Immune efficacy of an adenoviral vector-based swine influenza vaccine against antigenically distinct H1N1 strains in mice

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ABSTRACT

Avian-like H1N1 swine influenza viruses are prevalent in pigs and have occasionally crossed the species barrier and infected humans, which highlights the importance of preventing swine influenza. Human adenovirus serotype 5 (Ad5) has been tested in human influenza vaccine clinical trials and has exhibited a reliable safety profile. Here, we generated a replication-defective, recombinant adenovirus (designated as rAd5-avH1HA) expressing the hemagglutinin gene of an avian-like H1N1 virus (A/swine/Zhejiang/199/2013, ZJ/199/13). Using a BALB/c mouse model, we showed that a two-dose intramuscular administration of recombinant rAd5-avH1HA induced high levels of hemagglutination inhibition antibodies and prevented homologous and heterologous H1N1 virus-induced weight loss, as well as viral replication in the nasal turbinates and lungs of mice. Furthermore, a prime–boost immunization strategy trial with a recombinant plasmid (designated as pCAGGS-HA) followed by rAd5-avH1HA vaccine provided effective protection against homologous and heterologous H1N1 virus infection in mice. These results indicate that rAd5-avH1HA is an efficacious genetically engineered vaccine candidate against H1N1 swine influenza. Future studies should examine its immune efficacy in pigs.

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1. Introduction

Influenza A viruses are classified into 18 subtypes based on the hemagglutinin (HA) protein and 11 subtypes based on the neuraminidase protein (Tong et al., 2013). Eurasian avian-like H1N1 (EA H1N1) swine influenza virus (SIV) was first reported in Europe in the late 1970s, and then it spread to many European and Asian countries (Pensaert et al., 1981; Vincent et al., 2014). Sporadic infections of an EA H1N1 SIV in humans have also been reported in some European countries (de Jong et al., 1988; Myers et al., 2007; Rovida et al., 2017). In China, EA H1N1 viruses have become predominant in the epidemiology of influenza in swine (Yang et al., 2016) and have caused human infections in 2011 and 2013 as well (Qi et al., 2013; Wang et al., 2013), which highlights their potential threat to human health. Since 2009, genetic reassortment among the pandemic 2009/H1N1 virus, as well as EA H1N1 and

other swine H1N1, H1N2, and H3N2 viruses, have been repeatedly detected in pigs (Ducatez et al., 2011; Liang et al., 2014; Qiao et al., 2014; Watson et al., 2015) and occasionally in humans (Epperson et al., 2013; Zhu et al., 2016), which further accelerates the need to develop effective vaccines against SIVs.

Conventional whole-virus inactivated vaccines produced with H1N1 or H3N2 SIVs have been used in North America and Europe for many years (De Vleeschauwer et al., 2015; Kitikoon et al., 2013; Vincent et al., 2010). Other approaches to generate vaccines to supplement current egg-based production systems, have also been developed, which include recombinant subunit vaccines using a baculovirus (Hernandez et al., 2016), plasmid DNA vaccines (Gorres et al., 2011), virus-like particle vaccines (Pyo et al., 2012), alphavirus replicon vaccines (Erdman et al., 2010; Vander Veen et al., 2012), and replication-incompetent adenovirus vectors (Braucher et al., 2012). An adenoviral vector can mimic natural infections and induce long-term, cross-protective immunity toward influenza viruses. Many studies have evaluated the protective effects of adenovirus-vectored influenza vaccines against various influenza subtypes (Webby and Weaver, 2015), and some have reported the results of phase I clinical trials (Gurwith et al., 2013; Liebowitz et al.,

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2015). The HA protein plays critical roles in the early stages of virus infection by binding to viral receptors and mediating membrane fusion between viruses and cells (Skehel and Wiley, 2000). Therefore, the HA protein is an attractive target for influenza vaccine development.

In the present study, we generated a replication-defective recombinant adenovirus expressing the HA gene of an EA H1N1 SIV. By two different prime–boost strategies with the adenovirus-based vaccine alone or by combination with the plasmid pCAGGS-HA encoding the HA gene, we evaluated the immunogenicities and protective efficacies of these recombinants against homologous and heterologous H1N1 virus infections in BALB/c mice.

2. Materials and methods

2.1. Viruses, plasmids, and cells

Three SIVs were included in this study: the EA H1N1 virus A/swine/Zhejiang/199/2013 (ZJ/199/13), which was deposited in the GenBank database under the accession numbers MF001012 to MF001019, as well as the previously characterized triple reassortant H1N1 virus A/swine/Guangdong/306/2013 (GD/306/13) and the pandemic 2009/H1N1 virus A/swine/Heilongjiang/44/2009 (HLJ/44/09) (Chen et al., 2013; Yang et al., 2016). The genetic and antigenic analysis results of these viruses are summarized in Table 1. These viruses were propagated in the allantoic cavities of 10-day-old specific pathogen free embryonated chicken eggs and titrated to determine the 50% egg infective dose (EID_{50}) by the method of Reed and Muench (1938).

