Clinical evaluation of vaccines for pandemic influenza H5N1

Avian influenza (H5N1) was first associated with human respiratory disease in Hong Kong in 1997. In 2004 the virus re-emerged among poultry and migratory birds in Asia and has spread into Europe and Africa, raising concerns that an H5 pandemic is imminent.

Highly pathogenic avian influenza (H5N1) was responsible for six deaths among 18 infected people during poultry market outbreaks of the virus in Hong Kong in 1997. In 2003 the virus re-emerged in Hong Kong, and since 2004 it has spread throughout Eurasia and into Africa (World Health Organization (WHO), 2006). Molecular surveillance of H5N1 viruses circulating since 2004 reveals virus diversity with several antigenically distinct sublineages in geographically different areas. There have been more than 250 reported cases of human H5N1 infection. Overall the mortality exceeds 50%, demonstrating that the clinical course of H5N1 influenza is more aggressive than that of seasonal influenza.

Continuing opportunity for human infection raises concerns that H5N1 may develop pandemic potential by acquisition of the molecular determinants required for efficient human-to-human transmission. The A/H1N1 virus responsible for the 'Spanish' pandemic of 1918 was derived from an avian virus (Tumpey et al, 2005) and caused up to 50 million deaths. The consequences of an H5N1 pandemic could be worse. Vaccination will be central to our response, and the development of an effective vaccine is discussed here.

Seasonal interpandemic influenza vaccines

Large quantities of influenza virus are grown in the allantoic fluid of eggs, inactivated and then formulated into seasonal vaccines. Most commercial vaccines are 'split-product', produced from detergent-treated, highly purified virus, or 'subunit', containing purified haemagglutinin and neuraminidase. Whole-virus vaccines are considered more immunogenic in immunological naïve subjects, but are generally associated with increased adverse events and rarely used.

Seasonal epidemic vaccines contain 15 µg haemagglutinin each from three representative circulating strains (influenza A/H1N1, A/H3N2 and B). Vaccine efficacy of 70–95% in healthy adults is obtained when there is a good match between the vaccine and the circulating strains (Meiklejohn et al, 1978). However, they display reduced efficacy against antigenically drifted viruses and are considered ineffective against unrelated strains.

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Adjuvanted influenza vaccines

As the antibody response to split and subunit vaccines is generally lower in older people, various approaches have been used to try to enhance immunogenicity in this group. Influenza vaccines containing MF59, an oil-in-water emulsion adjuvant, have been demonstrated to induce significant increases in seroconversions and post-vaccination geometric mean titres compared with non-adjuvanted vaccines (Podda and Del Guidice, 2003). Virosomes comprise phospholipid membrane bilayers containing antigen such as influenza haemagglutinin and can be used as delivery systems for antigen. Improved antibody responses in elderly subjects have been achieved with virosomal influenza vaccines (de Bruijn et al, 2005). Both approaches have been licensed in several EU countries.

Licensure of seasonal vaccines

To achieve annual registration of existing influenza vaccines, or licensing of new seasonal vaccines, the vaccines must fulfil immunogenicity criteria in clinical trials, as measured by post-vaccination haemagglutinin-inhibition responses, set out by the EU Committee for Human Medicinal Products (CHMP) (*Table 1*, CHMP, 1996).

Vaccines for pandemic influenza H5N1

Development of vaccines against H5 proved initially challenging as these viruses are lethal to eggs in which influenza viruses are grown for vaccine production. In addition, the use of highly pathogenic viruses requires heightened biocontainment facilities to protect staff and the environment from contamination. In order to generate attenuated viruses suitable for influenza vaccine production, highly pathogenic strains are engineered to remove the amino-acid sequence in the haemagglutinin gene responsible for virulence, and then combined using reverse genetics with influenza viruses that grow well in

eggs (Webby et al, 2004). WHO plays a crucial role in coordinating surveillance of influenza H5N1 viruses and identifying strains that are suitable for provision to vaccine producers. It is essential that avian influenza isolates from poultry and humans from affected countries are shared in a timely fashion with WHO laboratories for prompt antigenic characterization.

