Infectimune™ enhances antibodies elicited by computational optimized broadly reactive antigen (COBRA) hemagglutinin influenza vaccine

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CIVICs Collaborative Influenza Vaccine Innovation Centers

Abstract

Infectimune™ is a non-viral vector/immune activator based on the proprietary cationic lipid R-DOTAP that can enhance the cell-mediated immune response, especially the CD8+ T cells responses. The computationally optimized broadly reactive antigen (COBRA) was designed to elicit broadly-reactive antibodies to influenza hemagglutinin (HA) immunogens. In this study, H1 and H3 COBRA HA vaccines were formulated as bivalent vaccine with or without Infectimune™ to evaluate enhancement of the immune response in mice. Experimental mice were administered with the HA in a dose response with and without Infectimune™ and mock vaccine. the HA plus Infectimune™ groups had significantly higher hemagglutination inhibition (HAI) titers against various H1 and H3 strains than the HA only group. Furthermore, after the lethal challenge, all mice, even in the group of lowest dose plus Infectimune™ survived with less than 5% body weight loss and no signs of morbidity. However, ~40% of mice vaccinated with the HA only vaccine survived challenge and none of mice in mock group survived. No virus in lungs was detected in all Infectimune™ groups, but the lungs collected from the HA only vaccine and mock groups had significantly higher virus lung titers. We conclude that, the formulation of Infectimune™ with COBRA HA vaccines can enhance antigen cross-presentation and immunogenicity and reduce the amount of vaccine required to protect against viral challenge.

Design and development of COBRAs

Full-length wild-type influenza virus H1N1 or H3N2 HA amino acid sequences were downloaded from online resource (GISAID). All sequences in each flu season were aligned multiple rounds according to the generated consensus sequence, eventually all candidates were tested, and the ones could elicit the widest antibody breadth were selected for further research.
NG2: COBRA H3N2 HA (May 2016 to April 2018)[1]
Y2: COBRA H1N1 HA (May 2014 to Sep 2016)[2]

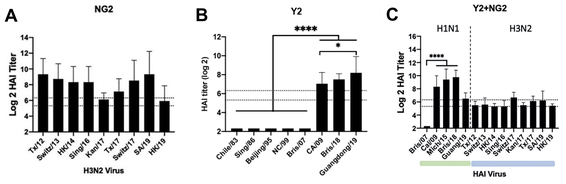
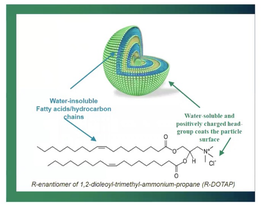
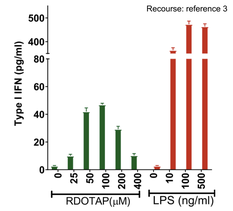


Figure A and B showed the serum post 3rd vaccination for naïve mice; figure C showed the serum post 2nd vaccination for pre-immune mice.
All vaccines were adjuvanted by AddaVax.

Infectimune™ (R-DOTAP)





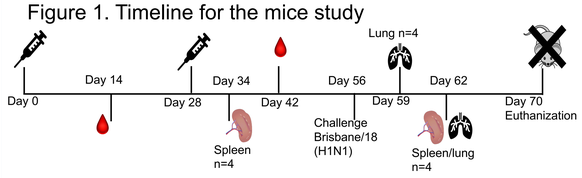
- Initially used to transport nucleic into cells for transfection. Equally efficient at transporting protein or peptides into cells.
- R-DOTAP activates the Type I interferon pathway[3].
- It is both safe and effective to induce the antibody, CD4+ and CD8+ T cells response. It has been utilized in a therapeutic HPV cancer vaccine and demonstrated to be both safe and effective for inducing CD8+ T cells response in a phase I clinical trial[4].

Experimental design

Table 1. The group design

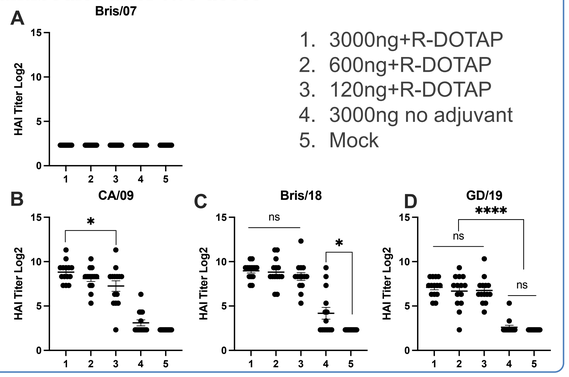
GROUP	VACCINE	Dose	Adjuvant	Dose	Route
1	Y2/NG2	3000ng	R-DOTAP	200ug	IM
2	Y2/NG2	600ng	R-DOTAP	200ug	IM
3	Y2/NG2	120ng	R-DOTAP	200ug	IM
4	Y2/NG2	3000ng	None	N/A	IM
5	None	N/A	None	N/A	IM

