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Review Article

Current and next generation influenza vaccines: Formulation and production strategies

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ABSTRACT

Vaccination is the most effective method to prevent influenza infection. However, current influenza vaccines have several limitations. Relatively long production times, limited vaccine capacity, moderate efficacy in certain populations and lack of cross-reactivity are important issues that need to be addressed. We give an overview of the current status and novel developments in the landscape of influenza vaccines from an interdisciplinary point of view. The feasibility of novel vaccine concepts not only depends on immunological or clinical outcomes, but also depends on biotechnological aspects, such as formulation and production methods, which are frequently overlooked. Furthermore, the next generation of influenza vaccines is addressed, which hopefully will bring cross-reactive influenza vaccines. These developments indicate that an exciting future lies ahead in the influenza vaccine field.

1. Introduction

Influenza viruses are negative stranded RNA viruses of the *Orthomyxoviridae* family. Three types of influenza viruses, influenza A, B and C, are capable of infecting humans, of which influenza A and B are the most common circulating types. Individuals infected with influenza virus generally display symptoms such as chills, fever, headache, muscle pain, fatigue, rhinitis and coughing. Progressed influenza infections can lead to severe complications including bronchitis, pneumonia, secondary bacterial infections, acute respiratory distress and cardiovascular complications, which all can lead to death if left untreated. Individuals with a weakened immune system, such as immunocompromised patients, elderly and young children [1–3], are particularly vulnerable to influenza infections and are thus classified as high-risk populations.

Global influenza epidemics emerge seasonally and typically occur during the winter seasons of the northern and southern hemispheres. The WHO estimates that there are 3–5 million cases of severe influenza infections annually, with 250.000–500.000 deaths globally. The reemergence of a pandemic H1N1 strain in 2009 [4], and the emergence of highly pathogenic avian H5N1 and H7N9 influenza viruses [5,6], has reaffirmed that influenza remains a global threat to this day.

Vaccination against influenza is the most cost-effective method to prevent influenza infections. Fast availability of influenza vaccines to the world population is one of the key factors for effective coverage against seasonal and pandemic influenza. Despite the fact that influenza vaccines are on the market since the 1930s, several limitations still exist involving both their availability and their effectiveness, which are listed in Table 1.

Current influenza vaccines are predominantly produced by egg-based production methods. Being dependent on the supply of vaccine-quality eggs, vaccine manufacturers cannot be flexible in the amount of doses produced. This can lead to vaccine shortages, especially during pandemic situations. Alternative production platforms, such as cell culture-based vaccine production, plant-based vaccine production or synthetic vaccines, could increase the flexibility of manufacturers. It is often thought that these novel production methods decrease the time needed to develop and release an influenza vaccine. However, the availability of strain-specific reagents for vaccine potency and release tests such as the single radial immunodiffusion (SRID) assay and subsequent clinical trials are the main factors that delay the commercial release of influenza vaccines.

Directly tied to the commercial release of influenza vaccines are the regulatory approval procedures. To speed up these procedures, mock-up vaccines are developed to generate a registration dossier, which can subsequently be used for the licensing of an actual seasonal or pandemic influenza vaccine.

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Table 1
Limitations of current influenza vaccines and potential solutions.

Limitation	Potential solution(s)
Dependence on egg-based production	Cell culture-based production of virus Recombinant antigens Synthetic vaccines
Regulatory approval procedures	Mock-up vaccines to generate regulatory dossier
Limited worldwide vaccine availability	Technology transfer of vaccine production methods Dose sparing by the addition of adjuvants or alternative administration routes Increase stability and shelf life of vaccines to prevent vaccine loss in unfavorable conditions
Limited efficacy in elderly and unprimed populations	Increase vaccine immunogenicity by increasing antigen dose, the addition of adjuvants or using alternative administration routes Increase breadth of immune response by the addition of adjuvants, alternative administration routes or by inclusion of novel antigens
Lack of cross-reactivity by current vaccines	Vaccines inducing stalk-reactive antibodies M2e-targeted vaccines T-cell inducing vaccines Heterologous prime-boost strategies with seasonal and cross-reactive vaccines

Limited vaccine availability is not only caused due to the inflexibility of the vaccine production capacity; especially not in developing countries. Technology transfer of production methods to developing countries increases the worldwide vaccine production capacity. Increasing the (heat) stability and shelf life of influenza vaccines negates the need of a cold chain, which is imperfect in developing countries. This prevents unnecessary vaccine loss. Furthermore, decreasing antigen dose by the addition of adjuvants can also increase the number of influenza vaccines. Development of stabile vaccine formulations and effective adjuvants is thus important.