Clinical experience with H5 vaccines

Inactivated split-virion and subunit vaccines

Following the 1997 H5N1 outbreak in Hong Kong, reverse-genetic generated vaccines were unavailable, but a non-pathogenic, antigenically well-matched virus, influenza A/duck/Singapore/97 (H5N3) was available for vaccine formulation (Nicholson et al, 2001; Stephenson et al, 2005) (*Table 2*). Three doses of subunit H5N3 vaccine each containing up to 30 μg haemagglutinin were poorly immunogenic in healthy adults and unlikely to protect against H5N1 infection, raising concerns about pandemic vaccine supply.

Reverse-genetic generated vaccine candidates against 2004 influenza H5N1 strains are currently undergoing clinical evaluation. In the US, the National Institutes of Health has sponsored a number of pandemic vaccine trials. A dose-ranging study of subunit A/Vietnam/1203/04 (H5N1) vaccine was conducted in healthy adults (Treanor et al, 2006a). Although the vaccine was well tolerated, immune responses were disappointing with only two doses, each containing 90 μg haemagglutinin, inducing antibody levels likely to confer protection against H5N1. It is clear that, in order to maximize the use of limited antigen during an emerging pandemic, 'dose-sparing' vaccine strategies are required.

Dose-sparing influenza vaccine strategies

During an emerging pandemic, the number of available egg-grown vaccine doses would be outstripped by demand, resulting in a significant worldwide shortfall in supplies. Savings made by the use of a monovalent rather than a trivalent vaccine would likely be offset by the need for a two-dose immunization schedule, demand for vaccine across all age groups, and difficulties with egg supply. Immunopotentiating effects of adjuvants and whole-virus vaccine may improve immunogenicity and allow dose reductions in haemagglutinin content, enabling maximum use of limited antigen.

Table 1. EU criteria for the licensing of annual interpandemic influenza vaccines

Committee for Human Medicinal Products criteria	Age population and criteria target	
	18–59 years	>60 years
Mean geometric increase in antibody	>2.5	>2.0
% subjects achieving seroconversion or significant rises in antibody (>4-fold increase in post-vaccination titre)	>40%	>30%
% subjects achieving a seroprotective haemagglutinin inhibition titre (≥1/40)	>70%	>60%

From Committee for Human Medicinal Products (1996)

Adjuvanted subunit influenza vaccines

Aluminum salts are relatively inexpensive and non-proprietary compounds are already licensed in some countries for use as adjuvants in influenza vaccines, although clinical studies have shown limited benefit in seasonal vaccines (Davenport et al, 1968). In Australia, 400 healthy adults received two doses of aluminium-phosphate adjuvanted or non-adjuvanted influenza H5N1 vaccine (Stephenson et al, 2006a). After two doses of non-adjuvanted vaccine containing 7.5 μg or 15 μg, 18% and 34% of subjects, respectively, developed seroconversions by neutralizing antibody. The addition of alum induced modest increases in immunogenicity, with 34% and 41% subjects, respectively, seroconverting. In France, alum-adjuvanted and non-adjuvanted split

Table 2. Frequency (%) of seroconversion to H5 by neutralizing antibody following two doses of H5 vaccine in published randomized clinical trials

			H5 haemagglutinin content of vaccine (μg)										
Vaccine type	Antigen	n	1.25	2.5	5.0	7.5	10	15	25	30	45	90	Reference
Non-adjuvanted subunit MF59 subunit MF59 trivalent vaccine	A/duck/Sing/97 (H5N3)	33 32 17				10 80		18 100 87		36 100			Nicholson et al (2001) Stephenson et al (2006b)
Non-adjuvanted subunit Alum-adjuvant subunit	A/Vietnam/1194/04 (H5N1)*	49 50				20 16		22 18		27 41			Bresson et al (2006)
Non-adjuvanted subunit	A/Vietnam/1203/04 (H5N1)*	393				7		20			41	53	Treanor et al (2006a)
Baculovirus	Recombinant H5	77							17		28	52	Treanor et al (2001)
Alum-adjuvant whole virus	A/Vietnam/1194/04 (H5N1)*	94	48	50	96		96						Lin et al (2006)

Seroconversion: defined as at least 4-fold rise in neutralizing antibody titres; *vaccines derived from reverse-genetic generated vaccine reference strains

influenza H5N1 vaccines were evaluated in a randomized trial (Bresson et al, 2006). Although the vaccines were well tolerated, immunogenicity was generally disappointing. Two 30 μg doses of alum-adjuvanted H5N1 vaccine induced seroconversion by neutralizing antibody in only 41% of recipients. There was no effect of alum adjuvant at lower doses compared with non-adjuvanted vaccine.

