Development of a VLP-based RSV vaccine targeting pre-fusion F-protein

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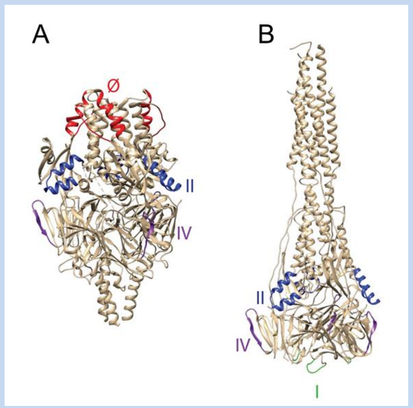
Abstract

Respiratory syncytial virus (RSV) is a human pathogen that is a predominant cause of acute lower respiratory tract infection and serious respiratory illness in both children and the elderly. There is no current FDA licensed vaccine for RSV infection and the only approved prophylactic treatment is the RSV neutralizing mAb palivizumab.

Agilvax's innovative vaccine technology is based on bacteriophage MS2 virus-like particles (VLPs). We have produced MS2 VLPs to display high-complexity, random sequence peptide libraries, which are used for affinity-selection on known RSV F-protein neutralizing antibodies discovered from patients that resolved RSV infection. Using this technology, we can identify epitope mimics of the selecting antibodies which are displayed in a multivalent format on the VLP surface. Immunization with the selected VLPs elicits long-lived, high titer antibody responses that mimic the neutralizing antibody.

Using the affinity-selection approach, Agilvax has identified and produced VLPs displaying antigenic RSV peptides. In our initial study, immunizations elicited significant levels of RSV neutralizing antibodies. To further increase the neutralization response, we have evaluated adjuvanted vaccine formulations and have identified new VLP candidates that bind to different antigenic regions of the pre-fusion F-protein. The results of these studies showed a significant increase in binding of immune sera to F-protein. Ongoing studies are measuring serum neutralization levels. Candidates will be further tested in the cotton rat model of RSV infection.

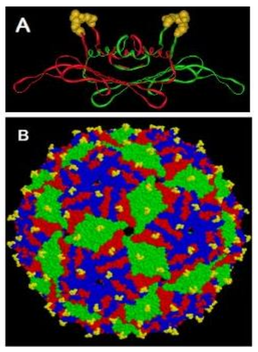
Pre-fusion and post-fusion RSV F-protein



Pre-fusion (A) and post-fusion (B) structures of RSV F. Neutralizing monoclonal antibodies used for affinity selection in our studies target the RSV pre-fusion F-protein and bind to distinct antigenic sites, including sites II and IV. Adapted from Widjaja *et al.* (2016), *Journal of Virology*, 90(13), 5965-5977.

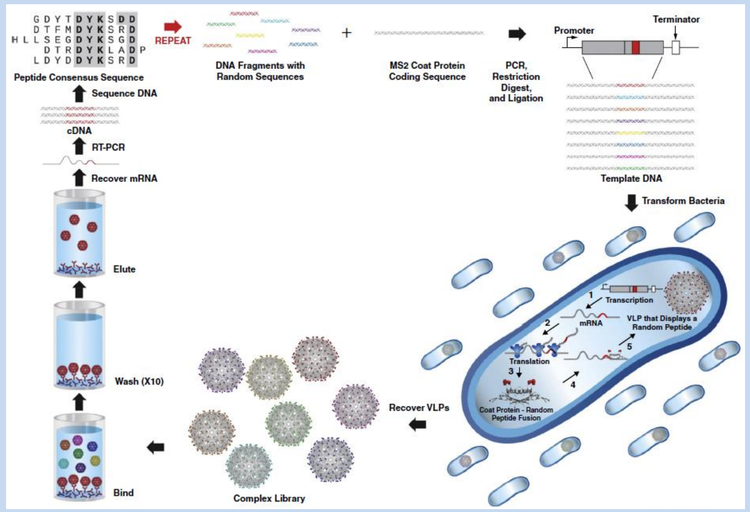
Agilvax's VLP Technology

- Highly immunogenic heterologous antigen display technology
- Antigen display by simple genetic insertion or chemical conjugation
- High-level production and scalable expression from *E. coli*
- Encapsulate endogenous TLR7 adjuvant (ssRNA)
- Dry powder formulation eliminates need for cold chain



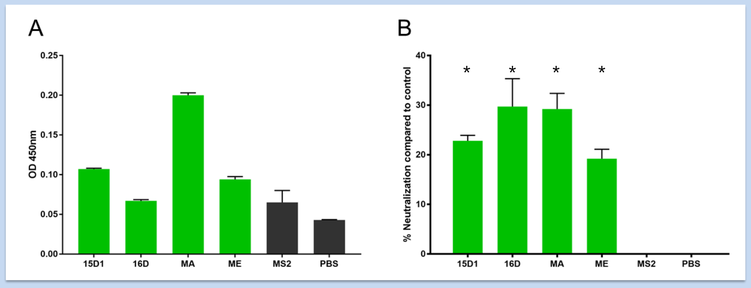
The structure of MS2. (A) The coat protein dimer with the two identical chains in red and green ribbons, and the AB-loops emphasized in yellow space fill. (B) The MS2 VLP with the AB-loops highlighted in yellow. Coat protein exists in three slightly different conformations, here shown in red, blue, and green. Adapted from Peabody *et al*. (2008), *J Mol Biol*, 380(1), 252-263.

