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Abstract # 5816

Adjuvanting of Fluzone® with JVRS-100 in Mice, Rabbits and Non-Human Primates Demonstrates Increased Immunogenicity and Dose-Sparing

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INTRODUCTION

JVRS-100 (lipid-DNA complexes) is a unique and promising adjuvant for vaccine applications that require high levels of antibody and T-cell immunity. The JVRS-100 adjuvant was mixed with a split influenza vaccine (Fluzone® , Sanofi Pasteur) and administered either subcutaneous to mice, or intramuscular to rabbits and non-human primates. Vaccination with JVRS-100-Fluzone® resulted in a significant increase in total IgG, IgG1 and IgG2a influenza antibodies. Furthermore, hemagglutination inhibiting (HAI) antibodies were higher compared with Fluzone® alone. Administration of decreasing amounts of Fluzone® mixed with JVRS-100 resulted in a ~50-fold dose-sparing effect based on HAI titer. In vitro stimulation of splenocytes from JVRS-100-Fluzone® vaccinated mice with Fluzone® demonstrated increased antigen-specific T cell responses (IFN-γ production) compared with Fluzone® alone. Splenocytes from JVRS-100-Fluzone® vaccinated mice responded to unmatched H1N1, H3N2, and influenza B viruses, suggesting induction of cross-reactive T cell responses to conserved viral antigens. Analysis of T-cell responses from vaccinated non-human primates demonstrated significant enhancement of both interferon-gamma positive and IL-2 positive cells (intracellular cytokine staining) indicating the enhancement of both primary and memory responses to influenza antigens.

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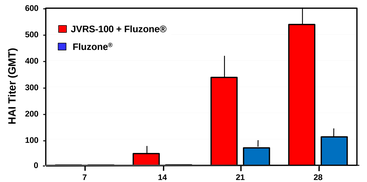
RESULTS

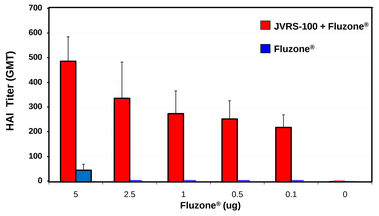
JVRS-100/Fluzone® Vaccination Mouse HAI Antibody Kinetics



Vaccination = Days 0 & 14

CD-1 mice were administered 5ug Fluzone® with or without 20ug JVRS-100 adjuvant on day 0 and 14. Sera were collected at day 7, 14, 21, and 28 for hemagglutination inhibition (HAI) antibody determination (using Fluzone® as the antigen)

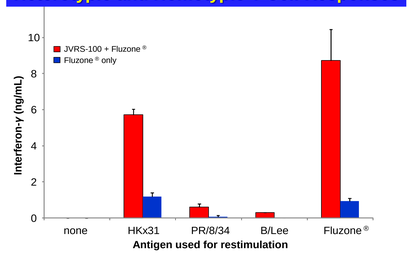
JVRS-100/Fluzone® Vaccination Fluzone® Dose Titration



Vaccination = Days 0 & 14
Assay = Day 28

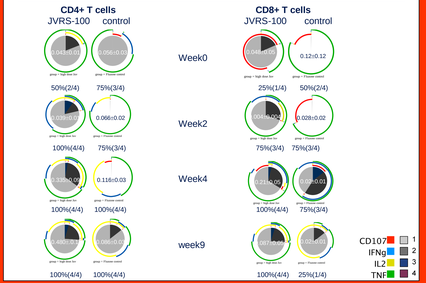
CD-1 mice were administered 20µg of JVRS-100 with 5.0, 2.5, 1.0, 0.5, and 0.1µg of Fluzone® on day 0 and 14. Sera were collected on day 28 for hemagglutination inhibiting (HAI) antibody determination (using Fluzone® as the antigen).

JVRS-100/Fluzone® Results in More Robust Heterotypic and Homotypic T-Cell Responses



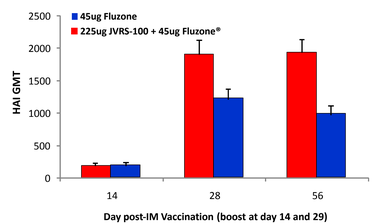
CD-1 mice were administered 5ug of Fluzone® with 20ug JVRS-100 on day 0 and 14. Splenocytes were isolated on day 28 and restimulated with either HKx31, PR/8/34, B/Lee or pediatric Fluzone®.

Fluzone specific T cell responses in high dose JVRS-100 and no adjuvant animal groups



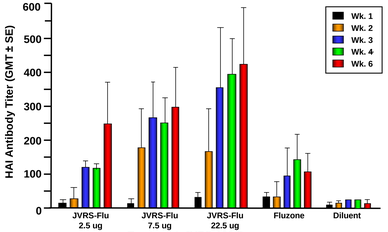
Mean influenza specific T cell responses in non-human primate PBMC before and after Fluzone® immunization with or without JVRS-100 adjuvant. The pie charts summarize the mean T cell responses and the extent to which the $CD4^+$ and $CD8^+$ responses were polyfunctional. For each pie chart the percentage (and proportion) responding is indicated below the chart. Mean frequency (± SEM) of each response is shown as a white number in the middle of the pie chart; only animals with a positive response are included. Each portion of a pie chart indicates the percentage of Fluzone-specific T cells that responded with one, two, three, or four functions; and the arcs around the pie show the function or combination of functions to which the specific response corresponds (see color legend).

JVRS-100 Enhances Anti-Fluzone® Responses in Rabbits



Vaccine was administered at day 0, 14, and 29 and HAI response quantitated at day 14, 28, and 56. The administration of JVRS-100 with Fluzone® increased the antibody response in rabbits both in magnitude and duration.

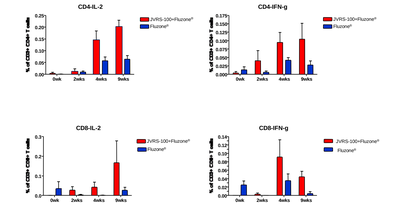
JVRS-100 (graded doses)/Fluzone® (22.5µg) Vaccination Results in Increased HAI Titers in Non-Human Primates



Immunization = days 0 & 14

A dose-ranging study was conducted in non-human primates (*Macaca mulatta*). Groups of macaques (n=4) were vaccinated on day 0 and 14 (IM) with graded doses of JVRS-100 adjuvant mixed with a fixed dose (22.5µg) of Fluzone® vaccine.

Fluzone® Specific T-cell Responses in High Dose JVRS-100 vs. No Adjuvant in Non-Human Primates



PBMC samples from non-human primates were restimulated with pediatric Fluzone®, blocked with brifeldin A and analyzed for intracellular accumulation of interferon-gamma or IL-2 as an indicator of primary or memory immune response.

CONCLUSIONS

These results suggest that use of the JVRS-100 adjuvant enhances immune responsiveness and reduced antigen doses needed for strong immune responses to a licensed flu vaccine. The JVRS-100 adjuvant has also been shown to potentiate immune responses to multiple viral and bacterial antigens, and could be a broadly applicable adjuvant for human and veterinary vaccines.