Vaccine xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

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

Infectious bronchitis virus poly-epitope-based vaccine protects chickens from acute infection

Lei Tan [a], Yuqiang Zhang [a], Fang Liu [a], Yanmei Yuan [a], Yuan Zhan [a], Yingjie Sun [a], Xusheng Qiu [a], Chunchun Meng [a], Cuiping Song [a], Chan Ding [a,b,*]

[a] Department of Avian Diseases, Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Shanghai 200241, PR China
[b] Jiangsu Co-innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou 225009, PR China

ARTICLE INFO

Article history:
Received 16 June 2016
Received in revised form 12 September 2016
Accepted 14 September 2016
Available online xxxx

Keywords:
Infectious bronchitis virus
Poly-epitope vaccine
Neutralization antibody
Cellular immunity
Challenge
Protection

ABSTRACT

Infectious bronchitis virus (IBV) causes major losses in the poultry industry. The safe and effective vaccine to control IBV spread is imperative. In the present study, we developed IBV S1 glycoprotein poly-epitope-based DNA vaccine *pV-S1B+S1T* consisting of SH1208 and Holte strain BF2-restricted T cell epitopes and Australian T strain dominant B cell neutralization epitopes. Specific pathogen-free chickens were vaccinated with *pV-S1B+S1T* and control plasmids twice to elicit strong humoral and cellular immune response, as indicated by viral neutralization titers and results of $CD8^+$ T cell proliferation assays. A lethal dose of IBV SH1208 strain used for protection and challenge experiments at two weeks post-booster immunization following challenge protection and virus shedding reverse transcription quantitative PCR assay, indicated that *pV-S1B+S1T* protected against IBV and significantly reduced viral excretion. These results demonstrated that the IBV poly-epitope-based vaccine effectively prevents infection and represents a potential IBV vaccine.

1. Introduction

Avian infectious bronchitis virus (IBV) belongs to the order of *Nidovirales*, family *Coronaviridae*, subfamily *Coronavirinae* and genus *Gammacoronavirus*. IBV mainly infects chickens of all ages and shows subtype-dependent tropism in renal tubuli, oviduct, and parts of the gastrointestinal tract. It causes respiratory disease, diarrhea, reduced weight gain and feeding, and decline in egg production and quality [1,2]. The extensive diversity of IBV strains worldwide hinders any preventative measures against infection in chickens, which in turn renders the disease critical for the poultry industry [3]. The vaccines targeting individual IBV serotypes show poor cross-protection, probably due to the presence of a large number of serotypes [1,4]. IBV shows extensive antigenic variation, reflecting mutations in the spike gene [5,6]. The IBV spike glycoprotein (S) is proteolytically cleaved into a 92-kDa S1 glycoprotein and a 84-kDa S2 glycoprotein, which play an important role in viral infection [7].

Mass, Australia T, Holte and Gray are the serotypes prevalent in China [8]. In recent years, the QX-like (also called LX4 like) IBV is the most significant type in China [9], which has rapidly increased from 11.7% to nearly 70%, whereas the Mass type IBV declined from 50.4% to 4.4% [10]. The QX-like IBV strains occur widely in Asia and Europe [11–14]. In this study, a QX-like IBV strain CK/CH/Shanghai/1208 (SH1208) that was previously isolated in a H120-vaccinated broiler flock in China was used for immune challenge experiment. The SH1208 strain showed 99%, 97%, and 96% homology of the IBV S1 nucleotide to field IBV strains SAIBK (GenBank Accession: DQ288927.1), SC021202 (GenBank Accession: EU714029.1) and YN (GenBank Accession: JF893452.1), respectively. Phylogenetic analysis showed that these four strains belong to the QX-like genotype and share a common ancestor, but cluster separately from the classic strains QX and LX4 isolated earlier [15,16]. The Australian T strain was the first documented

Abbreviations: IBV, infectious bronchitis virus; CMI, cell-mediated immunity; CTL, cytotoxic T lymphocyte; MHC, major histocompatibility complex; SPF, specific-pathogen free; ELD_{50}, chicken embryo lethal dose; EID_{50}, median embryo infectious dose; ATCC, American Type Culture Collection; DMEM, Dulbecco's Modified Eagle Medium; RPMI1640 medium, Roswell Park Memorial Institute; FBS, fetal bovine serum; PBS, phosphate-buffered saline; mAb, monoclonal antibodies; CFSE, carboxyfluorescein succinimidyl; VN, virus-neutralization; LSM, lymphocyte separation medium; dpc, days post challenge; C_t, cycle threshold; RT-qPCR, reverse transcription quantitative PCR; SEM, Standard Error Mean.

