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Prime–boost immunization with HA/C3d DNA followed by a recombinant pseudorabies virus boost enhanced protective immunity against H3N2 swine influenza virus in mice

Guo-Xin Li [a,b], Yan-Jun Zhou [a], Hai Yu [a], Zhi-Jun Tian [b], Li-Ping Yan [a], Qiang Zhang [c], Shou-Ping Hu [b], Guang-Zhi Tong [a,b,*]

[a] Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Sciences, No. 518 Ziyue Road, Minhang District, Shanghai 200241, China
[b] Division of Swine Infectious Diseases, National Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, No. 427 Maduan Street, Harbin 150001, Heilongjiang, China
[c] Technical Center for Animals, Plants and Foods Inspection and Quarantine Shanghai Entry-Exit Inspection and Quarantine Bureau PRC, No. 1208 Minsheng Road, Pudong New Area, Shanghai 200135, China

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ABSTRACT

DNA and recombinant virus vaccines against swine influenza virus (SIV) have been pursued with promising results, but induce poor immunogenicity. This study evaluated the effects of a vaccine regimen in mice including priming with three DNA vaccines expressing soluble HA (sHA), complete HA (tmHA), or sHA fused with three copies murine C3d (sHA–mC3d3) and boosting with recombinant pseudorabies virus expressing HA (rPRV–HA). Immune responses were monitored by ELISA, HI assays, and virus neutralization. Protective efficacy was evaluated by virus isolation from lungs, distribution in tissues, and pathology following challenge with H3N2 SIV. Priming with sHA–mC3d3 and boosting with rPRV–HA induced higher levels of HA-specific antibodies and yielded the most effective protection. This finding implied that priming with a DNA vaccine expressing C3d fused with antigen and boosting with a recombinant vector vaccine is an effective way to induce protective humoral immunity and prevent some infectious diseases.

1. Introduction

Swine influenza virus (SIV) causes severe respiratory disease characterized by an acute explosive outbreak of symptoms such as coughing, high fever, nasal discharge, anorexia, and weight loss (Olsen, 2002). Under field conditions, coinfection of SIV with other pathogens could result in a significant negative economic impact for the swine industry (Choi et al., 2003). Beyond veterinary implications, influenza virus infection in pigs also poses an important public health concern. Swine are referred to as a "mixing vessel" because of their susceptibility to both human and avian influenza viruses (Ito et al., 1998; Castrucci et al., 1993). Reassortment of avian and mammalian influenza viruses in swine may produce new viruses, some of which may have the potential to transmit to humans (Webster et al., 1992; Karasin et al., 2000). Therefore, it is important to develop effective strategies to control swine influenza to prevent virus replication in the swine "mixing vessel", thus decreasing the possibility of creating potentially pandemic influenza virus reassortments.

Inactivated vaccines are commercially available, but new vaccines that are capable of inducing virus-specific neutralizing antibody plus cell-mediated immunity will provide superior protection against acute influenza diseases (Wesley et al., 2004). In recent years, as an alternative to conventional swine influenza vaccines, DNA vaccines and recombinant virus vaccines have been pursued with some positive and promising results (Macklin et al., 1998; Wesley et al., 2004; Endo et al., 1991; Larsen et al., 2001; Tang et al., 2002). Our recent study showed that three repeated inoculations of DNA vaccines expressing different forms of hemagglutinin (HA) elicited specific immune responses and protected mice from homologous SIV challenge. Although the results were promising, the requirement for three injections makes the DNA vaccines more theoretical than practical for use in swine (Li et al., 2009). In another study, we constructed a recombinant pseudorabies virus expressing HA (rPRV–HA) which protected mice from homologous SIV challenge, although only weak antibody responses were induced (Tian et al., 2006).

Recombinant virus vaccines are not suitable for booster immunization when priming with vaccines produced in the same vector

* Corresponding author. Address: Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Sciences, No. 518 Ziyue Road, Minhang District, Shanghai 200241, China. Tel.: +86 21 54113138; fax: +86 21 54081818.
E-mail address: gztong@hvri.ac.cn (G.-Z. Tong).

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because the immunity induced by prior immunization may neutralize or inactivate the vector and interfere with antigen presentation. The main disadvantage of DNA vaccines is their poor immunogenicity, especially in large animals. Large quantities of DNA are required to induce only modest immunogenicity (Graham et al., 2006). To circumvent the above problems, we explored a combination vaccine strategy. The immune response was primed with an antigen delivered by one vector, and then boosted using the same antigen delivered by an immunologically distinct vector to augment immune response and protection (Newman, 2002). Several studies have shown that a prime–boost immunization regimen with a DNA plasmid and recombinant virus vaccine, both expressing the same antigen, can induce a strong immune response, including cell-mediated immunity (Schneider et al., 1998; Dégano et al., 1999; Amara et al., 2001). Furthermore, a regimen including a DNA prime and inactivated influenza vaccine boost induced stronger immune responses than did the prime–boost using inactivated vaccine or DNA vaccines alone (Larsen et al., 2001; Wang et al., 2008). However, it is unknown whether a DNA vaccine expressing soluble HA, complete HA, or a fusion of HA with a molecular adjuvant is more effective in heterologous prime–boost immunization regimens.

