



Review Article

Current and Next Generation Vaccines Against Influenza

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Abstract: Influenza is an infectious disease. In order to overcome various infectious diseases, many vaccines have been developed so far. Influenza vaccines have played an important role in prevention and control of influenza. However, current influenza vaccines are not perfect. Current influenza vaccines are produced by anticipating influenza viruses that may occur in annual epidemics or pandemics because influenza viruses have characteristic of antigen mutations. Furthermore, current subcutaneous or intramuscular inoculation of vaccine cannot sufficiently induce IgA that plays an important role in defending the entry of pathogens from the mucosa. In order to overcome the drawbacks of these current influenza vaccines, next generation vaccines are under investigation. One candidate is universal influenza vaccine using the antigens that are conserved among influenza virus strains. Another one is mucosal vaccine that can induce IgA on the mucosa of the upper respiratory tract. Here current and next generation influenza vaccines are described.

Keywords: Influenza, Hemagglutinin (HA), Universal Vaccine, Matrix Protein2 (M2), Mucosa

1. Introduction

Influenza remains a serious ongoing threat to public health. Influenza is an infectious disease that plagues us every year [1]. Furthermore, the new strains of influenza that occurs at intervals of several decades have caused many victims [2]. In either case, vaccination is the most cost-effective public health measure to prevent disease and mortality caused by influenza virus infection. Embryonated chicken egg or cell culture is used for production of influenza vaccines [3]. In case of pandemic influenza, the embryonated chicken egg cannot be used for vaccine production because chickens are influenced by pandemic influenza virus. Therefore, cell culture system of influenza vaccine has been promoted worldwide.

The majority of currently licensed influenza vaccines are inactivated influenza vaccines, based on purified viral protein components that are administered by intramuscular injection. The protein production and purification technologies vary according to manufacturer, but the general principle is the same for inactivated influenza vaccines. Indeed, the investigational influenza vaccines of the 1940s were produced in a markedly similar fashion to the egg-grown inactivated influenza vaccines of today. From the viewpoint that influenza

viruses are cultured, both methods are the same. Influenza vaccines by recombination DNA technology has also developed, but at present it is not yet mainstream. Especially, current influenza vaccines cannot suppress mutant virus and cannot protect invasion from respiratory mucosa [4, 5].

Recently, a novel therapeutic drug baloxavir marboxil that inhibit cap-dependent endonuclease, an enzyme essential for viral replication has been put on the market [6]. Under these circumstances, In order for the influenza vaccine to show its presence, it is necessary for novel vaccines to have such features as to protect against mutant viruses and to prevent invasion of viruses from respiratory mucosa. In this article current and next generation influenza vaccines are described.

2. Influenza Virus

Influenza viruses are enveloped, single-stranded, negative-sense RNA viruses of the family Orthomyxoviridae. Influenza viruses are classified into three antigenically distinct types: A, B and C, according to the antigenic differences in the viral nucleoprotein (NP) and matrix protein (M) [7]. Surface proteins of influenza virus are hemagglutinin (HA), neuraminidase (NA), and matrix protein 2 (M2). Influenza A viruses (Figure 1) are divided into subtypes based on HA and

NA. Up to date, 16 subtypes of HA (H1- H16) and 9 subtypes of NA (N1-N9) have been identified [8]. Since antibodies against HA can neutralize the virus, HA has been used as antigen for influenza vaccines.

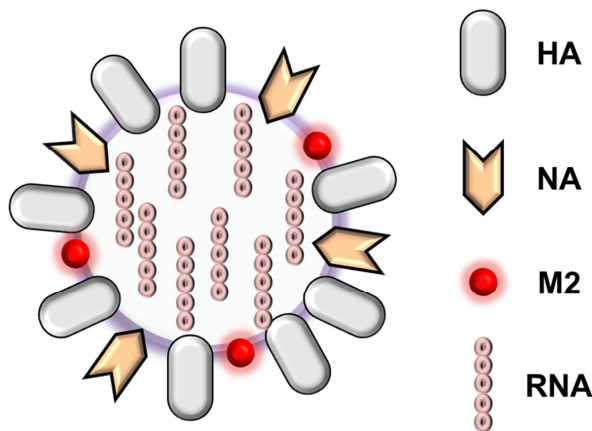


Figure 1. Schematic representation of Influenza A virus.

