Baculovirus surface display of influenza virus haemagglutinin and its potential as a vaccine

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1- Aims of the study

- To display Flu virus haemagglutinin (HA) on the Autographa californica multiple nucleopolyhedrovirus (AcMNPV) envelope surface
- To investigate the display of native HA vs truncated HA51 in *S. frugiperda* (Sf9) and *Trichoplusia ni* (T.ni Hi5) insect cell lines
- To compare immune responses in mice

2- What is a baculovirus?

Baculoviruses constitute a family of large circular, double-stranded DNA viruses with a genome size ranging from 80 to 180 kilo base pair, which mainly infect the insect orders Lepidoptera, Hymenoptera and Diptera. The life cycle of baculoviruses includes two distinct virion phenotypes in a single infection cycle. Budded viruses (BV) are produced during the late phase of the infection cycle (Figure 1 A) and occlusion derived viruses (ODV), which are produced in the very late phase of the infection in the nucleus, and are further packaged in a protein crystal called the polyhedron (Figure 1 B).

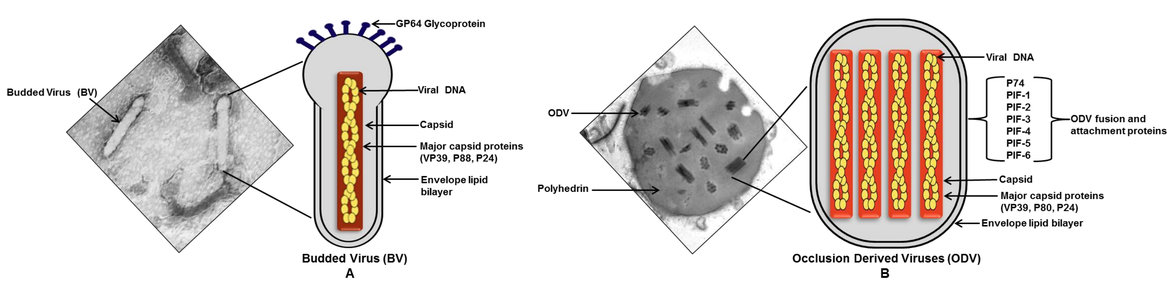

Figure 1: Schematic representation of the two structure forms of baculoviruses. A: The AcMNPV budded virus (BV) EM image and its model structure. The BV includes a single nucleocapsid that is enclosed in a lipid bilayer and it has GP64 envelope protein at the apical ends. **B:** AcMNPV occlusion body (OB) EM image including multiple ODV and their structures; the ODV includes multi nucleocapsids that are enclosed in a lipid bilayer.

3- Why Baculovirus for vaccines development?:

- Easy & simple to generate the recombinant virus
- High yield of protein within a short time
- Do not replicate in mammalian cells
- No pre-existing immunity or cytotoxicity

4- Construction of truncated & Native HA vector

- Two vectors were made to express a truncated HA51 or native HA under control of the *polh* promoter (Figure 2 A & B, respectively)
- Recombinant viruses AcSurf-HA51^{polh} or AcRP19HA^{polh} were constructed (Figure 2 C)