The purpose of the present study was to determine if a vaccine strategy including priming with HA-expressing DNA and boosting with rPRV–HA could enhance immune responses and protection efficiency against homologous SIV challenge. Our previous study showed that a DNA vaccine expressing a fusion of soluble HA with three copies of murine C3d (sHA–mC3d3) induced a stronger immune response than a DNA vaccine expressing soluble HA (sHA) or complete HA (tmHA) (Li et al., 2009). This study determined that priming with sHA–mC3d3 was more effective than priming with either sHA or tmHA when boosting with rPRV–HA.

2. Materials and methods

2.1. Viruses and cell cultures

SIV strain A/Swine/Heilongjiang/74/2000 (H3N2) (SwHLJ74) was provided by Dr. Li at Harbin Veterinary Research Institute, Harbin, China. PRV Bartha-K61 strain and recombinant pseudorabies virus expressed HA (rPRV–HA) were propagated and titrated in PK-15 or Vero cells as previously described (Tian et al., 2006). All cells were grown and maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal calf serum (FCS), 100 U/ml ampicillin, and 100 μg/ml streptomycin.

2.2. HA–DNA vaccines

The HA–DNA vaccines encoding complete HA (tmHA), soluble HA (sHA), or a soluble fused form of HA (sHA–mC3d3) were previously constructed from the H3N2 subtype of SIV (Li et al., 2009). tmHA expresses full-length wild-type HA. sHA was generated by deleting the transmembrane and cytoplasmic domains of HA and replacing the tissue plasminogen activator (tPA) leader sequence with a signal peptide. sHA–mC3d3 was generated by inserting three copies of murine C3d downstream of sHA (Fig. 1). All plasmids were amplified in *Escherichia coli* strain DH5α and were purified using anion-exchange resin columns (Qiagen).

2.3. Immunization of mice

Eight-week-old BALB/c female mice were obtained from the Laboratory Animal Center of Harbin Veterinary Research Institute for immunogenicity studies. Animal maintenance and experimental protocols were approved by the Animal Experiment Ethics Committee of the authors' institute. The mice (18 mice per group) received two immunizations at weeks 0 and 4 with different combination immunizations (Table 1). Animals were injected intramuscularly (i.m.) with 100 μg plasmid DNA or inoculated intranasally (i.n.) with 10^5 PFU of rPRV–HA as indicated in Table 1. Sera were collected at 0, 2, 4, 6, and 8 weeks after the primary immunization to detect specific antibodies. At week 8 after primary immunization, mice were challenged i.n. with 10^5 TCID50 of SwHLJ74 (H3N2).

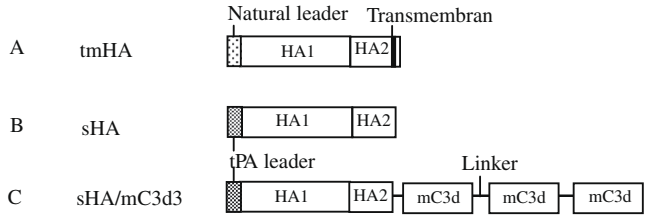

Fig. 1. Schematic representation of DNA vaccine constructs. (A) Structure of the wild-type, transmembrane form of HA. (B) Structure of extracellular part of HA, linked with a signal sequence of tPA encoding a secreted HA (sHA). (C) Structure of sHA linked with three copies of murine C3d (sHA–mC3d3). Two repeats of four glycines and one serine $\{(G_4S)_2\}$ as the linkers were inserted at the junctures of HA and mC3d and between each mC3d repeat.

2.4. Serological assay

An endpoint ELISA was performed to assess the titers of HA-specific antibody. Purified influenza virus was used to coat plates as described previously (Chen et al., 2007). Endpoint dilution titers from immunized mice that were twofold higher than sera from mice in control groups were considered positive. The hemagglutinin inhibition (HI) assay was conducted as previously described (Robinson et al., 1997) using four hemagglutination units of SIV virus. Neutralization assays were conducted as previously reported (Torres et al., 2000). Neutralization titers were reported as the highest dilution giving complete inhibition of replication of MDCK cells given a TCID50 of 100 in 50 μl DMEM medium. The presence of replicating virus in a well was scored by hemagglutination.

2.5. Lymphocyte proliferative responses

Lymphocyte proliferative responses using mouse splenocytes were detected as previously described (Li et al., 2009). Briefly, four weeks after boost immunization splenocytes were isolated from immunized mice, suspended in RPMI 1640, and seeded into 96-well plates at 4×10^5 cells per well (200 μl). The cultures were stimulated with either Con A (positive control), 20 μl of inactivated and purified swine influenza virus as the specific antigen, or nothing (negative control). The proliferative response was measured by adding 20 μl WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt) to each well with a further 5 h of incubation. The stimulation index was calculated as the ratio of the average OD values in wells containing antigen-stimulated cells to the average OD of wells containing only cells with medium.

2.6. IL-4 and IFN-γ release assay

At week 4 after boost immunization, the spleens from three mice from each group were harvested. Mouse splenocytes were prepared as described (Bounous et al., 1992) and incubated at 37 °C in 96-well plates at a concentration of 2×10^5 cells per well in the presence of 20 μl inactivated and purified SIV. After 72 h incubation, supernatants were harvested and the presence of IL-4