Refining the approach to vaccines against influenza A viruses with pandemic potential

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Abstract

Vaccination is the most effective strategy for prevention and control of influenza. Timely production and deployment of seasonal influenza vaccines is based on an understanding of the epidemiology of influenza and on global disease and virologic surveillance. Experience with seasonal influenza vaccines guided the initial development of pandemic influenza vaccines. A large investment in pandemic influenza vaccines in the last decade has resulted in much progress and a body of information that can now be applied to refine the established paradigm. Critical and complementary considerations for pandemic influenza vaccines include improved assessment of the pandemic potential of animal influenza viruses, proactive development and deployment of pandemic influenza vaccines, and application of novel platforms and strategies for vaccine production and administration.

Keywords

antigenic mismatch; avian influenza; pandemic influenza; prepandemic vaccine; prime boost

Influenza viruses are enveloped viruses belonging to the Orthomyxoviridae family. Influenza viruses are grouped into three types: A, B and C. Of these, influenza A and B viruses are responsible for epidemic human disease. Influenza A viruses are further divided into subtypes distinguished by antigenic properties of the viral surface proteins: hemagglutinin (HA) and neuraminidase (NA). These proteins are critical for entry into host cells and for release of mature, infectious progeny virus and are the main targets of the human immune response [1]. To date, 16 subtypes of HA and 9 subtypes of NA have been isolated from waterfowl and shorebirds, the natural hosts of influenza A viruses [2]. In addition, sequences of two novel influenza-like viruses have been identified in bats and classified as two novel subtypes: H17N10 and H18N11 [3,4]. A segmented RNA genome, error-prone RNA polymerase and the ability to infect many different species contribute to the substantial diversity of influenza A viruses in nature.
Two influenza A subtypes, H1N1 and H3N2, currently co-circulate with influenza B viruses in humans. Vaccination is the most effective strategy for prevention and control of influenza and its associated morbidity and mortality [5]. Strain selection, manufacture and deployment of seasonal influenza vaccines for the control of these viruses have become a routine component of national health programs in many countries. Antigenic drift in the HA protein necessitates annual reformulation of seasonal vaccines to maximize vaccine efficacy. Prediction of the influenza variants that will dominate a given influenza season is a challenging task that is based on global surveillance of circulating influenza viruses [6]. The lead time of this ‘reactive’ approach to control of seasonal influenza is several months. Between 1999 and 2009, four seasonal vaccine formulations selected for implementation in the northern hemisphere failed to adequately match the epidemic strain because a new antigenic variant emerged after the vaccine strain composition decision was made [7]. Mismatch events have occurred at a similar frequency in the southern hemisphere in recent years [8].

The assessment of the pandemic potential of animal influenza viruses is a complex task. There have been four pandemics of influenza in the last century. In addition, interpandemic periods have been punctuated by occasional epidemics caused by viruses with unusual properties, for example, enhanced pathogenicity or transmission in certain subgroups of the population [9]. Furthermore, several avian influenza viruses (AIV) have caused sporadic zoonotic infections in humans [10]. Although human-to-human transmission of these zoonotic viruses has not been efficient, their potential to acquire this property renders them a pandemic threat.

The public health response to the 2009 H1N1 pandemic (H1N1pdm) was rapid and included the development and deployment of monovalent H1N1pdm vaccines. However, production and distribution were not rapid enough to prevent the second wave of the pandemic [11]. Effective control of pandemic influenza may therefore require a different philosophical approach than the established paradigm for control of seasonal influenza viruses. A proactive pandemic vaccination strategy will rely on three critical elements: timely identification of viruses with pandemic potential, proactive development and characterization of vaccines, and development of improved vaccines. This article summarizes new developments and open questions in each of these areas.

**Improved identification of avian influenza viruses with pandemic potential**

Influenza pandemics occur when novel influenza viruses are introduced into susceptible human populations. When such a virus is capable of efficient human-to-human transmission, lack of pre-existing immunity facilitates rapid spread. Novel influenza viruses may be introduced into humans via reassortment between animal and human viruses, as in the case of the 1957 and 1968 pandemic viruses, or via direct zoonotic transmission, as in the case of the 1918 and 2009 H1N1pdm viruses [12,13]. As the natural hosts of influenza viruses, aquatic birds are the source of novel influenza viruses. Influenza viruses have also become enzootic to domesticated animals, most notably poultry and swine [14]. Molecular analysis of the four known pandemic influenza viruses (1918 H1N1, 1957 H2N2, 1968 H3N2 and 2009 H1N1) has revealed the contribution of AIV genes to pandemic viruses [12,13,15–17].
The continued rise in the number of animal and human cases of avian H5 and H7N9 infections and sporadic cases of infection with H6, H9 and H10 subtype viruses are a direct call to action for improved understanding of the public health threat posed by AIV [10].

