Vaccine 28 (2010) 8112–8119

Contents lists available at ScienceDirect

Vaccine

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

Construction and immunogenicity of a recombinant fowlpox vaccine coexpressing S1 glycoprotein of infectious bronchitis virus and chicken IL-18

Hong-Ying Chen[a,1], Ming-Fan Yang[a,1], Bao-An Cui[a,*], Pei Cui[b], Min Sheng[b], Guo Chen[b], Shu-Juan Wang[a], Jing-Wei Geng[a]

[a] College of Animal Science and Veterinary Medicine, Henan Agricultural University, Wenhua Road 95#, 450002 Zhengzhou, Henan Province, People's Republic of China
[b] Henan Center for Animal Disease Control & Prevention, Animal Husbandry Bureau of Henan Province, Zhengzhou 450008, Henan Province, People's Republic of China

ARTICLE INFO

Article history:
Received 18 November 2009
Received in revised form 8 September 2010
Accepted 23 September 2010
Available online 14 October 2010

Keywords:
Infectious bronchitis virus
S1 gene
Chicken interleukin-18 gene
Recombinant fowlpox virus

ABSTRACT

Infectious bronchitis virus (IBV) poses a major threat to the chicken industry worldwide. In this study, we developed a recombinant fowlpox virus (rFPV) vaccine expressing the IBV S1 gene and chicken interleukin-18 gene (IL-18), rFPV-S1/IL18. Recombinant plasmid pSY-S1/IL18 was constructed by cloning chicken IL-18 into fowlpox virus transfer plasmid containing S1 gene and transfected into the chicken embryo fibroblasts cell pre-infected with S-FPV-017 to generate rFPV-S1/IL18. Expression of the recombinant proteins was confirmed by RT-PCR and IFA. We also constructed the recombinant fowlpox virus rFPV-S1 without IL-18. One-day-old chickens were vaccinated by wing-web puncture with the two rFPVs, and the induced humoral and cellular responses were evaluated. There was a significant difference in ELISA antibody levels ($P < 0.05$) elicited by either rFPV-S1 or rFPV-S1/IL18. The ratios of $CD4^+$ to $CD8^+$ in chickens immunized with rFPV-S1/IL18 were significantly higher ($P < 0.05$) than in those immunized with rFPV-S1. All chickens immunized with rFPV-S1/IL18 were completely protected (20/20) after challenge with the virulent IBV HN99 strain 43 days after immunization, while only 15 out of 20 of the chickens immunized with the rFPV-S1 were protected. Our results show that the protective efficacy of the rFPV-S1 vaccine could be enhanced significantly by simultaneous expression of IL-18.

1. Introduction

Infectious bronchitis (IB) is an acute, highly contagious respiratory, renal, and urogenital disease of chickens caused by the coronavirus, infectious bronchitis virus (IBV). It is still a major health problem in the chicken industry worldwide. Vaccination to control IB has been practiced for over half a century [1–3]. Such conventional vaccines, although generally effective, do have some disadvantages. Attenuated vaccines, which generally induce long-lasting immunity, have a risk of insufficient attenuation and/or genetic instability [4]. The limitations of inactivated vaccines include high manufacturing costs and lack of long-term immunity. Thus, developing a vaccine to control this disease with higher efficacy and fewer side effects is highly desirable.

Since IBV was first described by Schalk and Hawn in the 1930s [5], numerous serotypes or variants have been identified worldwide, against which little or no cross-protection exists [6,7]. IBV was first detected in China in 1972, and numerous nephropathogenic strains have been isolated since 1982 [8]. In spite of extensive vaccine use, IBV outbreaks remain frequent in China. Infected broilers show clinical signs of depression, dehydration, and polyuria, with swelling of the kidneys and severe urate deposition, which results in death. Infected breeders or layers have decreased egg production. The HN99 nephropathogenic strain of IBV was isolated from one of a group of approximately 3-week-old broilers in Henan Province suffering from depression, dehydration, and polyuria. Because it is the most prevalent strain in China, we developed a recombinant anti-IBV vaccine based on this virus.

