Modulation of Natural Killer Cell Activity By Simian Immunodeficiency Virus Peptides

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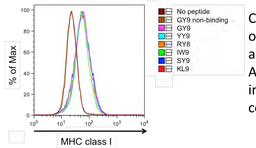
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Introduction

Natural killer (NK) cells recognize and kill virus-infected cells without prior antigenic stimulation. This recognition is achieved in part through the disruption of interactions between inhibitory killer-cell immunoglobulin-like receptors (KIRs) on NK cells and their MHC class I ligands on virus-infected cells. Interactions between inhibitory KIRs and their MHC class I ligands are often influenced by MHC class I-bound peptides[1-7]. The binding of a common rhesus macaque MHC class I molecule, Mamu-A1*002 (A1*002), to Mamu-KIR3DL05 (KIR3DL05) is stabilized by certain SIV peptides, but not others[8]. Here we use a cytotoxicity assay with primary NK cells and peptide-pulsed target cells to interrogate this stabilization and demonstrate that viral peptides presented by MHC class I can modulate the cytolytic activity of KIR-expressing 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



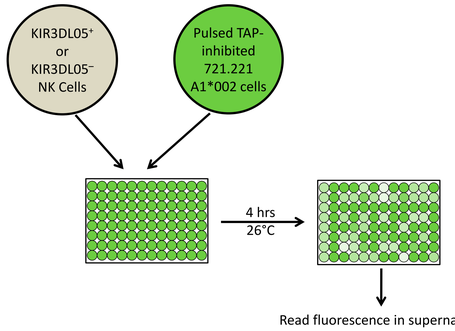
Cells pulsed with 25 uM of peptide at 26°C overnight. Staining with W6/32 pan MHC class I antibody confirms equivalent stabilization of A1*002 expression by six A1*002-binding epitopes in comparison to no peptide and non-binding controls.

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

Primary KIR3DL05 Sorted NK Cells

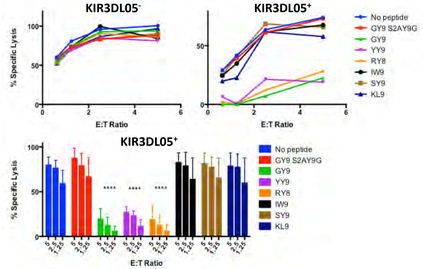
KIR3DL05$^+$ and KIR3DL05$^-$ subsets of primary NK cells were isolated using GY9 tetramer to stain KIR3DL05 and separating cells by FACS into CD3$^-$, NKG2A$^+$, KIR3DL05$^+$ or $^-$ subsets. Sorted subsets were expanded on K562 Clone 9.mbIL21 feeder cells[9].

Calcein AM Cytotoxicity Assay



Results

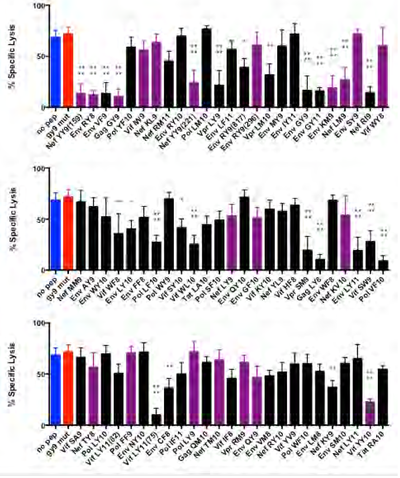
SIV Epitopes Affect NK Cell Activity



SIV Epitopes GY9, YY9, and RY8 inhibit KIR3DL05$^+$ NK cells when presented by A1*002

TAP-inhibited 721.221-A1*002 cells were pulsed overnight with indicated peptides and used as targets in a calcein AM cytotoxicity assay. Graph on bottom compiles results obtained in three independent experiments. ****p<0.001 by two-way ANOVA. Error bars indicate +1 SD.

A1*002 SIV Peptides

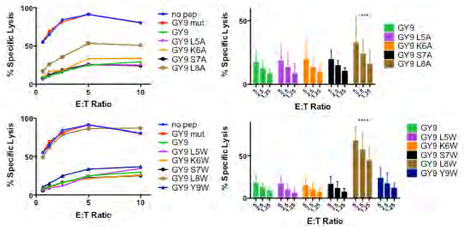


TAP-inhibited 721.221-A1*002 cells were pulsed overnight with between 1.25 µM and 50 µM of the indicated peptides so that each peptide stabilized A1*002 surface expression to approximately the same level as assessed by staining with an anti-MHC class I antibody W6/32. Pulsed cells were used as target cells in a calcein AM cytotoxicity assay with KIR3DL05+ NK cells at a 5:1 E:T ratio. SIV 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 ANOVA. Error bars indicate +1 SD.

