Virus enhancement following infection with antibody-coated infectious bursal disease virus (IBDV) in chickens

Rajesh Kumar & Shiv Charan*
Dept. of Veterinary Microbiology, College of Veterinary Sciences, Chaudhary Charan Singh Haryana Agricultural University, Hisar 125 004, India
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A novel concept of vaccination, employing virus-antibody complex has been reported for the control of infectious bursal disease in chickens. A comparison of virus replication, serum neutralizing antibody response and pathogenicity in chickens inoculated with the antibody coated virus, prepared by mixing virus and antibody in different ratios (1:1, 1:0.1, 1:0.01) and virus alone without antibody, has been made. Antibody coated virus (when mixed in certain crucial ratios) replicated to a higher magnitude in the target organ, caused more severe pathogenesis but induced a primary serum neutralizing antibody response almost comparable. The results may have important implications in understanding of pathogenesis and development of control strategies against infectious bursal disease virus, specially employing immune complex vaccine.

Infec­tious bursal disease virus (IBDV) mainly infects the B-cells of bursa of Fabricius. However, other lymphoid organs e.g. caecal tonsils, thymus, spleen and Harderian glands are also affected. The virus is known to produce severe combined B- and T-cell immuno-suppression.

To control IBD, both killed and live vaccines have been used. Recently, use of virus-antibody complex has been suggested as prophylactic measure. Although, the exact mechanism of induction of protection by the antibody complex vaccine has not been fully understood, it has been suggested that better immune response with antibody complex vaccine over the conventional vaccines is associated with delayed release of virus, lesser degeneration of lymphoid organs and higher localization of IBDV antigen on bursal and splenic follicular dendritic cells. In these studies we have investigated the replication kinetics of antibody coated virus vs virus alone and we report that the antibody-coated IBDV may lead to enhancement of the virus replication in vivo under certain conditions. This is probably the first report in IBDV on antibody mediated enhancement of virus replication in vivo. Therefore, these studies may have important implications in the field applications of immune-complex vaccines.

Chicks—Broiler chicks (18 days old; 80) from a farm with the history of no outbreak were procured. The chicks were tested for anti-IBDV antibody status. For raising polyclonal anti-IBDV antibodies 7-9 weeks old chickens were used.

Pre-immune status of chicks for IBD antibody—Although the chicks were obtained from a farm with the history of no outbreak of IBD, the chicks were tested for anti-IBDV antibody possibly for any maternal antibody. The sera were collected randomly from 5-6 chicks and pooled for the assessment of antibody employing SN test and the titer was found to be 1:160.

Virus—Georgia strain (intermediate) of IBDV was procured from M/s Indovax Ltd, Hisar (Haryana) and was grown and titrated in chickens embryo fibroblast (CEF).

Titration of virus—Micro-titration method was used for titration of the virus. Various dilutions of the virus ranging from $10^1$ to $10^8$ were made in the growth medium-199. 100 µl of each dilution of the virus was added to the well starting from highest dilution. This was followed by 100 µl of cell suspension $(1\times10^6$ cells/ml) to all the wells. Proper cell controls were also set up simultaneously. The plates were observed daily for CPE. After 4-5 days supernatant was discarded and the cells were stained with crystal violet (1%). The virus titer was calculated by the method of Reed and Munch (10). The titer of stock virus was found to be $5\times10^6$ TCID$_{50}$/ml.

Production and titration of hyper-immune serum—Antiserum against the Georgia strain of IBDV, propa-
gated in CEF, was raised in 7-9 weeks old 6 chickens by inoculating three doses of $1 \times 10^5$ TCID$_{50}$ (approx.) virus at 15 day interval by ocular-oral route. The chickens were bled 10 days after the last injection. Hyper-immune serum raised in White Leghorn chickens against Georgia strain grown in CEF was titrated by serum neutralizing test and titer was found to be $1:5000$ (approx.), whereas the serum from control chickens had a titer of less than $1:5$. The antibody titer of the hyper-immune serum against IBDV was further confirmed in a commercial ELISA and was found to be $5300$.

