Vaccine 27 (2009) 7046–7052

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

# Protection of chickens against infectious bronchitis by a recombinant fowlpox virus co-expressing IBV-S1 and chicken IFNγ

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ARTICLE INFO

*Article history:*
Received 7 July 2009
Received in revised form 7 September 2009
Accepted 16 September 2009
Available online 26 September 2009

*Keywords:*
Infectious bronchitis virus
S1 gene
Recombinant fowlpox virus
Chicken type II interferon

ABSTRACT

A fowlpox virus expressing the chicken infectious bronchitis virus (IBV) S1 gene of the LX4 strain (rFPV-IBVS1) and a fowlpox virus co-expressing the S1 gene and the chicken type II interferon gene (rFPV-IBVS1-ChIFNγ) were constructed. These viruses were assessed for their immunological efficacy on specific-pathogen-free (SPF) chickens challenged with a virulent IBV. Although the antibody levels in the rFPV-IBVS1-ChIFNγ-vaccinated group were lower than those in the attenuated live IB vaccine H120 group and the rFPV-IBVS1 group, the rFPV-IBVS1-ChIFNγ provided the strongest protection against an IBV LX4 virus challenge (15 out of 16 chickens immunized with rFPV-IBVS1-ChIFNγ were protected), followed by the attenuated live IB vaccine (13/16 protected) and the rFPV-IBVS1 (12/16 protected). Compared to those of the rFPV-IBVS1 and the attenuated live IB vaccine groups, chickens in the rFPV-IBVS1-ChIFNγ group eliminated virus more quickly and decreased the presence of viral antigen more significantly in renal tissue. Examination of affected tissues revealed abnormalities in the liver, spleen, kidney, lung and trachea of chickens vaccinated with the attenuated live IB vaccine and the rFPV-IBVS1 vaccine. In rFPV-IBVS1-ChIFNγ-vaccinated chickens, pathological changes were also observed in those organs, but were milder and lasted shorter. The lesions in the mock control group were the most severe and lasted for at least 20 days. This study demonstrated that chicken type II interferon increased the immunoprotective efficacy of rFPV-IBVS1-ChIFNγ and normal weight gain in vaccinated chickens although it inhibited serum antibody production.



## 1. Introduction

Avian infectious bronchitis (IB) is an acute, highly infectious viral disease affecting chicken respiratory systems. This disease can also affect chicken kidney function and decrease both egg production and quality. Chickens may develop this disease at different stages in their life, and when infected will typically die of respiratory or renal infection. The mortality rate of IB generally ranges from 20 to 30%, and may reach 40–90% [1]. Attenuated live and inactivated oil-emulsion vaccines are generally effective in controlling the disease. However, as the number of chickens being raised increases, IB outbreaks may occur despite the use of the vaccine. Moreover, new viral strains continually emerge and mutation and recombination occur frequently in attenuated live vaccines [2]. Because of these issues, traditional vaccines may not be able to control and eliminate IB virus (IBV) efficiently. Development of molecular techniques may bring a new hope to the agricultural community in combating diseases with multiple serotypes. For example, the development of live vector vaccines is promising in the field of animal disease control.

The major immunogen of IBV, S1 protein, has a molecular weight of ~90 kDa and is made up of 520–538 amino acids with 28–29 glycosylation sites. The S1 protein contains epitopes that can induce the production of specific antibodies capable of neutralizing the virus and inhibiting hemagglutination [3–5]. In addition, the S1 protein N-terminus plays an important role in tissue tropism and the degree of virulence of the virus [6]. The S1 protein can help the virus adhere to the cell membrane, thus facilitating cell fusion and infection. Therefore, this protein is important in making strategies for controlling IB and understanding the mechanism of IBV evolution. There are several reports on the production of genetically engineered vaccines using the S1 gene. Tomley et al. [7] reported that a recombinant vaccine virus expressing the S1 protein induced neutralizing antibodies in vaccinated mice. Wang et al. [8] also found that their constructed recombinant fowlpox virus expressing the S1 protein protected chickens against an infectious bronchitis virus

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doi:10.1016/j.vaccine.2009.09.065

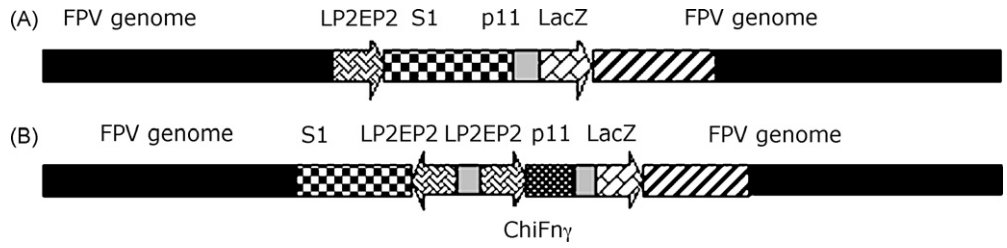


**Fig. 1.** Illustration of exogenous genes on fowlpox virus genome: rFPV-IBVS1 (A) and rFPV-IBVS1-ChIFNγ (B).

challenge. A recombinant fowl adenovirus expressing the S1 gene induced a 90–100% protection against either a homologous or heterologous IBV challenge [9]. These studies suggested that live viral vectors offer a promising vaccine alternative in fighting against IBV.

Fowlpox virus (FPV) vectors expressing foreign genes have become widely used in vaccine development. Several strains of FPV expressing major avian immunogenic genes have been successfully constructed, some of which are commercially available [10,11].

