Surface Conjugation to Ace-DEX Microparticles Augments Matrix-2 Ectodomain-Based Influenza Vaccine Efficacy

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Statement of Purpose: To reduce influenza disease burden, seasonal vaccines are administered annually, but efficacy is poor and variable. This is primarily because the immune response is focused on the head domain of the hemagglutinin protein, which readily changes due to antigenic drift and shift. Less variable conserved epitopes exist on the virus; however, they are generally immunosubdominant. One such epitope is the ectodomain of the matrix 2 protein (M2e). We have shown the immunogenicity of M2e is increased by encapsulating it in acetalated dextran (Ace-DEX) microparticles (MPs) and combining it with cyclic guanosine-adenosine monophosphate (cGAMP) MPs. [1] Optimal B cell stimulation and resulting humoral response can be achieved with surface presentation of antigen to B cell receptors. We hypothesized that surface display of M2e on Ace-DEX MPs would generate a greater humoral response and protection when combined with cGAMP MPs compared to soluble or encapsulated M2e.

Methods: Ace-DEX was synthesized and characterized as described previously.[2] Ace-DEX MPs with thiol-reactive surfaces were generated through a sonication emulsion method incorporating PLA-PEG copolymers with maleimide or pyridyl disulfide end groups. To create Ace-DEX-M2e, a previously described modified M2e peptide containing a c-terminal cysteine [3] was conjugated to the Ace-DEX MPs to form thioether (maleimide) or disulfide (pyridyl disulfide) bonds. Antigen conjugation was quantified by size-exchange chromatography with UV detection. M2e was encapsulated in Ace-DEX MPs using a double emulsion process.[1] Ace-DEX cGAMP adjuvant MPs were generated using electrospray. [3] cGAMP loading was determined by RP-HPLC with UV detection. Ace-DEX-M2e and adjuvant MPs were characterized by scanning electron microscopy, dynamic light scattering, and zeta potential. BALB/c mice were immunized intramuscularly with a prime-boost-boost schedule (Figure 1A). Antibody titers and splenocyte antigen recall of M2e antigen were evaluated. To determine protective efficacy, mice were challenged with a lethal dose of Influenza A/Puerto Rico/8/1934 (PR8).

Results: M2e was successfully conjugated to the surface of Ace-DEX MPs. Immunization with these MPs in combination of Ace-DEX cGAMP adjuvant MPs resulted in robust humoral responses. Encapsulated antigen elicited a lesser humoral response than surface-conjugated antigen (Figure 1B). Encapsulation or conjugation of M2e to MPs by a reducible disulfide bond both resulted in a greater antigen recall T cell response compared to protease-resistant thioether conjugation (Figure 1C). Each antigen formulation offered significantly greater protection against lethal influenza challenge compared to the soluble M2e with Ace-DEX cGAMP microparticle adjuvant.

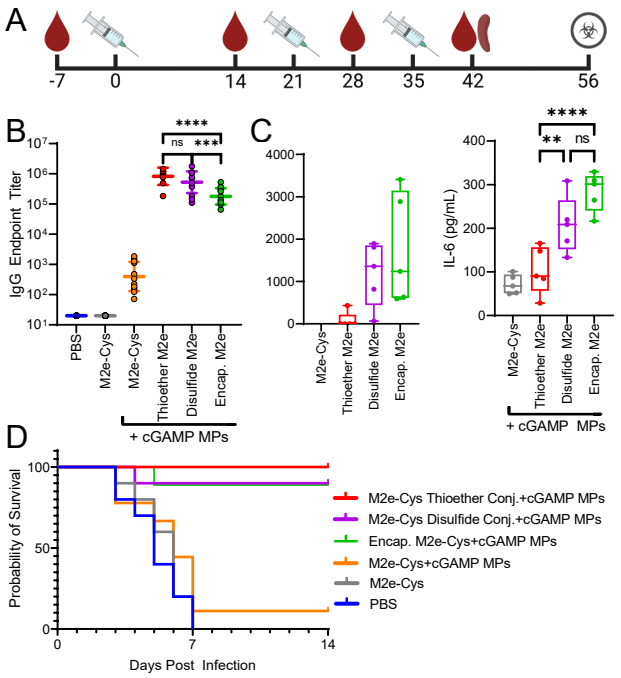

Figure 1. **(A)** Vaccination schedule. **(B)** Serum anti-M2e IgG titers on day 42. ****p<0.0001, ***p<0.001. **(C)** Secretion of IFN-γ and IL-6 by splenocytes stimulated with M2e on day 42. **(D)** Survival of mice after challenge with ~6x LD_{50} of PR8 on day 56.

Conclusions: Conjugation to the surface of Ace-DEX particles significantly augmented the humoral immunogenicity of M2e compared to encapsulated and soluble antigen. The greater T cell response induced by encapsulated and disulfide-conjugated antigens indicates that one or both of these antigen incorporation methods have the potential to engage both cellular and humoral arms of immunity. This study paves the way for evaluation of these M2e-based formulations in higher animal models of influenza infection, as well as presenting a generalized strategy for conjugation of thiol-containing antigens to the surface of Ace-DEX MPs to augment humoral immune responses.

References:

1. Chen N. et al. JCR. 2018; 289 p.114.
2. Kauffman KJ. Appl Mater Interfaces. 2012; 4 p.4149.
3. Watkins-Schulz R. et al. Biomaterials. 2019; 205 p.94.