Balancing Activation and Inhibition: CIML NK Cell Responses to JC Polyomavirus

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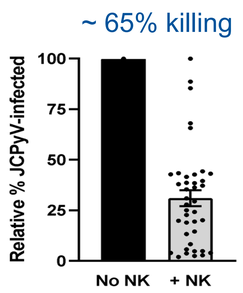
Background

JCPyV reactivation causes PML

- The human polyomavirus JCPyV is an opportunistic, double-stranded DNA virus infecting over 50% of humans worldwide. This virus characteristically establishes asymptomatic, persistent, and latent infection in the host.
- JCV has been described as a ubiquitous virus that has co-evolved wit the human population over time
- Latent JCV reactivates with immunosuppression to attack glial cells, leading to Progressive Multifocal Leukoencephalopathy (PML) in immunocompromised patients

Human Natural Killer (NK) cells target JCPyV-infected cells

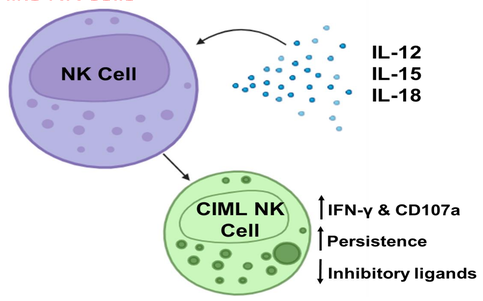
- NK cell response to JCPyV is detected in peripheral blood and extends beyond T cell response
- NK cells kill JCPyV-infected SVGA cells *in vitro*
- Antigen specific or memory-like NK cell response to JCPyV
- NK cell modulation as novel immunotherapy for PML



CIML NK Cells: A Strategy to Boost Antiviral Responses

Cytokine-induced memory-like NK cells

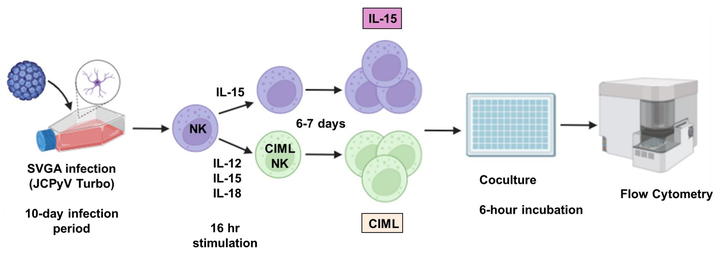
- long-lived memory cells post infection that are highly activated
- Cytokines (IL-12/15/18) mimic this memory-like phenotype



Research Aims: Enhancing NK Cell Function Against JCPyV

1. Characterize the functional advantages of cytokine-induced memory-like (CIML) NK cells against JCPyV-infected targets.
2. Define exhaustion and checkpoint receptor expression in CIML NK cells.
3. Determine how JCPyV infection modulates NK cell receptor–ligand interactions.

Methods



Results

CIML NKs Show Enhanced Functional Recall

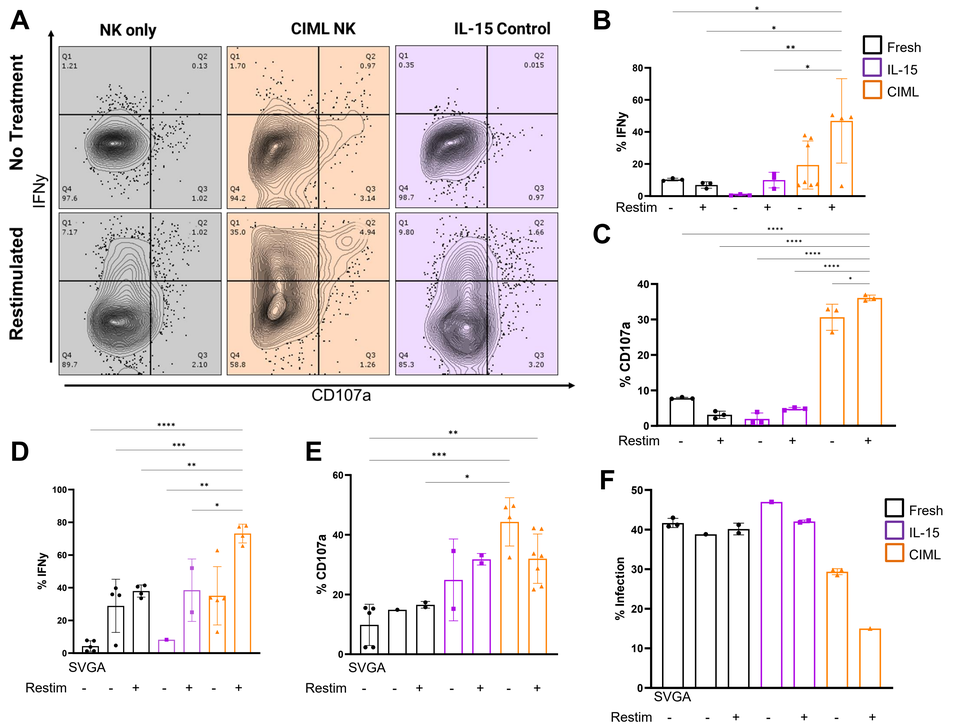


Figure 1. Assessment of NK cell activation and cytotoxicity. (**A-C**) NK cells were isolated, stimulated with cytokines, and assessed for their activation status using IFNγ and CD107a. NK cells were put into cocultured with infected and uninfected SVGA cells and assessed for **(D)** infectivity, and (**E-F**) activation status was measured by flow cytometry. One-way ANOVA. Result of 3 independent experiments

