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Protective efficacy of an inactivated Eurasian avian-like H1N1 swine influenza vaccine against homologous H1N1 and heterologous H1N1 and H1N2 viruses in mice

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ABSTRACT

Eurasian avian-like H1N1 (EA H1N1) swine influenza viruses are prevalent in pigs in Europe and Asia, but occasionally cause human infection, which raises concern about their pandemic potential. Here, we produced a whole-virus inactivated vaccine with an EA H1N1 strain (A/swine/Guangxi/18/2011, SW/GX/18/11) and evaluated its efficacy against homologous H1N1 and heterologous H1N1 and H1N2 influenza viruses in mice. A strong humoral immune response, which we measured by hemagglutination inhibition (HI) and virus neutralization (VN), was induced in the vaccine-inoculated mice upon challenge. The inactivated SW/GX/18/11 vaccine provided complete protection against challenge with homologous SW/GX/18/11 virus in mice and provided effective protection against challenge with heterologous H1N1 and H1N2 viruses with distinctive genomic combinations. Our findings suggest that this EA H1N1 vaccine can provide protection against both homologous H1N1 and heterologous H1N1 or H1N2 virus infection. As such, it is an excellent vaccine candidate to prevent H1N1 swine influenza.

1. Introduction

Eurasian avian-like H1N1 (EA H1N1) swine influenza virus (SIV) was first introduced into pigs in 1979; since then, it has been prevalent in European pig populations [1,2]. In China, EA H1N1 viruses were first detected in 1993, and have been repeatedly isolated from pigs [3–5]. Since 2009, an intensive surveillance program for swine influenza has been carried out in more than 20 provinces in China. This program found that EA H1N1 viruses were the predominant circulating viruses in pigs and formed two antigenic groups distinct from the classical swine H1N1 (CS H1N1) and the 2009/H1N1 viruses [6–8].

Sporadic human infection with the EA H1N1 SIV has been reported in Europe and China [9–11]. Moreover, our recent study showed that the EA H1N1 viruses have obtained traits that could cause a human influenza pandemic after long-term evolution in pigs [6]. Pigs, which are considered to be an intermediate host for potential pandemic influenza viruses, thus represent a source of a new threat to public health. Indeed, after the introduction of the pandemic 2009/H1N1 virus in pigs, multiple reassortant viruses derived from the pandemic 2009/H1N1, EA H1N1, CS H1N1, triple reassortant H1N2, and swine H3N2 viruses were detected in China [12–15].

Vaccination is an important and effective measure for controlling swine influenza, and conventional inactivated vaccines produced with H1N1 or H3N2 swine influenza viruses have been used in North America and Europe for many years [16]. Herein, we produced an inactivated EA H1N1 virus vaccine and evaluated its protective efficacy in mice against infection with homologous H1N1 virus, heterologous H1N1 and H1N2 viruses bearing the HA gene from EA H1N1, and heterologous 2009/H1N1 virus.

2. Materials and methods

2.1. Viruses

Five SIVs were included in this study: A/swine/Guangxi/18/2011(SW/GX/18/11), A/swine/Tianjin/42/2011(SW/TJ/42/11), A/swine/Guangdong/306/2013(SW/GD/306/13), A/swine/Guangxi/325/

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2012(SW/GX/325/12) and A/swine/Heilongjiang/44/2009(SW/HLJ/44/09). These viruses were previously isolated from pigs during surveillance activities conducted in China between 2009 and 2013 for swine influenza [6,7,13], and were used as challenge viruses in this study. These viruses were propagated in specific pathogen-free (SPF) embryonated chicken eggs and titrated to determine the 50% egg infective dose (EID_{50}) by the method of Reed and Muench [17].

2.2. Laboratory facilities

All experiments involving live H1N1 and H1N2 viruses were conducted within enhanced animal biosafety level 2 plus (ABSL2+) facilities at the Harbin Veterinary Research Institute (HVRI) of the Chinese Academy of Agricultural Sciences (CAAS). This study was carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the Ministry of Science and Technology of the People's Republic of China. All protocols were approved by the Committee on the Ethics of Animal Experiments of the HVRI, CAAS. All animal studies were approved by the Review Board of the HVRI, CAAS.

2.3. Vaccine preparation

The whole-virus inactivated vaccine was prepared as follows: First, the harvested allantoic fluid containing virus was inactivated by adding 0.2% formalin (v/v) and maintained at 37 °C for 24 h. Virus inactivation was confirmed by inoculating an aliquot of the formalin-treated viruses into embryonated eggs to verify that the allantoic fluid harvests were negative for hemagglutination. Then, the inactivated allantoic fluid was emulsified in Montanide ISA 15A VG (Seppic Company, Paris, France) at a w:w ratio of 85:15 virus-to-adjuvant.