**Standardized tools for assessment of pandemic potential**

Virologic surveillance in livestock, poultry and wild birds is a critical first step in cataloging circulating AIV, but tools for assessing their pandemic potential are needed to guide preparedness efforts. The development of standardized and objective criteria for pandemic risk assessment has been the subject of much debate. In 2012, the US CDC proposed a standardized influenza risk assessment tool (IRAT) for evaluating the pandemic potential of influenza viruses not currently circulating in humans [18,19]. This framework is based on ten key elements, including a spectrum of factors representing molecular and biological attributes of the virus, attributes of the population and the epidemiology of the virus, which are agreed upon by a panel of experts and weighted to reflect their relative importance. However, the IRAT framework must be updated as new understanding of the molecular determinants of transmission and pathogenesis of AIV emerges. One example is the multibasic cleavage site seen in some H5 and H7 avian influenza HAs that distinguishes highly pathogenic from low pathogenic AIV in poultry and is a virulence motif in mammalian models of influenza [20–22]. Another example is the recent identification of the minimum set of adaptive mutations necessary for airborne transmission of avian H5N1 viruses among ferrets [23,24]. These studies confirmed the importance of receptor binding specificity (an existing IRAT element) and polymerase activity, but also identified the acid stability of HA as a novel factor influencing transmissibility.

Some AIV defy simple classification with regard to pandemic risk assessment. For example, avian H9N2 viruses have been associated with only a handful of mild human infections and may be classified as having only intermediate pandemic risk. However, H7N9, H5N1, H6N1 and H10N8 AIV that have caused severe human disease derived their internal protein genes from H9N2 viruses [25–29]. In another example of such gene constellation effects, an H3N2 variant (H3N2v) virus that was the product of reassortment between circulating swine influenza viruses and the H1N1pdm virus caused mild disease in children in the USA between 2011 and 2012 and has only been detected sporadically in humans since 2012 [30]. Finally, virulence in humans is difficult to predict; for example, zoonotically transmitted avian H7N9 viruses cause no apparent disease in wild birds and poultry, but cause severe respiratory disease in humans [31–33]. Similarly, precursors of the H1N1pdm virus circulated for over a decade without causing apparent disease in swine, yet transmission to humans resulted in the first influenza pandemic of the 21st century [34].

AIV continue to evolve in nature and their diversity presents a major challenge in deciding which strains and subtypes should be targeted for vaccine development. The consequences, for humans, of antigenic drift in animal species are not well understood. Veterinary vaccination is a powerful tool that has been used to control AIV outbreaks in poultry [14]. However, large-scale vaccination of livestock is not affordable in some parts of the world. Furthermore, vaccination may confound serological surveillance efforts for early detection of AIV in domestic poultry unless the vaccine design and serologic detection assay are
directed against different viral proteins. This strategy is referred to as a Differentiating Infected from Vaccinated Animals (DIVA) approach [35]. However, the growing availability of AIV sequence data and increasingly widespread laboratory capacity for molecular surveillance has the potential to abrogate this problem. Finally, suboptimal vaccination via poor vaccination coverage or use of mismatched vaccines may result in nonsterilizing immunity, where morbidity and mortality in poultry are reduced but viral replication still occurs. This may drive viral escape and promote endemicity of escape variants. In this manner, suboptimal vaccination may drive antigenic drift and provide more opportunities for reassortment with human viruses, thereby potentially increasing the chances of zoonotic transmission [14,36–38].

