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Immune responses of poultry to Newcastle disease virus ☆

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ABSTRACT

Newcastle disease (ND) remains a constant threat to poultry producers worldwide, in spite of the availability and global employment of ND vaccinations since the 1950s. Strains of Newcastle disease virus (NDV) belong to the order Mononegavirales, family Paramyxoviridae, and genus Avulavirus, are contained in one serotype and are also known as avian paramyxovirus serotype-1 (APMV-1). They are pleomorphic in shape and are single-stranded, non-segmented, negative sense RNA viruses. The virus has been reported to infect most orders of birds and thus has a wide host range. Isolates are characterized by virulence in chickens and the presence of basic amino acids at the fusion protein cleavage site. Low virulent NDV typically produce subclinical disease with some morbidity, whereas virulent isolates can result in rapid, high mortality of birds. Virulent NDV are listed pathogens that require immediate notification to the Office of International Epizootics and outbreaks typically result in trade embargos. Protection against NDV is through the use of vaccines generated with low virulent NDV strains. Immunity is derived from neutralizing antibodies formed against the viral hemagglutinin and fusion glycoproteins, which are responsible for attachment and spread of the virus. However, new techniques and technologies have also allowed for more in depth analysis of the innate and cell-mediated immunity of poultry to NDV. Gene profiling experiments have led to the discovery of novel host genes modulated immediately after infection. Differences in virus virulence alter host gene response patterns have been demonstrated. Furthermore, the timing and contributions of cell-mediated immune responses appear to decrease disease and transmission potential. In view of recent reports of vaccine failure from many countries on the ability of classical NDV vaccines to stop spread of disease, renewed interest in a more complete understanding of the global immune response of poultry to NDV will be critical to developing new control strategies and intervention programs for the future.

1. Introduction

Despite the advances made in the diagnosis of and vaccination for Newcastle disease since it was first described in 1926, the disease continues to negatively impact poultry producers by infecting birds worldwide (Alexander et al., 2012; Goldhaft, 1980). From 2006 to 2009 the most widespread animal diseases, in terms of the number of countries affected, were rabies, Newcastle disease (ND) and Bovine tuberculosis (Anonymous, 2011). ND ranked as the fourth most important disease in terms of the number of livestock units lost for poultry species, behind highly pathogenic avian influenza, infectious bronchitis, and lowly pathogenic avian influenza (Anonymous, 2011). The disease is caused by only the virulent strains of avian paramyxovirus serotype-1 (AMPV-1) and APMV-1 is synonymous with Newcastle disease virus (NDV) (OIE, 2012). Strains are defined as virulent if they (1) have three or more basic amino acids at position 113–116 of the un-cleaved fusion protein cleavage site (F0) with a phenylalanine at position 117, or (2) obtain a intracerebral pathogenicity index (ICPI) value of $\geqslant$0.7 in day-old chickens (*Gallus gallus*) (OIE, 2012). Failure to demonstrate multiple basic amino acids necessitates an ICPI value be obtained for the isolate.

NDV is known to infect over 236 species of birds (Kaleta and Baldauf, 1988) and besides poultry species virulent NDV (vNDV) strains are commonly found in pigeons and double crested cormorants (Diel et al., 2012b; Kim et al., 2008; Pchelkina et al., 2013) and occasionally in some other wild bird species (Kaleta and Kummerfeld, 2012). Typically, the concern is that pigeons will transmit their vNDV strains of genotype VIb to poultry (Abolnik et al., 2004; Alexander and Parsons, 1986), however, poultry are able to transmit their vNDV strains to pigeons, as well (Merino et al., 2009). The incubation period and clinical disease observed with a NDV infection depends on multiple factors. The typical

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range is from three to six days depending on the species of host infected with the vNDV, the immunity of the host to NDV and the amount and strain of vNDV the host is exposed to (Alexander and Senne, 2008).

The clinical signs observed upon infection will be non-specific and can include depression, ruffled feathers, open mouth breathing, hyperthermia, anorexia, listlessness and hypothermia before death. In addition, since the lesions observed upon infection with vNDV are not pathognomonic, other diseases such as highly pathogenic avian influenza, infectious laryngotracheitis and mycoplasmosis should be considered (Alexander and Senne, 2008). However, if hemorrhage and necrosis of lymphoid tissues is present, especially of the intestine, spleen and thymus, viscerotropic vNDV should be suspected (Cattoli et al., 2011). Because layers receive multiple NDV vaccinations during their production cycle, and thus have persistent immunity, they may not show signs of infection except a drop in egg production (Bwala et al., 2012; Cho et al., 2008). Birds infected with neurotropic vNDV strains remain alert prior to developing neurological signs such as torticollis, ataxia or a wing or leg paralysis and gross lesions are usually absent (Cattoli et al., 2011).

All strains of Newcastle disease virus (NDV) belong to the order Mononegavirales, family Paramyxoviridae, and genus Avulavirus, are contained in one serotype and are also known as avian paramyxovirus serotype-1 (APMV-1) (Alexander and Senne, 2008). The virions are pleomorphic in shape, and consist of single-stranded, non-segmented, negative sense RNA genomes (Miller et al., 2010). There are at least three different genome lengths (15,186, 15,192 or 15,198), with six genes that produce six structural proteins in a 3′ to 5′ order: nucleocapsid (N), phosphoprotein (P), matrix (M), fusion (F), hemagglutinin-neuraminidase (HN) and the RNA dependent RNA (large) polymerase (L). Editing of P produces at least one other protein, the V protein, which has anti-interferon properties (Czegledi et al., 2006).

