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Short communication

In vitro characterization of the novel H3N1 reassortant influenza viruses from quail

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ABSTRACT

Quail is considered as an intermediate host for generation of the novel reassortant influenza A viruses (IAVs). In this study, we evaluated the replication ability of the three novel H3N1 reassortant viruses recovered from pandemic H1N1 2009 (pH1N1) and duck H3N2 (dkH3N2) co-infected quail generated from our previous study in embryonated chicken eggs, mammalian (MDCK) and human lung derived (A549) cells. Our study demonstrated that all of the reassortant viruses replicated efficiently in avian and mammalian cells, albeit with slightly lower titers than the parental viruses. Of note, all of the reassortant viruses showed enhanced replication in human lung derived A549 cells compared to their parental viruses. Interestingly, among the reassortant viruses tested, a reassortant virus (P(NA,NS)-DK) containing NA and NS genes derived from pH1N1 and the other genes from dkH3N2 exhibited the highest replication ability in all *in vitro* models, indicating a high level of gene compatibility of this reassortant virus. Our results highlight the potential role of quail as intermediate hosts for the generation of the viable reassortant viruses with ability to replicate efficiently in avian, mammalian, and particularly human lung derived cells. These findings emphasize the need for the continuous IAV surveillance in quail to prevent the risk of the emergence of the novel viable reassortant viruses.

1. Introduction

The genome structure of influenza A virus (IAV) allows gene reassortment among different IAV infections. Genetic reassortment event can generate the novel reassortant viruses with marked genotypic and phenotypic changes, facilitating crossing of species barriers and possibly leading to influenza pandemic. It is well established that reassortment event contributed to the emergence of human pandemics in 1957, 1968 and 2009 (Yen and Webster, 2009).

Genetic reassortment between avian and mammalian IAVs occurs in hosts susceptible to both viruses. As such, those hosts serve as mixing vessels or intermediate hosts for the generation of the novel reassortant viruses with pandemic potential (Webby and Webster, 2001). Quail are considered to be one of the intermediate hosts for generation of the new reassortant viruses, since they express receptors for both mammalian and avian IAVs and are susceptible to both avian and mammalian IAVs (Makarova et al., 2003; Wan and Perez, 2006). Recently, our previous study showed that the novel reassortant viruses could be generated in the respiratory tract of the experimental co-infected quail, supporting the role of quail as an intermediate host of IAV reassortment. Noteworthy, a high number of the novel H3N1 reassortant viruses were recovered from pandemic H1N1 2009 (pH1N1) and duck H3N2 (dkH3N2) co-infected quail (Thontiravong et al., 2012). However, whether these novel reassortant viruses would be viable for replication in avian, mammalian and human cells remain unknown. In this study, the replication characteristics of the three H3N1 reassortant viruses recovered from co-infected quails were evaluated in embryonated chicken eggs, mammalian and human cell lines compared with their parental viruses. Genetic characteristics of HA and NA genes of the reassortant viruses were also analyzed.

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2. Materials and methods

2.1. Viruses and cells

In previous study, the three novel reassortant viruses, including the H3N1 reassortant virus containing HA from dkH3N2 and the other genes from pH1N1 (designed DK(HA)-P), the H3N1 reassortant virus containing NA from pH1N1 on a dkH3N2 backbone (designed P(NA)-DK) and the H3N1 reassortant virus containing NA and NS from pH1N1 on dkH3N2 backbone (designed P(NA,NS)-DK), were found to be dominant following co-infection of pH1N1 and dkH3N2 in quail (Thontiravong et al., 2012). Therefore, these three novel dominant H3N1 reassortant viruses were selected for evaluation in this study (Fig. 1A). Two parental viruses used in this study were pandemic H1N1 (pH1N1) (A/swine/Thailand/CU-RA4/2009) and LPAI duck H3N2 (dkH3N2) (A/duck/Thailand/AY-354/2008) viruses. All reassortants and pH1N1 parental viruses were propagated in Madin-Darby canine kidney (MDCK) cells maintained in minimum essential medium (MEM) (Invitrogen, Carlsbad, CA) containing 1 μg/ml tosylsulfonyl phenylalanyl chloromethyl ketone (TPCK)-treated trypsin (Sigma–Aldrich, St. Louis, MO) as described previously (Thontiravong et al., 2012). The dkH3N2 parental virus was propagated in the allantoic cavity of 10-day-old embryonated chicken eggs. Virus propagation and handling were performed in a BSL-2 containment facility.

