CHARACTERIZATION OF TISSUE-RESIDENT NK CELLS IN A TISSUE MODEL OF HIV INFECTION

David Perea[1], Nerea Sánchez-Gaona[1], Ana Gallego-Cortés[1], Stefania Landolfi[2], Félix Pumarola[3], Núria Ortiz[3], Juan Lorente[3], Vicenç Falcó[4], Meritxell Genescà[1] and María J. Buzón[1]

[1]HIV Translational Laboratory, Infectious Diseases Department, Vall d'Hebron Research Institute (VHIR), Autonomous University of Barcelona, Barcelona, Spain; [2]Department of Pathology, Vall d'Hebron University Hospital, Barcelona, Spain; [3]Department of Otorhinolaryngology, Vall d'Hebron University Hospital, Barcelona, Spain; [4]Infectious Diseases Department, Vall d'Hebron University Hospital, Barcelona, Spain

338

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 have characterized NK cells present in relevant tissues, particularly their **memory-like and residency properties**, and assessed their **functionality** in killing HIV-infected cells in a **tissue model**.

HYPOTHESIS & AIMS

Tissue resident memory NK cells are important in controlling HIV replication

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

METHODS

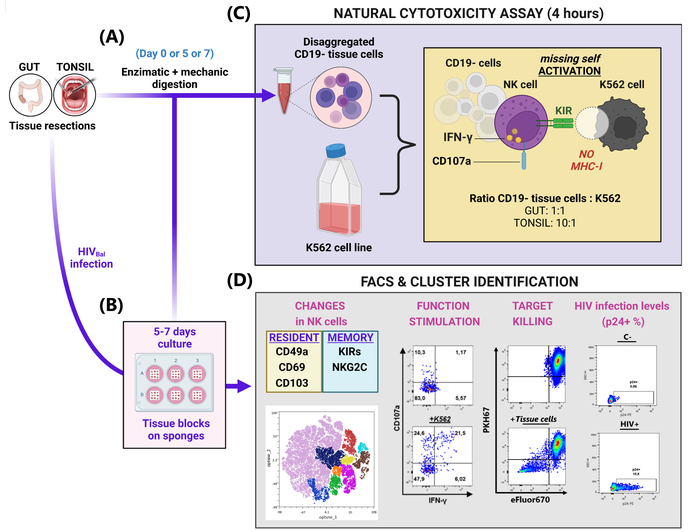

FIG 1| Workflow of the phenotyping and functional capacity determination of tissue NK cells. Human tissue resections from routine surgeries were minced into small pieces. **(A)** Part of the tissue was used for the functional assay at day 0 or **(B)** *ex vivo*-infected with HIV_{Bal} on sponges for 5-7 days in culture. Then, the infected tissue blocks were digested and processed as fresh tissue at day 0 and the same functional assay was performed. For the functional assay **(C)** to determine the natural cytotoxicity of tissue NK cells, CD19- tissue cells were cocultured with HLA-null K562 cells for 4h at 37 ºC. **(D)** Tissue resident memory NK cells were characterized by flow cytometry, as well as their functional capacity, HIV infection levels reached in the cultured tissues, and K562 target-killing ability. Dimensionality reduction analysis was applied to identify tissue NK cell clusters.

RESULTS. TRM fingerprints in tonsil and gut NK cells and reshaping by HIV infection

- Most tonsil and gut NK cells are **CD56+ CD16-**
- **CD16+ subset** more frequently expresses memory and residency markers

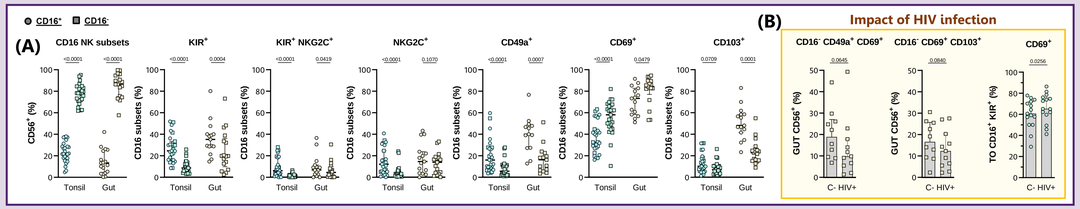

FIG 2| TRM phenotype of tonsil and gut NK cells. (A) Scatterplots of resident and memory markers expression on CD16+/- subsets of CD56+ tissue NK cells, and **(B)** impact of HIV *ex vivo* infection on tissue NK cell populations. *Median with interquartile range. Wilcoxon matched-pairs signed rank test. Circles: CD16+, squares: CD16-. Significant: p<0.05*

- High numbers of NK cells expressing **at least one residency marker** in both tissues
- **Major CD16- CD69+ population** in both tissues (**#1, #2, and #3**)
- **#4** resembling **TRM NKs** (CD16- KIR+ CD49a+ CD69+ CD103+) is **more abundant in gut**
- **#5, #7, and #8** NK cell clusters are **more abundant in tonsils** and show marked **NKG2C** expression

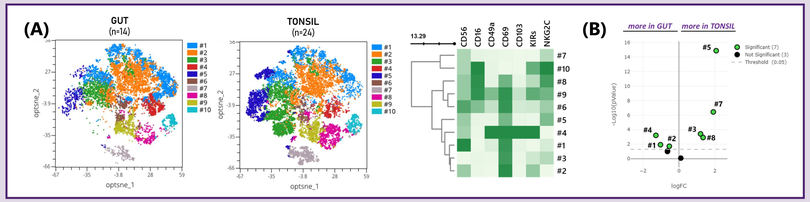

FIG 3| NK cell distribution in tonsil and gut. (A) Optimized-t-Distributed Stochastic Neighbor Embeddings (opt-SNEs) showing the abundance of NK cell clusters (#) in tonsil and gut. **(B)** Volcano plot comparing their abundances

