Improving antibody and T-cell responses to subunit influenza vaccines using supramolecular assembly and randomized T-helper epitopes

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Statement of Purpose: Inducing long-lasting and broadly protective immune responses using vaccines is crucial for global health. For pathogens with high mutation rates such as influenza, long-lived protection is challenging. The work reported here seeks to address the need for a highly protective influenza vaccine that steps toward universal protection to eliminate the need for seasonal vaccines. In addition to increasing the breadth of influenza vaccines, increasing protection from currently available seasonal vaccines is also of significant interest.[1] Here we explored two strategies for improving the breadth and efficacy of subunit vaccines based on the influenza antigen hemagglutinin (HA): supramolecular oligomerization and incorporation of additional CD4+ T-helper epitopes to strengthen anti-HA T-cell responses. We designed supramolecular peptide nanofibers incorporating HA using the Q11 peptide fibrillizing system and antigen bearing a β-tail fusion tag.[1] Additional T-helper epitopes were incorporated using KEYA-Q11, a fibrillizing peptide containing randomized peptide sequences of lysine, glutamic acid, tyrosine, and alanine inspired by glatiramoids.[2] Using this supramolecular approach we investigated how highly multivalent antigen display and randomized T-cell epitopes may be employed in combination to enhance both humoral and cellular responses to hemagglutinin-based subunit vaccines.

Methods: Peptides were synthesized using Fmoc solid phase peptide synthesis, purified via HPLC, and verified using MALDI. A/Hong Kong/1/1968 H3N2 expressing a β-tail tag (HA-β-tail) was expressed in MDCK cells and purified via nickel-immobilized affinity chromatography. To form nanofibers displaying HA and KEYA, Q11, KEYA-Q11, and HA β-tail protein were combined at 2mM peptide concentration in 1xPBS. Binding of HA after nanofiber conjugation was assessed using polyclonal sera from mice immunized with A/X-31 virus (X31); and monoclonal antibody 9H10 (a generous gift from NH). Eight-week old female C57BL/6 mice were immunized with soluble HA (HA), nanofibers bearing HA (HA-Q11), nanofibers bearing HA and KEYA (HA-KEYA-Q11), or PBS. All groups received 15 μg HA β-tail in 100 μL subcutaneous injections. Antibody responses were monitored by ELISA with weekly blood draws for 6 weeks prior to infection. Mice were infected with A/X-31 influenza virus and monitored for 2 weeks in a sublethal challenge. After infection, cellular responses were analyzed via ELISpot from splenic lymphocytes.

Results: We began by confirming that HA was inserted into the nanofibers via cryoTEM and found that HA successfully incorporated into the nanofibers during self-assembly. We next investigated if attachment to the nanofiber decreased HA binding to HA specific antibodies. We found that the conjugation of HA onto nanofibers using the β-tail scheme discussed previously did not significantly impact binding to X31 or 9H10 antibodies. Upon incorporation of high percentages of KEYA-Q11 however, binding to both antibodies was decreased. Based on this, 1% KEYA-Q11 was chosen to move forward to mouse experiments.

We found that HA-KEYA-Q11 immunization significantly increased anti-HA and IgG production over HA alone, while producing little antibody response against KEYA or Q11. To evaluate the T cell responses to these vaccines, we challenged mice against a sublethal infection of A/X-31 influenza virus. After 14 days mice were sacrificed, and splenic lymphocytes were harvested. Analysis via ELISpot showed an increase in Th1 (IFN-γ producing) CD4+ T cells for mice immunized with HA-KEYA-Q11 compared to HA alone when restimulated with HA or inactivated X31 virus (Figure 1).

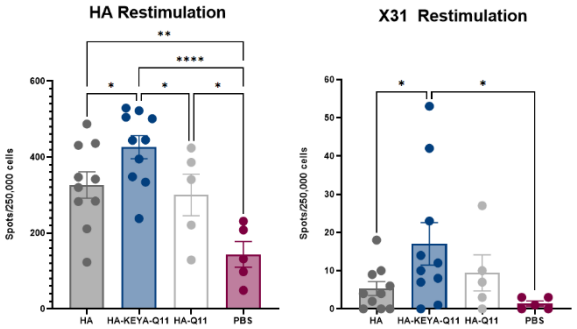

Figure 1. HA-KEYA-Q11 nanofibers raised stronger Th1 (IFN-γ producing) CD4+ T cell responses against HA (left) and X31 influenza virus (right) than unmodified HA antigen. *p<0.05; **p<0.01; ****p<0.0001 by ANOVA with Tukey post-hoc test.

Conclusions: These results demonstrate that multivalent display of HA on nanofibers also containing a universal T cell stimulating peptide increases both humoral and cellular responses to subunit flu vaccines. In the future, we will explore the breadth of the antibodies produced via multiplex HA binding to a wide variety of H3 and H1 hemagglutinins. We expect that antibodies from mice immunized with HA-KEYA-Q11 will demonstrate increased breadth compared to those immunized with soluble HA alone.

References: [1] Hudalla GA. Nat Mater. 2014;13:829-836. [2] Votaw NL. Biomaterials. 2021;273:120825.