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Characterization of the glycoproteins of bat-derived influenza viruses

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ABSTRACT

Recently found bat-derived influenza viruses (BatIVs) have hemagglutinin (HA) and neuraminidase (NA) gene segments distinct from those of previously known influenza A viruses. However, pathogenicities of these BatIVs remain unknown since infectious virus strains have not been isolated yet. To gain insight into the biological properties of BatIVs, we generated vesicular stomatitis viruses (VSVs) pseudotyped with the BatIV HA and NA. We found that VSVs pseudotyped with BatIV HAs and NAs efficiently infected particular bat cell lines but not those derived from primates, and that proteolytic cleavage with a trypsin-like protease was necessary for HA-mediated virus entry. Treatment of the susceptible bat cells with some enzymes and inhibitors revealed that BatIV HAs might recognize some cellular glycoproteins as receptors rather than the sialic acids used for the other known influenza viruses. These data provide fundamental information on the mechanisms underlying the cellular entry and host restriction of BatIVs.

Introduction

Influenza A viruses (IAVs), which belong to the family *Orthomyxoviridae*, have 8 segmented negative sense RNA genomes. IAV is one of the most important zoonotic pathogens, with high morbidity in humans, pigs, horses, and poultry. IAVs have two envelope glycoproteins, hemagglutinin (HA) and neuraminidase (NA), and are divided into subtypes based on antigenicity. IAVs of H1–16 HA and N1–9 NA subtypes have been isolated from water birds such as migratory ducks, the natural reservoir of IAVs (Fouchier et al., 2005; Kida and Yanagawa, 1979; Webster et al., 1992).

HAs are expressed as trimers on the virion surface (Wilson et al., 1981). HA is initially synthesized as an inactive precursor HA0 and subsequently cleaved into HA1 and HA2 subunits by trypsin-like proteases of host cells (Sakai et al., 2014). The proteolytic cleavage of the HA molecule is essential for IAVs to acquire infectivity (Lazarowitz et al., 1973; Wiley and Skehel, 1987). HA1 is responsible for virus binding to sialic acid receptors on the cell surface, and HA2 mediates membrane fusion under acidic conditions in endosomes, thereby delivering the viral genomic RNA into the cytoplasm of target cells (Matlin et al., 1981; Rust et al., 2004). NAs, expressed on the virion surface as tetramers, have sialidase activity that enables mature virus particles to be released from infected cells after budding (Colman, 1994; Webster et al., 1992).

Recently, IAV-like RNA genomes were detected in succession from 2 frugivorous bat species, little yellow-shouldered bats (*Sturnira lilium*) and flat-faced fruit bats (*Artibeus planirostris*) in Guatemala and Peru, respectively. The nucleotide sequences of the HA and NA of these bat-derived influenza viruses (BatIVs) were divergent from all previously known IAVs and new subtypes, H17N10 and H18N11, have been proposed (Tong et al., 2012, 2013). However, infectious viruses have not been isolated yet. Previous studies by others tried to rescue BatIVs using a reverse genetics approach, but failed to generate infectious BatIVs (Juozapaitis et al., 2014; Zhou et al., 2014). Thus, the information on the biological properties of BatIVs is mostly speculative and the possible functions of BatIV HAs and NAs are only hypothetical, based on structural analyses (Li et al., 2012; Tong et al., 2013; Zhu et al., 2012, 2013).

In this study, we utilized a vesicular stomatitis virus (VSV) pseudotype system, enabling us to directly analyze the biological functions of the BatIV glycoproteins, which presumably play important roles in the replication cycle and pathogenicity. We found some bat cell lines susceptible to VSVs pseudotyped with BatIV HAs and NAs. Our data suggest that BatIVs do not use sialic acids as a viral receptor and may have a limited host range, at least considering receptor engagement.

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Results

Generation of VSVs pseudotyped with BatIV HAs and/or NAs

To investigate cellular entry mediated by BatIV glycoproteins, VSVs pseudotyped with BatIV HAs and/or NAs (VSVΔG*-H17N10, -H18N11, -H17, -H18, -N10, and -N11) were generated as described in *Materials and Methods*. We first observed the virions of these pseudotyped VSVs using transmission electron microscopy (Fig. 1). We found that the virions of all of these pseudotyped VSVs showed characteristic morphology (i.e. a bullet-like shape) similar to parental VSVΔG*-G. It was noted that VSVs pseudotyped with BatIV HA and NA (Fig. 1A and B), HA alone (Fig. 1D and E), and NA alone (Fig. 1G and H) all had numerous spikes on their surfaces, as was the case with VSVs pseudotyped with IAV HA (H3) and NA (N2) (Fig. 1C), H3 HA alone (Fig. 1F), and N2 NA alone (Fig. 1I). Immune electron microscopy with anti-H17 HA and anti-N10 NA antibodies revealed that both BatIV HA and NA were efficiently incorporated into VSV particles (Fig. 1K and L). No difference was found in the overall morphology among these VSV virions. These data indicated that BatIV HAs and NAs were efficiently incorporated into the VSV particles.

