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Review

A universal flu vaccine

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Influenza is a global health concern. The single most effective way of protecting people against influenza infection and disease is vaccination. However, currently available vaccines against influenza induce only strain-specific immunity, and do not elicit long-lasting serum antibody titers. Therefore, they are ineffective in the case of possible pandemics. There is an urgent need for a new generation vaccine which would induce broad and long-lasting immune protection against antigenically distinct flu viruses. The paper presents recent achievements and the challenges in the field of universal vaccine construction.

Key words: universal vaccine, flu vaccine, influenza virus

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INTRODUCTION

Influenza is a global health concern, with the annual attack rate estimated at 5%–10% in adults and 20%–30% in children. Illness can result in hospitalization and death, especially among high-risk groups: the very young, elderly or chronically ill (Thompson *et al*., 2004; Zhou *et al*., 2012). It is estimated that each year influenza infections cause for 3 to 5 million cases of severe illness and 250 000 to 500 000 deaths worldwide (http://www.who.int/mediacentre/factsheets/fs211/en/). The European Centre for Disease Prevention and Control estimates that, on average, nearly 40 000 people die prematurely each year from influenza in countries of the European Union (EU) and the European Economic Area (Mereckiene *et al*., 2014). Given its disease-causing potential, the prevention of infection with influenza viruses is a high public health priority. Currently, the most effective single way of protecting people against influenza infection and disease is vaccination (Nicoll *et al*., 2013; Cox *et al*., 1999). The influenza virus belongs to the family Orthomyxoviridae (Lamb *et al*., 2001) and is divided into the A, B and C genera, which are distinguishable on the basis of antigenic differences between their matrix and nucleoproteins (M and NP), their host range, variations in surface glycoproteins, genome organization and morphology. Within the influenza virus genera, influenza A and B viruses are the most relevant clinically because they cause severe respiratory infections in humans (Hampson *et al.,* 2006). Of the two, type A viruses are more virulent, cause the most severe disease and are the primary pathogens responsible for seasonal and pandemic influenza outbreaks (Hayashida *et al*., 2001; Wright *et al*., 2001).

Type A viruses can be divided into different subtypes based on the serotypes of their main surface antigens: hemagglutinin (HA) and neuraminidase (NA) (Hayashida H *et al*., 1985). So far, 18 HA and 9 NA subtypes have been identified. Phylogenetically, HA subtypes are categorized into two groups (H1, H2, H5, H6, H8, H9, H11, H12, H13, H16, H17, and H18 in group 1, and H3, H4, H7, H10, H14, and H15 in group 2) (Medina *et al*., 2011; Huber, 2013; Tong *et al*., 2013; Pica *et al*., 2013). Historically, H1 (H1N1), H2 (H2N2) and H3 (H3N2) strains have caused influenza pandemics in humans and resulted in millions of deaths (Steel *et al*., 2010).

Influenza B viruses have diverged into two antigenically and phylogenetically distinct lineages that co-circulate in the environment (Rota *et al*., 1990; Hay *et al*., 2001; Kanegae, *et al*., 1990). Infections caused by influenza B viruses are less severe, but the pathogen can still cause outbreaks. The influenza C virus is of little concern for human infections, causing only a mild common cold-like disease in children (Moriuchi *et al*., 1991).

DISADVANTAGES AND LIMITATIONS OF CURRENTLY AVAILABLE VACCINES

Currently available vaccines against influenza are poorly immunogenic and do not induce long-lasting serum antibody titers. They provide protection only against a subset of strains circulating in the environment, namely, those closely related to the vaccine strains. This limited effectiveness is due to a mechanism called antigenic drift: the influenza virus undergoes genetic variations, allowing it to evade the pre-existing immune responses of the host. Therefore, immune responses mounted against earlier forms of the virus are less effective or completely ineffective against newer variants. Thus, a new vaccine must be reformulated and prepared every flu season. Besides, the vaccine has to be based on a surveillance of antigenic drift and predictions of the dominant strain for the upcoming flu season (Russell *et al*., 2008). The strains are selected by a network of experts several months in advance before the next influenza season regarding the duration of the manufacturing process. Although the process of antigenic drift is well studied, precise predictions of what strains will circulate in a given season remains problematic. Mismatches between vaccine strains and circulating viruses occur, resulting in a sharp drop in vaccine efficacy (de Jong *et al*., 2000; Ram Yogev, 2005; Carrat *et al*., 2007). Moreover, current vaccines appear to be less effective in the elderly (Centers for Disease