Proactive development & deployment of pandemic influenza vaccines

Mathematical modeling of the spread of a pandemic influenza virus suggests that intervention during the 2 weeks following initial recognition of a pandemic has the greatest potential for an effective response in terms of human morbidity and economic cost [39]. The production of licensed seasonal influenza vaccines takes 5–7 months [40]. There are several opportunities in the existing framework for influenza vaccine production for an accelerated response, which will be a critical consideration in the event of a pandemic (Figure 1). First, preclinical and clinical evaluation of the safety and immunogenicity of candidate pandemic influenza vaccines during the interpandemic period can enhance pandemic preparedness. Second, the creation of a library of vaccine seed stocks may substantially reduce the lead time for vaccine production [41,42]. Third, new technologies such as reverse genetics and synthetic biology can facilitate accelerated production of vaccine viruses [43]. Fourth, deployment of a vaccine prior to a pandemic (prepandemic vaccination) has been proposed to circumvent the delays associated with distribution of a new vaccine in the midst of a pandemic [44]. Such proactive approaches are not without precedent. Several H5N1 vaccines have been licensed in the USA and Europe; stockpiling of these vaccines is now a component of the WHO Pandemic Influenza Preparedness framework [45]. However, the safety and immunogenicity of vaccines against other subtypes would have to be established in clinical trials.

Is an exact match between vaccine virus & pandemic virus necessary?

Experience with seasonal influenza vaccination has demonstrated that antigenic drift, or the gradual accumulation of antigenic changes in the influenza virus surface glycoproteins, has a profound effect on vaccine efficacy [46]. The closer the antigenic match between vaccine strain and epidemic strain, the greater the efficacy of the vaccine. It is important to note that antigenic differences are determined using post-infection ferret antisera and there is increasing evidence that an individual’s immune history is an important determinant of the specificity of their antibody response. Therefore, the data from ferrets following primary infection may not accurately reflect what would happen in influenza-experienced humans [47–49].

A recent meta-analysis of vaccine efficacy studies estimated that mismatched vaccines can protect against laboratory-confirmed influenza with a vaccine efficacy of 60% (95% CI: 44–71) in the case of live-attenuated influenza vaccines (LAIV) and 56% (95% CI: 43–66) in
the case of trivalent inactivated influenza vaccine (IIV) compared with no vaccine [46]. Seasonal LAIV administered to children was superior to trivalent IIV in its ability to protect against drifted viruses not contained in the vaccine [50–52]. In contrast, the efficacy of seasonal IIV was equal or superior to LAIV in adults, possibly because adults were immunologically primed by previous influenza infections and vaccine [53,54]. However, neither LAIV nor IIV were efficacious against the mismatched circulating H3N2 virus in the 2014–2015 season [55]. Because seasonal influenza vaccines provide variable protection against drift variants, their composition is modified after new antigenic variants with epidemic potential are identified.

Antigenic drift will pose a challenge for novel antigens as well. Phylogenetic analysis of avian H5N1 HAs reveals diversification into multiple distinct clades [56] and similar diversity may occur in other AIV subtypes. In the past 3 years, eight new clades of H5N1 viruses, including fifth order subclades, have emerged [57]; it is not clear whether all clades pose equivalent pandemic risk. In the event of a pandemic, an H5 vaccine from a different clade may not prevent infection but may still prevent severe disease and death. It has been estimated that a pandemic vaccine with efficacy as low as 30% would have a substantial impact on limiting morbidity and mortality and would be more cost effective than a reactive pandemic response [58]. Furthermore, recent characterization of pandemic influenza vaccines and advances in immunology suggest that an exact match between vaccine strain and pandemic strain may not be necessary. These include the discovery of conserved epitopes in the influenza HA, an independent contribution of T-cell-mediated immunity to cross-protective immunity, empirical evidence from Phase I clinical trials of cross-reactive serum antibody responses elicited by pandemic vaccine candidates and improvements in vaccine formulations.

**Conserved epitopes can elicit cross-protective immunity**—The identification of a conserved epitope on the HA stalk that is the target of neutralizing antibodies supports the concept that a mismatched vaccine may provide some cross-protection [59,60]. Stalk-reactive antibodies have been detected in the sera of individuals following infection with H1N1pdm or immunization with seasonal influenza vaccine [59,61–63] or an adjuvanted H5N1 split virion vaccine [64].

Cell-mediated immunity elicited against conserved T-cell epitopes can also independently contribute to cross-protective immunity. Although cell-mediated immunity cannot prevent infection, T-cell responses enhance viral clearance and can protect from severe disease and death in preclinical models [65]. Syngeneic T-cell transfer and T-cell depletion studies in vaccinated or influenza virus-infected mice established a role for cell-mediated cross-protection from lethal challenge with heterologous influenza viruses [66–69]. In a human challenge study with an H1N1 virus, the presence of pre-existing cross-reactive influenza-specific CD4+ T cells correlated with protection from severe illness and decreased shedding [70]. In the 2009 pandemic, the presence of cross-reactive CD8+ T cells correlated with protection from influenza virus infection [71]. H1N1pdm and H5N1 vaccines elicited influenza-specific T cells with cross-clade and heterosubtypic reactivity [72,73]. Furthermore, a T-cell response to conserved epitopes in influenza proteins, such as the nucleoprotein, was associated with protection from severe illness [74].

