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Developing vaccines against pandemic influenza

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Pandemic influenza presents special problems for vaccine development. There must be a balance between rapid availability of vaccine and the safeguards to ensure safety, quality and efficacy of vaccine. Vaccine was developed for the pandemics of 1957, 1968, 1977 and for the pandemic alert of 1976. This experience is compared with that gained in developing vaccines for a possible H5N1 pandemic in 1997–1998. Our ability to mass produce influenza vaccines against a pandemic threat was well illustrated by the production of over 150 million doses of 'swine flu' vaccine in the USA within a 3 month period in 1976. However, there is cause for concern that the lead time to begin vaccine production is likely to be about 7–8 months. Attempts to reduce this time should receive urgent attention.

Immunogenicity of vaccines in pandemic situations is compared over the period 1968–1998. A consistent feature of the vaccine trials is the demonstration that one conventional 15 μg haemagglutinin dose of vaccine is not sufficiently immunogenic in naive individuals. Much larger doses or two lower doses are needed to induce satisfactory immunity. There is some evidence that whole-virus vaccines are more immunogenic than split or subunit vaccines, but this needs substantiating by further studies. H5 vaccines appeared to be particularly poor immunogens and there is evidence that an adjuvant may be needed.

Prospects for improving the development of pandemic vaccines are discussed.

Keywords: influenza; pandemic plan; avian influenza; vaccine

1. INTRODUCTION

Inactivated influenza vaccines are in worldwide use to protect special-risk groups. Although there have been some significant technical developments in vaccine production and standardization over the years, the process is still closely related to that developed in the mid-1940s (Wood & Williams 1998). The vaccine virus is grown in fertile hens' eggs, it is inactivated by chemical means, purified and usually disrupted by detergent or by ether. The vaccines are usually trivalent, containing representative influenza A (H1N1), A (H3N2) and influenza B strains. Influenza vaccine efficacy depends on the vaccine strains being regularly updated and this is achieved by a well-practised and efficient process, coordinated by the World Health Organization (WHO) (WHO 1996).

An influenza pandemic present enormous challenges for vaccine development.

(i) Can a vaccine be produced in time?
(ii) Is the pandemic strain safe to use?
(iii) How much antigen is needed to induce a protective immune response?
(iv) How will vaccine potency be standardized?

These issues have come to the fore in recent years following the H5N1 and H9N2 avian influenza incidents in Hong Kong. However, most of the issues are not new. They were prominent during the pandemics of 1957 (H2N2), 1968 (H3N2) and 1977 (H1N1), and the H1N1 pandemic alert in 1976. In this article, comparisons will be made between recent experiences of the H5N1 outbreak and those obtained from the past. Although there are many similarities, some of the issues were unique to the Hong Kong H5N1 situation.

2. VACCINE AVAILABILITY

During interpandemic periods, inactivated influenza vaccines are normally ready for use 8 months after the decision has been taken to update vaccine strains. Historically the first waves of a pandemic have spread to most continents within a 6 month period, but with increased international travel future pandemics are likely to spread much more quickly (Gust *et al.* 2001). It is therefore questionable whether the 8 months required for normal vaccine development would have any immediate impact on public health. What lessons can we learn from the past?

In figure 1, the response times for vaccine production in 1957, 1968, 1976 and 1998 are compared.

(a) *1957*

In 1957 the first isolates of the H2N2 virus were available to vaccine manufacturers in the USA in May and by mid-June small quantities of vaccine (inactivated, whole-virus) had been produced (Murray 1961). By August all the US vaccine manufacturers were working to maximum capacity and 10 million doses per month were produced. Unfortunately this was at a time when local outbreaks of Asian influenza had already begun in the USA, and by the time the epidemic first wave peaked in the USA (9

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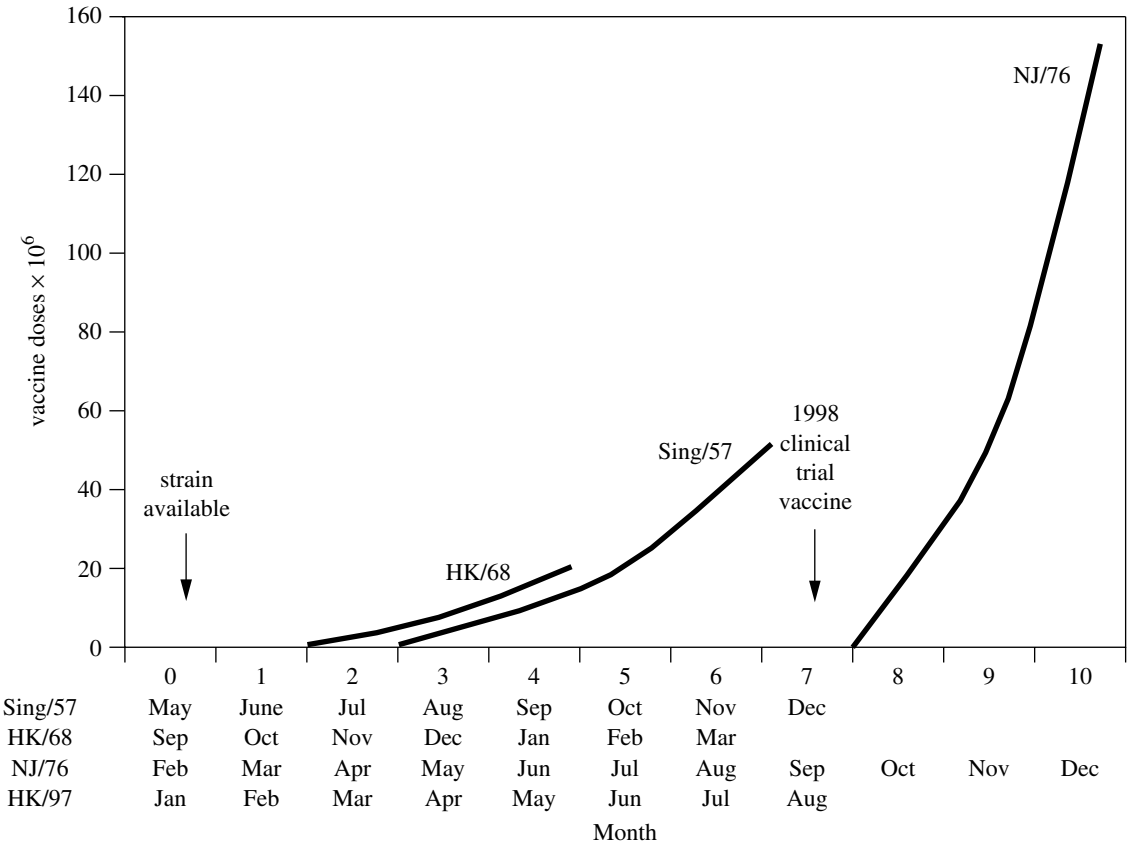

