Engineering Natural Killer Cells With NKp30-Based Chimeric Antigen Receptors (CARs) to Target HIV

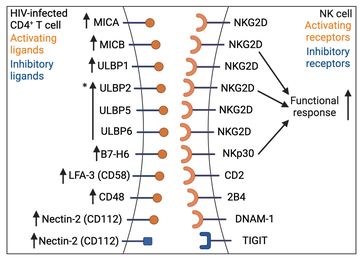
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BACKGROUND

Natural killer (NK) cells respond rapidly in early HIV infection. The function of NK cells is regulated through the interaction between NK cell receptors and their cognate ligands on target cells. In our previous work, we identified that B7-H6 (ligand of NKp30), MICB and ULBP2 (ligands of NKG2D) on CD4⁺ T cells facilitates the functional response of NK cells to autologous HIV-infected CD4⁺ T cells[1].

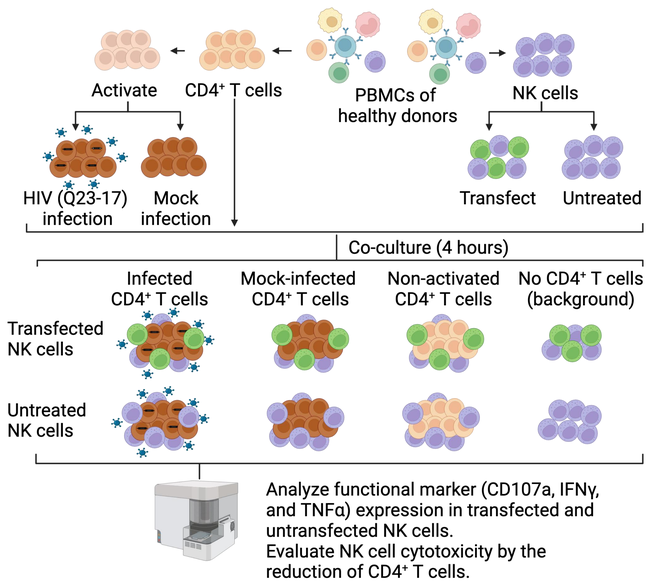


*The anti-ULBP2 flow cytometry antibody cross-reacts with ULBP5 and ULBP6.

We *hypothesized* that HIV-targeting function of NK cells can be enhanced by overexpressing NKp30 and NKG2D and further improved by NKp30 and NKG2D-based CARs.

METHODS

NK cells were transfected with mRNA complexed with charge-altering releasable transporter (CART) to overexpress NKp30, NKG2D and NKp30-based chimeric antigen receptors (CARs), and co-cultured with CD4⁺ T cells. Functional markers in NK cells and cytotoxicity on target CD4⁺ T cells were evaluated.

