Development of an effective diagnostic tool and novel vaccine against infectious bursal disease virus infection

Abdul Rahman Omar

Faculty of Veterinary Medicine
Universiti Putra Malaysia
43400 UPM, Serdang, Selangor
Malaysia

Telephone Number of Corresponding Author: 03-89468268
E-mail of Corresponding Author: aro@vet.upm.edu.my

Key words: very virulent IBDV, RFLP, VP2 subunit and DNA vaccine

Introduction

Infectious bursal disease virus (IBDV) infection is an economically important and highly contagious immunosuppressive disease of young chickens. In Malaysia very virulent (vv) IBDV was first diagnosed in the 1990's. IBD can be diagnosed using serology assays. These assays detect the presence of IBDV antigen and/or antobody but do not identify the serotype or antigenic subtype of the virus. Identification of IBDV serotypes/subtypes is essential since vaccination will not always induced cross protection. Some commercially available live attenuated vaccines have been shown able to induce protection against vvIBDV infections. However, the vaccines are still pathogenic and able to induce immunosuppression. Development of VP2 subunit and DNA vaccines probably able to produce a safer vaccines that able to induce a complete protective immunity following challenge with vvIBDV

Materials and Methods

Local IBDV isolates (6 different isolates) isolated during the outbreaks between 1991 to 1997 were analyzed by RFLP of the VP2 gene and pathogenicity studies in SPF-chickens. In addition, the putative full-length of the VP2 gene (~1.35 kb) were also sequenced and compared with other IBDV isolates characterized elsewhere. The origin of the local IBDV strains was later abalyzed by phylogenetic analysis. Two of the six IBDV isolates, each of typical and atypical vvIBDV based on RFLP and sequence analysis of the VP2 gene were further analyzed by complete sequencing of the entire genome (segments A and B). The VP2 gene from these two isolates were also cloned into different expression vectors; an inducible vector, pRSet for expression in bacteria as subunit vaccine and in pVAX and pcDNA-based vectors for expression in mammalian cells as DNA vaccine. The developed VP2 vaccines (subunit- and DNA-based) were then used in SPF-chickens experiments for determining the vaccines immunogenicity as well as ability to induce protection against development of lesions in the bursa and mortality

Results and Discussion

VP2 RFLP and sequence analysis revealed that all the isolates were of vvIBDV henotype based on the presence of SspI site. In addition, all the isolates lack markers of classical and attenuated/mild IBDV isolates and also positive for StyI site. Thus, all the isolates can be classified as vvIBDV. In addition, all the isolates except 94/273 can be classified as typical vvIBBDV. The 94/273 despite for having SspI site, do not has StyI site. In addition, based on the pathogenicity studies, all the isolates except 94/273 cause high mortality (> 70%) in SPF-chickens with the development of lesions in bursal and non-bursal tissues. The 94/273 caused only 10% mortality with bursal lesions. Sequence analysis indicated all the isolates have vvIBDV markers at the VP2 gene with amino acids (aa) substitutions at position P222A, V256I, L294I. However, the 94/273 also demonstrated aa substitutions as found in variant (G254S) and attenuated (A270E) strains of IBDV. Thus, all the isolates were classified as typical vvIBDv whereas the 94/273 was classified as atypical vvIBDV. Sequence analysis of the entire genome of both Immunogenicity in SPF-chickens indicated that chickens inoculated with the subunit vaccine developed IBDV-specific antibody as measured by ELISA. Likewise, chickens inoculated with VP2 DNA vaccine alone also developed specific-antibody.. However, both vaccines failed to provide 100% protection following challenged with vvIBDV

Conclusions

RFP analysis of VP2 able to differentiate IBDV subtypes. Based on the presence of several different RFLP markers (SspI and StyI), the RFLP classified viruses are phenotypucally distinct. VP2 subunit and DNA vaccine able to confer partial protection against challenged with vvIBDV

Benefits from the study

Future development of PCR-based diagnosis assay based on RFLP of VP2 for rapid identification and differentiation of IBDV based on virulence and pathogenicity. Future optimization on the VP2 subunit and DNA vaccine

