EVALUATION OF IMMUNE RESPONSES BY LIVE INFECTIOUS BURSAL DISEASE VACCINES TO AVOID VACCINATION FAILURES

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Infectious Bursal Disease (IBD) is a viral, contagious immunosuppressive disease posing an important threat to the commercial poultry industry. Evolution of highly virulent strains of IBD virus warranted the need for detailed characterization of the immune responses offered by the currently available vaccines. Two extensively used live vaccines of varied attenuation levels – intermediate and intermediate plus – strains were analyzed for the induction of immune responses. Both the vaccines induced protective antibody titers with the onset, quicker and higher with the intermediate plus vaccine. The intermediate plus strain vaccine was observed to induce higher levels of IFN- γ in the birds. These results were supported by immunophenotype analyses with an increase in CD8⁺ and simultaneous decrease in CD4⁺ cell population. Both vaccine strains conferred protective immunity against virulent challenge. The study warrants the use of intermediate plus vaccines in disease endemic regions and intermediate vaccines in non-endemic regions to prevent IBD infection.

Keywords: infectious bursal disease, live vaccines, IFN- γ , immunophenotyping

Introduction

Infectious bursal disease is one of the most common viral infections plaguing the world's poultry industry. Vaccination plays an important role in the successful control of the disease [1, 2]. Live attenuated vaccines form the most employed type of vaccine at field level. The emergence of variant or newer strains of the virus in the recent times has been reported to cause vaccination failures [2–4]. Moreover, the various attenuation levels of commercially available live vaccines for infectious bursal disease virus (IBDV) lead to varying levels of immunosuppression increasing the birds' vulnerability to various infections.

Control of IBDV poses one of the most challenging tasks in the poultry health management. Understanding the pathogenesis and immune mechanisms of protection against the virus is an important prerequisite for preventing the disease. Moreover, the efficiency of vaccination program is dependent on the time of vaccination, influenced by the levels of residual maternal derived antibodies [1]. Therefore, the safety and efficacy of these live vaccines still remains a major area of concern and forms a prime area of research. In the present study, we have evaluated the immune responses of commercially available live vaccines of different pathogenic indices with the aim of deciphering their protective efficacy against the disease.

Materials and methods

Chicken

White Leghorn day-old chicken free from hatchery vaccination was obtained from the Institute of Poultry Production and Management, TANUVAS (Chennai, India) and housed in the Central Animal House at Madras Veterinary College (Chennai, India).

IBDV vaccines

Commercially available live vaccines belonging to intermediate strain and intermediate plus strain of infectious bursal disease manufactured by Ventri biologicals (India) were used in the study.

Study groups and immunization

The birds procured were grouped into three treatment groups. Birds in group 1 were sham-immunized with sterile normal saline. Birds in group 2 were immunized with the recommended dosage of the intermediate strain live vaccine. Birds in group 3 were administered with inter-

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Table 1. Design of the experiment

Group	Immunogen Dose: EID ₅₀ ≥ 10 ² /0.03 ml	Immunization	Bleeding (Days post vaccination)
Group 1 (<i>n</i> = 10)	Normal Saline	Day-14	0, 7, 14, 21, 28
Group 2 (<i>n</i> = 17)	Intermediate Strain Vaccine	Day-14	0, 7, 14, 21, 28
Group 3 (<i>n</i> = 17)	Intermediate Plus Strain Vaccine	Day-14	0, 7, 14, 21, 28

mediate plus strain live vaccine. The vaccines were administered via the recommended intra-ocular route. The number of birds in each group and bleeding details are listed in *Table 1*.

Humoral immune response

Specific antibody titers to IBDV were quantified in serum samples using ProFLOK IBD ELISA kit (Synbiotics, USA). Birds in the treatment groups were bled prior to immunization and on 7, 14, 21, and 28 days post vaccination (DPV). The difference of the mean antibody titers was subjected to statistical analysis with Student's *t*-test.