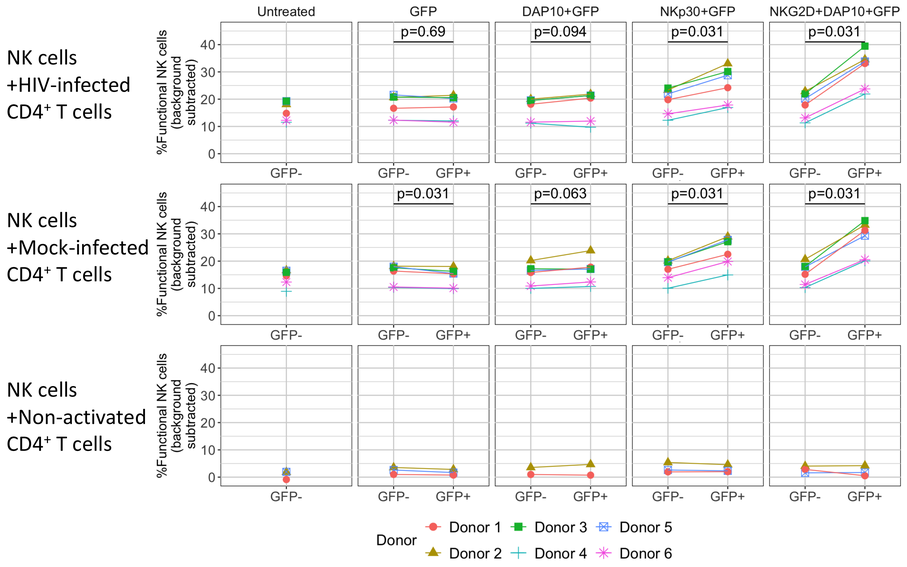


MAIN FINDING

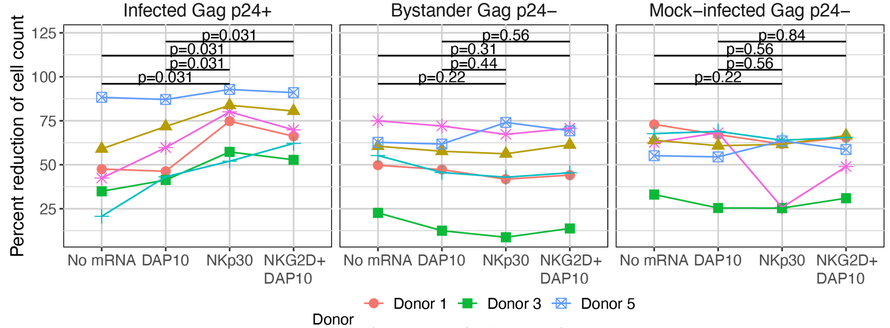
- **Overexpression of NKp30 and NKG2D enhances NK cell functional responses to CD4⁺ T cells and HIV-specific cytotoxicity.**
- **Expression of NKp30-based CARs outperformed unmodified NKp30 in elevating the function of NK cells.**

RESULTS

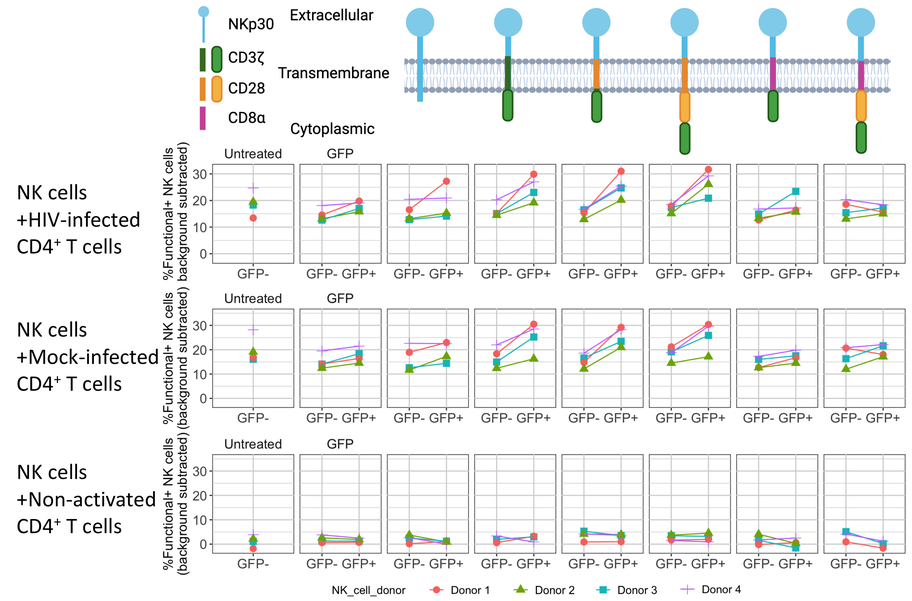
Overexpression of NKp30 and NKG2D Enhances the Expression of Functional Markers in NK Cells in Response to autologous CD4⁺ T Cells.



Overexpression of NKp30 and NKG2D Enhances HIV-Specific NK Cell Cytotoxicity on autologous CD4⁺ T Cells.



NKp30-Based CARs Enhances the Functional Response of NK Cells on allogeneic CD4⁺ T Cells.



CONCLUSIONS

- Together with our previous work, the results of our study indicate that receptor-ligand pairs including NKp30:B7-H6 and NKG2D:MICB/ULBP2 facilitate NK cell recognition of HIV-infected cells.
- Overexpression of NKp30 and NKG2D enhances NK cell functional response to both HIV-infected and mock-infected CD4⁺ T cells but significantly enhances cytotoxicity specifically to HIV-infected CD4⁺ T cells.
- Primary NK cells can be engineered with NKp30-based CARs for improved function of targeting HIV.
- Our work suggests that NK cell activation through NKp30 and NKG2D can be exploited for designing strategies of cell therapy to treat HIV infection.

ACKNOWLEDGEMENTS

This work was supported by the National Institutes of Health (NIH DP1 DA046089 and NIH R01 AI161803, C.A.B.), a pilot project through the Bill & Melinda Gates Foundation (OPP1113682, C.A.B.), and the National Science Scholarship from A*STAR Singapore (N.Q.Z.). The Marson lab support includes funding from NIH the HIV Accessory & Regulatory Complexes (HARC) Center (P50 AI150476), NIH/NIAID P01AI138962, Gilead, and The Simons Foundation. We thank Drs. Paul Wender, Gillian Sun and Harrison Rhan in Stanford University for providing the CART ONA used in this work. We thank Dr. Kristiaan Thielemans in the Vrije Universiteit Brussel, and Drs. Ronald Levy and Julian Lohmeyer in Stanford University for providing the plasmid for the preparation of mRNA used in this work. In addition, we thank the Human Immune Monitoring Core (HIMC) at Stanford University for the use of their Helios mass cytometer.

REFERENCE

1. NKp30 and NKG2D contribute to natural killer recognition of HIV-infected cells. Nancy Q. Zhao, Ruoxi Pi, David N. Nguyen, Thanmayi Ranganath, Christof Seiler, Susan Holmes, Alexander Marson, Catherine A. Blish. BioRxiv. 2024.

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