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ORIGINAL INVESTIGATION

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Experience with the clinical development of influenza vaccines for potential pandemics

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Abstract During normal interpandemic influenza seasons, immune responses to vaccines are quite predictable and meet the licensing criteria of the European Union (EU) Committee for Proprietary Medicinal Products (CPMP). In a pandemic situation, large sections, if not all of the community will be immunologically naïve and therefore new immunisation strategies will be needed. In 1976 and 1977 H1N1 vaccines were prepared and tested clinically. To stimulate 'protective' antibody responses, two doses of vaccine were needed in people below the age of 24 years (no previous experience of H1N1 virus), whereas one conventional dose was adequate in older people. In 1997, the highly pathogenic avian influenza H5N1 virus caused widespread concern when it infected man, with lethal effects. Due to safety concerns it was necessary to adopt new strategies for vaccine development and one such strategy was to produce vaccine from an avirulent H5N3 virus, A/Duck/Singapore-Q/F119-2/97. Clinical trials of a subunit vaccine prepared from A/Duck/Sing/97 virus revealed that even two doses of twice the normal vaccine concentration (i.e. 30 μg haemagglutinin) were poorly immunogenic, whereas an H5N3 vaccine adjuvanted with microfluidised emulsion (MF) 59 stimulated antibody levels that complied with CPMP criteria after two half strength doses (i.e. 7.5 μg haemagglutinin).

Keywords Influenza · Pandemic · Vaccine

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Introduction

A critical aspect of influenza vaccine development is the demonstration that immunisation is capable of inducing a protective immune response. In individuals who have been immunologically primed by exposure to related viruses by infection or by immunisation, a single dose of 15 μg haemagglutinin (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 [16]. Vaccines are usually prepared from split products or from purified subunits and occasionally from whole virions, but the immune responses to immunisation 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 performed in adult and elderly populations. The criteria for adult populations, prepared by the EU Committee for Proprietary Medicinal Products (CPMP) [4] are as follows:

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

In a pandemic situation, the immune status of the population is quite different. At the onset of the 1957 (H2N2), 1968 (H3N2) and 1977 (H1N1) pandemics, younger adults were immunologically naïve to the new strains, whereas older populations had been primed by previous infections of related strains [21]. In 1997/98 the

human infections with avian H5N1 and H9N2 viruses in Hong Kong gave cause for concern because there was worldwide naivety to these influenza subtypes. We therefore need to know whether any changes to immunisation protocols are necessary to protect people in pandemic situations. In this context, much valuable information can be gleaned from earlier studies performed in 1976 and 1977 and also from more recent experience with H5N1 and H9N2 influenza.

Results from 1976 and 1977

In 1976, the Fort Dix 'Swine Flu' outbreak caused by an H1N1 virus, triggered a pandemic alert and the United States of America (USA) responded by planning a nation-wide immunisation campaign. 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, the lead time for vaccine production in the USA was 7–8 months, as a result of legal problems, shortages of fertile hens' eggs, development of a high-growth reassortant and development of suitable quality control tests [3].

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 Single Radial Diffusion (SRD) test was used to standardise vaccine potency. Most of the trials were performed in the USA; Wright et al. [27] reviewed 11 trials and Parkmann et al. [19] reviewed 15 trials, whereas in the United Kingdom (UK) two trials were performed by the Medical Research Council (MRC) [15] and Jennings et al. [9]. A summary of the results of 28 whole virus vaccine trials is shown in Fig. 1. There were clear differences between unprimed populations (people under 24 years, as they were born after H1N1 viruses last circulated) and primed populations (over 24 years). There was a shallow dose-related increase in post-vaccination 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 [10, 17, 22] were very similar to those obtained with the A/New Jersey/8/76 virus.

