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Plant-derived H7 VLP vaccine elicits protective immune response against H7N9 influenza virus in mice and ferrets

S. Pillet[a,b], T. Racine.[c], C. Nfon[d], T.Z. Di Lenardo[b], S. Babiuk[d,e], B.J. Ward[a], G.P. Kobinger[c,e,f], N. Landry[a,*]

[a] Medicago Inc., 1020 Route de l'Église, Bureau 600, Québec, QC, Canada
[b] Research Institute of the McGill University Health Center, Montreal, QC, Canada
[c] Special Pathogens Program, National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, MB, Canada
[d] National Centre for Foreign Animal Disease, Canadian Food Inspection Agency, Winnipeg, MB, Canada
[e] Department of Immunology, University of Manitoba, MB, Canada
[f] Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA, USA

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ABSTRACT

In March 2013, the Chinese Centre for Disease Control and Prevention confirmed the first reported case of human infection with an avian influenza A H7N9 virus. Infection with this virus often caused severe pneumonia and acute respiratory distress syndrome resulting in a case fatality rate >35%. The risk of pandemic highlighted, once again, the need for a more rapid and scalable vaccine response capability. Here, we describe the rapid (19 days) development of a plant-derived VLP vaccine based on the hemagglutinin sequence of influenza H7N9 A/Hangzhou/1/2013. The immunogenicity of the H7 VLP vaccine was assessed in mice and ferrets after one or two intramuscular dose(s) with and without adjuvant (alum or GLA-SE^{TM}). In ferrets, we also measured H7-specific cell-mediated immunity. The mice and ferrets were then challenged with H7N9 A/Anhui/1/2013 influenza virus. A single immunization with the adjuvanted vaccine elicited a strong humoral response and protected mice against an otherwise lethal challenge. Two doses of unadjuvanted vaccine significantly increased humoral response and resulted in 100% protection with significant reduction of clinical signs leading to nearly asymptomatic infections. In ferrets, a single immunization with the alum-adjuvanted H7 VLP vaccine induced strong humoral and CMI responses with antigen-specific activation of CD3^{+} T cells. Compared to animals injected with placebo, ferrets vaccinated with alum-adjuvanted vaccine displayed no weight loss during the challenge. Moreover, the vaccination significantly reduced the viral load in lungs and nasal washes 3 days after the infection. This candidate plant-made H7 vaccine therefore induced protective responses after either one adjuvanted or two unadjuvanted doses. Studies are currently ongoing to better characterize the immune response elicited by the plant-derived VLP vaccines. Regardless, these data are very promising for the rapid production of an immunogenic and protective vaccine against this potentially pandemic virus.

1. Introduction

On 29 March 2013, the Chinese Center for Disease Control and Prevention confirmed the first reported case of human infection with an avian H7N9 influenza A virus. As of October 2014, 453 laboratory-confirmed human infections with H7N9 viruses, including 175 deaths, have been reported resulting in a case fatality rate of 38.6% [1]. In most of the laboratory confirmed cases, infection with this novel H7N9 strain resulted in severe pneumonia and acute respiratory distress syndrome (ARDS) that required intensive care [2]. Although human-to-human transmission remains highly controversial [3], the ability of the H7N9 hemagglutinin (H7) to bind weakly to alpha 2,6-linked sialic acid suggests that H7N9 viruses could pose a pandemic threat [4,5]. Moreover, A/H7N9 viruses appear to be transmissible between ferrets via respiratory droplets [6] adding to the concern of a possible pandemic threat as transmission of influenza virus in ferrets closely mimics that in humans [7].

The possible emergence of H7N9 is occurring in the context of the A/H1N1 2009 outbreak that challenged national and global pandemic plans. In particular, the H1N1 2009 experience reinforced the world's commitment to vaccination as the most-cost effective means to prevent infection and to control potential pandemics. Unfortunately, the full process from the identification of a new

* Corresponding author. Tel.: +418 658 9393; fax: +418 658 6699.
E-mail address: landryn@medicago.com (N. Landry).

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strain to release of the vaccine product is traditionally completed within 4–6 months. Seasonal and pandemic strains that kill or grow poorly in eggs can be even more challenging [8], highlighting some of the limitation of current egg-based vaccines. Despite substantial efforts to develop so-called 'universal' vaccines and/or to predict the nature of the next pandemic strain, the degree of homology between vaccine and the circulating strains remains a crucial determinant of vaccine efficacy as illustrated during the 2012–2013 season [9]. In the absence of a universal vaccine, there is a great deal of interest in technologies and platforms that can produce large amounts of strain-specific vaccine rapidly.

Among the most promising approaches to address these issues are nanoparticles vaccines including virus-like particles (VLP) and particularly VLP vaccines produced in plants [10–12]. VLP are assembled upon expression of specific viral proteins and can present an external surface that closely resembles that of the cognate virus (Fig. S1) but without any genetic material. Influenza VLP that incorporate one or more viral proteins resemble intact virions in structure and morphology, and contain functionally active and immunologically relevant structural proteins [13]. In particular, the highly immunogenic viral surface glycoproteins hemagglutinin (HA) and neuraminidase (NA) are usually in their native conformation within VLP due to the absence of possible modifications by fixatives or chemicals used to inactivate and/or split virions in other vaccines. The self-assembled macrostructure of VLP can therefore present native conformational epitopes of surface proteins to the immune system [13]. Furthermore, influenza viruses grown in embryonic eggs and, in a lesser extent mammalian tissue culture systems, have the potential to mutate for optimal growth in these environments. The number and location of mutations in egg- and tissue-culture-adapted strains varies widely from year to year [9]. The immune response generated by those vaccines could therefore diverge from the actual circulating strain particularly when mutations occur in critical areas of the surface proteins. In contrast, the proteins contained in VLP can be engineered to exactly match the amino acid sequence of the circulating wild-type strain. Finally, the non-infectious nature of VLP is also a desirable safety feature for a vaccine candidate.