2.2. Viral replication kinetics of the H3N1 reassortant viruses

The replication ability of the three novel H3N1 reassortant viruses and the parental viruses were evaluated by virus replication kinetics in embryonated chicken eggs and different cell lines (MDCK and A549 cells). The embryonated chicken eggs, MDCK and A549 cells were used as a representative model of avian, mammalian and human hosts, respectively. MDCK cells were chosen, since they are often used for studying the replication of different IAVs (Jackson et al., 2009; Octaviani et al., 2010). A549 cells were chosen, because they originated from the human respiratory epithelial cells, the primary target of human IAV infections and have been used to study the replication of human IAVs (Song et al., 2015). To determine virus replication kinetics in cell lines, monolayers of MDCK and A549 cells were inoculated in triplicate with each virus at a multiplicity of infection (MOI) of 0.01 at 37 °C. After 1 hour (h) of inoculation, cell monolayers were washed and overlaid with MEM (for MDCK cells) or DMEM (for A549 cells) (Invitrogen, Carlsbad, CA) supplemented with 0.3% bovine serum albumin fraction V (BSA) (Invitrogen, Carlsbad, CA) and 1 μg/ml TPCK-treated trypsin (Sigma–Aldrich, St. Louis, MO) and then placed at 37 °C. To determine virus replication kinetics in embryonated chicken eggs, 10-day-old embryonated chicken eggs were inoculated in triplicate with 10^3 $TCID_{50}$/ml of each virus and then incubated at 37 °C. Cell supernatants and allantoic fluid were

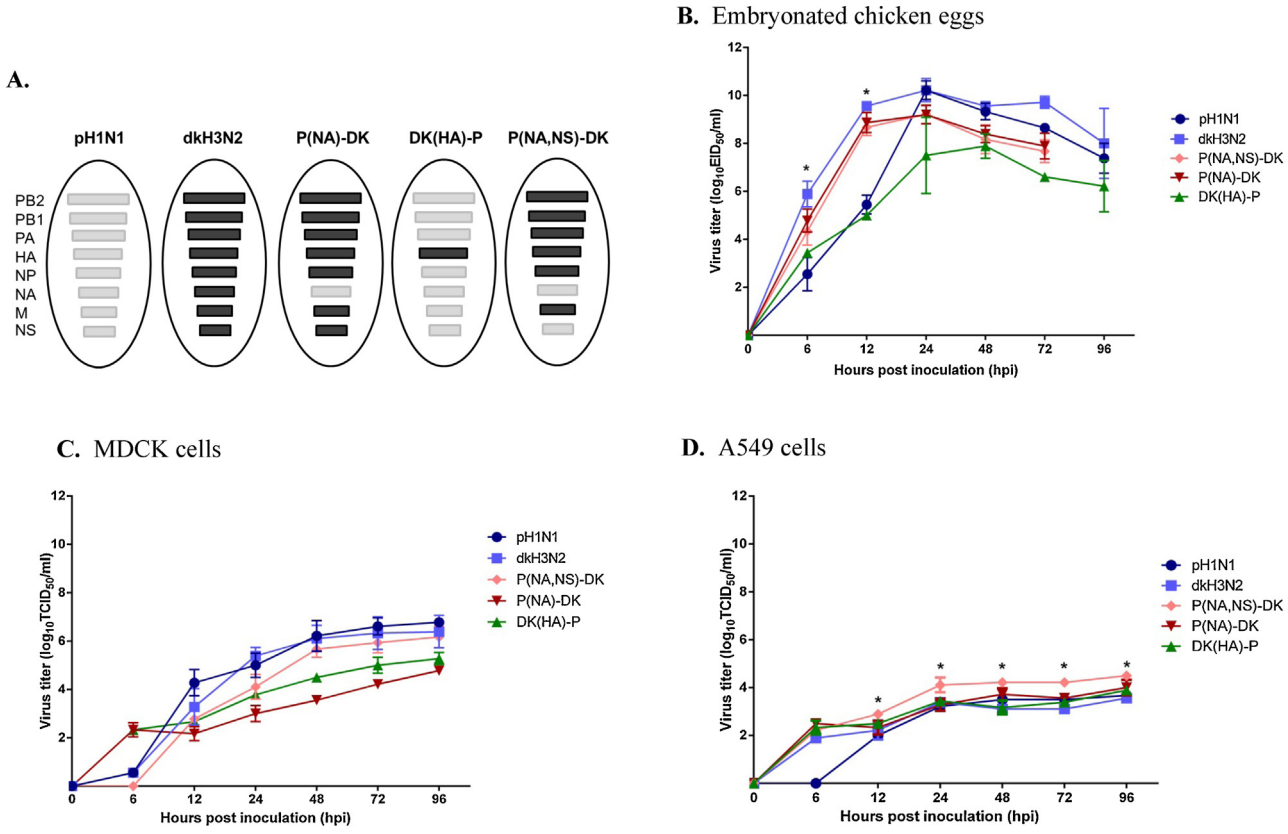

Fig. 1. Genotypes and replication kinetics of the novel H3N1 reassortant viruses and their parental viruses (A) Genotypes of the parental viruses (pH1N1 and dkH3N2) and the novel reassortant viruses (P(NA)-DK, DK(HA)-P, and P(NA,NS)-DK). Opened bars indicate gene from pH1N1 and filled bars indicate gene derived from dkH3N2. Replication kinetics of the novel H3N1 reassortant viruses and their parental viruses in (B) embryonated chicken eggs (C) MDCK and (D) A549 cells. Cells and eggs were infected with each virus at an MOI of 0.01 or 10^3 $TCID_{50}$/ml, respectively. Virus titers were determined by virus titration at the indicated time points. Each data point represents the mean ±standard deviation of three independent experiments. *, $P < 0.05$ (one-way ANOVA) for virus titers compared with the ressortant viruses and pH1N1 in eggs, as well as for virus titers compared with P(NA,NS)-DK, the parental viruses and other reassortant viruses in A549 cells at the indicated time points.