In H5N3 vaccine studies, the addition of MF59 had a striking and significant effect in boosting immune responses to H5 in comparison with non-adjuvanted vaccines (Nicholson et al, 2001). In this study, two 7.5 μg doses of MF59-adjuvanted vaccine were sufficient to fulfil CHMP licensing criteria for interpandemic vaccines. In addition, a third dose of MF59-H5 vaccine induced broadly cross-reactive antibodies to antigenically unrelated H5 variants (Stephenson et al, 2005). This effect could potentially be utilized in pre-pandemic priming of key workers.

Whole-virus vaccines

Although whole-virus vaccines generally display superior immunogenicity in immunologically-naïve subjects compared with split or subunit vaccines, their clinical use has been limited because of increased adverse effects, including febrile reactions, particularly in children (Nicholson et al, 1979).

Major influenza vaccine manufacturers, with facilities for bulk seasonal split-vaccine production, often rely on the splitting process to inactivate the vaccine virus strain and cannot easily switch production to whole-virus formulations. Changes in production methods would require significant investment in infrastructure, and impose regulatory and commercial uncertainties (Fedson, 2006). However, a dose-ranging study of a reverse-genetic alum-adjuvanted whole-virus H5N1 vaccine conducted in China found that two doses each containing 10 μg induced seroconversions in 78% and 96% of recipients by haemagglutinin-inhibition (HI) and neutralizing responses respectively (Lin et al, 2006). Moreover, the vaccine was well tolerated with no significant adverse effects.

Live attenuated vaccines

Live attenuated vaccines have the advantage of inducing mucosal responses and were licensed for clinical use against seasonal influenza in the US in 2003. They are well tolerated, genetically stable, replicate below body temperature (therefore attenuated) and are not transmissible between people. In addition, their growth characteristics in eggs are favourable for bulk production, as required for the response to pandemic influenza.

In mouse models, a single dose of live attenuated H5N1 vaccine protected against lethal viral challenge with homologous and antigenically distinct H5N1 variants (Lu et al, 2006) and human immunogenicity trials are underway.

Alternative approaches to H5N1 vaccines

Current inactivated H5 vaccines require a reliable egg supply for production. In the event of a pandemic, maintenance of the egg supply could be challenging, as H5N1 viruses are highly virulent to poultry. The effectiveness of any stockpiled vaccine may be compromised if the emergent strain is antigenically drifted from the pre-prepared vaccine. Therefore, as well as dose-sparing approaches, strategies to broaden the breadth of immune responses or reduce dependency on egg supply have to be considered.

Cell-grown vaccines

Cell-culture vaccines offer the potential for rapid surge responses required in an emerging pandemic by avoiding the need for eggs. In addition, cell-grown influenza viruses more closely resemble clinical isolates and may provide better-matched vaccines than egg-derived vaccines.

Baculovirus-expressed recombinant vaccine

Recombinant influenza haemagglutinin expressed in insect cells can be formulated into vaccine, reducing the dependency on egg-grown virus vaccine. In elderly adults, seasonal trivalent vaccine derived from baculovirus-expressed haemagglutinin is well tolerated and induces better antibody responses than conventional vaccine (Treanor et al, 2006b). However, recombinant influenza H5 vaccine was only modestly immunogenic, with 52% of participants developing a neutralizing antibody response after two doses of 90 μg (Treanor et al, 2001).

Adenovirus vector vaccines

Replication-incompetent adenoviral vectors can be grown to high titres in cell lines and delivered by parenteral or oral routes. In mice, an adenovirus vector vaccine containing influenza H5 haemagglutinin was protective against challenge with homologous and heterologous H5N1 influenza viruses (Hoelscher et al, 2006). One advantage of this strategy is induction of broad humoral and cellular immune responses that potentially confer cross-protection against continuously evolving H5N1 viruses.

