IMMUNOLOGICAL STUDIES WITH EGG ADAPTED INFECTIOUS BURSAL DISEASE VIRUS

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ABSTRACT

Egg-adapted infectious bursal disease virus (IBDV) was tested for its immunogenicity and pathogenicity in one day-old and four to six week-old susceptible chicks respectively. Passage six IBDV was not sufficiently adapted to chicken embryo for its use as a safe immunogen. However, passage ten IBDV was non-pathogenic to chicks and showed seroconversion in the susceptible chicks. Similarly passage ten IBDV had no immunosuppressive effect on the immune system of chicks.

INTRODUCTION

INFECTIOUS bursal disease (IBD) till recently unknown in India has caused a great concern especially among broiler farmers, since its recent outbreaks in Maharashtra. For the effective control of this disease the development of suitable immunizing agent should be the aim. One of the common procedures is adaptation to the chicken embryo. The present study was undertaken to assess the degree of attenuation of an egg-adapted IBDV at 6th (IBDV/6) and 10th (IBDV/10) passage level.

MATERIALS AND METHODS

Field isolate of IBDV[1] was adapted to embryonating fowl eggs by chorio-allantoic route was used at 6th and 10th passage level. Field isolate of IBDV was used for challenging the chicks. LaSota vaccine and Ranikhet disease (RD) virulent virus each were obtained from Institute of Veterinary Biological Products, Pune. Five randomly selected day-old chicks from each consignment were tested for IBD antibodies by Agar gel precipitin test (AGPT).

Effect of IBDV/6 inoculation in day-old chicks:

Thirty chicks of one-day age were grouped into A and B, having fifteen chicks in each. These chicks from both the groups were vaccinated against RD on 1st day of age. Two drops (0.02 ml) of 20% chorio-allantoic membrane (CAM) suspension of IBDV/6 was given intra-ocularly to the chicks from group A on 4th day of age. Both the groups were housed separately. On 28th day of age chicks from both the groups were challenged with 0.2 ml of 100 EID_{50} RD virulent virus (EID_{50} $10^{3\cdot5}$/0.2 ml) intra-muscularly and were observed for the symptoms of RD.

Immuneresponsiveness to IBDV/10 inoculation:

Forty, one-day old chicks were grouped as C and D having 20 chicks each. One drop of (0.02 ml) IBDV/10 (EID_{50} $10^{6\cdot38}$/0.2 ml) was given intra-ocularly to the chicks from group C on 1st day of age. Group D chicks were kept as control. All the chicks from group C and D were bled by jugular vein at weekly intervals for four weeks. The sera were subjected to radial immunodiffusion test (RID)[2]. Two chicks from each group C and D were scarificed after 48 hr and on 4th, 7th and 15th post inoculation days (PID) and the tissues (Bursa of Fabricius, thymus, kidney and spleen) were collected for histopathology in 10% formalin. On 28th PID, five chicks from each group C and D were challenged intra-ocularly by giving one drop of virulent IBDV. These chicks were housed separately and sacrificed 7 days after the challenge and the tissues were collected for histopathology as before.

Immunosuppressive effect of IBDV/10:

Sixty, one-day old chicks were grouped as E and F having 30 chicks in each. Maternal antibodies against RD virus were determined from 25 randomly selected chicks by Hemagglutination inhibition (HI) test[3] using eight hemagglutination units of LaSota strain of RD. Group E chicks were inoculated with IBDV/10 at day-old stage while chicks from group F were kept as control. On fourth day of age, chicks from both the groups were vaccinated against RD and were housed separately. All the chicks from group E and F were bled at weekly intervals and HI test was performed. Twentyeight days after RD vaccination chicks from both the groups were challenged with virulent RD and were observed for the symptoms of RD subsequently. *t* test was applied to compare mean HI titres in this trial.

Effect of IBDV/10 in susceptible age of chicks:

Thirtysix, four-week old chicks were inoculated

with IBDV/10 as before and housed separately. These chicks were sacrificed along with the control after 48 hr, 4 days, 7 days and 15 days PI and the tissues were collected for histopathology as before. At 5th and 6th week of age, another batch, each having eight chicks was inoculated with the same virus and the experiment was repeated.

