Review Article

Vaccines for Pandemic Influenza. The History of our Current Vaccines, their Limitations and the Requirements to Deal with a Pandemic Threat

Alan W Hampson,[1,2] *BSc, MSc, Hon MD*

Abstract

Fears of a potential pandemic due to A(H5N1) viruses have focussed new attention on our current vaccines, their shortcomings, and concerns regarding global vaccine supply in a pandemic. The bulk of current vaccines are inactivated split virus vaccines produced from egg-grown virus and have only modest improvements compared with those first introduced over 60 years ago. Splitting, which was introduced some years ago to reduce reactogenicity, also reduces the immunogenicity of vaccines in immunologically naïve recipients. The A(H5N1) viruses have been found poorly immunogenic and present other challenges for vaccine producers which further exacerbate an already limited global production capacity. There have been some recent improvements in vaccine production methods and improvements to immunogenicity by the development of new adjuvants, however, these still fall short of providing timely supplies of vaccine for all in the face of a pandemic. New approaches to influenza vaccines which might fulfil the demands of a pandemic situation are under evaluation, however, these remain some distance from clinical reality and face significant regulatory hurdles.

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Introduction

The ongoing epizootic of avian influenza due to A (H5N1) viruses, the growing count of associated human fatalities, and the fear that this may be the forerunner to a severe human pandemic have focussed new attention on the status, and in particular the shortcomings, of our current human influenza vaccines. It is now over 60 years since the demonstration that influenza viruses could be readily cultivated in the allantois of embryonated hens' eggs[1] and the subsequent application of this method of cultivation to prepare inactivated virus vaccines that were protective against infection.[2] Remarkably, despite the developments in viral vaccines for other illnesses including living attenuated vaccines, the application of large-scale cell culture and of recombinant technology,[3,4] there have been only modest improvements in the influenza vaccines. The bulk of those that are used currently are still inactivated virus vaccines prepared from egg-grown viruses. This, together with unique difficulties encountered with attempts to produce effective human A(H5N1) vaccines, has lead to grave concerns regarding the global vaccine supply in the event of a pandemic.[5]

The History of Influenza Vaccine Development

Early influenza vaccines were rather crude, impure preparations manufactured by methods such as adsoption to and elution from chicken erythrocytes and high speed centrifugation or freeze-thawing of virus-containing allantoic fluid harvests.[6] These induced a high incidence of both local and systemic reactions,[7] particularly in infants and children.[8] It was generally considered at that time that this was due to an inherent 'toxicity' of inactivated influenza virus.[7,9] However, the development of more highly purified products by the introduction of the continuous-flow zonal ultracentrifuge demonstrated that much of the reactogenicity had been due to impurities rather than the virus itself.[10,11] Nevertheless, infants and young children still displayed a high rate of systemic reactions to these more highly purified vaccines.[12,13]

Prior to the routine use of improved purification methods, it had been demonstrated that a vaccine prepared using disruption or 'splitting' of the virus with Tween-80 and ether was largely devoid of systemic reactivity compared with the standard vaccine, although, it was unclear whether

[1] Interflu Pty Ltd
[2] Honorary Senior Research Fellow, School of Applied Sciences and Engineering, Monash University
Address for Correspondence: Dr Alan William Hampson, 6 Monica Street East Doncaster Victoria 3109, Australia.
Email: Interflu@bigpond.net.au

the splitting *per se* or other aspects of the additional processing involved were responsible for the reduction in toxicity.[14] Because ether represents an explosion risk in large-scale manufacturing, various detergent treatments were introduced for the preparation of split vaccines. Sodium deoxycholate was adopted for preparation of a commercial vaccine,[15] based on animal studies demonstrating that treatment reduced pyrogenicity of influenza 'vaccines' in experimental animals.[16] However, the vaccines used in these animal studies had not undergone an inactivation step, hence, there is a real possibility that the demonstrated reduction in pyrogenicity simply represented detergent inactivation of the virus. Nevertheless, deoxycholate-treated vaccines were shown to have reduced reactogenicity in both adults and children.[15,17,18] In addition, it is known that deoxycholate dissociates bacterial endotoxins,[19,20] a common contaminant of egg-grown influenza vaccines[21] and a likely contributor to residual pyrogenicity for infants and young children in purified whole virus vaccines, and this may contribute to the observed benefit.