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Pandemic influenza vaccines can elicit cross-protective antibody responses—

Pandemic influenza vaccine candidates have been developed on several platforms and have been found to elicit cross-clade immunity in pre-clinical models and clinical studies. Inactivated and live attenuated H5N1 vaccines were highly effective in ferrets and mice against lethal challenge with homologous and heterologous H5N1 viruses from different clades [75,76]. Similarly, live-attenuated vaccines against H2, H3, H6, H7 and H9 viruses elicited subtype-specific cross-reactive serum antibodies and reduced the replication of homologous and heterologous challenge viruses in mice or ferrets [77–82]. An inactivated monovalent H7N7 vaccine candidate conferred protection from lethal challenge of mice with H7N9 influenza virus [83].

Pandemic influenza vaccines also induce antibody responses against heterologous viruses in humans. An adjuvanted subunit vaccine containing the HA of an avian H5N3 virus elicited serum neutralizing antibody responses against several H5N1 clinical isolates collected over a 7-year period [84]. Adjuvanted inactivated whole virion and split virion H5N1 vaccines elicited neutralizing antibodies against homologous virus and drifted H5N1 viruses representing distinct clades in children, adults and the elderly [85–88]. Furthermore, H1N1pdm vaccine elicited H5N1-reactive memory B-cell responses in the upper respiratory mucosa [89].

Novel formulations & platforms can enhance cross-protective immunity—

Enhancement of cross-protective responses is an ongoing challenge and can be seen as an important step in the development of universal influenza vaccines. Recent studies directly comparing different formulations of pandemic influenza vaccines have demonstrated that the choice of vaccine platform and inclusion of adjuvant can influence the breadth and quality of the immune response [90,91]. Improved vaccine formulations have the potential to elicit protection against a greater breadth of influenza virus subtypes [90,92]. Novel formulations may also elicit protective immunity by other mechanisms, such as antibody-dependent cellular cytotoxicity (ADCC) mediated by non-neutralizing antibodies [93]. As previously stated, there is growing evidence that exposure and immune history can also influence the immune response to influenza vaccines. Serological studies suggest that previous exposure does not constrain B-cell responses to novel antigens in the context of seasonal influenza vaccination [94]. In fact, it has been suggested that administration of a novel antigen preferentially elicits antibodies to conserved epitopes that are shared with existing seasonal influenza viruses [95]; the H1N1pdm vaccine elicited antibodies with broad cross-reactivity in adults [96].

Are pandemic influenza vaccines safe & immunogenic?

Safety—The manufacture of pandemic influenza vaccines poses some unique safety concerns. Manufacturing protocols must be carefully designed and implemented to reduce the risk of exposure to novel viruses. Two strategies proposed to mitigate this risk are the use of less pathogenic viruses of the same subtype and the production of vaccine using reverse genetics-derived viruses bearing a recombinant HA from which known pathogenicity determinants have been removed [97]. Pandemic LAIV (pLAIV) have a number of theoretical advantages over IIVs, including a superior ability to elicit mucosal
and cellular immune responses, needle-free administration and greater yield. Clinical studies in controlled settings have demonstrated that replication of pLAIV candidates was significantly restricted in healthy adults; vaccine virus was shed only at low titer from a subset of vaccine recipients [98–100]. However, because they contain replicating, albeit attenuated virus, there is a possibility that pLAIV virus could reassort with circulating influenza viruses; the outcome of such an event may be a virus with a novel HA and potential for spread in the community [101]. Therefore, pLAIV would only be used when recommended by public health officials and likely only when a pandemic is inevitable. However, proactive generation and evaluation of a library of pLAIV against a range of HA subtypes will allow rapid production of vaccine if needed.

Postmarketing surveillance following the mass vaccination efforts during the 2009 H1N1 pandemic demonstrated that unadjuvanted H1N1pdm vaccines licensed in North America and Europe had comparable safety profiles to seasonal influenza vaccines [102,103]. Several adjuvanted IIV formulations marketed in Europe were also found to be safe. Similarly, pLAIV have demonstrated acceptable safety profiles in small Phase I trials. pLAIVs carrying the HA and NA proteins of avian H5N1, H7N3 and H9N2 viruses elicited only mild, local reactogenicity that was comparable to seasonal LAIVs [98–100,104]. Inactivated H5N1, H7N1, H7N7 and H9N2 vaccines were also demonstrated to be safe in Phase I clinical trials [84,91,105–109]. Together, these data suggest that administration of novel influenza virus vaccines to unprimed hosts is generally safe and well tolerated.

