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Pathogenicity of swine influenza viruses possessing an avian or swine-origin PB2 polymerase gene evaluated in mouse and pig models

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ABSTRACT

PB2 627K is a determinant of influenza host range and contributes to the pathogenicity of human-, avian-, and mouse-adapted influenza viruses in the mouse model. Here we used mouse and pig models to analyze the contribution of a swine-origin and avian-origin PB2 carrying either 627K or 627E in the background of the classical swine H1N1 (A/Swine/Iowa/15/30; 1930) virus. The results showed PB2 627K is crucial for virulence in the mouse model, independent of whether PB2 is derived from an avian or swine influenza virus (SIV). In the pig model, PB2 627E decreases pathogenicity of the classical 1930 SIV when it contains the swine-origin PB2, but not when it possesses the avian-origin PB2. Our study suggests the pathogenicity of SIVs with different PB2 genes and mutation of codon 627 in mice does not correlate with the pathogenicity of the same SIVs in the natural host, the pig.

Introduction

Swine influenza caused by influenza A virus is one of the most important infectious diseases in pigs. To date, there are 16 hemagglutinin (HA) and 9 neuraminidase (NA) subtypes of influenza A virus, all of which have been isolated from aquatic birds (Alexander, 2000; Fouchier et al., 2005; Webster et al., 1992). Influenza A virus can also infect a large variety of mammalian species including humans, horses, dogs, cats, and sea mammals. Because host range is restricted, only certain subtypes of influenza A virus have been established and maintained in mammalian species; for example, only three subtypes (i.e., H1N1, H3N2, and H1N2) of influenza A viruses are consistently isolated from pigs worldwide. Pigs have been suggested to play an important role in transmission between birds and humans by acting as the "mixing vessel" for influenza viral reassortment (Ma et al., 2009; Scholtissek, 1994) because of the species' susceptibility to infection with both human and avian influenza viruses. This susceptibility may be due to the fact that porcine tracheal cells have viral receptors for mammalian and avian viruses (Ito et al., 1998).

With the recent introduction of genes from human and avian influenza viruses into swine influenza viruses (SIVs), the major viruses now circulating through North American swine herds are triple-reassortant viruses (i.e., H3N2, H1N1, and H1N2). These triple-reassortant SIVs have similar, conserved triple-reassortant internal genes, including the avian PA and PB2 polymerase genes, the human PB1 polymerase gene, and the swine nucleoprotein (NP), matrix (M), and nonstructural protein (NS) genes. The novel avian/human polymerase complex encoded by these genes can accept multiple HA and NA types and may endow a selective advantage to SIVs. The recently characterized novel swine H2N3 and swine human-like H1 viruses are examples of viruses that demonstrate the flexibility of the avian/human polymerase complex to accept various HAs and NAs (Ma et al., 2007; Vincent et al., 2009). The 2009 pandemic H1N1 virus is a reassortant virus that contains NA and M genes from Eurasian SIVs and the other 6 genes from North American triple-reassortant SIVs (Smith et al., 2009). The common feature of the pandemic H1N1 and North American triple-reassortant viruses is that these viruses have a novel polymerase complex (i.e., avian PA and PB2 and human PB1).

The polymerase complex is responsible for transcription and replication of the viral genome and is a major determinant of host range and pathogenicity (Naffakh et al., 2008; Neumann and Kawaoka, 2006). A single residue at position 627 in the PB2 protein is largely responsible for determining host range and pathogenicity of influenza viruses (Hatta et al., 2001; Subbarao et al., 1993). PB2 that is derived from human influenza viruses usually possesses a lysine (K) at position 627 whereas that derived from avian influenza viruses predominantly

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possesses a glutamic acid (E) at this position (Chen et al., 2006). In North American SIVs, almost all pre-1998 isolates are classical H1N1 viruses containing PB2 627K whereas most post-1998 isolates are triple-reassortant H1N1 or H3N2 viruses containing PB2 627E (Bussey et al., 2010). The pandemic H1N1 virus contains E at position 627 of its PB2 protein (Garten et al., 2009). It is unclear how the avian PB2 with 627E in SIVs affects viral replication, pathogenicity, and interspecies transmission. In this study, a reassortant virus (i.e., 1930-Tx/98PB2-627E) was generated by reverse genetics in the genetic background of the classical H1N1 A/Swine/Iowa/15/30 (1930) virus, which carries the PB2 gene from an H3N2 triple-reassortant A/Swine/Texas/4199-2/98 (Tx/98) virus. The PB2 gene of the Tx/98 virus is avian in origin (Zhou et al., 1999). In addition, reassortant viruses (1930-Tx/98PB2-627K, 1930-PB2-627E) with a single amino acid (aa) substitution at position 627 in the avian-origin or swine-origin PB2 were generated. The growth properties and pathogenicity profiles of reassortant viruses carrying avian-origin or swine-origin PB2 with 627 E or K were evaluated *in vitro* and *in vivo*.

Results

Growth Characteristics of the Wild-type and Reassortant Viruses

The avian-origin Tx/98 PB2 shows 96.8% homology at the nucleotide level and 95.5% homology at the aa level with the swine-origin 1930 virus PB2. There are 34 aa difference between the avian-origin Tx/98 and swine-origin 1930 PB2 proteins (Table 1). The reassortant 1930-Tx/98PB2-627E and 1930-Tx/98PB2-627K viruses formed similar size plaques as the wild-type 1930-PB2-627K virus did; however, the 1930-PB2-627E virus with the single substitution at position 627 formed smaller plaques than the other three viruses did (Fig. 1a). In MDCK cells, the virus carrying the avian-origin PB2 gene with 627K (1930-Tx/98PB2-627K) grew to significantly higher titers ($P<0.05$) than did the respective virus with PB2 627E (Fig. 1b). Similarly, the virus containing the swine-origin PB2 gene with 627K (1930-PB2-627K) grew to significantly higher titer ($P<0.05$) than did the respective virus with the swine-origin PB2 627E except for the time point 36 h post-inoculation (Fig. 1b).

