Mucosal NK cells are associated with reduced mucosal reservoir and systemic inflammation during acute HIV-1 infection

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Background

- The mucosa is the primary site of HIV transmission, early viral replication, and establishment of the persistent viral reservoir.
- Mucosal NK cells are among the first innate immune responders and contribute to early antiviral defense through cytotoxicity, cytokine production, and immune surveillance.
- The impact of acute HIV infection (AHI) and early ART on mucosal NK cell subsets and their relationship with the tissue-associated HIV reservoir remains poorly understood.

Question

How does acute HIV infection reshape the mucosal NK cell compartment, and what is the impact of early ART?

Methods

- 28 people living with HIV (PLWH) diagnosed in early acute HIV infection AHI (Fiebig I: 8 Fiebig II: 6; Fiebig III:14) underwent sigmoidoscopy at the time of diagnosis, as well as 6 and 24 months after early ART initiation.
- Comparison biopsies were obtained from 9 people living without HIV (PLWoH).
- Mucosal cell subsets were characterized using multiparameter flow cytometry.
- Tissue-associated HIV reservoir was quantified by total HIV DNA, and plasma cytokine levels were measured by ELISA.

RV254 cohort and study design

RV254/SEARCH010
Acute HIV Infection Cohort

Real-time screening of **629,404** samples in Thailand

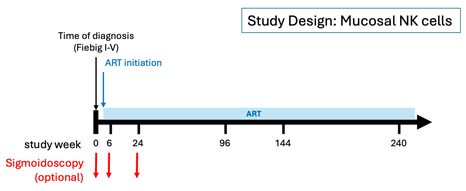
Acute HIV infection **(n=780 enrolled/1,037 detected)**

Immediate ART **(n=774)**

97% Male
Median 26 years old
73% CRF01_AE

Optional Procedures (N)
Sigmoid biopsy (286)
Leukapheresis (554)
Lumbar puncture (612)
Inguinal LN biopsy(257)
Brain MRI /MRS (1,052)

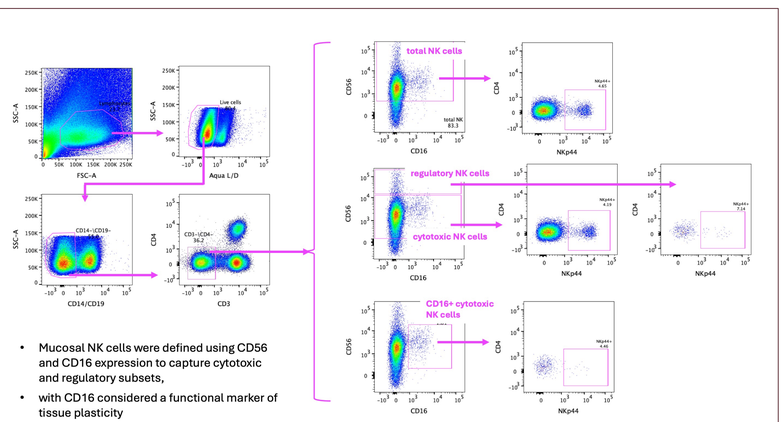
Study Design: Mucosal NK cells



- **Flow cytometric assessment of mucosal NK cells**
- **Assessment of total mucosal HIV DNA**
- **Soluble markers of inflammation in plasma**

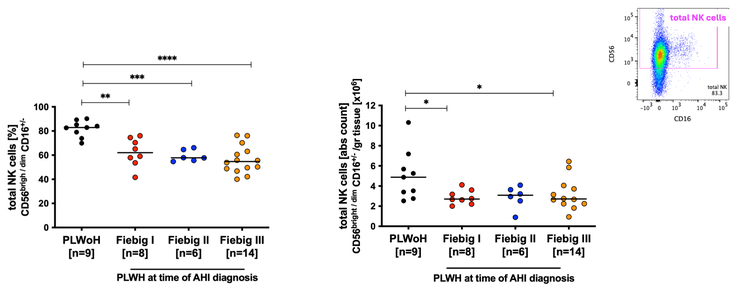
Comparison is done by age-, gender- and risk group-matched PWOH (RV304/SEARCH013)

