

Vaccines for an influenza pandemic: scientific and political challenges

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So far, most published results from clinical trials using various avian influenza virus vaccine formulations have been disappointing. Should the pandemic strike, we still do not have the ability to provide an efficacious pandemic vaccine in time and in sufficient quantities for the world. The H5N1 enzootic could potentially give rise to a pandemic at any time. Transcontinental air traffic could seed the pandemic virus to most corners of the globe within a few weeks/months. We still have a unique window of opportunity to stimulate and support academia and the pharmaceutical industry to accelerate the urgently needed vaccine research. The political inertia is surprising, particularly as politicians, if and when a pandemic eventuates, will be asked why,

despite repeated warnings, they did not take appropriate action in time. It is a governmental obligation – and not that of the WHO or the pharmaceutical industry – to protect their nationals. Moreover, when the poorer nations of this world realize that equitable quantities of the scarce supplies of vaccines, drugs and medical essentials will not come their way, the post-pandemic international scene will be one of even more deep distrust for many years. This scenario is not acceptable.

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Introduction

It is now nearly 40 years since the world's last influenza pandemic. The 1997 Hong Kong incident of H5 N1 avian influenza virus with associated human infections and death of six of the 18 confirmed cases raised a worldwide concern that a new pandemic was imminent. This episode showed unequivocally for the first time that avian influenza could be transmitted from infected poultry to man and cause serious systemic illness and death. The reappearance of highly pathogenic H5N1 in poultry in 2003, and the subsequent zoonotic cases in Asia, Europe and Africa have heightened the perceived risk of a pandemic. So far only limited human-to-human spread has been convincingly documented in a few cases.^{1,2} Although the apparent case-fatality rate is extremely high (approximately 60%), it may be an overestimate as less severe clinical cases may have gone unnoticed, although it has been claimed that this may not be the case.³ With close to 300 reported human cases of H5N1 avian influenza since late 2003, the situation today is causing alarm.⁴ The virus is genetically unstable and has evolved as a number of distinct genetic and antigenic clusters, complicating the selection of strains for clinical trials.^{5–7} In addition, the host range

appears to have widened.⁸ Moreover, a small number of human cases of H9N2 in Hong Kong and China, and in recent years zoonoses with the H7 subtype have also raised concern, especially as limited inter-human spread has been documented.^{9,10}

To initiate infection the haemagglutinin (HA) of avian influenza viruses preferentially binds to epithelial cell sialic acid receptors with a terminal α -2,3-galactose residue, whereas human strains prefer an α -2,6-galactose linkage. It has now been reported that avian receptors can be found only in the lower respiratory tract in man, possibly explaining why zoonotic cases up until now have been few.^{11–13} Structural studies have demonstrated that just one or two amino acid substitutions at the receptor-binding region of the HA could change the receptor preference, and this has been seen in the case of occasional isolates of H5N1 viruses from human cases.¹⁴ The adaptation of avian influenza to the terminal α -2,6-galactose receptor is presumably the first of several steps in the transition to a pandemic virus followed by others, for example, involving the NS1 and polymerase genes adapting the virus to efficient replication in human cells, and facilitating sustained human-to-human spread.¹⁵

Pandemic vaccines

About half a century's use of inactivated vaccine for seasonal influenza has provided clear evidence that serum antibodies against the HA antigen are associated with protection against illness and death.¹⁶ The current global annual vaccine output is approximately 350 million doses of trivalent doses containing 15 µg HA of each strain and egg-based production cannot easily be scaled up in an emergency, as access to quality-assured embryonated hens' eggs cannot be secured on short notice.¹⁷ Vaccine production based on cell culture is both easier to scale up and has the potential to maintain the genetic and antigenic authenticity of the seed virus, something which may be lost during growth in eggs.¹⁸

This article will discuss some of the recent human clinical trials and for the most part will not refer to animal studies. Table 1 shows an overview of clinical trials currently under way or recently completed. As of 17 October 2006, the International Federation of Pharmaceutical Manufacturers & Associations (IFPMA) listed 37 trials relevant for pandemic preparedness. It should be noted that not all trials are registered in this particular database (<http://www.ifpma.org/Influenza/index.aspx?42>). The greatest recent interest has been on vaccines for H5 influenza both because of the zoonotic outbreaks and in view of early studies, which indicated that the H5 HA is poorly immunogenic in man.

