Preparation and evaluation of an experimental iscom-based infectious bursal disease vaccine

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Received: 17 September 2007 / Accepted: 8 February 2008

Abstract The present study was conducted to develop and evaluate an experimental ISCOM-based infectious bursal disease (IBD) vaccine. The indigenous very virulent infectious bursal disease virus (vvIBDV) already attenuated and adapted to Vero cell line was used. After denaturation of viral proteins with sodium dodecyl sulphate (SDS), an IBD-ISCOM was constructed. The non-incorporated viral components were separated from ISCOM by centrifugation of dialysate. The pathogenicity and immunogenicity trials were conducted in 3-week-old broiler chicken. A commercial oil-emulsified vaccine (CEVAC IBD K) was used for comparison. There were no clinical signs of disease, gross or microscopic lesions in bursa of Fabricius in group G1 vaccinated with ISCOM-based vaccine and bursa to body weight ratio were comparable to un-vaccinated control group (G3). The virus-neutralizing antibody titers were significantly ($P<0.05$) higher in group G1 as compared with group G2 which was vaccinated with commercial vaccine. On challenge with vvIBDV, 100%, 75% and 0.00% protection was achieved in G1, G2 and G3, respectively. The results indicated that ISCOM-based IBD vaccine is safe and immunogenic.

Keywords Infectious bursal disease · ISCOM · Vaccine

Introduction

The importance of poultry farming in agricultural production of Pakistan hardly needs any emphasis. Poultry production has emerged as a good substitute for beef and mutton. In spite of all the efforts to improve management, feed formulation and disease preventive measures adopted in public and private sectors, overall poultry population has remained victim to a number of infectious and non-infectious diseases. Among infectious diseases, infectious bursal disease (IBD) also called Gumboro disease has been a great concern for the poultry industry worldwide. The disease was first reported in 1962, in Southern Delaware, USA [1]. Infectious bursal disease virus (IBDV), the causative agent of this disease is a dsRNA virus with a bisegmented genome enclosed within an icosahedral, non-enveloped capsid of 55–65 nm. It is a member of genus *Avibirnavirus* of family *Birnaviridae* [2, 3].

In Pakistan, incidence of disease has increased during the last ten years. It was reported as 0.065% in 1989 [4]. The relative mortality among six commercial broiler strains during an epidemic of IBD was observed in a vaccinated flock of 1500 birds. The mean ($\pm$ SD) mortality score ranged from 2.0 ± 1.0 (Arbor Acres) to 4.4 ± 3.6 (Lohmann) during 7–8 week [5]. The overall incidence was $3.05\pm0.04\%$ in 1994 through 1996. It was significantly ($p<0.05$) higher in broiler ($5.3\pm0.002\%$) than in breeder ($2.93\pm0.01\%$) and layer ($1.08\pm0.01\%$) [6]. As immunization is currently the most effective control measure to IBD, there is a continuous need to develop safe and efficacious vaccines for commercial poultry production. The indigenous IBDV isolates belong to virulent and very virulent strains [7]. The local very virulent infectious bursal disease virus (vvIBDV) has also been adapted and attenuated to Vero cell line [8].

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Over the past 10–15 years, a large research effort has attempted to find novel adjuvants with ability to induce a broad range of immune responses, including cell mediated immunity. The immunostimulating complex (ISCOM) is one adjuvant with multiple adjuvant properties. ISCOM was first described in 1984 [9]. These are open cage like complexes typically with a diameter of about 40 nm and contain saponins from the bark of the tree *Quillaia saponaria* Molina [10]. ISCOM, unlike many other vaccine adjuvants, appear to promote a broad immune response by simultaneously promoting high levels of antibody and strong T cell responses, including enhanced cytokine secretion and activation of cytotoxic T lymphocyte (CTL) responses [11, 12].

Experimental ISCOM vaccine formulations have induced protective immunity to a number of microorganisms encompassing viruses including retroviruses and bacteria in several species including primates [13, 14]. These ISCOM-based vaccines have also induced protection against chronic and persistent infections like human immunodeficiency virus (HIV). ISCOM vaccines have also shown immune response efficiently in murine and bovine species even in the presence of maternal antibodies while conventional vaccines have failed [15]. The present study was conducted to construct and evaluate an experimental ISCOM-based IBD vaccine using indigenous Vero cell adapted and attenuated vvIBDV.