Gating strategy: Mucosal NK cell subsets



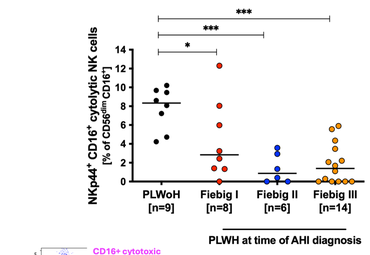
- Mucosal NK cells were defined using CD56 and CD16 expression to capture cytotoxic and regulatory subsets,
- with CD16 considered a functional marker of tissue plasticity

Decreased frequency and absolute count of total colonic NK cells during AHI



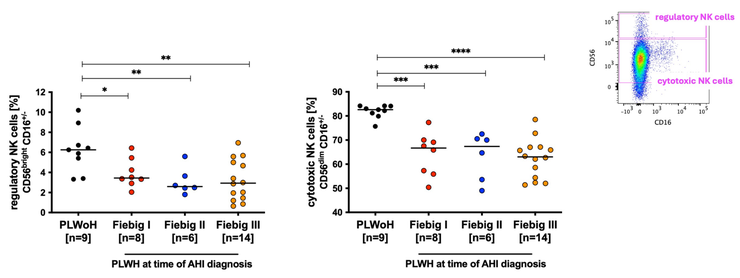
➢ Colonic NK cells decrease as early as Fiebig I during acute HIV infection (AHI)

Activated NKp44⁺ CD16⁺ cyotoxic effector NK cells appear to be selectively depleted during Fiebig I



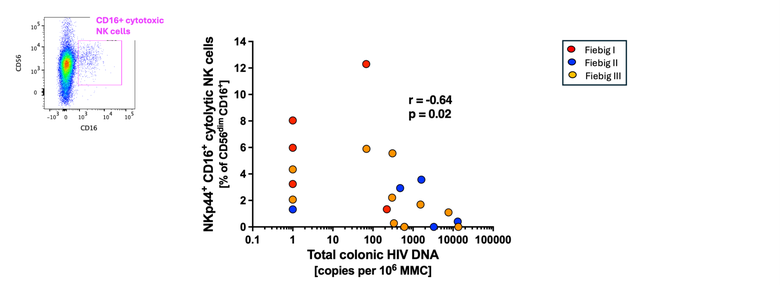
- Mediating direct elimination of HIV-infected target cells through perforin- and granzyme-dependent cytotoxicity *(Voskoboinik, Nat Rev Immunol, 2015)*
- Mediating Antibody-dependent cellular cytotoxicity *(ADCC; Iannello, J Leukoc Biol, 2008)*
- Rapid producers of pro-inflammatory cytokines, including IFN-γ and TNF-α, promoting antiviral immunity and activation of macrophages and dendritic cells *(Vivier, Nat Immunol, 2008)*

Regulatory and cytotoxic colonic NK cells are decreased as early as Fiebig I during AHI



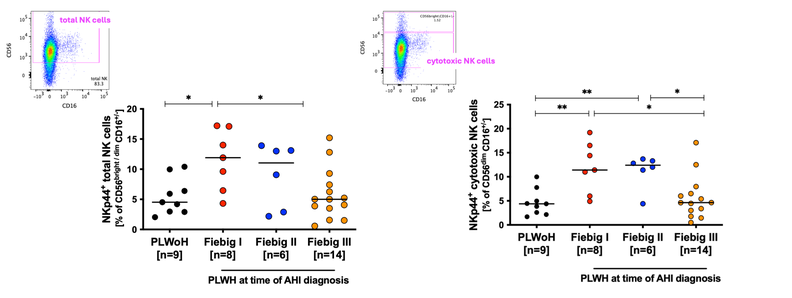
➢ Early decrease of colonic NK cells during AHI occurs in the regularoty and cytolytic NK cell subsets