Subvirion vaccines

Currently the great majority of vaccines against seasonal influenza are subvirion vaccines, either split-product or purified surface antigen. Trials using non-adjuvanted egg-grown

subvirion H5N1 vaccine or recombinant H5 HA both demonstrated that very high antigen doses (90 µg HA) given twice were required to elicit a reasonable immune response.^{19,20} For the egg-grown vaccine such high doses are clearly unacceptable in view of the difficulty of satisfying a pandemic demand. However, the well-tolerated recombinant protein vaccine, probably as an adjuvanted or virosomal formulation, offers some promise as scale-up procedures for recombinant proteins are considered far more feasible than egg-grown or even cell-grown vaccine production.

Adjuvanted vaccines

The use of adjuvants will most certainly be required to obtain satisfactory responses at acceptable antigen levels. Adjuvants have so far been used in 30 of the 34 inactivated virus vaccine trials registered by the IFPMA, mostly using non-proprietary aluminium salts. Overall, whole virus vaccines appear to perform better than subvirion vaccines when formulated with aluminium adjuvants. In a study using two doses of a putative pandemic vaccine containing H2N2 whole virus adjuvanted with alum it was shown that as little as 1.9 µg HA gave a seroprotection rate of 82% in immunologically naïve subjects, compared to 98% for a standard split and non-adjuvanted 15 µg HA vaccine.²¹ Similar results were obtained for an H9N2 vaccine.²² When the same H9 subtype was used in a two-dose clinical study with non-adjuvanted whole or split vaccines, the whole virus formulation performed slightly better.²³ However, this has not been the case with H5 antigens.

While a clinical trial with two doses of an alum-adjuvanted H5N1 whole virus vaccine containing 10 µg HA satisfied the CHMP requirements for licensure,²⁴ a split alum-

Table 1. Overview of clinical trials of pandemic vaccines underway or recently completed

Strain	No. trials	Inactivated			Live, attenuated	Substrate		Adjuvanted	
		Whole*	Split	Surface*		Egg	Cell	Yes	No†
H5N1	25	7	11	5	2	21	4	20	5
H5N3	2	–	–	2	–	2	–	2	–
H9N2	6	4	–	1	1	6	–	5	1
H7N7	1	–	1	–	–	1	–	1	–
H7N1	1	–	1	–	–	–	1	1	–
H2N2	1	1	–	–	–	1	–	1	–
M2	1	–	–	1	–	–	1	–	1
Sum	37	12	13	9	3	31	6	30	7

Summarized from data from the International Federation of Pharmaceutical Manufacturers & Associations (IFPMA), 17 October 2006. For further details, including the names of the vaccine companies, see the IFPMA Clinical Trials Portal (<http://www.ifpma.org/Influenza/index.aspx?42>). A recent Chinese trial with whole egg-grown alum-adjuvanted vaccine is not tabulated here, but is registered with the ClinicalTrials.gov (<http://clinicaltrials.gov/show/NCT00356798>).

*Virosomal vaccines are tabulated as 'whole' and the M2 vaccine as 'surface'.

†Live vaccines would not be adjuvanted.

adjuvanted egg-grown H5N1 vaccine required two doses of 30 μg HA to satisfy the licensing requirements for seasonal influenza vaccines.²⁵ Two European vaccine manufacturers have announced, but not yet formally published, their preliminary results from trials with adjuvanted H5N1 vaccines. One alum-adsorbed cell-grown whole virus vaccine was claimed to stimulate cross-reactive antibodies against a range of H5 variants after two doses of 3.8 μg HA.²⁶ Another company has announced that a split-virus formulation of egg-grown H5N1 (3.8 μg HA) with a novel proprietary adjuvant gave protective levels of anti-HA antibodies in more than 80% of the subjects.²⁷

The proprietary MF59 adjuvant has been successfully used both for seasonal²⁸ and for a candidate H5 pandemic vaccine based on the surface antigens from a non-pathogenic H5N3 duck strain.^{29,30} Two doses of an adjuvanted 7.5 μg HA vaccine and 30 μg HA of a non-adjuvanted formulation satisfied the CHMP licensing criteria when tested against a 1977 human H5N1 isolate. This cross-reactivity against a variant H5 strains is a particular useful quality and could offer a substantial benefit for an eventual pre-pandemic vaccine meant to prime the population in advance of a pandemic. An even more pronounced cross-reactivity was found after a third dose given 16 months after the second dose.³⁰

Intradermal dosing

Given the scarce availability of vaccine when the pandemic strikes, dose-sparing strategies using intradermal dosing of seasonal vaccines have been evaluated and have demonstrated that 20–30% of a standard 15 μg dose would give a satisfactory immune response and thus be a way to stretch a limited vaccine supply.^{31–33} However, it remains to be seen how this strategy will work when unprimed subjects are tested against a pandemic strain candidate. Furthermore, it is doubtful whether the more difficult intradermal injection procedure will be feasible in a mass-vaccination programme.⁸

