Revealing the Role of Tissue-Resident NK Cells in HIV Infection Using Tissue Explant Models

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445

BACKGROUND

Although **NK cells** are essential in controlling HIV infection, their **role in the main tissues where HIV persists**, such as the **secondary lymphoid tissue** and the **gastrointestinal tract**, is largely undefined. Here, we present an examination of NK cells in these relevant tissues, emphasizing their **memory-like and residency properties**, and **specific subset functionalities** in **killing HIV-infected cells** in a **tissue model**.

HYPOTHESIS & AIMS

Tissue-resident memory (TRM) NK cells are important in controlling HIV replication

1. What is the **phenotype** of tissue-resident memory (TRM) NK cells in human tissues?
2. Which NK cell populations stand out in **controlling HIV infection** in human tissues?

RESULTS. TRM fingerprints in tonsil and gut NKs

- **Predominantly cytokine-producers (CD56+ CD16-)**
- **Cytotoxic CD16+ subset** exhibits a **strong imprint of resident memory** NK cells
- **CD49a enriches for memory**-like resident NK population

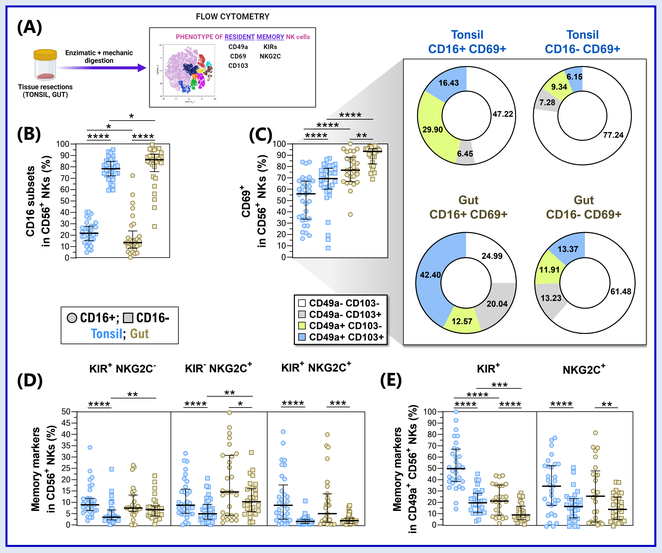


FIG 1| TRM phenotype of tonsil and gut NK cells. (A) Fresh human tissue samples were processed to characterize NK cells by flow cytometry. Expression of CD16 **(B)**, resident **(C)** and memory **(D, E)** markers on CD56+ tissue NK cells. *Median with interquartile range. Wilcoxon matched-pairs signed rank test. Significant: p*.*

RESULTS. Functionality of TRM NK cells

- **Similar basal activation levels** (% CD107a+) on tonsil- and gut-resident NK cells
- **K562 cells stimulate IFN-γ in CD49a+ NK cells coexpressing memory** markers ONLY in **tonsil**
- **Gut NK cells** were **hypofunctional** in this assay
- **Extracellular matrix** components **may confer distinct functions** to tonsil-resident NK subsets

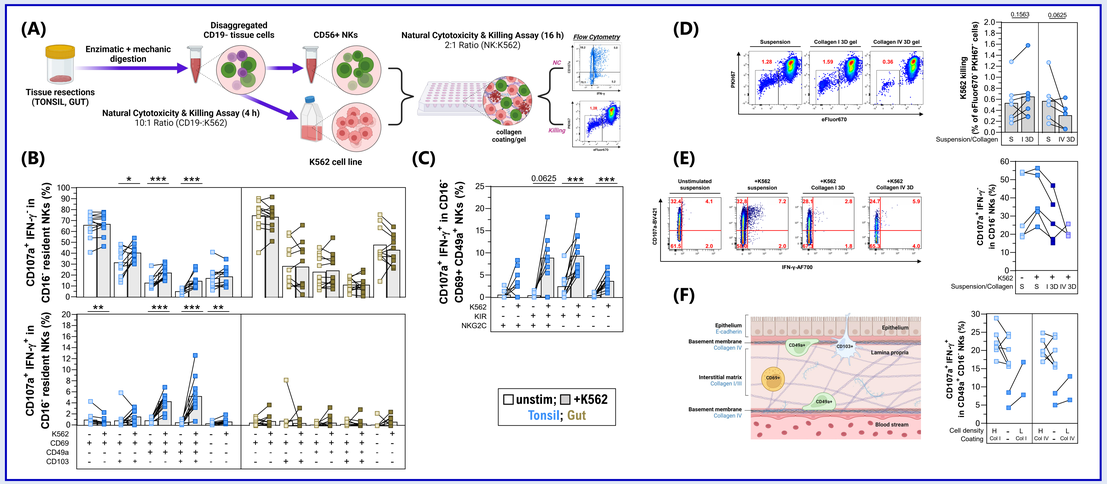


FIG 2| Functionality of tonsil and gut NK cells. (A) Experimental setting for the functional assay. Proinflammatory (IFN-γ+) and cytotoxic degranulating (CD107a+) **(B)** resident and **(C)** memory NK subsets. Effects of extracellular matrix (collagen) on **(D)** tonsillar NK cell-mediated killing of K562 targets, **(E)** degranulation and **(F)** IFN-γ. *Wilcoxon matched-pairs signed rank test. Significant: p*.*