Reduced frequencies of activated NKp44⁺ CD16⁺ cytotoxic effector NK cells are associated with increased total colonic HIV DNA



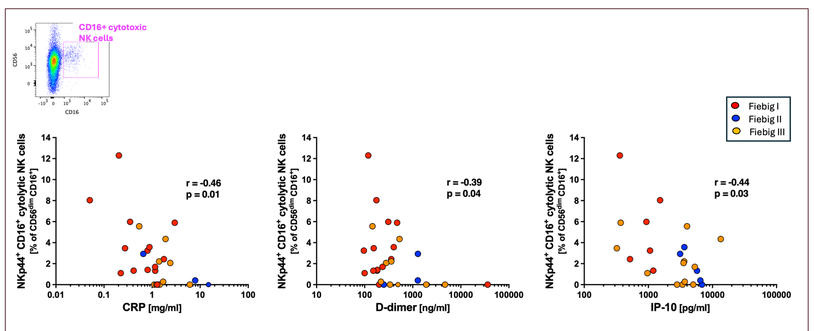
➢ The inverse association with total colonic HIV DNA suggests that activated NKp44⁺ CD16⁺ cytotoxic NK cells may contribute to limiting the mucosal HIV reservoir.

Increase in activated NKp44⁺ NK cells occurs during Fiebig I/II predominantly in the cytotoxic NK cell subset



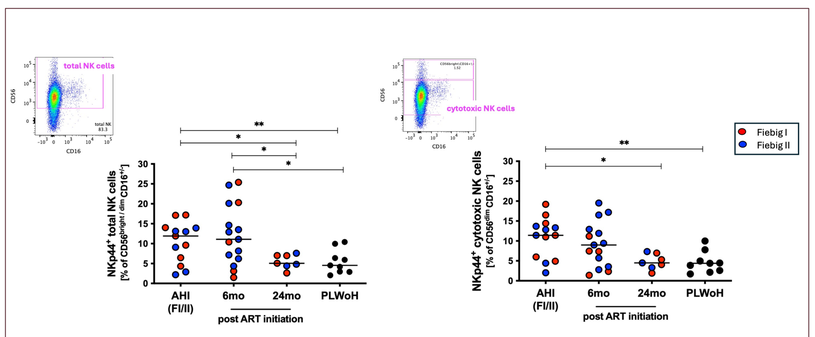
➢ Transient activation of cytotoxic colonic NK cells is observed during Fiebig I/II and returns to levels comparable to PLWoH by Fiebig III.

Decrease in activated NKp44⁺ CD16⁺ cyotoxic effector NK cells is associated with systemic inflammation



➢ The association between depletion of activated NKp44⁺ CD16⁺ cytotoxic NK cells and increased systemic inflammation suggests that these cells may contribute to maintaining mucosal immune homeostasis during acute HIV infection.

Activated NKp44⁺ cytotoxic NK cells remain elevated for up to 6 months following early ART initiation



➢ Following early ART initiation during Fiebig I/II, activated cytotoxic colonic NK cells remain elevated for up to 6 months before returning to levels comparable to PLWoH after 24 months

Summary

- Early acute HIV infection is characterized by profound disruption of the mucosal NK cell compartment
- Activated cytotoxic NK cell responses in the mucosa appear to be transient and normalize following prolonged ART
- Early loss of activated NKp44⁺ CD16⁺ cytotoxic effector NK cells is associated with increased colonic HIV DNA and systemic inflammation

Conclusion

These findings highlight the potential role of mucosal innate immune dysregulation in early HIV persistence and systemic inflammation and provide a rationale for further investigation of mucosal NK cells in HIV prevention and cure strategies.

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