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Muscovy duck reovirus infection rapidly activates host innate immune signaling and induces an effective antiviral immune response involving critical interferons

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ABSTRACT

Muscovy duck reovirus (MDRV) is a highly pathogenic virus in waterfowl and causes significant economic loss in the poultry industry worldwide. Because the host innate immunity plays a key role in defending against virus invasion, more and more attentions have been paid to the immune response triggered by viral infection. Here we found that the genomic RNA of MDRV was able to rapidly induce the production of interferons (IFNs) in host. Mechanistically, MDRV infection induced robust expression of IFNs in host mainly through RIG-I, MDA5 and TLR3-dependent signaling pathways. In addition, we observed that silencing VISA expression in 293T cells could significantly inhibit the secretion of IFNs. Remarkably, the production of IFNs was reduced by inhibiting the activation of NF-κB or knocking down the expression of IRF-7. Furthermore, our study showed that treatment of 293T cells and Muscovy duck embryo fibroblasts with IFNs markedly impaired MDRV replication, suggesting that these IFNs play an important role in antiviral response during the MDRV infection. Importantly, we also detected the induced expression of RIG-I, MDA5, TLR3 and type I IFN in Muscovy ducks infected with MDRV at different time points post infection. The results from *in vivo* studies were consistent with those in 293T cells infected with MDRV. Taken together, our findings reveal that the host can resist MDRV invasion by activating innate immune response involving RIG-I, MDA5 and TLR3-dependent signaling pathways that govern IFN production.

1. Introduction

Muscovy duck (*Cairina moschata*) reovirus (MDRV), the etiological agent of a disease discovered in South Africa in 1950 (Yun et al., 2013), was first isolated in France in 1972 (Gaudry et al., 1972). MDRV is a member of the Orthoreovirus genus of the Reoviridae family and shares common properties with other avian reoviruses (ARV). These properties include a genome of 10 segments of

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double-stranded RNA (dsRNA) that is packaged into a non-enveloped icosahedral double-capsid shell with a diameter of 70–80 nm, syncytium formation in cell culture and inability to haemagglutinate (Zhang et al., 2007a). The genomic segments can be divided into three size classes: large (L1–L3), medium (M1–M3) and small (S1–S4), which encode at least 10 structural proteins and 4 nonstructural proteins (Yun et al., 2013). However, MDRV is antigenically different from other ARV. For example, the homology of nonstructural proteins MDRV P10 (GenBank: KC571174.1) and ARV P10 (GenBank: AF330703.1) has only 34.53% similarity. Importantly, MDRV is a waterfowl highly pathogenic virus causing an epidemic and acute infectious disease in ducklings between 2 and 4 weeks of age, resulting in severe symptoms characterized by hepatic lesions and up to 50% mortality rate (Wang et al., 2013; Zhang et al., 2007b). Previous investigations have mainly focused on the pathogenesis of ARV (Chen et al., 2014; Rodríguez-Grille et al., 2014). However, the molecular mechanisms underlying MDRV–host interaction remain largely unknown and little information is available on host immune response to the MDRV infection.

The host innate immune response provides the first line of defense against virus infections. In the cellular response to pathogen invasion, the hosts identify some conserved non-self molecules, which are called pathogen-associated molecular patterns (PAMPs). These PAMPs can be recognized by pathogen-recognition receptors (PRRs), which triggers a series of signaling cascades (O'Neill and Bowie, 2010). Three types of PRRs are associated with the identification of viral infection: certain Toll-like receptors (TLRs); retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), including RIG-I and melanoma differentiation-associated gene 5 (MDA5); and nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) (Takeuchi and Akira, 2010). Among them, TLRs and RLRs, the two types of PRRs, are studied most for their importance to viral PAMPs. Virus-induced signaling adaptor (VISA), also known as IPS-1, MAVS, and Cardif, is a mitochondrial transmembrane protein essential for RLR-mediated response to cytosolic RNA. The primary role of NLRs is to regulate IL-1β mature by activating caspase-1 (Pétrilli et al., 2007). After binding to viral PAMPs, the conformations of TLRs and RLRs are changed, leading to the recruitment of the specific adapter protein located in the cytoplasm and initiating the signaling cascades, which results in the activation of transcription factors, including nuclear factor-κB (NF-κB) and IFN-regulatory factors (IRFs). These transcription factors, in turn, regulate the production of cytokines, chemokines, and interferons (IFNs) that induce the expression of IFN-stimulated genes (ISGs).

