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Manipulation of Virus-Like Particles for the Purpose of Optimizing Immunostimulation

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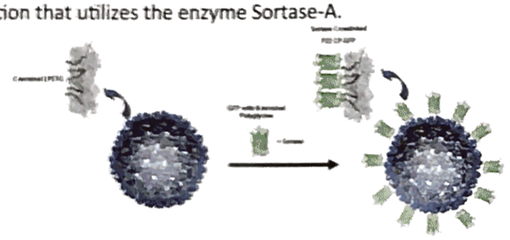
Introduction

Traditional vaccination methods rely on cultivation and attenuation of live viruses to prevent pathogenicity upon inoculation into live hosts. This presents the limitation that the vaccine will possess viruses with antigens that may not be conserved throughout other strains of the same virus, a problem yet to be overcome in the year-to-year mass production of the influenza virus. In addition, killing the virus may alter the structure of some proteins in such a way that they are no longer immunogenic.

Virus-like particles (VLPs) are proteins derived from those that self-assemble to form the capsid coat of the viral structure, are non-pathogenic, and yet elicit immune responses similar to the native virus. They are ideal as carries for immunogenic proteins, while serving as antigens themselves. They do not require attenuation. This can create a vaccine with multivalent display that creates an extremely targeted and efficient immune response. Here we evaluate the VLP derived from the P22 bacteriophage in the influenza virus as a scaffold for the construction of immunotherapeutic nanomaterials with the intent to elicit targeted immune responses in the live organism. The strategy is to attach immunogenic protein cargo to the exterior of the VLP for multivalent display using a method of protein ligation that utilizes the enzyme Sortase-A.

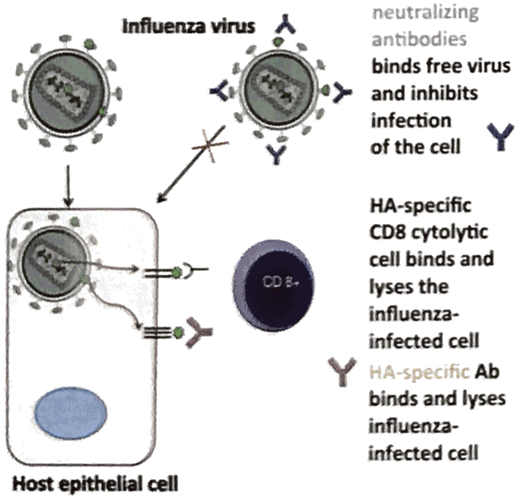
For proof of concept, we evaluate the attachment of Green Fluorescent Protein (GFP) to the exterior of P22. The goal is to attach Hemagglutinin, protein native to the influenza virus.



Targeting Influenza Immune Responses

-Certain immune cells (B-cells) produce neutralizing antibodies that prevent invading viruses from causing disease.

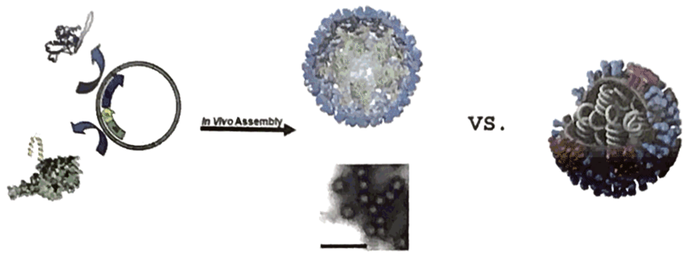
- Evading the initial immune defenses influenza virus will invade the healthy cell. Components of the influenza virus (antigens) are released inside the host cell, which are subsequently displayed on the exterior cell surface to signal infection. The immune system CD8+ cell target these foreign antigens to remove infected cells.



-Non-neutralizing antibodies specific to the viral antigens are also produce to recruit more immune cells in the fight.

-Virus-Like Particles (VLPs) can be used to mimic the molecular structure of viruses leading to immune responses produced by viruses without pathogenicity.

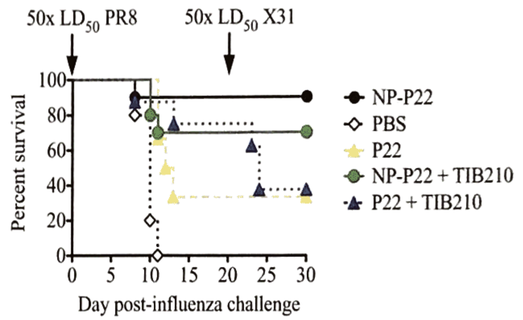
Evidence for P22 VLP Based Vaccines



-P22 VLPs can be used as scaffold to harbor protein antigens, such as the nucleoprotein of influenza (NP), on the interior of the VLP, mimicking the normal viral display.

