Human Vaccines & Immunotherapeutics 8:3, 411–414; March 2012; © 2012 Landes Bioscience

# Virus-like particle (VLP)-based vaccines for pandemic influenza

## Performance of a VLP vaccine during the 2009 influenza pandemic

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**The influenza pandemic of 2009 demonstrated the inability of the established global capacity for egg-based vaccine production technology to provide sufficient vaccine for the population in a timely fashion. Several alternative technologies for developing influenza vaccines have been proposed, among which non-replicating virus-like particles (VLPs) represent an attractive option because of their safety and immunogenic characteristics. VLP vaccines against pandemic influenza have been developed in tobacco plant cells and in Sf9 insect cells infected with baculovirus that expresses protein genes from pandemic influenza strains. These technologies allow rapid and large-scale production of vaccines (3–12 weeks). The 2009 influenza outbreak provided an opportunity for clinical testing of a pandemic influenza VLP vaccine in the midst of the outbreak at its epicenter in Mexico. An influenza A(H1N1)2009 VLP pandemic vaccine (produced in insect cells) was tested in a phase II clinical trial involving 4,563 healthy adults. Results showed that the vaccine is safe and immunogenic despite high preexisting anti-A(H1N1)2009 antibody titers present in the population. The safety and immunogenicity profile presented by this pandemic VLP vaccine during the outbreak in Mexico suggests that VLP technology is a suitable alternative to current influenza vaccine technologies for producing pandemic and seasonal vaccines.**

**Keywords:** influenza, pandemic, vaccines, vaccination, H1N1, virus-like particles, clinical trials

**Abbreviations:** VLPs, virus like particles; HA, hemagglutinin; NA, neuraminidase; M1, matrix 1 protein; HBV, hepatitis B capsid; PapMV, papaya mosaic virus; Sf9, *Spodoptera frugiperda* cells; HAI, hemagglutination inhibition; IMSS, Mexican Social Security Institute; COFEPRIS, Federal Commission for Protection Against Sanitary Risk

Submitted: 11/07/11

Accepted: 11/14/11

http://dx.doi.org/10.4161/hv.18757

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### The First Pandemic of the 21st Century

For many years, experts from around the globe have been warning of the risk of an influenza pandemic and the need for pandemic vaccines.[1] Although the world's attention had been focused for more than a decade on H5N1 viruses as a pandemic threat, in April–May 2009, a new influenza A(H1N1) virus caused an outbreak in Mexico and the US. By May 18, 40 countries had officially reported laboratory-confirmed cases of the new virus and on June 11, the first influenza pandemic of this century was declared.[2] In Mexico, the influenza pandemic occurred in three waves, all in 2009: the first in April–May, the second in June–July and the third in August–December.[3] Associated with these three waves, an 11.1 excess all-cause deaths per 100,000 population and 445,000 y of life lost was reported, with a pandemic mortality burden 0.6–2.6 times that of a typical influenza season. Comparison of these data with available estimates from other countries shows that Mexico experienced a higher 2009 A (H1N1) pandemic mortality burden than most other countries.[4]

In response to the emergency, the main influenza vaccine producers as well as new manufacturers from China, Thailand, India and South America developed pandemic H1N1 influenza vaccines using embryonated chicken eggs (egg technology). Despite these efforts, monovalent vaccines were not ready for distribution until September 2009 and initial supplies were insufficient to have an impact on the bulk of the cases that occurred in the northern hemisphere.[5] At the pandemic epicenter, the inactivated vaccine was not available until October 5 in the US[2] and until November 27 in Mexico (by which time the third wave was waning).[3,6] Thus, the 2009 influenza outbreak revealed the inability of the established global capacity

for egg-based vaccine production technology to provide enough vaccines for the population within an appropriate time.

