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Effective replication of human influenza viruses in mice lacking a major α2,6 sialyltransferase

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Abstract

The hemagglutinins of influenza viruses isolated from humans typically prefer binding to sialic acid in an α2,6 linkage. Presumably, the virus uses the presence of these receptors on the respiratory tract to gain entrance into the host cell. The ST6Gal I sialyltransferase knock-out mouse lacks the main enzyme necessary for the attachment of α2,6 sialic acid to N-linked glycoproteins on the cell surface. Yet even in the absence of detectable α2,6 sialic acid in the mouse respiratory tract, human influenza viruses can still infect these mice and grow to similar titers in the lung and trachea as compared to wild-type animals. This work demonstrates that the presence of a major α2,6 sialic acid on N-linked glycoproteins is not essential for human influenza virus infection in mice.

Keywords: Influenza virus; Sialyltransferase; Hemagglutinin; Receptor-binding specificity

1. Introduction

Influenza A virus (Family *Orthomyxoviridae*, genus *InfluenzavirusA*, species *Influenza A virus*) infects several hosts, including humans, birds, swine, and horses, but individual viruses are usually adapted to sustained infection in only one species. Viruses isolated from these different species bind sialic acid through their surface glycoprotein, hemagglutinin, and require this interaction for productive infection. Sialic acid (SA) is a nine-carbon sugar that terminates glycans found on glycoproteins and glycolipids. It is attached via carbon-2 to a variety of glycans in the underlying sugar chain, including galactose (Gal), *N*-acetylglucosamine (GlcNAc), *N*-acetylgalactosamine (GalNAc) or another sialic acid. It was observed that viruses isolated from human and swine sources prefer binding to sialic acid in a 2,6 linkage (SAα2,6), whereas viruses isolated from wild birds and horses bind more strongly to sialic acid in a 2,3 linkage (SAα2,3) (Connor et al., 1994; Gambaryan et al., 2005b; Rogers and D'Souza, 1989; Rogers and Paulson, 1983). This observation correlates grossly with the type of sialic acid detected on the surface of the affected organs. Human trachea predominantly contains SAα2,6, duck gut and equine respiratory tract express SAα2,3 and swine trachea contains both types of sialic acid (Baum and Paulson, 1990; Couceiro et al., 1993; Ito et al., 1998). This dichotomy in sialic acid expression has been proposed to play a role in host restriction of influenza viruses, suggesting that a virus would need to acquire changes in receptor binding specificity in order to adapt to a new host (Shinya et al., 2006; van Riel et al., 2006; Tumpey et al., 2007). This is supported by studies showing that an avian re-assortant virus that contained seven avian influenza virus genes and a human HA gene could only replicate in duck cells after mutations changed its binding preference to SAα2,3 (Naeve et al., 1984). Similar studies have also been conducted in horses and ferrets (Suzuki et al., 2000; Leigh et al., 1995).

A large number of sialyltransferases are needed for the addition of sialic acid to proteins and lipids. There are eight sialyltransferases identified in mice responsible for addition of α2,6 linked sialic acid to glycoproteins and glycolipids (Harduin-Lepers et al., 2001; Ikehara et al., 1999; Krzewinski-Recchi et al., 2003; Okajima et al., 1999; Takashima et al., 2002). Six of these sialyltransferases (ST6GalNAc I–VI) attach α2,6 sialic acid to GalNAc; ST6GalNAc I, II, and IV are involved in sialylation of glycans on O-linked proteins and ST6GalNAc III, V,

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and VI are involved in sialylation of gangliosides. Until recently, it was believed that only one enzyme, ST6Gal I, was responsible for addition of SAα2,6 to the Galβ1-4GlcNAc disaccharide found on the glycans of N-linked, and some O-linked glycoproteins (Weinstein et al., 1987). However, there is a second enzyme, ST6Gal II, which is expressed mainly in the embryo and brain that adds SAα2,6 to Galβ1-4GlcNAc on oligosaccharides, but has not been shown yet to use glycoproteins as a substrate (Takashima et al., 2003). There are orthologs of all the α2,6 sialyltransferases in humans, and those that have been cloned and characterized (ST6Gal I and II, ST6GalNAc I–IV) have similar properties in comparison to the mouse enzymes (Harduin-Lepers et al., 2001; Tsuchida et al., 2005).

Mouse genetics has allowed the study of animals missing various glycosyltransferases in an effort to better understand the physiological role of sialylation. The ST6Gal I knock-out mouse appears healthy and behaves normally (Hennet et al., 1998). However, the mouse is deficient in the Siaα2,6Galβ1-4GlcNAc trisaccharide which serves as the ligand for the B cell receptor, CD22. As a result, their B cells are not activated as efficiently in vitro and immunized knock-out mice produce reduced levels of antibodies (Hennet et al., 1998). We were interested in using the ST6Gal I knock-out mouse as a model for the importance of Siaα2,6Galβ1-4GlcNAc for human influenza virus infection.