The local very virulent infectious bursal disease virus (vvIBDV) adapted and attenuated to Vero cell line [8] was obtained from Department of Veterinary Microbiology, University of Agriculture, Faisalabad. The purified 9th passage virus was treated with 1% sodium dodecyl sulphate (SDS) and stored at -70°C till further use. The vaccine was prepared as described by Kersten *et al.* [13] with modifications. Briefly, a mixture of phosphatidyl choline (Sigma Chemical Co, St Louis, Mo.) and cholesterol (Sigma Chemical Co, St Louis, Mo.) in chloroform (Fisher Scientific Springfield, NJ.) in the ratio of 1:1 was dried under nitrogen in a round bottom flask. The dried lipid film was solubilized in Tris NaCl buffer (10mM Tris HCL and 0.9% NaCl, pH7.4). The purified SDS treated virus suspension was mixed in TN buffer containing 136 mM n-octyl β -D glucopyranoside (Sigma Chemical Co, St Louis, Mo.) for 2 h. This mixture was then added to previous mixture (lipid solution) at a protein (denatured IBDV) to lipid ratio of 1:10 (w/w). Next, saponins (Quil A, EM Sergeant Pulp Co, Clifton, NJ.) was added to the solution at a lipid to saponins ratio of 1:1 (w/w). The resulting mixture was dialyzed against 2 changes of 1L of TN buffer for 24 h at 4°C to form ISCOM. The non-incorporated viral components were separated from ISCOM by centrifugation of the dialysate in a 30% sucrose gradient at 122700 g for 3 h at 4°C [16].

One hundred and eighty 3-week-old broiler chicken were divided into three equal groups i.e. G1, G2, and G3. They were reared in isolated sterile rooms of laboratory animal house under strict hygienic and standard management conditions. The birds of group G1 were vaccinated with 0.2 ml of ISCOM-based IBD vaccine through subcutaneous route. A 0.2 ml of a commercial killed oil-emulsified (CEVAC IBD K) vaccine was injected intramuscularly to group G2. The birds of group G3 were kept as un-vaccinated control. Daily morbidity and mortality, if any, was recorded in each group up to 3-week post-vaccination. At days 3, 5, 7, 10, 14 and 21 post-vaccination, five birds from each group were randomly selected and sacrificed. Gross and microscopic lesions were examined in bursa of Fabricius and mean bursa to body weight ratio were calculated [17].

Blood was collected by puncturing wing veins from 10 birds at random in each group before vaccination, at 7, 14 and 21 days post-vaccination. Serum was separated and heat inactivated at 56°C in water bath (Eyela, Japan) for 30 minutes. It was examined for antibody response to different vaccines by the use of virus-neutralization (VN) test using constant virus diluting serum method [16, 18]. At 21 day post-vaccination, 50% of the remaining birds in each group were challenged. A 2 ml (10 time dose, 10^2 EID₅₀/dose) of live vvIBDV was given orally. Clinical signs, morbidity and mortality were recorded in each group to calculate the protection percentage. Data were analyzed by calculating geometric mean titers (GMTs) of antibody response. Mean $\pm$ SD., analysis of variance (ANOVA) technique followed by Duncan's multiple range (DMR) test.

The use of adjuvants has been well documented to increase and prolong the immune response of different vaccines. However, conventional adjuvants used in poultry vaccines have a number of disadvantages like less stability of vaccines, difficulty in inoculation, and granuloma formation at the site of injection and toxicity. In the present study local very virulent infectious bursal disease virus (vvIBDV) attenuated after 9 passages on Vero cell line was used to construct IBD-ISCOM vaccine. The use of ISCOM-based vaccines have induced protective immunity to a number of animal viruses, bacteria [13, 14]. In present study no untoward reaction was observed after vaccination in group G1 vaccinated with ISCOM-based vaccine. In group G2, few birds showed stress and granuloma at the site of injection. These findings showed that ISCOM-based formulations are completely safe and non-toxic. The results are in line with the findings of other scientists [10] who also described that ISCOM had reduced toxicity and reactogenicity compared with other formulations.

The results of mean bursa to body weight ratio at 3, 5, 7, 10, 14 and 21 days post-vaccination are given in Table 1.