2.4. Vaccination and challenge experiments

A total of 86 six-week-old female SPF BALB/c mice were used in this study. Prior to vaccination, mice were confirmed to seronegative for circulating SIVs by use of a hemagglutinin inhibition (HI) assay (data not shown). One group contained 40 mice that were vaccinated twice (with a three-week interval) with 50 μl of vaccine by intramuscular injection. Another group included 40 mice that received the same volume of phosphate-buffered saline (PBS) as a challenge control. The remaining six mice remained untreated and were used as normal controls.

Each week after vaccination, serum samples were randomly collected from the vaccine-immunized and PBS-inoculated mice for HI and virus neutralization (VN) antibody detection. To evaluate the protective efficacy of the SW/GX/18/11 vaccine against homologous SW/GX/18/11 and heterologous H1N1 and H1N2 SIVs, the two groups of 40 mice were each randomly divided into five subgroups ($n = 8$) and intranasally challenged with 10^6 EID_{50} of one of the viruses listed in Table 1 at two weeks after the boost-vaccination. On day 4 post-challenge (p.c.) three mice per subgroup were euthanized and their organs (including the nasal turbinate, lung, spleen, and kidney) were collected for virus titration in eggs. The remaining five mice per subgroup were observed daily for weight loss or clinical signs of infection for two weeks.

2.5. Serological tests

Sera from immunized mice were treated with Vibrio cholera (Denka-Seiken Company, Tokyo, Japan) receptor-destroying enzyme before being tested for the presence of HI and VN antibody following international standards (WHO Global Influenza Surveillance Network; Manual for the Laboratory Diagnosis and Virological Surveillance of Influenza; http://whqlibdoc.who.int/publications/2011/9789241548090_eng.pdf.). The homologous H1N1 virus SW/GX/18/11 and heterologous virus SW/HLJ/44/09, which represent two different antigenic H1N1 viruses, were both used as antigens in the HI and VN tests.

2.6. Virus titration

Virus titration in eggs was performed from clarified 10% (w/v) tissue homogenates in PBS. Each sample was serially diluted tenfold in PBS, and each different dilution was inoculated in a 0.1-ml volume into three 10-day-old embryonated eggs via the allantoic cavity. Allantoic fluids were tested for hemagglutinin activity after incubation at 37 °C for 72 h. The virus titers were calculated by the method of Reed and Muench [17], and expressed as the $\log_{10}$ EID_{50}/ml. Samples from which virus was not detected in the 0.1 ml of organ homogenate were assigned a numeric value of 0.5 as the limit of detection.

2.7. Statistical analysis

Antibody titers and viral titers were compared by use of the two-sided *t*-test. HI and VN antibody titers detected at two contiguous time points using an antigen were compared, and the titers at a time point using two different antigens were also compared. Viral titers in each organ between the vaccinated group and unvaccinated group were compared. $P < 0.05$ was considered to be a statistically significant difference, while $P < 0.01$ was considered to be an extremely significant difference.

Table 1
Protection of mice by the inactivated SW/GX/18/11 vaccine.[a]

Challenge virus	Amino acid sequence identity between the HA genes of the challenge viruses and the inactivated SW/GX/18/11 vaccine (%)	Percent weight-loss (%) of mice up to two weeks post-challenge		Virus replication ($\log_{10}$ EID_{50}/ml) at four days post-challenge[b]			
		Vaccinated	Unvaccinated	Lung		Nasal turbinate	
				Vaccinated	Unvaccinated	Vaccinated	Unvaccinated
SW/GX/18/11	100	–	14.5	<0.5	5.42	<0.5	3.25
SW/TJ/42/11	98.4	1.0	18.9	1.73 (2/3)	5.13	3.08	4.5
SW/GD/306/13	97.7	1.3	15.1	2.0 (2/3)	4.33	3.08	4.08
SW/GX/325/12	94.5	0.6	10.0	1.25 (2/3)	4.67	2.75	4.08
SW/HLJ/44/09	77.7	3.9	17.9	3.58 (2/3)	5.42	4.08	4.83

[a] Groups of six-week-old BALB/c mice were vaccinated with two-doses (three weeks apart) of vaccine, or PBS by intramuscular injection. The challenge experiment was conducted with 10^6 EID_{50} of SW/GX/18/11, SW/TJ/42/11, SW/GD/306/13, SW/GX/325/12, or SW/HLJ/44/09 virus two weeks after the second immunization.
[b] Three mice from each group were euthanized at four days post-challenge and virus replication in the lungs and nasal turbinates was determined by titration in chicken embryos. <0.5 indicates that no virus was titrated from the undiluted sample.

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