Preparation of Virus antibody complex—For preparing virus-antibody complex for immunization, appropriate dilutions of anti-serum were mixed in equal volume of virus $1 \times 10^5$ TCID$_{50}$. This virus antibody was kept at room temperature ($23^\circ$-$25^\circ$C) for 1 hr with frequent gentle mixing. Various preparations were prepared so as to give $1 \times 10^5$ TCID$_{50}$ of IBDV and unit activity of IBDV antibody so that the final ratio of $1:1; 1:0.1; 1:0.01$ was achieved between IBDV and unit activity of IBDV antibody (unit activity of antiserum has been defined as reciprocal of serum neutralizing antibody titer per milliliter of antiserum, ref 8).

Experimental design—Chicks (80) were divided into 5 groups of 16 birds each. These groups were inoculated with different ratio of virus and antibody i.e. Group I with 1:1, Group II with 1:0.1, Group III with 1:0.01, Group IV with virus with normal chickens serum, while Group V with phosphate buffered saline (PBS) alone. After 6 weeks these groups were re-inoculated with the virus via ocular-oral route. Various parameters i.e. clinical signs, mortality, gross lesions, bursal/body weight ratio (b:b ratio) including microscopic examination of organs (bursa, thymus, spleen) were recorded. In addition, virus isolation from bursa specimen was also done. Blood samples were collected at 0, 3, 6, 10, 15, 21 and 28 days after primary inoculation as well as 5 and 10 days after second inoculation for serum neutralization test.

Bursal preparation for virus titration—Bursal samples from 2-3 birds at various time intervals from Group I to V were pooled, ground in Dounce’s homogenizer in Hank’s balanced salt solution (HBSS) to make 10% (w/v) homogeneous suspension and subjected to antibiotic treatment for 1 hr at $37^\circ$C. The suspension was then subjected to three cycles of freezing and thawing. Finally, it was centrifuged at 2000g for 15 min. The supernatant was passed through 0.22 µ Millipore membrane. The titer was determined as described earlier and expressed as per g of bursal tissue.

Neutralization test (SN)—Constant virus varying serum method was followed for performing SN test in 96-well micro tissue culture plates (Nunc, Denmark). Sera were inactivated at $56^\circ$C for 30 min. in water bath. Test was run in triplicate. Two-fold dilutions of each serum was made starting from 1:10 to 1:5120 in 50 µl volume using medium M-199. IBDV was diluted to give titer of $100$ TCID$_{50}$/50µl and added to each well in 50 µl amount, except in wells marked for cell control. In the wells marked for cell control, 100 µl of growth medium (M-199 supplemented with 10% bovine serum, l-glutamine, sodium bicarbonate and antibiotics) was added. The plates were incubated at $37^\circ$C for 1 hr to allow serum to neutralize the virus. CEF cells were diluted in growth medium (M-199) to give a concentration of $1 \times 10^6$ cells/ml and added in 100 µl amounts in each well. The plates were incubated at $37^\circ$C in 5% CO$_2$ in air. After 5 days, the supernatant was discarded and cells were stained with crystal violet and examined.

Microscopic examination of bursa—Samples of bursal tissues were collected in 10% formal saline from each group at various time post inoculation and the tissue sections were examined after Haematoxilin and Eosin staining for comparison of disease severity.

Virus titration in the bursa—The results of virus titration in bursa (pooled from 2-3 birds) of chickens inoculated with IBDV antibody complex or virus alone at various time intervals have been shown in Fig 1. The maximum virus replication was found in Gp II inoculated with virus antibody ratio (1:0.1). The degree of virus replication was minimum in Gp I which had maximum amount of antibody. The virus replication in chickens inoculated with virus along with normal chickens serum (Gp IV) or with virus antibody ratio of 1:0.01 (Gp III) were almost similar. A direct correlation between concentration of antibodies and inhibition of virus replication in bursa was not seen in these studies, virus titers in bursae of Gp II chickens reached highest peak with virus titre $7 \log_{10}/g$ (approx.) of tissue and was detectable till day 28. Thus, in Gp II (1:0.1) maximum titre of virus in bursa suggests that probably this ratio of virus and antibody was very crucial and may support enhanced viral replication.

