Veterinary Microbiology 166 (2013) 59–67

Contents lists available at SciVerse ScienceDirect

Veterinary Microbiology

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

Protection of chickens against reticuloendotheliosis virus infection by DNA vaccination

Kai Li, Li Gao, Honglei Gao, Xiaole Qi, Yulong Gao, Liting Qin, Yongqiang Wang, Xiaomei Wang *

Division of Avian Infectious Diseases, State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, The Chinese Academy of Agricultural Sciences, Harbin 150001, PR China

ARTICLE INFO

Article history:
Received 30 January 2013
Received in revised form 25 April 2013
Accepted 30 April 2013

Keywords:
Reticuloendotheliosis virus
DNA vaccine
Codon optimization
WPRE

ABSTRACT

The present study was undertaken to evaluate the ability of DNA vaccination to protect chickens against reticuloendotheliosis virus (REV) infection and to determine whether codon optimization and the woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) could improve the immunogenicity of the DNA vaccines. The wild-type and codon-optimized gp90 genes of REV were cloned into pCAGGS vector, and designated as pCAGgp90 and pCAGoptigp90, respectively. Plasmids pCAGWgp90 and pCAGWoptigp90 containing WPRE were also constructed. To evaluate vaccine efficacy, 3-week-old specific pathogen free chickens were injected with the constructed plasmids twice at 3-week intervals and challenged with REV 3 weeks post boost. Plasmids pCAGoptigp90 and pCAGWgp90 elicited significantly higher humoral and cellular immune responses than pCAGgp90, while chickens immunized with pCAGWoptigp90 showed the highest immune responses among the groups. Chickens immunized with pCAGgp90, pCAGoptigp90, pCAGWgp90 or pCAGWoptigp90 had 53%, 67%, 73% or 87% protection, respectively, as evidenced by the absence of REV viremia, while the empty vector pCAGGS only conferred 13% protection against viremia. These results highlight the potential value of DNA vaccination in the prevention of REV infection and suggest that codon optimization and WPRE could increase the efficacy of DNA vaccines.

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1. Introduction

Reticuloendotheliosis virus (REV) is a member of gammaretrovirus with a variety of strains, which causes an oncogenic, immunosuppressive and runting syndrome in multiple avian hosts including chickens, turkeys, ducks, geese, pheasants, peafowl and some other bird species (Witter and Fadly, 2003; Bohls et al., 2006). REVs are distinct from the avian leukosis-sarcoma virus group (Kang and Temin, 1973) and are more closely related to mammalian retroviruses, both antigenically (Barbacid et al., 1979; Tsai et al., 1986) and at the genome level (Rice et al., 1981). The immunosuppression caused by REV infection increases the susceptibility to concurrent or secondary bacterial or viral infections and results in poor immune responses to other vaccines (Kawamura et al., 1976; Yoshida et al., 1981). REV can be transmitted vertically via eggs and horizontally by direct contact with infected birds (Witter and Salter, 1989), mechanically by insects (Davidson and Braverman, 2005), by integration into large DNA viruses, or accidentally by injection of contaminated vaccines (Hertig et al., 1997; Zhang and Cui, 2005). Serologic surveys conducted in several countries have detected antibodies against REV in 3.3–25% of chicken flocks (Cheng et al., 2007). Recent epidemiological studies demonstrated that REV infection is highly

* Corresponding author at: Harbin Veterinary Research Institute, 427 Maduan Street, Harbin 150001, PR China. Tel.: +86 451 85935004; fax: +86 451 82762510.
E-mail address: xmw@hvri.ac.cn (X. Wang).

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http://dx.doi.org/10.1016/j.vetmic.2013.04.031

prevalent in China, causing severe damages to our poultry industry (Zhao et al., 2003; Qin et al., 2010). The oncogenicity and the immunosuppressive ability of these viruses, their co-infection with other infectious viruses and their presence as contaminants in poultry biologics warrant development of a suitable vaccine (Calvert et al., 1993).

The genomic structure of REV consists of a group-specific antigen (gag), protease (pro), polymerase (pol), and envelope (env) regions flanked by long-terminal repeats (LTRs) (Witter and Fadly, 2003). The env gene encodes two glycoproteins, gp90 and gp20. The gp90 protein is the major component of the virion envelope and responsible for eliciting virus-neutralizing and protective antibodies, which is known to be the major candidate antigen for vaccines and disease serological diagnosis (Tsai and Oroslan, 1988; Davidson et al., 1996; Li et al., 2012a). Furthermore, the genetic sequences of different REV strains show only minor variations (Bohls et al., 2006), and the various strains of REV are antigenically similar (Purchase et al., 1973), which suggested that a suitable vaccine expressing the gp90 gene of a single REV isolate may provide protective immunity against numerous REV-associated diseases (Calvert et al., 1993).

