

# The Immunogenicity and Efficacy of a Virus-Like Particle Vaccine Candidate against Respiratory Syncytial Virus in Mice

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## Introduction

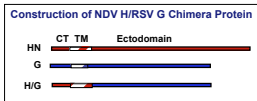
Human respiratory syncytial virus (HRSV) is the primary cause of serious respiratory disease in infants and young children worldwide. The virus is also increasingly recognized as a significant pathogen in elderly populations. Bronchiolitis or pneumonia results in 25-40% of HRSV infections of infants and between 0.5 and 2% of these infections result in hospitalization. Despite the incidence and severity of these infections, there is no HRSV vaccine available. Immunoprophylaxis remains the only effective defense against this viral disease.

Virus-like particles (VLPs) are increasing being considered as safe, effective vaccine candidates. VLPs are particles released from cells expressing the major structural proteins of a virus. These particles are structurally similar to authentic virus and possess native conformational epitopes displayed in an array typical of virus particles. However, VLPs do not contain a genome and are, therefore, incapable of a spreading infection.

We have previously demonstrated that cells expressing the major structural proteins of Newcastle disease virus (NDV) release VLPs very efficiently and these VLPs stimulate robust immune responses in mice.

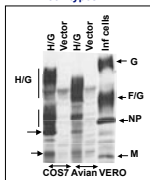
**Hypothesis:** The G protein of RSV can be incorporated into ND VLPs and these particles can be used to stimulate protective immune responses in a murine model system.

## Construction of ND VLPs containing RSV G protein



The NDV HN protein TM and CT domains were fused to the ectodomain of the RSV G protein to create an H/G chimera protein.

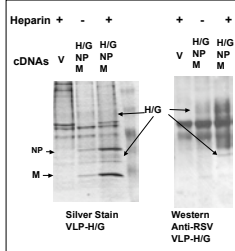
### Expression of H/G in different cell types



Expression of the chimera protein in different cell types resulted in different levels of glycosylation. Identical results were obtained with wild type G protein (not shown).

**Conclusion:** Chimera H/G protein can be expressed.

### ND VLPs containing H/G (VLP-HG)



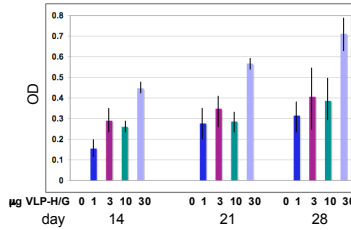
ND VLPs containing the NDV M and NP and the H/G were released from avian cells. Efficient release of particles required addition of heparin to transfected cells. The H/G chimera protein was approximately 15-20% of the total VLP protein. M and NP, NDV membrane and nucleocapsid protein; H/G, chimera protein; V, vector DNA.

**Conclusion:** ND VLPs containing the G protein ectodomain (VLP-HG) can be produced.

## Antibody Responses to VLP-H/G

**Protocol:** Mice were immunized with intraperitoneal inoculation (IP) with 1, 3, 10, or 30 µg total VLP-H/G protein (5 mice/group) or comparable amounts of UV inactivated, purified RSV. Amounts of G protein in VLP-H/G and UV-RSV were comparable. Another set of mice received live RSV intra-nasally (IN) ( $3 \times 10^6$  pfu). A third set of mice was injected with PBS. Serum was obtained from the tail vein at days 14, 21, and 28.

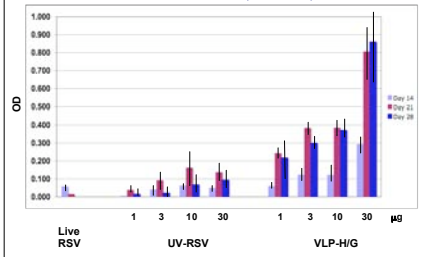
### Antibody Responses to Increasing Doses Of VLP-H/G



Antibody levels to the G protein were determined by ELISA assays using RSV infected VERO cell extracts as capture antigen. Average of results from each group of five mice are shown. Error bars indicate the standard deviation of responses within each group. Sera was diluted 1/100.

