A novel immunoproteomic approach to MHC class I-restricted peptide discovery for influenza CD8+ T cell adaptive vaccine development

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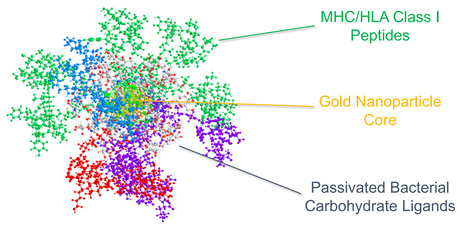
KEY FINDINGS

- This ***novel two-step approach to MHC class-I associated peptide isolation*** yielded a diversity of viral peptide identifications
- Peptides from ***NEG8***, a viral protein on the negative sense strand of segment 8 of the influenza A virus (IAV), were identified
  - To our knowledge, this is the first proteomic evidence of NEG8 in IAV strains
- These ***naturally processed and presented peptides*** can serve as potential candidates in the development of ***T cell adaptive vaccines for influenza***

Background & Purpose

- CD8+ T cell mediated response is a key component of the immune system against viral infection and disease [1]
- However, the repertoire of virus-specific peptides presented by MHC class I molecules on the surface of infected cells for T cell recognition remains ***largely undiscovered*** [2]
- Current algorithm-based prediction methods for MHC class I-restricted viral peptides ***do not provide adequate candidates*** for vaccine development [3]
- The Emergex immunoproteomics platform establishes improvements over existing methods for MHC class I ***immunopeptidomic discovery***
  - These ***naturally processed and presented*** MHC class I-restricted viral peptides (depicted in ***green*** below) will be used for ***novel T cell vaccine development***



Fully synthetic vaccine: Gold nanoparticles efficiently delivery MHC class-I associated peptides to the immune system

Methods

Cell Infection & Peptide Isolation

- Two influenza A strains utilized: (1) **A/Puerto Rico/8/1934 (PR8)** and (2) **A/Hong Kong/8/1968 (HK)**
- MHC class I-restricted peptides isolated from infected cells using a novel two-step procedure:
  1. **Step 1: Strip** – Naturally processed and presented viral peptides stripped from PR8- and HK-infected cells
  2. **Step 2: Immunoprecipitate (IP)** – Cells lysed; endogenous MHC I-restricted peptides captured by immunoprecipitation

LC–MS/MS Analysis

- Thermo UltiMate 3000 RSLCnano system coupled with Thermo Orbitrap Eclipse Tribrid MS operating in data-dependent analysis (DDA) mode
- 70-min separation gradient; mobile phase A: H2O + 0.2% FA; mobile phase B: 80% ACN + 0.16% FA
- MS1 resolution: 120k (FWHM at *m/z* 200); scan range: 275–1500 *m/z*; precursor ion charges 1–7; MS2 resolution: 15k (FWHM at *m/z* 200)
- Collision mode: stepped HCD (normalized collision energies of 25, 28, and 32%)

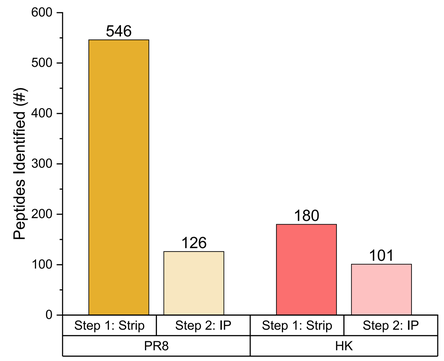
Database Searching

- Two programs utilized: (1) Proteome Discoverer and (2) MaxQuant
- Raw MS data searched against respective viral protein fasta databases
- Search engine: SEQUEST HT; digestion type: unspecific (non-enzymatic); PSM validation mode: fixed; charge-dependent XCorr thresholds: $z = 1$, 1.5; $z = 2$, 2.0; $z = 3$, 2.5; $z \geq 4$, 3.0; peptide length: 6–17 aa; precursor and fragment mass tolerances: 10 ppm and 0.02 Da; dynamic modifications: N-terminal acetylation and methionine oxidation
- Final data combined across Proteome Discoverer and MaxQuant searches

Results: *Overall Peptide Identifications*

- Greater number of peptides identified in **Step 1: Strip** compared with **Step 2: IP** for both **PR8** and **HK** strain samples (Fig. 1)
  - **PR8:** 546 (Step 1: Strip) vs. 126 (Step 2: IP)
  - **HK:** 180 (Step 1: Strip) vs. 101 (Step 2: IP
- Large differences in number of peptide identifications between PR8 and HK strain samples likely due to differences in cell infectivity
- Percentage of overlapping peptides identified in both steps were < 3% of the total peptides detected for both **PR8** (Fig. 2) and **HK** (Fig. 3) strain samples

Fig. 1



Figs. 2 & 3. Unique and common peptides identified across Step 1: Strip and Step 2: IP for PR8 and HK strain samples.