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Abbreviations: HA, hemagglutinin; NA, neuraminidase; bnAbs, broadly neutralizing antibodies; VLPs, virus like particles; HBc, hepatitis B core antigen; KLH, keyhole limpet hemocyanin; MVA, Modified vaccinia virus Ankara; PB1, polymerase basic protein 1

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It is also important to note that currently available vaccines do not protect against possible pandemics resulting from genetic variations between different subtypes of the influenza virus concurrently infecting the same host (Shapshak *et al.*, 2011). Additionally, it takes at least 6–8 months to develop, test and produce conventional vaccines against emerging viruses (Krammer *et al.*, 2014). The reformulation of the annual flu vaccine is also an expensive undertaking that costs consumers and the global health system more than $4 billion each year (Hoag, 2013).

Despite these drawbacks, influenza vaccination has been demonstrated to be highly cost-efficient and cost saving (Nichol *et al.*, 2007; Scuffham *et al.*, 2002). In December 2009, the European Council unanimously recommended that EU countries adopt and implement national action plans to achieve 75% influenza vaccination coverage in all at-risk groups by the influenza season of 2014/15 (Official Journal of the European Union L348: 71–72). The US Centers for Disease Control Prevention recommended universal influenza vaccination for all persons at least 6 months of age (Fiore *et al.*, 2010). Despite the availability of safe and relatively effective vaccines and the recommendations of official health sources, seasonal influenza vaccination coverage rates remain suboptimal and generally well below the WHO and US targets. During the 2012/2013 flu season in the USA, flu vaccination coverage was 45% (http://www.cdc.gov/flu/fluvaxview/coverage-1213estimates.htm#estimated). According to information from the National Institute of Public Health, in the 2012/2013 flu season, 3.75% of the Polish population was vaccinated against influenza. It demonstrated that even the 2009/2010 A/H1N1 pandemic had little effect on the coverage rates for the seasonal influenza vaccine (Blank *et al.*, 2012).

EFFICACY OF CURRENT INFLUENZA VACCINES

The efficacy rate of available in the USA influenza vaccines is approximately 59% for adults (Osterholm *et al.*, 2012; Jefferson *et al.*, 2010). Yet, vaccine effectiveness against H3N2, the main flu strain circulating during the 2012/2013 season, proved to be only 46% for adults aged 18–49, 50% for those aged 50–64, and a dismal 9% for people aged over 65, who represent a vulnerable group (Centers for Disease Control and Prevention 2013; http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6207a2.htm?s_cid=mm6207a2_w.; Kissling *et al.*, 2014). However, the suboptimal vaccine effectiveness for the H3N2 component during the 2012/2013 season was related to mutations in the egg-adapted IVR-165 vaccine strain (Skowronski *et al.*, 2014).

The low effectiveness of currently available flu vaccines results, among other reasons, from the low use of the seasonal influenza vaccine. This is caused by the necessity of yearly vaccine re-administration, related cost issues, lack of influenza vaccine acceptance in the general population, and unknown efficacy for a given season. Sensational media coverage and public debate concerning vaccine effectiveness, which depends on the match between the circulating virus and the vaccine strains, can negatively impact vaccination coverage (Kissling *et al.*, 2011; Kissling *et al.*, 2011). Another problem, especially in European countries, is the public attitude to the adjuvants present in vaccines. Thus, in the event of a pandemic, vaccination could be ineffective. To overcome the limitations of seasonal influenza virus vaccines and enhance our pandemic preparedness, we need a vaccine that provides universal and durable protection.

