Pandemic vaccines: promises and pitfalls

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ABSTRACT

- Prototype vaccines against influenza A/H5N1 may be poorly immunogenic, and two or more doses may be required to induce levels of neutralising antibody that are deemed to be protective. The actual levels of antibody required to protect against a highly pathogenic virus that potentially can spread beyond the large airways is unknown.
- The global capacity for vaccine manufacture in eggs or tissue culture is considerable, but the number of doses that can theoretically be produced in a pandemic context will only be sufficient for a small fraction of the world's population, even less if a high antigen content is required.
- The safety of new pandemic vaccines should be addressed in an internationally coordinated way.
- Steps are underway through the Therapeutic Goods Administration to evaluate mock-up vaccines now, so that the time to registration of a new product can be minimised.
- It will be 3–6 months into the pandemic before an effective vaccine becomes available, so other control measures will be important in the early stages of a pandemic.
- The primary goal of a pandemic influenza vaccine must be to prevent death, and not necessarily to prevent infection.

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The threat of another influenza pandemic has galvanised governments, industry, the World Health Organization, academia and others to address this global threat. Many issues are being addressed, and here we focus on key questions related to vaccination:

- Can an effective vaccine be produced?
- What dosage will be required?
- Could enough of it be made in time?
- How will it be produced?
- How can safety be optimised?

Vaccination is one of the key components of Australia's pandemic plan: contracts with influenza vaccine manufacturers have been drawn up to guarantee supply, and funding provided to accelerate research on influenza vaccines relevant to a pandemic. A vaccine would ideally achieve disease prevention, and, at the very least, partial immunity to the pandemic influenza strain in the population. This could prevent infection altogether, or reduce morbidity and mortality in infected people. A vaccine that prevents death but does not necessarily prevent infection could still dramatically reduce the impact of a pandemic.

Front-line health care and other essential workers will be first in line, but it is envisaged that vaccination will be extended to other sections of the community. The extent of coverage achievable will depend on the dose of haemagglutinin antigen required to achieve immunity. Seasonal influenza vaccine contains 15 μg of antigen for each of three strains. An adult who has received previous vaccination requires a single dose each year, matched to circulating strains. Children being vaccinated for the first time require two doses, 1 month apart.

Vaccine manufacturers have been developing prototype vaccines against influenza A/H5N1 in anticipation of an emergent pandemic, but initial control measures such as social distancing and antiviral prophylaxis will be important because of the anticipated delays in vaccine production. A prototype vaccine will not be a perfect match for an emergent virus, as we will not know the exact antigenic constitution of the pandemic strain until the pandemic actually occurs. However, a prototype vaccine may provide a degree of protection and be useful as a stop-gap measure until a matched vaccine is produced, 3–6 months into the pandemic.[1]

Influenza vaccines are currently grown in fertile hens eggs, making it a slow and labour-intensive production process. The highly pathogenic avian H5N1 virus may be lethal to or grow poorly in eggs, thus compromising production capacity. Furthermore, it is likely that two doses of pandemic vaccine at a higher dose than seasonal vaccine will be required to optimise protection in humans.[2-4] Minimising the amount of viral antigen needed per dose of vaccine to compensate for these factors will be essential to provide sufficient yields of life-saving vaccines. Early in 2006, the United States government committed more than US$1 billion to support research by five different pharmaceutical companies into improving cell culture as an alternative to cultivation in fertile eggs.

H5N1 vaccine trials

Late in 2005, in the US, Sanofi Pasteur conducted an early trial of an unadjuvanted vaccine for H5N1, derived from a genetically modified strain. In this trial, 90 μg (six times the standard influenza vaccine dose) of H5 haemagglutinin was required to induce acceptable immunogenicity[2] — a worrying finding if there is to be any hope of producing enough vaccine to protect the wider population. Reassuringly, vaccination was well tolerated.

