Engineering and Evaluation of a Fully Synthetic Universal Influenza-A Vaccine Based on M2e-Conjugated Gold Nanoparticles

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Statement of Purpose: Influenza is a highly contagious respiratory disease caused by influenza virus. Due to the remarkable mutability of its major surface glycoproteins hemagglutinin (HA) and neuramindase (NA) the new virus strain evades recognition by existing anti-influenza antibodies. Therefore, the vaccine formulation has to be updated each year. We postulated that a universal influenza vaccine could perhaps be designed by conjugating the highly conserved extracellular region of the matrix 2 protein (M2e) of influenza A virus to gold nanoparticles (AuNPs) to create nanoconjugates with a high surface density of M2e, which in the presence of an adjuvant CpG oligonucleotide may significantly enhance the immunogenicity of M2e and protect against various lethal influenza virus strains.

Methods: Citrate-reduced AuNPs were synthesized and characterized by transmission electron microscopy and dynamic light scattering. M2e (acetylated-SLLTEVETPIRNEWGSRSNDSSDC-amidated) was chemically synthesized (AAPPTec, LLC). Thiol-gold interaction of M2e-AuNPs was analyzed and confirmed using x-ray photoelectroscopy (XPS) and UV spectroscopy. Mice were intranasally vaccinated with M2e alone, M2e with CpG, M2e-AuNP conjugate, and three different dosages of M2e-AuNP conjugates with CpG (a single stranded oligonucleotide). Serum antibodies (IgG) against M2e were determined using enzyme-linked immunosorbent assay (ELISA). IgG subtypes were also determined. Finally, weight loss and survival were monitored after mice were challenged with four different influenza viruses including 2009 pandemic strain and highly pathogenic avian influenza A strain.

Results: Citrate-reduced AuNPs with a diameter of 12 nm were successfully synthesized. Stable conjugation of M2e-AuNP solution was indicated by a slight increase (indicating increase in size) of the UV absorbance wavelength compared with bare AuNPs. Results from ELISA showed that M2e alone was poorly immunogenic and even addition of soluble CpG to M2e did not significantly increase the immune response. However, conjugation of M2e to AuNPs significantly increased anti-M2e IgG antibodies in mice serum (Figure 1). Addition of soluble CpG to M2e-AuNP conjugates further increased the immune response and stimulated a balanced IgG1/IgG2a response. Upon challenge with 5 times 50% lethal dose of PR8-H1N1 influenza virus, the M2e-AuNP group was only partially protected, however, addition of adjuvant CpG to the formulation resulted in complete protection. In contrast, naïve mice and mice vaccinated with M2e alone or with soluble CpG were unprotected. Mice, to which half dosage of M2e with AuNP or washed M2e-AuNPs (no free M2e antigen in solution) were administered, were only partially protected from PR8 challenge. Finally, 100% protection of mice was observed in mice vaccinated with the full dosage of M2e-AuNP with CpG, following challenge with 2009 pandemic strain (H1N1) and highly pathogenic avian influenza A (H5N1) viruses, and 92% protection was observed following challenge with a H3N2 virus (Figure 2).

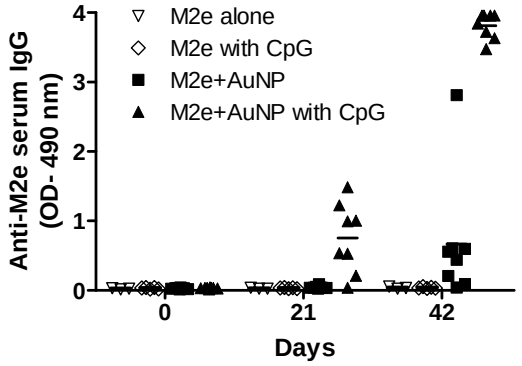

Figure 1. Serum anti-M2e IgG response (serum dilution 1:200).

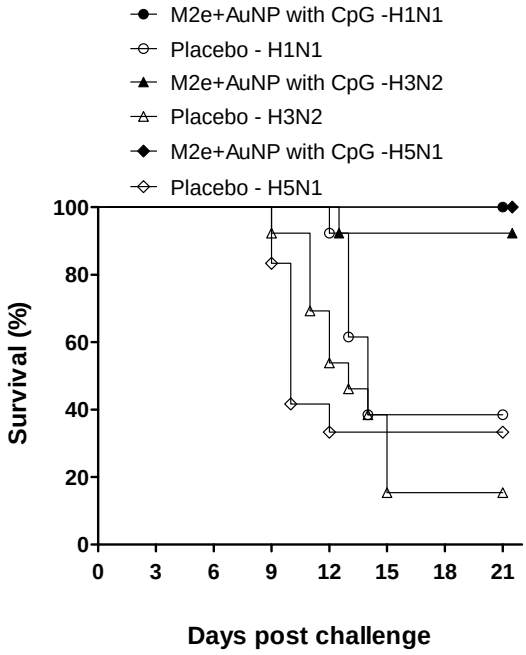

Figure 2. Survival of mice after lethal challenge with different influenza viruses.

Conclusions: The AuNP-M2e nanoconjugate formulated with soluble CpG as an adjuvant has potential for development as a universal influenza A vaccine.