However, rare and unexpected adverse events may not be seen until vaccines are administered to a large population. One formulation of adjuvanted, split-virion, inactivated H1N1pdm vaccine marketed in Europe was associated with an increased incidence of narcolepsy in children and young adults [110–117]. Interestingly, a different formulation of AS03-adjuvanted monovalent H1N1pdm vaccine [118,119] and other monovalent H1N1pdm vaccines were not associated with narcolepsy [102,120,121]. It is possible that differences in antigen processing or adjuvant formulation contributed to the observed differences [119,122]. Host factors could have also influenced the safety outcomes observed with AS03-adjuvanted vaccines; narcolepsy has been associated with genetic loci that influence antigen presentation and T-cell differentiation. Age was also identified as a risk factor for the development of narcolepsy following exposure to H1N1pdm via either immunization or infection [123]. The complexity of the narcolepsy-pandemic influenza issue was recently reviewed [124]; further studies will be necessary to clarify these issues.

Effective control of a pandemic by vaccination will require the rapid development, evaluation and deployment of safe and efficacious products. Novel vaccine targets, platforms and adjuvant formulations offer great promise, but do not have the benefit of the decades of clinical experience of licensed vaccines. Postmarketing surveillance for adverse events provides critical safety data.

Immunogenicity—A clear understanding of the determinants of a protective immune response facilitates the rational development and evaluation of influenza vaccines. The immunogenicity of candidate pandemic influenza vaccines is currently evaluated according to criteria developed by the US FDA and the Committee for Medicinal Products for Human
Use in Europe. The Committee for Medicinal Products for Human Use guidelines recommend that candidate vaccines elicit a serum hemagglutination inhibition (HAI) titer of 1:40 in 70% of vaccinated individuals [125]. Although it is clear that serum antibody correlates with protection from influenza, sole reliance on serum HAI titers for the evaluation of vaccines is problematic for a number of reasons. First, this correlate is only relevant for influenza vaccines that contain the globular head of the HA protein, which mediates hemagglutination activity. HAI assays fail to detect potentially functional antibodies that bind HA stem epitopes or other viral proteins. In addition, serum antibody titers ignore the contributions of cell-mediated immunity, which is required for clearance of virus, and innate immune responses, which have been proposed to play a role in influenza pathogenicity. Finally, the HAI titer is not a robust correlate of protection for LAIVs [126,127].

It is not known whether the licensing criteria applied to seasonal influenza vaccines are appropriate for pandemic influenza vaccines. Different criteria may be appropriate for different populations [128]. Furthermore, there is no known correlate for immunologic priming; this would be important for novel pandemic vaccines, which may not meet the classical criteria of HAI for immunogenicity, but prime for robust responses following administration of a booster vaccine. A critical research priority is therefore the identification and validation of additional correlates of protection for influenza. An attractive approach for the identification of potential correlates is a large-scale, high-throughput analysis of antibody repertoires. Convalescent serum from survivors of AIV infection could be used to probe a library of peptides representing the viral genome (antigenic fingerprinting) in order to identify key viral antigenic epitopes [129]. Furthermore, the development and application of ADCC [93] and cellular immunity assays will also enhance understanding of vaccine-mediated protection.

The H1N1pdm LAIV vaccines developed in the USA and Russia elicited comparable serologic responses to seasonal LAIVs. In Phase I/II clinical trials in the USA, monovalent H1N1pdm LAIV elicited seroconversion rates of 14.9% in adults aged 18–49 after two doses of vaccine [130]. Phase I/II clinical trials conducted with the Russian formulation of monovalent H1N1pdm LAIV elicited similar serum HAI antibody responses. A modest increase in the frequency of virus-specific CD4+ and CD8+ memory T cells was reported in the peripheral circulation of healthy adults [131]. With both formulations, immunogenicity was slightly higher in children below 18 years of age compared with adults. Several adjuvanted and unadjuvanted IIV were also characterized in the USA and Europe and found to be robustly immunogenic. A single dose of unadjuvanted H1N1pdm IIV was immunogenic in all age groups over 3 years of age. Inclusion of an adjuvant in H1N1pdm IIV formulations permitted dose sparing; the equivalent of a quarter dose of HA antigen was comparable in immunogenicity to unadjuvanted formulations [11].