Regulatory challenges surrounding pandemic vaccinese

The use of reverse genetics to manipulate highly pathogenic influenza viruses to generate attenuated vaccine reference strains is well established in a number of WHO-affiliated laboratories and is reliable for the manipulation of H5N1 viruses, although vaccine manufacturers may need to obtain appropriate licences for intellectual property rights for this technology (Webby et al, 2004). In some countries, guidelines governing 'genetically modified organisms' impose additional regulation on the use of reverse-genetics generated viruses.

Currently, different countries have specific influenza vaccine licensing regulations. Harmonizing regulatory processes would allow approval of pandemic vaccines for clinical use without time-consuming duplication of immunogenicity trials in different countries. The European Medicines Agency and US Federal Drug Administration recognize that approval for pandemic vaccines may be granted during the early stages of an evolving pandemic.

The European approach has been the development of 'core dossiers' containing preclinical and safety data pertinent to existing influenza vaccines. Dossiers can be submitted in advance, and the strain or subtype variation added when the pandemic strain is known. Safety and immunogenicity assessments of novel vaccine preparations should be conducted during interpandemic periods. Robust post-marketing surveillance must be an integral part of fast-track licensing procedures.

Immunogenicity assessment

Standard haemagglutinin-inhibition tests used for seasonal influenza serology are generally insensitive for the detection of antibody to avian influenza haemagglutinin. Alternative assays for detection of anti-H5 antibodies include modified haemagglutination inhibition using horse erythrocytes, and virus neutralization tests (Rowe et al, 1999; Stephenson et al, 2003). However, poor laboratory reproducibility and the lack of recognized correlates of immunity pose challenges to developing immunological endpoints for clinical trials and criteria for pandemic vaccine licensing.

To allow comparative analysis of vaccine studies, WHO has recommended that priority be given to the generation of international standards for assessing serological responses to H5N1 antigen.

Pandemic vaccine supply will be limited

The global manufacturing capacity of trivalent influenza subunit vaccine is approximately 300 million doses per 6 months (Fedson, 2006). If monovalent pandemic vaccine were similarly formulated with 15 μg haemagglutinin per dose, then 900 million vaccines would be available. Assuming a two-dose regimen, this would provide vaccine sufficient for 450 million people. However, trials suggest that the quantity of antigen required to induce satisfactory immune responses is 2–6 times higher than that for seasonal vaccines. Furthermore, for reasons that are unclear, egg yields of antigen from reverse-genetic engineered H5N1 viruses are 30–40% lower than the average of seasonal influenza viruses, further limiting available antigen. In the event of pandemic influenza, global vaccine demand will soar and it is clear that there will be significant shortfalls in availability.

Conclusions

Although pandemic planning has advanced since the first H5 outbreak in 1997, our ability to respond rapidly with effective vaccine remains less than optimal. Vaccines containing H5 haemagglutinin seem less immunogenic in humans than vaccines based on H1, H2 and H3 antigens. Enhancement with adjuvants such as MF59 and whole-virus vaccines may optimize antigen use. There is an urgent need for dose-sparing vaccine formulations in healthy adult, elderly and paediatric populations. **BJHM**

Conflict of interest: Dr Stephenson has received grants for scientific research, speaker's honoraria and sponsorship for travel to international meetings from drug companies who make influenza vaccines, including Novartis, GlaxoSmithKline and Sanofi-Pasteur.

KEY POINTS

- Avian influenza H5N1 activity in poultry is associated with sporadic human infections and poses a pandemic threat.
- Vaccination will be the principal means of combating pandemic influenza.
- Attenuated H5 vaccine reference strains can be generated from highly pathogenic viruses by molecular engineering (reverse genetics).
- Conventional subunit or split influenza H5N1 vaccines are poorly immunogenic compared with seasonal influenza vaccines.
- Immunogenicity of H5 vaccines can be increased by the use of adjuvants or whole-virus vaccine formulations.
- New methods of influenza vaccine production may be needed to provide for surges in demand imposed by an emergent pandemic.
- Fast-track licensing of H5 pandemic influenza vaccines is complicated by lack of immunogenicity criteria.
- Global influenza vaccine manufacturing capacity is unlikely to meet pandemic demands, leaving a significant shortfall.

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