Virus Research 211 (2016) 46–57

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Lactobacillus plantarum vaccine vector expressing hemagglutinin provides protection against H9N2 challenge infection

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ARTICLE INFO

Article history:
Received 3 June 2015
Received in revised form 27 August 2015
Accepted 4 September 2015
Available online 9 September 2015

Keywords:
Hemagglutinin
Dendritic cell-targeting peptide
Lactobacillus plantarum
H9N2 influenza virus
Mucosal vaccination

ABSTRACT

Hemagglutinin (HA) has been demonstrated as an effective candidate vaccine antigen against AIVs. Dendritic cell-targeting peptide (DCpep) can enhance the robustness of immune responses. The purpose of this study was to evaluate whether DCpep could enhance the immune response against H9N2 AIV when utilizing *Lactobacillus plantarum* NC8 (NC8) to present HA-DCpep in mouse and chicken models. To accomplish this, a mucosal vaccine of a recombinant NC8 strain expressing HA and DCpep that was constructed in a previous study was employed. Orally administered NC8-pSIP409-HA-DCpep elicited high serum titers of hemagglutination-inhibition (HI) antibodies in mice and also induced robust T cell immune responses in both mouse and chicken models. Orally administered NC8-pSIP409-HA-DCpep elicited high serum titers of hemagglutination-inhibition (HI) antibodies in mice and also induced robust T cell immune responses in both mouse and chicken models. These results revealed that recombinant *L. plantarum* NC8-pSIP409-HA-DCpep is an effective vaccine candidate against H9N2 AIVs.

1. Introduction

Despite extensive vaccination efforts, low pathogenic avian influenza (LPAI) H9N2 viruses have prevailed in chicken populations in China (Zhang et al., 2012). With a genetically diverse population, LPAI H9N2 viruses have infected both wild and domestic species of birds and mammals (Blair et al., 2013; Peng et al., 2013; Shanmuganatham et al., 2013; Wang et al., 2014; Zhou et al., 2014; Zhu et al., 2013) and have also contributed their six internal gene segments to the H7N9 viruses (Bi et al., 2014; Gao et al., 2013). Thus, there is a great deal of interest in developing vaccines to prevent the transmission and control the ubiquity of H9N2. Because H9N2 viruses gain host entry via mucosal surfaces of the respiratory tract, there is an urgent need to develop vaccines that elicit effective mucosal immune responses against them. The properties of mucosal immunity and its manipulation in immunization are at the foreground of novel vaccine development, and targeted vaccination is one of the most promising methods available to prevent and control H9N2 infection.

Although vaccination does not provide complete immunity, it is one of the most effective control measures for H9N2. The HA protein of H9N2 contains the cell receptor-binding site of the virus and is a neutralizing epitope; as a consequence, it is the primary antigenic determinant of AIV. In previous studies, we confirmed that oral administration of recombinant *Lactobacillus plantarum* NC8 expressing HA could protect against AIV H9N2 in BALB/c mice (Shi et al., 2014).

A previous report described a strategy that specifically targeted a hepatitis C viral antigen to dendritic cells (DCs) using 12-mer peptides, which induced efficient antigen-specific immune responses in vivo (Mohamadzadeh et al., 2008). The peptide also recognizes the conserved region of its ligand on avian DCs (Owen et al., 2013). In the current study, we aimed to identify a DCpep that would

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http://dx.doi.org/10.1016/j.virusres.2015.09.005
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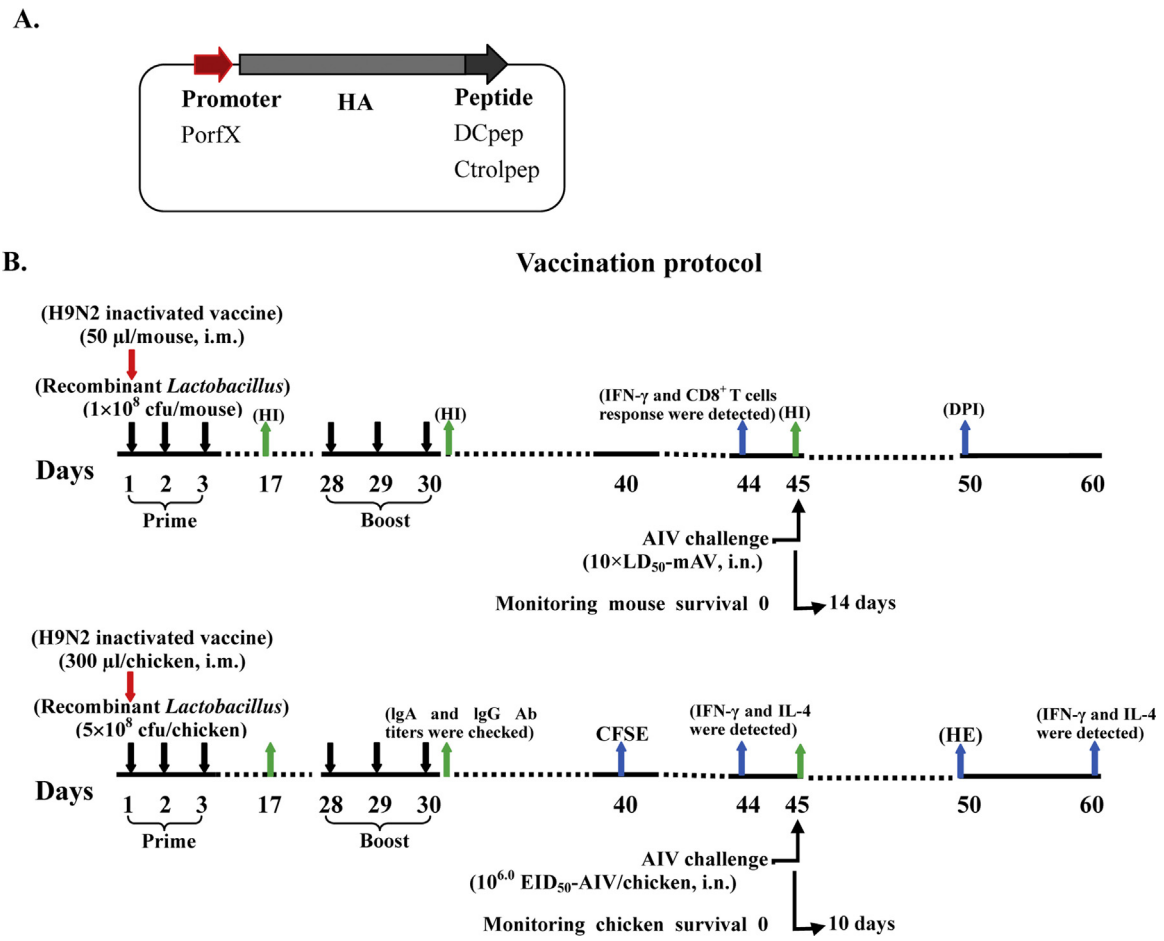

