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T. Hamouda¹, A. Myc², N. Mank², J. Knowlton², J. Sutcliffe¹, and J. Baker Jr^{1,2}
¹NanoBio Corp. and ²University of Michigan, Ann Arbor, MI, US

For additional information contact:
John Coffey, Jr.
Phone: (734) 302-9107
E-mail: john.coffey@nanobio.com

ABSTRACT

Background: W₈₀5EC is an oil-in-water emulsion adjuvant composed of high-energy nanometer-sized droplets stabilized by surfactants. W₈₀5EC kills enveloped viruses on contact via membrane destabilization, thereby inactivating the virus. Incorporation of protective epitopes into nanodroplets stabilizes them and co-delivery of the antigen-adjuvant enhances uptake of viral antigens into mucosal dendritic cells.

Methods: Mice were immunized intranasally with influenza A/Puerto Rico/8/34 (H1N1) virus inactivated and adjuvanted with W₈₀5EC. Animals received different doses of virus inactivated with varying doses of the adjuvant on two occasions, four weeks apart. Sera from different time points were tested for the presence of specific antibodies using hemagglutination inhibition assay and ELISA. Spleens were harvested and tested for cytokine production following stimulation with PR virus.

Results: Based on antibody response, the optimal vaccine formulations were achieved using 5 x 10⁶ pfu PR virus and adjuvant concentrations from 5-20%. Cytokine analysis showed that IFN- γ , IL-2, IL-17, IL-13 and GM-CSF responses were enhanced 10- to 100-fold upon virus stimulation as compared to PBS-stimulated controls. TNF- α , IL-10, IL-4, IL-6, IL1a, IL-5, KC, and MIP-1a increased more than two-fold over baseline, but the biological significance is unclear.

Conclusions: The W₈₀5EC-adjuvanted influenza viral vaccine provided an exceedingly robust and dose-dependent immune response. Although other adjuvanted vaccines have been shown to have a Th1-bias, W₈₀5EC-adjuvanted influenza vaccine uniquely enhanced both Th1 and Th17 responses. W₈₀5EC is novel in its ability to inactivate influenza virus and evoke a more balanced systemic response to the pathogen.

BACKGROUND

Influenza can be a severe viral infection that results in significant morbidity and mortality, especially among children, the elderly and people with chronic debilitating diseases (1). Although current vaccines are effective, there is still a need for a more effective vaccine to protect against influenza infection in the high-risk populations.

Adjuvants have been used to augment the immune response to antigens for more than 80 years (2). Aluminum salts, the only adjuvant approved by the FDA, generally provide enhanced Th2-type humoral immunity, but they are unable to elicit effective cell-mediated immunity, specifically cytotoxic T lymphocyte responses (3, 4), making them less or not effective against intracellular pathogens.

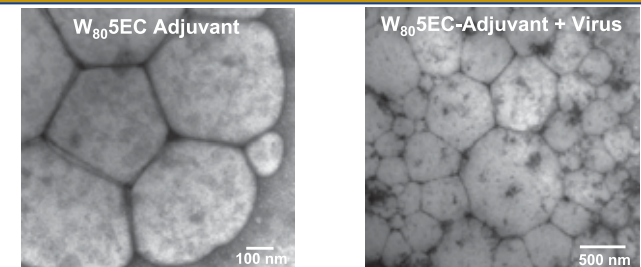
We have developed an oil-in-water nanoemulsion (NE)-based adjuvant, W₈₀5EC, consisting of ingredients that are on the FDA inactive ingredient list for approved drug products. Studies with the prototype NE formulations X8P and 20N10 showed that these NEs inactivated whole influenza virus, augmented the immune response when given intranasally to mice and conferred protection when mice were challenged with homologous influenza virus (5). In subsequent studies, a second-generation NE adjuvant, W₂₀5EC, was used to inactivate live vaccinia virus, and the combination was used as a vaccine to produce both systemic and mucosal immune responses in mice (6). The W₂₀5EC adjuvant has also been combined with purified antigens such as recombinant anthrax protective antigen and HIV gp120, providing a Th1 systemic response with neutralizing serum antibodies and mucosal IgA when administered intranasally in mice or guinea pigs (7). Recently, a more optimized formulation, W₈₀5EC, was combined with hepatitis B surface antigen and produced an enhanced immune response with no signs of inflammation in the nasal cavity or histopathological changes in key organs in four animal species (8).

