MOLECULAR CHARACTERISATION OF NEWCASTLE DISEASE AND INFECTIOUS DISEASE VIRUSES

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Introduction

Newcastle disease virus (NDV) and infectious bronchitis virus (IBV) are two major pathogens of poultry with worldwide distribution which are controlled through vaccination. However, these diseases occur occasionally even among vaccinated flocks. Any effort to develop an effective vaccine against these RNA viruses would require a detailed understanding of their molecular biology and mechanism of infection. The haemagglutinin-neuraminidase (HN) and fusion (F) proteins of NDV are immunogenic and protects the chicken against ND and these proteins are involved in viral pathogenesis. Their molecular characterisation in our local strains/isolates would benefit in the development of subunit vaccine against NDV. In addition, the multiplicity of IBV strains and serotypes has made it difficult to develop efficacious vaccines against IBV. Therefore the objectives of this project were: (1) to amplify and study specific regions in the genomes of NDV and IBV; (2) to clone the glycoprotein genes (F and HN) into *Escherichia coli* and Baculovirus vectors and (3) to analyse the expressed proteins.

Materials and Methods

Viruses in the study included 12 IBV field isolates, the reference strains M41, Beaudette and H120; 20 NDV field isolates, reference strains S, AF2240, V4(UPM), V4(QUE) and F. The viruses were grown in embryonated chicken eggs or in tissue culture, purified by centrifugation and their genomic RNAs were extracted using the hot phenol or TRIZOL methods. These were either used in diagnostic studies or for cloning work. Primers were constructed for the polymerase chain reaction (PCR) in both genomes. The PCR products were analysed by restriction fragment length polymorphism (RFLP), single strand conformational polymorphism (SSCP), denaturing gradient gel electrophoresis (DGGE) and sequencing. In addition, specific genes from our local isolates, IBV strain MH5365/95 and NDV strain AF2240, were studied in detail. The spike genes of strain MH5365/95 as well as the M, F and HN genes of strain AF2240 were sequenced. The HN and F genes of strain AF2240 were then cloned into the Baculovirus expression system. The expressed gene products were then analysed in detail.

Results and Discussion

Diagnosis of IBV and NDV. The complete sequence of the S gene of the Malaysian isolate MH5365/95 was determined. Universal primers were then constructed to amplify the S1 fragment of 12 local field isolates including the reference strains and their pathogenicity was analysed. The PCR products were then cloned into TA cloning vector (Invitrogen) and sequenced. Three of the isolates were similar to the vaccine strains but the remaining nine isolates had sequences which were different. Our technique was able to distinguish the different strains/isolates of IBV. The cleavage site of the fusion (F) protein gene of NDV isolates were analysed by sequencing, restriction fragment length polymorphism (RFLP), single-strand conformational polymorphism (SSCP) and denaturing gradient gel electrophoresis (DGGE). It was possible to distinguish between the various isolates. Our sequencing results confirmed that the cleavage sites of the velogenic isolates were more basic than those of the other pathotypes. A PCR-ELISA technique has been developed to detect NDV. Sequence determinations of M, F and HN genes of NDV. The sequences of all three genes of velogenic-viscerotropic NDV strain AF2240 have been sequenced and each given EMBL/GenBank database accession numbers. The HN gene sequence has been published (Yusoff et al. 1998) . In addition, the HN gene of vaccine strain V4(UPM) was sequenced and compared to that of V4(QUE). It was found that the HN gene of the heat stable V4(UPM) as well as strain AF2240 had deletions at the Arg 403 residue. Based on these sequences, we can classify strain AF2240 in group C in phylogenetic studies. In addition, strain AF2240 has a new class of HN protein. Cloning and expression of HN and F genes of NDV. The HN gene of strain AF2240 was cloned into pAMP1 vector and transformed into *E. coli* DH5↦. The HN protein was expressed and detected by Western blot analysis. The HN gene was amplified by one-step RT-PCR and cloned into PCR ™ Bac baculovirus transfer vector (Invitrogen). The recombinant pAF2240-1 was co-transfected with triple cut, linearised Bac-N-Blue ™ AcMNPV (Invitrogen) DNA into *Spodoptera frugiperda* (SF9) insect cells using cationic liposomes mediated transfection method. Similarly, the F gene was cloned into the pBlueBacHis2 A, B and C vectors (Invitrogen) and transformed into TOP10F' cells before transfection into SF9 cells. The recombinant proteins which are expressed in the Baculovirus systems are currently being analysed. The recombinant HN protein has been shown to be immunogenic and seemed to give some protection against NDV. However, more work has to be carried out for confirmation.

Conclusions

The NDV and IBV isolates could be distinguished using PCR and other techniques. The PCR-ELISA technique for NDV is available for commercialisation. The M, F and HN genes of local challenge NDV strain AF2240 and S gene for local nephropathogenic IBV strain M5365/95 were sequenced and their sequences have been given EMBL/ GenBank database accession numbers. Strain AF2240 appears to be different from the other NDV strains. The F and HN genes of NDV strain AF2240 were also cloned into the Baculovirus expression system and are being developed as subunit vaccines.

References

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