The IBV gene encoding the virus surface glycoprotein spike protein 1 (S1) was a logical choice for inclusion in a recombinant candidate vaccine vector. The S1 protein is considered to be a primary inducer of protective immunity [9]. For example, four intramuscular administrations of immuno-affinity purified S1 induced 78% protection against IBV challenge [10]. Likewise, recombinant baculovirus expressing the S1 gene of a Korean nephropathogenic strain of IBV protected 50% of inoculated chickens against IBV challenge, as assessed by examination of their kidneys [11]. Overall, the results of these studies indicate that the S1 glycoprotein would be a useful candidate for inclusion in alternative IBV vaccines.

Fowlpox virus (FPV) has a large double stranded DNA genome and a host range limited to avian species [12]. FPV has been developed as an effective live viral vector, successfully expressing

* Corresponding author. Tel.: +86 371 63558878; fax: +86 371 63558878.
E-mail addresses: cuibaoan127@163.com, baoancui@henau.edu.cn (B.-A. Cui).
[1] These two authors contributed equally to this work.

0264-410X/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.vaccine.2010.09.106

protective foreign genes from various poultry pathogens, including Newcastle disease virus, avian influenza virus, IBV, infectious laryngotracheitis virus, and Marek's disease virus. Fowlpox virus has a number of advantages as a vector, but its side effects cannot be ignored: its effects on weight gain and on immune function [13]. It has been confirmed that some cytokines can relieve these side effects, and some of these cytokines are effective immunomodulators in animal models or clinical trials. Cytokine adjuvants have been widely used to promote the induction of immune responses and enhance the immunoprotective effects of vaccines against bacteria, viruses, or parasites [14]. IL-18 is one possible option, and is known as interferon-gamma (IFN-γ)-inducing factor because of its ability to stimulate T helper 1 (Th1) cells to secrete IFN-γ [15]. Therefore, the purpose of the present study was to construct two recombinant fowlpox viruses (rFPVs) expressing the S1 gene of IBV and co-expressing the S1 gene of IBV and the chicken IL-18 gene, which was included to overcome the FPV-induced inhibition of weight gain, and increase the efficacy of immunization.

2. Materials and methods

2.1. Virus, experimental animals and plasmids

Fertilised White Leghorn specific-pathogen-free (SPF) eggs were purchased from Shangdong Institute of Poultry Science, Shandong, PR China. Chickens were hatched and housed in a SPF environment at the Laboratory Animal and Resources Facility, Henan Agricultural University. The nephropathogenic strain HN99 of IBV was propagated in the allantoic cavities of 10-day-old SPF embryonated chicken eggs, and the allantoic fluid was harvested 48 h after inoculation. The median embryo infective dose (EID_{50}) was determined by inoculating a 10-fold dilution series of the virus into 10-day-old SPF embryonated chicken eggs.

The parental fowlpox virus, S-FPV-017, was a less attenuated FPV strain (a kind gift from Dr. Hua-Lan Chen, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences).

Recombinant plasmids pGEM-T-S1 and pGEM-T-IL18 were used in this study. A cDNA fragment encoding the full-length S1 gene was amplified from the RNA of IBV HN99 strain and cloned into the pGEM T-Easy vector. The resulting plasmid, pGEM-T-S1 was sequenced (GenBank accession no. AY775551). The chicken IL-18 gene was obtained from chicken splenocytes by reverse transcriptase-polymerase chain reaction (RT-PCR) and cloned into pGEM T-Easy, and the resultant recombinant plasmid, pGEM-T-IL18, was sequenced (GenBank accession no. AY775780).

