Modulation of Natural Killer Cell Activity By Simian Immunodeficiency Virus Peptides

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Introduction
Natural killer (NK) cells recognize and kill virus-infected cells without prior antigenic stimulation. The recognition is achieved partly through the disruption of interactions between inhibitory class I MHC molecules and inhibitory NK-cell receptor ligands on virus-infected cells. Interactions between inhibitory KIRs and their MHC class I ligands are often influenced by MHC class I-bound peptides.1 The binding of a common chain may stabilize MHC class I molecules. Mann et al.2 (A1*002, A1*003) in murine KIRs (KIRDS05) is stirred by certain SIY peptides, but not others. Here we use a cytotoxic assay with primary NK cells and peptide-pulsed target cells to interrogate this stabilization and demonstrate that SIY peptides present on MHC class I can modulate the cytolytic activity of HLA-restricted NK-cell populations. Our results suggest that immunodeficiency viruses may acquire changes in epitopes that increase the avidity of MHC class I ligands for inhibitory KIRs as a mechanism of immune evasion.

Materials and Methods
TAP-Inhibited 721.221-A1*002 Cells
Cells pulsed with 5 µM of peptide at 37°C overnight. Storing with 4°C for 48 h allows the equal distribution of A1*002 expression by an A1*002 binding epitope in comparison to peptide and non-binding controls.

Primary KIRDS05 Sorted NK Cells
KIRDS05 and KIR3DL05 subsets of primary NK cells were isolated using GY9 tetramers to stain KIRDS05 and separating cells by FACS into CD3−, NKGA−, KIR3DL05+ or− subsets. Sorted subsets were expanded on K562 cell IL21 feeder cells.

Calcein AM Cytotoxicity Assay

Results

SIV Epitopes Affect NK Cell Activity

TAP-inhibited 721.221-A1*002 cells were pulsed overnight with two inhibitory peptides (between 12.5 µM and 50 µM) of the indicated peptides so that each peptide stabilized A1*002 surface expression to approximately the same level as assessed by staining with an anti-MHC class I antibody M4-71. Pulsed cells were then used as target cells in a 4-h, 5173 γ-irradiated cytotoxicity assay with KIRDS05− NK cells at a 1:1 E:T ratio. SIY peptides are shown in order of A1*002 binding affinity. Data compiled from three independent experiments. *p<0.05, **p<0.01, ***p<0.001 by two-way ANOVA. Error bars indicate ±SD.

C-Terminal Peptide Residues Critical

Inhibitory Peptide Dominant

Target cell line presenting a specific MHC class I peptide complex.

Primary KIR3DS05 Sorted NK Cells
KIRDS05 and KIR3DL05 subsets of primary NK cells were isolated using GY9 tetramers to stain KIRDS05 and separating cells by FACS into CD3−, NKGA−, KIR3DS05+ or− subsets. Sorted subsets were expanded on K562 Clone 9.mbIL21 feeder cells.

Calcein AM Cytotoxicity Assay

Dilution of NY9 at Constant Concentration of GY9 8W

In this study, we have:

- Developed a method for interrogating the role of MHC class I-bound peptides in modulating interactions with KIR
- Identified 27 A1*002-binding SIY peptides that facilitate interaction with KIRDS05
- Characterized peptide residues 6 through 9 as important for ability to facilitate or disrupt the A1*002-KIRDS05 interaction
- Demonstrated that the effect of an inhibitory peptide, GY9, is dominant over the effect of a non-inhibitory peptide, GY9 8W, even when present at a small fraction of total peptide concentration

Literature Cited


Acknowledgements
We are grateful to Emmanuel Wietz at University Medical Center Utrecht for providing the 721.221 EMK-pulsed cell line. We also thank Shawn Lee from the University of Texas for providing the K562 Clone 9.mbIL21 cells. This work was supported by NIH Public Health Service grants AI050884 and AI041089. Additional support was provided by PHS grants AI051100 and AI010849.