Targeted Acidosis Mediated Delivery of Antigenic MHC-Binding Peptides

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Abstract

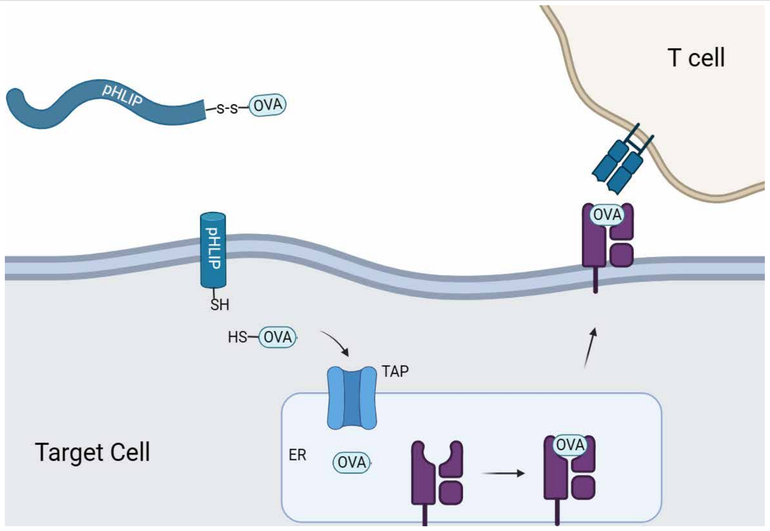
Objective

One of the immune system's critical mechanisms for eliminating cancer is to target cancer-specific peptide antigens presented on the major histocompatibility complex (MHC).[1] However, cancers with low mutational burdens often manage to avoid immune clearance due to the limited presence of cancer-specific peptide antigens.[2] The lack of immunogenic peptides for display on MHC to immune cells greatly hinders the ability of the immune system to recognize and eliminate cancer.[3] To counteract this mechanism of immune evasion, we aimed to:

- Specifically deliver antigenic MHC binding peptides to cancer cells
- Demonstrate improved immune activation against targeted cancer cells

Background

Targeted Delivery of Antigenic Peptide



pH(low) insertion peptides (pHLIP) selectively insert into the membrane of cells in acidic microenvironments – a hallmark of cancer biology.

SIINFEKL (OVA) is a model peptide antigen from the protein ovalbumin that readily binds to MHC and activates the immune system.

Disulfide release enables OVA to enter the antigen presentation pathway.

Presentation of OVA on MHC alerts T cells to activate a cytotoxic response

References

1) Schappert, A. et al. *Life Sci*. **2018.** 209: 255-258
2) Jardim, D. et al. *Cancer Cell*. **2021.** 39: 154-173
3) Zou, X. et al. *Front. Immunol*. **2021**. 12: 689076

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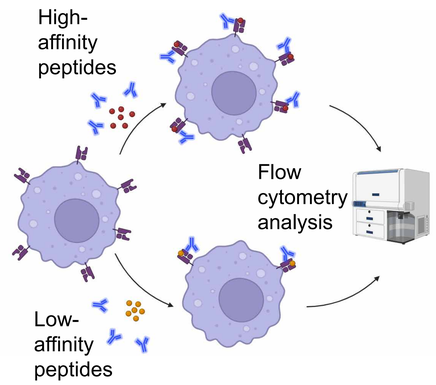
Methods and Results

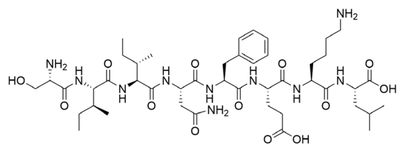
RMA-S Stabilization Assay is a Well-Established Method to Measure MHC Affinity

RMA-S cells display empty MHC molecules that can bind exogenous peptide.

Peptide-MHC affinity can be quantified by the amount of pMHC complexes stabilized on the surface of the cell.

