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# Influenza A virus infection of primary differentiated airway epithelial cell cultures derived from Syrian golden hamsters

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**Abstract**

The ability of several different influenza A virus strains to infect and replicate in primary, differentiated airway epithelial cell cultures from Syrian golden hamsters was investigated. All virus strains tested replicated equivalently in the cultures and displayed a preference for infecting nonciliated cells. This tropism correlated with the expression of both α2,3- and α2,6-linked sialic acid on the nonciliated cells. In contrast, the ciliated cells did not have detectable α2,6-linked sialic acid and expressed only low amounts of α2,3-linked sialic acid. In contrast to clinical isolates, laboratory strains of influenza A virus infected a limited number of ciliated cells at late times post-infection. The presence of α2,3- and α2,6-linked sialic acid residues on the same cell type suggests that Syrian golden hamsters and differentiated airway epithelial cell cultures derived from hamsters may provide a system for studying the reassortment of influenza A virus strains which utilize different forms of sialic acid as a primary virus receptor.


*Keywords:* Influenza; Sialic acid; Respiratory epithelial cells; Airway epithelium; Ciliated cells; Clara cells; Goblet cell; Virus receptors; Virus tropism

## Introduction

Influenza virus infection is estimated to be responsible for approximately 36,000 deaths and 100,000 hospitalizations annually in the United States alone (Thompson et al., 2003, 2004). These numbers would increase considerably in the event of an influenza pandemic, which would result from the introduction of an influenza A virus strain encoding a hemagglutinin (HA) gene that was not present in viruses circulating recently in the human population (Barnett et al., 2005; Bartlett and Hayden, 2005; Wilson et al., 2005). Pandemic influenza A virus strains are generated through one of two mechanisms: reassortment or adaptation. Since the influenza A virus genome consists of eight distinct RNA segments (Lamb and Krug, 2001), infection of one cell with two influenza A virus strains can lead to the exchange of genetic material, a process called reassortment, resulting in a new virus strain possessing biological properties derived from either parental viruses (Horimoto and Kawaoka, 2005). The presence of influenza A virus strains bearing various combinations of the 16 HA and 9 neuraminidase (NA) serotypes in a range of animal reservoirs provides a pool of virus strains that could be the source of the next influenza pandemic (Fouchier et al., 2005; Rohm et al., 1996; WHO, 1980). The influenza pandemics of 1957 and 1968 resulted from reassortment of human and avian influenza A viruses (Webster et al., 1997). In contrast, the influenza pandemic of 1918 is believed to have been caused by an avian influenza A virus which acquired the ability to infect and efficiently spread in the human population through adaptation, not reassortment (Reid et al., 2004; Taubenberger et al., 2005; Tumpey et al., 2005).

The primary, but certainly not the only, hurdle influenza A virus must overcome when crossing a species barrier is believed

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to be utilization of host cell receptors (Ito, 2000). Influenza A virus uses sialic acid (SA) residues present at the terminus of oligosaccharide modifications of host cell glycoproteins as attachment receptors (Chu and Whittaker, 2004). SA residues are usually linked to the penultimate carbohydrate group via α2,3-, α2,6-, or α2,8-linkages, and most influenza A virus strains characterized to date utilize either α2,3- or α2,6-SA as a primary receptor (Glaser et al., 2005; Matrosovich et al., 2000). Avian influenza A virus strains preferentially bind to α2,3-SA since this form of SA is abundant in the gastrointestinal tract of various avian species (Gambaryan et al., 2002, 2004; Kim et al., 2005; Matrosovich et al., 2000). However, while the human respiratory tract contains both α2,3- and α2,6-SA (Matrosovich et al., 2004; Shinya et al., 2006; Slepushkin et al., 2001; van Riel et al., 2006; Zhang et al., 2005), most human influenza A virus strains show a binding preference for α2,6-SA as a receptor (Baum and Paulson, 1990; Gagneux et al., 2003; Matrosovich et al., 2000, 02004).

In mammals, the primary tissues infected by influenza A virus are the trachea as well as the upper and lower bronchial tubes (Zambon, 2001). In rare cases, virus infection of the alveolar epithelial cells occurs, leading to viral pneumonia. Several animal models have been used to study influenza A virus pathogenesis, most notably the laboratory mouse (Lu et al., 1999; Novak et al., 1993). However, most human influenza A virus strains must be adapted for efficient replication in mice (Brown et al., 2001; Lu et al., 1999). Human influenza A virus clinical isolates replicate and cause disease in ferrets (Govorkova et al., 2005; Herlocher et al., 2001; Maher and DeStefano, 2004; Reuman et al., 1989; Sweet et al., 2002; Zitzow et al., 2002), Syrian golden hamsters (Ali et al., 1982; Daly et al., 2003; Murphy et al., 1997; Potter and Jennings, 1976; Renis, 1977; Snyder et al., 1989; Stein-Streilein and Guffee, 1986), cotton rats (Ottolini et al., 2005) and non-human primates (Rimmelzwaan et al., 2001), but with the possible exception of ferrets, none of these animal models is used extensively.

