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Adenoviral-expressed recombinant granulocyte monocyte colony-stimulating factor (GM-CSF) enhances protective immunity induced by inactivated Newcastle Disease Virus (NDV) vaccine

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ABSTRACT

Although vaccination has been hugely successful in protecting birds against infection by the New castle disease virus (NDV), newly-emerged highly virulent strains have been found to overcome established immune protection and threaten the poultry industry. The need to improve the immunization efficacy is, therefore, urgent. Here, we tested the potential immunostimulatory adjuvant activity of the adenoviral-expressed recombinant chicken granulocyte monocyte colony stimulating factor (rchGM-CSF) in an inactivated Newcastle Disease Virus (NDV) vaccine. 126 commercial layer chicks, divided into six groups, were first vaccinated at day 7, followed by a subsequent boost and later an intramuscular challenge at day 21 and 35 respectively. rchGM-CSF expressed by adenovirus raised NDV-specific hemagglutinin-inhibition (HI) titers from 10 to 12 (log2) and significantly upregulated the production of interferon α/β/γ (IFN-α/β/γ), interleukin-4 (IL-4) and major histocompatibility complex II (MHC-II) in spleens. Crucially, chicks inoculated with the inactivated NDV vaccine plus the rchGM-CSF adjuvant displayed only mild clinical signs, lower tissue viral loads, fewer tissue lesions, and decreased mortality and viral shedding than those in the group immunized with the vaccine alone. Our present work has demonstrated that chicken GM-CSF may act as an enhancer in the orchestration of host immune responses induced by the inactivated NDV vaccine. The molecule, expressed by an adenovirus, has the potential to be used as an immune adjuvant to improve protection by NDV vaccination.

1. Introduction

Newcastle disease (ND) is one of the most contagious infectious diseases affecting both domestic and wild avian species. First described in 1926, the disease remains a major threat to the worldwide poultry industry. In fact, ND was one of the three most widespread animal diseases from 2006 to 2009 and one of the four most important diseases for poultry species in terms of livestock unit lost (Seal et al., 2000). The etiology of the disease is the virulent strains of Newcastle Disease virus (vNDV) (Alexander et al., 2012). The virus, also known as avian Paramyxovirus type-1 (AMPV-1), belongs to the order *Mononegavirales*, family *Paramyxoviridae* and genus *Avulavirus*. Although genetically diverse, all NDV strains belong to only one single serotype (Alexander and Senne, 2008) which can be further divided into class I and class II; with the latter having 18 known genotypes (Diel et al., 2012). Most of the class I NDVs reported are isolated from wild birds and they are of low virulence. On the other hand, class II NDVs of genotype II and X are the most commonly found low virulent strains infecting wild birds, while viruses of genotypes III–IX, and XI–XVI are all virulent (Diel et al., 2012); of which genotypes V, VI, and VII are the predominant ones circulating worldwide (Miller et al., 2010, 2009). However, genotype VII is of particular importance given that it has been associated with many of the most recent outbreaks in Asia, Africa, and the Middle East (Liu et al., 2003; Miller et al., 2010; Wang et al., 2006; Yu et al., 2001). Genotype IX is a unique group that includes the first virulent outbreak in China reported in 1948. Members of this genotype has since then continued to be occasionally isolated in the country (Wang et al., 2006).

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In general, vNDVs are characterized by the composition of the amino acids at the cleavage site of the F_0 fusion protein. The site is usually made up of three or more basic residues at positions 113–116 and a phenylalanine at position 117 (Miller et al., 2011). However, the current accepted gold standard for pathotyping is the determination of the intracerebral pathogenicity index (ICPI). The Office International Des Epizooties (OIE) recommends the use of index values $\geq$ 0.7 in day-old chickens as the standard (OIE, 2012).

Vaccination is the most commonly used control strategies for ND in many countries. Conventional NDV vaccines, including live, inactivated and genetically engineered virus vaccines have all shown to provide protection to varying degrees against the virulent ND challenge. Both live and inactivated vaccines were developed to deal with rapid spread of ND in the poultry industry during the 1960s and 1970s (Beard and Easterday, 1967; Butterfield et al., 1973). Advantages and disadvantages of these vaccines have previously been reviewed elsewhere (Senne et al., 2003). In brief, the former vaccines are inexpensive, easily mass-administered via water or aerosol and offer multi-route vaccination opportunities. On the other hand, the risk of the live virus reverting back to its virulent state poses a safety concern that cannot be entirely overlooked. Worldwide, the most commonly employed live NDV vaccine strains are B1 and La Sota. Although the latter is more immunogenic, it also suffers from relatively higher pathogenicity. Nevertheless, this strain is still used more widely in China simply due to the widespread of the disease in the country.

