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Heterologous prime-boost immunization of Newcastle disease virus vectored vaccines protected broiler chickens against highly pathogenic avian influenza and Newcastle disease viruses

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ABSTRACT

Avian Influenza virus (AIV) is an important pathogen for both human and animal health. There is a great need to develop a safe and effective vaccine for AI infections in the field. Live-attenuated Newcastle disease virus (NDV) vectored AI vaccines have shown to be effective, but preexisting antibodies to the vaccine vector can affect the protective efficacy of the vaccine in the field. To improve the efficacy of AI vaccine, we generated a novel vectored vaccine by using a chimeric NDV vector that is serologically distant from NDV. In this study, the protective efficacy of our vaccines was evaluated by using H5N1 highly pathogenic avian influenza virus (HPAIV) strain A/Vietnam/1203/2004, a prototype strain for vaccine development. The vaccine viruses were three chimeric NDVs expressing the hemagglutinin (HA) protein in combination with the neuraminidase (NA) protein, matrix 1 protein, or nonstructural 1 protein. Comparison of their protective efficacy between a single and prime-boost immunizations indicated that prime immunization of 1-day-old SPF chicks with our vaccine viruses followed by boosting with the conventional NDV vector strain LaSota expressing the HA protein provided complete protection of chickens against mortality, clinical signs and virus shedding. Further verification of our heterologous prime-boost immunization using commercial broiler chickens suggested that a sequential immunization of chickens with chimeric NDV vector expressing the HA and NA proteins following the boost with NDV vector expressing the HA protein can be a promising strategy for the field vaccination against HPAIVs and against highly virulent NDVs.

1. Introduction

Influenza viruses belong to the family *Orthomyxoviridae* with segmented, negative sense, single-strand RNA genomes [1]. Among the five genera, the *Influenzavirus* A genus contain eight gene segments and encode at least 10 proteins: polymerase basic 1 (PB1), PB2, polymerase acid (PA), hemagglutinin (HA), nucleoprotein (NP), neuraminidase (NA), matrix 1 (M1), M2, nonstructural 1 (NS1) and 2 (NS2). All the avian influenza viruses (AIVs) are classified into the genus *Influenzavirus* A. Among the 18 known HA subtypes of Influenza A viruses, H5 and H7 subtypes have caused high levels of morbidity and mortality in chickens and other terrestrial poultry. Vaccines have been used to control AIV infections in the fields [2]. Inactivated, oil adjuvanted, whole virus vaccines are the most common vaccines available for AIV. However, poor quality vaccines and inappropriate application have led to vaccine failures in the field. The use of attenuated live influenza vaccines in poultry is not recommended due to the potential risk of reassortment or mutations [3].

Newcastle disease virus (NDV) is also an economically important respiratory pathogen of poultry. NDV strains are classified into three pathotypes: lentogenic (avirulent), mesogenic (moderately virulent), and velogenic (virulent) [4]. Avirulent NDV strains, such as LaSota and B1, are used as live attenuated vaccines to control Newcastle disease in poultry worldwide [5]. Furthermore, they have been used as promising vaccine vectors for human and veterinary uses [6]. NDV can be an ideal vaccine vector for development of an AI vaccine, since NDV replicates efficiently and induces strong local and systemic immune responses at the respiratory tract [7]. However, pre-existing immunity to the vector due to vaccination against NDV has limited the protective efficacy of NDV vectored vaccines in the field [8]. As an alternative strategy, we previously evaluated vaccine efficacy of an antigenically chimeric NDV vector in which the ectodomains of F and HN proteins were replaced by those of serologically distinct avian paramyxovirus serotype-2

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(APMV-2) [9]. The chimeric NDV vector stably expressed the HA protein *in vivo*, did not cross-react with NDV, was attenuated, and provided a partial protection of chickens against HPAIV challenge, indicating its potential use for early protection of chickens. In this study, to enhance the protection efficacy, we further generated chimeric NDVs expressing the HA protein (chimeric NDV/ HA) in combination with NA protein, M1 protein, or NS1 protein. The protective efficacy of our vaccine candidates were evaluated in both specific pathogen-free (SPF) and broiler chickens.

2. Materials and methods

2.1. Construction and characterization of chimeric NDV vectored vaccines

The H5N1 NA, M1 and NS1 were individually inserted between the M and F genes in the chimeric NDV containing the HA gene (Fig. 1A). The recombinant viruses were recovered as previously described [10]. Cytopathic effect (CPE) of the chimeric NDV vaccine viruses was determined by immunostaining [9]. The multicycle growth kinetics of the chimeric NDVs was evaluated in DF1 cells [10,11]. The expression of H5N1 proteins was evaluated by Western blot. The pathogenicity of chimeric NDVs was determined by the mean death time (MDT) test in 9-day-old SPF embryonated chicken eggs and by the intracerebral pathogenicity index (ICPI) test in 1-day-old SPF chicks [4]. The genetic stability of vaccine viruses was evaluated by passaging the viruses five times in the respiratory tract of 1-day old chicks using an oculonasal infection. After the final passage, the viral genome RNA was isolated and RT-PCR was performed for the sequence analysis.

