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OVERVIEW OF CURRENT ADVANCES IN THE DEVELOPMENT OF SUBUNIT AND RECOMBINANT VACCINES AGAINST NEWCASTLE DISEASE VIRUS

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Review paper

Abstract: Newcastle disease virus (NDV) is one of the most important viral pathogens of avian species and the causative agent of atypical fowl plague, a highly contagious and economically important disease characterized by high mortality rates and reduction of egg production. The HN and F proteins are the main targets for immune response to NDV. Vaccination of poultry with live and inactivated NDV vaccines is the most effective method of control and prevention of Newcastle disease, however due to their disadvantages, efforts are being invested into developing subunit vaccines. To this end, the NDV HN and/or F protein have been expressed using different viruses as vectors, but have also been expressed using transgenic plant systems, yeast and lactic acid bacteria in order to produce the NDV subunit vaccine. Many authors have investigated the possibility of preparation of vaccines from purified and biologically active NDV subunits with HN and F glycoproteins, purified from nucleocapsids, viral ribonucleic acid (RNA) and pyrogens. The above mentioned viral glycoproteins with preserved antigenic structure and biological activities can be used as subunit vaccinal antigens due to their immunogenic properties.

Key words: NDV, HN, F, subunit vaccines, recombinant vaccines

Introduction

Newcastle disease virus (NDV) is one of the most important viral pathogens of avian species and the causative agent of atypical fowl plague, a highly contagious and economically important disease characterized by high mortality rates and reduction of egg production (*Westbury, 2001; Ganar et al., 2014*). This is an enveloped virus with negative-sense single-stranded RNA and is classified in the genus *Avulavirus* of the subfamily *Paramyxovirinae* in the family

Paramyxoviridae (*Mayo, 2002; Kapczynski et al., 2013*). The viral genome contains six open reading frames (ORF) which encode the nucleoprotein (NP), the phosphoprotein (P), the matrix protein (M), the fusion protein (F), the haemagglutinin-neuraminidase (HN) and the large protein (L) (*Steward et al., 1993*). However, the F gene and the HN gene encodes essential proteins for virulence determination. The fusion (F) protein is responsible for mediating fusion of the viral envelope with cellular membranes while the HN protein is involved in cell attachment and release (*Milić et al., 2001; Milić et al., 2003; Nišavić et al., 2007; Heiden et al., 2014; Qiu et al., 2014; Jaganathan et al., 2015*). The HN and F proteins are the main targets for immune response to NDV (*Morgan et al., 1992; Arora et al., 2010; Chaturvedi et al., 2011; Kumar et al., 2011*). Newcastle disease virus strains were classified according to pathotyping assays to three classes: virulent - velogenic, moderately virulent - mesogenic, and non-virulent – lentogenic virus strains (*Dortmans et al., 2011; Susta et al., 2015*). Lentogenic NDV strains sometimes cause subclinical infections with mild respiratory or enteric disease and are considered as low-virulent. Mesogenic NDV strains are of intermediate virulence causing respiratory infection with moderate mortality (< 10%), while velogenic NDV strains are highly virulent causing mortality rates up to 100% (*Beard and Hanson, 1981*). The ND virus spreads horizontally between healthy and infected birds through direct contact with bodily secretions from infected birds (*Alexander, 2009*). The diagnosis of Newcastle disease virus infection is performed by the application of standard methods including virus isolation in chicken embryos, hemagglutination (HA test) and hemagglutination inhibition tests (HI test) as well as molecular methods based on Reverse transcription polymerase chain reaction - RT-PCR (*Nišavić et al., 2007; Milić et al., 2012*).