pLAIV for H5, H7 and H9 AIV have shown promise in preclinical studies. The first target of a pLAIV was an H9N2 virus. The vaccine was immunogenic in healthy, seronegative adults; two doses elicited seroconversion in >90% of vaccine recipients [98]. Similarly, LAIVs on two different backbones targeting H7N3 and H5N2 AIV were immunogenic in healthy seronegative adults, eliciting seroconversion rates of >45% in those who received
two doses of vaccine [100,132]. These Phase I clinical trials suggest that pLAIV-bearing novel antigens derived from AIV can elicit serum antibody responses. However, all pLAIV were not equally immunogenic. Two H5N1 pLAIV representing distinct viruses, which had demonstrated great promise in pre-clinical studies, failed to elicit robust serum neutralizing antibody responses in Phase I clinical trials [99]. Recombinant H5 HA and unadjuvanted H5N1 IIV were also poorly immunogenic in humans [133,134]. Notably, however, both LAIV and recombinant HA vaccines primed for a robust response to subsequent IIV [135,136].

The way forward: improved vaccine formulations & platforms

Novel vaccine formulations and combinations of existing vaccine platforms offer great promise for enhanced immunogenicity and expanded breadth of immunity, critical characteristics of an ideal pandemic influenza vaccine.

‘Second generation’ prime-boost strategies

Mass vaccination in response to the ‘swine flu’ outbreak of H1N1 in 1976 provided a clear demonstration of the impact of priming on the immunogenicity of a novel vaccine. A single dose of split or subunit vaccine was immunogenic among older individuals, who had been primed by previous exposure to related H1N1 viruses during their lifetime. However, a higher dose of antigen (administered either as additional doses of vaccine or a single dose of vaccine formulated with more antigen) was necessary to elicit comparable responses in immunologically naive, younger individuals [137–141].

Prime-boost schedules for pandemic vaccines have been evaluated in numerous clinical studies. Two doses of a live attenuated H9N2 prototype vaccine were highly immunogenic in seronegative individuals [98], but this was not typical for other candidate pLAIV [101]. However, the combination of different vaccine platforms demonstrates promise for improving the immunogenicity of prototype pandemic vaccines. This has been illustrated clearly in the evaluation of H5N1 pandemic vaccines. Sequential use of inactivated vaccine following priming with DNA [142,143], recombinant HA [134,136], vectored HA [144], LAIV [135] or inactivated H5N1 vaccine [145–149] elicited robust neutralizing antibody responses. Booster vaccination with an H5N1 IIV could rapidly elicit serum antibody responses, even when the interval between primary and secondary vaccination was >5 years [135,136]. Similar results were found with H7N7 influenza vaccines with an interval of 18–22 months between pLAIV and pIIV [150].

Furthermore, these prime-boost strategies elicited cross-reactive serum antibodies. Primary vaccination with pLAIV, DNA or vectored H5N1 vaccine followed by a homologous H5N1 IIV elicited antibodies with cross-clade neutralization activity [135,142–144,150]. Similarly, a booster dose of H5N3-inactivated vaccine delivered 16 months after primary vaccination elicited cross-clade antibody responses [151,152]. Sequential vaccination with antigenically mismatched vaccines also elicited serum antibody with cross-clade neutralizing activity. In individuals primed with an H5N3-inactivated vaccine, boosting with an inactivated H5N1 vaccine elicited high titers of cross-clade neutralizing serum antibody within 7 days [153]. Administration of a recombinant, baculovirus-expressed HA subunit vaccine primed for
robust cross-clade recall responses upon boosting with an antigenically distinct H5N1 inactivated subvirion vaccine up to 8 years later [134,136].

Finally, sequential administration of antigenically mismatched vaccines may be used to modulate antibody responses to the HA stalk. It has been suggested that sequential administration of antigenically distinct HA antigens can selectively elicit a more robust antibody response to the more conserved, but less immunodominant, HA stalk or stem [96]. These ‘stem antibodies’ often have potent and broad cross-neutralization activity, but are poorly elicited by vaccination with monovalent inactivated vaccines [154]. In contrast, priming with pLAIV or DNA vaccines prior to vaccination with pandemic IIV efficiently elicited high affinity stem antibodies [142,155,156].