Plant-derived VLP vaccines address several of the serious limitations of currently licensed vaccines [13]. First, the protein(s) included in the VLP is/are based on the genetic sequence of circulating human influenza viruses rather than influenza strains adapted for optimal growth in embryonated eggs or tissue culture. Moreover, among the novel recombinant antigen production platforms, Agrobacterium infiltration-based transient expression in *Nicotiana benthamiana* has shown unprecedented speed and productivity, each kilogram of infiltrated leaves potentially producing approximately 1500 vaccine doses, based on a theoretical human dose of 30 μg of antigen [13,14]. Finally, plant-based manufacturing systems avoid some of the scalability issues associated with tissue-culture and egg-based production platforms as well as other recombinant technologies (i.e. available capacity in large-scale bioreactors). In 2012, Medicago demonstrated its capacity to produce 10 million doses of a plant-based H1N1 influenza vaccine within a month [15].

Here, we described the rapid development of an H7N9 plant-derived VLP candidate vaccine based on the HA sequence of A/Hangzhou/1/2013. When an adjuvant was incorporated, a single intra-muscular (IM) injection of this plant-made H7 VLP vaccine induced a robust HI antibody response in both mice and ferrets. We also demonstrated clear induction of a strong, Ag-specific, T-cell response after immunization in ferrets. These humoral and cell-mediated immune (CMI) responses were observed in conjunction with 100% protection after a lethal challenge with H7N9 A/Anhui/1/2013 in mice, as well as a significant reduction of clinical signs and virus load in ferrets. Two doses of the unadjuvanted vaccine achieved an even better level of protection in the mouse model.

2. Material and methods

2.1.1. Plant-derived VLP vaccine production and characterization

The vaccine production and characterization is described in supplementary methods and results sections. The vaccine was ready for production 19 days after obtaining the H7 sequence. By mid-May 2013, we performed our first pre-clinical trials in mice and ferrets demonstrating the immunogenicity of the H7 VLP vaccine (Fig. 1).

2.2. Animal immunizations

Mice: Female BALB/c mice 6–8 weeks old (Charles River, Saint-Constant, QC) were immunized IM using a 27G1/2 needle with one or two dose(s) of 3 μg H7 VLP vaccine (lot #500-32-020) based on HA content (see supplementary methods for details) with or without either Alhydrogel® (alum, 0.5 mg/dose, Cedarlane Laboratory, Burlington, ON, USA) or the toll-like receptor 4 (TLR-4) agonist glucopyranosyl lipid adjuvant-stable emulsion (GLA-SE, 0.005 mg/dose, Immune Design, Seattle, WA, USA) adjuvant 21 days apart. Placebo groups received IM injection of the saline buffer used to dilute the vaccine with either alum or GLA-SE adjuvant. All mouse procedures performed at the Research Institute of the McGill University Health Centre prior to challenge were approved by the McGill University Animal Care and Use Committee.

Ferrets: Male ferrets (*Mustela putoris furo*) 10–14 weeks old (Marshall Farms, North Rose, NY, USA) were immunized IM using a 25G5/8 needle with one or two dose(s) of 15 μg H7 VLP vaccine (lot #500-32-020) based on HA content with or without either alum (0.5 mg/dose) or GLA-SE (0.005 mg/dose) adjuvant 21 days apart. All ferret procedures prior to challenge were performed in the Centre National de Biologie Expérimentale (Laval, QC, USA) after approval by the Institutional Animal Care and Use Committee.

2.3. Immune response assessment in mice and ferrets

Hemagglutination inhibition (HI) assay: HI assays were performed as previously described according to the WHO recommendations using turkey's red blood cells and H7 VLP as the target antigen [16,17].

CMI response in ferrets: Fourteen days after the first immunization (prime), ferret's peripheral blood was collected in potassium EDTA-containing Vacutainer blood collection tubes (BD). Peripheral blood mononuclear cells (PBMC) were isolated as described previously [18] and seeded at 10^6 cells/well in 96-well plate in RPMI supplemented with 0.1 mM MEM non-essential amino acid (Invitrogen), 1 mM sodium Pyruvate (Invitrogen), 10 mM HEPES, 2 mM L-glutamine (Invitrogen), 55 μM β-mercaptoethanol, 10% FBS, 100 IU/mL penicillin and 100 μg/mL streptomycin (Complete RPMI) for cell proliferation and flow cytometry assays. *Flow cytometry analysis*: PBMC were incubated at 37 °C in a 5% CO_2 atmosphere with H7 or H5 VLP in complete RPMI during 18 h. After incubation, 1 μl/mL of Brefeldin A solution (GolgiPlug, BD) was added to the PBMC according to the manufacturer's recommendation, and cells were incubated for an additional 5 h at 37 °C in a 5% CO_2 atmosphere. PBMC were then washed, fixed, permeabilized and stained with FITC-conjugated mouse anti-human CD3 (Santa Cruz Biotechnology) and PE-conjugated mouse anti-bovine IFN-γ [18]. *Lymphocyte proliferation*: PBMC were incubated at 37 °C in a 5% CO_2 atmosphere with H7 VLP in complete RPMI for 48 h. Mitogen (2.5 μg/mL concanavalin A, Sigma-Adrich)-stimulated PBMC were included as a positive control. After incubation, cells were pulsed with 1 μCi of ^{3}H-TdR/well (MP Biomedical, Solon, OH, USA) and