Figure 1. Production of influenza vaccines for pandemics or pandemic alerts from 1957–1998. Data from Murray (1961, 1969) and Barry *et al.* (1977).

November 1957) only 48.8 million doses had been produced. Thus within a 6 month period, not enough vaccine had been produced to be useful in the first epidemic wave.

(b) *1968*

The situation was even worse in 1968. Although vaccine production in the USA began within 2 months of the new H3N2 strain becoming available (1 month earlier than in 1957), only 20 million doses were available when the Hong Kong influenza epidemic reached its peak in the USA (Murray 1969). In this instance, the first epidemic wave was at its peak in the USA only 5 months from the recognition of the Hong Kong H3N2 strain and 4 months from the start of vaccine production.

(c) *1976*

In 1976, the Fort Dix 'swine flu' outbreak triggered a pandemic alert and the USA responded by planning a nationwide immunization campaign. Once the manufacturers had begun to produce vaccine, the rate of production (10 million doses per month) was far in excess of earlier attempts in 1957 and 1968. This was largely due to the use of a high-growth reassortant prepared from the A/New Jersey/76 (H1N1) virus. However, a further factor was the assurance by the US Government of a market for the vaccine, so that manufacturers were able to operate at maximum capacity, 24 hours per day, 7 days a week, and ultimately they produced 150 million doses of vaccine (Barry *et al.* 1977). It was a remarkable achievement that the manufacturers produced enough monovalent vaccine for the whole population of the USA within a 3 month period.

However, assurances of vaccine sales came at a price; there was a 1–2 month delay while indemnification legislation was passed in the USA. There would also have been delays in securing fertile hens' eggs, as the normal vaccine production season was 3–4 months earlier. Other delays in 1976, that were probably not significant in 1957 or 1968, were due to increased awareness of vaccine safety and vaccine standardization. Since 1968, vaccine purification had improved and microbial contamination of vaccines could be controlled by use of a test for bacterial endotoxin. Similarly, tests of vaccine potency had improved from the chick cell agglutination (CCA) test in use in 1968 to the single radial immunodiffusion (SRD) test. This was a significant development as the CCA test was unable to reliably measure the potency of newly developed split and subunit vaccines. Vaccine manufacturers were thus able to produce a safer vaccine with more confidence in vaccine potency. This confidence was reinforced by independent tests performed by the Food and Drug Administration of the USA. Such independent testing added an extra 2–3 weeks to the time of vaccine availability (Barry *et al.* 1977). Thus the total lead time for vaccine production in the USA was 7–8 months.

(d) *1997*

In 1997, there was also a pandemic alert when the H5N1 virus appeared in man. However, this time there was a more measured response. In Hong Kong, although there were 18 human cases of H5N1 infection, there was

no evidence of person-to-person transmission and thus it was not thought appropriate to begin mass vaccine production, particularly as the cull of chickens in Hong Kong had removed the immediate threat. This action is consistent with the phases of the newly developed WHO Pandemic Preparedness Plan (WHO 1999). However, despite the lack of full-scale vaccine production in 1997–1998, most of the early stages of vaccine development took place, so it is appropriate to make comparisons with the experience of earlier years. The most striking difference is the fact that it took 7 months to produce the first lots of H5 inactivated vaccine for clinical trial. This is much longer than in 1957, 1968 and 1976 and it is important to examine the reasons for the delay.

The single most influential factor in 1997–1998 was the high degree of H5N1 virus pathogenicity. The virus was highly pathogenic for poultry, caused death in a third of the 18 documented human infections (Gao *et al.* 1999) and was even lethal for fertile hens' eggs. It was necessary to handle the Hong Kong virus under at least BSL 3+ containment and there were important public health and veterinary regulations to observe and permits to obtain before work could begin. Three principal strategies for H5N1 vaccine development were adopted in several laboratories throughout the world.