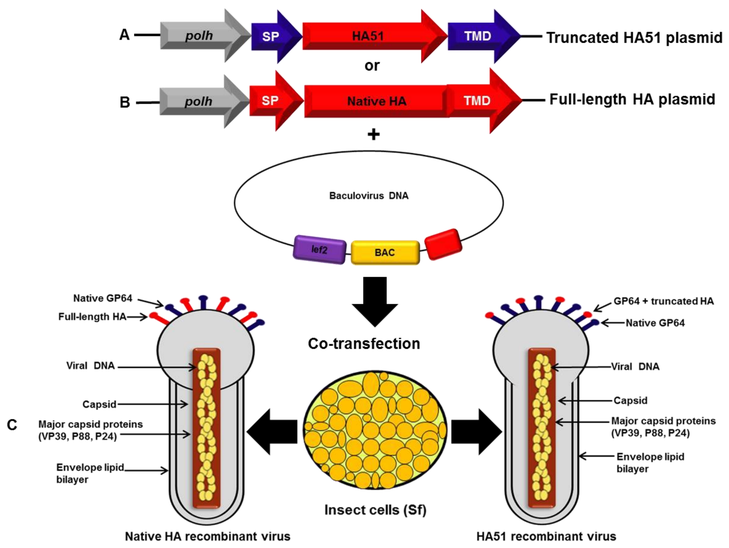

Figure 2: Schematic representation of the construction of recombinant baculoviruses A: Illustrates surface display technology where the truncated HA51 gene inserts into baculovirus GP64 envelope protein sequences between the signal peptide (SP) and the transmembrane domain (TMD); the BV includes both the wild-type GP64 & the recombinant one. **B:** Illustrates the cloning of full-length HA under *polh* promoter using its own SP & TMD. **C:** co-transfection of insect cells to produce recombinant viruses.

5- Biological activity test of the HA51 & HA proteins

- The biological activity of HA protein was tested with AcSurf-HA51^{polh} or AcRP19-HA^{polh} in Sf9 cells
- Infected cells showed clear evidence for haemadsorption of chicken red blood cells (RBCs) on the cell surface (Figure 3 C & D, respectively)

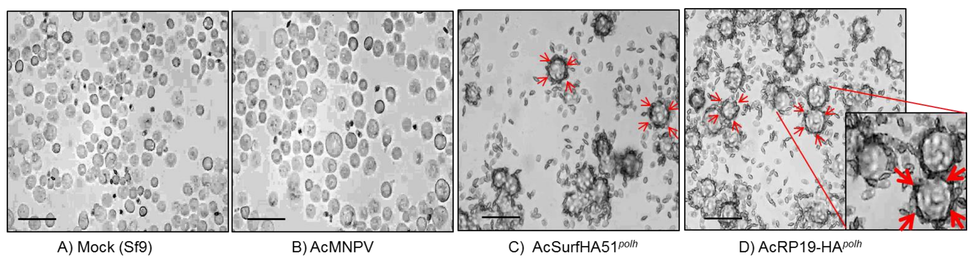

Figure 3: Haemadsorption assays of HA protein. Sf9 cells were infected with corresponding recombinant viruses and incubated at 28°C for 48 hpi, then washed with PBS before 0.5 ml of 0.5% freshly prepared RBCs were added. Cells were examined under the light microscope to show RBCs haemadsorption (100X). HA51 and native HA viruses infected Sf9 cells showed haemadsorption activity on the surface indicated with red arrows..

6- Translocation of HA51 or HA to the Sf9 cell surface

- Sf9 cells were infected with AcSurfHA51^{polh} or AcRP19-HA^{polh} to be examined by confocal microscopy
- The results confirmed that both HA51 & native HA were able to present at the plasma membrane of infected cells (Figure 4)

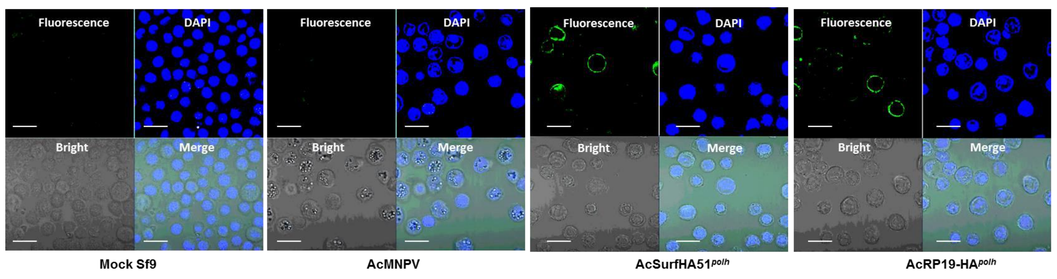

Figure 4: Characterization of HA-baculoviruses in Sf9 cells by confocal microscopy. Sf9 cells infected with AcSurf-HA51^{polh} or AcRP19-Ha^{polh} recombinant viruses. Negative controls were mock- and AcMNPV-infected Sf9 cells. Infected cells were fixed at 48 hpi and stained by indirect immunofluorescence using an anti-HA antibody. A secondary antibody conjugated to an Alexa-fluor 488 was used to visualise the HA translocated into infected cells plasma membrane using a confocal microscope. Scale bar 20µm.