Fig. 2

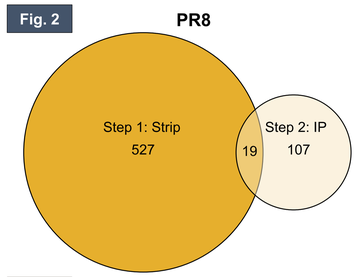
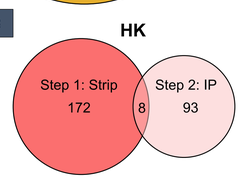


Fig. 3



Results: *Peptide Length Distributions*

- **PR8** (Fig. 4):
  - Step 1: Strip – 13 aa peptides dominant (290 peptides)
  - Step 2: IP – 8 aa peptides dominant (38 peptides)
- **HK** (Fig. 5):
  - Step 1: Strip – 6 aa peptides dominant (54 peptides)
  - Step 2: IP – 9 aa peptides dominant (25 peptides)
- Theoretical MHC class I peptide length range is between 8–12 aa; high abundance of peptides within this range observed

Fig. 4

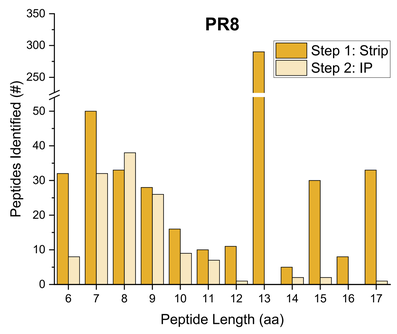
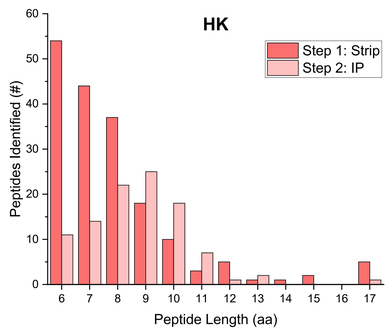


Fig. 5



Results: *Peptide Identifications by Protein*

- **PR8** (Fig. 6):
  - Step 1: Strip – NS1, HA, PA peptides dominant
  - Step 2: IP – PB1 peptides dominant
- **HK** (Fig. 7):
  - Step 1: Strip – NS1, PB1, HA peptides dominant
  - Step 2: IP – NP peptides dominant
- Examining protein origin allows for the selection of peptide candidates from ***highly conserved regions***, enabling the creation of virus ***mutation-resistant vaccine products***
- Peptides from NEG8, a negative sense strand viral protein, were also identified
  - NEG8 is highly conserved across flu strains, but has only been predicted at the genomic level
  - To our knowledge, this is the ***first proteomic evidence*** of NEG8 in IAV strains

Fig. 6

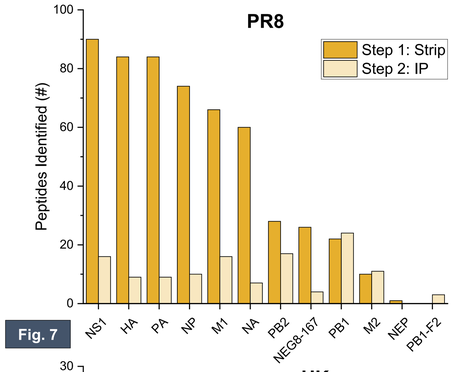
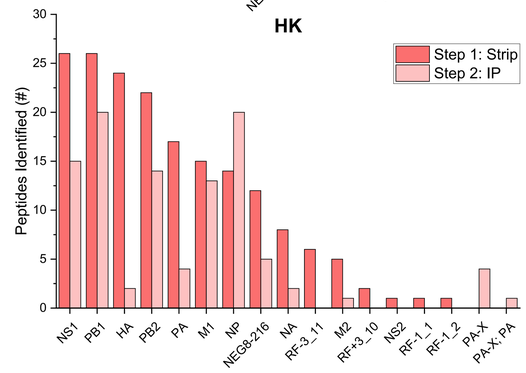


Fig. 7



HA = Hemagglutinin; NA = Neuraminidase; M1 = Matrix protein 1; M2 = Matrix protein 2; NEG8-167 = Negative stand segment 8 (167); NEG8-216 = Negative stand segment 8 (216); NEP = Nuclear export protein; NP = Nucleocapsid protein; NS1 = Non-structural 1; NS2 = Non-structural 2; PA = Polymerase; PA-X = Polymerase X; PB1 = Polymerase basic protein 1; PB1-F2 = Polymerase basic protein 1 F2; PB2 = Polymerase basic protein 2; RF-1_1 = Reverse frame-1 (segment 8 potential novel ORF 1); RF-1_2 = Reverse frame-1 (segment 8 potential novel ORF 2); RF+3_10 = Reverse frame-3 (segment 8 potential novel ORF 10); RF-3_11 = Reverse frame-3 (segment 8 potential novel ORF 11)

References

- [1] Yewdell, J. W. (2022). MHC Class I Immunopeptidome: Past, Present, and Future. *Molecular & cellular proteomics: MCP, 21*(7), 100230. https://doi.org/10.1016/j.mcpro.2022.100230
- [2] Bullock, T. N. J., Mullins, D. W., & Engelhard, V. H. (2003). Antigen Density Presented By Dendritic Cells In Vivo Differentially Affects the Number and Avidity of Primary, Memory, and Recall CD8+ T Cells. *J Immunol*, *170*(4), 1822–1829. https://doi.org/10.4049/jimmunol.170.4.1822
- [3] Wilson, E. A., Hirneise, G., Singharoy, A., & Anderson, K. S. (2021). Total predicted MHC-I epitope load is inversely associated with population mortality from SARS-CoV-2. *Cell reports. Medicine, 2*(3), 100221. https://doi.org/10.1016/j.xcrm.2021.100221