Identification of vaccine candidates



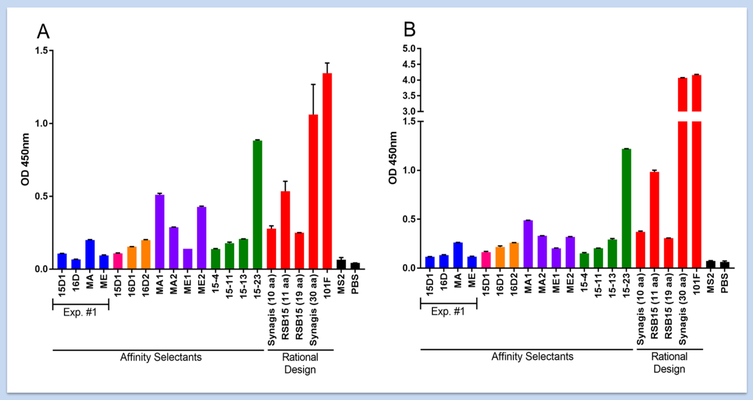
VLP Affinity Selection. We have produced nine different VLP libraries of greater than 10^{10} members displaying random peptide sequences ranging from 6 to 15 amino acids into the AB-loop of MS2. After an initial round of affinity selection using a monoclonal antibody, RNA, containing the peptide sequence, is isolated and amplified from VLPs by RT-PCR and then cloned into the MS2 coat protein expression vector. After 3 or 4 rounds of affinity selection, individual VLP plasmids are isolated and peptide inserts are sequenced. Adapted from O'Rourke *et al.* (2015), *Current Opinion in Virology*, 11; 76-82.

F-protein binding and RSV neutralization



Affinity selected VLPs elicit antibodies that bind to pre-fusion F-protein by ELISA and neutralize RSV infection *in vitro*. (A) DS-Cav1 was bound to a nickel coated plate and then probed with pooled immune sera (1:50 dilution). Data shown is the average of duplicate measures and the error bars represent SEM. (B) Pooled immune sera (1:30 dilution) was used for *in vitro* microneutralization assays. Data shown is the average of triplicate measures and the error bars represent SEM. All VLP immune sera showed significant neutralization compared to mice immunized with wild-type MS2 or with PBS (Student *t*-test; $P<0.05$).

RSV VLPs elicit antibodies that bind to F-protein



Affinity selected and rational design VLPs elicit antibodies that bind to pre- and post-fusion F-protein by ELISA. (A) DS-Cav1 or (B) RSV-F Protein (Sino Biological) was bound to nickel coated plates and then probed with pooled immune sera (1:50 dilution). Data shown is the average of duplicate measures and the error bars represent SEM.

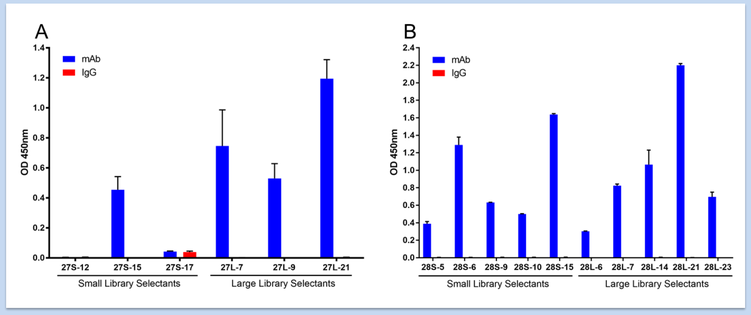
Next generation RSV vaccine candidates

Selecting mAb	# Unique Sequences	# Producing VLPs	# Binding mAb
27-small	4/24	3	1
27-large	8/24	4	3
28-small	15/24	11	10
28-large	20/22	19	17

Selecting mAb	# Unique Sequences	# Producing VLPs	# Binding mAb
28-small	16/24	14	9
28-large	7/24	7	5

Affinity selections were performed using 2 anti-RSV neutralizing antibodies. After rounds 3 (top table) and 4 (bottom table) of selection, we sequenced 24 clones from each population. Small and large indicates selections performed with random VLP libraries displaying peptides ranging from 6-9 or 10-15 amino acids in length, respectively.

RSV VLPs bind to selecting mAbs



Affinity selected VLPs bound to selecting monoclonal antibodies, but not to human IgG control. Capture ELISA. (A) mAb 27 and human IgG or (B) mAb 28 and human IgG were bound to a plate and then probed with individual VLPs. Data shown is the average of duplicate measures and the error bars represent SEM.

Further development of AX14

- RSV *in vitro* neutralization assays using selected immune sera will be conducted in the laboratory of Dr. Mark Peeples
- Efficacy and safety studies of RSV VLP vaccine candidates using the cotton rat (*Sigmodon hispidus*) model of RSV infection will be performed at Sigmovir Biosciences
- Dose escalation, adjuvant, and route of immunization studies will be completed to determine the optimal AX14 vaccine formulation

Conclusions

- Affinity selection has identified RSV VLP candidates that may bind to different antigenic regions of pre-fusion F-protein
- RSV VLP immunization elicited antibodies that bound to pre- and post-fusion F-protein
- RSV VLP immunization generated neutralizing antibodies
- Adjuvanted vaccine formulations enhanced the F-protein antibody response
- AX14 is based on a well established VLP platform/technology that has shown efficacy and safety in the clinic

Acknowledgements and Funding

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