7- Analysis of HA51 vs native HA display in different cell lines

- Sf9 and Tni Hi5 cells were infected with either AcSurf1HA51^{polh} or AcRP19HA^{polh}. The native HA showed best incorporation into BV envelope in both Sf9 and Tni Hi5 infected cells (Figure 5)

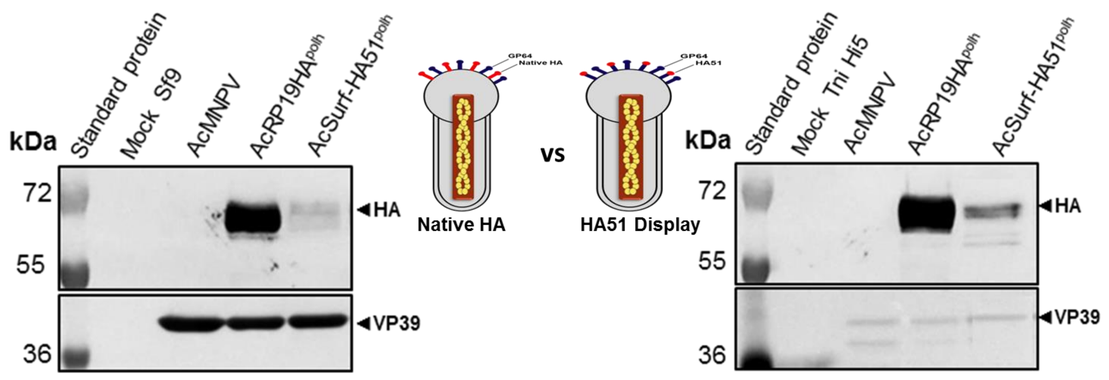

Figure 5: Comparison of native HA vs HA51 incorporation into budded virus produced in Sf9 and Tni Hi5 cells. The cells were infected with the corresponding recombinant viruses. Culture media were harvested at 72 hpi and the BV fractions were separated by SDS-PAGE gel (10%) and analysed by immunoblotting. VP39 was used as indicator for BV amount in culture media.

8- Effect of MOI and cell culture system on BV production & HA display

- Low & high MOI in addition to monolayer & shaking cultures were examined
- Shaking cultures showed the best BV production & HA display in both MOI (Figure 6). However, HA showed two different size bands, which could be due to AcRP19HA^{polh} cathepsin proteolytic activity

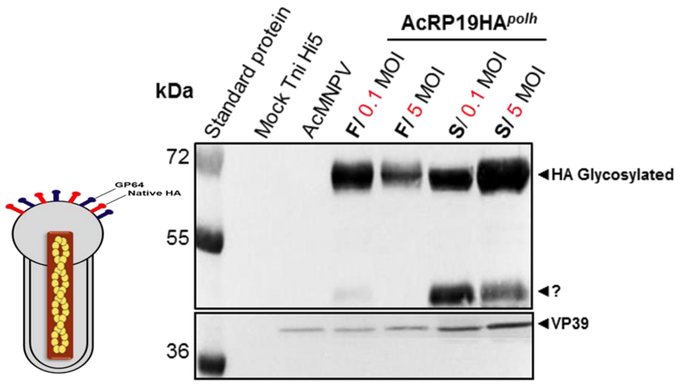

Figure 6: Analysis of HA from BV produced in TnHi5 cells infected with low (0.1 pfu/cell) or high (5 pfu/cell) MOI in either monolayer (F) or shake cultures (S). Tni Hi5 cells were seeded in 35 mm dishes or 25 ml flasks and infected with an AcRP19HA^{polh} virus using 0.1 or 5 MOI. Proteins from BV faction samples were separated by SDS-PAGE gel (10%) and analysed by immunoblotting. VP39 was used as indicator for BV amount in culture media.