2.2. Homologous recombination and screening of the recombinant virus

The plasmid pSY-S1/IL18 was constructed as described previously [16]. Briefly, cDNA encoding the whole S1 gene of IBV was amplified by PCR from the plasmid pGEM-T-S1 using the forward primer 5′-ATGAGGATCCAATGTTGGTGAAG TCACT-3′ and the reverse primer 5′-ATGCG GATCCATA ACTAACATAAGGGCA-3′ (*Bam*H I restriction enzyme site is shown by an underline on the sense and antisense primers). The PCR product was digested with *Bam*H I and cloned into similarly digested plasmid pSY538 under the control of the early-late LP_2EP_2 promoter of FPV. The LacZ gene fragment with the P11 late promoter of vaccinia virus from the plasmid pSC11 was digested with *Pst* I and *Xba* I, and also cloned into the *Sma* I site of the pSY538 containing the S1 gene. The fragment containing the S1 and LacZ genes was cloned into a *Not* I site between the homologous arms of the poxvirus gene in the FPV transfer vector pSY681, resulting in the plasmid pSY-S1. For the construction of plasmid pSY-S1/IL18, the chicken IL-18 gene was amplified by PCR from pGEM-T-IL18 using the primers 5′-CCCGAATTCATGAG CTGTGAAG AGATC-3′ and 5′-CGGGGAATTCTCATAGGTTGTGCCTTT-3′ (*Eco*R I restriction enzyme site is shown by an underline on the sense and antisense primers). The PCR product was digested with *Eco*R I and cloned into similarly digested pSY538 under the control of the early-late LP_2EP_2 promoter of FPV. Finally, pSY-S1/IL18 was constructed by the insertion of the fragment containing the LP_2EP_2 promoter and the IL-18 gene into pSY-S1.

The rFPVs were generated by homologous recombination using published procedures [17]. Briefly, two rFPVs (rFPV-S1 and rFPV-S1/IL18) were generated by transfecting into chicken embryo fibroblasts (CEF) with the corresponding recombinant plasmids in six-well plates which had been infected with the parental fowlpox virus S-FPV-017 at multiplicity of infection (m.o.i.) of 0.01 two hours before transfection. Parental fowlpox virus S-FPV-017 infected CEF cells were used as an infection control. The viruses were collected after cytopathic effect (CPE) appeared, and rFPVs were screened for beta-galactosidase activity in the presence of 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-gal) (TaKaRa, Dalian, China). After eight rounds of blue plaque purification, the two rFPVs were obtained and cultured in CEF cells. Insertion of the recombinant gene into the FPV genome was confirmed by PCR, and expression of S1 and IL-18 confirmed by RT-PCR and indirect immunofluorescence assay (IFA) as described below.

2.3. PCR analysis of the rFPVs

The genomic DNA of rFPVs, extracted using SDS-proteinase K-phenol, was used as PCR template and amplifications were performed with TaKaRa Ex *Taq* DNA polymerase and the primers described above.

2.4. RT-PCR analysis

After infection for 48 h, the cells were harvested and total cellular RNA was prepared from the cells using Trizol reagent (Gibco BRL, USA). The reverse transcription (RT) reaction was performed using 20-μl volumes; the reaction mixture contained 5× Strand buffer, 25 mM of each deoxynucleoside triphosphate (dNTP; Amersham Biosciences Corp., Piscataway, NJ, USA), 2.5 U of RNase inhibitor (Promega Corporation, Madison, WI, USA), 50 pmol/ml random hexamers, Moloney murine leukemia virus (MMLV) reverse transcriptase (Invitrogen Life Technologies, Carlsbad, CA, USA), and 5 μl of total cellular RNA and diethyl pyrocarbonate (DEPC)–water. RT was performed at 42 °C for 60 min and at 75 °C for 10 min. Polymerase chain reaction (PCR) was then amplified with specific primer sets for the S1 gene and IL-18 as described above.

2.5. Indirect immunofluorescence assay of rFPV infected cells

After infection for 48–72 h, cells were washed with phosphate-buffered-saline (PBS) and fixed with cold methanol for 10 min. Cells were blocked with 1% bovine serum albumin (BSA) in PBS for 30 min at 37 °C. The fixed cells were incubated at 37 °C for 1 h with an IBV specific-chicken antiserum at a dilution of 1: 50. After three washes for 5 min each with PBS, the cells were incubated for 45 min at 37 °C with secondary fluorescein isothiocyanate (FITC)-labeled rabbit anti-chicken antibody (Sigma Chemical Co., St. Louis, USA) at a dilution 1:800. Cells were washed three times with PBS, and then examined with a fluorescent microscope (Model AX70, Olympus).

2.6. Immunization of chickens with the rFPVs

Eighty one-day-old White Leghorn SPF chickens were randomly allocated into four groups of 20. Chickens in groups 1 and 2 were immunized with rFPV-S1/IL18 or rFPV-S1, respectively. Chickens in