UNIVERSAL VACCINE

The development of a universal vaccine is one of the major goals in global pandemic preparedness plans. A universal flu vaccine could provide protection regardless of the strain or subtype of the circulating virus, allowing the vaccine to be prepared in advance in appropriate amounts, and be ready to use "off-the-shelf" in the event of a pandemic (Epstein *et al.*, 2010).

It is expected that a universal flu vaccine would have several advantages over currently available seasonal vaccines. The universal vaccine would require less frequent administration, ideally only once. This would reduce the exposure of vaccinated individuals to adjuvants and would eliminate the recurring cost of yearly vaccination. These features could increase public acceptance of vaccination against the flu and thereby augment flu vaccine coverage.

CONSERVED INFLUENZA ANTIGENS

The development of universal vaccines relies on the utilization of highly conserved antigenic targets (Epstein, 2003; Heiny *et al.*, 2007). However, conserved antigen epitopes are usually less exposed to the host immune system, and as such, are naturally weakly immunogenic. The goal of a universal vaccine is to augment their immunogenicity sufficiently to induce protective immunity.

Several proteins encoded by the influenza virus have been evaluated as promising candidate antigens for the development of a universal vaccine. Among them are the HA, M (M1 and M2e), NP and NA proteins. All of the listed antigens have highly conserved regions that are potential immunogens for a universal vaccine.

STRUCTURE OF THE HA ANTIGEN

Hemagglutinin, the major envelope glycoprotein of influenza A viruses, is the target of almost all neutralizing antibodies. HA is synthesized as an immature polypeptide chain called HA0, which is activated upon cleavage by host proteases to yield two subunits, HA1 and HA2. HA2 creates a helical chain "stem" that is anchored in the viral lipid membrane. The HA1 subunit of HA forms a globular "head" that contains receptor binding sites and the majority of the virus antigenic sites (Wiley *et al.*, 1981). Because HA1 loops are highly variable, antibodies targeting these regions are strain-specific, explaining why immunity by natural exposure or vaccination is typically restricted to the currently circulating strains. It has been estimated that human seasonal H3 and H1 viruses have undergone between 2.1% and 3% amino acid changes per drift variant between 1999 and 2010.

In contrast to HA1, the HA2 subunit is highly conserved among viruses belonging to the same phylogenic group. It also undergoes mutations, although at a much lower rate. It underwent only 3 different amino acid changes in this region in the H1 and H3 strains in the same period of time (Han *et al.*, 2011). Furthermore, HA2 is also immunogenic (Russ *et al.*, 1987). Indeed, the stem region of HA represents a promising target for

universal vaccine design eliciting broadly cross-reactive neutralizing antibodies directed against an epitope in this region of HA (Gerhard *et al.*, 2006; Krystal *et al.,* 1982).

THE PURSUIT OF AN ANTIBODY THAT TARGETS CONSERVED REGIONS

The first report describing an antibody cross-reactive with the HA stem was published by Yoshinobu Okuno (Okuno *et al.*, 1993). The mouse antibody C179 neutralizes the H1, H2, H5, H6, and H9 subtypes of the virus (Okuno *et al.*, 1993; Sakabe *et al.*, 2010; Smirnov *et al.*, 1999). The idea of cross reactive antibodies became popular when, five years ago, six independent groups began to publish data on existence of human antibodies capable of neutralizing many different subtypes of the influenza virus. Broadly neutralizing antibodies (bnAbs) neutralizing viruses belonging to HA phylogenetic group 1 (mAb CR 6261 and F10) (Sui *et al.*, 2009; Throsby *et al.*, 2008; Kashyap *et al.*, 2008; Ekiert *et al.*, 2009), group 2 (CR 8020, CR8043) (Ekiert *et al.,* 2011; Friesen *et al.*, 2014), and group 1 as well as group 2 (mAb FI6) (Corti *et al.*, 2011; Russell*,* 2011; Clementi *et al.*, 2011) were identified. The identification of antibodies that can bind to both influenza virus groups is important, as the influenza A viruses responsible for human pandemics derive from both group 1 (H1N1 and H2N2) and group 2 (H3N2). In addition, zoonotic viruses from both groups can infect humans and have the potential to trigger future pandemics (including H5N1 and H9N2 from group 1 and H7N7 from group 2). Consequently, future universal therapies based on the FI6 antibody have the potential to provide protection against both group 1 and group 2 influenza viruses (Corti *et al.*, 2011).