Table 1 Mean bursa to body weight ratio at different days post-vaccination

Group	Days post-vaccination					
	3	5	7	10	14	21
G1	5.51a±0.39	5.54a±0.37	5.89a±0.35	5.01a±0.49	5.42a±0.47	5.61a±0.50
G2	3.17b±0.16	2.23b±0.39	2.01c±0.27	1.91b±0.21	1.75b±0.12	1.32c±0.09
G3	5.49a±0.35	5.41a±0.43	5.36b±0.37	5.04a±0.12	5.39a±0.40	5.26ab±0.33

Mean values for five birds. Values within a column followed by different lowercase letters are significantly ($P<0.05$) different.

G1 = ISCOM-based IBD vaccine

G2 = Commercial (oil-emulsified CEVAC IBD K)

G3 = Un-vaccinated control

Table 2 Mean Virus-neutralizing antibody titers before and at 7, 14 and 21 days post-vaccination

Group	Days post-vaccination			
	Before vaccination	7	14	21
G1	23.98±1.32	1256.02a±1.69	2041.73a±1.81	3311.31a±1.73
G2	25.70±1.41	769.13b±1.27	1172.20b±1.75	1659.58b±1.37
G3	27.50±1.55	14.86c±1.58	11.22c±1.46	2.29c±1.60

Geometric Mean values for ten birds. Values within a column followed by different lowercase letters are significantly ($P<0.05$) different.

G1 = ISCOM-based IBD vaccine

G2 = Commercial (oil-emulsified CEVAC IBD K)

G3 = Un-vaccinated control

In group G1 vaccinated with ISCOM-based vaccine, bursa to body weight ratio were high and comparable to group G3 i.e. un-vaccinated control throughout the study period. Group G2, vaccinated with commercial oil-emulsified vaccine had significantly ($P<0.05$) small bursae as indicated by low bursa to body weight ratio as compared to control group. There were no gross lesions on bursae of Fabricius in group G1 and G3 at each collection up to 21 days post-vaccination. However, bursae in group G2 were initially enlarged and hemorrhagic and later on filled with pus in few birds. Similarly, no microscopic lesions were seen on histopathology of bursae in group G1 and G3. Both groups had large, intact and active follicles filled with lymphoid cells. While inflammation with necrosis of lymphocytes and atrophy of follicles were observed microscopically in bursae of group G2. As evident from the results that ISCOM-based vaccine is safe and non-pathogenic compared to commercial killed oil-emulsified vaccine.

The results of virus-neutralization (VN) test are shown in Table 2. The geometric mean titers (GMTs) were similar in all three groups before vaccination i.e. 23.98, 25.70 and 27.50 in group G1, G2 and G3, respectively. These titers clearly indicates that maternally derived antibodies are completely catabolized by the end of 3rd week in broiler chicken and this is the most susceptible age for clinical infection with IBDV. Similar findings were described earlier [19]. The antibody titers then increased

considerably from day 7 to day 21 post-vaccination. The GMTs were significantly ($P<0.05$) higher in group G1 than G2 throughout the study period. The maximum GMT was 3311.31 in group G1 at 21 day post-vaccination. Previously very high VN-antibody titers with live attenuated and inactivated BGM-70 cell culture passaged IBDV has also been reported [20]. The indigenous live attenuated Vero cell adapted vvIBDV also showed very high i.e. 1345.86, 2187.76 and 3090.29 GMTs at 7, 14 and 21 days post-vaccination [8]. The overall results of immunogenicity showed that ISCOM-based vaccine had highest GMTs post-vaccination.

On challenge with vvIBDV, 100% birds in group G1 were protected. The bursae in group G1 had no gross or microscopic lesions with 0.00 mean lesion score up to 20 days post-challenge. In group G2 75% birds were protected against challenge with vvIBDV and remaining 25% had gross and moderate to marked microscopic lesions (3.15 ± 0.21) at 10 day post-challenge. The un-vaccinated challenged group (G3) showed sever gross and microscopic bursal lesions (4.27 ± 0.30) in all birds at 10 day post-challenge. It is evident from the results that vaccines prepared from indigenous IBD virus protects the birds against challenge with vvIBDV, while commercial vaccines only give partial protection. Based on these findings, it may be concluded that ISCOM-based IBD vaccine prepared from local Vero cell attenuated vvIBDV is safe, non-pathogenic

and more immunogenic in terms of VN-antibody titers as compared to commercial vaccine. In future we hope, this vaccine will help to boost and prolong immune response to give better protection against IBD particularly in commercial layers.

