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H7 virus-like particles assembled by hemagglutinin containing H3N2 transmembrane domain and M1 induce broad homologous and heterologous protection in mice

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ABSTRACT

Influenza A H7N9 virus has caused five outbreak waves of human infections in China since 2013 and posed a dual challenge to public health and poultry industry. There is an urgent need to develop an effective vaccine to reduce its pandemic potential. In the present study, we evaluated the biochemical characteristics and immunogenicity of two H7 virus-like particles (VLPs) composed of the matrix 1 (M1) and hemagglutinin of wild-type (HA-WT) or hemagglutinin of whose transmembrane domain replaced by that from H3N2 subtype (HA-TM). H7 VLPs-WT and H7 VLPs-TM could assemble and release into the supernatant of Sf9 cells and they had similar morphological characteristics. However, compared to H7 VLPs-WT, H7 VLPs-TM had more trimeric HA proteins and could better resist thermal changes. In mice H7 VLPs-TM induced higher titers of HI, IgG, IgG2a and IFN-γ, and provided better protection against homologous and heterologous H7N9 viruses (no matter belonging to Yangtze River Delta or Pearl River Delta) challenge with less weight loss and higher survival rate. In summary, H7 VLPs-TM represents a potential strategy for the development of H7N9 vaccines.

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1. Introduction

A novel reassortant avian influenza H7N9 virus emerged in China in February 2013 and caused human infection (WHO). Until February 28, 2018, there were 1625 laboratory confirmed human cases of infection [1], with a fatality rate of approximately 38%, causing an unprecedented threat to public health and poultry industry [2,3]. Although human-to-human transmission hasn't been reported, H7N9 virus has caused five epidemic waves in China, evolving into two main diversified lineages: Yangtze River Delta (YRD) and Pearl River Delta (PRD) lineages based on hemagglutinin (HA) genes [4–6]. Viruses of the YRD lineage reacted less well with post-infection ferret antiserum raised against the PRD candidate vaccine [7], indicated a remarkable difference of antigenicity between the two lineages. Highly pathogenic (HP) H7N9 virus belonging to the PRD lineage was first reported in late February 2017, indicated the continued evolution of H7N9 virus [8–10].

Current influenza vaccines mainly rely on trivalent/quadrivalent inactivated or live attenuated vaccine, and embryonated chicken eggs are needed for the proliferation of influenza viruses (IVs) [11]. However, HPAIV is poorly proliferate in embryos and egg-based technology is a time-consuming cumbersome process that can hardly satisfy the urgent demand of vaccines. As an alternative to egg-based technology, virus-like particles (VLPs) have many incomparable advantages such as special security, effective immunity, easy operation and have been used for prevention of IV, hepatitis E virus (HEV), human papillomavirus (HPV), human immunodeficiency virus (HIV), severe acute respiratory syndromes (SARS), and so on [12–16].

Influenza vaccines only confer protection against closely related strains and have to be frequently reformulated to change candidate vaccine virus (CVV) for influenza antigenic variants. This highlights the urgency and importance of vaccines with cross-protection that could protect a wide variety of influenza subtypes. Our group has reported recombinant HA (rHA) has a transmembrane domain (TM) of H3N2 subtype had more trimeric proteins and tended to be more stable to thermal changes. Moreover, mice vaccination with rHA-TM could increase the cross-protection of inter-clade and subtypes [17–19].

In this study, we intended to explore whether the H7 VLPs composed of matrix 1 (M1) and HA of wild-type (WT) or HA with the replaced H3N2 TM could be constructed and compare the differences of characteristics and immunological properties between the two H7 VLPs.

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2. Materials and methods

2.1. Cells and viruses

Spodoptera frugiperda Sf9 insect cells were maintained as suspension in serum-free SF900II medium (Invitrogen Carlsbad, CA) at 27 ± 0.5 °C in spinner flasks at a speed of 90–100 rpm. The HA segments from the following eight H7N9 strains were synthesized:

A/Chicken/Guangdong/53/2014(H7N9) (H7N9-53);
A/Chicken/Guangdong/GSB/2014(H7N9) (H7N9-GSB);
A/Chicken/Guangdong/38/2014(H7N9) (H7N9-38);
A/Hangzhou/1/2013(H7N9) (H7N9-HZ);
A/Anhui/1/2013(H7N9) (H7N9-AH);
A/Chicken/Zhejiang/S01/2014(H7N9) (H7N9-ZJ01);
A/Chicken/Guangdong/MCX/2014(H7N9) (H7N9-MCX);
A/Chicken/Guangdong/ZSM/2017(H7N9) (H7N9-ZSM).

The first seven isolates belong to YRD lineage and the last one isolate belongs to PRD lineage (Fig. S1). All viruses used for assessment of the effect of humoral and cellular immunity were purified as described previously [20]. Furthermore, H7N9-53, H7N9-MCX and H7N9-ZSM were used for challenge.

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.vaccine.2018.07.004.