* Corresponding author at: Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Sciences, No. 518, Ziyue Road, Minhang District, Shanghai 200241, PR China.
E-mail address: shoveldeen@shvri.ac.cn (C. Ding).

http://dx.doi.org/10.1016/j.vaccine.2016.09.022
0264-410X/

nephropathogenic IBV strain causing regional epidemics in Australia [17]. In recent years, the Mass-type IBV and IBV closely related to Australian classical strains were also identified, indicating that IBVs with genetic variation were co-circulating in China [18]. Holte strain was first isolated in Canada, which induced nephritis and nephrosis in chickens [19]. This type of strain is mainly epidemic to North America, but also emerged in China. The Australian T, Holte and SH1208 strains are nephropathogenic and cause severe kidney damage. Most QX-like isolates also show strong affinity for the respiratory tract, with pronounced clinical signs [20].

Phylogenetic analysis suggested that the disease outbreaks in China might have been caused by infection with multiple strains of IBV [21] suggesting the need for a safe and effective IBV vaccine. Most of the currently used commercial vaccines, such as H120 and M41, belong to the Mass type [8]. However, vaccines specific to QX-like, T and Holte types are still not commercially available in China. Recently, epitope-based vaccines that are safe, non-infective, stable, and low cost have been developed, which may be improved by the inclusion of multiple epitopes such as those of T cells, B cells, or T helper cells, to develop poly-epitope vaccines [22–24]. Naturally occurring immune responses do not recognize all the possible epitopes, and instead only recognize common epitopes [25]. The immunodominant epitope response to a given pathogen activates T-cell-mediated immunity (CMI) and antibody-specific humoral immunity.

T cell epitopes combined with restricted major histocompatibility complex (MHC) class I complex activate cytotoxic T lymphocyte (CTL) response against virus-infected cells. Antibodies (Abs) that are mainly produced by B cell recognize the receptor-binding sites of antigens and those of neutralizing pathogens, including bacteria and viruses [26,27]. Naïve B cells are initially activated by specific B cell epitopes following co-stimulation by T-lymphocytes and differentiate into plasma cells that produce antibodies [28,29]. B cell epitopes contain contiguous linear epitopes and mostly discontinuous conformational epitopes [28]. Neutralizing antibodies are generally produced by B cell conformational epitopes [30], which have a high capacity to bind antibodies to neutralize the biological activity of antigens and cross-protect against different serotypes of homologous viruses [6,31,32].

The S protein also contains multiple epitopes that induce neutralizing antibodies. The IBV neutralizing epitopes reside within the hypervariable region of S1 at amino acid residues positions 24–61 and 132–149 and outside the hypervariable region at 291–398. The S2 subunit anchored to the viral membrane by a small hydrophobic transmembrane segment forms the stalk of the spike [4,7,33–35]. Furthermore, the S1 subunit of S protein harbors several T cell epitopes [24,36]. Our previous study showed that the S1 subunit BF2-restricted T cell epitope vaccine induced a high-level CMI and protects 90% of chickens against a lethal dose of IBV [37]. Ignjatovic et al. defined peptide regions within the S1 and S2 subunit of S protein and nucleocapsid (N) proteins to elicit humoral, cell-mediated, and protective immune responses. Two S1 subunit and one S2 subunit peptides protect kidney tissues against virulent challenges. None of the N peptides imparts a protective effect against virulent challenges. These results suggest that the S1 subunit of S protein carries antigenic regions that may be immunoprotective [36]. Thus, the S1 subunit is primarily considered to develop effective anti-IBV vaccines. In this study, we used the S1 subunit of three IBV strains S protein (SH1208, Australian T and Holte) to design a poly-epitope DNA vaccine. The S1 subunit nucleotide sequences of SH1208 strain S protein showed 80.7% and 79.4% homology to Australian T (GenBank Accession: AY775779.1) and Holte strain (GenBank Accession: L18988.1), respectively.