Similarly, antibodies (CR8033 and CR8071) were identified as recognizing conserved epitopes in the HA head region of influenza B (Dreyfus *et al.*, 2012). Furthermore, an antibody (CR9114) that recognizes epitopes in the HA stem of both influenza A and influenza B, and which protects against lethal challenges from both of these genera, was discovered (Dreyfus *et al.*, 2012). CR9114 is the most broadly neutralizing antibody identified so far.

In addition to cross-reacting antibodies which bind to the conserved regions of HA2, broadly neutralizing antibodies that recognize regions on HA1 were also identified (Ohshima *et al.*, 2011; Ekiert *et al.*, 2012; Lee *et al.*, 2012; Tsibane *et al.*, 2012).

It has been shown that such broadly cross-reactive HA stem antibodies provide protection through passive transfer (Ekiert *et al.*, 2011; Corti *et al.*, 2010; Sui *et al.*, 2009; Wang *et al.*, 2010; Corti *et al.*, 2013).

UNIVERSAL VACCINES BASED ON HA ANTIGEN

The identification of bnAbs against influenza viruses has raised hopes for the development of the universal vaccines for influenza. It was shown that bnAbs recognizing the HA stem, can be elicited after influenza infection in humans, although they are produced at low levels (Ohshima *et al.*, 2011; Sui *et al.*, 2011; Corti *et al.*, 2013). The natural occurrence of bnAbs has inspired construction of a vaccine that would exclusively induce bnAbs, i.e., a universal vaccine. Such a vaccine could potentially provide a long-lasting protection; a recent study showed that a high titer stem-reactive antibodies induced by an influenza virus vaccine were detectable after more than 30 years (Miller *et al.*, 2013).

Two approaches are used when developing a universal vaccine based on the HA antigen. One approach involves the use of full-length HA, and the other focuses on the HA conserved stem domain. Both of these approaches are associated with low level of neutralizing antibodies that recognize conserved regions on the HA stem. When using full-length HA, considerably lower levels of immunological response are achieved to the HA stem than to the head because the HA head physically masks the stem region on the influenza virion (Kwong *et al.*, 2009). There have been some attempts to use full length HA to elicit a broad neutralizing response.

In one such approach adenovirus vectors expressing centralized consensus influenza antigens representing putative HA ancestors were used. Centralized HA antigens were obtained from synthetic full-length HA sequences within a subtype or among different subtypes. The proposed vaccine provided protection that was limited to viruses within the same subtype (Weaver *et al.*, 2011). Another approach using the full-length HA antigen is DNA vaccine technology (Chen *et al.*, 2008).

It is challenging to induce immunological responses to conserved regions that are weakly immunogenic. There are currently several strategies employed for stem-oriented antigen design that eliminate the dominant immune response to the HA head. One such strategy is the use of a truncated HA that lacks the globular head domain, but still maintains the integrity of the stem region. Such a headless HA antigen derived from H2N2 was expressed in CV-1 cells and detected with the C179 antibody that neutralizes all H1 and H2 subtypes. Mouse experiments revealed that the mice were protected from the homologous virus and partially protected from the H1N1 virus (Sagawa *et al.*, 1996).