(i) Attenuate the A/Hong Kong/97 (H5N1) virus so that it was no longer lethal for poultry and other animals. An 'attenuated' H5 haemagglutinin (HA) protein and the NI neuraminidase (NA) gene were then rescued into suitable viruses by reverse genetics to produce H5N1 reassortants that were suitable for vaccine production.
(ii) Select a surrogate apathogenic H5N1 virus. The most suitable strain was A/duck/Singapore-Q/F119-2/97 (H5N3), which was antigenically similar to the H5N1 strain.
(iii) Express the H5 HA in baculoviruses by recombinant technology.

The first attenuated reassortants were produced in about 3 months from the date of the second human case in Hong Kong (K. Subbarao, Center for Disease Control Atlanta, GA, USA (CDC), personal communication; M. Tashiro, National Institute for Infectious Diseases, Tokyo, Japan (NIID), personal communication) and were demonstrated to be safe and protective in animals within 7 months (Li *et al.* 1999; Takada *et al.* 1999). However, there were concerns about their safety for man, so it was difficult to begin large-scale virus production without the security of biological containment facilities. This was a problem in 1998, when the threat of a pandemic was diminishing and the risks to man and the environment were great. However, such risks may have assumed only minor importance if the H5N1 virus had begun to spread around the world, a pandemic was imminent and vaccine was urgently needed. Despite such difficulties, one such vaccine was produced for clinical evaluation in Japan (Takada *et al.* 1999).

The A/duck/Singapore/97 strain possessed an antigenically suitable H5 HA, but in other respects it was unsuitable for vaccine production. It possessed an N3 NA and grew poorly in hens' eggs. Despite repeated attempts in several laboratories to produce high-growth reassortants in eggs, none was successful. Two variants of A/duck/Singapore/97 with better growth properties were produced, but neither was suitable for large-scale vaccine production. One such variant, NIB-40, was used for preclinical and clinical evaluation of H5 vaccines. By 9 months, experimental whole-virus vaccines had been shown to be protective in animals (Wood *et al.* 2000) and split vaccines had been produced in Italy and tested clinically in the UK by 17 months (Nicholson *et al.* 2001). One interesting feature of the clinical trial is that use was made of an adjuvant (MF-59), in order to provide experience of adjuvants in a pandemic situation.

The first lots of recombinant H5 HA were available for clinical use within 2–3 months of the second Hong Kong case (Treanor *et al.* 2001). Thus speed of production was a significant advantage of this approach. A second important feature was that of safety. There was no need to handle infectious virus, so no need for containment.

Another aspect of pandemic vaccine development that played a part in 1998 was the need for reagents to standardize H5 vaccine potency. In interpandemic years, reagents for vaccine standardization are produced to new vaccine strains and it takes about 2–3 months for their development. Reagents were produced to standardize the inactivated A/duck/Singapore/97 vaccines and also to standardize experimental vaccines produced from the pathogenic A/Hong Kong/97 (H5N1) strain. However, it took 6 months before the H5 reagents were available. This was due to (i) safety issues and the need to work under containment and to obtain permits for the work, (ii) poor growth of A/duck/Singapore/97 virus, and (iii) the fact that the immediate threat of a pandemic had disappeared. It is important to improve on this performance nonetheless, so that the availability of reagents is not a rate-limiting step in pandemic vaccine production.

In view of the difficulties encountered in 1998, it is fortunate therefore that H5 vaccine was not needed on a large scale. It is likely that the lead time for H5 vaccine production would have been in excess of the 7–8 months needed in 1976.

(e) *Conclusions from experience of vaccine development*

The 1976 experience in the USA demonstrated that manufacturers were capable of producing sufficient monovalent H1N1 vaccine for the whole population of the USA within 3 months. This was an admirable achievement and it may not be possible to improve on this performance. Comparisons between 1976, 1957 and 1968 illustrate the enormous benefit derived from the use of high-growth reassortants in 1976. The technology developed in the late 1960s by Professor E. Kilbourne (Kilbourne 1969) allowed manufacturers in 1976 to produce four times the amount of vaccine produced in 1957 and 1968. Reassortants have been in regular use over the past 30 years and their development would be a prominent feature of pandemic vaccine production.

What gives cause for concern is the long lead time before vaccine production started. In 1957 this period was 2–3 months; in 1968 it was 1–2 months; in 1976 it was 7–8 months; and in 1998 it may have been in excess of 7–8 months, should there have been an H5N1 pandemic. This

is a critical period when: high-growth reassortants are produced; reagents to standardize vaccine potency are produced; supplies of eggs are secured; negotiations about possible indemnity take place (in 1976); discussions are taking place about logistics of vaccine production, arrangements for clinical trials, arrangements for vaccine testing and licensing. It is important to plan ahead so that time can be saved by dealing with some of these issues beforehand.

One important aspect of modern vaccine production is the care taken to ensure product quality and safety. Modern vaccines are undoubtedly safer and more reliable in quality than those produced in 1957, 1968 and probably in 1976. However, the implementation of these measures demands extra time during vaccine production. In a pandemic situation, when speed is of the essence, careful decisions must be made so that vaccines can be produced quickly, without sacrificing quality or safety.