## 2009 Influenza Pandemic Vaccines Produced Using Egg Technology

The current pandemic influenza A (H1N1)2009 vaccines approved by the US Food and Drug Administration and the European Medicines Agency were produced using egg technology and are composed of adjuvanted or non-adjuvanted inactivated detergent-split virion subunits of hemagglutinin (HA), purified HA and neuraminidase (NA), or of inactivated whole virion, or of live attenuated virus.[5] Several studies around the world tested the immunogenicity, safety and tolerability of these vaccines. A meta-analysis of these studies that pooled data from randomized clinical trials registered up to April 2011 (including more than 20,000 subjects) showed that for all A(H1N1) 2009 split/subunit inactivated vaccines, a course of two doses was highly immunogenic and fulfilled the seroconversion criteria. After one dose, all split/subunit vaccines were immunogenic (≥ 70%) in adults and adolescents, while only non-adjuvanted vaccines at high doses and oil-in-water adjuvanted vaccines showed acceptable results for children and the elderly. Vaccines with oil-in-water adjuvants were more immunogenic than either non-adjuvanted or aluminum-adjuvanted vaccines.[7]

Nevertheless, a recent analysis of studies of the performance of egg technology influenza vaccines from the past 44 years questions the evidence for the effectiveness of these vaccines and points out the need for better vaccines.[8] Furthermore, because of the inability of egg-based manufacturing systems to produce an adequate vaccine supply for a pandemic outbreak, and given that a pandemic could threaten the availability of egg substrate, it has been suggested that alternative influenza vaccines that do not rely on egg substrates should be developed. Therefore, several other technologies for developing influenza vaccines have been proposed; among these, nonreplicating VLPs represent an attractive option because of their characteristics.[9]

## Virus-Like Particles

VLPs are nanoparticles composed of a noninfectious subset of viral components that mimic the wild-type virus structure but lack viral genetic material, thereby presenting a whole but inactive virus particle to the host. VLP molecular arrays are also exploited as platforms for heterologous antigen expression for viruses including hepatitis B capsid (HBV), papillomavirus, hepatitis E virus, cowpea mosaic virus, alfalfa mosaic virus, bacteriophage Qβ capsid, bacteriophage MS2–2MS2, flock house virus, tobacco mosaic virus and papaya mosaic virus (PapMV) (reviewed by Plummer and Manchester).[10]

## Immunogenicity of and Immunity Induced by VLP Vaccines

VLPs express a repetitive and organized molecular array not found in host tissues and therefore suitable for activating the host immune system. The VLP proteins, or the molecules attached to the VLP surface, can bind pattern recognition receptors on innate immune cells, triggering innate immunity. The ligand-receptor interaction is favored by the molecular array presented by the VLP structure.[11] VLP platforms have been identified as efficient tools for translating innate responses into long-lasting antibody responses.[12] Efficient activation of innate responses often leads to efficient antigen processing and presentation to T cells, promoting efficient antigen-specific T-cell responses. T-cell responses help the development of B cell and antibody responses.[13] In addition, these molecular arrays are potent inducers of type 2 T-independent B-cell responses by efficiently cross-linking B-cell receptors. Thus, these kinds of molecular arrays favor the development of robust immune responses.[14]

However, despite the strong immunogenicity shown by these VLPs, not all are able to induce long-lasting immunity. To achieve this, VLPs must contain the correct antigens in the right combination. In addition, other factors such as VLP particle size, geometry, the density of epitopes expressed on the surface, the presence of pathogen-associated molecular patterns, the route of immunization and the pharmacokinetics and pharmacodynamics of the VLP vaccine have been proposed to be important in inducing long-lasting immunity.[15] These factors should be considered when generating efficient VLP vaccines that maximize immunogenicity without compromising safety and tolerability.

