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Chicken Infectious Disease Virus Recombinant DNA Constructs and its Efficacy in Special Pathogen Free Chickens

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Abstract: In order to determine the effective region of large segment genome of infectious bursal disease virus for the preparation of DNA vaccine, four-recombinant DNA constructs with various combination of VP2 and VP5 fragments have been inserted into plasmid pcDNA3.1 were constructed. They were designated as VP5-5.1 + VP2-3.4 (A), VP5-5.2 + VP2-3.4 (B), VP2-2.5 + VP2-3.4(C) and VP2-5.3 + VP2-3.3 (D) respectively. Immunization assays was conducted using 1-week-old SPF chickens and DNA constructs were delivered in two ways, intramuscularly injections or via the gene gun bombardments. Chickens were primed with 100ug DNA, then boosted were given at 7days interval for two times. The chickens were finally challenged with a IBDV HK46 strain. After analyzing the neutralizing antibody and bursa-body weight ratios, the recombinant construct VP5-5.2 + VP2-3.4 was found to be the most effective when given through intramuscularly injections or via the gene gun bombardments.

Key words: DNA vaccine, Infectious Bursal Disease Virus and HK46 strain

Introduction

Infectious bursal disease, also known as Gumboro disease, is caused by infectious bursal disease virus (IBDV), it may cause up to 20% mortality in chicken three weeks of age or older and prolonged immunosuppression of chickens infect at early age (Lukert and Saif, 1996).

This problem is compounded by the emergence of antigenic variants, new antigenic viruses continue to emerge through molecular changes in the hypervariable region of the VP2 gene, these changes may allow the new viruses to escape neutralization from antibody produced against commercial vaccine viruses (Jackwood and Summer, 1999; Snyder *et al.*, 1986). Vaccination is the primary means to reduce IBDV-associated problems, the DNA vaccine technology has been developed in the past few years (Wolff *et al.*, 1990), since DNA vaccine is very stable in chemical nature and it can elicit both humoral and cellular immunity, as a alternative way, it may have better efficacy in the prevention of IBD outbreak.

IBDV viral genome has two segments, designated as A and B (Azad *et al.*, 1987and Spies *et al.*, 1987), segment A encodes structural protein VP2, VP3 and protease VP4 of IBDV, VP5 is overlapped with VP2. While segment B encodes the functional protein VP1. VP2 is the major host-protective immunogen of IBDV, which contains the antigenic sites responsible for the induction of neutralizing antibodies (Azad *et al.*, 1987; Becht *et al.*, 1988; Fahey *et al.*, 1991 and Synder *et al.*, 1992). Sequences of VP2 in various strains are highly conserved, except the central Accl-Spe I restriction fragment which is designated as the hypervariable region (Bayliss *et al.*,1990). VP5 has a regulatory function and could play a key role in virus release and dissemination (Mundt *et al.*, 1995). Due to the apoptotic activity and conformation dependent of VP2, DNA vaccine incorporating the whole VP2 sequences had been confirmed not totally effective protect chicken from infection, the appropriate region in fragment A fits for the preparation of DNA vaccine remains to be solved. In this study, 4 combination of various size of VP2 and VP5, derived from a IBDV HK46 strain, was determined in the SPF chicken immunization test and two kinds of DNA delivery methods was also compared.

Materials and Methods

Animals: The SPF broiler embryos, from a Shandong SPF farm flock, were separated into 5 groups. Chickens were given a standard cornsoy broiler feed and water ad libitum.

Virus. IBDV HK46 strain was a very virulent strain which was isolated and identified by our lab previously, it was propagated and titrated in SPF embryonated chicken eggs as described (Boon-leong *et al.*,1999).

Construction and Preparation of DNA Vaccines. Four size inserts ampplified from IBDV HK46 strain with a RT-PCR were cloned into pcDNA3.1 respectively according to the protocol described in molecular cloning, after characterization of the recombinants with restriction enzyme digestion and sequencing, propagate it in large scale and extract plasmid by using a Giga plasmid preparation kit (Qiagen).

Experimental Protocol:1-week-old SPF chicken was separated into 5 treatment groups.6chickens per group. In the first group, chickens were given pcDNA3.1,

Table 1: Primers used in this study

Primer name	Sequence	Primer combination	Product size
1.VP2-5.2	CGAGAAGCTTCGATGTACTGCAGGCTAGTG AGTCG	1+3(A)	1178
2.VP2-3.3	GGCGAATTCTCATACTAGTGTGACGGGACGGA	2+4(B)	439
3.VP2-3.4	GGCGAATTCTCATCCTGAAGCAGCCTGTGTCT		
4.VP2-5.3	CGAGAAGCTTCGATGTACACCATGTACACCAT AACTGCAGCCGA		
5.VP5-5.1	AGAGAAGCTTCGTCCAGGATGGAACTCCTCCT	5+3(C)	1505
6.VP5-5.2	GGAGAAGCTTCCGATGGTTAGTAGAGATCAGA	6+3(D)	1468

Table 2: Efficacy of Four IBDV DNA vaccines given to SPF broilers by intramuscularly injection

Treatment group	No. Atrophied bursae	% Protection	Vaccine content
Saline (nonchallenged)	0	--	
Saline (challenged)	6	0	
Vaccine A	1	83	VP5-5.1+VP2-3.4
Vaccine B	0	100	VP5-5.2+VP2-3.4
Vaccine C	2	67	VP2-2.5+VP2-3.4
Vaccine D	2	67	VP2-5.3-VP2-3.3