Shortcomings of Split Inactivated Virus Vaccines

While split vaccines demonstrated similar immunogenicity to whole virus vaccines in immunologically 'primed' individuals[22] studies conducted in children at the time of the perceived pandemic threat from swine influenza in 1976, and then with the re-emergence of the H1N1 subtype in 1977, demonstrated the inferiority of split vaccines compared to whole virus preparations for immunising individuals who had not been immunologically primed by exposure to the same viral subtype and also demonstrated the need for 2 vaccine doses as would be the case in a future pandemic.[23-25] This together with observations with influenza B vaccine in children[13] and response studies in mice[26] foreshadowed potential problems in vaccinating against a future pandemic. However, these observations went largely unheeded for the next 2 decades.

Of the 2 immunologically distinct types of influenza viruses, A and B, responsible for major outbreaks and severe disease in humans, only influenza A has been known to be associated with pandemic influenza. Influenza viruses have 2 exposed glycoprotein antigens on their surface, the haemagglutinin (HA) which is the major antigen, and an enzymically active neuraminidase (NA). The haemagglutinin is responsible for attachment to cell receptors to initiate infection and the neuraminidase has a role in release of the virus from the cell by receptor removal. It is generally accepted that immunity to infection in humans, particularly that acquired by vaccination with the current inactivated virus vaccines, correlates closely with the level of circulating antibodies against the viral haemagglutinin;[27,28] it is type and sub-type specific and also largely strain specific.[29] For both influenza A and influenza B the 2 surface antigens display gradual, ongoing antigenic variation, referred to as *antigenic drift*, which allows the viruses to escape immunity acquired through infection or vaccination.[29] This necessitates global surveillance to track these antigenic changes and twice yearly updating of vaccine formulations to provide effective vaccines against seasonal influenza.[30]

In addition, influenza A viruses with 3 quite antigenically distinct haemagglutinins and 2 distinct neuraminidases have circulated in the human population since laboratory studies commenced and the influenza A viruses have been divided into subtypes based primarily on their HA proteins.[31,32] Last century saw the appearance of new influenza A HA subtypes in the human population in 1957 (H2 subtype) and 1968 (H3 subtype) which were associated with pandemic influenza and there is evidence that the 1918-19 Spanish Influenza pandemic, and possibly those late in the nineteenth century, were also associated with the emergence of new HA subtypes. This is referred to as *'antigenic shift'*[33] and it is known to occur when an influenza A virus emerges in the human population with a new HA, with or without a new NA, derived from an avian influenza virus. While there may be some evidence of weak, short-lived cross-subtype immunity in humans following infection,[34] there is no evidence that current inactivated vaccines confer such hetero-subtypic immunity.

Supply Issues for Pandemic Vaccines

The whole process of preparing egg-grown influenza vaccines is a lengthy one, taking up to 6 months or more to meet the demand for seasonal influenza vaccine.[35] Once the antigenic characteristics required for a vaccine virus have been established based on the World Health Organization (WHO) surveillance programme,[36] a suitably qualified strain must be selected and adapted to provide a satisfactory yield. For influenza A viruses, this is currently achieved by a long-accepted *ad-hoc* genetic reassortment involving co-infection with a laboratory strain of virus.[37] On occasions, the availability of a suitable strain or high-yield reassortant has proven limiting for vaccine availability.[38] However, efficient 'reverse genetics' procedures using cloned DNA copies of the virus gene segments to construct directed reassortants now offer several potential advantages.[39] Reagents, including a calibrated reference antigen and a hyper-immune serum against highly purified haemagglutinin from the vaccine strain, must be prepared for vaccine standardisation which is based on an immunological assay;[40,41] calibration of the reference antigen is performed by collaborative assays carried out by international regulatory bodies and this process may, on occasions, contribute to delays in vaccine availability. Additional limitations on seasonal vaccine availability are

imposed by the need to plan 6 months ahead for availability of sufficient numbers of embryonated eggs with the correct characteristics, the fact that egg-handling capacity is usually not readily scalable, and that isolation and passage of influenza viruses in eggs can adversely alter their immunogenicity.[42]

