining long-term outcomes.
- NK cells play an important role in controlling HIV-1, but the dynamics and diversity of their responses to different subtypes have not been previously described.
- Here, we studied the evolution of NK cell responses during AHI in a unique cohort of patients with different HIV-1 subtypes.

**Fig. 1. Acute HIV-1 infection.** Representative viral load (VL) and CD4⁺ T-cell counts during AHI. Individuals who control HIV without ART can maintain low VL and reestablish pre-infection CD4⁺ T cell levels.

## STUDY COHORT & METHODS

### A. Study cohort




### B. Experimental procedure

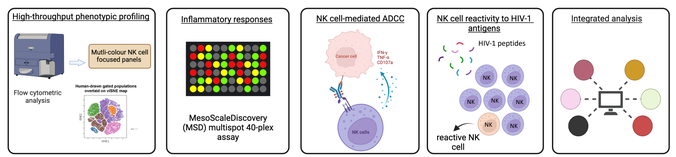


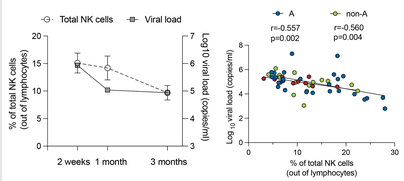

**Fig. 2. Study cohort and experimental design. A)** Participants with AHI were sampled longitudinally in four different sub-Saharan African sites, under "IAVI" protocol C. Samples were collected at three time points as indicated. **B)** Experimental procedures for in depth phenotypic and functional characterisations of NK cell responses.

### Table.1. Cohort characteristics.

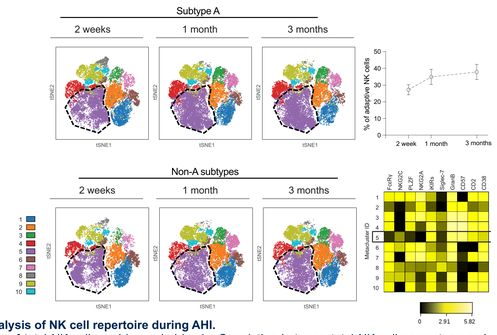
| | Subtype A | Subtype C,D, or R (non-A) |
|---|---|---|
| Age (Year), range | 28.5 (21-51) | 31.5 (17-51) |
| Gender (Female:Male) | 6:22 | 5:23 |
| Country (Rwanda: Uganda: Kenya: Zambia) | (9:3:16:0) | (1:6:8:13) |
| Days post estimated date of infection (range) | Visit 1: 32 (19-42)<br>Visit 2: 95 (72-254) | Visit 1: 38 (24-42)<br>Visit 2: 92 (81-117) |
| Viral Load (copies/ml) median (range) | Visit1: 83400 (612-19840000)<br>Visit 2: 73000 (615-1068000) | Visit 1: 69646 (442-1360000)<br>Visit 2: 79350 (51-2710682) |
| CD4 count (cells/ul) median (range) | Visit 1: 536.5 (189-1759)<br>Visit 2: 511 (267-2000) | Visit 1: 488 (245-1041)<br>Visit 2: 503.5 (264-1090) |

## RESULTS :1. Expansion of adaptive NK cells early in AHI correlates with set-point viral load.

### A. NK cells correlate negatively with HIV-1 viral load.




### B. Expansion of adaptive NK cells at the early stage of infection.




### C. Early expansion of adaptive NK cells correlate negatively with set-point VL.

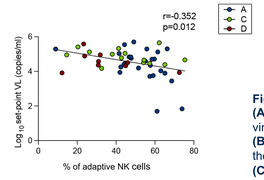
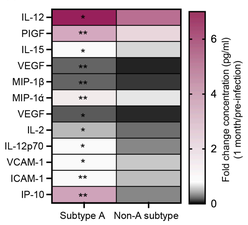



**Fig. 3. Longitudinal analysis of NK cell repertoire during AHI.**
**(A)** Longitudinal analysis of total NK cells and $log_{10}$ viral loads. Correlation between total NK cell percentage and viral load at 1 month post-infection.
**(B)** vi-SNE projections of CD56$^{dim}$ NK cells. The circle shows the adaptive NK cell cluster. Longitudinal analysis of the percentage of adaptive (FcεRγ$^{-}$CD57$^{+}$) NK cells.
**(C)** Correlation between the percentage of adaptive NK cells and $log_{10}$ set-point VL.