By contrast, in July 2006, the Chief Executive Officer of GlaxoSmithKline claimed that their new, adjuvanted preparation was immunogenic in humans given only 3.8 μg (a quarter of the standard dose). This is a greater than 20-fold turnaround in potency if the results are reproducible.[5] Was it just an effect of the adjuvant? A surprising number of variables may differ between vaccine studies, in addition to adjuvant use and type. These factors include:

- Antigen content;
- Use of whole inactivated virion or detergent-split virus preparation;
- Growth in eggs or cell culture;
- Sex and age of subjects (the GlaxoSmithKline trial had a ceiling age of 40 years, whereas the Sanofi Pasteur trial included a large proportion of subjects aged 40–65 years, in whom immunogenicity will be less);
- Prior exposure to seasonal human influenza or vaccination; and
- More subtle differences, like whether methods include recruiting subjects with prior positive involvement in vaccination trials (a variant of the "healthy volunteer effect") and the type of assay used to measure protection.

A recent European trial compared doses of 7.5 μg, 15 μg and 30 μg of haemagglutinin with or without aluminium hydroxide adjuvant in 300 healthy volunteers aged 18–40 years. This study found that a two-dose regimen of 30 μg induced the highest response, with

adjuvanted vaccine being more immunogenic than the non-adjuvanted vaccine, but only at the highest dose.[3] Australian manufacturer CSL Ltd has also completed trials of an H5N1 vaccine candidate, but the results are not available. Other vaccine research in ferrets, the closest animal model of relevance to humans, shows that a two-dose regimen of vaccine in an immunologically naïve population is not only immunogenic against the target virus, but also provides cross-protection against antigenically distinct H5N1 strains.[4] Cross-protection mediated by cellular immune responses to internal conserved antigens (called heterosubtypic protection) may also play a role, but more research in this area is needed.

Prototype vaccines for stockpiling in Australia

The Australian Government decided in 2005 to stockpile up to five million doses of prototype H5N1 vaccine, provided evidence is produced of safety and efficacy (ie, likely protection based on antibody responses). A prototype vaccine will not be a perfect match for an emergent virus (remembering that the pandemic virus may not even be H5N1). However, it may be of some benefit as a stop-gap measure until a matched vaccine is produced.[4] The Australian Government has also announced it would acquire up to 50 million doses of "pandemic strain" vaccine from two suppliers (CSL; and Sanofi Pasteur, Paris), if a pandemic occurs.[6]

The WHO advised in August 2006 that the choice of H5N1 strains for development of candidate vaccines should be representative of the distinct groups (clades) of viruses that have been afflicting humans recently; this equates to recommending that in addition to clade 1 H5N1 virus (eg, the 2004 Vietnam strain used in trials reported above), examples of clade 2 (a variant of H5N1 now circulating in Indonesia) should also be included. The recent 2005–2006 outbreaks in Indonesia due to clade 2 H5N1 viruses have already resulted in more than 50 human deaths and afflicted poultry in 28 of Indonesia's 33 provinces.[7] This raises the need for a whole new swathe of studies to assess safety, immunogenicity, priming, cross-reactivity and cross-protection of vaccines against a clade 2 H5N1 strain.

The WHO has also called for an enhanced role in collecting information on the safety of candidate pandemic vaccines. They suggest that both efficacy and safety testing should be performed forthwith in individual and cluster-randomised (CR) clinical trials. CR trials use a group as the unit of randomisation and so are able to measure both direct protection of individuals and indirect protection of unvaccinated people within the cluster that is offered vaccination. It might also be possible to address the relative merits of different manufacturers' products in head-to-head immunogenicity comparisons, an endeavour considered impossible to date, but perhaps achievable given enough collective political will to overcome the reservations of pharmaceutical companies.

Safety in pregnancy needs to be addressed, and the WHO recommends use of congenital malformation registries and database linkage to address safety in mothers and infants. Noting that unexpected adverse events may occur after vaccination (such as Guillain–Barré syndrome), the WHO recently reviewed the data on use of "swine flu" epidemic vaccine in the US during the mid 1970s. Having appropriate baseline data regarding potential vaccine-related adverse events, stratified by age, will be important before pandemic vaccines are used.[8] Testing of new influenza vaccines, whether for seasonal or pandemic disease, only involves a maximum of a few hundred subjects. Less common adverse events can only be detected through a system of sensitive post-marketing surveillance.

