Novel gene identification, from an influenza virus infection model using multi-omics approach, reveals potential CD8+ T cell adaptive vaccine candidates

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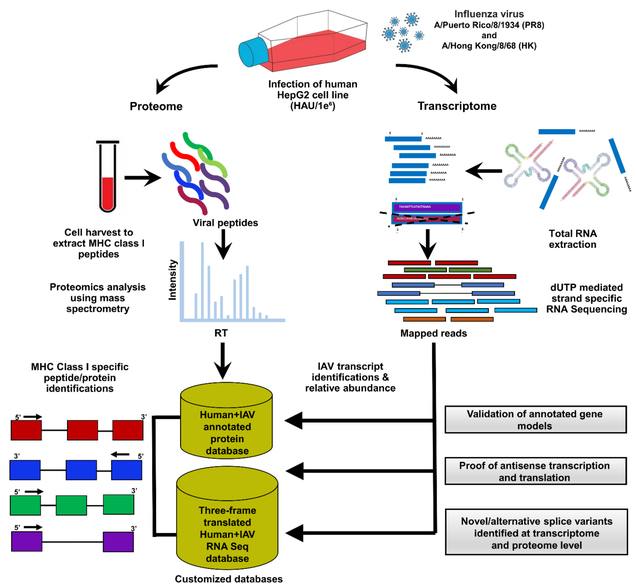
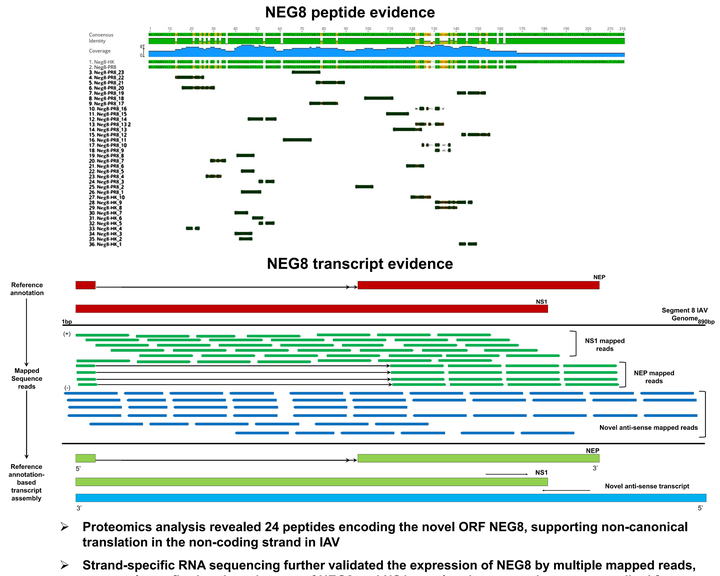
Background & Purpose

- Influenza virus can cause acute respiratory infections in humans; Among multiple viral strains (A-D) known, A and B are primarily responsible for annual outbreaks
- Annually, ~3-5 million cases of severe illness and 290-650K respiratory deaths from influenza virus are reported
- Influenza A virus (IAV) elicits a robust CD8+ T cell response, governed through the presentation of MHC class I-restricted, virus-specific peptides (ViPs) on the surface of an infected cell [1]
- Negative strand viruses like IAV provide an opportunity for non-canonical translation from non-AUG start sites, or mRNA splicing, thereby forming a potential source of immunogenic peptides
- ViPs can serve as antigens for the development of next generation T cell adaptive vaccine technology that may provide broad and durable immunity for future pandemic strains
- Using both immunoproteomics and RNA sequencing approaches, we have identified and validated the existence of a novel non-canonical open reading frame (ORF) called NEG8 (NEGative strand segment 8), expanding the discovery potential of novel antigenic candidates for CD8+ T cell vaccine development

Multi-omics workflow for MHC class I peptide identifications



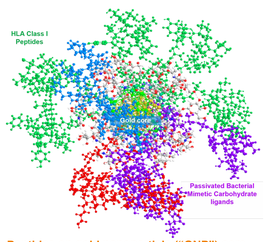
Evidence for the expression of IAV NEG8 ORF



- Proteomics analysis revealed 24 peptides encoding the novel ORF NEG8, supporting non-canonical translation in the non-coding strand in IAV
- Strand-specific RNA sequencing further validated the expression of NEG8 by multiple mapped reads, accurately confirming the existence of NEG8 and NS1, overlapping genes that are transcribed from opposite strands