For a pandemic vaccine, particularly should the cause be an H5N1 virus, the shortcomings of the current vaccines and their production processes would be further amplified. Recent estimates place global production capacity for influenza vaccines at around 300 to 350 million doses of trivalent vaccine, at the standard dose of 15 μg of HA antigen per strain over a 6 to 12 month period.[5,43] This would translate to around 1000 million doses of a monovalent vaccine or sufficient to fully immunise 500 million people. However, it must be remembered that past pandemics have spread essentially worldwide within around 6 months and it may be anticipated that a future pandemic would travel at least as quickly.[44] The currently circulating H5N1 viruses, considered as a potential pandemic precursor, are highly pathogenic for domestic poultry and for embryonated chicken eggs, a property conferred by the multibasic cleavage site in the viral haemagglutinin.[45] The viruses also represent a threat to vaccine production staff; hence, it is not feasible to produce egg-grown vaccines without modifying the haemagglutinin using reverse-genetics.[46] However, the use of this technology has intellectual property implications and may also impose additional regulatory requirements.[47]

The prospect of increasing pandemic vaccine supplies by the use of a reduced antigen content vaccine was demonstrated in clinical trials of vaccines against the re-emergent H1N1 influenza in 1977[48] and with alum adjuvanted formulations of H2N2 and H9N2 whole virus vaccines.[49] Unfortunately, early human trials with an adjuvanted vaccine,[50] and with a recombinant antigen vaccine,[51] both demonstrated poor responses to the H5 haemagglutinin suggesting that the human response to this subtype may be atypical. In addition, measuring the serological response to H5 has been complicated by the apparent insensitivity of the conventional haemagglutination-inhibition test for H5 antibody.[52] The poor response to the H5 HA has subsequently been borne out in trials with conventional split virus vaccines[53,54] although the immunogenicity of a whole virus vaccine, as shown earlier for the H1N1 subtype, does appear more acceptable.[55] Adding alum adjuvants to these vaccines has not produced major improvements[54,55] while the recently registered oil in water adjuvant MF-59 did display significant enhancement of antigenicity.[56] Therefore, the probability of formulating effective lower antigen content vaccines seems unlikely. In fact, we may face the prospect of needing to increase antigen content. Yet a further impediment to H5N1 vaccine production is the poor yields, reported by vaccine manufacturers, for the available vaccine strains prepared by reverse genetics.[57]

Clearly, our current pandemic preparedness and response is compromised by this restricted capacity to produce sufficient vaccine to meet the probable global demand. This also raises ethical issues regarding equity of supply for developing countries[43]and the responses that this may provoke.[58] Even if the capacity to produce current vaccines was greatly increased, cost may still represent a barrier to access for many populations.[5] One short-term response to the H5N1 threat, by both WHO and national governments, has been to stockpile vaccines prepared from available H5N1 isolates;[59-61] however, there are currently multiple lineages of antigenically diverse H5N1 viruses circulating[62,63] and the importance of a close antigenic match has been repeatedly demonstrated with seasonal influenza vaccines.[64] Nevertheless, a contributing factor to the severity of pandemic influenza is certainly the immunological naivity of the population at large and of individuals. Cross-reactivity of antibodies to the various clades of H5N1 have been demonstrated in human trials, particularly with adjuvanted vaccines,[56,65] and the potential value of antigenic priming for subsequent vaccination against an antigenically drifted H5 variant has recently been reported,[66] which is similar to results obtained for H3N2 subtype vaccination in immunologically naïve infants.[67] The potential value of either stockpiling or priming the population[61] in the face of a pandemic threat should not be underestimated.[68] However, as demonstrated by the ill-fated 'swine influenza' vaccination programme undertaken in the USA in 1976, there are a number of legal and ethical issues associated with vaccination ahead of ongoing human-to-human transmission of an influenza virus. In particular, the potential of litigation for adverse events is something that needs to be addressed by national authorities.[69,70] In addition, neither stockpiling nor pre-pandemic vaccinations will be an option if a subtype, other than H5, should appear without warning as in the 1957 and 1968 pandemics. Recent human infections with H7 and H9 influenza viruses[71] and earlier studies showing serological evidence of human infection with a number of avian influenza subtypes highlight such a possibility.[72]

