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Construction of recombinant baculovirus vaccines for Newcastle disease virus and an assessment of their immunogenicity

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ABSTRACT

Newcastle disease (ND) is a lethal avian infectious disease caused by Newcastle disease virus (NDV) which poses a substantial threat to China's poultry industry. Conventional live vaccines against NDV are available, but they can revert to virulent strains and do not protect against mutant strains of the virus. Therefore, there is a critical unmet need for a novel vaccine that is safe, efficacious, and cost effective. Here, we designed novel recombinant baculovirus vaccines expressing the NDV *F* or *HN* genes. To optimize antigen expression, we tested the incorporation of multiple regulatory elements including: (1) truncated vesicular stomatitis virus G protein (VSV-GED), (2) woodchuck hepatitis virus post-transcriptional regulatory element (WPRE), (3) inverted terminal repeats (ITRs) of adeno-associated virus (AAV Serotype II), and (4) the cytomegalovirus (CMV) promoter. To test the *in vivo* efficacy of the viruses, we vaccinated chickens with each construct and characterized the cellular and humoral immune response to challenge with virulent NDV (F48E9). All of the vaccine constructs provided some level of protection (62.5–100% protection). The F-series of vaccines provided a greater degree of protection (87.5–100%) than the HN-series (62.5–87.5%). While all of the vaccines elicited a robust cellular and humoral response subtle differences in efficacy were observed. The combination of the WPRE and VSV-GED regulatory elements enhanced the immune response and increased antigen expression. The ITRs effectively increased the length of time IFN-γ, IL-2, and IL-4 were expressed in the plasma. The F-series elicited higher titers of neutralizing antibody and NDV-specific IgG. The baculovirus system is a promising platform for NDV vaccine development that combines the immunostimulatory benefits of a recombinant virus vector with the non-replicating benefits of a DNA vaccine.

1. Introduction

Infectious diseases, including Newcastle disease (ND), cause major economic hardship in the poultry industry. ND is caused by Newcastle disease virus (NDV; Maas et al., 2003), and is characterized by acute morbidity and high mortality (Lam et al., 2011). A means of controlling the spread of NDV is an urgent unmet need in the global poultry industry.

Current strategies for preventing ND utilize inactivated and attenuated vaccines. However, it is always possible that immune failure can occur and there will be a resurgence in virulent NDV (Kattenbelt et al., 2006). The F protein is one of the major protective antigens in NDV (White et al., 2008; Yin et al., 2006). It acts as a fusion protein and contributes to viral adsorption (Lamb and Jardetzky, 2007). The HN protein is the other major protective antigen in NDV. The HN protein combines hemagglutinin (HA) and neuraminidase (NA) activities (Takimoto et al., 2002). Previous studies have achieved a 100% rate of protection by immunizing chickens using a recombinant NDV vaccine containing the *F* and *HN* gene using the avian paramyxovirus type III virus (APMV 3) as the vector (Kumar et al., 2011). Similarly, a subunit vaccine developed by Lee et al. (2008) using recombinant F and HN protein elicited a good immune response, and the protection rates were 100% and 80%, respectively (Lee et al., 2008). However, it is likely that conventional vaccines will be replaced by genetically engineered vaccines.

Compared with other recombinant expression systems, the baculovirus system has distinct advantages. For example, it can accommodate large fragments of exogenous genes (Sakaguchi et al., 1998) and post-translationally modify products without causing cytotoxic effects (Li et al., 2009). In addition, baculovirus systems can express multiple genes simultaneously at high levels (Mahonen et al., 2007). The expressed products also retain their biological activity (Hu, 2008). Most notably, the baculovirus expression sys-

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tem is generally considered a very safe way to express exogenous genes.

The baculovirus system has been modified in many different ways to optimize the expression of exogenous genes. For example, mammalian cell promoters such as simian virus 40 (SV40), cytomegalovirus (CMV), and CMV early enhancer/chickenβactin promoter (CAG) are utilized to optimize the efficiency of exogenous gene expression (Hu, 2006). The CMV promoter is a particularly strong promoter that controls expression from recombinant baculovirus expression platforms in mammalian and poultry cells (Krishnan, 2000). The woodchuck hepatitis virus post-transcriptional regulatory element (WPRE) added to the 3′ untranslated region of the expressed gene can also improve the expression efficiency of target gene expression (Donello et al., 1998; Mahonen et al., 2007). The transduction efficiency of the baculovirus system can be increased by displaying a truncated vesicular stomatitis virus G protein (VSV-GED) (Kaikkonen et al., 2006) on the surface of the baculovirus. Finally, inverted terminal repeats (ITRs) from AAV extend the length of time that target genes are expressed *in vivo*. Sustained expression has been observed for up to 90 days *in vivo* from a CMV expression cassette containing adenovirus ITRs (Wang et al., 2006). Thus modified baculovirus systems have the potential to produce large amounts of recombinant proteins in a sustained manner, suggesting they could be an ideal platform for NDV vaccine development.

The aim of this study was to investigate the effects of the VSV-GED, WPRE, and ITRs regulatory elements on expression of the NDV *F* and *HN* genes controlled by the CMV promoter in a recombinant baculovirus vaccine for NDV. To assess the efficacy of the vaccine we assessed the humoral and cellular immune response *in vitro* to the F and HN proteins in the presence and absence of each regulatory element. We also assessed the level of target protein expression. Finally, the *in vivo* efficacies of the constructs were tested *in vivo*, and the humoral and cellular immune response to vaccination was characterized.