In several population groups, such as unprimed young children, the elderly and immunocompromised individuals, influenza vaccines have limited efficacy. Unprimed individuals have a reduced response to influenza vaccines, whereas elderly, due to immunosenescence, and immunocompromised individuals generally suffer from a declined immune function. Increasing the immunogenicity and breadth of the immune response elicited by influenza vaccines might improve vaccine efficacy in these vulnerable groups.

Current influenza vaccines induce neutralizing antibodies against the viral membrane surface proteins hemagglutinin (HA) and neuraminidase (NA). Due to antigenic shift and drift of HA and NA genes, neutralizing antibodies elicited by influenza vaccines lack cross-reactivity against non-matching influenza strains. While seasonal adjustments to the vaccine strains are made to cope with this problem, it is not as convenient and fast as a potential cross-protective influenza vaccine. Thus, the identification of alternative correlates of protection (CoPs) against influenza is an important step toward the development of cross-reactive influenza vaccines.

The aforementioned limitations of current influenza vaccines may be resolved through the implementation of new technologies in the field of influenza production and vaccine formulation. Novel antigens often require novel production methods, which carry their own advantages and disadvantages. Additionally, these novel antigens often need to be formulated with excipients and adjuvants to be sufficiently immunogenic. While important, the development of alternative administration methods and devices for influenza vaccines is not within the scope of this current review, and has been thoroughly reviewed by Amorij et al. previously [7]. In this review, we will discuss advances in immunological, formulation and production aspects for current and promising novel influenza vaccine antigens, and discuss their potential to solve the limitations of influenza vaccines today.

2. Immune responses against influenza

The efficacy of current influenza vaccines is determined by the presence of adequate HI- or VN-titers in vaccinated individuals. HI titers indicate antibody responses against HA, which are not cross-reactive, and do not protect against mismatching influenza strains. Ideally, an influenza vaccine would protect against all strains, uninfluenced by antigenic changes. VN titers indicate antibody responses that are able to neutralize influenza virus, and thus can potentially be applied for cross-reactive vaccines. Nonetheless, identification of alternative CoPs, such as cross-reactive antibodies or T cell responses would significantly aid the development of universal vaccines [8].

Induction of immune responses against novel and more conserved epitopes, other than the variable epitopes of HA, has come under the attention in recent years (Fig. 1). These include vaccines that induce antibodies directed against stalk regions of HA and matrix protein 2 ectodomains (M2e), and vaccines that induce cellular responses against internal influenza proteins. These vaccine could potentially be the basis of a universal influenza vaccine.

2.1. HA-specific antibodies

Antibodies against HA can be divided into categories: those reactive against the globular head domain, and those reactive to the stalk domain. Current influenza vaccines induce mainly antibodies directed against the head domain, which is highly variable due to antigenic drifts. In contrast, the stalk domain is more conserved, which makes it an attractive target for the induction of a cross-reactive humoral response. Certain stalk-reactive antibodies, such as globular head-reactive antibodies, inhibit the virus attachment to cell membranes [9], thereby preventing infection (Fig. 1A). Other stalk-reactive antibodies disrupt viral membrane fusion (Fig. 1B), preventing endosomal escape of the virus. Indeed, several monoclonal antibodies directed against these stalk domains proved to be effective, and are currently in the development to provide therapeutic treatment of acute influenza infections [10].

Several HA stalk-directed vaccines are currently under development, which proved effectiveness against both influenza A group 1 and 2 viruses [11], as well as influenza B. However, the potential side effects of these antibodies still need to be carefully evaluated. Khurana et al. showed that HA2 stalk-reactive antibodies promoted viral fusion and respiratory disease symptoms by pH1N1 influenza in pigs [12], indicating that the induction of stalk-reactive antibodies is not without risk. Further clinical studies should determine whether stalk-reactive antibodies are suitable for protection against influenza infection.

2.2. Matrix protein 2 ectodomain-specific antibodies

Matrix protein 2 (M2) is a tetrameric transmembrane protein that acts as a proton-selective ion channel. It plays a crucial role in the acidification and subsequent destabilization of the viral membrane, which facilitates the release of the genetic material of the virus into the host cell. The M2 protein is, except in low amounts in WIV and LAIV vaccines, not included in current seasonal vaccines; M2-specific antibodies are generally not detected in subjects vaccinated with seasonal influenza vaccines. Nonetheless, it possesses a sequence of amino acids that is highly conserved among influenza subtypes, located on the N-terminal ectodomain.

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