RESULTS. TRM NK cells controlling HIV replication

- **CD69+CD49a-CD103- KIR+ tonsillar NK cells** correlate with reduced *ex vivo* HIV infection
- Higher frequency of **CD16- CD103+ NKG2C+ gut NK cells** after infection indicate lower HIV levels
- Specific tissue-resident NK subsets are represented in similar frequencies as CD4+ T subsets
- **Total KIR+ NK cell fraction had lower HIV-killing capacity** than KIR- counterpart in tonsils
- **CD69+CD49a-CD103-** tonsillar NK cells shown the **highest** anti-HIV activity and **CD103+** the **lowest**

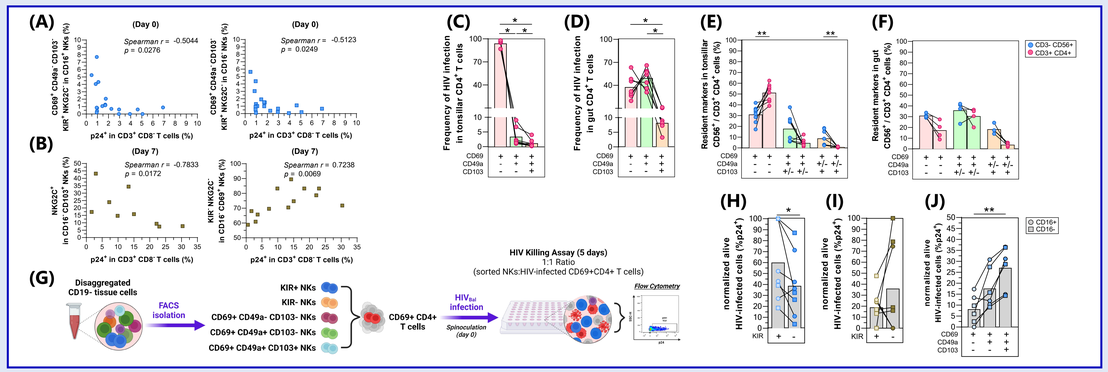


FIG 3| TRM NK cells restrain HIV infection in a tissue model. Spearman correlations indicating specific tissue NK cells associated with lower HIV levels in **(A)** tonsils at day 0 and **(B)** gut 7 days after *ex vivo* infection. Frequency of HIV infection within **(C)** tonsillar and **(D)** gut TRM CD4+ T cells. Paired frequencies of **(E)** tonsillar and **(F)** gut TRM NK and CD4+ T subsets. **(G)** Experimental setting to determine HIV-killing capacity of distinct TRM NK subsets **(H-J)**. *Wilcoxon matched-pairs signed rank test. Significant: p*.*

RESULTS. TRM NK cell reshaping by HIV infection

- **Expansion** of **KIR+ CD16+ CD69+ CD49a+ CD103-** and **CD16- CD103+** NK cells was found in **tonsils** and **gut** after *ex vivo* **HIV infection**, respectively
- HIV infection rendered **CD69+ CD49a+** and **CD49a- CD16- NK subsets hyperreactive** to new target cells

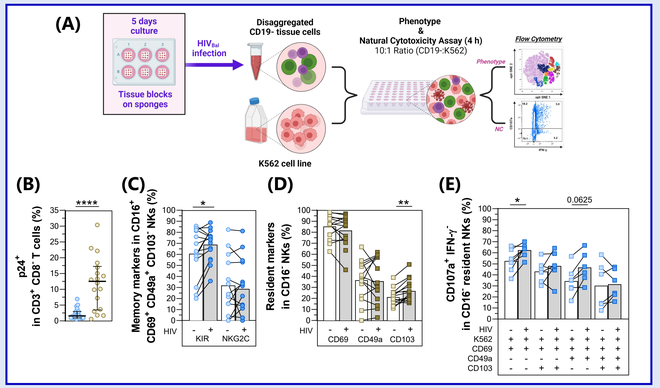


FIG 4| HIV infection alters the dynamics of TRM NK cells. **(A)** Experimental setting for the functional assay. **(B)** HIV *ex vivo* infection levels after 5 days in tonsil and 7 days in gut. Expression of memory markers on **(C)** CD16+ tonsil-resident NK cells and resident markers on **(D)** CD16- gut NK cells in the infected culture. **(E)** HIV increases the maximum degranulation capacity (% of CD107a+ IFN-γ- cells) of tonsil-resident CD16- NK cells. *Significant: p*.*

CONCLUSIONS

Expansion and/or boosting of resident memory NK cells in tissues may have an impact on reducing HIV persistence

- **CD16- KIR+ CD49a+ CD69+ CD103+ tonsillar NK cells** exhibit **high stimulatory potential**, while gut NK cells are unresponsive
- **HIV** infection **increased intraepithelial** (CD103+) **gut CD16- NK cells** and expanded **memory-like** (KIR+) **cytotoxic** (CD16+) **CD49a+ tonsillar** NK cells
- **Tonsil CD16- CD69+ CD49a- CD103- NK cells** outperformed **reducing HIV levels** and the infection boosted their cytotoxicity
- Expression of **KIR** in **tonsils**, and **NKG2C** in the **gut**, define resident memory NK cells correlating with **HIV control**

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