As an alternative approach for the prevention and control of disease, DNA vaccines have several advantages, which employ genes encoding for viral antigenic proteins in the absence of intact viruses, thus problems associated with conventional vaccines such as reverted virulence, divergent mutants, and the possibility of environmental contamination can be minimized (Dhama et al., 2008). Regarding veterinary practice, the last few years have seen numerous trials of DNA vaccines against a variety of bacteria, viruses and parasites (Fan et al., 2002; Oshop et al., 2002; Ding et al., 2005). However, vaccination with plasmid DNAs encoding protective antigens of REV has not been studied. The objective of this study was to evaluate the ability of DNA vaccine to protect chickens against REV infection and to determine whether codon optimization and the woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) could improve the immunogenicity of DNA vaccines.

2. Materials and methods

2.1. Viruses, cells and plasmids

REV HLJR0901 strain (GenBank accession number GQ415646) was isolated and stored at Harbin Veterinary Research Institute (HVRI) of the Chinese Academy of Agricultural Science (CAAS) at −70 °C. Primary chicken embryo fibroblasts (CEF) cells were prepared from 10-day-old specific-pathogen-free (SPF) chicken embryos provided by HVRI, CAAS. The immortalized CEF cell line (DF-1) was cultured in Dulbecco's modified Eagle's medium (DMEM) (GIBCO/BRL, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS). The eukaryotic expression vector pCAGGS was kindly provided by Dr. J. Miyazaki (University of Tokyo, Tokyo, Japan).

2.2. Antibodies and primers

The anti-gp90 monoclonal antibody (MAb) was prepared in our laboratory following standard procedures. All primers used were synthesized by Invitrogen (Beijing, China).

2.3. Animals

SPF White Leghorn Chickens were obtained from the Experimental Animal Center of HVRI, CAAS and housed in the negative-pressure-filtered air isolators. Animal experiments were approved by the Animal Ethics Committee of the Institute and performed in accordance with animal ethics guidelines and approved protocols.

2.4. Construction of DNA vaccines

The wild-type gp90 gene of REV HLJR0901 was amplified by PCR from DNA extracted from REV-infected CEF cells with a pair of primers (gp90F: 5′-GACGAATTCGCCGCCACCATG-GACTGTCTCACCAACCTC-3′ and gp90R: (5′-TTTATCGATT-CACTTATGACGCCCAGCGGTGTACTCG-3′). The optigp90 gene with codons optimized for chicken usage was designed by reverse translation of the amino acid sequence of gp90 protein of REV HLJR0901 using DNAStar software (DNAStar, Madison, USA) and synthesized by GenScript (Nanjing, China). The optigp90 gene shares 100 and 76.6% homology at the amino acid level and nucleotide level, respectively, with the wild-type gp90 gene. The wild-type gp90 and optigp90 genes were cloned into pCAGGS vector under control of chicken β-actin promoter, and designated as pCAGgp90 and pCAGoptigp90, respectively. To construct pCAGWgp90 and pCAGWoptigp90, the WPRE element (nucleotides 1093–1685 of GenBank: J04514) was synthesized and inserted into pCAGgp90 and pCAGoptigp90 between the gp90 or optigp90 gene and the polyA sequence using the XhoI and BglII sites. The constructed plasmids were confirmed with right orientation by sequencing and purified by Qiagen EndoFree Plasmid Giga Kit (Qiagen, Santa Clarita, CA). Plasmid concentration was determined by spectrophotometry at 260 nm.

2.5. Transfection

Monolayers of 80–90% confluent DF-1 cells in six-well plates were transfected with 4 μg of pCAGgp90, pCAGoptigp90, pCAGWgp90, pCAGWoptigp90 or empty vector pCAGGS using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Briefly, DNA and transfection reagent were mixed (10 μl lipofectamine 2000 and 4 μg DNA), incubated at room temperature for 30 min, and added to the cells. Six hours later, the DNA-transfection reagent mixture was replaced by DMEM containing 10% FBS. All plasmids were transfected in triplicates. In the mock-treated cells, only the transfecting reagent was used.

2.6. In vitro expression of the constructed plasmids

The expression of encoded gp90 protein from these plasmids was confirmed by indirect immunofluorescence

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