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Pathogenicity and tissue tropism of infectious bronchitis virus is associated with elevated apoptosis and innate immune responses

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ABSTRACT

To establish a characteristic host response to predict the pathogenicity and tissue tropism of infectious bronchitis viruses (IBV), we investigated innate immune responses (IIR) and apoptosis in chicken embryo kidney cells (CEKC) and tracheal organ cultures (TOC) infected with three IBV strains. Results showed nephropathogenic IBV strains 885 and QX induced greater apoptosis in CEKC than M41, which induced greater apoptosis in TOCs compared to 885 and QX. Elevated IIR is associated with tissue tropism of different IBV strains. Compared to M41, 885 and QX caused greater induction of toll like receptor 3 (TLR3), melanoma differentiation associated protein 5 (MDA5) and interferon beta (IFN-β) in CEKC. In contrast, M41 infection caused greater expression of these genes than 885 or QX in TOCs. In summary, greater levels of apoptosis and elevated levels of TLR3, MDA5 and IFN-β expression are associated with increased pathogenicity of IBV strains in renal and tracheal tissues.

Introduction

Infectious bronchitis (IB) is an acute and highly contagious disease caused by a gamma coronavirus that affects chickens of all ages and is characterized by lesions in respiratory and urogenital organs (Cavanagh, 2007; Dolz et al., 2006). Avian infectious bronchitis virus (IBV) continues to cause serious economic losses to global chicken production. Along with highly pathogenic avian influenza (HPAI) and velogenic Newcastle disease (ND), IB is the most economically important viral respiratory disease affecting poultry industry worldwide (Cook et al., 2012). Vaccination has been considered as the most reliable approach for IBV control (Meeusen et al., 2007) however current vaccines have proved to be inadequate due to constant emergence of new variant viruses (De Wit, 2000; de Wit et al., 2011). Concurrent circulation of both classic and variant IBVs has been identified in most parts of the world, raising major challenges to the current IBV prevention and control strategies.

Current IBV isolates present high antigenic diversity (Hofstad, 1975), and emergent strains that differ in antigenic properties, tissue tropism and pathogenicity are continuously being reported across the world (Gelb et al., 1991; Jackwood, 2012; Shaw et al., 1996; Zanella et al., 2000). While all IBV strains appear to initially infect chickens *via* the respiratory tract, vireamia enables spread to secondary sites for further replication and persistence. An example is IBV strain M41, which replicates primarily in the respiratory tract and subsequently spreads and replicates in a range of other tissues. In contrast, strains of IBV such as strain QX and IS/885/00 are primarily nephropathogenic (Benyeda et al., 2009; Meir et al., 2004). IS/885/00, referred to as a nephropathogenic infectious bronchitis virus (NIBV), was first isolated from a severe outbreak of renal disease in several broiler farms in Israel (Meir et al., 2004) and has been detected in many other Middle East countries (Abdel-Moneim et al., 2012; Awad et al., 2014; Mahmood et al., 2011). IBV QX was first isolated in China from chickens with proventriculitis (YuDong et al., 1998) but was later found to cause renal, (Ganapathy et al., 2012; Liu and Kong, 2004; Terregino et al., 2008; Worthington et al., 2008), respiratory and reproductive lesions (Ducatez et al., 2009; Terregino et al., 2008) in chicken flocks in Europe, Asia, Africa and Middle East (Beato et al., 2005; Domanska-Blicharz et al., 2006; Gough et al., 2008; Jackwood, 2012).

Differences in tissue tropism and thus differences in the pathogenicity of IBV strains have been hypothesized to be associated with differences in the binding properties of their spike proteins (Casais et al., 2003; Wickramasinghe et al., 2011). While the ability to bind to

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susceptible host cells is the first step in the viral life cycle, host innate immune responses could also be a major contributing factor to the pathological outcome of IBV infection. Variant IBVs continually emerge and the host determinants of IBV pathogenicity are not yet fully understood. Early cellular and innate immune responses of virus infected cells *in vitro* could act as useful indicators for predicting the pathological outcome of viral infection *in vivo*. For example, the three different genotypes of Newcastle disease viruses (NDV) produce distinct host response patterns in chicken spleenocytes, which is useful to differentiate the NDV genotypes (Hu et al., 2012). In order to establish the characteristic host response to predict the tissue tropism and pathogenicity of IBVs, we investigated apoptosis and innate immune responses in chicken embryo kidney (CEK) cells and tracheal organ cultures (TOCs) following infection with IS/885/00-like, QX-like and M41 IBV strains.

Results

CEK cells infected with IBV strains 885, QX or M41 were subjected to immuno-cytochemical staining of viral nucleoprotein (NP) at 6 h post-infection (hpi). The dose (MOI 1.0) used resulted in a similar level of infection in CEK cells across all the three virus strains (Fig. 1).