Inactivated virus oil-emulsion vaccines produced from infective allantoic fluid, on the other hand, have to be injected individually to each bird. Not only is the procedure arduous and costly, immunized layer chickens may also suffer from atypical ND (Yu et al., 2001) because of the lack of strong cell-mediated (Senne et al., 2003) and/or adequate humoral immune response (Han et al., 2017). However, with the aids of more concentrated antigens and adjuvants, these vaccines are still given widely to the chicken flocks due to the reduced subclinical infection losses (such as, egg drops in laying hens) conferred by the higher humoral antibody levels as indicated by high hemagglutinin-inhibition (HI) titers. Although anti-ND HI titers higher than 4 (log2 (HI)) have previously been reported to be necessary for the survival of a vNDV challenge, field data has demonstrated that protection against ND-related egg drops only took place at much higher HI titer values of greater than 13 (log2 (HI)) (field observation). In fact, our recent works showed that laying hens immunized with inactivated La Sota vaccine required HI titers of more than 14 (log2) to prevent clinical signs, *e.g.* egg drops and viral shedding, caused by the F48E9 challenge (Han et al., 2017). The same vaccine is also used in the current study. However, due to such factors as the influence of materially-derived antibodies (MDAs) or pre-immunized antibodies, secondary infections, vaccine competition, and incomplete vaccination coverage, rarely can such high HI titers be achieved in the field with current commercial inactivated vaccines. The incentive to improve current vaccines for hens is therefore clear and the use of a more potent immunostimulating adjuvant is among the most promising strategy pursued.

Cytokine/molecular adjuvants like granulocyte monocyte colony stimulating factor (GM-CSF), IL-18 and CD40L have been extensively used and proven to improve vaccine efficacy in different types of live vector or subunit vaccines in mammals (Kwa et al., 2015; Yue et al., 2015; Zhang et al., 2015). GM-CSF is a potent cytokine effecting on granulocyte and macrophage maturation from hematopoietic precursors, and it can also amplify the response of the mature immune system to antigen (das Graças Sasaki et al., 2003). Moreover, the molecule as an adjuvant effector has been shown *in vitro* to augment the expression of the T cell costimulatory molecule B7-1 and B7-2 on antigen presenting cells (APCs) (Dranoff, 2002). And increased in the expression of MHC-II molecules in APCs (Deresinski, 1999) has also been attributed to GM-CSF. In fact, an improvement in the kinetics and magnitude of antibody response was observed in a recent chicken vaccination trial using GM-CSF as an adjuvant (Zeshan et al., 2011). Our present study therefore sought to improve the immune efficacy of an inactivated vaccine of NDV with the aid of the imunostimulating GM-CSF. The cytokine adjuvant, expressed by adenovirus, was able to enhance convincingly the immune response of inactivated NDV vaccinated chicks.

2. Materials and methods

2.1. Eggs, chicks and ethnical statement

Fertilized specific-pathogen-free (SPF) chicken eggs were purchased from Merial-vital Laboratory Animal Technology (Beijing, China) and used for passage and titration of ND virus.

The NDV-free healthy one-day-old layers chicks (n = 126) were obtained from a local poultry hatchery and were tested by the supplier to be free of immunosuppressive diseases and other critical avian pathogens. All chicks were kept in separated incubators (biological security level 3) with free access to water and food. After one week of adaption to the rearing environment, they were subjected to subsequent immunization and challenge experiments.

All experimental procedures involving animals were reviewed and approved by the Ethics Committee at Northwest A&F University. Guidelines from the independent Animal Care and Use Committee in Shaanxi Province, China, were strictly adhered to.

2.2. Virus, NDV vaccine and cells

F48E9, the virulent gold standard NDV strain commonly used for the evaluation of vaccine efficacy in China, was obtained from a reference laboratory at China Institute of Veterinary Drugs Control. This genotype IX, Class II virus with an ICPI of 1.89 at day-1 was isolated from a chicken in China in 1948. However, tropism of the virus has not been accurately determined.

F48E9 and LaSota were propagated in nine-day-old SPF chicken embryos and titered in the chicken fibroblast DF-1 cell line. The inactivated NDV vaccine with oil adjuvant derived from La Sota strain, were obtained from Yebio (Qindao, China, Catalog Number (2015) 150132241).

Adenovirus expressing GM-CSF was constructed as described before (Wang et al., 2012; Zeshan et al., 2011). Adenoviruses were propagated and titered in HEK-293A cells (ATCC CRL1573). HEK-293A and DF-1 cells were cultured in DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin.

2.3. Animal experiment

A total of 126 chicks were randomly divided into six groups of 21 each. The relevant treatments for each group were listed in Table 1. Primary immunization was conducted at day 7 and boosted 2-weeks later. Immunization of one inoculation/bird was conducted by intramuscular injection to the pectoralis. Adenovirus expressing rchGM-CSF (10^7 $TCID_{50}$/mL) was mixed with the vaccine (v/v = 1:1) by vortex.

To reduce operational differences and accurately quantify the viral load, all chicks were challenged by injecting 0.2 ml of 10^8 $TCID_{50}$ F48E9 into the chest muscle at day 14 post-boost, *i.e.* at day