Development of an
Australian pandemic vaccine

Introduction

Although influenza pandemics have plagued mankind for centuries, a pandemic resulting from the current H5N1 threat would be a unique experience in many ways. Greater awareness and increased surveillance have allowed us to watch events unfolding and have facilitated early detection and containment of disease episodes. Well developed strategies for containment and availability of the antiviral drugs Relenza and Tamiflu for both therapy and prophylaxis further strengthen our capabilities in retarding development of a pandemic and give us hope of a delay in rapid dissemination at an early stage of a pandemic.

However, none of these improvements can offer protection of the world’s communities once a real pandemic is established. Their net effect is that we have an understanding of the virus which presents the threat and potentially gives us time to develop the only defence which could offer long-term and effective protection – vaccines. It is indeed fortunate that we have had some time, because, as we will discuss below, the H5N1 virus offers many challenges to vaccinology that we have not encountered before.

A most difficult virus

The H5N1 viruses which are currently circulating have unique properties which make vaccine development difficult. Many of these result from the presence of the much discussed ‘poly basic cleavage site’. This series of basic amino acids (R-R-K-K-) facilitates rapid haemagglutinin (HA) activation and can result in multi-organ pathology. For further discussion on this, see article by Glenn Marsh in this issue. The ready proteolytic cleavage of this site by a multitude of proteases means that, not only is the virus highly pathogenic for its host, but it will also kill the embryonated hens’ eggs used in vaccine production, hence the need for eradication of this sequence in production seed virus. This is best accomplished by reverse genetics which introduces two issues for the manufacturer:

- Access to the reverse genetics IP for large scale manufacture – discussion of this is beyond the scope of this article.
- That the virus becomes a genetically modified organism (GMO). A major consideration is therefore the upgrade of the manufacturing facility to comply with the exacting requirements of the Office of the Gene Technology Regulator (OGTR). This task is proceeding at CSL, with partial financial support of the Department of Health and Ageing (DoHA).

The requirement for reverse genetics has also delayed availability of candidate strains for vaccine manufacture. Vaccine development over the past 2 years worldwide has used one of two closely related strains from Clade 1 of the H5N1 virus – an RG reassortant of A/Vietnam/1194/2004 prepared by NIBSC (NIBRG-14) or a closely related alternative from A/Vietnam/1203/2004 prepared by St Judes Children’s Research Hospital, Memphis, Tennessee. The US has used the latter and the rest of the world NIBRG-14.

It has become clear that these viruses give a low yield of the principal antigen, HA. Manufacturers have found yields to be approx 30% of expected – not a good base for rapid manufacture. The problem appears to be associated with a low content of HA at the surface of the virion, not low levels of replication of virus [personal communication John Wood/Jim Robertson, NIBSC]. We cannot yet say if other H5 reassortants will give similar problems. Four more candidates have been developed (A/turkey/Turkey/1/2005, A/Whooping Swan/Mongolia/2004/2005, A/Indonesia/5/2005, A/Bar Headed Goose/Qinghai/IA/2005), but have not yet been widely distributed – suitability for production remains unknown.

A further unfortunate characteristic of the virus is that it appears to be poorly...
immunogenic. It is to be expected that any novel virus in a naïve population will require a priming followed by a booster dose. Normal seasonal influenza vaccine requires a two dose regime in a naïve subject, after which good response is observed (e.g. paediatric use). Other potential pandemic strains (H7 and H9) give acceptable responses after two doses 7.

For the H5 virus, two 15μg doses (each equivalent to normal vaccine) results in very poor responses 9, 10. Studies at NIH using the RG reassortant for A/Vietnam/1203/2004 required two doses of 90μg (i.e. total of 12 times an annual vaccination dose) to approach acceptable response 11 – again not a good outcome for rapid vaccine supply. As discussed later, a higher dose/lower production output must be weighed against wider community coverage.

Approach to vaccine development

The primary requirements for a development programme in the current situation are:

- A product must be developed quickly to ensure a process is available for manufacture in an emergency situation in the near-term.
- The product must be suitable for mass vaccination i.e. its risk of reactogenicity must be low.
- The product must be effective in preventing disease or at least greatly reducing morbidity and mortality. This is not possible to test directly for a pandemic vaccine (see article in this edition by Grohmann).
- The product must be able to be produced as rapidly as possible in a pandemic situation and distributed quickly.
- The number of doses should be minimised, given the logistical challenge of vaccinating millions of people.