Recent Improvements in Influenza Vaccines and their Manufacture

What then, is required to rectify the deficiencies of our current vaccines and what progress have we made to date? There are a number of features that would be highly desirable in an influenza vaccine for both seasonal and pandemic use including simplified, more rapid production, lower cost and ability to induce broad long-lasting protective immunity, preferably with heterosubtypic protection.

Although seasonal influenza vaccines play a valuable role in protecting individuals, particularly those at high risk of serious illness, there have been attempts to overcome the reduced effectiveness due to immunological senescence in the elderly,[73] and the effects of ongoing antigenic drift,[64] by the use of immunological adjuvants.[74-79] While studies in immunologically naïve animals usually show a significant adjuvant effect, the results in an immunologically primed human population have been marginal for seasonal influenza vaccines.[74,79] The potential benefits of adjuvants in a pandemic situation are more akin to the animal studies and this is where they could prove valuable, possibly reducing the required antigen content by 4-fold or more for H5N1 vaccines and improving the breadth of immunity.[56,65] However, while the antigen-sparing potential of new adjuvants may significantly increase vaccine supply using egg-grown antigens, this alone will not be sufficient to ensure global pandemic availability in a timely fashion, nor does it seem likely that it will reduce vaccine cost. The capacity to produce the adjuvants in sufficient quantity, and their cost, will become an important consideration.

The potential benefits of developing cell culture based influenza vaccines, particularly in the pandemic context, have been recognised for many years.[80] Manufacturers have evaluated a variety of cell lines for this purpose[81,82] and several vaccines for seasonal influenza are close to commercialisation. In addition, recent encouraging results have been reported for the large-scale growth of H5N1 virus in Vero cell culture.[83] There is no doubt that cell culture overcomes a number of the difficulties associated with egg-grown vaccines including bacterial contamination, bio-containment of viruses pathogenic for production staff, rapid start up of production and probably, ease of scalability. Nevertheless, production capacity will still be constrained by economic considerations and cost will remain an important limitation for widespread access to vaccine. Another very promising approach to improved vaccine production, under development for several years[84] and is now close to fruition,[85] is the use of a baculovirus expression system to produce recombinant viral haemagglutinin in insect cell culture. This method has been shown to produce immunogenic haemagglutinin antigen for a wide range of influenza viruses.[86] It is claimed that trivalent vaccines containing 45 µg of haemagglutinin antigen, 3 times the normal vaccine level for each strain, are more immunogenic than standard egg-grown vaccines. Hence, they may provide broader protection than standard vaccines,[87] and that "*these doses are well within the production capacity of the system at an economically and logistically feasible scale*".[85] Although early trials with an H5 haemagglutinin vaccine[51] demonstrated poor immuno-genicity, it was subsequently found that the results were essentially the same as those for conventional split egg-grown vaccines. The claimed economics and logistics of production, together with the fact that production neither requires modification of the H5 haemagglutinin, nor handling large quantities of living virus, offer substantial potential advantages. This should be further enhanced if the vaccine could be formulated with one of the newer adjuvants.