Registration of pandemic influenza vaccines

Australia is fortunate in having a long tradition in the production of influenza vaccines. In Australia, the Therapeutic Goods Administration (TGA) has responsibility for ensuring that all regulatory requirements for vaccines are met; performing pre-market evaluations; assessing quality, safety and efficacy; then post-market, batch release testing and adverse event monitoring. For influenza vaccines, the TGA is involved in selecting vaccine virus strains and calibrating and supplying reagents used to establish the potency of the vaccine. The TGA has a responsibility, with industry, to ensure that all regulatory and good manufacturing practice requirements are met to ensure vaccine safety and efficacy.

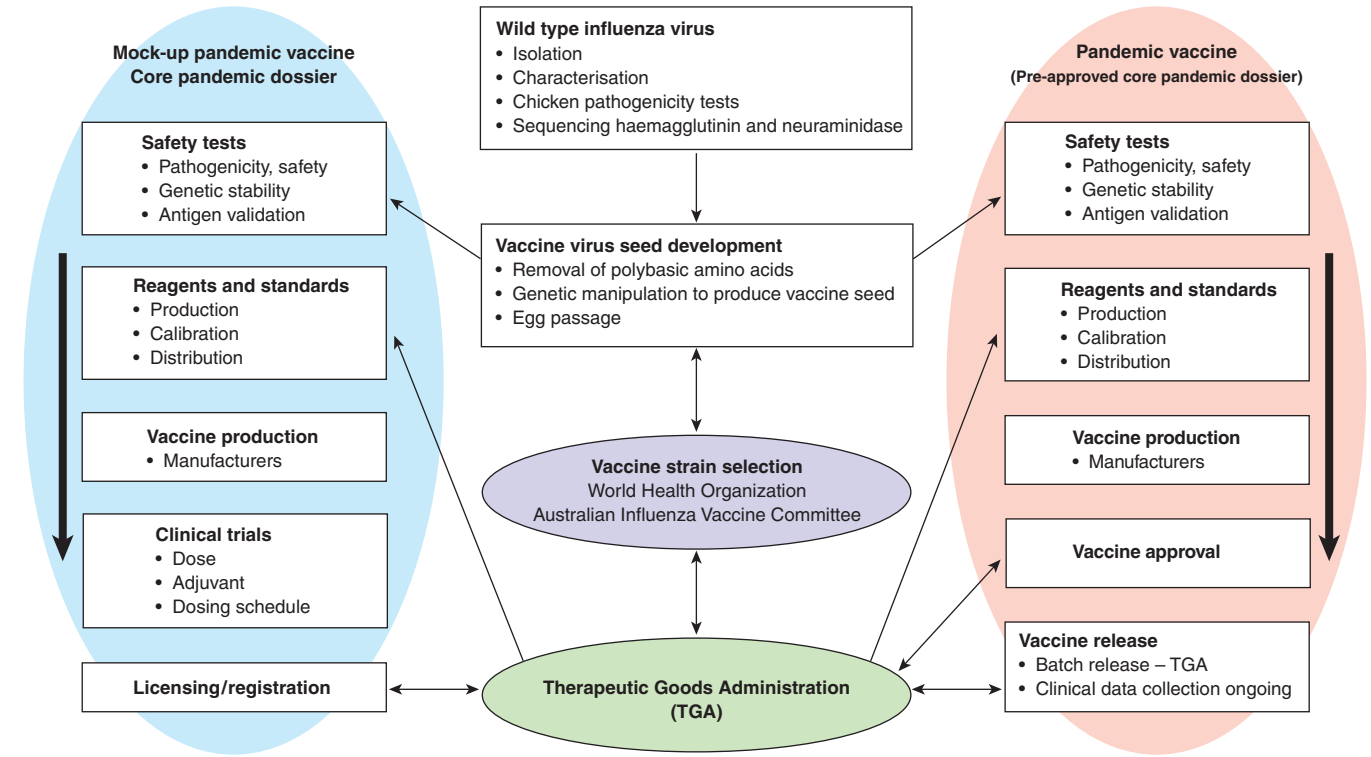
The immediate problem if a pandemic is declared by the WHO will be to produce large quantities of vaccine. Authorities need to decide on the vaccine strain to be used, have access to reverse genetics or other techniques to modify the virus to remove the sequence responsible for lethality to embryonated eggs, and have appropriate animal safety data, before production can begin. Safety and clinical data will be required for the pandemic vaccine before its release. This will take time: inter-pandemic vaccine generally has a timeline involving 7–11 months. When cultured in embryonated eggs, there is often a poor viral yield and, depending on the characteristics of the virus, the haemagglutinin content may be low, resulting in manufacturing difficulties (the current WHO prototype H5N1 vaccine virus, NIBRG-14, is poorly immunogenic). Egg-based vaccines offer a long history of safety with few viral and prion safety concerns and are generally efficacious, but there are limits to the quantities that can be produced.