2.2. Expression and purification of H7 VLPs-WT/TM by recombinant baculoviruses

H7N9-53 HA containing the TM of H3N2 HA (HA-TM) was obtained by overlap PCR based on the H7N9-53 HA segment (HA-WT) and the TM of A/swine/Guangdong/01/1998(H3N2) HA. Two H7 VLPs were automatically assembled by HA-WT/TM and M1 proteins from H7N9-53 expressed by Bac-to-Bac expression system and named H7 VLPs-WT and H7 VLPs-TM. Briefly, HA-WT/HA-TM and M1 were cloned into the pFastBacDual vector (Invitrogen Carlsbad, CA), and the recombinant bacmids were transfected into Sf9 insect cells to get recombinant baculoviruses (rBVs) WT and recombinant baculoviruses (rBVs) TM. After three passages of the rBVs-WT/TM in Sf9 cells, H7 VLPs-WT/TM were yielded from 500 ml of Sf9 cells infected with the third passage of rBV-WT/TM. H7 VLPs-WT/TM were harvested at 72 h post-infection from the supernatant and purified through 20%–40%–60% of sucrose density gradients ultra-centrifugation.

2.3. Characterization of H7 VLPs-WT/TM

Purified H7 VLPs-WT and H7 VLPs-TM were negatively stained for electron microscopy (JEM-100CX-II, JEOLLTD, Japan) observation. The cleavability of precursor HA (HA0) in H7 VLPs into HA1 and HA2 subunits was determined by escalating concentrations of TPCK-trypsin (Sigma, Darmstadt, Germany) as previously described [21]. The thermal resistance of purified H7 VLPs-WT and H7 VLPs-TM was conducted in a Peltier Gradient Thermal Cycler (AB2720, Waltham, USA) at a temperature of 37, 46, 48, 50, 52, 54, 56 and 58 °C for 30 min, then the HA titers were measured after cooled down to room temperature.

2.4. Hemagglutination (HA) and hemagglutination inhibition (HI) assays

A series of two-fold dilutions of the purified H7 VLPs-WT and H7 VLPs-TM in PBS at 50 μl were prepared and incubated for 30 min with 50 μl of 1% chicken red blood cells (RBC). The HA titer was calculated as the highest dilution factor that produced a positive reading. HI assay was conducted as previously described [22]. In brief, the receptor destroying enzyme (RDE, Seiken, Japan) was used for treating mice serum at 37 °C 18–20 h followed by inactivated the RDE at 56 °C for 0.5–1 h, and 4 HA units of the eight inactivated H7N9 viruses working as antigen. Antibody titers were expressed as log 2 of the highest dilution giving complete hemagglutination inhibition.

2.5. Mice vaccination and challenge

A total of ninety-nine 6–8-week-old female BALB/c mice were divided into three groups (n = 33 per group) and receiving H7 VLPs-WT, H7 VLPs-TM or PBS. Mice were subcutaneously immunized twice at a two-week interval with 50 μl of VLPs containing 1 μg of HA formulated with Freund's adjuvant. Serum samples were taken two weeks after the boost vaccination for serological test (a total of 18, n = 6 per group). Three weeks after the boost, twenty-seven mice in each group were randomly divided into three groups of nine mice and challenged intranasally with 100 $\times$ MLD_{50} of H7N9-53, H7N9-MCX and H7N9-ZSM, respectively. Three days post challenge, three mice were sacrificed for determination of IV replication in the lungs. The remaining six mice were monitored daily for weight loss and death for 14 days. Infected mouse lost $\geq$20% body weight was humanely euthanized and regarded as dead.

2.6. ELISA and ELISPOT assays

ELISA assay was used to assess IgG total antibody titer, IgG isotypes titers and anti-M1 antibody titer in the serum of the immunize mice. The eight purified H7N9 viruses from 2.1 or M1 protein of H7N9-53 virus was coated at a concentration of 1000 ng/ml (100 μl/well), incubated with serial dilutions of each serum samples and detected as previous described [18].

The ability of splenocytes secreting IFN-γ or IL-4 under the stimulation of different H7N9 viruses was evaluated by ELISPOT (EZ-Sep, DAKEWE, China). In brief, freshly isolated splenocytes from the immunized mice were seeded in 96-well plates (5×10^5/well) that pre-coated with anti-mIFN-γ or -mIL-4, then the three purified inactivated IVs (H7N9-53, H7N9-MCX, H7N9-ZSM) were added to each well at a concentration of 10 μg/ml and incubated at 37 °C for 16–20 h. After processing, the number of spots was counted by ImmunoSpot ELISPOT reader (Bioreader 4000, BIO-Sys, Germany).

2.7. Lung viral titers

The virus titers in lungs were performed by the plaque assay using Madin–Darby Canine Kidney (MDCK) cells as described previously [23]. The detection limit of this assay was a titer of 10^1 pfu/lung, and lung samples with titers less than 10^1 were assigned a value of $10^{0.9}$ pfu/lung which represents the undetectable level of virus.

2.8. Statistics analysis

Statistics analysis were performed using GraphPad Prism 6. Unpaired Student's t-tests or ANOVA followed by Tukey's multiple comparison tests were used for statistical comparisons and statistics analysis. Statistical difference between two groups was indicated by $^{*}(p < 0.05)$, $^{**}(p < 0.01)$, $^{***}(p < 0.001)$, $^{****}(p < 0.0001)$.

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