Vaccination of poultry with live and inactivated NDV vaccines is the most effective method of control and prevention of Newcastle disease (*Senne et al, 2004*). However, previous experience in immunoprophylaxis of atypical fowl plague has shown that vaccination with both mentioned vaccine types has both advantages, as well as disadvantages and that routine vaccinations are insufficient to control this disease given the increasing number of outbreaks in commercial poultry flocks worldwide (*Arora et al., 2010; Kang et al., 2016*). Live NDV vaccines generally induce protective immunity in vaccinated poultry, but circulating live vaccine viruses present additional risks such as reversion of virulence and recombination with wild-type strains. Furthermore, the immune response of wild birds induced by infection with vaccinal strains may provide selective pressures resulting in viral antigenic drift or increased virulence (*Lee et al., 2012; Palya et al., 2012; Read et al., 2015; Devlin et al., 2016*). Also, vaccines prepared from inactivated NDV strains often have a weaker immunogenic effect in immunized poultry, compared to live vaccines, and can cause local inflammation after the application of oil-emulsion inactivated vaccines (*Homhuan et al., 2004*).

Other types of vaccines that have been developed include the subunit and recombinant vaccines (*Boursnell et al., 1990; Nagy et al., 1991; Peeters et al., 2001*). Another major drawback of all currently used whole-virus-based live and inactivated NDV vaccines is that vaccinated animals cannot be distinguished from infected animals with standard serological tests, such as hemagglutination inhibition (HI test) or virus neutralization (VN test). A different concept for the development of a marker vaccine is based on the use of subunit vaccines and it has been achieved for many antigens involved in inducing protective immunity, including the two glycoproteins F and HN of NDV (Morgan et al., 1992). The above mentioned viral glycoproteins with preserved antigenic structure and biological activities can be used as subunit vaccinal antigens (*Milić et al., 1996; Tanabayashi and Compans, 1996; Milić et al., 2001; Arora et al., 2010; Milić et al., 2015)*. Furthermore, birds vaccinated with the recombinant fowlpox-NDV HN subunit vaccine can now be distinguished from the naturally infected ones by their antibody responses to the subunit vaccine on ELISA plates coated with recombinant baculovirus-NP protein as the coating antigen. However, such tests can only be useful if the current live or inactivated vaccines are replaced by recombinant subunit vaccines (*Yusoff and Tan, 2001*).

Theoretically, the genes encoding any protein can be cloned and expressed in bacteria, yeasts or mammalian cells. A number of genes encoding surface antigens from viruses, bacteria and other single celled pathogens have been cloned in expression systems and the expressed antigens have been used as vaccines (*Arntzen and Mason, 1995*). Efforts are being invested into developing subunit vaccines because of the disadvantages presented by the existing traditional vaccines. To this end, the NDV HN and/or F protein have been expressed using different viruses as vectors, but have also been expressed using transgenic plant systems, yeast and lactic acid bacteria in order to produce the NDV subunit vaccine.

As an initial approach to the development of novel anti-NDV vaccines, *Berinstein et al. (2005)* demonstrated that NDV F and HN proteins can be correctly expressed in transgenic potato plants. Specific anti-NDV antibodies recognize them and they are immunogenic in mice after parenteral administration or as edible vaccines, stimulating, in the latter case, the production of specific IgA in the gut. *Shahriari et al. (2015)* studied the application of tobacco hairy roots for expression of the F and HN epitopes of Newcastle disease virus. The authors have suggested that since plant-based systems possess a number of drawbacks in recombinant vaccine production, these might be overcome by using transient expression systems like tobacco hairy roots as they have proved to be an efficient tool for expression of these viral antigens.

Kang et al. (2016) demonstrated the potential of F protein of NDV expressed by the methylotrophic yeast *Pichia pastoris* (*P. pastoris*) as a subunit vaccine candidate when administered with flagellin as the adjuvant. The

aforementioned protein was efficiently expressed in the *P. pastoris* system and verified by sodium dodecyl sulfate polyacrylamide gel electrophoresis and western blotting. The F protein induced strong humoral and cell-mediated immune response in experimental mice when administered i.p. with *Salmonella* flagellin as adjuvant. *Khulape et al. (2015)* attempted to express the HN protein of NDV in a yeast expression system. The authors found that *Saccharomyces cerevisiae* was a better expression system for HN protein than *Pichia pastoris* as determined by codon usage analysis. The yeast cells were able to generate glycosylated HN protein with proper folding and antigenicity. The recombinant HN (rHN) protein was characterized by western blot and purified by affinity column purification and it was concluded that it could be further used as subunit vaccine. Since lactic acid bacteria are naturally associated with mucosal surfaces, particularly the gastrointestinal tract, they have also been considered as promising mucosal delivery vesicles to produce protective antigens (Shaw et al., 2000). Jiang et al. (2015) constructed a recombinant *Lactobacillus plantarum* (RLP) expressing HN protein of NDV. Oral administration of RLP significantly increased the production of secretory immunoglobulin A (SIgA) and the percentages of CD3+CD4+ T cells in chickens, providing at least partial protection in the NDV challenge experiment. The immunization with HN resulted in 40% survival rates in experimentally infected chicken. One of the possible explanations of only partial protection is that the selected HN protein performs less effective with regard to protection results compared to another glycoprotein, the fusion protein F, according to reports of *Meulemans et al. (1986), Kumar et al., (2011)* and *Kim et al., (2013).*

