

EVALUATING THE EFFECT OF FORMULATION ON THE UPTAKE OF A ZIKA SUBUNIT VACCINE CANDIDATE BY ANTIGEN-PRESENTING CELLS.

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A major issue with vaccination for Zika, Dengue and other flaviviruses is the potential for antibody-dependent enhancement (ADE) of disease, caused by the generation or boosting of infection-enhancing antibodies. To address this concern, a subunit vaccine is being developed against the Zika virus using a modified version of the envelope protein as the antigen which has been modified with glycan residues to mask the fusion loop region of the protein (Figure 1), which is a cross-reactive and immunodominant site strongly implicated in the generation of antibodies capable of ADE. With this subunit vaccine approach, there is a need to formulate with an appropriate adjuvant to enhance the immunogenicity of the modified envelope protein. In this study we have evaluated a range of adjuvants using flow cytometry and fluorescence microscopy and have determined the relative uptake by human Antigen-presenting cells (APCs). Various combinations of clinically acceptable adjuvant materials: Alhydrogel®, 3D-(6-acyl) PHAD™ (a synthetic analogue of MPL) and QS21, were tested using liposomal formulations. In addition, the modified Zika envelope protein was compared to that of wild type Zika antigen, similarly formulated.

A further, more-general, issue in human vaccine development is the development of human-relevant test systems that might be used to better predict immunogenicity of vaccine antigens in combination with adjuvant formulations. To this end, dendritic cells were differentiated from human peripheral blood mononuclear cells (PBMCs) using a cytokine cocktail and matured with lipopolysaccharide (LPS). These differentiated dendritic cells were used as antigen presenting cells (APC) for antigen uptake performance evaluation. In brief, the cells were incubated with the vaccine formulations and antigen uptake was evaluated using flow cytometry and immunofluorescence. The antigen was detected using a fluorescently labelled anti-Zika envelope protein monoclonal antibody following cell permeabilization, and where applicable Alhydrogel uptake was evaluated using the aluminium specific fluorescent probe lumogallion. Uptake efficiency was evaluated in a time course fashion and at various amounts of antigen/adjuvant quantities. Fluorescent signal was correlated with the amount of antigen incorporated in the APCs. This approach allowed to compare the efficacy of multiple vaccine formulations in a human-relevant biological environment. The results demonstrated that all formulations were internalized by the APCs with equivalent uptake of the modified envelope protein and the wild type protein. Moreover, the liposomal formulations without Alhydrogel were internalized at a slower rate than those comprising liposomes alone.

Figure 1: Ribbon diagram showing the modified envelope protein with attached glycan.