Cell lines susceptible to VSVs pseudotyped with BatIV glycoproteins

Since previous studies have suggested that cell lines commonly used for IAV propagation are nonpermissive for BatIVs, we screened various cell lines, including bat-derived cells, for susceptibility to pseudotyped VSVs (Table 1) (Fig. 2). VSVs pseudotyped with HAs and NAs of BatIVs and well-characterized IAV strains, A/WSN/1933 (H1N1) (WSN) and A/Aichi/2/1968 (H3N2) (Aichi), were generated and treated with trypsin before use, since BatIV HAs, like WSN and Aichi HAs, have a cleavage site potentially recognized by trypsin-like proteases (Tong et al., 2012, 2013). We found that VSVΔG*-WSN, -Aichi, and -VSV G infected all cell lines tested (Fig. 2A, B, and E). On the other hand, VSVΔG*-H17N10 and -H18N11 infected bat cell lines YubFKT1, IndFSPT1, and SuBK12-08, but not the other cell lines tested, except MDCK cells, which were much less susceptible than these bat cells. Since IndFSPT1 cells showed the highest susceptibility to VSVΔG*-H17N10 and

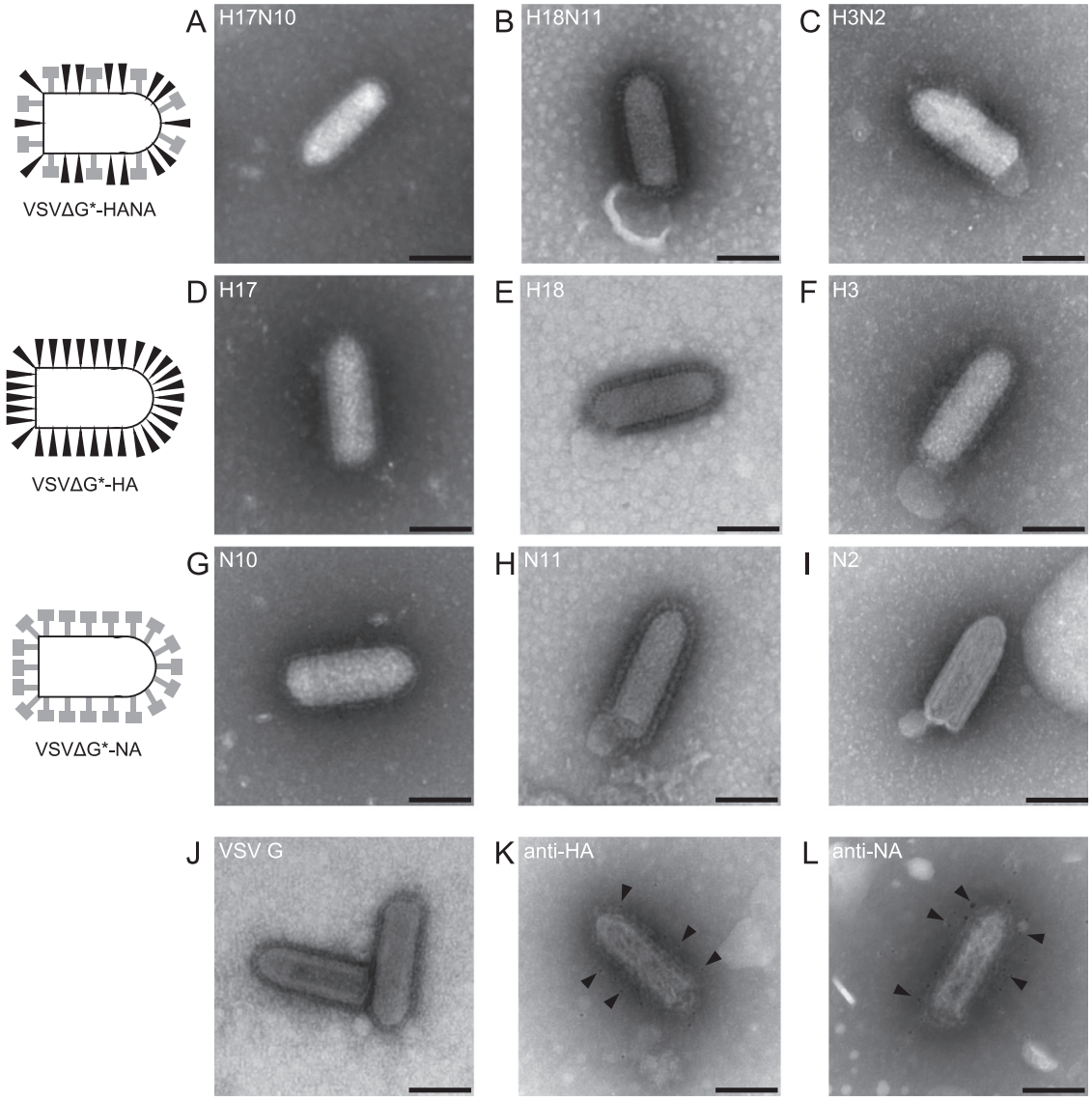

Fig. 1. Transmission electron microscopy of pseudotyped VSVs**.** VSVΔG*-H17N10 (A), -H18N11 (B), -H3N2 (C) -H17 (D), -H18 (E), -H3 (F), -N10 (G), -N11 (H), -N2 (I) and VSVΔG*-G (J) were fixed and stained as described in Materials and Methods. For immune transmission electron microscopy of VSVΔG*-H17N10, anti-HA2 monoclonal antibody (K) and anti-N10 NA mouse serum (L) were used. Scale bars represent 100 nm. Arrowheads indicate gold particles.