## 2. Higher levels of inflammation induced by subtype A contribute to adaptive NK cell expansion.

### A. Subtype A induces a stronger inflammatory response




### B. IL-15, IL-12 and IP10 at 1 month post-infection correlate with adaptive NK cells

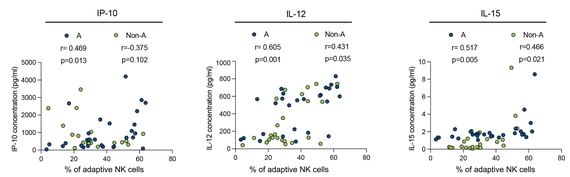
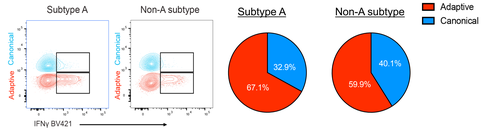


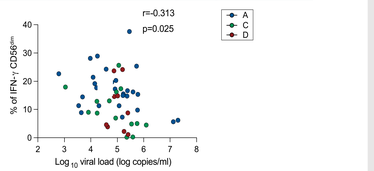

**Fig. 4. Associations between soluble inflammatory markers and adaptive NK cells.**
**(A)** Heatmap showing fold change in inflammatory markers.
**(B)** Correlation between the percentage of adaptive (FcεRγ$^{-}$CD57$^{+}$CD56$^{dim}$) NK cells and plasma concentration of IP-10, IL-15, and IL-12.

## 3. Enrichment of adaptive NK cells during the first month of infection is associated with enhanced ADCC capacity and better viral control.

### A. Adaptive NK cells exhibit an enhanced ADCC capacity during AHI.




### B. ADCC responses correlate negatively with HIV-1 VL.




### C. Reactivity of Adaptive NK cells against HIV-1 antigens.

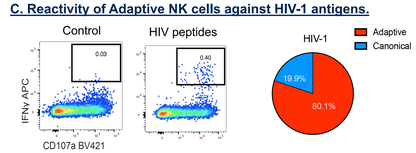
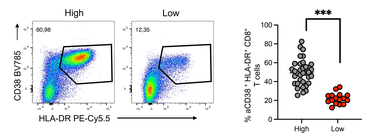


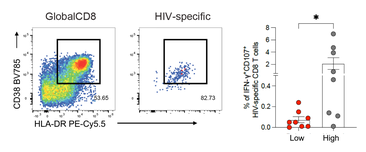

**Fig. 5. Longitudinal assessment of functional responses of adaptive NK cells during AHI.**
**(A)** Flow plots and paired analysis of IFN-γ production in canonical (FcεRγ$^{+}$CD57$^{+}$) and adaptive (FcεRγ$^{-}$CD57$^{+}$) NK cells.
**(B)** Correlation between of frequency of IFN-γ-producing NK cells and and $log_{10}$ viral load at 1-month post-infection.
**(C)** Representative flow plots and pie chart showing IFN-γ$^{+}$ production following HIV-1 peptide stimulation in adaptive and canonical NK cells.