Serum neutralization (SN) antibody response—After primary immunization, SN antibodies appeared

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between day 10 and 15 post-inoculation and declined to basal level between day 28 and 35 in all the virus inoculated groups (I-IV). After secondary immunization, there was rapid increase in the level of SN antibody titer in I, III, and IV groups, whereas in Gp II where virus antibody ratio was (1:0.1), SN antibody titers remained low (20) at day 5 and 10 days post secondary immunization. This was the same group where there was maximal replication of virus.

Gross and microscopic lesions—The group inoculated with IBDV alone showed mild lesions characterized by petechial haemorrhages in thigh muscles. However, the lesions were more severe in the groups inoculated with virus antibody complex. The highest degree of the haemorrhages in thigh and pectoral muscle and splenomegaly was found in Gp II at 15 day post infection. This was the same group in which virus replication was maximal and SN antibody titers were lowest after secondary immunization.

Similarly, histopathological lesions in the group of chickens inoculated with virus alone, showed milder reaction of heterophil infiltration and depletion of lymphocytes in follicles of bursa of Fabricius between day 3 and 6, as compared to the groups inoculated with virus antibody complex which showed moderate to severe lesions characterized by atrophy of follicles and necrosis of lymphocytes, thickening of interfollicular space due to connective tissue proliferation and infiltration of reticular cells and leucocytes. The maximum lesions were also found on day 15 as was the virus titers in bursa. With the virulent strain of IBDV, generally the maximum lesions and virus replication in the bursae have been reported around day 4. This delayed reaction in the present studies may be because of the use of vaccine virus and due to the effect of coating with antibodies in these experiments.

In the present study, the virus replication, SN antibody responses and pathogenesis of the live IBDV alone (as in case of a conventional live vaccine) and after mixing with the antibodies (as in case of an immune-complex vaccine) were compared. It was observed that in chickens (Gp II) inoculated with the virus coated with the antibody, there was about 100-fold more virus replication and the severity of the lesions was markedly higher than in chickens inoculated with the virus alone. Primary SN antibody responses, however, were almost comparable in the groups of chickens whether the virus was given alone or together with the antibodies. Interestingly, in the chickens showing higher virus replication there was a remarkably suppressed secondary SN antibody response. Such a remarkably lower secondary antibody response may be attributable to degeneration of bursal cells after primary inoculation since the target cells for the replication of IBDV are bursal B-cell leading to poor priming of B and/or T cells during immunization. The immunosuppressive feature of IBDV has been well documented with respect to B-cells resulting into lower antibody responses against a number of infectious agents and vaccines in chickens which have been previously exposed to IBDV. Suppression of T cells responses as a result of IBDV infection has been reported more recently and is of transient nature.

Possibility of such phenomenon was recognized since the findings that the sera from human immune deficiency virus (HIV) infected individual can enhance HIV infection in vitro as well as in vivo where antibody dependent enhancement could be demonstrated at high dilutions (up to 1:65,000) while SN antibody activity can rarely be demonstrated at dilutions higher than 1:1000. Similarly, in studies with feline immunodeficiency virus (FIV) enhancement of FIV infection has been reported. The cats vaccinated with FIV-env showed accelerated acute infection, early appearance of viraemia and greater viral load after challenge than the control cats. In our studies, an enhanced virus replication in the group of chickens inoculated with virus antibody ratio of 1:0.1 may reflect a similar phenomenon. To the best of our knowledge this appears to be the first report with respect to IBDV.
Thus, the present studies highlight the importance of routine monitoring the level of IBDV antibodies in commercial flocks since lower levels of SN antibodies may rather support replication of IBDV. Therefore, acceleration or enhancement of IBDV infection by antibodies during field vaccination employing live vaccines and more particularly when immune-complex vaccine are used could be very crucial. The results of these studies will therefore provide a novel basis for understanding pathogenesis and formulating efficient vaccination program against IBDV critically.

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