Although the fowlpox virus has proven to be a successful vector and a dozen of recombinant fowlpox viruses expressing various protective antigens have been confirmed to be safe and effective, some studies have demonstrated that vaccinated animals do not gain adequate weight following fowlpox virus and pigeon poxvirus administration [12–15]. The interferon has been proven to suppress stress reactions induced by poxvirus administration [16–18].

In this study, a fowlpox virus expressing the IBV S1 gene (rFPV-IBVS1) and a fowl poxvirus co-expressing the IBV S1 gene and the chicken type II interferon gene (rFPV-IBVS1-ChIFNγ) were constructed and their immunological efficacy investigated by immunizing SPF chickens, respectively.

## 2. Materials and methods

### 2.1. Viruses, cells, and chicken embryos

The parental virus used to construct the recombinant FPV, S-FPV-017, was kindly provided by Dr. Yilma, the University of California, Davis (USA). The parental FPV was propagated in monolayers of specific-pathogen-free (SPF) chicken embryo fibroblasts (CEF) (Center of Laboratory Animals, Harbin Veterinary Research Institute, China) in DMEM supplemented with 5% fetal calf serum. The LX4 strain of the IBV, a virus capable of inducing renal pathology [19], was used to test the vaccine efficacy. The vesicular stomatitis virus (VSV) Indiana strain and L929 cells provided by Dr. Sun Chengqun, Harbin Veterinary Research Institute, China were used to evaluate the anti-viral activity of IFNγ in this study.

### 2.2. Construction of recombinant fowlpox viruses

The amplification primers (forward primer 5′-ATA TGG ATC CCA TGT TGG GGA AGT CAC TGT-3′ and reverse primer 5′-TAT GGA TCC AAT GCC AAC TAT ATT GCC ACC-3′) were designed based on the S1 gene sequence from the IBV LX4 strain (Accession no. AY338732 and AY223860) and the S1 gene (Accession no. AY189157) was subsequently amplified using a reverse transcription polymerase chain reaction (RT-PCR) [20]. The obtained product was inserted into a *Bam*HI site downstream of the LP2EP2 promoter in the pSY538 plasmid [11]. The S1 expression cassette in pSY538 was digested with restriction enzymes and the produced fragments were ligated into a *Not*I site between the homologous arms of the poxvirus gene in the pSY681 plasmid to generate a pSY681-IBVS1 plasmid [11]. The chicken type II interferon gene (ChIFNγ; nucleotides 1–504 bp) was cloned into the *Eco*RI site at the LP2EP2 promoter in the pSY538 plasmid and the P11 promoter-LacZ gene expression cassette of the vaccinia virus was inserted into the *Sma*I site. The DNA fragment containing the ChIFNγ expression cassette and LacZ expression cassette was obtained by using *Pst*I cleavage and ligated into the *Not*I site of the pSY681-IBVS1 plasmid that had been partially cut with *Not*I to generate the transfer vector pSY-ChIFNγ-S1 (Fig. 1). Plasmid DNA was purified with a Wizard®PureFection Plasmid DNA purification system (Promega, Madison, WI, USA) and transfected into CEF that had already been infected with the parental fowlpox virus, S-FPV-017. The CEFs were cultured at 37 °C in 5% $CO_2$ for 2 h and the culture medium was then replaced with maintenance medium. When 80% of the cells had developed lesions, all CEFs were collected and freeze-thawed three times. After inoculation onto another CEF monolayer, these cells were subjected to plaque purification. By consecutively purifying the culture eight times, clones that formed stable blue-colored plaques were expanded. The recombinant FPV containing full-length S1 and ChIFNγ was confirmed by PCR and named rFPV-ChIFNγ-S1. Expression levels of the IBV S1 gene and ChIFNγ gene in rFPV-ChIFNγ-S1 were also analyzed.

The LacZ expression cassette was inserted into the *Sma*I site of the pSY681-IBVS1 plasmid to construct a transfer vector pSY-IBVS1. Through screening and identification, the recombinant fowlpox virus that was confirmed to contain the IBV S1 gene was named rFPV-IBVS1.

### 2.3. Immunization and challenge of SPF chickens

Eighty-four-week-old SPF white Leghorn chickens from the Animal Breeding Center of Harbin Veterinary Research Institute, China were divided into five groups with 16 chickens in each group. Chickens were raised in five separate Specific Free Pathogen (SPF) isolators with negative pressure. Three groups were vaccinated by subcutaneous wing injection of $5 \times 10^6$ PFU of the S-FPV-017, rFPV-IBVS1 and rFPV-IBVS1-ChIFNγ. The fourth group was vaccinated with H120 vaccine according to the manufacturer's instructions (Harbin Weike Biotechnology Development Company, Harbin, China). The fifth group was injected with PBS. Four weeks later, chickens from all groups were challenged with $10^{3.8}$ EID50 per chicken in 200 μl of the IBV LX4 strain. Any clinical signs including death from day 2 post-challenge were recorded. Blood samples from vaccinated chickens were collected once a week until day 21 post-challenge when all experimental chickens were euthanized with sodium pentobarbital. On days 2, 4, 6, 8, 10, 12, and 14 post-challenge, throat swabs were collected and taken for virus isolation from five chickens randomly selected from each group. On days 7, 10, 13 post-challenge, one chicken randomly selected from each group was euthanized to obtain its trachea, liver, spleen, kidney, lung, pancreas and glandular stomach for pathological examination. Each chicken was weighed on the day before vaccination (when they were 28 days old), day 15 post-vaccination (43 days old), the day before challenge (56 days old) and day 15 post-challenge (71 days old). Body weight gains were analyzed by ANOVA using the STATISTICA software (*version 6.1*.StatSoft, Inc., Tulsa, OK).