* Total number of birds is 6 per group

** Challenge virus was a variant HK46 strain

Table 3: Efficacy of Four IBDV DNA vaccines given to SPF broilers by gene gun bombardment

Treatment group	No. Atrophied bursae	% Protection	Vaccine content
Saline (nonchallenged)	0	--	
Saline (challenged)	6	0	
Vaccine A	0	100	VP5-5.1+VP2-3.4
Vaccine B	0	100	VP5-5.2+VP2-3.4
Vaccine C	0	100	VP2-2.5+VP2-3.4
Vaccine D	2	67	VP2-5.3-VP2-3.3

* Total number of birds is 6 per group

** Challenge virus was a variant HK46 strain

whereas in group 2, 3, 4 and 5 were given one of the four DNA constructs (A, B, C, D), DNA constructs were delivered in two methods, gene gun bombardment or needle injection. primed the chicken with 100ug DNA/chicken at first week, then booster with the same dosage at a 1week interval for 3 times, challenged chicken with HK46 at 10^3 mean chicken infective dose by eye and nasal drop at fifth week, at the end of fifth week, birds were euthanatized and bursa weights and body weights were taken, chicken serum was analyzed with a neutralization assay.

Statistical Design: For the challenge infection, statistics described by Giambrone (Giambrone *et al.*, 2001) were used to analyze differences in the bursa and body weight data among groups. Briefly, A ratio was calculated by following formula: bursa weight in grams/body weight in grams X 1000. The mean and standard deviation were calculated for all groups. Data were derived from SAS statistical analysis system. A challenged bird with a bursa-body weight ratio two standard deviations below the mean of the control group was scored as lacking protected (atrophied bursa). A percentage of birds protected (not having an atrophied bursa) were then calculated for each challenged flock.

Results

Data for the first experiment are listed in Table 2 and Fig.1. All four IBDV DNA vaccines caused neutralization antibody response when given to SPF broilers via intramuscularly injection, among them, Vaccine B had the best protection and highest neutralization antibody titer.

In the second experiment (Table 3 and Fig.2), when these DNA vaccine were given by gene gun bombardment, DNA vaccine A, B, C had a full protection, whereas vaccine D only had a protection of 67%, the neutralization antibody response was the same as intramuscularly injection group.

Discussion

Data herein demonstrated which fragment of large segment genome of IBDV is the most effective region when preparing a DNA vaccine, after intake DNA vaccine by the cell, the effectiveness of DNA vaccine

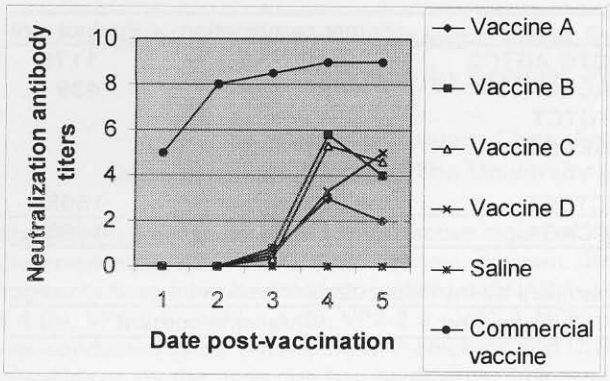

Fig.1: Neutralization antibody response in SPF broilers caused by 4 IBDV DNA vaccines given by intramuscularly injection

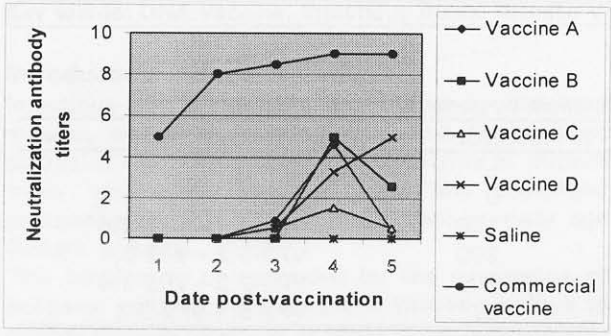

Fig.2: Neutralization antibody response in SPF broilers caused by 4 IBDV DNA vaccines given by gene gun

depends on its ability to elicit humoral or cell-mediated immunity by the expression of antigenic determinant encoded by the DNA strand, since the protective epitopes in VP2 protein is well known to be highly conformation dependent (Heine *et al.*, 1994), therefore, the right region is critical for the efficacy of DNA vaccine, VP2 gene has been expressed in a number of systems(Macreadie *et al.*, 1990; Pitcovski *et al.*,1996; Heine and Boyle, 1993; Sheppard *et al.*,1998) and a Purdue group(Chang *et al.*, 2003) has found one of the region for DNA vaccine, our results confirmed their findings and provided an alternative way for the preparation of IBDV DNA vaccine.

The effect of DNA delivery route on immunity and clinical protection was compared in this study, DNA vaccine delivered by gene gun bombardment is better than intramuscularly injection, whereas Vaccine B had a protection of 100% when delivered by either of the methods, therefore, it is thought to be the best region for the preparation of DNA vaccine, it is well known that the efficacy of DNA vaccines varies not only according to the route of delivery, but also to the animal species and pathogen and delivery of DNA at mucosal sites may have a higher expression.

Neutralization antibody titer to IBDV was low compared with commercial vaccine, but protection was achieved, this confirmed the mechanism of protective immunity induced by DNA vaccine differs from conventional IBD vaccines; it is identical with other groups research.

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