## VLPs as Pandemic Influenza Vaccines

VLPs formed from non-influenza proteins expressing influenza peptides have been used to generate a candidate influenza vaccine. Platforms including HBV, AP205 bacteriophage and PapMV expressing influenza M2 peptides have been used in the search for a universal vaccine.[10,16] However, these approaches have the limitation that they include only a restricted array of peptides and could exclude several important antigens from these proteins that are important in inducing long-lasting immunity. To avoid epitope immunogenicity restrictions, other VLP technologies such as immune stimulating complexes, liposomes and virosomes use complete influenza proteins to induce immunity.[10]

VLPs can also be constructed with influenza proteins (influenza VLPs) by a self-assembly process incorporating structural proteins into budding particles.[17] These VLPs have been produced using insect cells [derived from the ovaries of the fall armyworm *Spodoptera frugiperda* (Sf9)] infected with recombinant baculovirus carrying the HA, NA and Matrix 1 (M1) influenza genes, leading to the expression of these proteins and VLP formation. These systems yield high expression levels of recombinant proteins and allow subsequent large-scale manufacturing of a vaccine.[18] VLPs produced in insect cells are considered safer than other expression systems because baculoviruses are found in vegetables and are not able to replicate in mammalian cells. In addition, the Sf9 cells are usually maintained in a serum-free, animal product-free medium, can be identified by karyotype and isoenzyme analysis, are free of contaminating microorganisms, adventitious agents, retroviruses and have been shown to be non-tumorigenic.[18] These production characteristics of VLPs support the view that this technology is safe.

Several pandemic recombinant influenza VLP vaccines have been generated in Sf9 cells infected with baculovirus vectors expressing multiple influenza components,[19] either with the structural influenza genes HA, NA, M1 and M2,[20] with HA, NA and M1 genes cloned into a single baculovirus construct[19,21-24] or with HA and M1 proteins only.[24,25] In addition, VLPs that express the HA, NA and M1 of various clades of the H5N1 subtype of avian influenza with pandemic potential have been produced.[21] These VLPs have been shown to be efficient inducers of antibody and T-cell responses and immunity in animal models;[26] in ferrets, a single immunization with a pandemic influenza VLP vaccine induced higher levels of antibodies than two doses of the commercial split vaccine.[27]

Pandemic influenza VLP vaccines have also been produced in tobacco plants. With this technology, a VLP vaccine can be developed within three weeks of the release of the virus sequence information.[28] A plant-made VLP pandemic influenza vaccine containing the HA protein of H5N1 influenza (A/Indonesia/5/05) was generated using transient expression of influenza glycoproteins in *Nicotiana benthamiana*. These VLPs were adjuvanted with alum and their administration induced cross-reactive antibodies, prevented pathology and reduced viral loads following heterotypic lethal challenge in ferrets. A phase I clinical study of this VLP vaccine was performed in healthy adults 18–60 y of age who received two doses 21 d apart of 5, 10 or 20 μg of alum-adjuvanted plant-made VLP vaccine or alum as placebo. The vaccine was well tolerated at all doses. Immunogenicity results showed that seroconversion (16.7%, 25% and 58%, respectively) and seroprotection (16.7%, 25% and 50%, respectively) were achieved only after the second dose. These data suggest that plant-based VLP vaccines could be an alternative method for developing seasonal and pandemic vaccines.[28]

## Pandemic Influenza VLP Vaccine Tested at the Pandemic Epicenter during the 2009 Outbreak

The central question for vaccines against pandemic viruses is whether they will work during a pandemic emergency. The on-time availability of supplies of pandemic influenza vaccine, its safety and immunogenicity profile, its efficacy—particularly for high-risk groups such as children, pregnant women and the elderly—and the duration of vaccine-induced protection during a pandemic situation are important parameters that remain to be evaluated.