Influenza viruses have two major mechanisms of antigenic evolution: antigenic drift and antigenic shift [9]. Antigenic drift occurs when the virus accumulates mutations at antigenic sites during replication through the actions of the inherently error-prone RNA polymerase, producing variant viruses that can escape existing immunity. This phenomenon is common to both influenza A and B viruses. More dramatically, antigenic shift occurs when a virus acquires an antigenically novel HA through reassortment, a property made possible due to the segmented nature of the viral genome. Although both influenza A and B viruses undergo reassortment, antigenic shift is a feature of influenza A viruses only, due to the animal sources of antigenically distinct HA genes. Some of the most virulent type A influenza viruses have been responsible for serious human pandemics. Since the Spanish flu outbreak of 1918, which claimed millions of human lives, the influenza pandemics are appearing with intervals of 10 to 15 years.

3. Vaccine Production Technologies

There are different licensed technologies of influenza vaccines. Majority of the current licensed influenza vaccines are made using embryonated chicken eggs. The Egg-based production has been used for more than 70 years for influenza vaccine, and it is still the most extensively used technology. This production method requires large numbers of chicken eggs to produce vaccine and may take longer than other methods used to produce vaccine. The production process has depended on a continuous supply of eggs. In the case of pandemic outbreaks, this mode of production might be problematic because of a possible drastic reduction in the egg supply and the low flexibility of the manufacturing process resulting in a lack of supply of the required vaccine doses in a timely fashion.

Other production technologies using mammalian or insect cell cultures have developed to overcome the limitations of the egg-based production system [10, 11]. These industrially well-established production systems have been primarily selected for a faster and more flexible response to pandemic

threats. In contrast to egg-based production processes, cell-based production technology allows manufacturers to respond to market needs faster and in shorter production cycles and also allows a greater surge capacity, greater process control, and a more reliable and well-characterized product. In addition, cell culture, unlike embryonated eggs, can be cryopreserved, reconstituted, and scaled up at any time. To date, the continuous mammalian cell lines Vero (monkey kidney cells) and MDCK (canine kidney cells) and human-derived PER.C6 cells have been used successfully to prepare seasonal and pandemic influenza vaccines [12-14].

Insect cell and baculovirus expression vector (BEVS) systems have been extensively used for recombinant protein production. BEVS technology allows the expression of influenza virus antigens, such as monomeric or multiple HA, and also auto-assembled fully folded proteins that can form virus-like particles (VLP). This is the first recombinant HA influenza vaccine [15].

Developments of plant-produced recombinant influenza vaccines are also undergoing [16]. *Nicotiana benthamiana* plants are vacuum infiltrated by agrobacterium harboring HA gene of influenza virus. Such plants can produce VLP displaying HA (Figure 2). Plants could become a promising biofactory for expression of recombinant proteins due to the low final cost and inherent safety of products resulting from the absence of pathogens common to plants and animals. Plant production systems have emerged as a promising alternative for manufacturing of vaccine antigens. Plants possess the eukaryotic post-translational protein modification machinery broadly similar to that of mammals, do not harbor human pathogens, and can be rapidly scaled up as economic biomass generator. The plant-produced recombinant HA is in clinical study [17].

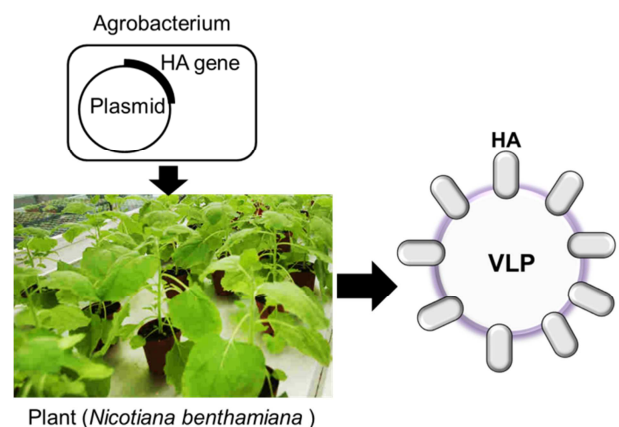


Figure 2. Schematic representation of plant-produced HA-VLP.

4. Universal Influenza Vaccine

Although influenza vaccines offer an available protection against seasonal or pandemic influenza strains, current influenza vaccines are not perfect. HA of influenza virus is characterized by a high level of plasticity and the ability to rapidly acquire mutations, causing antigenic drift and a need for regular updates to the vaccine.

Influenza A and B viruses accumulate mutations due to no proof-reading activity. When these mutations occur in the HA

and NA, they lead to antigenic drift, which over time results in escape from earlier immune responses. Influenza A can also undergo antigenic shift, whereby a strain with a new HA subtype enters and transmits readily in an immunologically naïve population. Antigenic shift is possible through reassortment resulting from the exchange of gene segments between two or more strains. Reassortment plays a significant role in the evolution of Influenza A in the natural reservoir and during the emergence of pandemic strains. Because the variations of seasonal influenza viruses and pandemics can be unpredictable, current vaccines may not provide effective protection against them.