These studies suggest that a proactive prime-boost approach to pandemic preparedness should be considered. A booster vaccine could be administered to a vaccine-primed population when a pandemic threat materializes. Prepandemic vaccine priming could potentially be coupled with seasonal influenza vaccination. It has been suggested that while concurrent administration of H1N1pdm and seasonal trivalent IIV did not negatively affect the safety or immunogenicity of either vaccine, sequential administration could slightly attenuate the immunogenicity of inactivated H1N1pdm vaccine [157,158]. The underlying immunological mechanism for this, and whether it would apply to pandemic vaccines containing completely novel HA subtypes, is not known. However, the results of these studies, as well as the Phase I/II evaluations of prototype pandemic vaccines described above, suggest that robust serum antibody responses are achievable despite prior seasonal influenza vaccination. Prime-boost strategies for delivery of pandemic influenza vaccines show promise even in older age groups, in which effectiveness of seasonal influenza vaccines is suboptimal. At least two formulations of inactivated H5N1 vaccines have been shown to prime for a rapid and robust serum-neutralizing antibody response to heterologous inactivated booster vaccine in elderly cohorts [86,159].

New production methods & formulations

Production of an adequate supply of vaccine in a short period of time is a major challenge for a number of reasons, including the need for facilities that meet good manufacturing practice (GMP) standards, a dependence on eggs for production and the lead time required to generate vaccines on existing platforms. The first step is the selection and characterization of a ‘vaccine seed virus.’ This is amplified in embryonated eggs or cell culture to produce antigen. Dependence on chicken eggs for amplification is a critical weakness if the seed virus is pathogenic to and/or grows poorly in eggs. Finally, the vaccine must be formulated, purified and prepared for distribution. Egg-based amplification of vaccine seed stocks can be improved by introduction of mutations that enhance amplification in embryonated eggs without compromising antigenicity [160]. Indeed, there is growing evidence that some of these substitutions, particularly those that influence the stability of the HA, can enhance the immunogenicity of live attenuated avian influenza candidate vaccines in preclinical models [161]. However, adaptive mutations introduced through amplification of influenza viruses in eggs or mammalian cell lines may impair the immunogenicity of the vaccine; therefore thorough characterization of vaccine seed stocks is necessary [162].
Novel platforms that are not dependent on infectious virus can circumvent the need for egg-based amplification and mitigate risk to humans during vaccine production. Proof of principle for cell-based amplification of virus for LAIV and IIV has been established. A vaccine for seasonal influenza produced in a mammalian cell line, Flucelvax®, was licensed by the FDA in 2014 [5]. Mammalian cell culture derived H5N1 vaccines have also been shown to be well-tolerated and immunogenic [163–166]. Other candidates, including an H7N9 vaccine, show promise in preclinical models [167]. These vaccines are likely to be safe for individuals who are allergic to eggs. Furthermore, recombinant influenza virus proteins have been expressed in a variety of cell lines, including insect cells and plant cells, as soluble protein or noninfectious virus-like particles. This approach offers the opportunity to modulate immune responses by genetic engineering of the influenza virus antigens.

DNA vaccines are another alternative to traditional influenza vaccine platforms; they are delivered subcutaneously or intramuscularly and rely on host cells at the injection site to express and present viral proteins to the immune system [168]. DNA vaccines can be produced in large quantities and are very stable, facilitating the stockpiling of such vaccines. Alternatively, vaccine technologies that deliver viral antigens via replication-defective vectors such as adenovirus or modified vaccinia virus Ankara have been demonstrated to be safe and immunogenic in humans [144,169]. These platforms share the advantages of a pLAIV in that they can stimulate robust cell-mediated and antibody-mediated immune responses without the risk of introduction of novel pandemic influenza genes into seasonal influenza viruses. Influenza vaccine candidates using other vectors, including adenovirus-associated viruses [170], alphaviruses [171], Newcastle disease virus [172] and vesicular stomatitis virus [173] demonstrate promise for delivery and expression of influenza antigens in a variety of preclinical models, but have not yet been evaluated in humans.