In another example, mice were vaccinated with a combination of DNA and Virus Like Particles (VLPs) expressing a headless HA construct derived from H1N1 and H3N2 viruses. Immunization elicited antisera that were cross-reactive against multiple group 1 subtypes of hemagglutinin, and provided protection against homologous lethal challenges (Steel *et al.*, 2010). Schneemann *et al.* used a multivalent display of a 20-residue A-helix from HA2 on icosahedral VLPs derived from the capsid of the Flock House virus to immunize mice. The 20-residue A-helix of HA2 is the major component of the epitopes of the broadly neutralizing antibodies CR6261, F10, and others. It was shown that immunization with VLPs displaying 180 copies/particle of the A-helix elicited antibodies recognizing multiple HA subtypes from group 1, but not from group 2. However, the elicited antibodies did not neutralize the influenza virus (Schneemann *et al.*, 2012). In another attempt, a stable trimeric influenza hemagglutinin stem domain was produced through the T4 bacteriophage fibritin foldon fusion at the C-terminus of the HA stem domain (Lu *et al.*, 2014).

Selected peptides corresponding to HA conserved regions can be used to construct a universal vaccine. They can be fused with carrier proteins, such as keyhole limpet hemocyanin (KLH), for improved antigen presentation, and enhanced immunogenicity based on the adjuvant function of the carrier proteins. It was shown that KLH fusion protein comprising an HA2 synthetic peptide from an H3 virus conferred heterosubtypic protection against H5 and H1 viruses (Wang *et al.*, 2010). Bommakanti *et al.* designed an HA2-based immunogen derived from the sequence of H3N2, which was expressed in *Es-*

cherichia coli and refolded from inclusion bodies. The obtained antigen provided protection against a homologous H3 viral challenge and also provided cross-strain protection within the subtype (Bommakanti *et al.,* 2010).

Vaccines based on the HA antigen aiming to elicit an immunological response against the HA conserved domain have the potential to be developed. Even if the immunological response to the HA stem is weak, this response could be augmented by boosting with vaccines that exclusively induce bnAb HA stem antibodies. This theory is supported by data demonstrating that individuals infected with pandemic 2009 IAV experienced a boost in virus-neutralizing antibodies specific to the HA stem (Pica *et al*., 2012). This phenomenon has also been confirmed in a mouse model of sequential infection (Krammer *et al*., 2012; 2014).

In accordance with these results, it may be necessary to preimmunize individuals naïve to the influenza virus (such as children) before vaccinating them with a universal vaccine.

Additional aspect is the safety of next-generation influenza vaccines based on HA stem domain antigens. Generally, antigens induce antibody responses including both neutralizing and non-neutralizing antibodies. Recent studies demonstrated that non-neutralizing antibodies may be associated with enhanced infectivity (To *et al*., 2012). It was reported that whole inactivated H1N2 virus vaccination and subsequent challenging with H1N1 resulted in more frequent and more severe vaccine-associated pneumonia. (Khurana *et al*., 2013). It was suggested that elicited non-neutralizing anti-stalk antibody might promote H1N1 infection by enhancing H1N1 virus membrane fusion activity. As these results imply, it is of great importance from the safety point of view that we ought to understand fully the molecular basis for neutralization of influenza viruses in polyclonal responses *in vivo*.

UNIVERSAL VACCINES BASED ON M2e ANTIGEN

The extracellular domain of the M2 protein (M2e) may be the most explored target for a universal influenza vaccine. Interest in this protein as a vaccine target was triggered by observations that anti-M2 antibodies, while lacking neutralizing activity, reduce plaque size (Zebedee *et al*., 1988), and the level of replication of a challenge virus in the lungs of mice (Treanor *et al*., 1990; Wang *et al*., 2008). Moreover, this protein is relatively well conserved across viral strains. As an example, the N (amino)-terminal epitope SLLTEVET (residues 2-9) in M2e was found to be 100% conserved among human influenza A virus and over 99% among all influenza A subtypes (Fiers *et al*., 2004; Liu *et al*., 2005).