RESULTS. Functional potential of tissue NK cells and reshaping by HIV infection

- **CD107a basal production** prevails in CD16- clusters with **high CD69 expression** (**tonsil #2; gut #2, #9, and #12**)
- Gut NK cells are less stimulable than tonsil counterpart; **tonsil #1** (CD16- KIR+ CD49a+ CD69+ CD103+) **increases** the CD107a and IFN-γ production, while **gut #1** (NKG2C+) the IFN-γ production

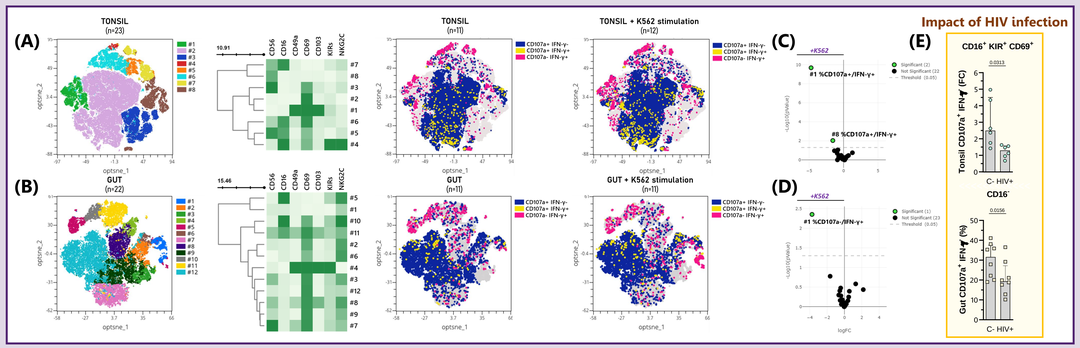

FIG 4| Basal function of tonsil and gut NK cells and HIV reshaping. Opt-SNEs showing the expression of functional markers (CD107a and IFN-γ) on identified **(A)** tonsil and **(B)** gut NK cell clusters at basal day 0. Volcano plots of the fold-change on the frequency (%) of CD107a and IFN-γ expression on identified **(C)** tonsil and **(D)** gut NK cell clusters. **(E)** Impact of HIV infection on the functionality of tissue NK cells. *Median with interquartile range. Wilcoxon matched-pairs signed rank test. Significant: p<0.05*

RESULTS. Tissue NK cells controlling HIV replication

- **Higher basal frequencies of tonsil TRM NKs** correlate with **better control of HIV** in *ex vivo*-infected tissues
- An **increased killing of K562** targets tends to be associated to **higher production of CD107a and IFN-γ** in **tonsil CD16- KIR+ resident NK cells**
- A **higher frequency of memory NK cell populations** (KIR+/- NKG2C+) found **after gut and tonsil *ex vivo* infection** is associated to **reduced HIV infection**

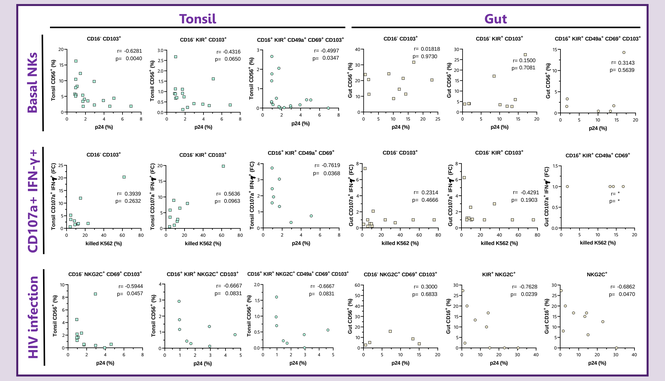

FIG 5| Specific tissue NK cells in the control of HIV infection and the killing of K562 targets. *Spearman correlation. Significant: p<0.05*

CONCLUSIONS

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

- **TRM** NK cells are **more abundant in gut**
- CD16- KIR+ CD49a+ CD69+ CD103+ tonsil NKs **(#1)** exhibit **high stimulatory potential**, while gut NK cells are more unresponsive
- **In tonsil, higher basal function and expansion of TRM NK cells** correlate with **better control of HIV** replication
- **HIV alters some specific NK cell populations** in both tissues, expanding or lowering their stimulatory potential
- **Tonsil CD16- KIR+ resident NK cells** outperformed **reducing HIV levels** in our tissue model and killing K562 targets
- High frequency of **tonsil CD16- CD103+** NKs may be important in the setting of HIV infection, while **abundant CD16+ KIR+ NKG2C+ CD103+** NKs stand out **after 5 days of infection**

ACKNOWLEDGEMENTS

Funding. The project has received funding from the Spanish Secretariat of Science and Innovation and FEDER funds (RTI2018-101082-B-100 and PID2021-123321OB-I00) and from "la Caixa" Foundation (ID 100010434). D.P. is supported by the VHIR Fellowship program and M.B. is supported by the Miguel Servet program funded by the Spanish Health Institute Carlos III (CP17/00179 and CPII22/00005)
Acknowledgments. VHIR's flow cytometry technician Dr. Joan P. Ortiz
Corresponding author address. mariajose.buzon@vhir.org