2. Materials and methods

2.1. Ethics

All animal experiments were carried out in accordance with the Guidelines for Animal Experiments of the National Institute of Infectious Diseases (NIID, Japan). Experimental protocols were reviewed and approved by the Animal Ethics Committee of Harbin Veterinary Research Institute of the Chinese Academy of Agricultural Sciences (CAAS) and the Animal Ethics Committee of Heilongjiang Province (SYXK (H) 2006-032).

2.2. Virus, plasmids, and cells

The virulent NDV strain F48E9 was purchased from the China Veterinary Microbiology Culture Collection. The plasmids pLM(−), pLM, pLM-ITRs, pTYL-HA-F, and pNDV-HN were obtained from the Key Laboratory of Microbiology in the College of Life Science at Heilongjiang University. The F and HN genes were obtained from plasmids pTYL-HA-F and pNDV-HN using Xho I/Sal I and Xho I/Sph I, respectively. Plasmid pLM(−) contains the CMV promoter and simian virus 40 (SV40) poly(A); Plasmid pLM contains the elements of WPRE, VSV-GED, CMV promoter, SV40 poly(A) and gp64 signal peptide (gp64sp) sequence; Plasmid pLM-ITRs contains the elements of ITRs, WPRE, VSV-GED, CMV promoter, SV40 poly(A) and gp64sp sequence. The chicken embryo fibroblast cells and Sf9 insect cells were maintained in our laboratory.

2.3. Construction of baculovirus vectors

Six (6) plasmids were constructed: (1) pLM(−)-F, (2) pLM-F, (3) pLM-ITRs-F, (4) pLM(−)-HN, (5) pLM-HN and (6) pLM-ITRs-HN. The *F* or *HN* genes were individually amplified with primers that also inserted a the His tag (The forward primer for the *F* gene was 5′-ATCCTCGAGATGGGCTCCAGACCTTCTACC-3′). The reverse primer for the *F* gene was: 5′-GGCGTCGACTCA*ATGATGATGATGATGATG*CATTTTTGTAGTGGCTCTCATC-3′. The forward primer for *HN* was: 5′-ATCCTCGAGATGGACCGCGCAGTTAGC-3′ and the reverse primer for *HN* was: 5′-CGGGCATGCCTA*ATGATGATGATGATGATG*ACCAGACCTGGCTTA-TCTAACCTAT-3′). F and HN genes were inserted into the plasimids pLM(−), pLM and pLM-ITRs with *Xho* I/*Sal* I and *Xho* I/*Sph* I endonuclease, respectively.

The *E.coli* DH10 Bac competent cells were prepared using the SEM method (Rong et al., 2002). The plasmids (pLM(−)-F, pLM-F, pLM-ITRs-F, pLM(−)-HN, pLM-HN and pLM-ITRs-HN, pLM(−), pLM, and pLM-ITRs) were transformed into *E. coli* DH10 Bac competent cells. Positive colonies were identified by blue-white screening and the recombinant Bacmid (rBac) DNA was extracted using the alkaline lysis method. The recombinants were identified as rBac-LM(−)-F, rBac-LM-F, rBac-LM-ITRs-F, rBac-LM(−)-HN, rBac-LM-HN, and rBac-LM-ITRs-HN, rBac-LM(−), rBac-LM, and rBac-LM-ITRs (Fig. 1) by PCR amplification with the M13 primer (CGCCAGGGTTTTCCCAGTCACGAC).

2.4. Cell culture

Sf9 insect cells were cultured in suspension at 27 °C in Sf900II SFM medium containing 10% FBS (Gibco, CA, USA) and 1% antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin) in a 0 mL volumes. Primary avian cells were cultured in a 6-well plate at 37 °C in 11-day-old embryonated specific-pathogen-free (SPF) chicken eggs. The primary avian cells were prepared according to a standard protocol (Spector et al., 1988) and were maintained in Dulbecco's modified Eagle medium (DMEM, Hyclone, Logan, USA) supplemented with 10% FBS (Gibco, CA, USA), 2 mM glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin.

A third passage (P_3) baculovirus (Section 2.5) was used to infect chicken embryo fibroblasts (CEFs) at 80% confluence using a multiplicity of infection (MOI) of 100 in the presence of 10 mM sodium butyrate in 2 mL of DMEM for 12 h. The media containing virus was replaced by fresh DMEM containing 10% FBS and the cells were incubated for an additional 48 h. The cells were pelleted by centrifugation and washed three times with PBS then lysed using 200 μL/well cell lysates buffer (Beyotime, Shanghai, China) in a 6-well culture plate.

2.5. Generation and titering of recombinant baculovirus

Sf9 cells were transfected with the individual baculovirus transfer vectors, such as rBac-LM(−)-F, rBac-LM-F, rBac-LM-ITRs-F, rBac-LM(−)-HN, rBac-LM-HN, and rBac-LM-ITRs-HN, rBac-LM-ITRs, rBac-LM(−), rBac-LM and rBac-LM-ITRs using the liposome-mediated method (Whitt et al., 2001). The co-transfection supernatants were collected after 72 h culture. The viruses were passaged 3 times in Sf9 cells to obtain high titer viral stocks. The viruses were allowed to infect the Sf9 cells (2×10^6/mL) at room temperature for 30 min, then cultured at 27 °C with agitation (70 r/min).The culture supernatants from infected cells were collected once 80% of the cells had been infected The same process were repeated to obtain second and third passage virus. The viral genome was extracted and amplified with the M13 universal primer, and each reverse primer, to confirm that the target genes were correctly inserted into the recombinant baculoviruses.