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Effects of Newcastle disease virus vaccine antibodies on the shedding and transmission of challenge viruses

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ABSTRACT

Different genotypes of avian paramyxovirus serotype-1 virus (APMV-1) circulate in many parts of the world. Traditionally, Newcastle disease virus (NDV) is recognized as having two major divisions represented by classes I and II, with class II being further divided into sixteen genotypes. Although all NDV are members of APMV-1 and are of one serotype, antigenic and genetic diversity is observed between the different genotypes. Reports of vaccine failure from many countries and reports by our lab on the reduced ability of classical vaccines to significantly decrease viral replication and shedding have created renewed interest in developing vaccines formulated with genotypes homologous to the virulent NDV (vNDV) circulating in the field. We assessed how the amount and specificity of humoral antibodies induced by inactivated vaccines affected viral replication, clinical protection and evaluated how non-homologous (heterologous) antibody levels induced by live NDV vaccines relate to transmission of vNDV. In an experimental setting, all inactivated NDV vaccines protected birds from morbidity and mortality, but higher and more specific levels of antibodies were required to significantly decrease viral replication. It was possible to significantly decrease viral replication and shedding with high levels of antibodies and those levels could be more easily reached with vaccines formulated with NDV of the same genotype as the challenge viruses. However, when the levels of heterologous antibodies were sufficiently high, it was possible to prevent transmission. As the level of humoral antibodies increase in vaccinated birds, the number of infected birds and the amount of vNDV shed decreased. Thus, in an experimental setting the effective levels of humoral antibodies could be increased by (1) increasing the homology of the vaccine to the challenge virus, or (2) allowing optimal time for the development of the immune response.
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1. Introduction

Infection of birds with virulent strains of Newcastle disease virus (NDV) causes one of the most important infectious diseases of poultry, Newcastle disease (ND), which is found worldwide and leads to economic losses from mortality and condemnation of carcasses. In 2010, seventy countries reported ND in domestic species to the World Organization for Animal Health (OIE) (www.oie.int.wahis/public.php?page=disease_status_lists) and many countries have endemic NDV, with outbreaks occurring year after year. Also known as avian paramyxovirus serotype-1 (APMV-1) virus, NDV is a member of the genus Avulavirus in the *Paramyxoviridae* family (Mayo, 2002a,b).

Antigenic similarity is shared among all NDV strains and isolates will cross-protect against challenge with any other NDV isolate. It is this immunological stimulation that serves as the basis of vaccination with live low virulent NDV (loNDV) to protect against virulent NDV (vNDV). Genetically, ND viruses are diverse and sixteen different genotypes have been already described (Courtney et al., 2012; Diel et al., 2012). Early studies have shown antigenic differences between strains of NDV using virus neutralization assays, hemagglutination inhibition (HI) assays with monoclonal antibodies, and by evaluating sequences of neutralizing epitopes (Panshin et al., 2002; Russell and Alexander, 1983; Schloer et al., 1975). The antigenicity of classes and genotypes can also be differentiated by cross HI assays, which correlate to differences in vaccine protection as measured by virus shedding after challenge (Gu et al., 2011; Li et al., 2010; Miller et al., 2007, 2009). While information regarding the avian immune response to NDV is limited, both antibodies and cell-mediated immunity (CMI) play a role in protection and clearance of NDV following infection (Reynolds and Maraqa, 2000a,b). Antibodies can be detected against NDV approximately 6–10 days post infection, while

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stimulation of antigen specific cytotoxic T-cells (CTLs) generally require about 7–10 days. Because the mean death time following infection with vNDV is 2–6 days, the presence of preexisting antibodies prior to infection appear to be most critical to protection from clinical disease (Kapczynski and King, 2005a). Antibodies produced against the hemagglutinin (HN) and fusion (F) trans-membrane surface glycoproteins are able to neutralize NDV upon subsequent infection (Boursnell et al., 1990a,b; Edbauer et al., 1990). In contrast, CTLs help clear from the host cells that are already infected and cannot stop disease progression. Since the pathology for loNDV is less than vNDV, the existence of preexisting immunity is not as critical to inhibit disease, and makes them excellent vaccine candidates.

Infection of chickens with vNDV results in rapid death of immunologically naïve birds, and thus the contribution of cell-mediated immunity is likely negligible since most birds are dead by 5–10 days post inoculation (Kapczynski and King, 2005; Kapczynski and Tumpey, 2003; Reynolds and Maraqa, 2000b). In contrast, infection with loNDV strains in immunologically naïve birds results in a limited, local infection, in which both humoral antibodies and antigen specific T-cells are generated. Clinical signs of infection are generally not observed in limited infections with loNDV, whereas the presence of secondary pathogens and/or immunosuppression can exacerbate clinical disease. In addition, mucosal immunoglobulin A (IgA) is produced in the respiratory tract and intestinal tract of chickens (Al-Garib et al., 2003a,b). Immunoglobulin G (IgG) can also be detected on mucosal surfaces and is believed to contribute to the overall local immunity as well (Chimeno Zoth et al., 2008). This mucosal antibody stimulation appears to aid in reduction of viral shedding, and will further aid in reducing viral infection following secondary exposure to NDV.