Since Syrian golden hamsters can be infected with influenza A virus and infected hamsters can transmit virus to uninfected animals, we characterized the ability of influenza A virus strains to infect primary, differentiated trachea epithelial cell (TEC) cultures derived from Syrian golden hamsters. Our results demonstrate that Syrian golden hamsters and hamster TECs represent an attractive system for studying infection of nonciliated airway epithelial cells, investigating the reassortment of influenza A virus strains which have dissimilar receptor preferences and generating influenza A virus strains with altered or expanded receptor utilization.

## Results

### *Hamster TECs support influenza A virus replication*

The ability of influenza A viruses to replicate in well-differentiated hamster TEC cultures grown in Transwell tissue culture inserts was determined by infecting the cultures with recombinant influenza A virus strains A/WSN/33 (rWSN; H1N1) and A/Udorn/72 (rUdorn;H3N2), and the clinical isolates A/California/7/2004 (H3N2), A/Memphis/14/96 (H1N1) and A/Memphis/5/98 (H3N2). Infectious virus titers in the supernatants of virus-infected cells were determined by plaque assay at various times post-infection. Hamster TEC cultures are routinely infected between days 10 and 14 after initiation of an air liquid interface (ALI), a time when the culture is fully differentiated into a polarized, heterogeneous cell population (Rowe et al., 2004). Both recombinant strains and clinical isolates displayed similar patterns of replication, consisting of a peak in viral titer at 1 to 2 days post-infection, and then a decline to undetectable amounts by 4 to 5 days post-infection (Figs. 1A, B). Virus was detected in the apical but not the basolateral supernatants over the time course of infection (Rowe and Pekosz, 2006), and proteases produced by the cells were responsible for cleavage of the HA protein into its active subunits (Rowe et al., 2004). Thus, laboratory strains and clinical isolates of influenza A virus replicate to equivalent infectious virus titers in hamster TECs.

The cell tropism associated with influenza A virus infection was determined by immunostaining virus-infected hamster TEC cultures for expression of the influenza A virus HA protein and

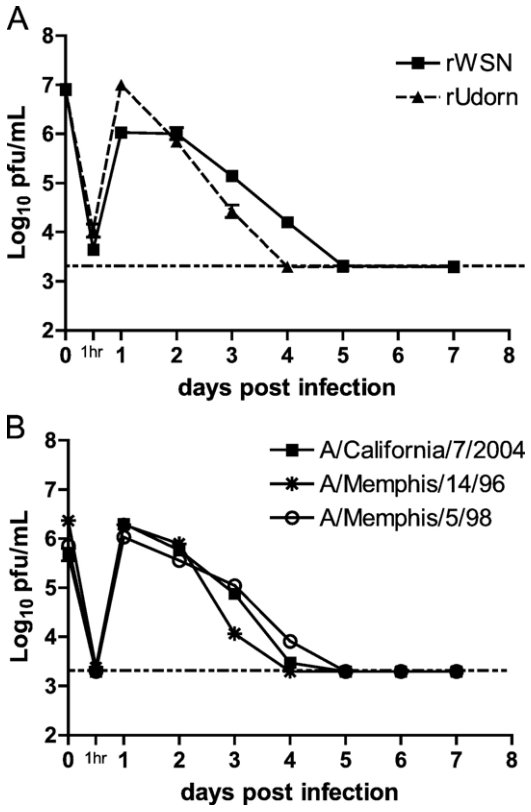


Fig. 1. Influenza A virus replicates in hamster TEC cultures. (A) Recombinant viruses rWSN and rUdorn and (B) clinical isolates A/California/7/2004, A/Memphis/14/96, and A/Memphis/5/98 were used to infect hamster TEC cultures at day 10 ALI with an MOI of 3. Infectious virus titers were determined at the indicated days. Virus titers from the apical chamber are shown as no infectious virus was detected in the basolateral chamber. The titer of the input virus is graphed at time = 0 and the 1 hr time point represents the initial inoculum that was remaining after washing. The horizontal, dashed line indicates the limit of detection, 2000 PFU/mL.