Identification of SIV peptides bound by A1*002 that suppress the cytolytic activity of KIR3DL05$^+$ NK cells

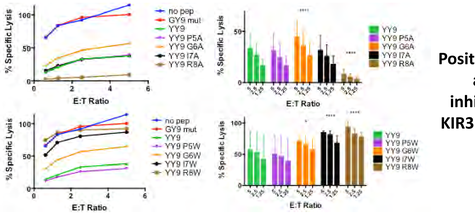
C-Terminal Peptide Residues Critical

GY9 Variants



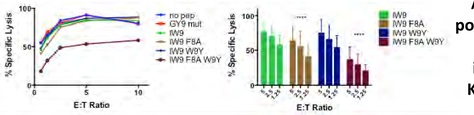
Position 8 is critical for GY9 inhibition of the KIR3DL05$^+$ NK cells

YY9 Variants



Positions 6, 7 and 8 affect YY9 inhibition of the KIR3DL05$^+$ NK cells

IW9 Variants

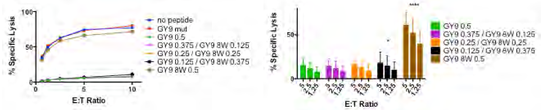


Alteration of positions 8 and 9 allows IW9 inhibition of KIR3DL05$^+$ NK cells

TAP-inhibited 721.221-A1*002 cells were pulsed overnight with the indicated peptides and used as target cells in a calcein AM cytotoxicity assay with KIR3DL05+ NK cells. Graph on right compiles data from three independent experiments. *p<0.05, ***p<0.005, ****p<0.001 by two-way ANOVA. Error bars indicate +1 SD.

Inhibitory Peptide Dominant

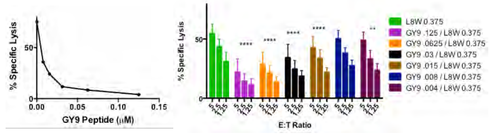
GY9/GY9 8W Peptide Ratios



The inhibitory peptide GY9 is dominant over non-inhibitory GY9 8W

TAP-inhibited 721.221-A1*002 cells were pulsed overnight with the indicated peptides at a total concentration of 0.5 µM and used as target cells in a calcein AM cytotoxicity assay with KIR3DL05+ NK cells. Graph on bottom compiles data from three independent experiments. *p<0.05, ****p<0.001 by two-way ANOVA. Error bars indicate +1 SD.

Dilution of GY9 at Constant Concentration of GY9 8W



At low concentrations in a peptide mixture with GY9 8W, GY9 can inhibit KIR3DL05$^+$ NK cells

TAP-inhibited 721.221-A1*002 cells were pulsed overnight with a mixture of 0.375 µM of GY9 8W and the indicated concentration of GY9. Pulsed cells were used as targets in a calcein AM cytotoxicity assay with KIR3DL05+ NK cells. Graph on left representative of results obtained in three independent experiments, which are summarized on right. **p<0.01, ****p<0.001 by ANOVA.

Conclusions

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 SIV peptides that facilitate interaction with KIR3DL05
- Characterized peptide residues 6 through 9 as important for ability to facilitate or disrupt the A1*002-KIR3DL05 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

[1] Malnati MS, et al. (1995) Peptide specificity in the recognition of MHC class I by natural killer cell clones. Science 267:1016–1018.
[2] Zappacosta F, Borrego F, Brooks AG, Parker KC, Coligan JE (1997) Peptides isolated from HLA-Cw*0304 confer different degrees of protection from natural killer cell-mediated lysis. Proc Natl Acad Sci USA 94:6313–6318.
[3] Maenaka K, et al. (1999) Killer cell immunoglobulin receptors and T cell receptors bind peptide-major histocompatibility complex class I with distinct thermodynamic and kinetic properties. J Biol Chem 274:28329–28334.
[4] Stewart CA, et al. (2005) Recognition of peptide-MHC class I complexes by activating killer immunoglobulin-like receptors. Proc Natl Acad Sci USA 102:13224–13229.
[5] Thananchai H, et al. (2007) Cutting Edge: Allele-specific and peptide-dependent interactions between KIR3DL1 and HLA-A and HLA-B. J Immunol 178:33–37.
[6] Fadda L, et al. (2010) Peptide antagonism as a mechanism for NK cell activation. Proc Natl Acad Sci USA 107: 10160–10165.
[7] Fadda L, Korner C, et al. (2012) HLA-Cw*0102-restricted HIV-1 p24 epitope variants can modulate the binding of the inhibitory KIR2DL2 receptor and primary NK cell function. PLoS Pathog 8(7): e1002805.
[8] Colantonio AD, et al. (2011) KIR polymorphisms modulate peptide-dependent binding to an MHC class I ligand with a Bw6 motif. PLoS Pathog 7(3): e1001316.
[9] Denman CJ, et al. (2012) Membrane-bound IL-21 promotes sustained ex vivo proliferation of human natural killer cells. PLoS ONE 7(1): e30264.

Acknowledgements

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