Universal vaccine can potentially provide broad protection against various types of influenza virus, so even if predictions are wrong, the vaccine will still be effective. Universal influenza vaccine approaches attempt to overcome the drawbacks of the highly changing nature of influenza viruses. The objective of universal vaccines is to induce cross-protective broadly neutralizing immunity, which depends on stimulating both humoral and cell-mediated arms of the immune system. Universal vaccines rely on the concept of developing immune responses against conserved viral epitopes. To develop universal influenza vaccines, conserved sequences that are shared by different influenza viruses must be used as vaccine antigens. M2 has remained fairly conserved since the 1918 influenza outbreak, and thus it is an attractive target to develop a universal influenza A vaccine.

However, M2 is the protein in very small numbers on the virus surface and is poorly immunogenic. To enhance the immunogenicity of M2 various approaches have been employed including fusion of M2 to different carriers such as hepatitis B virus core protein (HBc), bacterially-derived outer membrane vesicle (OMV) as shown Figure 3 [18, 19]. Each construct can display M2 on HBc particle or E.coli, respectively. Many M2 vaccines have been successfully tested for efficacy against a panel of divergent influenza viruses in animal models. Clinical studies have been conducted with M2e vaccine candidates, which demonstrated their safety and immunogenicity in humans. [20].

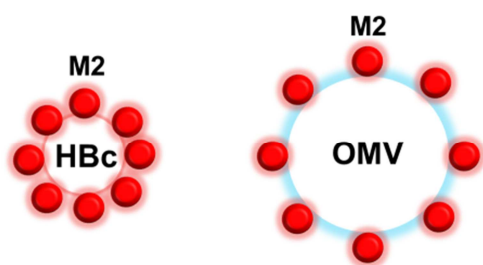


Figure 3. Schematic representation of M2 on HBc particle or OMV.

On the other hand, it is known that cytotoxic T-lymphocyte (CTL) populations directed against internal antigens of influenza A virus are broadly cross-reactive to influenza virus subtypes. Liposomal conjugates with CTL epitope peptides derived from highly conserved internal antigens of influenza viruses were evaluated for their ability to protect against infection with influenza viruses. Liposomal conjugates with peptide M1₅₈₋₆₆ was shown to inhibit viral replication of H1N1 or H3N2 in mice in the lung of immunized mice [21]. Their results suggest that liposome-coupled CTL epitope peptides

derived from highly conserved internal antigens of influenza viruses might be applicable to the development of vaccines that induce protection against infection with heterosubtypic influenza viruses.

5. Protection of Influenza Virus on Mucosa

Many infectious agents enter the body through mucosal surfaces. Mucosal immunity is being recognized to be important for providing effective protection against pathogens entering mucosal surfaces [22]. Influenza is a respiratory disease by influenza virus that enters through mucosal surface. Mucosal immunity acts as the front line of host defense by blocking influenza virus from infecting the upper respiratory tract and spreading to the lower respiratory tract. In the mucosal immunity IgA plays an important role. Therefore, efficacious influenza vaccine should induce IgA in the respiratory tract. In order to induce IgA in the mucosa, it is necessary to administer viral antigens via the mucosa with appropriate adjuvants.

Intranasal vaccination is an attractive route as a needle-free vaccine delivery method. When inactivated influenza vaccines were administered intranasally with an appropriate adjuvant, IgA was induced in the respiratory tract [23]. Several preclinical studies on adjuvant-combined, nasal-inactivated vaccines showed protection against infection of influenza virus [24].

Furthermore, studies on influenza vaccine combining universal vaccine and mucosal administration have been conducted. Intranasal vaccination with VLP displaying M2 could induce humoral and cellular immune responses conferring cross-protection against heterosubtypic influenza viruses [25].

6. Conclusion

Current and next generation influenza vaccines were described in this article. The majority of currently licensed influenza vaccines are inactivated influenza vaccines, based on purified viral protein components that are administered by intramuscular injection. Those current influenza vaccines cannot respond to virus mutants and cannot protect invasion of the virus from mucosa. In order to overcome the drawbacks, it is necessary for next generation vaccines to have features covering viral mutations and preventing viral invasion from upper respiratory mucosa. Universal influenza vaccine having conserved region among influenza viruses and mucosal influenza vaccines secreting IgA in mucosa would be promising candidates of next generation vaccines.

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