Mouse Pathogenicity of Reassortant SIVs with PB2 Mutations

Mice inoculated with 1930-Tx/98PB2-627K or the wild-type 1930-PB2-627K virus developed clinical disease (i.e., weight loss, dyspnea, rough fur, and lethargy); this disease resulted in death of most of the inoculated mice (Table 2). Mice inoculated with the 1930-Tx/98PB2-627E virus containing the avian-origin PB2 with 627E developed similar clinical illnesses but without mortality. In contrast, mice inoculated with the 1930-PB2-627E virus did not show any clinical signs and appeared similar to the mock-inoculated mice. Mice in either the 1930-Tx/98PB2-627E group or the 1930-Tx/98PB2-627K groups who survived the infections did not fully recover from them by the end of the experiment. Compared to mock-inoculated controls, four inoculated groups had significantly greater ($P<0.05$) microscopic lung lesions (Table 2). The 1930-PB2-627K, 1930-Tx/98PB2-627K and 1930-Tx/98PB2-627E caused significantly greater microscopic lung lesions ($P<0.05$) than did the 1930-PB2-627E (Table 2). No significant difference in microscopic lung lesion was evident between the 1930-Tx/98PB2-627K-inoculated mice and the 1930-Tx/98PB2-627E-inoculated mice (Table 2). Although viral RNA was detected in the lung homogenate from each infected mouse, virus was only isolated from the lungs of mice inoculated with the wild-type 1930-PB2-627K (mean virus titer $= 10^{3.97}$ $TCID_{50}$) and from the lungs of 9 mice inoculated with 1930-Tx/98PB2-627K (mean virus titer $= 10^{2.83}$ $TCID_{50}$). The viral RNA

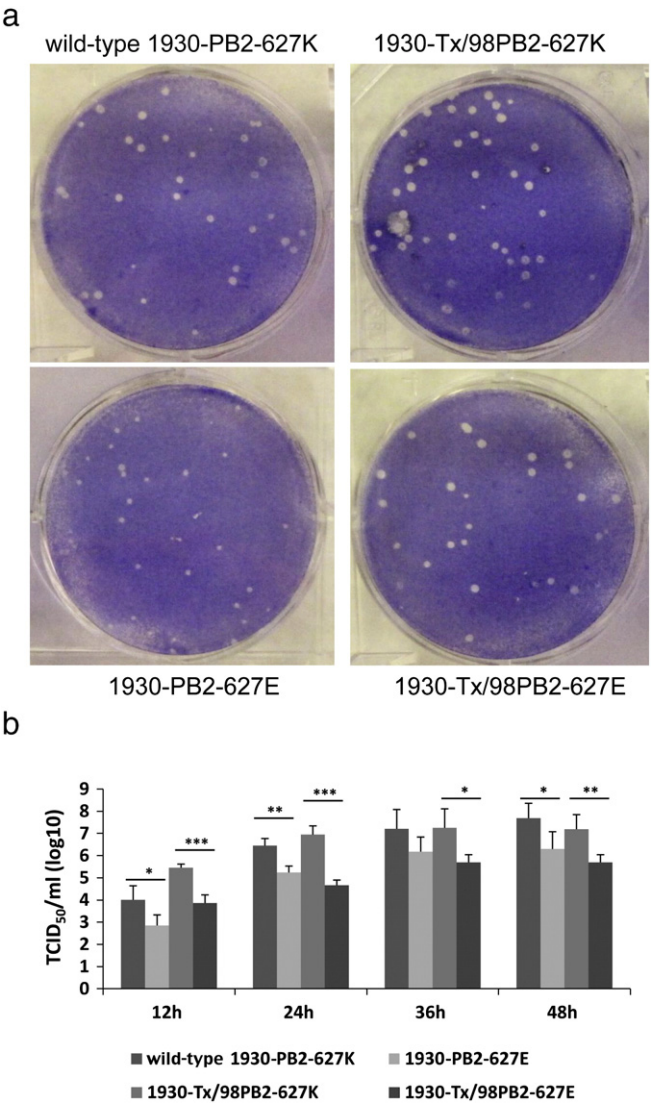

Fig. 1. Characterization of reassortant and PB2 single-substitution viruses *in vitro*. (a) Plaque size in MDCK cells 2 days after infection; (b) virus growth in MDCK cells at different time points. The results are representative of four independent experiments and values represent the $\log_{10}$ geometric mean $TCID_{50}$/ml ± SEM (*$P<0.05$; **$P<0.01$; ***$P<0.001$).

Table 1
Comparison of the avian-origin Tx/98 and swine-origin 1930 PB2 at the amino acid level.

	22[a]	64	65	66	82	114	122	136	147	184	199	225	243	265	271	312	382
Tx/98 PB2	K	M	D	M	N	V	V	R	T	T	A	G	M	N	A	E	I
1930 PB2	R	I	E	T	S	I	A	G	I	M	S	S	L	S	T	K	V
	421	467	468	475	478	480	539	588	590	591	627	637	645	660	661	684	702
Tx/98 PB2	V	M	I	L	I	V	I	T	S	R	E	T	L	K	A	A	K
1930 PB2	I	L	T	M	V	I	V	A	G	Q	K	A	M	R	T	T	R

[a] Position of the amino acid.