Cell-mediated immune response

The cell-mediated immune responses to vaccination with live vaccines of IBDV were assessed by interferon (IFN)- γ quantification. Peripheral blood mononuclear cells (PBMCs) were isolated from birds in the treatment groups on 0, 7, 14, 21, and 28th DPV. The PBMCs from each group were pooled separately and cultured with RPMI-1640 (Invitrogen, USA) at a seed rate of 10⁵ cells per well. These PBMCs were stimulated for 48 h with 10 μ g/ml of the respective IBDV vaccine antigens. Ten micrograms per milliliter of concanavalin A (Sigma-Aldrich, USA) was used as the positive control for the test (data not shown). Cells grown with media alone were the negative control for the test and were used for the normalization of the IFN- γ estimates. The cell culture supernatants were assayed for IFN- γ levels with Cytoset™ Chicken IFN- γ ELISA based kit (Invitrogen, USA).

Immunophenotyping

The assessment of CD4⁺ and CD8⁺ subset distribution post vaccination was analyzed by flow cytometry (BD FACS caliber, USA). Peripheral blood mononuclear cells (PBMCs) were isolated from birds on 0, 7, 14, 21, and 28th DPV. The PBMCs from each group were pooled as an analyte for each day of analysis. Briefly, PBMCs were washed with plain RPMI-1640 medium and suspended in FACS buffer (BD, USA). These PBMCs are then incubated with 2.5 μ l of Mouse Anti-Chicken CD4: FITC monoclonal antibody conjugated to fluorescein isothiocyanate (Abcam, UK) and 5 μ l of Mouse Anti-Chicken CD8: Cy5

monoclonal antibody conjugated to Cy5 (Abcam, UK) for 45 min on ice. These treated cells are washed, and then data was generated for 10,000 events with a flow cytometer and analyzed using CellQuest® software. The results were expressed as the average percent of cells stained with each monoclonal antibody, compared between vaccinated and sham immunized control groups of chickens at each sampling.

Challenge studies

Six chicken from each group were challenged on 28th DPV, through intraocular administration of 0.05 ml (10% w/v) bursal suspension containing 10² CID₅₀/0.05 ml of IBDV reference isolate maintained at Vaccine Research Centre (TANUVAS, Chennai). The birds were observed for 7 days post challenge for survival or mortality.

Results and discussion

Despite widely practiced vaccination, IBDV outbreaks are common resulting in heavy monetary losses. Currently, vaccines for IBDV are selected solely on the ability to induce neutralizing antibodies. A basic understanding of the protective immune responses offered by these vaccines at molecular and cellular levels greatly complement the control mechanisms for IBD. With this aim, two commercial IBD vaccines differing in their magnitude of pathogenicity – intermediate and intermediate plus strains have been administered to experimental chicken devoid of maternal derived antibodies.

Antibody response in IBDV vaccinated birds

An antigen specific indirect ELISA provided a clear insight of the induction of humoral immunity produced against different strains of IBDV under study. The control group of birds showed no detectable IgG antibodies for IBDV in the study period. Birds belonging to group 2, administered with intermediate strain of IBDV, induced an average serum antibody titer of 762.12 ($\pm$ 41.98) on 7th day, 2549 ($\pm$ 97.87) on 14th day, 2815.4 ($\pm$ 37.77) on 21st day, and 3330.1 ($\pm$ 52.79) on 28th day. Group 3 birds, vaccinated with intermediate plus strain of IBDV, exhibited an average serum antibody titers of 2033.3 ($\pm$ 48.33) on 7th day, 2949.5 ($\pm$ 124.43) on 14th day, 3727.1 ($\pm$ 211.2) on 21st

Fig. 1. ELISA titers (mean $\pm$ SE) of sera from birds immunized with intermediate and intermediate plus strains of infectious bursal disease virus in comparison with the unvaccinated birds. The assay was performed using ProFLOK IBD ELISA kit (Synbiotics, USA) to estimate the serological antibody titers against the virus. Series 1: unvaccinated group; Series 2: intermediate group; Series 3: intermediate plus group

day, and 4187.3 ($\pm$ 52.63) on 28th day (Fig. 1). Significant difference ($P < 0.001$) in the onset of ELISA titers starting from day 7 post vaccination revealed the importance of usage of intermediate plus strains of IBDV for vaccination, especially in regions where high levels of maternal derived antibodies (MDA) would interfere with the efficacy of the IBDV vaccines. Our results were in accordance with the findings of similar experiments with IBD vaccines [6, 7].