M2 is a tetrameric integral membrane protein that functions as a pH-dependent proton channel, and is a minor component of the virus envelope. The protein is essential for proper maturation of the HA, for uncoating the virus after viral entry, and for releasing the viral genome into the cytoplasm (Lamb *et al*., 1985; Schnell *et al*., 2008). M2e, while exposed on the virion surface, in 1 to 3 copies, is masked by HA and NA proteins (Song *et al*., 2011). These factors may explain why anti-M2 or anti-M2e antibody levels are very low in influenza-infected humans and animals. However, mouse model studies showed that anti-M2 antibodies have a protective nature; animals vaccinated with baculovirus-derived M2 were protected from lethal challenge with H1N1 and H3N2 influenza viruses (Slepushkin *et al*., 1995). Unfortunately, the extension of these studies to other laboratory animals, including ferrets and primates, was not encouraging (Fan *et al*., 2004). Nevertheless, studies in most animal models indicate that M2e-based vaccines reduce morbidity levels, but do not confer immunity to infection. However, pigs immunized with M2e-derived human or avian viruses showed no protection against challenge with a swine virus, although the anti-M2 antibody levels were increased (Heinen *et al.,* 2002; Hikono *et al.,* 2012). Differences in the M2e sequences between the vaccine and the challenge virus could explain why no protection was observed in this case.

M2e in its virion-bound form is poorly immunogenic (Rossman *et al*., 2011). Therefore, several approaches have been proposed to improve the immunogenicity of M2, including the addition of adjuvants (Slepushkin *et al*., 1995; Wu *et al*., 2007; Wu *et al*., 2009), fusing the peptide to known highly immunogenic carrier proteins, and employing genes that target and improve immune function (hepatitis B core antigen (HBc), KLH (Tompkins *et al*., 2007), bacterial outer membrane complex (Fu *et al*., 2009), and flagellin (Huleatt *et al*., 2008), VLPs (human papillomavirus L protein VLPs (Ionescu *et al*., 2006), phage Qβ-derived VLPs (Bessa *et al*., 2008)), or liposomal platforms (Ernst *et al*., 2006).

In one approach, to improve hetero-subtypic cross-protection, VLPs were used. The expressed tandem repeats of M2e peptides containing two human, two avian and one swine origin M2e sequences fused to the HA transmembrane and cytoplasmic domains (Kim *et al*., 2013). The M2e proteins incorporated into these VLPs were 100 times more abundant than they were in influenza virions. This study showed that sera from mice immunized with such chimeric VLPs reacted with a range of influenza viruses, including H1N1, H3N2 and H5N1 strains.

In another approach, different forms of VLPs based on the M2e antigen fused to HBc were shown to induce high levels of anti-M2e antibody responses (Fiers *et al*., 2004; Neirynck *et al*., 1999; de Filette *et al*., 2006; de Filette *et al*., 2005; Heinen *et al*., 2002). Nevertheless, the protection against infection mediated by M2e was not complete. There is also ongoing research directed at understanding the mechanism of M2e-specific immunity. It was shown that antibodies recognizing M2 do not neutralize the virus. Several theories explaining the protection mechanism were proposed, including antibody-dependent cell cytotoxicity, antibody-dependent natural killer cell activity, and complement-mediated lysis (Jegerlehner *et al*., 2004; Tompkins *et al*., 2007; El Bakkouri *et al*., 2011).

Research on M2e-based universal vaccines has produced several minor successes. For example, in a double-blind, placebo-controlled phase I clinical trial, the safety and immunogenicity of M2e-HBc VLPs combined with adjuvant, derived from recombinant cholera toxin A1, were evaluated in humans, and the results demonstrated that this approach was promising for further clinical studies. Another candidate, STF2.4×M2e, a fusion protein of M2e with the TLR5-ligand domains from *Salmonella typhimurium* flagellin flj B, also completed a phase I clinical trial, and was found to be safe and immunogenic (Huleatt *et al*., 2008; Rupp *et al*., 2011; Talbot *et al*., 2010).

It is of great importance to test the effectiveness of new universal flu vaccine strategies in clinical trials, as it is almost impossible to mimic the human situation using animal models.