Antibodies to the HN and F glycoprotein of NDV are critical for virus neutralization and thus protection from vNDV (Reynolds and Maraqa, 2000a). Antibodies against the HN are responsible for blocking viral attachment, while antibodies against the F glycoprotein can inhibit viral fusion with the host cell membrane. Interestingly, even low levels of antibodies can provide protection of chickens against vNDV challenge (Gough and Allan, 1973).

Interest in the amount of vNDV shed into the environment by vaccinated birds has arisen as a potential indicator of vaccine efficacy (Miller et al., 2007, 2009). The ND experiments have shown that by using vaccines formulated with a NDV with the same (homologous) genotype of the vNDV challenge virus, for both genotype II and genotype V NDV isolates, it is possible to decrease not only the number of birds shedding vNDV, but also the amount of vNDV shed from individual birds by evaluating oropharyngeal and cloacal swab material (Miller et al., 2007, 2009). However, in those studies, the amount of virus shed from the birds vaccinated with vaccines heterologous to the genotype of the challenge virus was also decreased, but at lower amounts.

There is considerable controversy regarding the issue of vaccine failure on NDV control. Some argue that vaccine failure is mainly caused by inadequate application (Dortmans et al., 2012). However, others have suggested that vaccines formulated with genotypes homologous to the genotype of the challenge virus that reduce viral shedding should be a critical component of disease control (Hu et al., 2011). It is unknown if the use of higher doses of classical vaccines, which should induce higher antibody levels, would be sufficient to prevent ND caused by vNDV from genotypes more distant from vaccine strains, or which genotypes are more likely to fail vaccination with classical vaccines formulated with genotypes I and II NDV strains. In addition, it remains to be determined whether these older vaccines can significantly reduce viral shedding from challenge with newer isolates. In the present study, we vaccinated birds with a live LaSota vaccine and then challenged them with the heterologous vNDV (CA/2002) (defined as a virus of a different genotype) at different days post-vaccination (PV) to evaluate the amount of virus shed from each group and to subsequently determine how successful that amount of virus was transmitted to other birds. In addition, we further examined the seroconversion of chickens vaccinated with different genotypes of inactivated NDV and challenged with homologous and heterologous genotypes of vNDV to determine vaccine efficacy and humoral immunity on viral shedding. Our data indicate that vaccination with NDV vaccines formulated with antigens homologous (of the same genotype) of the challenge virus significantly reduces shedding compared to heterologous antigen, and that a correlation exists between antibody response after challenge with transmission potential to susceptible cohorts.

2. Materials and methods

2.1. Viruses

Working stocks of virus isolates used were obtained from the SEPRL repository and include US/LaSota/1946 (LaSota), gamefowl/USA (California)/212676/2002 (CA/2002), poultry/Peru/1918-13/2008 (Peru), Malaysia/1041/2008 (Malaysia) and Zoomat/Mexico (Chiapas)/2010 (Mexico). All viruses were propagated in 9–11 days old SPF embryos by chorioallantoic sac inoculation (Alexander and Swayne, 1998). The virulent CA/2002 strain (genotype V) was isolated as the etiological agent responsible for the last outbreak of ND in the US. The widely used LaSota vaccine (genotype II) was compared to recent vNDV viruses from Malaysia (genotype VIId), Mexico (genotype V) and Peru (genotype XII), a novel and highly divergent genotype related to recent African and Asian viruses in vaccine efficacy studies (Diel et al., 2012). Pools of infective allantoic fluid were clarified via centrifugation at 1000*g* for 15 min. Infectivity titers of the pools were determined by titration in SPF embryos prior to being stored at −70° C for use as live vaccine virus using hemagglutination (HA) assays.

2.2. Chickens and vaccine preparation

For all experiments, mixed-sex SPF chickens were obtained from the Southeast Poultry Research Laboratory SPF flocks and transferred to a BSL2 or BSL3E facility for vaccination and a BSL3E facility for challenge. Birds were maintained in Horsfal isolation units with feed and water administered *ad libitum*. In experiment II (below), four experimental NDV inactivated vaccines were produced with the LaSota, Mexico, Peru and Malaysia isolates following growth in SPF eggs and harvesting of allantoic fluid. Oil emulsion-adjuvanted vaccines were prepared as described by Stone et al. (1978). Following BPL-inactivation (Miller et al., 2007) of allantoic fluid, each vaccine virus was diluted to provide a concentration of approximately $10^{9.5}$ EID_{50}/dose (0.5 ml). Sham vaccine was prepared as above with normal allantoic fluid harvested from SPF embryos.

2.3. Challenge experiment I

Birds were vaccinated with a live vaccine and challenged with virulent CA/2002 at 3, 10 or 21 days post vaccination (Table 1). Birds were given 100 μl of a live LaSota vaccine ($10^{6.5}$ EID_{50}) with half the dose given onto the right eye and the other half into the choanal cleft. Sham vaccines for the controls and non-vaccinated contacts consisted of sterile BHI and were given as described above. The birds were challenged with the selected CA/2002 virus with the specified (Table 1) mean 50% embryo infectious dose (EID_{50}) of 100 μl per bird, half administered in 50 μl into the right eye and half in 50 μl into the choana. At 48 h post challenge, the 10