**Conclusions:** VLP-H/G stimulated antibody responses to the fully glycosylated G protein in infected VERO cells. Responses to all concentrations of VLPs were detected with increasing responses with larger doses of antigen.

### Comparison of Antibody Responses to RSV G protein After immunization with VLP-H/G, UV-RSV, and Live RSV



To compare antibody responses to the G protein after immunization with VLP-H/G or RSV, ELISA assays were accomplished using as capture antigen extracts from avian cells transfected with cDNA encoding the RSV G protein. As determined by Western analysis, the amount of G protein in the avian extract used was the same as the amount of G protein in the RSV infected VERO cell extracts used in the figure above. Error bars indicate standard deviation of responses within each group of five mice.

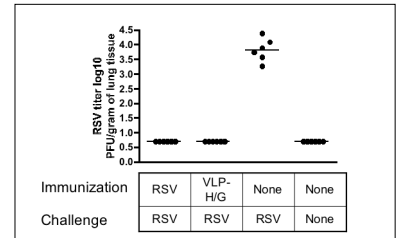
### Conclusions:

1. Antibody responses to RSV G protein after immunization with VLP-H/G were better than responses to live RSV or comparable amounts of UV-RSV.
2. Antibody responses to VLP-H/G were similar using as capture antigen the fully glycosylated G protein in infected VERO cell extracts or the under glycosylated G protein in avian cell extracts.

**Not shown:** The differences in antibody responses cannot be attributed to the under glycosylation of G protein in avian cells. A similar difference in antibody levels to the G protein in serum from Live RSV, UV-RSV and VLP-H/G immunized animals was also seen using as capture antigen extracts from COS-7 cells transfected with the RSV G protein cDNA.

The low level of responses to the G protein in RSV immunized animals does not reflect a failure of the virus to stimulate murine antibodies. Immunization of mice with either live or UV-RSV stimulated significant antibody responses to proteins present in RSV infected VERO cells.

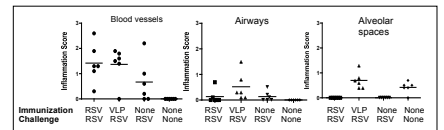
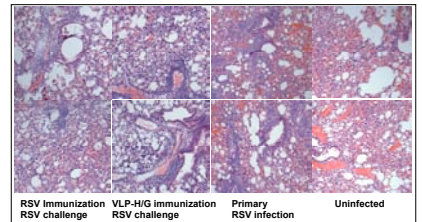
## VLP-H/G immunization prevents virus replication in the lungs of challenged mice



Mice immunized with 30 µg VLP-H/G and boosted with 10 µg of VLP-H/G (IP) were challenged with  $3 \times 10^6$  pfu of RSV by intranasal inoculation (IN). Control mice were immunized and boosted with live RSV ( $3 \times 10^6$  pfu IN). Another set of mice received no immunization. Four days after challenge, the titer of virus in lungs was determined by plaque assay. Virus was detected only in mice not previously immunized. Values shown in immunized mice are the limit of detection in the assay.

**Conclusion:** VLP-H/G immunization protected mice from RSV replication in lungs.

## Histology of lung tissue from mice after RSV challenge



Tissue sections obtained at 4 days after RSV challenge were stained with H and E. Representative fields are shown. At least 10 fields for each group were blindly scored for inflammation of blood vessels, airways, and alveolar spaces. The scores for each mouse are shown in the tables. Differences between RSV and VLP immunized mice were not statistically significant.

**Conclusion:** RSV challenge after VLP-H/G immunization did not result in inflammatory responses significantly different from those after prior RSV infection.

## Conclusions

1. The ectodomain of the RSV G protein can be efficiently incorporated into ND VLPs.
2. VLP-H/G stimulate a better antibody response to RSV G protein than a comparable amount of UV inactivated RSV or live RSV.
3. VLP-H/G immunization protects mice from viral replication in the lungs.
4. There is no evidence of enhanced immunopathology after RSV infection of VLP-H/G immunized mice.