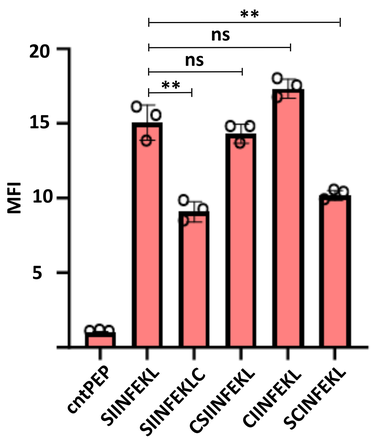


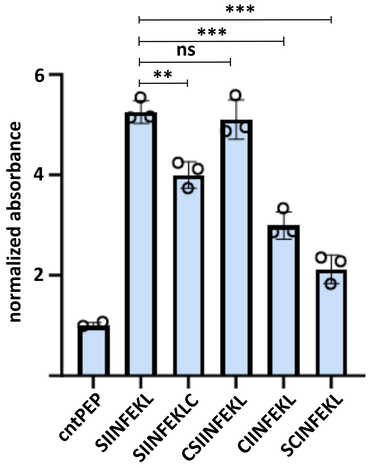
CSIINFEKL Retains Potent MHC Binding and T Cell Activation

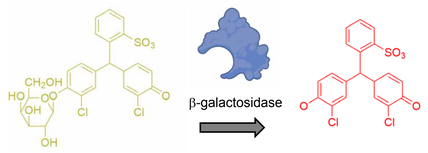


We synthesized a library of SIINFEKL derivatives to identify sites amendable to cysteine modifications.

Modifications near the N-terminus were well tolerated for MHC binding.





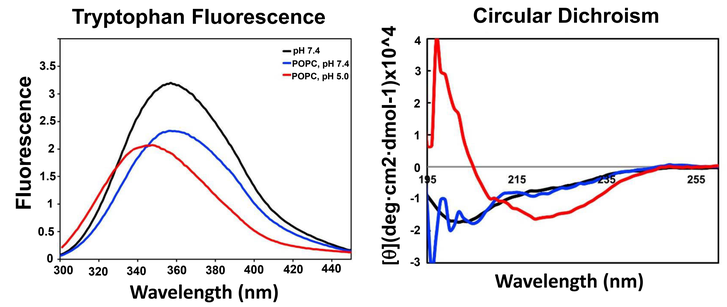


Reporter T cell line with OVA-specific T cell receptor measured T cell activation.

CSIINFEKL (CysOVA) proved to be least disruptive to T cell activation.

Results

pHLIP-CysOVA Selectively Inserts into Lipid Bilayers at Low pH

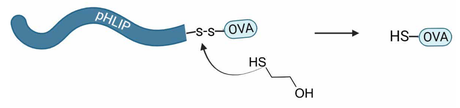


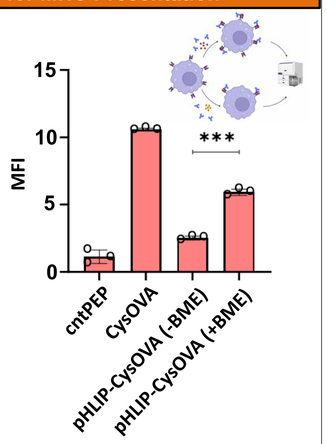
Synthesized pHLIP-CysOVA conjugate and confirmed pH dependent membrane anchoring using tryptophan fluorescence and CD spectrometry.

Disulfide Reduction is a Necessary Step for MHC Presentation

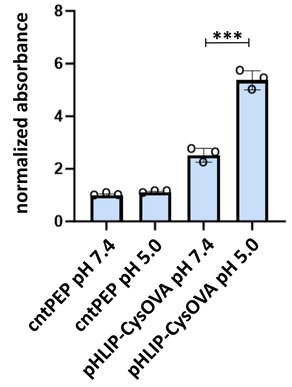
RMA-S cells display empty MHC molecules when incubated at 26ºC that can bind exogenous peptide.

Peptide-MHC affinity can be quantified by the amount of pMHC complexes stabilized on the surface of the cell.

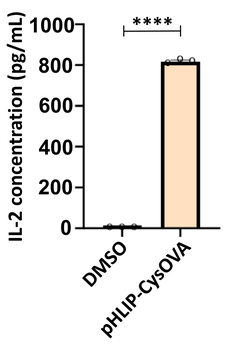




pHLIP-CysOVA Improves Activity of Antigen-Specific T cells



Treatment of pHLIP-CysOVA at low pH improves T cell activation.



ELISA results also confirmed proinflammatory IL-2 secretion