Antigen delivery systems

It is widely recognized that whole virion vaccines are more immunogenic than the split and subunit formulations. However, most seasonal influenza vaccines used today are not of the whole virus type. To generate better immune response against seasonal influenza, particularly among elderly subjects, trials using purified viral proteins in reconstituted virus-like particles have been completed with promising results.^{34–36}

Another approach has been the use of a 'biovector' for intranasal delivery. The formulation uses a bioadhesive delivery system incorporating a lipid micro-micelle system carrying vaccine antigens and a modified *Escherichia coli* enterotoxin. In a clinical trial with such an intranasal triva-

lent vaccine containing 7.5 μg HA from influenza H3N2, H5N3 and B strains, the H5 antigen, in contrast to the H3 and B components, failed to induce significant levels of neutralizing serum antibodies, but at the same time elicited a substantial mucosal IgA response.³⁷ While the vaccine was well tolerated, it only satisfied the CHMP regulatory requirements for the B component, whereas the 15 μg MF59 adjuvanted parenteral comparator passed the test for all strains. The longevity of the anti-H5 mucosal IgA response, and to what degree the H5 component elicited a long-lasting mucosal memory, remains to be evaluated.

'Universal' vaccine?

The vaccine industry has learned to cope with the challenge of updating the antigenic formulation of seasonal influenza vaccines. However, the need for annual vaccination and a close match between the antigens of the vaccine viruses is far from ideal. A vaccine not sensitive to antigenic drift in the HA of the circulating strains would therefore be a major breakthrough. This would even more important in the face of a pandemic where the exact antigenic nature of the new virus could not be anticipated, particularly if stockpiling pre-pandemic vaccine is contemplated. A broadly protecting vaccine is, therefore, highly desirable. While other antigens have been considered, the highly conserved trans-membrane M2 protein, the ion channel of influenza A, is a favoured candidate antigen. Immunity to M2 will not neutralize virus but rather reduce the clinical severity of the infection. The M2 protein in the virion itself is only poorly immunogenic. However, as the M2 is expressed on the surface of infected cells, it is a relevant target provided such an immune response could be initiated in the first place. To date several promising preclinical studies have been completed demonstrating a heterosubtypic response.^{38,39} A clinical trial in humans is now underway (Table 1).⁴⁰

DNA vaccines

The DNA vaccine concept should offer many advantages in a pandemic situation. This could be its rapid manufacturing process and scaling-up potential, its heat resistance and ease of strain adjustments, as well as presenting the immunogen in its authentic form and possibly also the ability to elicit a CTL response. A clinical trial with an influenza H3 DNA construct has been performed, and as little as 1 μg DNA satisfied one of the three CHMP criteria for serum antibody response, whereas all three requirements were met for the higher doses (2 and 4 μg) 8 weeks post-vaccination.⁴⁰ A rapid vaccine response is especially important for a pandemic situation, and higher DNA dosages may be required to achieve this. However, the extent to which any previous H3 memory aided the DNA vaccine response is not known. Any planned large-scale use of DNA vaccines would certainly focus attention on the particular safety concerns raised

regarding injecting nucleic acid material. That concern should be balanced against the public health implications of not offering immune prophylaxis of any kind.

Another cautionary note is also in place. Pigs vaccinated with a DNA construct containing the M2 gene sequence were not protected when challenged with live virus and suffered a more serious clinical outcome than the unvaccinated control animals.⁴² This could possibly be a consequence of an eventual Th1-biased immune response, contributing to the excessive inflammatory reaction in the challenged vaccinated animals.

Pre-pandemic 'priming'

As the H5N1 strains continue to evolve, the selection of appropriate vaccine strains has become increasingly difficult.⁷ In view of the large dosages and repeated vaccinations required to fulfil current licensing requirements for currently registered inactivated vaccines, as well as the risk of not having a pandemic vaccine available in time, pre-pandemic priming has been proposed.^{43–45} It is promising that preliminary data from a recent clinical data have showed that individuals primed with an H5N1 clade 3 vaccine strain 8 years earlier, substantially improved the immune response to a subsequent H5N1 clade 1 vaccine when compared to immunologically naïve subjects.⁴⁶ For a pandemic situation, the assumption is that a primed individual will, when infected with an antigenically variant pandemic virus (of the same subtype), generate a rapid cross-reactive immunity that will lessen the clinical impact.