Recombinant vaccines based on viral coat protein subunits represent an efficient tool as a substitute for conventional, attenuated virus based vaccines (*Makela, 2000). Boursnell et al. (1990)* have investigated the expression of hemagglutinin-neuraminidase (HN) gene from the Beaudette C strain of NDV in a recombinant fowlpox virus vector. When the recombinant fowlpox virus was inoculated into chickens by intravenous or wing-web routes, specific antibodies against HN antigen from purified NDV virions were produced. Protective immunity to NDV was generated all experimental chickens and at the highest dose of vaccine 100% of the tested chickens were protected against challenge with a virulent strain of NDV. Recombinant baculoviruses containing the fusion (F) and hemagglutinin-neuraminidase (HN) glycoprotein gene of the viscerotropic velogenic (vv) NDV isolate, Kr-005/00, and a lentogenic La Sota NDV strain were constructed in an attempt to develop an effective subunit vaccine to the recent epizootic of vvNDV in Korea (*Lee et al., 2008*). The authors evaluated the protective effect of individual recombinant glycoproteins derived from velogenic and lentogenic NDV strains. The recombinant glycoproteins from the virulent strain produced complete protection after the second immunization, whereas those from the lentogenic strain had a slightly lower protective effect. A synergistic effect of the combined F and HN glycoprotein was noted and it was concluded that

the use of a subunit vaccine composed of the two glycoproteins can offer good protection against NDV. *Ge et al. (2016)* designed novel recombinant baculovirus vaccines expressing the NDV F or HN genes. The F-series of vaccines provided a greater degree of protection (87.5–100%) than the HN series (62.5–87.5%). The authors concluded that 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. Kumar et al. (2011) 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. These vaccines were used to immunize 2-week-old chickens by the oculonasal route in order to evaluate the contribution of each protein to the induction of NDV-specific neutralizing antibodies and protective immunity. Protective immunity was evaluated by challenging the immunized birds 21 days later with virulent NDV and the obtained results indicated that F and HN proteins are independent neutralization and protective antigens, but that the contribution of F antigen in protection is greater. *Palya et al. (2014)* investigated the onset and long-term duration of immunity provided by a single vaccination with a turkey herpesvirus vector Newcastle disease (rHVT-ND) vaccine in commercial layers up to 72 weeks of age. Assessment of protection was done based on the prevention of clinical signs and reduction of challenge virus shedding via the oronasal and cloacal routes. Single vaccination with the rHVT-ND vaccine at one day of age provided complete or almost complete (95–100%) clinical protection against NDV challenges from 4 weeks of age up to 72 weeks of age when the latest challenge was done. Shedding of challenge virus both by the oronasal and cloacal route was significantly reduced compared to the controls.

Many authors have investigated the possibility of preparation of vaccines from purified and biologically active NDV subunits with HN and F glycoproteins, purified from nucleocapsids, viral ribonucleic acid (RNA) and pyrogens. The objective of the work of *Milić et al. (2015)* was to investigate some biological characteristics of purified glycoprotein subunits of PHY-LMV.42 Newcastle disease virus strain isolated from pigeons for the purpose of vaccine production. Testing for the immunogenicity of the viral subunits was carried out in a biological experiment on 75 Tetra SSL laying hens and 25 chickens Isa Brown after an artificial infection with Hertz 33 strain of NDV. The subunit vaccines of 256 and 128 HAU/0.5 ml induced a protective immune response in all vaccinated animals. Based on the obtained results it was concluded that the examined purified viral subunits of the PHY-LMV.42 strain of NDV, separated from nucleocapsids (NP proteins with viral RNA), large polymerase protein (L) and smaller fragment of F protein (F_2) can be used for a new potential vaccine. The study of *Arora et al. (2010)* concerned the immunization potential of purified HN and F glycoproteins of the Indian vaccinal NDV strain R_2B. This investigation indicates the role of