UNIVERSAL VACCINES BASED ON NP AND M1 ANTIGENS

Phylogenetic analysis of virus strains isolated from different hosts indicates that the NP and M1 genes are relatively well conserved, with a maximum amino acid difference of less than 11% for NP (Shu *et al*., 1993), and only about 5% for M1 (Reid *et al*., 2002). Therefore, they are attractive candidates for a broad-spectrum influenza vaccine (Shu *et al*., 1993; Heiny *et al*., 2007; Price *et al*., 2009). The NP protein is able to elicit subtype cross-reactive cytotoxic T lymphocyte immunity to speed up viral clearance in mice and humans (McMichael *et al*., 1983; Ulmer *et al*., 1998). It was also demonstrated that NP induces non-neutralization antibodies, which play a role in heterosubtypic immunity in mice (Carragher *et al*., 2008). Recently, multi-antigen constructs employing NP and M1 antigens have shown promise in conferring broad protection against influenza subtypes.

Modified vaccinia virus Ankara (MVA) vectors expressing various combinations of NP, M1, HA and NA have been evaluated in animal models (Boyd *et al*., 2013; Brewoo *et al*., 2013). Phase I and II clinical studies of an MVA expressing NP+M1 indicate that this approach is safe and might be efficacious for preventing influenza infection in humans (Berthoud *et al*., 201; Lillie *et al*., 2012).

Furthermore, MVA vectors expressing influenza NP alone or co-expressed with other conserved influenza proteins (e.g., the stem region of HA, proteins M1 and M2, the viral polymerase basic protein 1 (PB1), or the HA stem fused to a quadrivalent M2e) protect mice against lethal challenges with H5N1, H7N1 and H9N2 viruses by a mechanism involving influenza-specific CD4+ and CD8+ T cell responses (Hessel *et al*., 2014).

UNIVERSAL VACCINES BASED ON NA ANTIGEN

Although a vaccine based on NA is also being developed, this antigen alone is considered to have little potency in preventing infection (Johansson et al., 2011).

Nevertheless, it has been shown that NA-specific antibodies restrict viral replication by preventing the release of progeny from infected cells, which limits viral spread and shortens the severity and duration of illness (Powers *et al*., 1996; Kilbourne *et al*., 1968; Murphy *et al*., 1972; Couch *et al*., 1974; Webster *et al*., 1988). It was shown that the administration of N1-VLP particles induced the production of NA antibodies that confer significant cross protection against H5N1 and H1N1 (Wu *et al*., 2012). The immunization of mice with VLPs containing the N1 NA antigen induced the production of an antibody recognizing H1N1 and H3N2 viruses as well as protected against lethal infection by the homologous H1N1 and heterosubtypic H3N2 (Quan *et al*., 2012).

CONCLUDING REMARKS

The development of a universal vaccine against influenza viruses is challenging. Although promising research is in progress, there is still no commercially available vaccine protecting against a wide spectrum of influenza viruses. In 2013, Jesse Goodman, Chief Scientist at the U.S. Food and Drug Administration, predicted that a universal flu vaccine was still 5 to 10 years away. Given the disease-causing potential of the type A and B virus strains, vaccination against both of these virus types is a high public health priority. The ideal universal vaccine will protect against all subtypes of influenza A viruses and both lineages of influenza B. Nevertheless, influenza A and B have significant genetic and antigenic differences, and the construction of a single vaccine that provides protection against both genera seems to be difficult (Subbarao *et al*., 2013). Although, the possibility of designing a vaccine against both lineages has emerged due to recently identified, broadly neutralizing antibodies. It is thought that immunity induced against conserved antigens may not necessarily provide protection against infection, but it could decrease the severity of disease, accelerate virus clearance, and reduce morbidity and mortality during the initial stages of a pandemic outbreak until a strain-matched vaccine becomes available (Epstein *et al*., 2010). It has also been suggested that immunization with such a vaccine could reduce virus transmission from vaccinated, infected animals, thus reducing the size of epidemics. This hypothesis was confirmed in an experimental model in which immunization with a recombinant adenovirus expressing NP and M2, conserved antigens from the influenza virus, significantly reduced the transmission of the virus to co-housed, unimmunized mice (Price *et al*., 2014).

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Conflict of interest statement

The authors declare no competing financial interests.

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