RESULTS

Effect of IBDV/6 inoculations:

Fortyeight hours after the challenge with virulent RD chicks from group A exhibited the symptoms of RD, while those from group B did not show any symptoms. All the chicks from group A succumbed to the infection after 72 hr, indicating that passage six virus had not lost its virulence and hence it exhibited the immunosuppressive effect.

Immuneresponsiveness to IBDV/10 inoculation:

The chicks from group C did not show any gross lesions at autopsy apart from slight bursal enlargement, when sacrificed in the post-inoculation period of IBDV/10. However, histopathologically no abnormality was found. In RID antibodies against IBDV/10 were present in chicks of group C on 11th day PI. A wider zone was seen on 18th day PI. On 25th day the zone was slightly decreased and no antibodies were detected on 28th day.

The chicks from group C that were challenged with virulent IBDV did not show any symptoms of IBD, while those from group D showed bursal enlargement at autopsy. Histopathological examination revealed the lesions specific for IBD, such as severe lymphoid necrosis affecting the bursal follicles resulting in cystic degeneration. This was followed by atrophy of bursal follicles.

Immunosuppressive effect of IBDV/10:

The maternal HI antibody titre against RD was 64 units. The HI titres of group E and F did not differ significantly from one another (table 1). All the chicks from both the groups withstood the challenge with virulent RD.

Table 1 *Average HI titres against R.D.*

Day post-vaccination	Mean HI titres group E (IBDV/10)	Mean HI titres group F (Control)
7	179.2	204.8
14	204.8	230.4
21	230.4	243.2
28	179.2	179.2

The t value in all cases was ≥ 0.05.

Effect of IBDV/10 in susceptible age of chicks:

No macroscopic or microscopic IBD-specific lesions were observed when IBDV/10 was given to the chicks at four, five and six weeks of age.

DISCUSSION

Since the immunosuppressive effect was shown to be severe and of a permanent nature when the agent was given in the first week of life[4], the egg-adapted virus was given at one day of age in all trials except the one with IBDV/6 in which it was given on 4th day of age to assess this effect.

The results of IBDV/6 exposure indicate that the passage six virus was not sufficiently attenuated for its use as a safe immunogen and hence it exhibited its immunosuppressive effect on the immune system of chicks like a non-attenuated virus. This effect was assayed in terms of serological response to R.D. vaccination. The susceptibility of chickens to R.D. challenge despite prior vaccination was demonstrated, confirming the earlier reports[5,6].

IBDV/10 did not produce any lesions in the chicks and all the chicks withstood the challenge of virulent IBDV. Egg-adapted virus at eight passage level was shown to protect the chicks against virulent IBDV[7]. The criteria used to assess the protection were the absence of clinical symptoms and lesions in the post-inoculation period upto 10 days. In the present studies the criteria used to evaluate the immunity were the absence of clinical symptoms and lesions after post-inoculation period of 15 days and after the challenge period of 7 days. No precipitating antibodies were detected in AGPT. To overcome this difficulty in demonstrating precipitins, RID was performed. The precipitating zones were demonstrated 11 days after inoculation. The antibodies were not detected after 28 day post-inoculation. In this trial, chicks withstood the challenge with virulent IBDV indicating that specific neutralizing antibodies were developed in these chicks. The studies on immunosuppressive effect of IBDV/10 indicate that although the HI titres from IBDV inoculated groups (group E) were slightly low, they did not differ significantly from the control ones. These birds also withstood the challenge with virulent RD indicating that the virus was well adapted to the chicken embryo and hence did not produce im-

munosuppressive effect on the immune system of chicks.

Since the susceptible age for IBDV infection is 3 to 6 weeks, IBDV/10 was given to the chicks at this age to assess its effect. The inoculated chicks neither showed any symptoms in post exposure period nor the lesions when sacrificed at certain intervals after the exposure of virus.

In the light of the present observations it can be concluded that IBDV 10 inoculation produces seroconversion in the chicks without affecting the immunocompetence of the chicks. However, further field trials are essential to substantiate these findings.

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