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Construction and comparison of different source neuraminidase candidate vaccine strains for human infection with Eurasian avian-like influenza H1N1 virus

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Abstract

Human infections with Eurasian avian-like swine influenza H1N1 viruses have been reported in China in past years. One case resulted in death and others were mild case. In 2016, the World Health Organization recommended the use of A/Hunan/42443/2015(H1N1) virus to construct the first candidate vaccine strain for Eurasian avian-like swine influenza H1N1 viruses. Previous reports showed that the neuraminidase of A/Puerto Rico/8/34(H1N1) might improve the viral yield of reassortant viruses. Therefore, we constructed two reassortant candidate vaccine viruses of A/Hunan/42443/2015(H1N1) by reverse genetic technology, with (6+2) and (7+1) gene constitution, respectively. The (6+2) virus had hemagglutinin and neuraminidase from A/Hunan/42443/2015, and the (7+1) one had hemagglutinin from A/Hunan/42443/2015, while all the other genes were from A/Puerto Rico/8/34. Our data revealed that although the neuraminidase of the (7+1) virus was from high yield A/Puerto Rico/8/34, the hemagglutination titer and the hemagglutinin protein content of the (7+1) virus was not higher than that of the (6+2) virus. Both of the (7+1) and (6+2) viruses reached a similar level to that of A/Puerto Rico/8/34 at the usual harvest time *in vitro*. Therefore, both reassortant viruses are potential candidate vaccine viruses, which could contribute to pandemic preparedness.

Keywords: Influenza virus; A (H1N1); Eurasian avian-like influenza; Reverse genetic; Vaccine

1. Introduction

Influenza viruses circulate in animal and human hosts. Sometimes animal influenza viruses such as avian H5N1, avian H7N9 and swine H1N1 spread across species to infect humans [1−3]. Swine H1N1 includes two lineages, classical H1N1 and Eurasian avian-like (EA) H1N1. Human infections with avian-like swine H1N1 have been sporadically reported worldwide, but increasing numbers of infections have been reported in China since 2013 [4−7]. One case resulted in death and others were mild case [4−7]. At present, the most effective measure against influenza virus infection is vaccination. The human influenza vaccination coverage in China is less than 2% [8] and the seasonal trivalent inactivated influenza

Abbreviations: EA, Eurasian avian-like; CVV, candidate vaccine virus; HN42443, A/Hunan/42443/2015; HA, hemagglutinin; NA, neuraminidase; PR8, A/Puerto Rico/8/34; MDCK, Madin−Darby canine kidney; $TCID_{50}$, 50% tissue culture infective dose; p.i., post-infection; HI, hemagglutination inhibition; K_m, Michaelis−Menten constant; V_{max}, maximal velocity.

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vaccine did not substantially improve the level of cross-reactive antibodies to EAH1N1 [6]. Due to the lack of an existing candidate vaccine virus (CVV) representing these EA lineage viruses, the World Health Organization recommended the use of A/Hunan/42443/2015 (HN42443) virus as an influenza A(H1N1) variants vaccine virus for pandemic preparedness in 2016 [7,9].

Reverse genetics technologies are often used in the development of high-yield candidate vaccine viruses. Candidate (6+2) reassortant viruses with hemagglutinin (HA) and neuraminidase (NA) derived from the wild virus in high-yield A/Puerto Rico/8/34 (PR8) virus backbone were obtained by reverse genetics technologies, which is the main method for vaccine candidate composition. However, previous reports showed that the NA of PR8 or wild NA chimeras containing PR8 NA sequences might improve the viral yield of reassortant viruses [10–13]. If the antigenicity of reassortant viruses is maintained with good replication in culture medium, (7+1) reassortment viruses might be alternative vaccine candidates [11].

In this study, we used the EA virus HN42443 [7], which the World Health Organization recommended as an A(H1) variants CVV in 2016 [9], to construct (6+2) and (7+1) reassortant virus as CVVs. The (6+2) virus was created in which the HA and NA segments were derived from the HN42443 with the remaining six segments from PR8. The (7+1) reassortant virus had HA from the HN42443 virus and the rest of the segments were from PR8. We constructed two reassortant viruses, (6+2) and (7+1), and compared the characteristics of them *in vitro* with PR8 as a control virus. We hope that both might contribute to pandemic preparedness in the future.

