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Mechanisms of Immunogenicity Provided by Eilat Virus-Based Vaccines

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Objective: Explain potential mechanisms of enhanced immunogenicity provided by the Eilat virus vaccine vector platform.

Background: Eilat virus (EILV) is an alphavirus isolated from a pool of mosquitoes collected in Israel. It replicates efficiently in insect cells but is unable to replicate in vertebrate cells.[1] EILV is host-restricted in at least two points in its replication cycle: 1) attachment/entry, and 2) viral RNA replication. Our central hypothesis is that a chimeric alphavirus containing the non-structural protein genes of EILV and the structural protein genes of pathogenic alphaviruses, such as chikungunya (CHIKV) or Venezuelan equine encephalitis viruses (VEEV), will acquire the ability to attach and enter vertebrate cells via receptor-mediated endocytosis and deliver an RNA genome that is only capable of replication in insect cells, and provide for a safe and effective vaccine.

Methods: To test this hypothesis, we generated chimeric EILV/CHIKV and EILV/VEEV infectious cDNAs as well as various luciferase-based reporters using standard cloning techniques, and rescued the viruses in insect cells. We then performed cryo-electron microscopy (EM) of purified particles to assess structural characteristics, thin-section EM of EILV chimera-infected vertebrate cells to capture early entry events, luciferase-based reporter assays to evaluate genome delivery in vertebrate cells, and safety and immunogenicity studies in mice comparing EILV chimeras to formalin-inactivated and UV-inactivated preparations of vaccines.

Results and Conclusion: We found that EILV chimeras are structurally identical to their pathogenic counterparts, can enter vertebrate cells via clathrin-mediated endocytosis, deliver RNA, and provide superior immune responses to formalin-inactivated virus in mice while retaining a host-restricted replication phenotype in vivo. Finally, we performed an immunogenicity and efficacy study in non-human primates vaccinated with a single dose of EILV/CHIKV and demonstrated rapid seroconversion with high titer neutralizing antibody titers followed by complete protection against challenge with CHIKV.

Reference:

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P7

Probing the Humoral Immune Response against Respiratory Syncytial Virus to Guide Rational Vaccine Design

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Objective: Discuss how structure-based design has led to the development of promising RSV vaccine antigens and how characterization of the humoral response against RSV can lead to novel or improved vaccine candidates.

Background: Respiratory syncytial virus (RSV) causes acute lower respiratory tract infections that result in substantial morbidity and mortality in infants and the elderly. We believe that vaccine development can be guided by characterizing the spectrum of antibodies that each target population can elicit, and then designing vaccine antigens to preferentially induce the most protective antibodies. For RSV, it is generally agreed that an effective vaccine should elicit neutralizing antibodies against the viral fusion glycoprotein (RSV F). However, we are only just beginning to understand the breadth and properties of antibodies that the human immune system can generate against RSV F. Previously, we determined X-ray crystal structures of the prefusion (pre-F) and postfusion (post-F) conformations of RSV F[1,2], and using the structural information we engineered a soluble, prefusion-stabilized RSV F protein that elicited high neutralizing titers in mice and rhesus macaques, and provided proof-of-principle for structure-based vaccine design.[3]

Methods: To inform the design of new or improved antigens, we have begun characterizing the antibody response to RSV F at a molecular level. Using pre-F and post-F to sort memory B cells from RSV-infected infants and adults, we have cloned and characterized hundreds of RSV F-specific antibodies representative of responses

to natural infection. For each antibody, we have determined its neutralization potency and its binding affinity for pre-F and post-F.
Results and Conclusion: From these data, we have learned that infants can generate potent neutralizing antibodies, including some that lack somatic hypermutation. Competition mapping studies have identified several novel neutralizing antigenic sites in addition to the five known sites[4,5] on RSV F that could serve as new targets for epitope-focused vaccine antigen design. The results from these studies are expected to guide the development of safe and effective RSV vaccines and create a platform of reagents and technologies to rapidly assess vaccine performance in future clinical trials.

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Analytics for High Throughput (HT) Vaccine Antigen Characterization

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Objective: Identify two novel analytical technologies suitable for HT Vaccine formulation development.
Background: Our goal is to improve the vaccine formulation development process by incorporating high throughput formulation development (HTFD) into the early vaccine development program. Vaccine HTFD will increase the ability to screen for stability dramatically more vaccine formulations (> 250 formulations) in much less time (< 1M) with significantly less antigen. HTFD alone cannot confirm the optimal vaccine drug product formulation for both efficacy and stability. However, by rapidly examining increased amounts of formulation conditions and excipients, HTFD should give a better understanding of the formulation input factors that impact vaccine drug product stability and identify a vaccine formulation design space that might result in optimal stability.
Methods: We have focused on two HT analytical technologies, differential scanning fluorimetry (DSF) and dynamic light scanning (DLS). These technologies are particularly well suited for quickly comparing the thermal stability of a large number of protein antigen formulations. DSF, with extrinsic fluorescence dyes such as Sypro Orange™ and Proteostat™, is an excellent method to establish the unfolding (Tm) and aggregation temperatures (Tagg) of protein antigens. Proteostat™ can even measure Tagg in the presence of formulation surfactants. The vaccine formulation composition can influence both Tm & Tagg. Formulations with high Tm & Tagg are typically the most thermally stable.
Results and Conclusion: Screening utilizing DSF pH and ionic strength screens were successfully completed in 96 well plates for three model antigens (AgA, AgC, and AgC). DLS temperature scans were used to directly measure the aggregation onset temperature of a model antigen with or without surfactants. DLS technology provided an excellent orthogonal HT stability assay to DSF measurements of antigen unfolding and aggregation. We have shown that these HT analytical technologies are well suited for vaccine formulation development.

Reference:

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