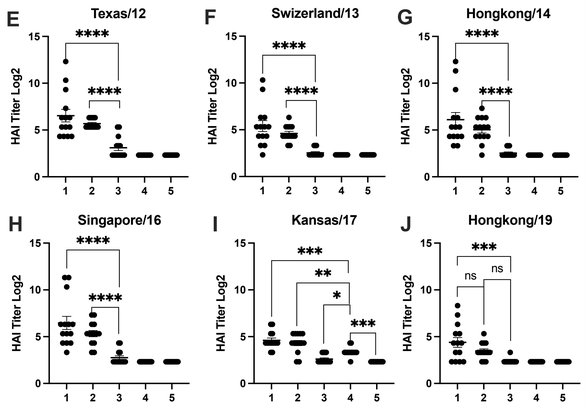
Figure 1. Timeline for the mice study



Results

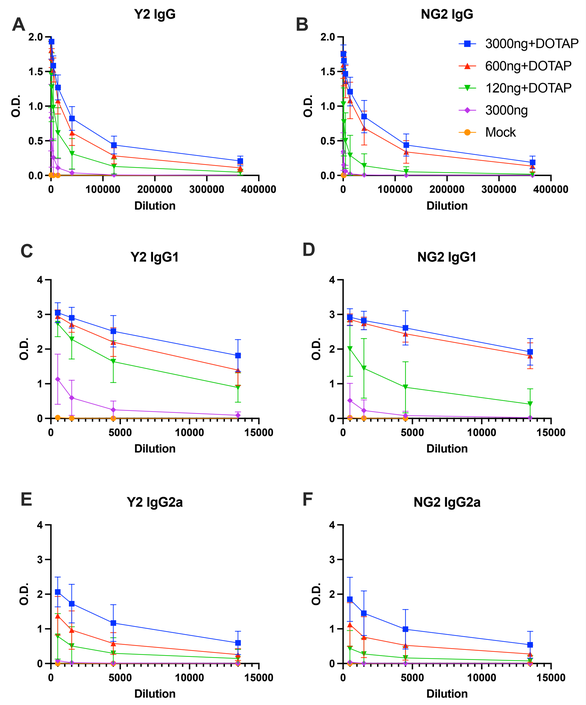
Result 1: COBRA adjuvanted with Infectimune™ (R-DOTAP) elicited expected broadly reactive antibodies after two doses





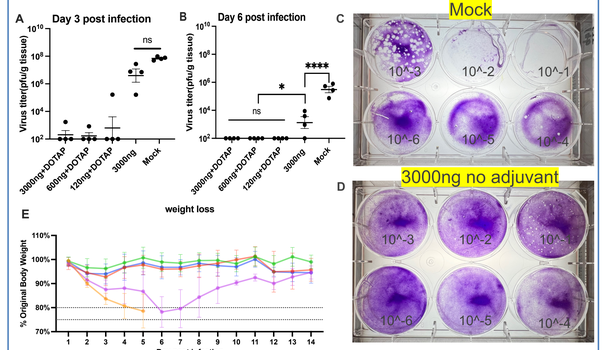
- The Brisbane/07 is a distant strain and a negative control.
- Figure A,B,C,D shows the HAI activity against H1N1 strains; Figure E,F,G,H,I,J shows HAI activity against H3N2 strains.

Result 2: COBRA adjuvanted with Infectimune™ (R-DOTAP) stimulated the antibody isotype switch and activated the Th1 pathway after two doses



- The addition of adjuvant significantly enhanced both IgG1 and IgG2a, which means it activated both Th1 and Th2 pathway. The IgG1 showed much higher value than the IgG2a because the DBA/2J mice are Th2 biased.

Result 3: COBRA adjuvanted with Infectimune™ (R-DOTAP) prevented the virus replication in mice lungs and protected vaccinated mice from lethal infection



- In the rHA alone group, one mouse (#115) showed undetectable viral titer 6 days post challenge and the HAI titer against Brisbane/18 was 1: 160, but another mouse in the same group who had higher HAI titer (1:320) showed much higher viral titer. This proved that the antibody is not enough to prevent infection.

Conclusions

1. Infectimune™ can significantly increase the efficacy of COBRA HA vaccine to elicit the antibody response.
2. Infectimune™ can extremely improve the protection against challenge with lower dose of vaccine.
3. The bivalent COBRA vaccine can function as well as the monovalent against H1N1 and H3N2, respectively.

Future study

1. Primary and memory B cells analysis: FluoroSpot assay
2. T cells activity analysis: ELISpot

Acknowledgements

This project has been funded by the National Institute of Allergy and Infectious Diseases, a component of the NIH, Department of Health and Human Services, under the Collaborative Influenza Vaccine Innovation Center (CIVIC) Contract 75N93019C00052

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