3. VACCINE IMMUNOGENICITY

A critical aspect of vaccine development is a demonstration that immunization is capable of inducing a protective immune response. In individuals who have been immunologically primed by exposure to related viruses by infection or by immunization, a single dose of 15 μg HA per strain is considered to give high levels of protective immunity in younger adults and to prevent severe consequences of infection in the elderly (MMWR 1999). Vaccines are usually prepared from split products or from purified subunits and occasionally from whole virions, but the immune responses to immunization in primed populations are considered equivalent for each type of vaccine. In the European Union (EU), there are regulatory criteria for satisfactory immunogenicity of influenza vaccines in annual clinical trials. These criteria, prepared by the EU Committee for Proprietary Medicinal Products (CPMP) (CPMP 1996) are as follows:

(i) Number of seroconversions or significant increase in anti-HA antibody $> 40\%$.
(ii) Mean geometric increase in antibody > 2.5.
(iii) Proportion of subjects achieving a haemagglutination inhibition (HI) titre 40 or single radial haemolysis (SRH) titre $> 25\,\mathrm{mm}^2$ should be $> 70\%$.

In a pandemic situation, the immune status of the population is quite different. At the onset of the 1957, 1968 and 1977 pandemics, younger adults were immunologically naive to the new strains, whereas older populations had been primed by previous infections of related strains (Potter 1998).

In 1997–1998, however, the human infections with the H5N1 virus gave cause for concern because there was worldwide naivety to this new subtype.

We therefore need to know whether any changes to immunization protocols are necessary, in order to protect people in pandemic situations. In this context, much valuable information can be gleaned from earlier studies.

(a) *1968*

Several clinical trials were performed with newly produced whole-virus vaccine from the A/Hong Kong/68 (H3N2) strain (Tauroso *et al.* 1969; Sonnoguchi 1969; Mostow *et al.* 1969; Monto *et al.* 1969; Waldman *et al.* 1969; Glezen *et al.* 1969; Brandon *et al.* 1969). Somewhat surprisingly, one dose of vaccine appeared to stimulate good immune responses and clinical protection. In fact, when compared with modern CPMP criteria for vaccine serology, most of the vaccines complied. In some trials two doses were administered and improved antibody responses were seen. These results must, however, be judged against the methods of vaccine standardization used in 1968. The CCA test used in 1968 was subsequently found to be unreliable, which means that direct comparison with results from later trials is quite difficult.

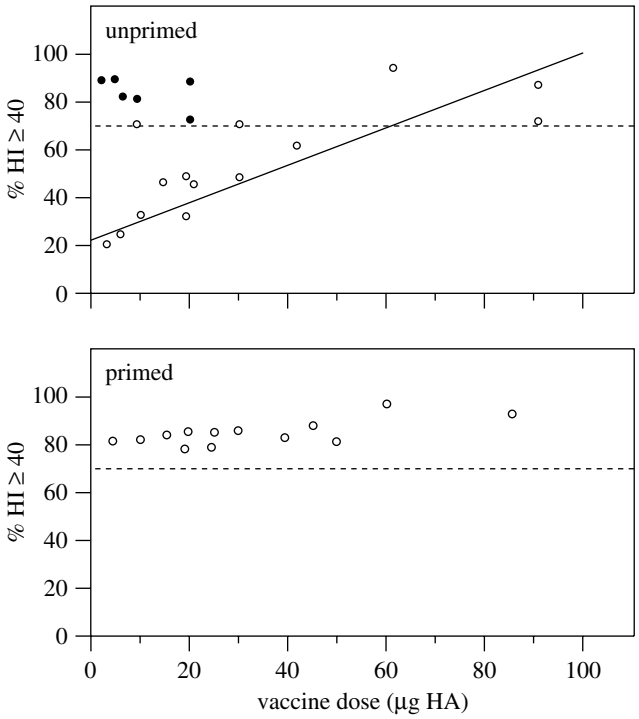

Figure 2. Immunogenicity of A/New Jersey/8/76 (H1N1) whole-virus vaccine in 1976 clinical trials. The incidence (%) of postvaccination HI antibody ⩾ 40 stimulated by influenza vaccines of different potencies (μg HA) in primed and unprimed populations is shown for 28 clinical trials performed in the USA and UK. Open circles, one dose; filled circles, two doses; dashed line, CPMP criteria. Data from Wright *et al.* (1977), Parkman *et al.* (1977), MRC (1977) and Jennings *et al.* (1981).

(b) *1976, 1977*

The Fort Dix outbreak gave rise to the largest, most intensive series of influenza vaccine trials ever conducted. The vaccines examined were either whole-virus, split or purified subunit vaccines produced from A/New Jersey/8/76 (H1N1) virus and the newly developed SRD test was used to standardize vaccine potency. Most of the trials were performed in the USA; Wright *et al.* (1977) reviewed 11 trials and Parkmann *et al.* (1977) reviewed 15 trials, whereas in the UK two trials were performed (MRC 1977; Jennings *et al.* 1981). A summary of the results of 28 whole-virus vaccine trials is shown in figure 2. There were clear differences between unprimed populations (under 24 years) and primed populations (over 24 years). There was a shallow dose-related increase in postvaccination antibody to one dose of

vaccine in unprimed populations and relatively high antigen concentrations (over 50 μg HA) were needed to meet CPMP criteria. If two vaccine doses were given to unprimed populations or one vaccine dose given to primed populations, much lower antigen concentrations (5 μg HA) were sufficient. When whole-virus and split or subunit vaccines were compared, the degree of immunological priming also had an effect. In unprimed populations, whole-virus vaccines were more immunogenic, whereas in primed populations no differences could be detected.