2. Materials and methods

2.1. Plasmids, viruses and cells

Plasmids containing the eight segments from PR8 (pHW2000-PB2, pHW2000-PB1, pHW2000-PA, pHW2000-HA, pHW2000-NP, pHW2000-NA, pHW2000-M, and pHW2000-NS) were used. The HA and NA genes of HN42443 were synthesized by GenScript (Nanjing, China) and cloned into a pHW2000 vector. They were named pHW2000-HN42443HA and pHW2000-HN42443NA, respectively. PR8, (6+2) and (7+1) reassortant influenza viruses were generated by a reverse genetics approach. To obtain the above viruses, we co-transfected plasmids containing HA and NA or only the HA gene from HN42443 as well as plasmids containing the rest of the genes from PR8 into Human Embryonic Kidney 293T and Madin–Darby Canine Kidney (MDCK) co-cultured cells. MDCK cells and 293T cells were grown in Dulbecco's modified Eagle's medium with 10% fetal bovine serum. One day post co-transfection, the culture media was changed to Opti-Minimal Essential Medium containing 1 μg/ml TPCK-treated trypsin. All transfections were performed in 6-well plates using TurboFect Transfection Reagent (ThermoFisher) according to the manufacturer's instructions. Three days after transfection, the supernatant in the co-culture was inoculated into 9-day old specific pathogen free eggs for virus propagation. Then, allantoic fluids with positive HA titers were collected at 72 h post-infection (p.i.) and stored at −80 °C. We sequenced viruses with our laboratory-sequencing platform. Briefly, viral RNA extracted from allantoic fluid was amplified using MBTUni12/13 primers as previously described [14]. DNA libraries were prepared using Nextera XT DNA Library Prep kits (Illumina) and a MiSeq v2kit (Illumina) was used to produce 2150 paired-end reads. Sequence reads were analyzed using CLC Genomics Workbench software (version 7.5.1). Finally, we used BioEdit (Version 7.1.3.0) to analyze the gene source of viruses.

2.2. Antigenic analysis of reassortant viruses by hemagglutination inhibition (HI) assay

HI assay was performed as described previously [15] to determine reassortant viral antigenicity. Specific antiserum against wild-type HN42443 virus and 1% Turkey red cells were used in the HI assay.

2.3. Growth properties in vitro

The 50% tissue culture infective dose ($TCID_{50}$) was determined in MDCK cells incubated with semi-log serially diluted viruses at 37 °C for 72 h. The $TCID_{50}$ was calculated by the Reed–Muench method [16]. MDCK cell monolayers were infected at a multiplicity of infection of 0.001 and then incubated for 1 h at 37 °C and 5% CO_2. Then the inoculum was replaced with DMEM lacking serum but containing 1 μg/ml of TPCK-treated trypsin. Supernatant fluids were collected at each time point (24, 48, 72, and 96 h p.i.) and stored at −80 °C until processed. The quantity of virus at each time point was determined by hemagglutination assays.

2.4. Virus purification and SDS-PAGE analysis

Viruses were purified by density gradient ultracentrifugation and the total protein content of purified viruses protein was determined by Nano Photometer NP60 (Software Version 1.1.11147, Germany). Purified virus proteins were adjusted to the same concentration and approximately 1 μl of a 1/50 dilution of PNGase F enzyme was added into the sample as described previously [17]. The proteins were separated on a 12% SDS-PAGE precast gel (Mini-proteaN TGX, Bio-Rad) and gels were stained with Coomassie brilliant blue. The proportion of HA protein band was determined as previous reported using Image lab software 3.0 [12,18].

2.5. Neuraminidase enzyme kinetics assay

The characterization of reassortant viruses of NAs was determined using a fluorometric assay as previously described [19], using 2′-(4-methylumbelliferyl)-α-D-N- acetylneuraminic acid (Sigma) as the substrate. Input viruses were

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