In an attempt to help speed up the regulatory process, the European Medicines Agency (EMEA) has provided scientific guidance to manufacturers and regulators, suggesting that a "core pandemic dossier" be supplied during the inter-pandemic period. Such a dossier would contain manufacturing, quality control, safety and efficacy data for a "mock-up" vaccine, where virus strains with pandemic potential or related viruses are used to generate safety and efficacy data (eg, H5, H9, H7, H2 and H1 viruses). The TGA has accepted the EMEA guideline.

The mock-up vaccine would include viral antigens to which humans are immunologically naïve, and the antigens would be different from those in the inter-pandemic influenza vaccines. Clearly, clinical data from inter-pandemic vaccine cannot be extrapolated to a pandemic situation, and a variation from inter-pandemic vaccine (which contains three viruses given as one dose containing three 15 μg concentrations and is non-adjuvanted) into a pandemic vaccine (one strain, likely different antigen content and adjuvanted, possibly preserved, and with a different dosing schedule) is not scientifically justifiable.

Ideally, the mock-up vaccine would be produced in the same way as intended for the pandemic vaccine, whether from cell culture or egg; comparisons would be made between whole virion and split or subunit vaccines; it would have similar antigen content as any future pandemic vaccine; and it would have the same adjuvant system (if used) as the future pandemic vaccine. The dossier containing preclinical (quality, safety and immunogenicity) and clinical data would be submitted to the TGA for evaluation. In the event of a pandemic, an application for a product variation would be submitted, containing the manufacturing and quality control data relating to the pandemic influenza strain and a commitment from the sponsor to gather clinical information during the pandemic (Box). This would permit a faster approval process.

Pandemic influenza vaccine development for Australia



With a pre-approved core pandemic dossier (blue), pandemic vaccine development (pink) is accelerated. Only the approval of a variation (pandemic virus strain) is required. ◆

In the battle against pandemic influenza, vaccines will be essential, and their production and availability will rely on strong partnerships between industry, regulators, the WHO and national health authorities.

Addressing the poor immunogenicity of H5N1 vaccines

Lessons to date with H5N1 prototype vaccines indicate that a suitable adjuvant will be essential to enable relatively low doses of vaccine to be used and therefore allow more people to be vaccinated with available viral antigen. Many studies, including several funded under the urgent research scheme of the National Health and Medical Research Council (NHMRC), have addressed novel adjuvants, but the question remains whether these will satisfy the regulatory requirements for use in humans and the practical requirements for large-scale production.

Certain experimental adjuvants allow vaccine delivery by non-parenteral routes, and intranasal and transdermal delivery may have advantages, particularly for mass vaccination scenarios.