IFNs consist of three types of cytokines: type I IFNs mainly include IFN-α and IFN-β; type II IFN is IFN-γ; and type III IFNs consist of three members in humans, IFN lambda1 (IFN-λ1), IFN-λ2, and IFN-λ3, which are also named interleukin-29 (IL-29), IL-28A, and IL-28B, respectively (Wang et al., 2014; Wei et al., 2014). Type I and type III IFNs are often considered the antiviral classes (Borden et al., 2007). Type I IFNs are proteins with pleiotropic functions, such as antiviral, antiproliferative, and immunomodulatory activities (Schmeisser et al., 2014) and type III IFNs share many similarities to type I IFNs, including antiviral and antiproliferative characteristics (Zheng et al., 2012). In fact, viral infection induces two main phases in type I IFN expression and regulation. In the early phase of viral infection, the phosphorylation of IRF-3 and IRF-7 happens at specific serine residues leading to the homodimerization or heterodimerization of IRF-3 and IRF-7. Then the dimers translocate to the nucleus and induce the production of small amounts of type I IFNs. In the late phase of infection, infected cells release and produce progeny viruses. Simultaneously, the type I IFN receptor (IFNAR) is bound to newly synthesized IFNs and the expression of numerous ISGs is induced *via* Janus-activated kinase/signal transducer and activator of transcription (JAK/STAT) pathway. The IFNs also activate the transcription of the IRF-7 gene, which results in an increase in the expression of type I IFNs and contributes to the production of antiviral proteins, such as ISG15, interferon-induced transmembrane protein 3 (IFITM3) and MxA (Ouyang et al., 2014; Tan et al., 2012).

Although IFNs play a critical role in combatting viral infections, little is known about MDRV-triggered innate immune signaling pathways. In this study, we examined the expression and the roles of type I and type III IFNs during MDRV infection. We found that the mRNA levels of type I and type III IFNs, especially IFN-β and IL-28A/B, increased markedly in MDRV infected 293T cells. Strikingly, MDRV infection affected the expression of IFN-β, IL-28A/B and IL-29 by altering the expression and/or activation of RIG-I, MDA5, TLR3, VISA, IRF-3, IRF-7 and NF-κB. Disrupting the expression of PRRs, VISA, IRF-7 or inhibiting the activation of NF-κB significantly inhibited production of IFN-β, IL-28A/B and IL-29. Furthermore, our experiments demonstrated that expressions of RIG-I, MDA5, TLR3, and type I IFN were induced by MDRV infection in Muscovy ducks. These results establish that MDRV infection can rapidly activate host innate immune signaling and induce an effective antiviral immune response involving IFNs.

2. Materials and methods

2.1. Ethics statement

The animal protocol used in this study was approved by the Research Ethics Committee of College of Animal Science, Fujian Agriculture and Forestry University (Permit Number PZCASFAFU2014002). All duck experimental procedures were performed in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals approved by the State Council of China.

2.2. Reagents

The following antibodies were used in this study: anti-ISG15, anti-NF-κB p65 (Santa Cruz Biotechnology, Santa Cruz, CA); anti-IFITM3 (Proteintech, Chicago, US); and anti-β-actin (Abcam, Cambridge, UK). The pharmacological NF-κB inhibitor BAY11-7082 was purchased from Merck (Darmstadt, Germany). Recombinant human IL-28A and IFN-β were purchased from PeproTech (Rocky Hill, NJ).