In 1977, when the A/USSR/92/77 (H1N1) virus emerged, vaccine was produced for clinical trial and results from eight trials performed in the USA and the UK (La Montagne *et al.* 1983; Nicholson *et al.* 1979; Potter *et al.* 1980) were very similar to those obtained with the A/New Jersey/8/76 virus.

One common finding in the H1N1 vaccine trials was the lower incidence of vaccine-associated reactions when split or subunit vaccines were used. In general, the incidence of adverse reactions also increased with vaccine dose. Thus in unprimed populations, high dose levels of whole-virus vaccines were most immunogenic, but also were most reactive.

Despite the overall low incidence of adverse reactions to the 'swine flu' vaccine in 1976, it was the recognition of a rare complication of influenza immunizations, Guillain–Barré syndrome, that ultimately halted the mass immunization campaign (Langmuir *et al.* 1984). This illustrates very well that rare events can take on significant proportions when huge numbers of individuals are concerned and public interest is high.

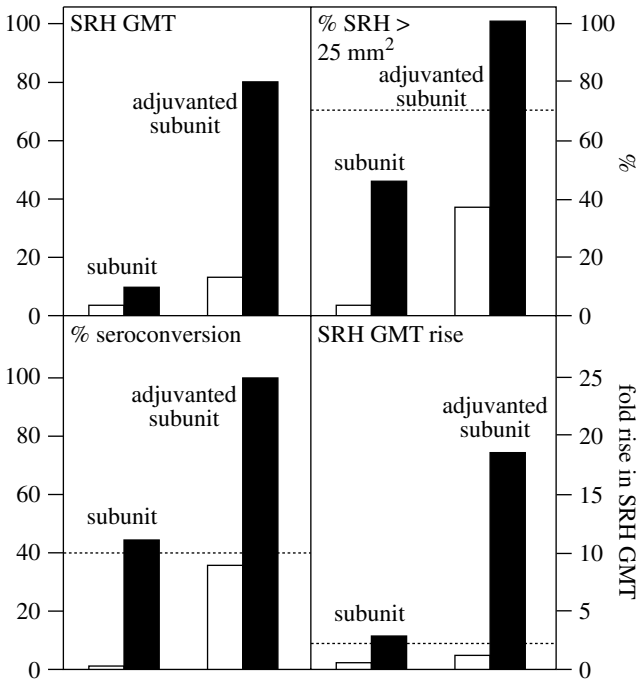

Figure 3. Immunogenicity of A/duck/Singapore/97 (H5N3) subunit and MF59 adjuvanted vaccine. SRH geometric mean titres (GMT), the incidence (%) of SRH antibody above 25 mm^2, percentage seroconversion and the SRH GMT rise in postvaccination antibody are indicated. Open bars, one dose of 15 μg HA; solid bars, two doses of 15 μg HA; dashed line, CPMP criteria. Data from Nicholson *et al.* (2001).

(c) *1997*

Despite the fact that H5 vaccines were developed in several laboratories throughout the world, very few were evaluated clinically. The first clinical trial was conducted with the recombinant HA protein, only 2–3 months after the second human case in Hong Kong. Results from this and subsequent trials have recently been summarized (Treanor *et al.* 2001) and they are generally disappointing. One of the problems with evaluating H5 vaccine immunogenicity in man was the insensitivity of the HI test. It was therefore necessary to do serology using alternative tests such as virus neutralization (VN) or SRH. Two 25 μg doses of recombinant HA stimulated a protective VN titre of 1:80 in only 17% of recipients and higher doses of 90 μg were required to induce a better response (52% responders).

Subunit vaccines produced from A/duck/Singapore/97 vaccine were assessed in a dose-escalating trial in comparison with subunit vaccines adsorbed with MF59 adjuvant (Nicholson *et al.* 2001). The SRH results (figure 3) demonstrate that a conventional 15 μg HA dose of conventional subunit H5 vaccine was poorly immunogenic and results after one dose did not comply with CPMP criteria. Even after two doses, SRH responses were barely acceptable and the vaccine would probably not have been protective. Higher doses of 30 μg HA (data not shown) did not improve immunogenicity. However, the MF59 vaccine was much more immunogenic and doses as low as 7.5 μg HA (data not shown) stimulated a satisfactory immune response even after one dose. All the A/duck/Singapore/97 vaccines were well tolerated and results from VN tests were generally in agreement with those of SRH.

Inactivated, split H5N1 vaccines produced by reverse genetics were evaluated by clinical trials performed in Japan. An H5N1 reassortant vaccine virus was produced from the modified H5HA of A/Hong Kong/156/97 virus and the remaining genes from an avirulent avian virus, A/duck/Hong Kong/836/80 (H3N1) (Takada *et al.* 1999). Although the vaccine was well tolerated, the VN antibody responses, even after two doses, were barely above background levels (S. Itamura, NIID, personal communication).