9- HA stability in AcRP19HA^{polh} & FBUHA^{polh}

- Cathepsin mutant FBUHA^{polh} virus was constructed to investigate HA degradation in the culture media
- Clear evidence of Cathepsin proteolytic activity was observed in AcRP19HA^{polh} BV samples before & after storage @ 4˚C (Figure 7 A & B)
- HA from FBUHA^{polh} BV samples was stable and showed one band only at expected size (Figure 7 A & B)

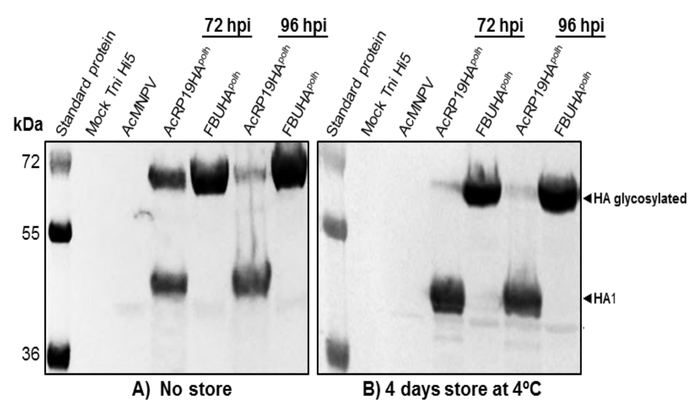

Figure 7: Analysis of the proteolytic activity of HA protein in BV produced in Tni Hi5 cells before storage at 4ºC (A) and after storage at 4ºC for 4 days (B). The cells were infected with AcRB19HA^{polh} or FBUHA^{polh} at an MOI of 5 pfu/cells. Culture media were harvested at two different time points (72 and 96 hpi). Proteins from BV fraction samples were separated by SDS-PAGE gel (10%) and analysed by immunoblotting for proteolytic activity and stability.

10- Post-vaccination serum antibody analysis

- Purified FBUHA^{polh} BV (BV-HA) & FBUHA^{polh}-infected Tni Hi5 cells (Tni-HA) were evaluated as potential vaccines
- Mice vaccinated with BV-HA elicited a high level of total HA-specific antibodies on day 21 post vaccination with an OD value of 2.02 compared to Tni-HA OD value of 1.67 (Figure 8 A)
- Sera antibodies from day 31 showed a significant increase in the OD values for both BV and infected cells (2.52, 2.24), respectively compared to day 21(Figure 8 B)

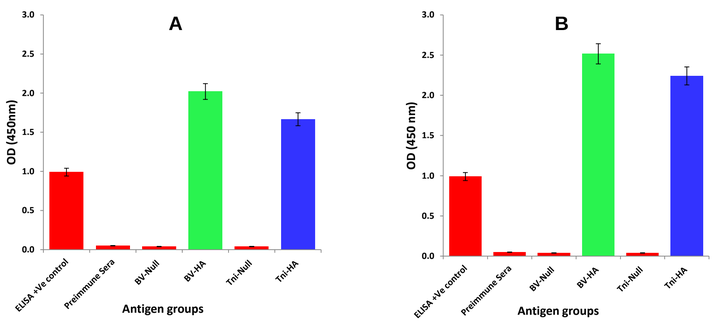

Figure 8: Analysis of post-vaccination sera antibodies using an indirect ELISA. To examine HA-specific antibodies in sera collected from vaccinated mice on 21 (A) and 31 dpv (B), a 96-well ELISA plate was coated overnight with 20 ng/well of native influenza H1N1 antigen prepared in coating buffer. Subsequently, 1:10000 dilution from each serum was examined in triplicate against the antigens. Each antigen group is represented by the average value of data obtained from four group-specific mice. Group 1: BV-Null, Group 2: Tni-Null, Group 3: BV-HA, Group 4: Tni-HA. HA antibody (Abcam Ltd) and pre-immune sera were included as ELISA positive (+ve) control and as HA-specific antibodies negative control, respectively. Error bars represents mean ± SD (n=4).

11- Conclusions

- HA51 & native HA were biologically active and able to localise within the plasma membrane of Sf9 cells
- Tni Hi5 was more efficient for HA display with BV compared to Sf9 cells
- Using baculovirus GP64 SP & TMD appear to be detrimental to HA surface display
- BV-HA & Tni-HA both elicited a strong immune response from the first immunization