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Pathogenicity testing of influenza candidate vaccine viruses in the ferret model

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ABSTRACT

The development of influenza candidate vaccine viruses (CVVs) for pre-pandemic vaccine production represents a critical step in pandemic preparedness. The multiple subtypes and clades of avian or swine origin influenza viruses circulating world-wide at any one time necessitates the continuous generation of CVVs to provide an advanced starting point should a novel zoonotic virus cross the species barrier and cause a pandemic. Furthermore, the evolution and diversity of novel influenza viruses that cause zoonotic infections requires ongoing monitoring and surveillance, and, when a lack of antigenic match between circulating viruses and available CVVs is identified, the production of new CVVs. Pandemic guidelines developed by the WHO Global Influenza Program govern the design and preparation of reverse genetics-derived CVVs, which must undergo numerous safety and quality tests prior to human use. Confirmation of reassortant CVV attenuation of virulence in ferrets relative to wild-type virus represents one of these critical steps, yet there is a paucity of information available regarding the relative degree of attenuation achieved by WHO-recommended CVVs developed against novel viruses with pandemic potential. To better understand the degree of CVV attenuation in the ferret model, we examined the relative virulence of six A/Puerto Rico/8/1934-based CVVs encompassing five different influenza A subtypes (H2N3, H5N1, H5N2, H5N8, and H7N9) compared with the respective wild-type virus in ferrets. Despite varied virulence of wild-type viruses in the ferret, all CVVs examined showed reductions in morbidity and viral shedding in upper respiratory tract tissues. Furthermore, unlike the wild-type counterparts, none of the CVVs spread to extrapulmonary tissues during the acute phase of infection. While the magnitude of virus attenuation varied between virus subtypes, collectively we show the reliable and reproducible attenuation of CVVs that have the A/Puerto Rico/9/1934 backbone in a mammalian model.

1. Introduction

Influenza viruses cause seasonal epidemics and occasional pandemics in humans, with the severity of disease ranging from mild respiratory illness to acute respiratory disease and death. The continued circulation of influenza viruses in animal hosts represents a constant threat to human health should a virus jump the species barrier and cause human infection (Swayne, 2016); detection of novel influenza viruses in humans of avian and swine origin in recent years underscores that possibility (Uyeki et al., 2017). Vaccines represent the most effective public health tool to protect against influenza virus infection, but the prolonged lead time in vaccine virus development and manufacture make them difficult to employ in the early stages of a pandemic. In response, the WHO supports the generation of pre-pandemic candidate vaccine viruses (CVVs); CVVs designated by the WHO currently encompass seven virus subtypes.

The ferret is the preferred mammalian model for the study of influenza virus pathogenesis and transmission, due to similarities in lung physiology, distribution of influenza virus receptors throughout the respiratory tract, and numerous shared clinical signs and symptoms of influenza virus infection (Belser et al., 2011). As such, pathogenicity and transmissibility data obtained from ferrets is included in the Influenza Risk Assessment Tool (IRAT), a multi-attribute model that supports decision-making efforts regarding the selection of appropriate candidate vaccine viruses (Cox et al., 2014). The WHO recommends the demonstration of attenuation of CVVs in ferrets prior to distribution to vaccine manufacturers (WHO, 2005b). While there have been several isolated studies showing CVV attenuation in ferrets

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(Dong et al., 2009; Robertson et al., 2011; Webby et al., 2004), no comprehensive analysis of the relative degree of attenuation among numerous strains/subtypes of influenza viruses with pandemic potential has been reported. A better understanding of strain-specific variation compared with parameters uniformly present among all safety-tested viruses is critical when evaluating safety profiles of CVVs against influenza viruses with pandemic potential. This is of particular importance as demonstration that CVVs possess reduced pathogenicity is needed to confirm the virus can be safely handled at the biosafety levels present during large-scale manufacturing of influenza vaccines. Similar to the current influenza vaccine production methods, the manufacturing process for CVVs includes virus concentration, inactivation, and purification procedures.

Here, we examined the mammalian virulence of six recently isolated wild-type influenza viruses from avian and swine reservoirs which have either been associated with human infection or are considered to pose a threat to human health, and determined the safety profile of each paired CVV using multiple parameters of the ferret model. Among ferrets inoculated with CVVs, we found strain-specific impacts of the HA and/or NA on morbidity and virus replication in the upper respiratory tract compared with the relevant wild-type virus, but high uniformity in the ablation of virus spread beyond the respiratory tract among all viruses analyzed. This study underscores the importance of aggregating safety-related in vivo data to demonstrate both the reliability and reproducibility of the A/Puerto Rico/8/1934 (PR/8) based CVV safety profile in mammals and as a resource when new potentially pandemic viruses emerge.