Patent(s), if applicable:

Nil

Stage of Commercialization, if applicable:

Nil

Project Publications in Refereed Journals

1. Hoque, M.M, Omar, A.R., Chong, L.K, Hair-Bejo, M. and Aini, I. 2001. Pathogenicity of SspI positive infectious bursal disease virus and molecular characterization of VP2 hypervariable region. Avian Pathology 30: 379-390

2. Chong, L.K., Omar, A.R., Yusoff, K., Hair-Bejo, M. and Aini, I 2001. Sequence and phylogenetic analysis of the A segment of a highly virulent infectious bursal disease virus. Acta Virologica 24: 217-226

3. Hoque, M.M, Omar, A.R., Hair-Bejo, M. and Aini, I. 2002. Sequence and phylogenetic analysis of the VP2 gene of very infectious bursal disease virus isolates *Journal of Biochemistry, Molecular Biology and Biophysics*. 6: 93-99

Project Publications in Conference Proceedings

1. Omar, A.R., Hair-Bejo, M., Aini, I., Hoque, M.M., Chong, L.K. and Phong, S.F. 2000. Pathogenicity and molecular characterization of *Ssp*I positive very virulent infectious bursal disease virus. In: *Proceedings of the 1st Joint Meeting of the Solvenian Society for Microbiology and the Hungarian Society for Microbiology*, 24-26 August 2000, Keszthely, Hungary

2. Hair-Bejo, M., Hafiza, H., Phong, S. P., Omar, A. R. and Aini, I. 2000. Pathogenesis and pathogenicity of Malaysian isolates of infectious bursal disease virus. In: Proceedings of the 1st Joint Meeting of the Solvenian Society for Microbiology and the Hungarian Society for Microbiology, 24-26 August 2000, Keszthely, Hungary

3. Hoque, M.M., Omar, A. R., Chong, L. K., Hair-Bejo, M. and Aini, I. 2001. Pathogenicity of very virulent infectious bursal disease virus and molecular characterisation of the VP2 gene. In : Proceedings 12th International Congress of the World Veterinary Poultry Association, 17-21 September 2001, Cairo, Egypt. p. 172

4. Hoque, M.M., Omar, A.R., Hair-Bejo, M. and Aini. I. 1999. Pathogenicity and molecular characterization of infectious bursal disease virus of the Malaysian and Bangladeshi isolates. In: National Congress on Animal Health and Production, 3-5 September 1999, A'Famosa Resort, Malacca, p. 105-109.

5. Hoque, M.M., Omar, A.R., Hair-Bejo, M. and Aini. I. 1999. Restriction fragment length polymorphism in the VP2 gene of infectious bursal disease viruses from Malaysia and Bangladesh. In: Proceedings of the 11th National Biotechnology Seminar, 22-24 November 1999, Century Mahkota Hotel, Malacca, p. 84-86.

6. Hoque, M.M., Omar, A.R., Hair-Bejo, M. and Aini, I. 2000. Molecular characterization of VP2 hypervariable region of infectious bursal disease virus of Malaysian and Bangladeshi isolates. In: Proceedings of the 22nd Malaysian Society of Animal Production, 29 May – 1 June 2000, Shangri-La's Tanjung Aru Resort, Kota Kinabalu, Sabah, p. 229-230.

7. Hoque, M.M., Omar, A.R., Hair-Bejo, M. and Aini, I. 2000. Characterization of infectious bursal disease virus of the Malaysian and Bangladeshi isolates. In: *The 7th Asia-Pacific Electron Microscopy Conference*, 26-30 June, 2000, Suntec City, Singapore, p. 318-319.

8. Hoque, M.M., Omar, A.R., Hair-Bejo, M. and Aini, I. 2000. Molecular pathogenicity of SspI positive very virulent infectious bursal disease virus from Malaysia in specific-pathogen-free chickens. In: Proceedings of the 12th Veterinary Association Malaysia Scientific Congress, 1-4 September 2000, MS Garden Hotel, Kuantan, Pahang, p. 13-14.