We show here that the ST6Gal I knock-out mice expresses barely detectable amounts of Siaα2,6Gal in the respiratory tract. These mice can be lethally infected with a mouse-adapted virus and human influenza viruses can replicate to high titers in their respiratory tract.

2. Materials and methods

2.1. Viruses and cells

Influenza A/Moscow/10/99 (H3N2), A/New Caledonia/20/99 (H1N1), and A/duck/Ukraine/1/63 (H3N8) viruses were grown in the allantoic cavity of 10 day old chicken eggs (Charles River) at 37 °C. Allantoic fluid was removed 72 h post-infection and titered by serial dilution on Madin–Darby canine kidney epithelial (MDCK) cells in the presence of 1 μg/ml trypsin (Sigma). Influenza A/WSN/33 (H1N1) virus was grown in MDCK cells and titered as above. MDCK cells were maintained in Eagle minimum essential media (BioWhittacker) supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin, 2 mM L-glutamine, and 0.15% $NaHCO_3$ (MEM) and virus was grown in MEM supplemented with 0.6% bovine albumin.

2.2. Infection of mice

ST6Gal I sialyltransferase knock-out mice on a 129/Sv background back-crossed at least three generations onto a C57BL/6 background were obtained from the Consortium for Functional Glycomics (www.functionalglycomics.org, Director James Paulson) and bred via homozygous mating for experiments. C57BL/6 mice were obtained from Jackson Laboratories. Six to seven week old mice were anesthetized with an intraperitoneal injection of 100 μg of ketamine and inoculated intranasally with indicated amounts of a human influenza virus or 10^3 PFU of the mouse-adapted influenza virus, A/WSN/33 in 50 μl PBS. To obtain viral titers from mouse lung and trachea, three to five mice from each group were euthanized on the specified day post-infection and the lungs and trachea were dounce homogenized in 1 or 0.5 ml phosphate buffered saline (PBS), respectively, and titered on MDCK cells in the presence of 1 μg/ml trypsin. After infection with A/WSN/33 influenza virus, five mice from each group were monitored and euthanized after a greater than 25% weight loss.

2.3. Re-sialylation of chicken red blood cells

Chicken red blood cells (CRBCs) were re-sialylated essentially as described before (Nobusawa et al., 2000). Briefly, CRBCs (CBT Farms) were washed twice with PBS and treated with 50 mU of Vibrio cholerae neuraminidase (Roche) for 1 h at 37 °C to remove sialic acid. Absence of hemagglutination of influenza virus was used to assess removal of sialic acid. Following two PBS washes, the de-sialylated CRBCs were re-sialylated with 0.5 mU rat α2,3-(*N*)-sialyltransferase (Calbiochem) for 1 h at 37 °C or 1 mU rat α2,6-(*N*)-sialyltransferase (Calbiochem) for 2 h at 37 °C. Re-sialylation was assessed by the ability of the CRBCs to agglutinate avian or human influenza virus.

2.4. Hemadsorption assay

MDCK cells grown to confluence in a 24-well tissue culture dish were infected at a multiplicity of infection (MOI) of 5 with the indicated influenza virus and incubated at 37 °C. Seven hours post-infection cells were moved to 4 °C for 30 min to inhibit neuraminidase activity. 200 μl of cold 0.5% re-sialylated or untreated CRBCs were added to each well and incubated for

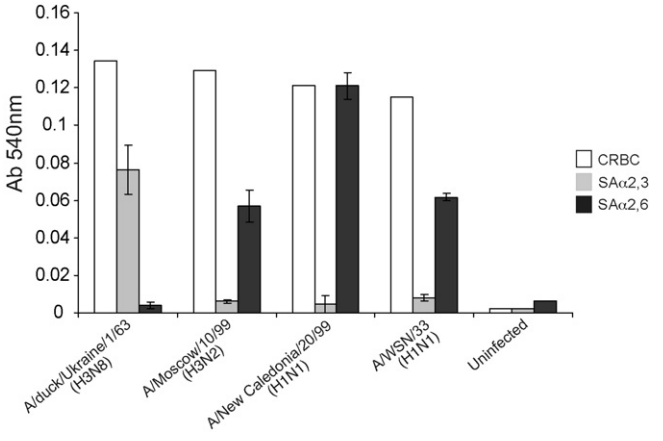

Fig. 1. Receptor binding specificity of mouse-adapted and modern human influenza viruses used in this study. Hemadsorption assay using CRBCs re-sialylated exclusively with either α2,3 linked sialic acid or α2,6 linked sialic acid. MDCK cells infected with an avian virus, A/duck/Ukraine/1/63 hemadsorb SAα2,3 re-sialylated CRBCs, better than SAα2,6 re-sialylated CRBCs. The mouse-adapted virus, A/WSN/33, and the human viruses, A/New Caledonia/20/99 and A/Moscow/10/99 hemadsorb SAα2,6 re-sialylated CRBCs more efficiently. Absorbance of hemoglobin from lysed CRBCs is measured at 540 nm. For experimental details, see Section 2.