A vaccine prototype using baculovirus expression system for the control of Avian Influenza Virus

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Introduction

Influenza virus causes an important disease worldwide. Avian Influenza LP and HP represent a high risk for poultry producers, and its control is a major burden according to sporadic outbreaks which are related to the distribution of HP variants. The virus causes disease in several continents. The control of AIV, HS subtype, remains as a main challenge and different kind of vaccines are available. The use of insect cells and baculovirus to produce the HS HA offers the versatility, simplicity, scale-ability and yields of a very efficient vaccine producing process.

Materials and Methods

A clade 1 sequence of H5 haemagglutinin from an Asian Avian H5N1 isolate was used as a template to chemically synthesize a codon-optimized version for expression in insect cells. When insect cells are infected with the mutK + HS HA Baculovirus, the polyhedrin promoter will subsequently direct a high level of expression of the mutK+HS HA protein. After plaque purification and subsequent scale-up in SF(+) cells, the supernatant of the recombinant baculovirus containing the mutK + HS HA gene was put down as a MSV and designated as “mutK + HS HA Baculovirus DB Master Seed Virus”.

Characterization

Stability analysis shows that the genetic construct and the protein are possible to be detected after 5 serial passages of the Master Seed virus. HS-specific primers were used to detect the 1.7 kb fragment (A), sequencing is ongoing. A monoclonal HS HA-specific antibody was used to detect the mono, di, and trimeric forms of the protein (B) whether from the first, fifth passage, or from a representative harvest (lane 2, harvest amount used to be obtained after 4 dpi).

Results

Infection with MOI of 0.5 (Green, purple) or 0.1 (Blue, Red). Cell density (A), cell viability (B), and antigen yield (C). Cell population reaches densities above 2x10^6E, and the antigen yields is the same, suggesting that the Optimal Peak Cell Density (OPCD2) must be between 2 and 3x10^6E.

Conclusions

• A vaccine prototype based on the H5 HA sequence expressed in the baculovirus/insect cells system is available.

• Process parameters have been optimized to achieve reproducible and reliable yields in the scale of 0.5 L.

• The expressed antigen triggered an immune response when administered as an inactivated oil-emulsion to 3 weeks old chickens.

• The antigen was stable upon combination with BEI-inactivated NDV and elicited a protective immune response demonstrated by challenge with NDV infectious virus.

References