Cell-mediated immune response in IBDV vaccinated birds

Though the antibody response is considered important in defense against virulent IBDV, cell-mediated immunity also appears to play a critical role for protection against

the infection. IFN- γ , a type-2 IFN, is mainly produced by activated T cells and natural killer cells and is considered an important indicator of Th-1 type immunity [8–10]. The induction of cell-mediated immunity by the live vaccines of IBDV studied was evident by quantifying the IFN- γ levels from the stimulated PBMCs. A significant increase in the levels of IFN- γ was demonstrated in both the vaccinated group of birds as compared to the unvaccinated group, indicating their ability to induce cellular immunity. The two vaccinated groups in the present study showed gradual increase in interferon- γ levels from 14 DPV till 21 DPV, and thereafter, it declined (Fig. 2). Birds belonging to group 2 vaccinated with intermediate strain showed a peak expression of 2740 pg/ml on day 21 DPV, and the birds belonging to group 3 vaccinated with intermediate plus vaccine showed a peak expression of 5840 pg/ml on

Fig. 2. IFN- γ levels quantified using Cytoset™ Chicken IFN- γ ELISA based kit (Invitrogen, USA) among IBD vaccinated birds compared to unvaccinated birds. C: unvaccinated control group; I: intermediate vaccine; H: intermediate plus vaccine

Fig. 3. Flow cytometric analyses of the T-cell subpopulations in IBD vaccinated birds. Panel a) shows the distribution of CD4⁺ cell population in the peripheral blood pool of the study groups. Panel b) presents the similar analyses of CD8⁺ cells. Series 1: unvaccinated group; Series 2: intermediate group; Series 3: intermediate plus group

day 21 DPV. Therefore, the birds vaccinated with intermediate plus strain of vaccine were observed to induce significantly higher levels of the IFN- γ , suggesting higher participation of both innate and adaptive pathways to provide protective immunity to the birds against the virus.

Immunophenotype analysis

The Th1–Th2 immune response polarization due to vaccination with IBDV would provide an insight of the cell-mediated immune responses [11]. The circulating peripheral PBMCs were immunophenotyped in an attempt to understand the direction of T-helper responses and thereby the cell-mediated immune responses in IBDV vaccinated

chicken. *Figure 3* summarizes the distribution of T-helper cell population of the study groups over different time points. There was a slight decrease observed in the circulating CD4⁺ cell population in the vaccinated birds as compared to the unvaccinated birds. Previously, studies on IBDV vaccines either reported a decrease or no appreciable changes in the percent of CD4⁺ cell population [12]. The analysis of CD8⁺ cell populations from the study was observed to show a slight increase in the percent of cells in the vaccinated birds than the unvaccinated birds. The less attenuated intermediate plus vaccine was found to induce higher CD8⁺ cell populations in the peripheral blood pool. These observations confirm the increased role of cell-mediated immune responses in the vaccinated birds against IBDV infection. A thorough literature search dur-

ing the study reported marked differences in the immunophenotype of T cells infiltrating the bursa, but not in the peripheral blood pool of IBDV vaccinated birds [12, 13].

Protective efficacy of vaccines against virulent challenge

All the birds tested from group 1 administered with sterile normal saline succumbed to the virulent virus resulting in 100 percent mortality. The vaccinated birds from both the groups 2 and 3 proved efficacious by protecting the birds against the challenge virus resulting in 0 percent mortality.

Conclusion

The observations in the study warrant the use of intermediate strain in absence of MDA in chicken. The intermediate plus strain is advocated to be used in IBDV endemic regions, where there is a high and unpredictable circulation of MDA. The humoral immune responses, therefore, effectively qualify the vaccines to provide protective efficacy against the disease. However, the functionality of the cellular immunity in IBDV vaccinated birds needs further analysis and understanding.

Authors' contributions

Padmaja Jakka executed the work as part of MVSc thesis and drafted the article. Y. Krishnamohan Reddy planned the work and is the major advisor for the project. J. John Kirubakaran and N. Daniel Joy Chandran supervised the work and participated in scientific discussion.

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