Overview of Emergex T cell adaptive vaccine platform

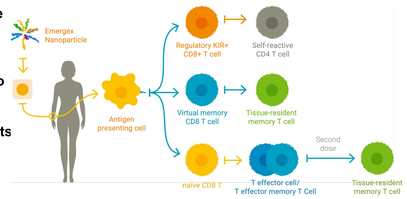
1. Naturally processed & presented infection model paired with novel immunoproteomic approach for identification of MHC Class I T cell pathogen-specific epitope candidates (ligandome library)
2. Generation of multivalent & multiallelic T Cell adaptive vaccine formulations utilizing the pathogen-specific ligandome candidate epitopes paired with a gold nanoparticle (GNP) core
3. Delivering the Adaptive T Cell Vaccine formulation via intradermal delivery to specialized immune cells to elicit functional, long-term CD8+ T cell protection



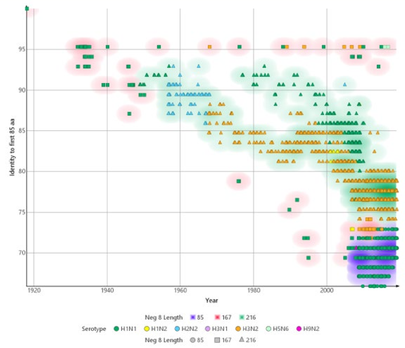
Peptides on gold nanoparticle ("GNP") core form the vaccine

Emergex vaccines elicits cellular immune response by delivering synthetic MHC class I viral peptides to APCs, which 'prime' CD8+ T cells

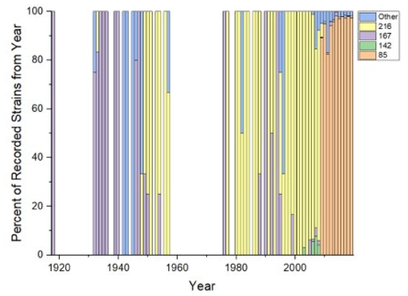
- Antigen presenting cells (APCs) 'prime' naïve CD8+ T cells to recognize specific viral peptides expressed by infected cells – these become antigen-specific 'effector' T cells
- The priming leads to CD8+ memory T cells which are ready to abort an infection prior to active viral replication [2]
- Subsequent exposure to the pathogen results in a rapid cellular immune response; providing comprehensive immunologic protection
- CD8+ T memory cells have been shown to be active for decades (SARS-1 infection) [3,5]



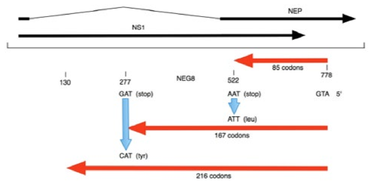
Evolutionary evidence for a novel gene (NEG8) associated with human IAV viruses



Timeline analysis of human influenza virus NEG8 ORFs showing variable codon length (85-216aa) across pandemic strains



Neg8 length variation specific to human IAV (167, 216, 85aa)



Overview of genome segment 8 annotation of IAV: Bioinformatics evidence of a novel influenza A virus ORF [4]

Is there a twelfth protein-coding gene in the genome of influenza A?

- There is an unusually long ORF on the genomic (negative) strand of segment 8 of current human IAVs [4,6]
- A very high degree of conservation of this ORF in the N-terminus region across pandemic strains has been observed
- The pre-dominant association of this ORF with human IAV indicates that an expressed protein may be enhancing viral fitness in the human host

Conclusion & future prospectives

- Integrative multi-omics approach provided novel experimental evidence on NEG8 expression in IAV infected cells, highlighting the potential of antisense targets for vaccine studies
- The high degree of conservation of NEG8 across pandemic strains over several years emphasizes its potential role in anti-viral immunosurveillance
- Further experimental evaluation of the NEG8 peptides and their MHC binding capabilities will reveal a potential role in antigen presentation and T cell activation/priming for vaccine development
- With a significant unmet clinical need for improved vaccines, antigens (NEG8 MHC I peptides) derived from the NEG8 ORF may potentially contribute to a vaccine formulation protective against future pandemics

References

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[6] Heather D. Hickman, Jacqueline W. Mays, James Gibbs, Ivan Kosik, Javier G. Magadán, Kazuyo Takeda, Suman Das, Glennys V. Reynoso, Barbara F. Ngudiankama, JiaJie Wei, John P. Shannon, Daniel McManus, Jonathan W. Yewdell; Influenza A Virus Negative Strand RNA Is Translated for CD8⁺ T Cell Immunosurveillance. *J Immunol* 15 August 2018; 201 (4): 1222–1228.