2.2. Immunogenicity and protective efficacy of the chimeric NDV vectored vaccines in SPF and broiler chickens

Groups (n=10 per group) of 1-day-old SPF chickens were immunized by the oculonasal route with 10^6 pfu/ml of LaSota/HA and chimeric NDVs with HA, HA-NA, HA-M1, and HA-NS1. An additional group of birds (n = 6) was left uninfected. Each group of

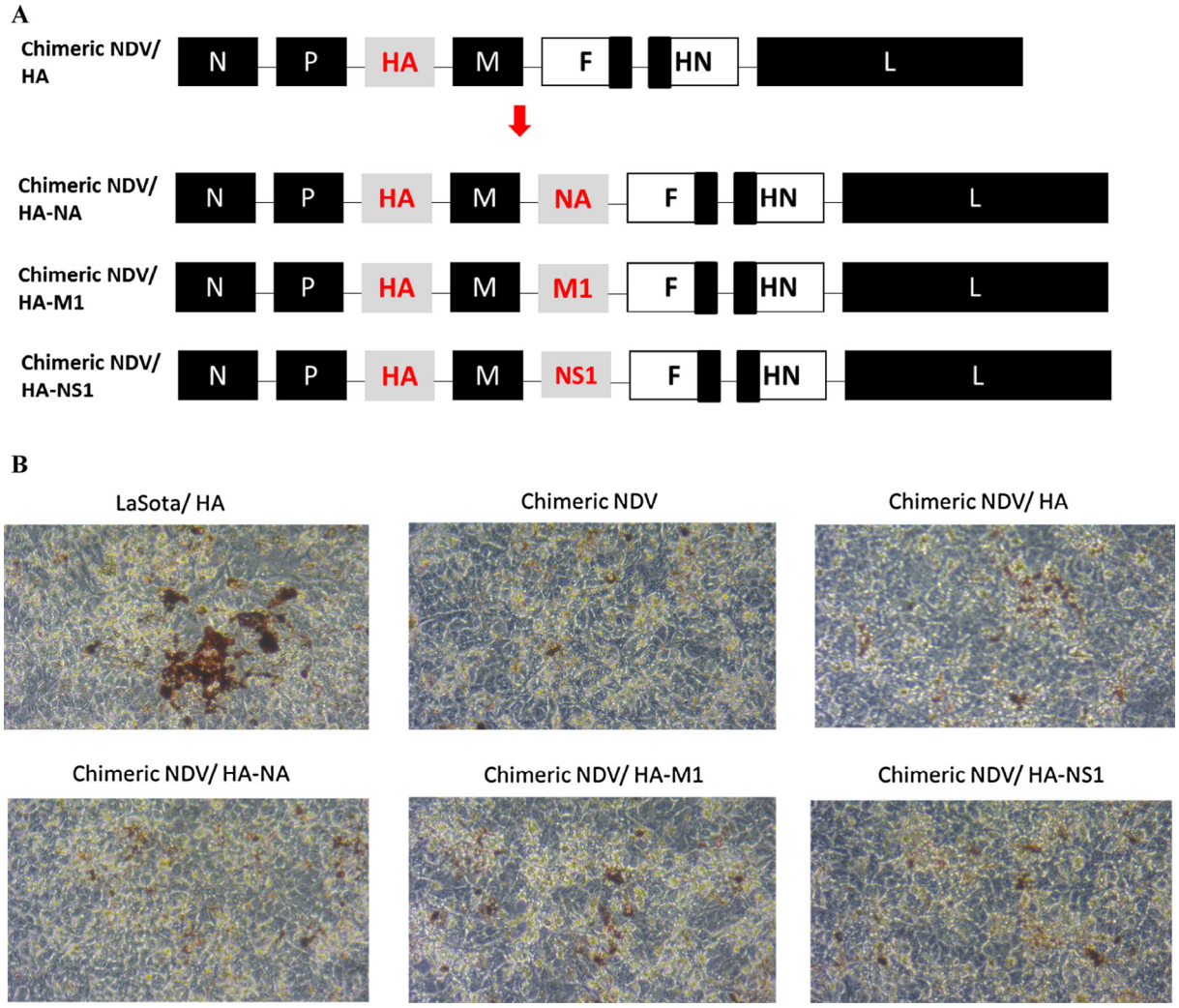

Fig. 1. Generation of chimeric NDV vectored vaccine viruses expressing the H5N1 HPAIV HA gene in combination with NA, M1, or NS1. (A) A full-length antigenomic cDNA of chimeric NDV/HA was modified by inserting each gene flanked by the NDV gene-start and gene-end signals into the intergenic region between the M and F genes. Ectodomains of the F and HN genes derived from APMV-2 are shown as white rectangle. (B) To evaluate the syncytium formation of chimeric vaccine viruses, DF1 cells in six-well plates were infected with the indicated viruses at a multiplicity of infection (MOI) of 0.01 PFU/cell, incubated for 72 h, and conducted immunoperoxidase staining using antiserum against the N protein of NDV, with viral antigen stained red. (C) The growth kinetics was determined by infecting DF1 cells with each virus at an MOI of 0.01. (D) Expression of H5N1 HPAIV HA, NA, M1, and NS1 proteins by NDV vectors in DF1 cells were analyzed by Western blot. DF1 cells were infected with each virus at MOI 1, and cell lysates were collected at 24 h post-infection for Western blot analysis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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