The adenovirus shuttle plasmid pDC315 and the backbone plasmid pBHGloxΔE1, E3Cre were purchased from Microbix Biosystems (Mississauga, ON, Canada). Plasmid pIRES2-EGFP and the eukaryotic expression vector pCAGGS under the control of the chicken β-actin promoter were kindly provided by Professor Zhigao Bu of the Harbin Veterinary Research Institute (HVRI). A recombinant plasmid, designated as pCAGGS-HA, was constructed by inserting the HA gene of ZJ/199/13 into the expression plasmid pCAGGS as reported previously (Jiang et al., 2007). The HA gene was polymerase chain reaction (PCR)-amplified using the primers P1 (5′–GCAATCGATATGGAAACAAAACTATTTGTATTA–3′) and P2 (5′–GCAAGATCTTTAAATGCATACTCTGCATTGC–3′). Artificial ClaI and BglIIrestriction sites (underlined) allowed the direct cloning of the amplification product into plasmid pCAGGS.

Human embryonic kidney 293 (HEK293) cells were cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum.

2.2. Generation of recombinant adenovirus rAd5-avH1HA

First, the enhanced green fluorescent protein (EGFP) gene fused to an internal ribosome entry site sequence (IRES) was amplified by PCR using plasmid pIRES2-EGFP as the template with the primers P3 (5′–AATTCAAGCTGCTAGCAAGGATCCGCCCCTCTCCCTCCC–3′)

and P4 (5′–ATAAACAAGTTGCTCGAAGTCGACTTACTTGTACAGCTCG TCCATG–3′).

Artificial BamHI and SalI restriction sites (underlined) permitted direct cloning of the amplification product into the adenovirus shuttle plasmid pDC315, and the resulting plasmid was designated as pDC315-EGFP. Next, the H1 HA gene of SW/ZJ/199 was PCR-amplified using the primers P5 (5′–CACCGTAGAACGCAGATC GAATTCATGGAAACAAAACTAT–3′) and P6 (5′–TCGACAAGCTG GATCCTTGCTAGCTTAAATGCATACTCTGC–3′). Artificial EcoRI and NheI restriction sites (underlined) allowed the direct cloning of the amplification product into pDC315-EGFP. Finally, the resultant plasmid was designated as pDC315-H1HA-EGFP.

HEK293 cells were co-transfected with pDC315-H1HA-EGFP and the adenovirus backbone plasmid pBHGloxΔE1, E3Cre at a ratio of 6:1 using Lipofectamine™ 2000 reagent (Invitrogen, Carlsbad, CA, USA). The adenovirus vector pFG140 was used as a control in the transfection experiments. The cells were examined under a fluorescence microscope 12 h after transfection. When the fluorescence intensity increased in the infected cells and typical cytopathic effects appeared, the cells were harvested and the recombinant adenovirus was further screened by empty plaque screening.

To detect the HA gene in the recombinant virus, total DNA was extracted from recombinant virus-infected HEK293 cells with the Wizard SV Genomic DNA Purification System (Promega, Madison, WI, USA) and then subjected to PCR detection using the primer pair P5/P6 as described previously. Cell lysates of recombinant virus-infected HEK293 cells were tested for HA expression in western blot analysis using mouse antiserum against the ZJ/199/13 virus.

The recombinant adenovirus was purified and concentrated using the ViraTrap™ Adenovirus Purification Maxiprep Kit (Biomiga, San Diego, CA, USA). The 50% tissue culture infective dose ($TCID_{50}$) was determined by titrating the purified virus on HEK293 cells using the Quick™ Adenovirus Titer ELISA Kit (Cell Biolabs, San Diego, CA, USA).

2.3. Immunization and challenge of mice

Six-week-old female BALB/c mice ($n = 96$) were purchased from Vital River Laboratories (Beijing, China) and divided randomly into four groups of 24. Mice were intramuscularly prime immunized with 10^8 $TCID_{50}$ of rAd5-avH1HA, 100 μg of plasmid pCAGGS-HA, 10^8 $TCID_{50}$ of rAd5-EGFP, and 50 μL of phosphate-buffered saline (PBS), respectively. Three weeks later, the mice received an intramuscular inoculation boost with 10^8 $TCID_{50}$ of rAd5-avH1HA, 10^8 $TCID_{50}$ of rAd5-avH1HA, 10^8 $TCID_{50}$ of rAd5-EGFP, and 50 μL of PBS, respectively. Two weeks after the second immunization, the mice in each group were divided randomly into three subgroups (eight per subgroup), which were subsequently challenged with 10^6 EID_{50} of SW/ZJ/199, SW/GD/306, or SW/HLJ/44, respectively. All mice were monitored daily by clinical examination and body weights were measured each day for 2 weeks after challenge. Four days post-challenge (4 dpc), three mice from each subgroup were euthanized and tissues, including the nasal turbinates, lungs,

Table 1
Genetic and antigenic analysis results of the viruses.

Viruses	Amino acid sequence identity (%)[a]	HI antibodies of chicken antiserum against[b]			
		ZJ/199/13	GD/306/13	HLJ/44/09	GX/18/11
ZJ/199/13	100	<u>1024</u>	64	128	512
GD/306/13	93.8	256	<u>512</u>	32	32
HLJ/44/09	77.8	64	16	<u>1024</u>	16
GX/18/11	98.8	1024	64	128	<u>512</u>

[a] The amino acid sequence identity (%) between the HA gene of each virus with that expressed by the rAd5-avH1HA virus.
[b] Antiserum was generated by inoculating specific pathogen-free chickens with an inactivated vaccine derived from the indicated viruses. Homologous titers are underlined.