To a very large extent, these requirements dictated the development path for the Australian product. To minimise both development time and risk of reactogenicity, the vaccine had to be similar to the existing product which CSL has manufactured since 1968 and to which there is extensive safety data, i.e. a split virion rather than whole virus or purified sub unit. A change, e.g. to the whole virus, would require time to develop, optimise and validate the new process and then may also be more reactogenic, especially in children.

The evidence 9-11 suggests that an adjuvant will be required. If so, it would have to be one which was already widely used, with a good safety profile and rapid speed of manufacture of substantial quantities. Aluminium based adjuvants are the only option which satisfy these criteria. CSL chose AlPO₄₃ based largely on encouraging experience with other antigens. Antigen content should be as low as possible to facilitate rapid manufacture. This and the number of doses required could only be determined by clinical study.

The product would have to be in a multidose vial (rather than the prefilled syringe used for the inter-pandemic product) to avoid a bottleneck in filling and to facilitate both distribution logistics and stockpiling of filling components – stockpiling 40-60 million specialist syringes is not practical.

Virus manufacture

Production for the first clinical study of H5N1 vaccine was undertaken at a very small scale in a newly constructed seed lab. Production for the second study was performed at a larger pilot scale in the manufacturing facility after modification to gain OGTR approval.

The virus used was NIBSC’s NIBRG-14 (A/Vietnam/1194/2004 reassortant). After modification to remove the polybasic cleavage site, this virus underwent extensive testing at NIBSC (PCR to demonstrate deletion of the polybasic site, intravenous chicken pathogenicity 13, ferret pathogenicity 14 as well for lethality in eggs) to demonstrate safety 12. Nevertheless, extensive precautions were taken during vaccine manufacture to prevent exposure of the environment to the virus; the operators to the virus and; the H5 virus to other influenza virus (this last precaution was to obviate the theoretical risk of operator-introduced human virus reassorting with the vaccine strain to form a new threat).

Clinical studies

Clinical studies were designed to evaluate the safety and immunogenicity of a
number of formulations covering a range of antigen contents as well as assessing the benefit of addition of aluminium adjuvant and the necessity for more than one dose. All vaccines were prepared using NIBRG-14\textsuperscript{12} and presented as a split virion vaccine in a 0.5mL dose administered intramuscularly.

Immunogenicity was assessed by testing sera by haemagglutination inhibition (HAI) with turkey erythrocytes, HAI with equine erythrocytes \textsuperscript{8} and by the microneutralisation test. Turkey HAI was performed at NIBSC and other tests by Dr M Zambon at the Health Protection Agency, Colindale, UK. Equine erythrocytes were used because previous reports \textsuperscript{8} suggest the commonly applied HAI test using turkey or chicken cells failed to measure responses with H\textsubscript{5} virus – one more challenging characteristic of this virus.

Clinical assessment is being conducted in two trials. In the first of these, 400 healthy human volunteers were recruited across two trial sites in Melbourne and Adelaide. All participants received two doses of vaccine, 3 weeks apart. The trial had four arms, each of 100 participants, and explored different concentrations of antigen (7.5 and 15\textmu g) with and without aluminium phosphate as an adjuvant. All participants were monitored closely for side effects. A third dose was administered after 6 months.

This trial was conducted at the Murdoch Children’s Research Institute in conjunction with Melbourne University and the Royal Adelaide Hospital, with Professor Terry Nolan as Principal Investigator. Final data following the third dose will be available shortly. Preliminary results following the first two doses suggest an adjuvant will be required and that about half the participants would be protected following two doses of the standard antigen content of 15\textmu g of HA.

A second study has now commenced comparing formulations at 30\textmu g and 45\textmu g with aluminium and includes a broader population and age range. These data should also be available in early 2007.

**Where to from here?**

It is expected that the trials underway should provide the data required to determine the most appropriate strategy for a pandemic situation. This decision may be based on balanced assessment of (i) faster supply rate with lower protection for low antigen dose and (ii) higher protection/slow supply rate for high antigen content. It may then be a choice of complete protection for some (an assessment of individual responses) or a community value assessment with lower protection for a broader section of the community.

The clinical trial programme has been designed to provide a broad body of data which will allow TGA and DoHA to formulate policy. Ideally, by further development and availability of sufficient time, planning and investment (e.g. in stockpiling of vaccine), the entire population of Australia may be protected for the first time in an influenza pandemic.

**References**