Even though all strains of NDV are contained in one serotype, there are phylogenetic differences found when comparing genome relatedness. Strains are divided into two classes, class I and class II, with class II further divided into 16 genotypes (Diel et al., 2012a). Class I viruses are typically isolated from wild birds and all reported strains are of low virulence except for one strain, chicken/Ireland/1990 (Alexander et al., 1992). Class II, genotype I NDV are all of low virulence except for the vNDV that caused the ND outbreak in 1998 in Australia (Gould et al., 2001). Class II, genotype II viruses contain NDV of low virulence, some of which (B1, LaSota, VG/GA) are used as NDV vaccines, and vNDV that are not commonly isolated (Miller et al., 2010). NDV strains of class II, genotypes III–IX, and XI–XVI are all virulent (Courtney et al., 2012; Diel et al., 2012a). Isolates of class II, genotype X are of low virulence and most often found in wild birds, but some have been isolated from some poultry species (Diel et al., 2012a; Miller et al., 2011).

While humoral immunity from vaccination is critical to ND control, another important aspect that is not a new concept, but is often neglected, is the differences in resistance to ND due to genetic variation (Albiston and Gorrie, 1942). In addition, it is known that there is a negative correlation between a primary antibody response to NDV and favorable production traits (Lwelamira et al., 2009). Genetic resistance to ND has been observed with various lines within a breed for chickens (Cole and Hutt, 1961; Gordon et al., 1971) and turkey (Tsai et al., 1992) and among breeds of chickens (Hassan et al., 2004; King, 1996) and ducks (Shi et al., 2011). Concerning this topic it is important to note that each Newcastle disease virus may be better adapted to grow in one species versus another, like what is seen with PPMV1 (pigeon NDV) strains in chickens (Pearson et al., 1987). Another example of this can be seen with the variability in the bird infectious dose 50 of one NDV for chickens, turkeys and ducks (Aldous et al., 2010). While improving genetic resistance to ND through breeding more resistant bird strains appears to be feasible, logistically it is very difficult due to the involvement of multifactorial components. Perhaps when the efficiency of producing transgenic birds is improved, more disease resistance breeds can be used for this purpose (Zhang et al., 2012).

Another important factor for ND control in developing countries is the lack of a "cold chain" or reliable source to keep the vaccines at 4 °C. Even the best live vaccine will not induce an immune response if it is not viable due to improper storage during the distribution process. Progress has been made with the thermostable I-2 strain of NDV and has been put into place in some developing countries (Bensink and Spradbrow, 1999; Harrison and Alders, 2010; Illango et al., 2005; Nasser et al., 2000). Continued improvement and utilization of thermostable NDV strains is necessary to improve controls in countries where vNDV isolates are endemic and the cold chain is unreliable.

2. Innate immune response to NDV infection in poultry

The innate immune response comprises factors that exist prior to the advent of infection, and are capable of exclusion or rapid response to microbes. The primary components of innate immunity of poultry are (1) physical and chemical barriers, such as feathers and skin, epithelia and production of mucus; (2) phagocytic cells, including macrophages and natural killer cells; (3) complement proteins and mediators of inflammation; and (4) cytokines. Overall, the innate immune response to virus infection is an immediate reaction designed to control and inhibit virus growth and spread and aid in developing pathogen-specific protection through the adaptive immune response. The early reactions of the innate immune system use germ-line encoded receptors, known as pattern recognition receptors (PRR's), which recognize evolutionarily conserved molecular markers of infectious microbes, known as PAMP's (pathogen-associated molecular patterns). Recognition of PAMPs by PRRs, either alone or in heterodimerization with other PRRs, (toll-like receptors (TLR); nucleotide-binding oligomerization domain proteins (NOD); RNA helicases, such as retinoic acid-inducible gene 1 (RIG-I) or MDA5; C-type lectins), induces intracellular signals responsible for the activation of genes that encode for pro-inflammatory cytokines, anti-apoptotic factors, and antimicrobial peptides. The virus is first recognized by host sentinel proteins, including TLR and NOD proteins, producing rapid signaling and transcription factor activation that lead to production of soluble factors, including interferon and cytokines, designed to limit and contain viral replication.

NDV infection *in vitro* results in nitric oxide (NO) induction in chicken heterophils and peripheral blood mononuclear cells, interferon alpha (IFN-α) and beta (IFN-β) mRNA detection in macrophages, and gamma (IFN-γ) mRNA production in peripheral blood mononuclear cells (Ahmed et al., 2007; Sick et al., 2000, 1998). In addition, infection of chicken heterophils decreased the ability to phagocytose bacteria, resulting in impaired heterophil function, and making birds more susceptible to secondary infection (Lam et al., 1996). Constitutive low-level expression of NO in the vascular endothelium plays a beneficial role in maintaining blood vessel homeostasis, but high levels of NO produced by macrophages in response to pathogens can have toxic effects on the host (Palmer et al., 1987).

In mammalian systems, such as cultured murine macrophages, NDV induced both IFN-α and IFN-β (Hoss et al., 1989; Zawatzky et al., 1991). The functional significance of the interferon regulatory factor genes (IRF)-3 and IRF-7 was examined in mouse macrophages derived from deletion knock out (KO) animals (Wilden

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