Randomized Peptide Assemblies for Improving the Efficacy of Epitope-Based Influenza Vaccines

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Statement of purpose: The yearly burden of the influenza virus goes beyond the estimated 12-60,000 annual deaths[1] in America to cause anywhere from 140-710,000 hospitalizations[1] and, especially in these unprecedented times, there is a need to reduce this burden with an effective vaccine. Currently, broadly protective vaccines being developed are attempting to either induce antibodies or cross-reactive T-cell response to conserved viral proteins[2]. Here we employ both strategies by using peptide epitopes combined on self-assembling nanofibers to raise strong B- and T-cell responses against conserved epitopes. We apply a newly developed technology termed $(KEYA)_{20}Q11$ that acts as a nano-scale adjuvant without causing inflammation. $(KEYA)_{20}Q11$was developed by synthesizing self-assembling peptides terminated with randomized libraries of peptides containing Lys, Glu, Tyr, and Ala. KEYA modifications serve as strong B-cell and T-cell epitopes *in vivo*, enhancing immune responses against epitopes relevant to influenza and improving peptide-based influenza vaccines.

Methods: $(KEYA)_{20}Q11$ was created by mixing the four amino acids at specific ratios during Fmoc solid phase peptide synthesis and appending it to Q11, a self-assembling β-sheet peptide. Nanofibers were prepared by dissolving lyophilized peptide in sterile water overnight and diluting the solution into 1x PBS at a 2mM concentration. C57BL6 mice were immunized subcutaneously, blood was analyzed with ELISA, and lymphocytes purified from the spleen were evaluated with ELISpot. Mice were infected with 60 PFU of influenza intranasally, weighed daily, and euthanized if they fell below 75% initial body weight.

Results: Mice were immunized with **(**$KEYA)_{20}Q11$ separately combined with three influenza epitopes: a CD4+ T cell epitope from the nucleoprotein termed NP, a CD8+ T cell epitope from the polymerase acidic protein termed PA, and a B cell epitope from the M2 protein termed M2e. First, combining **(**$KEYA)_{20}Q11$ with NP-Q11 resulted in stronger NP-specific T cell responses (Fig 1A). Mice were immunized and boosted once with nanofiber formulations containing NP-Q11 and either $(KEYA)_{20}Q11$ or Q11. Spleens were harvested at week 3.5, and purified lymphocyte populations were re-stimulated with peptide-NP for ELISpot. NP-Q11 by itself induced only weak T-cell responses, but the addition of $(KEYA)_{20}Q11$ to the nanofibers significantly enhanced pNP T-cell responses without altering the balance between cells producing IL-4 or IFNγ (Fig 1A). Next, combining **(**$KEYA)_{20}Q11$ with PAQ11 did not interfere with the PA specific response and trends toward a stronger T cell response (Fig 1B). Mice were immunized and boosted once with nanofiber formulations containing PAQ11 and either $(KEYA)_{20}Q11$ or Q11. An ELISpot was performed as described above and PA-Q11 alone or combined with $(KEYA)_{20}Q11$ induced a PA specific response (Fig 1B). Last, combining $(KEYA)_{20}Q11$ with M2e-Q11 resulted in dramatically higher antibody titers against the M2e epitope (Fig 1C). In peptide nanofibers, B-cell epitopes require co-assembled T-cell epitopes to break immune tolerance,[3] so NP-Q11 was included in both formations to provide T cell help in the absence of

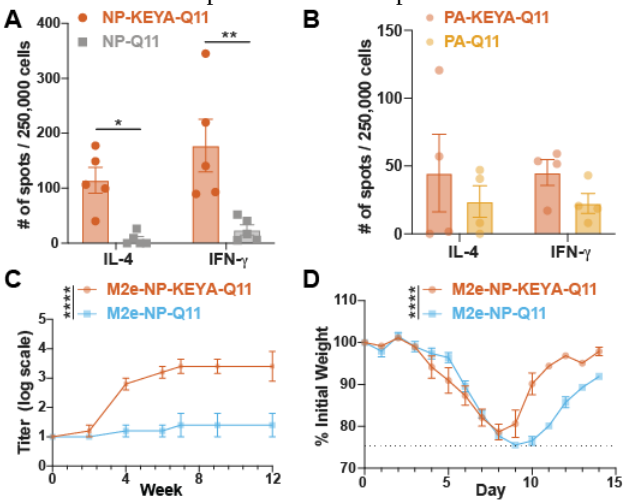

Figure 1. $(KEYA)_{20}Q11$ enhances responses to influenza-specific epitopes. (**A**) ELISpot shows the spot count from cells re-stimulated with peptide-NP. (**B**) ELISpot shows the spot count from cells re-stimulated with peptide-PA. (**C**) ELISA shows M2e-specific antibody titers. (**D**) Weight loss after an influenza challenge of mice in (C) given at week 10. Two-way ANOVA (A,B,C) with Tukey's post hoc test (A,B). Mixed-effects model (REML) with type III fixed effects (D). Mean +/− s.e.m. shown. *n* = 5 experimental replicates per group.

$(KEYA)_{20}Q11$. Mice were immunized and boosted every 2 weeks with nanofiber formulations containing M2eQ11, NPQ11, and either $(KEYA)_{20}Q11$ or Q11. Serum was purified from blood for ELISAs, and pM2e-specific antibody titers indicate that the addition of $(KEYA)_{20}Q11$ increases the M2e antibody response (Fig 1C). These mice were given a lethal influenza challenge and weighed daily (Fig 1D). Mice receiving immunizations containing $(KEYA)_{20}Q11$ recovered significantly faster than those without. Overall these findings indicate that $(KEYA)_{20}Q11$ is effective for augmenting responses to co-assembled T-and B-cell epitopes which corresponds to increased protection against a lethal influenza challenge.

Conclusions: We developed a novel immune-modulating nanomaterial, $(KEYA)_{20}Q11$, with the ability to enhance the response to flu-specific peptide epitopes and increase efficacy of a peptide-based vaccine. We separately boosted the response to three flu-specific epitopes that target different cell types and components of the influenza virus. Next, we plan to combine these components and hypothesize we will further increase the efficacy of this vaccine without the need for adjuvants.

References: [1]Rolfes, MA. Influenza Other Respi Viruses. 2018;12:132–7. [2]Mezhenskaya, D. J. Biomed Sci. 2019:26-76. [3]Hengartner, H. Sci 1993;262:1448–51.