2. Methods

2.1. Viruses

Influenza A viruses included in the analyses in this study are listed in Table 1. All viruses were propagated in the allantoic cavity of 10–11 day old embryonated chicken eggs at 35–37 °C for 24–48 h as described previously (Maines et al., 2009, 2005). Pooled allantoic fluid was clarified by centrifugation and aliquots were stored at −80 °C until use. Stock titers were titered for 50% egg infectious dose (EID_{50}) or plaque forming units (PFU) in MDCK cells as described previously (Reed and Muench, 1938; Zeng et al., 2007). All experiments with avian-origin viruses were conducted under biosafety level 2 or 3 containment, including enhancements required by the U.S. Department of Agriculture and the Division of Select Agents and Toxins/CDC (Chosewood et al., 2009).

Reassortant vaccine candidate viruses (GLP candidate vaccine viruses) were generated by transfecting reverse genetics plasmids encoding the HA and NA surface gene segments of the virus of interest along with reverse genetics constructs encoding the PB2, PB1, PA, NP, M, and NS gene segments of PR/8 influenza virus (plasmids described in Ridenour et al. (2015a)) into certified Vero cells (O'Neill and Donis, 2009). HA and NA Genbank accession numbers: EU258939, EU258937 (RG27); JN401974, CY062485 (RG29); CY103897, CY098760 (RG30); KF021597.1, KF021599.1 (RG32A); KP307984.1, KP307986.1 (RG43A). In some cases, HA and NA genes may be synthetically produced based on genetic sequence information (Dormitzer, 2015). For hemagglutinin gene segments of highly pathogenic avian H5N1 influenza viruses, constructs were generated that delete the region coding for polybasic amino acids juxtaposed to the HA1/HA2 protease cleavage site to create a mono-basic amino acid cleavage site characteristic of low pathogenic viruses (Dong et al., 2009; O'Neill and Donis, 2009; Subbarao et al., 2003; World Health Organization Global Influenza Program Surveillance, 2005). Automated sequencing was performed using an Applied Biosystems 3130 genetic analyzer to confirm both a matching genetic sequence with the parental viruses, and to verify the lack of a polybasic sequence at the HA cleavage site. Sterility testing of each CVV for bacterial (aerobic, anaerobic) and fungal contaminates was performed (WHO, 2005b).

2.2. Ferret experiments

Male Fitch ferrets (Triple F Farms, Sayre, PA), 6–12 months of age and serologically negative by hemagglutination inhibition assay to currently circulating influenza viruses, were used in this study. Animal research was conducted under the guidance of the Centers for Disease Control and Prevention's Institutional Animal Care and Use Committee in an Association for Assessment and Accreditation of Laboratory Animal Care International-accredited animal facility.

Table 1
Wild-type and CVVs used in this study.

Virus	Abbreviation	Subtype	Virulence in ferrets		Reference
			Wt loss[a]	Temp[a]	
A/swine/Missouri/2124514/2006	sw/MO/06	H2N3	13.7	1.7	(Pappas et al., 2015)
A/swine/Missouri/2124514/2006 (H2N3)-PR8-IDCDC-RG27	RG27	H2N3	5.9	1.3	This study
A/Egypt/N03072/2010	Egypt/10	H5N1	9.1	1.8	(Pearce et al., 2016)
A/Egypt/N03072/2010(H5N1)-PR8-IDCDC-RG29	RG29	H5N1	1.2	1.0	This study
A/duck/Vietnam/NCVD-1206/2012	dk/VN/12	H5N1	6.5	1.9	(Pearce et al., 2016)
A/Hubei/1/2010(H5N1)-PR8-IDCDC-RG30	RG30	H5N1	3.8	0.8	This study
A/Anhui/1/2013	Anhui/13	H7N9	11.0	1.5	(Belser et al., 2013)
A/Shanghai/2/2013 (H7N9)-PR8-IDCDC-RG32A	RG32A	H7N9	1.5	1.5	(Ridenour et al., 2015a)
A/gyrfalcon/Washington/41088-6/2014	gyr/WA/14	H5N8	3.5	1.3	(Pulit-Penaloza et al., 2015)
A/gyrfalcon/Washington/41088-6/2014(H5N8)-PR8-IDCDC-RG43A	RG43A	H5N8	1.2	0.2	This study
A/northern pintail/Washington/40964/14	np/WA/14	H5N2	0.5	1.4	(Pulit-Penaloza et al., 2015)
IDCDC-RG47B (A/gyrfalcon/Washington /41088-6/2014 (H5N8)-PR8-IDCDC-RG43A-like HA, A/turkey/Minnesota/15-014110-1/2015 NA) × PR8	RG47B	H5N2	0.3	1.2	This study

[a] Mean maximum weight loss (expressed as percentage) and rise in pre-inoculation temperature (reported as °C above baseline) collected during the first 9 days p.i.