Fig. 1. Plasmids constructed for HA-DCpep fusion expression and vaccination schedule. (A) Schematic representation of pSIP409-HA-DCpep (~7.2 kb). PorfX: inducible promoters; HA: hemagglutinin gene; Peptide: DCpep or Ctrolpep. (B) The upper figure shows the mouse protocol, and the lower figure shows the chicken protocol. Groups of mice and chickens were immunized orally with NC8-pSIP409-HA-DCpep, NC8-pSIP409-HA-Ctrlpep, NC8-pSIP409 (1×10^8 cfu in 200 μl for mouse, 5×10^8 cfu in 500 μl for chicken) or PBS (200 μl for mouse and 500 μl for chicken) on days 1–3, 28–30. H9N2 inactivated vaccine (50 μl/mouse and 300 μl/chicken) was injected intramuscularly on the first day (red arrows). Fourteen days after the final boost, the mice and chickens were challenged i.n. with H9N2 mAV ($10 \times LD_{50}$) and with AIV H9N2 virus ($10^{6.0}$ EID_{50}), respectively. The mice and chickens were monitored for weight loss and percent survival until day 14 or 10, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

increase antigen immunogenicity and enhance naive T cell priming. Importantly, the use of targeted vaccines should decrease the need for adjuvants to enhance or induce local mucosal immunity and systemic responses (Owen et al., 2013). Compared to the majority of vaccines used today, which are parenteral vaccines, oral mucosal vaccines have considerable advantages, including increased patient compliance, reduced cost, ease of administration, needle-free delivery, improved practicality for mass vaccination (due to a lack of requirement for trained personnel), and better accessibility (Lycke, 2012). Recent progress in understanding the molecular and cellular components of the mucosal immune system has facilitated the development of a novel mucosal vaccine platform that uses a specific DCpep in combination with orally administered lactobacilli to elicit efficient antigen-specific immune responses to prevent infection. For example, dendritic cell targeting using a *Bacillus anthracis* protective antigen expressed by *Lactobacillus acidophilus* protects mice from lethal challenge (Mohamadzadeh et al., 2009).

We previously constructed recombinant NC8-pSIP409-HA-DCpep and confirmed that it expressed HA-DCpep. In the present study, an m-AV strain with enhanced pathogenicity was used to show that recombinant NC8-pSIP409-HA-DCpep produced complete protection in immunized BALB/c mice and also greatly improved the immune responses of immunized chickens, protecting the chickens against homologous virus challenge.

2. Results

2.1. Activation of DC costimulatory molecules by NC8-pSIP409-HA-DCpep

To detect the effect of the NC8-pSIP409-HA-DCpep on DCs, mice were orally inoculated once with *L. plantarum* expressing HA-DCpep. The mice were humanely euthanized at 24 h after NC8-pSIP409-HA-DCpep inoculation. PP and MLN cells were collected and then stained to determine the frequency and level of DC activation induced by NC8-pSIP409-NP-M1-DCpep vaccination. A significant increase in the surface expression of $CD11c^+CD80^+$ and $CD11c^+CD86^+$ PP DCs was observed in mice that were immunized with NC8-pSIP409-HA-DCpep compared to other controls (Fig. 2). Moreover, the surface expression of $CD11c^+CD80^+$ and $CD11c^+$ $CD86^+$ MLN DCs was not significant (data not shown).

2.2. Serum HI titers in mice

Serum was isolated from each group of mice at 14, 28 and 42 days after initial immunization and was analyzed by an HI assay. As shown in Fig. 3, there were no significant differences in antibody titers between mice treated with NC8-pSIP409-HA-DCpep and those treated with inactivated H9N2 vaccines on