(d) *1999*

The events in Hong Kong stimulated much discussion about pandemic planning, not only in the public sector, but also within the vaccine manufacturing industry. One such company (Glaxo Smith Kline, Dresden, Germany (GSK)) began to make plans which involved preparing containment facilities for a possible pandemic strain and for the clinical evaluation of pandemic vaccines. The model vaccine strain chosen was the 1957 H2N2 strain, A/Singapore/57. H2N2 strains last circulated in the mid-1960s, so that in 1999, individuals below the age of 30 years would be immunologically naive. The split H2N2 vaccine was tested in populations below and above 30 years, and results essentially similar to those described in 1976 and 1977 were obtained: one dose of 15 μg HA was not sufficiently immunogenic in unprimed populations, but two doses of 15 μg HA in unprimed populations

Table 1. Minimum immunogenic dose of H1N1, H2N2 or H5N3 vaccines.

(Data are from many clinical trials from 1976 to 1999, as described in the text.)

vaccine type	virus subtype	population	minimum dose to comply with CPMP criteria (μg HA) one dose	two doses
whole-virus	H1N1	unprimed	40–50	5–10
split	H1N1	unprimed	> 65	20
subunit	H1N1	unprimed	> 18	9
split	H2N2	unprimed	> 15	15
subunit	H5N3	unprimed	> 30	> 30
adjuvant and subunit	H5N3	unprimed	7.5	7.5
whole-virus	H1N1	primed	5	NT[a]
split	H1N1	primed	5	NT
subunit	H1N1	primed	5	NT
subunit	H2N2	primed	< 15	< 15

[a] Not tested.

stimulated antibody responses that complied with CPMP criteria (N. Hehme & H. Engelmann, GSK, personal communication).

(e) *Conclusions from immunogenicity studies*

Unfortunately the 1968 studies must be excluded from this summary, due to unsatisfactory vaccine standardization. However, the remaining trials covering a 23 year period and three influenza subtypes show some remarkable similarities (table 1).

(i) In unprimed populations high antigen concentrations (at least 50 μg HA) were needed to stimulate a satisfactory immune response. In trials comparing whole-virus and split or subunit vaccines, whole-virus vaccines were more immunogenic.
(ii) With the exception of H5N3 vaccines, in unprimed populations two doses of 5–20 μg HA were satisfactory.
(iii) In primed populations one dose of 5 μg HA was generally satisfactory.

However, vaccines prepared from the H5 subtype (subunit A/duck/Singapore/97, recombinant H5 HA and the H5N1 reassortant vaccine produced by reverse genetics) were less immunogenic than vaccines prepared from H1N1 and H2N2 subtypes. It is possible that, as the A/duck/Singapore/97 and recombinant H5 HA vaccines were prepared from purified virus proteins, this had an effect on immunogenicity. Poor immunogenicity of subunit influenza vaccines has also been reported for H5N1 vaccines in animals (Rimmelzwaan *et al.* 1999) and for H9N2 vaccines in animals (Major *et al.* 2001). Alternatively the HA from avian viruses may be inherently less immunogenic than the HA from human viruses (Treanor *et al.* 2001). A dramatic improvement in immunogenicity was seen when the MF59 adjuvant was used with the A/duck/Singapore/97 vaccine and this approach may also be needed for other types of vaccines prepared from the H5 subtype. It is possible that a whole-virus H5 vaccine may be more immunogenic than a subunit or split vaccine and such comparisons will be useful for future planning.

4. PROSPECTS FOR THE FUTURE

The WHO has developed a Pandemic Preparedness Plan (WHO 1999), which indicates some key steps to be taken at international and national levels. It is almost inevitable that, during the first wave of a pandemic, vaccine will be in short supply. The WHO has recommended that an estimation of vaccine production capacity should be made and plans developed to make best use of the vaccine. Decisions must be taken on whether to attempt mass vaccination or to vaccinate only key risk groups. The role of antivirals in times of vaccine shortage must also be addressed. Whatever the decision, it will be vital to produce a safe effective vaccine in as short a time as possible. The following are some recommendations for future action based on experiences in the past.

(a) *Safe, productive vaccine strain*

High virus yields can be obtained by use of high-growth reassortants, but experience with the A/duck/Singapore/97 strain demonstrated how unreliable this process can be. More experience should be gained in producing such reassortants by reverse genetics. This has the potential for more reliable selection of influenza virus genes and for attenuating highly pathogenic viruses, as was achieved with the A/Hong Kong/97 strain. Developing new vaccine strains even with new technology could take up to 2 months, and with a pandemic sweeping across the world we may need more immediate action. One possibility would be to develop a library of high-growth reassortants from selected influenza subtypes and to use the most appropriate reassortant for vaccine against the first pandemic wave. Vaccines derived from the pandemic virus could be used for a second and more extensive immunization campaign. Even though such a reassortant library may not provide an exact antigenic match with a new pandemic strain, there should be sufficient antigenic similarity to protect against the major clinical consequences of a pandemic.

(b) *Vaccine potency reagents*

Reagents for the SRD test usually take 2–3 months to produce, but in 1998 much longer was needed. In an emergency, it may be necessary to use alternative estimates of vaccine potency, e.g. protein content, before reagents become available. It would be helpful now to accumulate comparative data between the SRD test and alternative tests, in order to validate more unconventional approaches. It should also be possible to develop libraries of SRD reagents which could be used for (i) potency estimates of vaccines produced from reassortant libraries, and (ii) potency estimates of vaccines produced from pandemic viruses.