Other vaccine strategies being explored use the considerable intrinsic immunogenicity of whole influenza virus particles. These strategies include:

- Whole formalin-inactivated virions;
- Highly attenuated or replication incompetent influenza viruses, including those lacking or defective in critical components necessary for efficient in-vivo growth, such as the nuclear export protein,[9] the interferon antagonist protein NS1[10] or the M2 ion channel;[11] and
- Influenza virosomes, which are reconstituted viral envelopes devoid of the viral genome.[12]

Whether these have any significant advantage over the conventional inactivated split-virion approach is unknown.

Addressing vaccine yield

Most prototype H5N1 vaccine seeds grow poorly in eggs, and even a twofold increase in virus yield would provide a significant increase in doses of vaccine produced. This is being addressed in studies examining the strain of eggs used, parameters surrounding egg inoculation, manipulation of the immune response of the chick embryo, and genetic modification of the vaccine virus.

Strategies that do not rely on egg-grown virus and could potentially supplement these include:

- Mammalian tissue culture grown split-virus vaccines;[13]
- Baculovirus-expressed viral proteins in adjuvant or in the form of virus-like particles;[14] and
- DNA vaccines.[15]

Vaccines to protect against severe disease and death rather than infection

The merit in vaccinating now with a current H5N1 virus isolate, despite the likelihood of its significant variation from a future pandemic strain, is under debate. The current vaccine strain may induce sufficient cross-reactive antibody to curtail, although not effectively prevent, infection by the pandemic strain.[4]

Future vaccines may depend on strategies that also target the internal conserved proteins of the virus and elicit heterosubtypic immunity (ie, common to all influenza A viruses). Such broadly cross-reactive responses, not induced by current inactivated virus preparations, can be mediated by $CD8^+$ cytotoxic T cells that kill virus-infected cells and secrete antiviral cytokines. These vaccines cannot prevent infection, but lessen the severity and duration of disease and reduce viral shedding. DNA vaccines encoding genes for internal proteins no doubt work principally by this method. Other strategies involving delivery of conserved $CD8^+$ T cell epitopes[16] and the use of adjuvants that may boost cross-reactive T cell responses are the subject of NHMRC-funded studies. The advantage of boosting heterosubtypic immunity is that vaccines can be delivered without prior knowledge of the emerging strain.

Attempts to address protective immunity

Clinical testing of candidate vaccines to ascertain immunogenicity is critical, but this does not provide information about protective efficacy. Levels of antibody said to be required to prevent H5N1 disease are largely a "best guess", and extrapolation from endemic human influenza virus infection may be misleading.

Data from appropriate animal models can often inform interpretation of clinical trial data and affect trial design. The model of choice for influenza infection is the ferret. Unlike mice, these animals are susceptible to human influenza viruses without further genetic adaptation. Few countries have the capacity to perform ferret studies with lethal H5N1, as these require a high level of biocontainment. Australia has recently developed this capacity at the Australian Animal Health Laboratory in Geelong, and experimental H5N1 vaccines are now under investigation for protective efficacy in this model. Optimisation of dosage and schedule may provide important pre-clinical data for human trial design.

Practical considerations

The most likely candidate, although not the only one, for pandemic influenza is H5N1. Licensed pre-pandemic vaccine may soon be available, and consideration should be given to its anticipatory use in essential and high-risk workers. When a pandemic comes, it will be important to have a sufficiently trained corpus of public health staff to efficiently deliver vaccination, maintain the cold chain and address other operational issues beyond the scope of this article. Developing a vaccine is not sufficient for protection — vaccination is necessary.

Conclusion

Much has been achieved in a short time, but if a pandemic strikes soon, much more effort will be required. We can legitimately expect that a vaccine of at least partial and life-saving efficacy will be widely available no earlier than 3 months into the pandemic. Clearly, other measures will be required in the interim, such as social distancing and antiviral prophylaxis.

Competing interests

Robert Booy has received support to attend scientific meetings from CSL, Sanofi, Roche and Wyeth. Raina MacIntyre is an investigator on an ARC Linkage grant in which Roche is a partner, and has had a conference registration fee paid by CSL.

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