- P22 VLPs containing NP on their interior, NP-P22, elicited strong protective immune responses when tested in mice inoculated with 50 times the lethal dose of influenza.

- Mice inoculated with NP-P22 had 90% survival at 30 days post-influenza challenge, whereas controls (PBS) showed to 100% mortality at 12 days out.



Manipulating the Virus-Like Particle

-The NP-P22 experiment validated the P22 VLP as a scaffold for stimulating immune response pathways, however the NP targeted pathway did not prevent infection.

- Developing methodology for external display of antigens from P22 is hypothesized to provide a means toward activating neutralizing antibody immune response, when antigens such as Hemagglutinin (HA) are used to decorate the VLP.

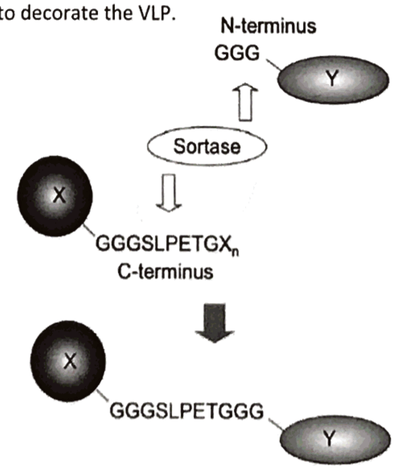
-The C-terminus of P22 coat proteins have been shown to be exposed on the exterior of the P22 VLP and can be exploited for antigen display.

-Attachment of antigens to P22 VLP is hypothesized through a Sortase-A mediated ligation.

-Sortase-A is an enzyme that covalently attaches the peptide sequence G-LPETG to another protein containing an N-terminal poly-glycine sequence.

- The LPETG sequence has been added to our P22 VLP providing attachment points for model antigens, such as GFP and HA, modified with N-terminal poly-glycine sequences

- The addition of Sortase-A in the presence of these proteins is hypothesized to cause covalent linking between the two proteins.



Protein-Protein Fusion Catalyzed by Sortase A

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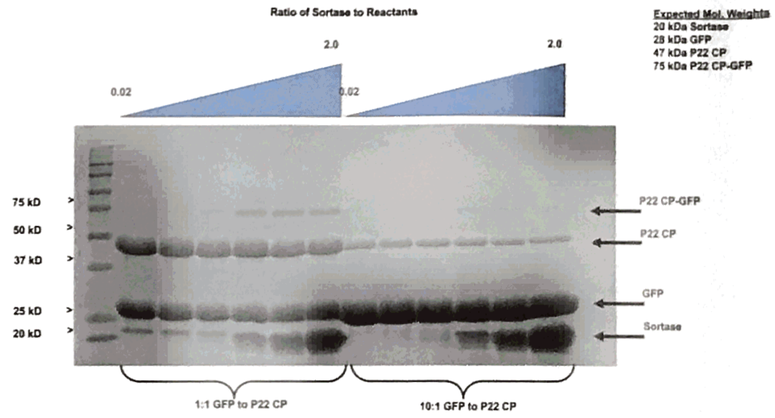
P22-Antigen Sortase Mediated Ligation

-E. coli cells were inoculated with plasmid DNA encoding P22 VLP and poly-gylcine-GFP.

-Cells were cultured and induced to express the P22 VLP and poly-glycine-GFP proteins, which were subsequently purified by nickel affinity chromatography.

-The purified proteins were evaluated for their ability to be ligated via Sortase A.

-Confirmation of successful ligation was indicated by SDS-PAGE, which allows separation of proteins by size and visualization upon staining of proteins.

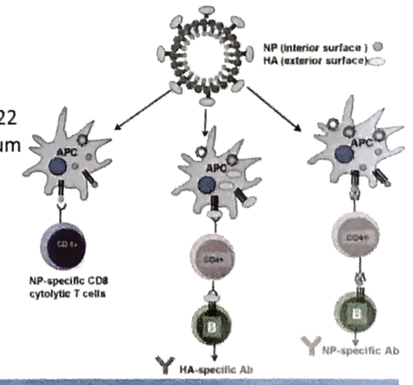


Future Work

-Characterize the ligated GFP-P22 further via Western Blot antibody screening, as well as electron microscopy.

-Successfully perform ligation to create HA-P22

-Screen vaccination in mice and evaluate serum antibody response



Acknowledgements

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