CIML NKs susceptible to exhaustion or checkpoint inhibition

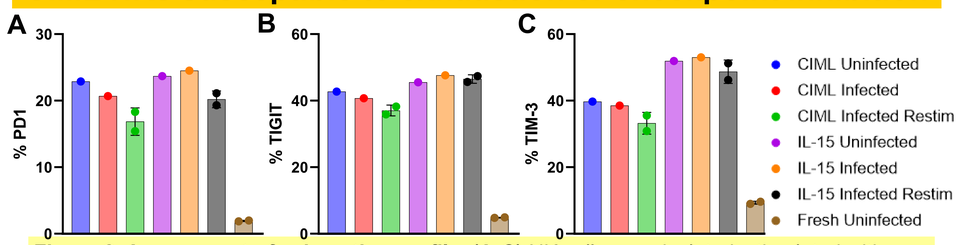
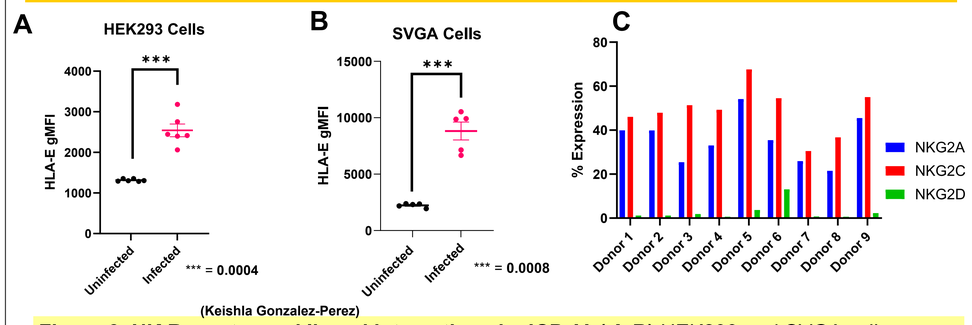


Figure 2. Assessment of exhaustion profile. (A-C) NK cells were isolated, stimulated with cytokines, and assessed for their levels of exhaustion using PD-1, TIGIT, and TIM-3, using fresh, unstimulated NK cells as controls.

JCPyV infection alters NK cell receptor-ligand interactions

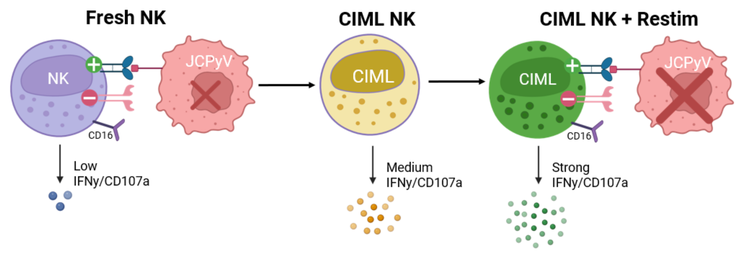


(Keishla Gonzalez-Perez)

Figure 3. NK Receptor and ligand interactions in JCPyV. (A-B) HEK293 and SVGA cells were infected with JCPyV and assessed or their expression level of HLA-E. (**C**) PBMCs from 9 healthy donors were collected and analyzed for their baseline expression of NKG2A, NKG2C, and NKG2D. Paired t test.

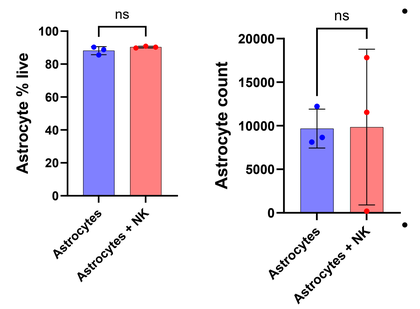
Conclusions

- CIML NK cells show enhanced IFNγ production, degranulation, and antiviral activity compared to Fresh and IL-15 NKs.
- JCPyV infection upregulates HLA-E
- NK receptor profiles vary between donors, potentially shaping JCPyV responses
- CIML NKs show elevated exhaustion marker expression, which may reflect high activation rather than true functional exhaustion



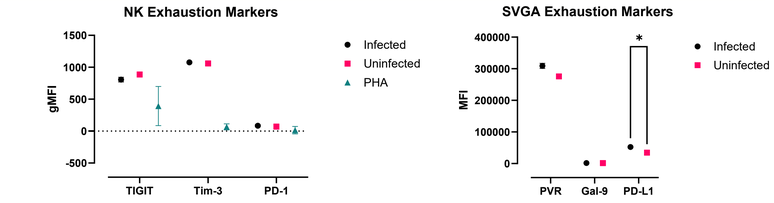
Future Directions

Enhancing CIML NK Function Through Restimulation



- Increase "n" for CIML NK and do restimulation experiments
  - Fresh NKs = muted
  - CIMLs = better
  - CIML + Restim = optimal, with enhanced degranulation, IFN-γ, and better killing of infected targets
- CIML NK experiments with primary cells (Astrocytes)

Explore NK Cell Exhaustion



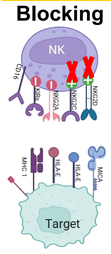
Each data point n=3
No significance across data sets.

Each data point n=3
* = 0.000599

(Allison Rux)

Map out mechanistic checkpoints that control JCPyV-specific NK cell responses

Blocking



- NKG2D, NKG2A, and NKG2C are key regulators of JCPyV-specific NK responses
- 27-color flow will define baseline expression across 15 healthy donors
- Blocking experiments will clarify receptor-specific contributions to cytotoxicity

Acknowledgements

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