9. Hoque M.M., Omar, A.R., Hair-Bejo, M. and Aini, I. 2000. Sequence and phylogenetic analyses of Malaysian and Bangladeshi very virulent infectious bursal disease virus VP2 gene In: Proceedings of the 12th National Biotechnology Seminar, 12-15 November 2000, Damai Laut Country Resort, Lumut, Perak, p. 46-48.

10. Chong, L.K., Omar, A. R., Yusoff, K., Hair-Bejo, M. and Aini, I. 2000. Sequence and phylogenetic analyses of a Malaysian highly virulent infectious bursal disease virus VP2-VP3-VP4 polyprotein In: Proceedings of the 12th National Biotechnology Seminar, 12-15 November 2000, Damai Laut Country Resort, Lumut, Perak, p. 55-57.

11. Omar, A. R. 2001. VP2 gene of infectious bursal disease virus as restriction fragment length polymorphism markers for virulency. In: Recent Advances in Animal Health and Production No. 24, 26 February 2001, Faculty of Veterinary Medicine, UPM, Abstract, p.12-13.

12. Kong, L.L., Omar, A.R., Hair-Bejo, M. and Aini, I. 2001. Sequence analysis of the segment A of an atypical very virulent infectious bursal disease virus. In: The 2nd International Congress/13th VAM Congress and CVA-Australasia/Oceania Regional Symposium. 27-30 August 2001, MINES Exhibition Centre, Kuala Lumpur, p. 77-79.

13. Chong, L. K., Omar, A.R., Yusoff, K., Hair-Bejo, M. and Aini, I. 2001. Expression of the VP2 protein of a very virulent infectious bursal disease virus in a prokaryotic expression system. In: The 2nd International Congress/13th VAM Congress and CVA-Australasia/Oceania Regional Symposium, 27-30 August 2001, MINES Exhibition Centre, Kuala Lumpur, p. 80-81.

14. Omar A. R. 2001. Virulent markers from infectious bursal disease virus. Hasil Penyelidikan dan Pembangunan (P&P) Rancangan Malaysia ke-7, UPM, Abstract p. 133.

15. Omar A. R. 2001. Development of a subunit vaccine against very virulent infectious bursal disease virus. Hasil Penyelidikan dan Pembangunan (P&P) Rancangan Malaysia ke-7, UPM, Abstract p. 134.

16. Omar, A. R., Aini, I., Hair-Bejo, M., Yusoff, K., Jamaluddin, A.A., Hassan, S. S., Darus A. and Kono, Y. 2001. Molecular characterization of avian immunosuppressive viral diseases. In: Persidangan Kebangsaan Penyelidikan dan Pembangunan Institusi Pengajian Awam, 25-26 October 2001, Putra World Trade Centre, Kuala Lumpur, Abstract p. 158.

17. Kong, L. L., Omar, A. R., Hair-Bejo, M., Aini, I. and Seow, H. F. 2001. Sequence analysis of segment A of typical and atypical very virulent infectious bursal disease virus isolated in Malaysia. In: Proceedings of the 13th National Biotechnology Seminar, 10-13 November 2001, Bayview Beach Resort, Penang, p. 54-57.

Graduate Research

Name of Graduate	Research Topic	Field of Expertise	Degree Awarded	Graduation Year
Mahfuzul Hoque.	Pathogenicity and Molecular Characterization of the VP2 gene of IBDV	Virology	PhD	2001
Chong Lee Kim	Molecular Characterization of a highly virulent strain of infectious bursal disease virus (IBDV) and development of VP2 recombinant protein	Immunology	PhD	2003
Zulkefley Othman	Development Of A PCR-Based Diagnostic Method For The Detection And Differentiation Of Oncogenic And Vaccine Strains Of Marek's Disease Virus In Chickens	-	Final Year Project (Biomedical)	2002

IRPA Project number: 01-02-04-0297
UPM Research Cluster:AFF