(c) *Vaccine substrates*

The availability of fertile hens' eggs is a significant factor in vaccine production. There would be severe delays if a pandemic strain appeared shortly after the

normal vaccine production period. There has been much recent development of vaccines produced on mammalian cell culture (Brown *et al.* 1999), but as yet, none of these vaccines is licensed. MDCK and Vero cells appear to support the growth of a variety of influenza strains including avian strains (T. Mabrouk, Biochem Vaccines, Montreal, Canada, personal communication; O. Kistner, Baxter Immuno, Vienna, Austria, personal communication) and they could theoretically be available any time of the year for vaccine production. If the problems encountered with immunogenicity can be overcome, recombinant protein vaccines also offer a more flexible approach to vaccine production.

(d) *Regulatory issues*

In the EU, it normally takes 4–6 weeks for the clinical trials required for relicensing of vaccines, about 2–3 weeks for the licensing process and 2–3 weeks for independent national authority batch release tests. Although the details will differ in other parts of the world, the regulatory process usually takes 8–12 weeks. Contingency plans should be prepared for fast-track licensing and testing of vaccines, including new approaches to influenza immunization. In view of some of the clinical trial results reported earlier, it may also be useful for manufacturers to have a whole-virus vaccine licensed.

(e) *Vaccine immunogenicity*

Many clinical trials have been reviewed in this article and some conclusions about vaccine immunogenicity can be made. However, we should gain more experience in comparing whole-virus, split, subunit and adjuvanted vaccines with as many subtypes as are feasible. Hopefully a consensus will emerge and the most antigen-sparing strategies for immunization can then be used. Development of alternative immunization strategies such as DNA vaccines, vaccines produced by reverse genetics and cell culture vaccines, should be encouraged, particularly using novel subtypes. Opinion seems to be divided on the role of live influenza vaccines in a pandemic. Are such vaccines too risky because of the limited time available for safety tests and the possibility of their use promoting early release of a novel subtype into the community? Alternatively the benefits of live vaccines include the fact that they can be produced quickly, more doses can be achieved per egg than inactivated vaccines and more complete protection may be achieved. Discussion about the above issues should be part of future planning for pandemics.

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REFERENCES

Barry, D. W., Mayner, R. E., Meisler, J. M. & Seligmann Jr, E. B. 1977 Evaluation and control of vaccines for the National Influenza Immunization Program. *J. Infect. Dis.* **136**, S407–S414.

Brandon, F. B., Cox, F., Quinn, E., Timm, E. A. & McLean Jr, I. W. 1969 Influenza immunization. Clinical studies with ether-split subunit vaccines. *Bull. WHO* **41**, 629–637.

Brown, F., Robertson, J. S., Schild, G. C. & Wood, J. M. 1999 Inactivated influenza vaccines prepared in cell culture. *Dev. Biol. Stand.* **98**.

CPMP 1996 Note for guidance on harmonization of requirements for influenza vaccines. CPMP/BWP/214/96. Circular No. 96-0666, 1–22. (http://www.emea.eu.int/pdfs/human/bwp/021496en.pdf)

Gao, P., Watanabe, S., Ito, T., Goto, H., Wells, K., McGregor, M., Cooley, A. J. & Kawaoka, Y. 1999 Biological heterogeneity, including systemic replication in mice, of H5N1 influenza A virus isolates from human in Hong Kong. *J. Virol.* **73**, 3184–3189.

Glezen, W. P., Loda, F. A. & Denny, F. W. 1969 A field evaluation of inactivated, zonal-centrifuged influenza vaccines in children in Chapel Hill, North Carolina, 1968–69. *Bull. WHO* **41**, 566–569.

Gust, I. D., Hampson, A. W. & Lavanchy, D. 2001 Planning for the next pandemic. *Rev. Med. Virol.* **11**, 59–70.

Jennings, R., Potter, C. W., Massey, P. M. O., Duerden, B. I., Martin, J. & Bevan, A. M. 1981 Responses of volunteers to inactivated influenza virus vaccines. *J. Hyg.* **86**, 1–16.

Kilbourne, E. D. 1969 Future influenza vaccines and the use of genetic recombinants. *Bull. WHO* **41**, 643–645.

La Montagne, J. R., Noble, G. R., Quinnan, G. V., Curlin, G. T., Blackwelder, W. C., Smith, J. I., Ennis, F. A. & Bozeman, F. M. 1983 Summary of clinical trials of inactivated influenza vaccine—1978. *Rev. Infect. Dis.* **5**, 723–736.

Langmuir, A. D., Bregman, D. J., Kurland, L. T., Nathanson, N. & Victor, M. 1984 An epidemiologic and clinical evaluation of Guillain–Barré syndrome reported in association with the administration of swine influenza vaccines. *Am. J. Epidemiol.* **119**, 841–879.

Li, S., Liu, C., Klimov, A., Subbarao, K., Perdue, M. L., Mo, D., Ji, Y., Woods, L., Hietala, S. & Bryant, M. 1999 Recombinant influenza A virus vaccines for the pathogenic human A/Hong Kong/97 (H5N1) viruses. *J. Infect. Dis.* **179**, 1132–1138.

Major, D. L., Newman, R. W., Dunleavy, U., Heath, A. B., Ploss, K. & Wood, J. M. 2001 Evaluation of H9N2 influenza vaccines in Balb/c mice. Options for the control of influenza: IV. (In the press.)