Acknowledgement I am thankful to the Vice Chancellor, University of Sargodha on providing funds under the University Research Promotion Fund (URPF) for the successful completion of this study. I am also grateful to the Chairman, Department of Pharmacy and Dean, Faculty of Science and Technology for their technical and moral help.

References

1. Cosgrove AS (1962) An apparently new disease of chicken-avian nephritis. *Avian Dis* 6:385–389
2. Van den Berg TP (2000) Acute infectious bursal disease in poultry: a review. *Avian Pathol* 29:175–190
3. Lukert PD and Saif YM (2003) Infectious bursal disease. In: *Diseases of poultry* (Saif YM eds). Ames Iowa State University Press. pp 161–179
4. Bhatti BM (1989) Incidence of poultry diseases and their importance in poultry production in Pakistan. *Pak Vet J* 9: 194–197
5. Akhtar S, Zahid S and Hussain A (1992) Relative susceptibility of six broiler chicken strains to infectious bursal disease (Gumboro) during an epidemic. *Pak Vet J* 12:18–20
6. Farooq M, Durrani FR, Faisal S, Asghar A and Khurshid A (2000) Incidence of infectious bursal disease among birds submitted to a diagnostic laboratory in NWFP, Pakistan. *Pak Vet J* 20:77–80
7. Zahoor MA, Hussain I and Khan QM (2005). Two outbreaks of vvIBDV in Pakistan. *Vet Rec* 157:813
8. Rasool MH and Hussain I (2006) Preparation and evaluation of Vero-cell infectious bursal disease vaccine in Pakistan. *Vaccine* 24:2810–2814
9. Morein B, Sundquist B, Hoglund S, Dalsgaard K and Osterhaus A (1984) Iscom, a novel structure for antigenic presentation of membrane proteins from enveloped viruses. *Nature* 308:457–460
10. Sjolander A, Cox JC and Barr IG (1998) ISCOMs: an adjuvant with multiple functions. *J Leukocyte Biol* 64:713–723
11. Barr I and Mitchell GF (1996). ISCOMs (immunostimulating complexes): The first decade. *Immunol Cell Biol* 74: 8–25
12. Claassen I and Osterhaus A (1992) The iscome structure as an immune enhancing moiety: experience with viral system. *Res Immunol* 143:531–541
13. Kersten GFA, Teealink T and Derks HJ (1988) Incorporation of the major outer membrane proteins of *Neisseria gonorrhoea* in saponins- lipid complexes (ISCOMs): Chemical analysis, some structural features and comparison of their immunogenicity with three other antigen delivery system. *Infect Immunol* 56:432–438
14. Deliyannis G, Jackson DC, Dyer W, Bates J, Coultr A, Harling LM and Brown LE (1998) Immunopotential of humoral and cellular responses to inactivated influenza vaccines by two different adjuvants with potential for human use. *Vaccine* 16:2058–2068
15. Morein B, Hu KF and Abusugra I (2004) Current status and potential application of ISCOMs in veterinary medicine. *Adv Drug Deliv Rev* 56:1367–1382
16. Chavez IRR, Rosenberger JK, Cloud SS and Pope CR (2002) Characterization of the antigenic, immunogenic and pathogenic variation of infectious bursal disease due to propagation in different host systems (bursa, embryo, and cell culture). II. Antigenicity at the epitope level. *Avian Pathol* 31:473–483
17. Abdel-Alim GA and Saif YM (2001) Pathogenicity of cell culture derived and bursa derived infectious bursal disease viruses in specific-pathogen-free chicken. *Avian Dis* 45:844–852
18. Rosenberger JK, Saif YM and Jackwood DJ (1998) Infectious bursal disease. In: *isolation and identification of avian pathogens*. Am Assoc of Avian Pathol. p.215–218
19. Skeels JK, Lukert PD, Fletcher OJ and Leonard DJ (1979) Immunization studies with a cell culture adapted infectious bursal disease virus. *Avian Dis* 23:456–465
20. Hassan MK, Natour MQ, Ward LA, and Saif YM (1996) Pathogenicity, attenuation and immunogenicity of infectious bursal disease virus. *Avian Dis* 40:567–571