IBV infections resulted in significant increase in apoptosis of CEK cells and TOCs

CEK cells and TOCs were infected with IBV strains 885, QX, M41 or mock infected, cell metabolic activity and percentage of apoptotic cells were evaluated at 24 and 48 hpi by MTT assay and Annexin V binding assay, respectively. A significant ($p < 0.05$) reduction in cell metabolic activity was found in IBV infected CEK cells, compared with mock infected cells, both at 24 and 48 hpi (Fig. 2A). A significantly ($p < 0.05$) greater level of apoptosis was also found in IBV infected CEK cells at 24 and 48 h after virus infection (Fig. 2B). Total apoptotic cells in IBV or mock infected TOCs were evaluated by TUNEL assay (Fig. 3). It was found that IBV infection resulted in significant increase in total apoptotic cells in TOCs when compared with mock infected controls at 24 and 48 hpi (Fig. 3C). Notably, infection of CEK cells with IBV strains 885 and QX resulted in significantly ($p < 0.05$) greater level of cell death, as shown by reduced metabolic activity (Fig. 2A) and increased apoptosis (Fig. 2B) when compared to M41 infected cells both at 24 and 48 hpi. In contrast, significantly higher levels of apoptosis were observed in M41 infected TOCs compared with those infected with 885 or QX (Fig. 2C). Infection with IBV strains 885 or QX resulted in significantly ($p < 0.05$) lower infectious virus production from CEK cells at 24 and 48 hpi when compared with M41 infected cells (Fig. 2D). In sharp contrast, significantly ($p < 0.05$) lower infectious virus production was observed from M41 infected TOCs compared with 885 or QX infected TOCs (Fig. 2E).

Infection of CEK cells with IBVs resulted in significantly higher up-regulation of TLR3 and MDA5

CEK cells were infected with IBV strains 885, QX, M41 or mock infected at a MOI of 1.0, and expression of LGP2, MDA, TLR1, TLR2, TLR3 and TLR7 were analysed at 9 and 24 hpi. It was observed that expression of LGP2 (Fig. 4A) and MDA5 (Fig. 4B) was significantly up-regulated at 9 hpi but down-regulated at 24 hpi in IBV infected CEK cells compared with mock infected cells ($p < 0.05$). However, at 9 hpi, infection with IBV strains 885 and QX resulted in significantly ($p < 0.05$) greater MDA5 expression than M41 in CEK cells (Fig. 4B).

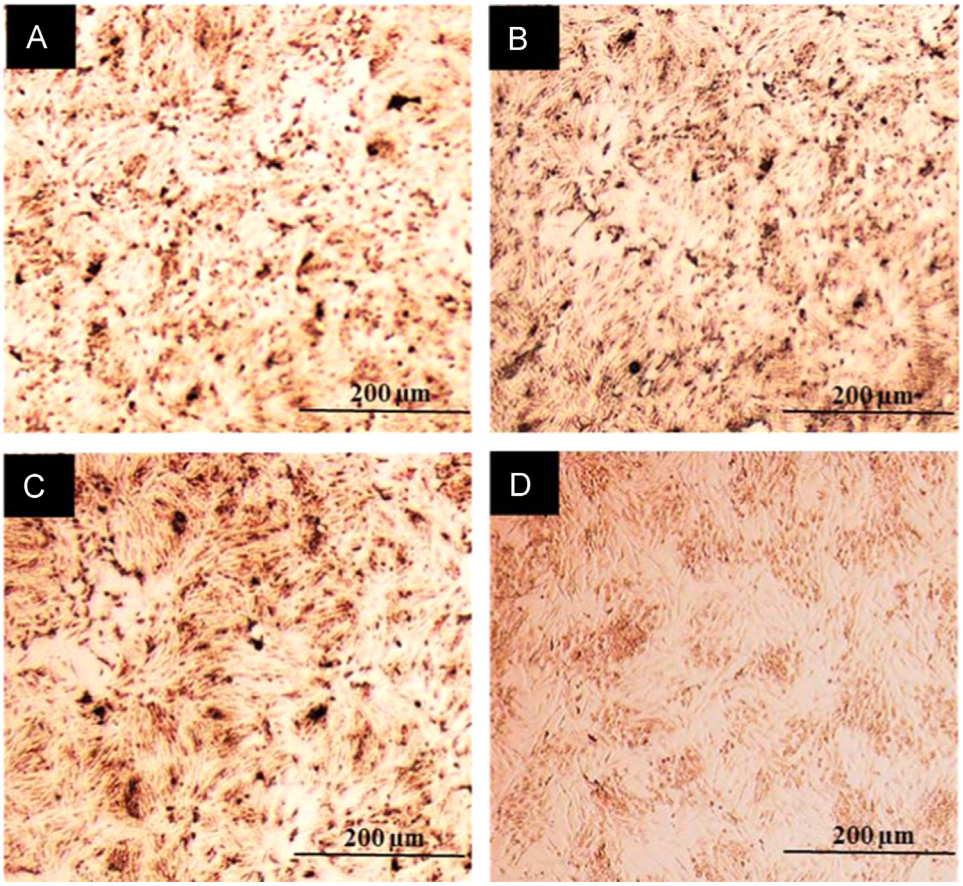

Fig. 1. Chicken embryo kidney (CEK) cells infected with (A) 885, (B) QX (C) M41 or (D) mock-infected and immuno-cytochemical staining of viral NP at 6 hpi (hours post-infection), revealing similar levels of infection of all the three viruses.