This study investigated different concentrations of the W₈₀5EC nanoemulsion for its ability to inactivate the influenza A/Puerto Rico/8/34 H1N1 virus and enhance the immune response in mice.

References

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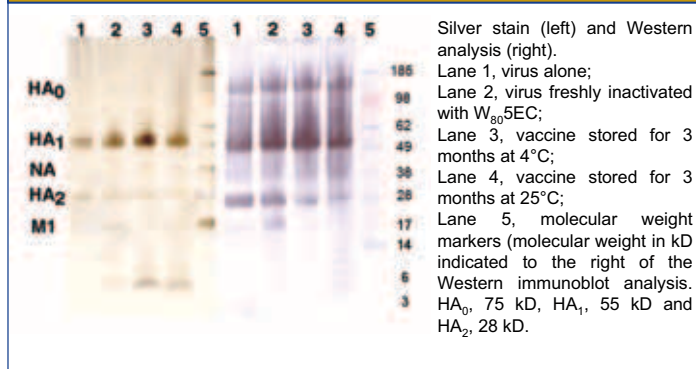
Influenza Vaccine Particle Size is Stable after Storage for Three Months at 4°C and 25°C



Nanoemulsion Adjuvanted Vaccine Droplet	Particle Size (nm)	
	4°C	25°C
● Cetylpyridinium chloride	337 ± 183	337 ± 183
○ Polysorbate	361 ± 113	338 ± 112
● Oil	331 ± 112	344 ± 102
● Antigen	322 ± 132	326 ± 112

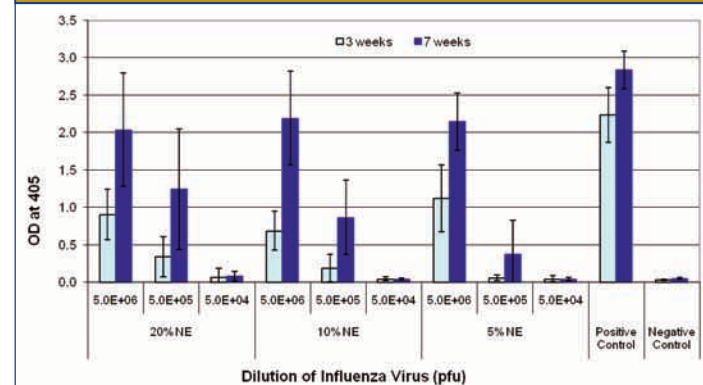
Age of Vaccine: Time 0, 1 Week, 1 Month, 3 Months

Hemagglutinin Antigen is Stable for Up to Three Months at 4°C and 25°C



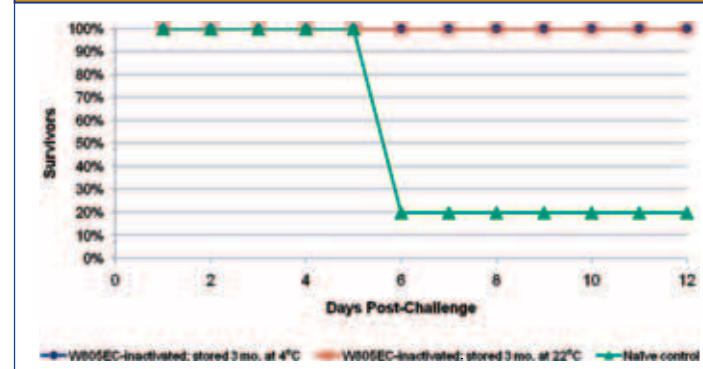
RESULTS

Mice Vaccinated Using Different Concentrations of Influenza Virus and W₈₀5EC Adjuvant Gave a Significant IgG Response



Sera were diluted 1:100. Positive control: sera from mice that survived infection with live virus; negative control: sera from naive mice.