Finally, inclusion of adjuvants in vaccine formulations has great potential to enhance the magnitude of serum antibody responses, facilitate the stimulation of mucosal and cell-mediated immunity, and to enhance the longevity of serum antibody responses following vaccination [174]. Inclusion of MF59 in subunit H5N1 vaccine formulations enhanced the quality and breadth of the serum antibody responses [92]. Furthermore, inclusion of adjuvant permits dose sparing, where a lower antigen dose can elicit a response sufficient to meet immunogenicity criteria, thereby increasing the number of doses of available vaccine in the event of a pandemic. Two formulations of pandemic vaccine that were deployed in efforts to control the 2009 H1N1 pandemic were adjuvanted with AS03, an oil-in-water emulsion, and were found to permit dose sparing and to enhance the magnitude and persistence of serum HAI titers relative to those elicited by unadjuvanted vaccine. However, the association with narcolepsy in some countries illustrates the safety and regulatory challenges of novel vaccine formulations and platforms for pandemic influenza vaccines.

**Conclusion**

A proactive approach to pandemic influenza vaccination requires international collaboration and a shared commitment to surveillance, research and characterization of pandemic vaccines. The foundation has already been laid for such an approach and much progress has been made over the past decade. The establishment of a formal pandemic influenza pre-
paredness plan in the 1970s illustrates a shift toward proactive pandemic preparedness in the USA. This plan called for increased support for influenza research, established a framework for interagency collaboration, and recommended increased surveillance of influenza viruses in animal populations and has since undergone periodic refinement [175]. An increasing recognition of the fundamental overlap between veterinary and human public health is embodied in the ‘One Health’ initiative, formally launched in 2011, and is illustrated by a growing number of countries with influenza surveillance programs in animal populations [176]. International collaboration of experts has resulted in the refinement of pandemic preparedness plans; notable among these is the WHO’s Pandemic Influenza Preparedness framework, which has provided a framework for sharing of influenza virus sequence data and access to diagnostic tests and vaccines since 2011 [45]. New tools for pandemic risk assessment and novel platforms and strategies for vaccination are paving the way for improved pandemic preparedness.

Future perspective

In the next 5–10 years, it is likely that AIV that are already enzootic in Asia will become established in avian species in other parts of the world. This will prompt a re-examination of agricultural practices, a greater emphasis on biosecurity and development of vaccines for veterinary and human use. In the near term, discussions will focus on the pros and cons of novel vaccine formulations, especially adjuvanted and vectored vaccines, and schedules with sequential use of vaccines based on different platforms. In the longer term, newer technologies and approaches to develop vaccines that induce broader immunity will progress, with the ultimate goal of a universal influenza vaccine. However, the regulatory path to licensure of vaccines that do not mediate protection through induction of antibodies to the HA head is not yet clear. Successful implementation of novel or, ultimately, universal influenza vaccines will require the development of assays to measure correlates of protection for such vaccines, with parallel advances in regulatory science.

References


EXECUTIVE SUMMARY

Timely pandemic response requires a philosophical shift from reactive to proactive pandemic preparedness

- The established paradigm for seasonal influenza vaccination may not be appropriate for pandemic influenza vaccination.
- Pandemic preparedness plans have been established by national and international agencies.
- Pandemic preparedness will require a combination of surveillance in wildlife and livestock animal populations, a new approach to development of vaccine and an enhanced understanding of the mechanisms by which vaccines protect from influenza.

Emerging frameworks permit risk assessment of the pandemic potential of avian influenza viruses

- Influenza viruses with pandemic potential are derived from animal influenza viruses.
- The discovery of molecular signatures associated with human transmission and pathogenicity of avian influenza viruses has permitted the development of standardized tools for assessment of pandemic risk.

Prepandemic development & deployment of vaccines is a promising approach

- A proactive pandemic preparedness strategy relies on development and characterization of pandemic vaccines.
- Pandemic vaccines could be either stockpiled for rapid deployment in the event of a pandemic or deployed during the prepandemic period.
- Pandemic vaccines developed on a number of platforms are safe and immunogenic in humans and elicit broad cross-protection in preclinical models.
- Some pandemic vaccines have been shown to elicit broadly cross-reactive serum antibody in humans.

Novel vaccination platforms & deployment strategies facilitate the development of promising pandemic influenza vaccines

- Prime-boost strategies combining different vaccine platforms and formulations can enhance immunogenicity of pandemic vaccines.
- Emerging vaccine technologies have the potential to facilitate rapid and safe production of pandemic vaccine.
Figure 1. Timeline for influenza vaccine development and production, identifying steps where pandemic influenza vaccine development can be accelerated

Seasonal influenza vaccines are formulated using already licensed technology and processes. The timeline for this existing framework, from strain selection to seasonal vaccine deployment, is 5–7 months. Steps in this process at which development and deployment of pandemic vaccines may be accelerated are indicated by arrows.