Molecular Characterisation of Newcastle Disease and Infectious Bronchitis Viruses*

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Introduction

Newcastle disease virus (NDV) and infectious bronchitis virus (IBV) are economically important poultry viruses, which are controlled through vaccination. The haemagglutinin-neuraminidase (HN) and fusion (F) proteins of NDV are immunogenic as well as protective against ND. Their molecular characterisation is important in the development of subunit vaccine against NDV. The multiplicity of serotypes in IBV has made it difficult to develop any efficacious vaccines. Diagnostic techniques are therefore required to distinguish these viruses. The objectives of this project are to amplify and study specific regions in the viral genomes (for the development of diagnostic kits); to clone and express the F and HN genes into *Escherichia coli* and *Baculovirus* vectors (for the development of subunit vaccines); and to analyse the expressed proteins.

Materials and Methods

Viruses in the study included several Malaysian IBV and NDV field isolates and reference strains. The viruses were grown, purified and their genomic RNAs were extracted. Primers were constructed to amplify specific regions in the genome by RT-PCR. The amplified products were analysed by restriction enzyme analysis and sequencing. The S gene of IBV strain MH5365/95 as well as the NP, P, M, F, HN and parts of the L genes of NDV strain AF2240 were cloned and sequenced. Diagnostic kits for IBV and NDV identification were developed using (i) universal primers for RT-PCR and (ii) nested PCR-ELISA respectively. The HN and F genes of NDV strain AF2240 were subcloned into the *Baculovirus* expression system. The expressed gene products were then studied in detail. Specific peptides sequences which bind to NDV were determined through biopanning with a phage display library. Various chimaeras and mutants are currently being constructed and their biological functions are determined.

Results and Discussion

Diagnosis of IBV and NDV. Universal primers were developed to detect IBV by RT-PCR. The complete sequence of the S gene of the IBV MH5365/95 was determined. Sequencing of the cloned S1 regions showed that some of the Malaysian IBV isolates were similar while others were to the vaccine strains. The different IBV isolates could be distinguished. Similarly, various NDV isolates could be distinguished by sequence analysis of the cleavage site of the F protein gene. A nested RT-PCR ELISA diagnostic kit has been developed for the determination and identification of NDV. This kit is more sensitive and specific then the current serological tests.

Sequence determinations of the various genes of NDV and their expressions. The sequences of all the genes of velogenic-viscerotropic NDV strain AF2240, except the L gene, have been completely determined and each given EMBL/GenBank database accession numbers. The HN, F, NP and P gene sequences have been published and the remaining gene sequences are in the process of being published. The HN gene of the heat stable V4(UPM) as well as strain AF2240 had deletions at the Arg 403 residue. However, this may not be entirely responsible for heat stability. The NP and P gene sequences have been filed for patents in Malaysia and US. The NP protein was expressed in *E. coli* as ring and herringbone-like structures. These structures were shown to be able to carry extra peptide fragments at the C-terminal end.

Cloning and expression of HN and F genes of NDV. The HN and F genes of strain AF2240 were amplified by RT-PCR and cloned into *E. coli* and PCR™ Bac *baculovirus* transfer vectors (Invitrogen). The recombinant plasmids were co-transfected with triple cut, linearised Bac-N-Blue ™ AcMNPV (Invitrogen) DNA into *Spodoptera frugiperda* (SF9) insect cells using cationic liposome mediated transfection method. The recombinant proteins expressed in the *Baculovirus* system are currently being analysed. The recombinant HN protein has been shown to be immunogenic. The HN genes of V4(UPM), V4(QUE) and AF2240 have also been cloned into *Baculovirus* and the expressed recombinant proteins were studied for the heat stability. In addition, the HN and F genes have also been cloned into *Pichia pastoris and eukaryotic* expression vectors for the development of alternative recombinant vaccines. Some positive results have been obtained for these recombinant proteins expressed as DNA vaccines. The expression of these proteins in *E. coli* are being studied in detail.

NDV proteins interactions: Work on the protein-protein interactions of the recombinant proteins are being carried out to determine the mechanism(s) of virus-cell interactions. The NDV receptors are being studied through biopanning with a phage display library. Two anti-NDV peptides have been constructed and shown to inhibit NDV replication. Chimaeras comprising various NDV protein segments with the NP protein have been constructed and their immunogenicity tested. These results are in the process of being published and a patent is being filed in Malaysia and the US.

Conclusions

Diagnostic tests for IBV and NDV have been developed. The complete sequences of the NP, P, M, F and HN genes of NDV strain AF2240 and S gene of IBV strain M5365/95 were determined and given EMBL/GenBank database accession numbers. Strain AF2240 differed in the length of the HN protein and the absence of the Arg (403) residue in the F protein. The F and HN genes of NDV strain AF2240 were cloned and expressed in the *Baculovirus*, *E. coli*, yeast and other expression systems. Anti-NDV peptides have been developed. The receptor and protein-protein interactions of the NDV proteins are being studied in detail. The NP protein can be expressed as a ring structure in *E. coli* and may be suitable as a carrier in future drug delivery system.

Benefits from the study

Development of diagnostic kits and subunit vaccines for NDV and IBV; patents for the PCR-ELISA kit and NP and P gene sequences; and training of molecular biologists.

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None.

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