W₈₀5EC-adjuvanted Vaccine Provides Complete Protection against Viral Challenge



METHODS

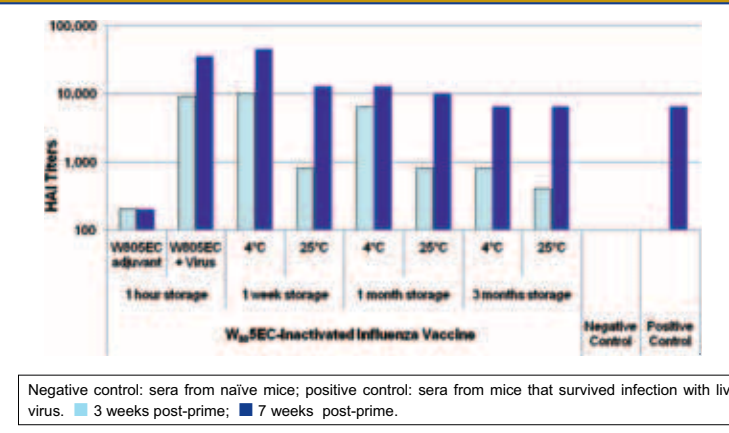
Nanoemulsion adjuvant. Concentrated W₈₀5EC nanoemulsion adjuvant (NE) was supplied by NanoBio Corporation (Ann Arbor, MI.) The NE is an oil-in-water formulation manufactured from components that are included on the FDA's list of inactive ingredients in drug substances. The concentrated emulsion was made by high-speed emulsification of Tween 80, ethanol, soybean oil, cetylpyridinium chloride and water. The mean droplet diameter was approximately 350 nm. Particle size was determined for the nanoemulsion adjuvant and vaccine samples using the Beckman-Coulter LS230.

Viral purification and limit of viral detection. Mouse-adapted influenza A/Puerto Rico/8/34 (H1N1) (PR) was purchased from ATCC. Virus was purified by sucrose gradient after 48 hrs of growth in pathogen-free hen eggs. The limit of detection was 11 pfu using the plaque assay (MDCK cells), 1.1 pfu using the CPE method, and 1.1 pfu using the EID₅₀ assay.

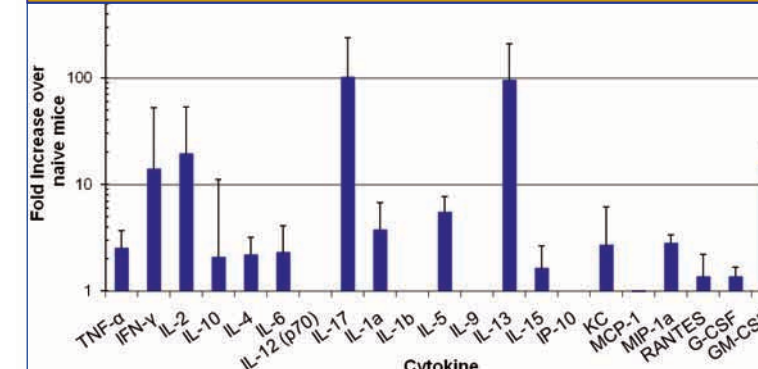
Intranasal immunization of mice and vaccination schedule. Mice were immunized intranasally with influenza A/Puerto Rico/8/34 (H1N1) virus inactivated and adjuvanted with W₈₀5EC. Animals received different doses of virus inactivated with varying doses of the adjuvant on two occasions, four weeks apart. Sera from different time points were tested for the presence of specific antibodies using hemagglutination inhibition (HAI) assay and ELISA. Spleens were harvested and tested for cytokine production following stimulation with PR virus.

HAI and splenocyte activation. The antibody titer was quantitated using the HAI assay by standard methodology. Four weeks after the second vaccination, the spleens were harvested from the mice, processed for splenocyte cultures and plated at 4 x 10⁶ cells/well in a 24-well plate. The splenocytes were activated with either DPBS (control), phytohemagglutinin (activation control), or live influenza virus at a multiplicity of infection (MOI) of 0.01. The splenocytes were incubated at 37°C/5% CO₂ for three days, after which the medium from the cells was harvested and tested for cytokine levels using a LINCplex kit according to the supplied protocol.

Immunogenicity of the Influenza Vaccine after Storage at 4°C and 25°C is Retained



Cytokine Profile Shows a Robust Cellular Immune Response with Enhanced Th1 and Th17 Immunity



CONCLUSIONS

• W₈₀5EC-adjuvanted influenza virus vaccination provided robust HAI titers and protection in mice.

• W₈₀5EC adjuvant inactivates influenza virus, stabilizes the HA antigen and eliminates the strict need for refrigeration.

• A robust cellular immune response with enhanced Th1 and Th17 immunity provided balanced immunity against both intracellular and extracellular forms of the virus.

• W₈₀5EC is a unique intranasal adjuvant and has been advanced to a Phase 1 clinical trial.