Living attenuated influenza vaccines based on cold-adapted master strains have been in use for some time in Russia[88] but only more recently in the USA[89] and have been shown to give improved breadth of protection than inactivated split virus vaccine against drifted influenza strains.[90] To date, the vaccines have been produced by growth in eggs and while they yield more vaccine doses per egg than inactivated vaccine, production capacity and cost are adversely affected by the current regulatory requirement for the use of specific pathogen free eggs for manufacture.[89,91] Thermal stability of the vaccines and some safety aspects have been identified as requiring further attention.[92] The cold-adapted phenotype can be quickly and reproducibly engineered into seasonal or potential pandemic vaccine strains by reverse genetics and a candidate H5N1 vaccine has demonstrated broad immunity in animals.[93] It remains, however, to be demonstrated that this will be the case in humans. There are perceived risks in pre-pandemic administration of living vaccines containing novel haemagglutinin subtypes to humans as this might potentially lead to a reassortment event and generation of a pandemic virus. Therefore, such use needs to be carefully controlled. There also remain regulatory concerns regarding the generation of vaccine strains by reverse genetics in some jurisdictions.[91] Under current constraints, living attenuated vaccines have had little impact on control of seasonal influenza and do not show immediate promise for pandemic control. However, the relaxation of the regulatory requirement for specific pathogen free eggs or, alternatively, a move to cell culture-based production, could significantly alter this situation.

A Universal Vaccine – The Ultimate Goal

Beyond these more immediate potential improvements to influenza vaccines and their production, there are a range of other longer-term options under evaluation and development. Induction of effective levels of anti-haemagglutinin immunity by delivery as a plasmid DNA vaccine has been claimed[91] and, while the production of DNA may be both rapid and inexpensive, economical delivery systems have yet to be described. The potential for use of plant cells for influenza virus haemagglutinin vaccine production has also recently been reported.[94,95] Regardless of the progress with conventional surface antigen vaccines, the ultimate quest for influenza virologists is a vaccine that will not only protect against antigenic drift but also against the antigenic shifts in influenza A that are associated with

pandemic influenza. This would require a vaccine to be more effective than the natural immunity acquired by infection as it appears that there is only limited heterosubtypic immunity induced by infection in humans.[34] It has been proposed that such vaccines could exploit either cell-mediated immunity (CMI)[96] or antibody-based[97] immunity directed against highly conserved regions of the viral proteins in influenza A viruses. The principal targets for a cell-mediated approach are a number of epitopes on the nucleoprotein and matrix (M1) protein which are highly conserved across human influenza A viruses.[96,98] However, while CMI alone may protect against severe illness and death, it will not protect against infection.

The favoured targets for an antibody-based vaccine are conserved regions on the haemagglutinin, which are found on the HA2 stem of the molecule or around the loop region where proteolytic cleavage takes place, and the ectodomain of the influenza A virus M2 protein (i.e., that short region of 23 amino acids on the outer viral surface) which occurs in small copy numbers in viral particles but in larger numbers on the surface of infected cells.[97,99] Currently, the greatest attention and progress appears to be focussed on vaccines based on the M2 ectodomain (M2e) employing a variety of constructs, adjuvants and delivery systems,[97,100] including M2e-hepatitis B core antigen[90,99] and flagellin constructs[101] and virus-like particles.[102] Clinical trials of at least 2 M2e vaccines are currently in progress;[103] however, animal studies have demonstrated that, as for CMI-based vaccines, M2e vaccine prevents severe illness and death but not infection and this will present both difficulties in evaluation of clinical efficacy and regulatory hurdles.[91] It may be that a vaccine combining the conserved epitope approach with conventional surface antigens of contemporary circulating viruses may represent the best answer to these difficulties. Nevertheless, for these highly mutable viruses the potential effects of increased immunological pressure on epitopes that have been relatively conserved to date remain unknown.