## 4. Adaptive NK cells contribute to optimal CD8⁺ T-cell activation and virus-specific responses.

### A. Lower CD8⁺ T-cell activation in a subset of patients




### B. Activated CD8⁺ T cells are HIV-specific.




### C. Adaptive NK cells drive optimal CD8⁺ T-cell activation.

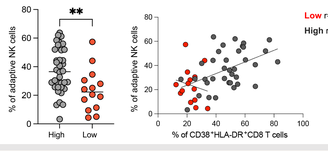
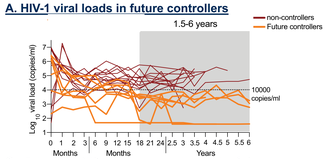


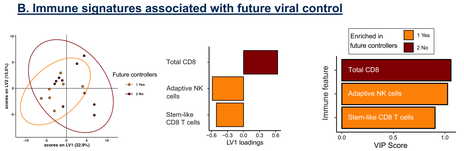

**Fig. 6. Delayed CD8⁺ T-cell activation during AHI is associated with a suboptimal adaptive NK cell response.**
**(A)** The frequencies of activated (CD38$^{+}$HLA-DR$^{+}$) CD8$^{+}$ T cells at 1 month post-infection in patients with high or low CD8$^{+}$ T-cell activation.
**(B)** The proportion of CD38$^{+}$HLA-DR$^{+}$ CD8$^{+}$ within global and HIV-specific CD8$^{+}$ T cells. Frequencies of HIV-specific CD8$^{+}$ T cells in individuals with low or high T-cell activation.
**(C)** Frequencies of FcεRγ$^{-}$CD57$^{+}$CD56$^{dim}$ NK cells in the two patient groups. Correlation between the percentage of CD38$^{+}$HLA-DR$^{+}$ CD8$^{+}$ T cells and FcεRγ$^{-}$CD57$^{+}$CD56$^{dim}$ NK cells.

## 5. The presence of adaptive NK cells during the first month of infection was associated with long-term viral control.

### A. HIV-1 viral loads in future controllers




### B. Immune signatures associated with future viral control




### C. Adaptive NK cells are enriched in future controllers

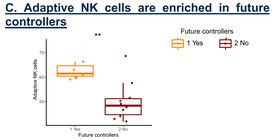
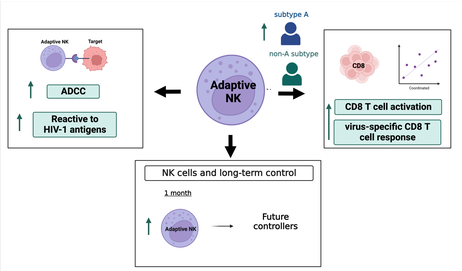



**Fig. 7. Adaptive NK cells during the first month of infection distinguish future viraemic controllers from non-controllers.**
**(A)** $Log_{10}$ viral loads of future viraemic controllers and non-controllers. Future viraemic controllers maintain levels of HIV-1 viraemia <10,000 copies/ml within 1.5-3.5 years.
**(B)** PLSDA following LASSO analysis. Scores plot showing the separation between controllers and non-controllers. Bar plot showing the contribution of the selected features to the PLS-DA latent variables (LV1) scores. Bar plot showing VIP scores of the selected features.
**(C)** The frequency of adaptive NK cells in controllers and non-controllers

## Conclusions




### Main findings:

1. Early expansion of adaptive NK cells after peak HIV-1 VL.
2. Adaptive NK cells exhibited an enhanced capacity for ADCC and reactivity against HIV-1 antigens.
3. The accumulation of adaptive NK cells during the first month of infection was associated with the optimal CD8⁺ T-cell activation, promoting virus-specific responses.
4. Early enrichment of adaptive NK cells was linked with future viral control.

**These findings provide a unique insight into the environment that promotes the optimal expansion of adaptive NK cells and subsequent CD8⁺ T-cell activation following HIV-1 exposure, laying the groundwork for new integrated approaches to promote long-term viral control through coordinated responses.**

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References:
**1** Noah Kiwanuka *et al.,* J Infect Dis. 2008
**2** Matt A Price *et al.,* J Infect Dis. 2019

CROI Conference on Retroviruses and Opportunistic Infections
وزارة التعليم Ministry of Education
NUFFIELD DEPARTMENT of MEDICINE
avi International AIDS Vaccine Initiative
BHIVA British HIV Association
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