A Novel M2e-Based DC-Targeting Influenza Universal Vaccine

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Introduction: The continuous emergence of the influenza virus results in over 650,000 deaths annually despite the annual vaccination, thus, calling for a novel universal influenza vaccine**.** Interestingly using Ebola glycoprotein (EboGP) to target dendritic cells (DCs) is highly immunogenic. We, therefore, developed novel DC-targeting influenza antigens by fusing the EboGP DC-targeting domain (EΔM) to conserved influenza hemagglutinin stalk regions and the ectodomain of matrix protein (M2e) (HM2e) generating EΔM-HM2e or four copies of M2e (tM2e) generating EΔM-tM2e. Likewise, the co-infection of influenza and Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV)-2 remains overwhelming. We also developed influenza and SARS-CoV-2 bivalent vaccines.

Methods: In this study, the EΔM-HM2e or EΔM-tM2e was incorporated into virus-like particles (VLPs), and recombinant vesicular stomatitis virus (rVSV) for DC-targeting ability, and antigenicity investigations against influenza respectively. The rVSV-EΔM-tM2e was then incorporated with the SARS-CoV-2 spike protein (SP) (having C-terminal deleted and I742A point mutation) (EM2e/SPΔC1), SP (having S2 deleted) (EM2e/SPΔC2) or the receptor binding domain (EM2e/RBD). We then characterized their immunogenicity property against influenza or SARS-CoV-2 infection in mice or hamsters.

Results: Our results revealed that VLP-EΔM-HM2e or EΔM-tM2e could target DCs/macrophages *in vitro*. Furthermore, rVSV-EΔM-tM2e or EΔM-HM2e intranasally or intramuscularly immunized mice had significantly high levels of induced immune responses and had 100% protection against the H3N2, and 60% or 100% protection against H1N1 respectively. Our Antibody-Dependent Cellular Cytotoxicity assay revealed that the survival rate is antibody-dependent. Furthermore, the EΔM-tM2e in the bivalent vaccines induced robust immune responses and protected against H1N1 in mice with reduced viral loads. While these vaccine candidates also protected against SARS-CoV-2 infection in ferrets.

Conclusion: Using our novel strategy, we developed a universal influenza vaccine by fusing influenza-conserved epitopes with EΔM to induce a broader immune response against influenza infections. While the EΔM-tM2e bivalent vaccines also protected against influenza and SARS-CoV-2.