Other scientific challenges

For reasons that have been inadequately determined, many contemporary vaccines for H5 influenza have not consistently elicited levels of circulating antibodies that will satisfy current licensing criteria. Live attenuated vaccines⁴⁷ and M2-derived vaccine formulations will similarly fail to meet current antibody-based criteria. As field efficiency trials of pandemic vaccine candidates cannot be undertaken, we face a regulatory predicament. However, clinical trials with live attenuated H5N1 virus open up the possibility of undertaking controlled challenge studies in volunteers having been immunized with a candidate pandemic vaccine. This point was especially mentioned in the Sixth Framework Programme of the European Union, having specifically called for such challenge trials.⁴⁸

The respiratory distress syndrome and multiorgan failure frequently seen in H5N1 patients is sometimes attributed to the consequence of a 'cytokine storm'^{1,49–51} and said to resemble to clinical picture observed for many of the 1918–1919 pandemic victims. Whether this inflammatory dysregulation is a consequence of the host's vigorous immune

response or an effect of the rapidly replicating virus is not clear.⁴ If the eventual pandemic virus swiftly attacks the cells of the lower respiratory tract, there may be a risk of immunopathological events, potentially worsened if the offending pandemic virus should boost a CTL-memory induced by a pre-pandemic vaccine. It therefore raises the pertinent question whether a pandemic vaccine should aim at stimulating a cytotoxic response in addition to the required humoral response. There are suggestions that whole virus and virosomal vaccine formulations will stimulate a CTL response by way of fusion of the viral/virosomal and endosomal membranes, allowing the peptides also to be presented on MHCI.³⁴ While the M2 protein offers interesting possibilities as a vaccine antigen, some caution must be exercised in view of the harmful outcome experienced in a pig model when M2 was delivered using the DNA vaccination approach which induced both antibody and cell-mediated responses.⁴²

Political challenges

While there seems to be a consensus regarding the need for improving pandemic preparedness in general, and in particular for the rapid development of new vaccine strategies, progress has been slow. Who is going to pay for the basic and applied research efforts and for the clinical trials? While development of vaccines is clearly within the remit of what may be expected of the pharmaceutical industry, the threat of an imminent pandemic has made it clear that private–public partnerships, and possibly more public than private, will be required to speed up vaccine development.^{17,52,53}

Following the H5N1 events in Hong Kong in 1997, and the subsequent zoonotic cases since 2003, the scientific community and the pharmaceutical industry envisaged a much needed flow of fresh research and development grants to address the many unknowns regarding the virus itself and the many challenges of preparing an efficacious pandemic vaccine. However, with very few exceptions, the academia, research establishments and vaccine industry had to settle for meagre and sometimes no public support at all. The inability of the scientific community and industry to lobby for more substantial financial support is remarkable when only a fraction of what is financially needed has been earmarked for pandemic preparedness.⁵⁴

Faced with a pandemic, antiviral drugs (the adamantanes and neuraminidase inhibitors) could be used both prophylactically and therapeutically to alleviate the situation, provided that drug resistance does not make them redundant. However, the global production of neuraminidase inhibitors may be inadequate and also too expensive for most nations. The consensus is that a pandemic vaccine will be our best option. The influenza vaccine market is dominated

by a small number of companies, most of them located in Europe, providing about 70% of the current global output of approximately 350 million trivalent doses of inactivated vaccine for seasonal influenza. For a pandemic scenario the industry will deliver too few doses too late, at least for the first pandemic wave. International sale and distribution of such a life-saving and scarce global commodity could be severely restricted or even prohibited by political interventions. Even if a fair distribution of vaccine between countries were agreed upon, the current industrial output would only cover a fraction of the soaring demand.⁵⁵ As the equity dilemma is still unresolved, the WHO can only urge for an increased use of seasonal influenza vaccine and call for new vaccine production facilities, so that the gap between global production capacity and the eventual pandemic surge will be lessened.⁵⁶

Remembering the 8000 SARS cases in 2003 and the resulting financial losses, the impact of an influenza pandemic on world trade could be dramatically more disruptive. The globalized industry today operates with extremely small stocks of spare parts and/or raw materials, depending on a 'just in time' and uninterrupted flow of domestic and especially international goods to keep the industrial wheels turning.⁵⁷ In this respect, the trade situation during the last pandemic in 1968 was strikingly different. Today, one can only assume that an eventual global financial depression and stagnant trade would severely obstruct any *ad hoc* emergency attempts by governments and industry to procure essential goods both during and also after the pandemic. The financial sequel would itself have a negative effect on global health.

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