The purpose of the present study was to design a recombinant IBV poly-epitope DNA vaccine based on B cell neutralizing epitopes and BF2-restricted T cell epitopes, which simultaneously elicit humoral and cellular immune responses. We investigated its protective effect on specific pathogen-free (SPF) chickens subjected to an IBV challenge. The findings of the present study provide an alternative strategy for the development of a cost-effective and extensively immunoprotective vaccine for the control of IBV infection.

2. Materials and methods

2.1. Virus, cells, animals, plasmid and ethics statement

IBV SH1208 strain used in cross virus-neutralization and immune-challenge experiments was stored at −70 °C. Viral titers were determined by inoculating 10-fold dilutions into groups of five 10-day-old embryonated chicken eggs. The median embryo lethal dose (ELD_{50}) and median embryo infectious dose (EID_{50}) were calculated based on the method of Reed and Muench [38]. HeLa cells were purchased from ATCC (American Type Culture Collection, Manassas, VA, USA). Fertile white Leghorn SPF embryonated eggs were purchased from Beijing Merial Vital Laboratory Animal Technology Co., Ltd. The plasmid pV-S1 encoded full length S1 subunit of SH1208 was previously constructed and stored at −70 °C [37].

All experiments involved standard procedures with the formal approval of the Ethics and Animal Welfare Committee of Shanghai Veterinary Research Institute, China [Approval No. SHVRI (SH2015-0118)].

2.2. Reagents

Lipofectamine™ 2000, TRIzol, Dulbecco's Modified Eagle Medium (DMEM), Roswell Park Memorial Institute (RPMI) 1640 medium, Opti-MEM® medium, Fetal Bovine Serum (FBS), *pVAX1* vector, and TOPO® XL PCR Cloning Kit were purchased from Life Technologies (Carlsbad, CA, USA). Fluorescein isothiocyanate (FITC)-labeled mouse anti-chicken $CD8^+$ T cell antibody was provided by SouthernBiotech (Birmingham, AL, USA). Rabbit anti-IBV S1 polyclonal Abs against SH1208 strain were prepared in our laboratory. HRP-labeled goat anti-rabbit IgG (H&L) secondary Abs were purchased from Abcam (Cambridge, MA, USA). Restriction endonucleases (*Nhe I*, *Spe I*, *Hind III*, and *Xho I*) as well as the pMD19-T vector were acquired from Takara (Dalian, China). Easy-A High-Fidelity PCR Cloning Enzyme was supplied by Agilent Technologies (Santa Clara, CA, USA). The QuantiNova Probe RT-PCR kit was from QIAGEN (Hilden, Germany). Streptavidin-alkaline phosphatase, and carboxyfluorescein succinimidyl amino ester (CFSE) were acquired from Sigma (St. Louis, MO, USA). The chicken lymphocyte separation medium was obtained from the Hao Yang Biological Manufacturing Co., Ltd. (Tian Jin, China).

2.3. Design of IBV poly-epitope DNA vaccine

In the present study, we designed two IBV poly-epitope vaccines, one exclusively containing neutralizing epitopes of the IBV S1 subunit protein known as *pV-S1B*, and the other a combination of neutralizing epitopes and BF2-restricted T cell epitope box (S1T) designated as *pV-S1B+S1T*. The amino acid residues used in the construction included T cell epitopes $P8_{SRIQTATDP}$, $P9_{SRNATGSQP}$ (SH1208 strain), $P18_{GAYAVVNV}$, $P19_{SRIQTATQP}$ (Holte strain) and neutralizing epitopes (Australian T strain) $P1_{NYVYYYQSAFRPSGGWHLHGGAYAVVNVSQETSNAGS}$, $P2_{RIAAMKQGGNGPSDLFY}$, and $P3_{QTYQTQTAQSGYYNFNFSFLSGFVYKEFNFMYGSYHPKCNFRPENINNGLWFNSLSVSLAYGPLQGGCKQSVFHGRATCCYAYSYLGPRLCKGVYSGELTQQFECGL$. The amino acid residues of the neutralizing epitopes were converted into corresponding nucleotide sequences. The Kozak sequence (GCCACCATGG) was

Please cite this article in press as: Tan L et al. Infectious bronchitis virus poly-epitope-based vaccine protects chickens from acute infection. Vaccine (2016), http://dx.doi.org/10.1016/j.vaccine.2016.09.022