these glycoprotein subunits in the elicitation of protective immune response against NDV. Similarly, Meulemans et al. (1986) reported higher protective response of the F glycoprotein which could be explained by the fact that specific anti-F antibodies block cell-fusion activity thus preventing the spread of infection.

Conclusion

Nowadays, the development of subunit vaccines is based on the expression of HN and F proteins of NDV using viruses, plant-based systems, yeast and lactic acid bacteria as vectors in order to prepare recombinant immunogens. Some of the developed vaccines stimulate a satisfactory immunological response against NDV and have proved to be successful in protection of vaccinated animals in challenge experiments. The advantage of NDV subunit vaccines comparing to live and inactivated vaccines is in their safety for vaccinated animals and the fact that there are no unwanted postvaccinal effects. The subunit vaccine production procedure enables the recovery of a larger concentration of vaccinal antigens, thus a greater number of doses of the vaccine compared to live or inactivated vaccines. Some of the abovementioned vaccines like VectorVax FP-N, Trovac-NDV and Innovax-ND have been licensed in certain countries. However, vaccinated animals may have acquired immunity to certain vaccinal vectors which could be unfavourable regarding the development of the immune response to HN and F antigens contained in the vaccine. Additionally, most vectors used for the preparation of subunit recombinant vaccines are potential pathogens for the population of vaccinated animals which raises the question of vaccine application in field conditions. A large number of NDV subunit vaccines are prepared form genetically modified live viruses which must pass rigorous testing before vaccine registration. Aside from that, the use of other expression systems like transgenic plants may cause a biological safety problem. Subunit NDV vaccines can also be prepared from purified and biologically active NDV subunits with HN and F glycoproteins, purified from nucleocapsids with viral ribonucleic acid (RNA) and pyrogens which elicit a strong immunological response in vaccinated animals with no unwanted postvaccinal effects.

Pregled savremenih saznanja o razvoju subjediničnih i rekombinantnih vakcina protiv virusa Newcastle bolesti živine

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Rezime

Virus Newcastle bolesti je jedan od najznačajnijih patogena u populaciji ptica i domaće živine koji izaziva atipičnu kugu živine, kontagiozno oboljenje koje prati visoka stopa morbiditeta i mortaliteta, što ima za posledicu i velike ekonomske gubitke u živinarstvu. Glikoproteinski HN i F antigeni virusa atipične kuge živine su najznačajniji prilikom razvoja imunološkog odgovora prijemčivih jedinki. Vakcinacija živine živim i inaktivisanim vakcinama protiv virusa Newcastle bolesti predstavlja najefikasniji metod kontrole i prevencije navedenog oboljenja, međutim klasične vakcine imaju izvesne nedostatke i iz tog razloga se sve više istraživanja se usmerava na razvoj subjediničnih vakcina. U cilju razvoja subjediničnih vakcina u današnje vreme se za ekspresiju HN i F proteina virusa Newcastle bolesti koriste različiti vektori kao što su virusi, transgene biljke, kvasci i mlečnokiselinske bakterije. Pored toga, mnogi autori su ispitivali mogućnosti pripremanja subjediničnih vakcina od prečišćenih i biološki aktivnih subjedinica, odnosno HN i F glikoproteina pomenutog virusa, oslobođenih od nukleokapsida sa virusnom ribonukleinskom kiselinom (RNK) i pirogena. Virusni glikoproteini sa očuvanom antigenskom strukturom i biološkim aktivnostima se zbog svojih imunogenih svojstava mogu koristiti kao subjedinični vakcinalni antigeni.

Ključne reči: NDV, HN, F, subjedinične vakcine, rekombinantne vakcine

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