The 2009 influenza outbreak provided the opportunity to test a pandemic VLP vaccine in the midst of the pandemic and at the outbreak epicenter. During the first months of the 2009 pandemic influenza outbreak, the lack of a vaccine and the high mortality revealed in early figures prompted Mexican health authorities to search for alternative vaccine technologies. In a collaborative effort, the Mexican Social Security Institute (IMSS) (a Mexican health-care institution that is attended by approximately 40% of the Mexican population), Avimex (a Mexican company that produces influenza vaccines for veterinary use) and Novavax (a US biotechnology company) developed a research protocol to test an A(H1N1) 2009 VLP pandemic influenza vaccine developed by Novavax. The vaccine was developed within 12 weeks of the release of the virus sequence, using Sf9 insect cells infected with a recombinant baculovirus, and was composed of 120 nm diameter purified recombinant H1N1 VLPs, which morphologically resembled influenza virions and exhibited HA and NA activities. These VLPs were immunogenic and induced protection in ferrets.[29]

The vaccine clinical research protocol was approved by the IMSS National Research Committee and by the Mexican Ministry of Health through the Federal Commission for Protection Against Sanitary Risk (COFEPRIS). From October 19, 2009, to March 5, 2010 (covering most of the third pandemic wave in Mexico), the safety and immunogenicity of one or two doses of A(H1N1)2009 influenza VLP vaccine composed of HA and NA derived from A/California/04/2009(H1N1) and M1 protein derived from A/Indonesia/05/2005 H5N1 strain were evaluated in a two-stage, phase II, randomized, double-blind, placebo-controlled study conducted in 4,563 healthy adults, 18–64 y of age.[30] In Part A, 1,013 subjects were randomized into four treatment groups (5, 15, or 45 μg HA VLP vaccine or placebo) and vaccinated twice 21 d apart, with sera collected on days 1, 14 and 36 for hemagglutination inhibition (HAI) testing. After review of safety and immunogenicity data from Part A, in Part B additional subjects were immunized with a single dose of 15 μg VLP vaccine (n = 2,537) or placebo (n = 1,011) and assessed for safety. Results showed that the H1N1 2009 VLP vaccine was safe and well tolerated. The VLP vaccine groups generated robust HAI immune responses after a single vaccination, with high rates of seroprotection (≥ 40 HAI titer) in 82–92% of all subjects and in 64–85% of subjects who were seronegative at the time of immunization.

The vaccine HAI reverse cumulative distribution was similar for all doses tested and in both young and elderly individuals, a finding that differs from other pandemic clinical trials.[7] Seroconversion was higher in the group without preexisting antibodies than in the group with preexisting antibodies; by contrast, seroprotection titers were higher in the group with preexisting antibodies than in the group without. The high rate of preexisting antibodies in the study subjects (36–41%) is an important characteristic of the herd immunity process elicited by the pandemic virus. Despite this, the VLP vaccine could induce seroconversion in 43% (5 μg dose) to 71% (45 μg dose) and seroprotection in 88% (5 μg dose) to 95% (45 μg dose) of people with preexisting antibody titers. These data suggest that the VLP vaccine efficiently boosted preexisting antibody responses and that these antibodies did not neutralize the vaccine effect. Interestingly, no clear evidence of a further boost was observed after the second dose of vaccine. This was also observed using other pandemic vaccines in adolescents, adults and elderly individuals but not in clinical trials with children.[7] Further studies are required to analyze these phenomena.

This study has particular characteristics that distinguish it from others: it was performed in the midst of the 2009 influenza pandemic at the outbreak epicenter and is one of the largest clinical

trials performed testing an influenza A (H1N1)2009 pandemic vaccine.

Overall, these data showed that by using different expression systems, a safe and immunogenic VLP vaccine to pandemic influenza could be rapidly developed (within 3–12 weeks) from influenza virus genetic sequences to supply the vaccine after the onset of a pandemic. In addition, the pandemic influenza A(H1N1)2009 VLP vaccine safety and immunogenicity profile revealed in the clinical trial performed during the outbreak suggests that this VLP technology is a suitable alternative to current influenza vaccine technologies to produce seasonal and pandemic vaccines.


### Financial Disclosure

C.L.M. is supported by IMSS grant: FIS/IMSS/PROT/PRIO/11/013.


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