Regulatory Processes and Pandemic Vaccines

Regardless of other factors, a key element in the availability of pandemic vaccines, both in the short-term and the long-term, will be national regulatory agencies and regulatory processes. Vaccines for annual seasonal influenza present a unique regulatory problem due to the regular updating of the vaccine strain composition.[104] While licensing processes have been adapted to meet this difficulty, they are far from uniform across jurisdictions; this means that vaccine shortages can go unfilled, as occurred in the USA in 2004-2005.[105] The USA has recently published a guidance document that outlines both the 'traditional approval' and the 'accelerated approval' process for new seasonal influenza vaccines, previously not licensed in the USA to improve seasonal vaccine supply in the US market.[106]

However, it has become clear that pandemic influenza vaccines will differ by more than simply the virus strain(s) compared to those used for seasonal influenza, that this will impact on licensing and will pose additional challenges for regulators.[104] To deal with this regulators have developed pandemic vaccine licensing strategies. The European Agency for the Evaluation of Medicinal Products (EMEA) adopted a strategy based on a 'mock up' dossier, in which vaccine formulation, safety and immunogenicity are determined for a 'pandemic-like' vaccine then, for the pandemic vaccine, only the virus strain would need to be updated and licensing quickly achieved;[68,107] nevertheless issues such as individual national requirements for labelling and product leaflets may still contribute to delays.[108] More recently the EMEA has provided additional guidelines to cater for the potential use of a pre-pandemic vaccine in the event that national authorities may wish to conduct pre-emptive vaccination in the face of a potential pandemic, but prior to a pandemic being declared.[109,110] The USA has also recently published guidelines, a series of 'non-binding recommendations', for the licensure of pandemic influenza vaccines.[111] For vaccines made by processes currently licensed for seasonal vaccines in the US, these describe the process for changing rapidly from the currently-licensed seasonal vaccine to a new pandemic vaccine by supplementing the existing license. For new vaccines, made by a process not currently licensed in the US, they define pathways for both traditional and accelerated approval approaches similar to that for new seasonal vaccines. Accelerated approval allows for evaluation based on biological indicators likely to demonstrate effectiveness such as the immune response to the vaccine, however, the difficulty in measuring the immune response to H5N1 viruses[52] may require some modification to the usual immunogenicity test requirements.

Eventually, our capacity to respond effectively to the pandemic threat will require the introduction of new vaccines. These will require new technologies to prepare the viral antigens, novel adjuvants to improve immunogenicity, new delivery such as DNA vaccines or new approaches such as a universal influenza vaccine.[112] Regulatory requirements for new vaccines have traditionally been very demanding[105,113] and those for biotechnology-derived vaccines[114] and new vaccine adjuvants[115] are still under development. There will be a need to balance carefully the risks of rare adverse events against the potential life-saving benefits of pandemic vaccines. Otherwise, the cost of undertaking the necessary development and clinical trials to meet stringent regulatory requirements may prove a serious disincentive for vaccine manufacturers.[112,116]

Conclusions

The WHO has formulated a 'Global Pandemic Influenza Action Plan' to increase vaccine supply which requires an investment of 3 to 10 billion US dollars and sustained commitment over a period of 5 to 10 years.[117] But the WHO has recently announced that the global influenza vaccine supply could achieve 4.5 billion pandemic courses by 2010.[118] However, this appears to be based on the assumption of universal access to the most favourable production and formulation technologies and ability to pay for the final product, something that is highly desirable but yet to be achieved. Also, we must not forget that the production and administration of vaccine will be a race against the spread of the pandemic, particularly if there has not been any prior development and assessment of candidate strains of the viral subtype involved.[119,120] Regardless of recent progress, the ability to respond globally and equitably to a future pandemic will require much more rapid and high-yielding vaccine production capacity than currently available, preferably at a much reduced cost and this will be influenced by the regulatory environment; needle-less administration could also be a distinct advantage.[121] The development of a truly universal vaccine, protective against all influenza A subtypes, would present the potential opportunity for both reducing the threat of a pandemic and the impact of seasonal influenza.

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