MRC 1977 Antibody responses and reactogenicity of graded doses of inactivated influenza A/New Jersey/76 whole virus vaccine in humans. *J. Infect. Dis.* **136**, S475–S483.

MMWR 1999 Prevention and control of influenza: recommendations of the Advisory Committee on Immunization Practices (ACIP). *Morb. Mortal. Wkly. Rep.* **48**, 1–28.

Monto, A. S., Davenport, F. M., Napier, J. A. & Francis Jr, T. 1969 Effect of vaccination of a school-age population upon the course of an A2/Hong Kong influenza epidemic. *Bull. WHO* **41**, 537–542.

Mostow, S. R., Schoenbaum, S. C., Dowdle, W. R., Coleman, M. T. & Kaye, H. S. 1969 Studies with inactivated influenza vaccines purified by zonal centrifugation 1. Adverse reactions and serological responses. *Bull. WHO* **41,** 525–530.

Murray, R. 1961 Some problems in the standardization and control of influenza vaccine in 1957. *Am. Rev. Resp. Dis.* **83**, 160–167.

Murray, R. 1969 Production and testing in the USA of influenza virus vaccine made from the Hong Kong variant in 1968–69. *Bull. WHO* **41**, 495–496.

Nicholson, K. G. (and 11 others) 1979 Clinical studies of monovalent inactivated whole virus and subunit A/USSR/77 (H1N1) vaccine: serological responses and clinical reactions. *J. Biol. Stand.* **7**, 123–136.

Nicholson, K. G., Colegate, A. E., Podda, A., Stephenson, I., Wood, J., Ypma, E. & Zambon, M. C. 2001 Confronting a potential H5N1 pandemic: a randomised controlled trial of conventional and MF59 adjuvanted influenza A/duck/

Singapore/97 (H5N3) surface antigen vaccine. *Lancet* **357**, 1937–1943.

Parkman, P. D., Hopps, H. E., Rastogi, S. C. & Meyer Jr, H. M. 1977 Summary of clinical trials of influenza virus vaccines in adults. *J. Infect. Dis.* **136**, S722–S730.

Potter, C. W. 1998 Chronicle of influenza pandemics. In *Textbook of influenza* (ed. K. G. Nicholson, R. G. Webster & A.-J. Hay), pp. 3–18. Oxford: Blackwell Science.

Potter, C. W., Clark, A., Jennings, R., Schild, G. C., Wood, J. M. & McWilliam, P. K. A. 1980 Reactogenicity and immunogenicity of inactivated influenza A (H1N1) virus vaccine in unprimed children. Report to the Medical Research Council committee on influenza and other respiratory virus vaccines. *J. Biol. Stand.* **8**, 35–48.

Rimmelzwaan, G. F., Claas, E. C. J., van Amerongen, G., de Jong, J. C. & Osterhaus, A. D. M. E. 1999 ISCOM vaccine induced protection against a lethal challenge with a human H5N1 influenza virus. *Vaccine* **17**, 1355–1358.

Sonnoguchi, T. 1969 Haemagglutination-inhibiting antibody response to and efficacy of inactivated Hong Kong influenza vaccine. *Bull. WHO* **41**, 517–523.

Takada, A. (and 11 others) 1999 Avirulent avian influenza virus as a vaccine strain against a potential human pandemic. *J. Virol.* **73**, 8303–8307.

Tauroso, N. M., Gleckman, R., Pedreira, F. A., Sabbaj, J., Yahwak, R. & Madoff, M. A. 1969 Effect of dosage and route of inoculation upon antigenicity of inactivated influenza virus vaccine (Hong Kong strain) in man. *Bull. WHO* **41**, 507–516.

Treanor, J. J., Wilkinson, B. E., Masseoud, F., Hu-Primmer, J., Battaglia, R., O'Brien, D., Wolff, M., Rabinovich, G., Blackwelder, W. & Katz, J. M. 2001 Safety and immunogenicity of a recombinant haemagglutinin vaccine for H5 influenza in humans. *Vaccine* **19**, 1732–1737.

Waldman, R. H., Bond, J. O., Levitt, L. P., Hartwig, E. C., Prather, E. C., Baratta, R. L., Neill, J. S. & Small Jr, P. A. 1969 An evaluation of influenza immunization. Influence of route of administration and vaccine strain. *Bull. WHO* **41**, 543–548.

WHO 1996 WHO influenza surveillance. *Wkly. Epidemiol. Rec.* **71**, 353–357.

WHO 1999 *Influenza pandemic preparedness plan. The role of WHO and guidelines for national and regional planning.* Geneva: WHO.

Wood, J. M. & Williams, M. S. 1998 History of inactivated influenza vaccines. In *Textbook of influenza* (ed. K. G. Nicholson, R. G. Webster & A.-J. Hay), pp. 333–345. Oxford: Blackwell Science.

Wood, J. M., Major, D., Daly, J., Newman, R. W., Dunleavy, U., Nicolson, C., Robertson, J. S., Schild, G. C. & Lee-Lin, Y. N. 2000 Vaccines against H5N1 influenza. *Vaccine* **18**, 571–580.

Wright, P. F., Thompson, J., Vaughn, W. K., Folland, D. S., Sell, S. H. W. & Karzan, D. T. 1977 Trials of influenza A/New Jersey/76 virus vaccine in normal children: an overview of age-related antigenicity and reactogenicity. *J. Infect. Dis.* **136**, S731–S741.