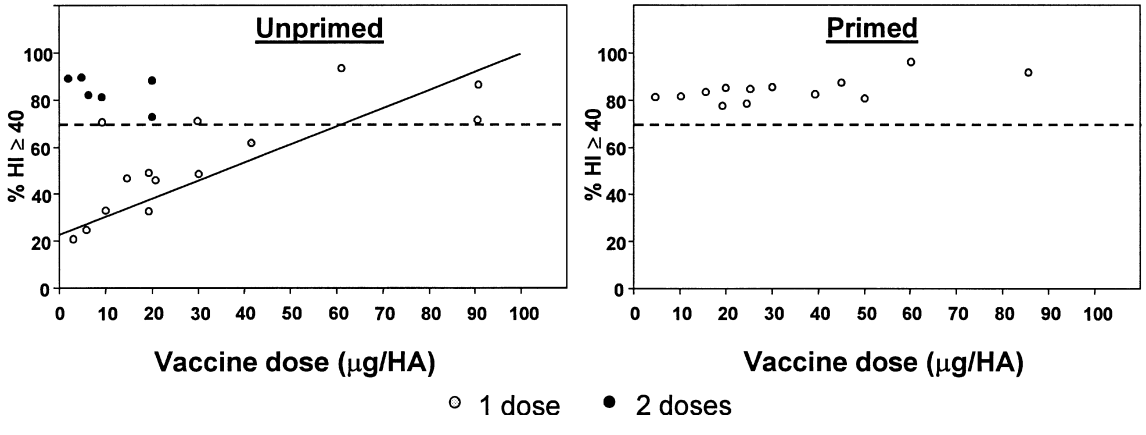
One common finding in both the 1976 and 1977 H1N1 vaccine trials was the lower incidence of vaccine-associated reactions when split or subunit vaccines were used. In general the incidence of adverse reactions also increased with vaccine dose. Thus, in unprimed populations, high dose levels of whole virus 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 immunisations, Guillain-Barré syndrome (GBS), that ultimately halted the mass immunisation campaign [11]. This illustrates very well that rare events can take on significant proportions when huge numbers of individuals are concerned and public interest is high.

Fig. 1 Immunogenicity of A/New Jersey/8/76 (H1N1) whole virus vaccine in 1976 clinical trials. The incidence (%) of post-vaccination 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. *Filled circles* one dose; *empty circles* two doses; *dashed line* criteria of the CPMP (*HA* haemagglutinin, *HI* haemagglutination-inhibition, *CPMP* Committee for Proprietary Medicinal Products) (data from [9, 15, 19, 27])

Results from 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 WHO Pandemic Preparedness Plan [25]. Experimental H5N1 vaccines were developed, despite practical difficulties due to the high degree of virus pathogenicity. The virus was highly pathogenic for poultry, caused death in a third of the 18 documented human infections [6, 28] and was even lethal for fertile hens' eggs. It was necessary to handle the Hong Kong virus under at least biosafety level (BSL) 3+ containment and there were important public health and veterinary regulations to observe and permits to obtain before work could begin. Three principle strategies for H5N1 vaccine development were adopted in several laboratories throughout the world:

- 'Attenuate the A/Hong Kong/97 (H5N1) virus so that it is no longer lethal for poultry and other animals'. An 'attenuated' H5 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.
- 'Select a surrogate apathogenic H5N1 virus'. The most suitable strain was A/Duck/Singapore-Q/F119-2/97 (H5N3), whose HA was antigenically similar to those of the H5N1 strains.
- '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 CDC USA; M. Tashiro NIID personal communication) and were demonstrated to be safe and protective in animals within 7 months [12, 23]. However, there were concerns about their safety for man, so that it was difficult to begin large-scale virus production, without the security of biological containment facilities.

The apathogenic A/Duck/Sing/97 virus was used to develop conventional whole virus and subunit egg-grown inactivated vaccines. When mice were immunised with two doses of whole virus vaccine containing the normal human dose of 15 μg HA, high levels of HI antibody were produced. After challenge with a lethal dose of Hong Kong H5N1 virus all the immunised mice survived, whereas control mice did not [26]. This demonstrated the potential of the A/Duck/Sing/97 virus for use as a vaccine against H5N1 influenza. Subsequently, a subunit inactivated vaccine was produced for clinical evaluation in an observer-blind, phase I, randomised trial in comparison with microfluidised emulsion (MF) 59-adjuvanted subunit vaccine. Two doses of 7.5, 15 or 30 μg HA were given 3 weeks apart to healthy subjects and safety, tolerability and immunogenicity were assessed [18].

Antibody responses to the A/Duck/Sing/97 vaccine were assessed by three different tests, HI, virus neutralisation (VN) and SRH. One of the problems faced by investigators of the Hong Kong outbreak was the insensitivity of the HI test for detection of human antibody to H5 HA. This was confirmed by the A/Duck/Sing/97 vaccine trials, where VN and SRH tests detected substantially more H5 antibody than did HI. Although all doses of non-adjuvanted vaccine were well tolerated, they were poorly immunogenic and the geometric mean

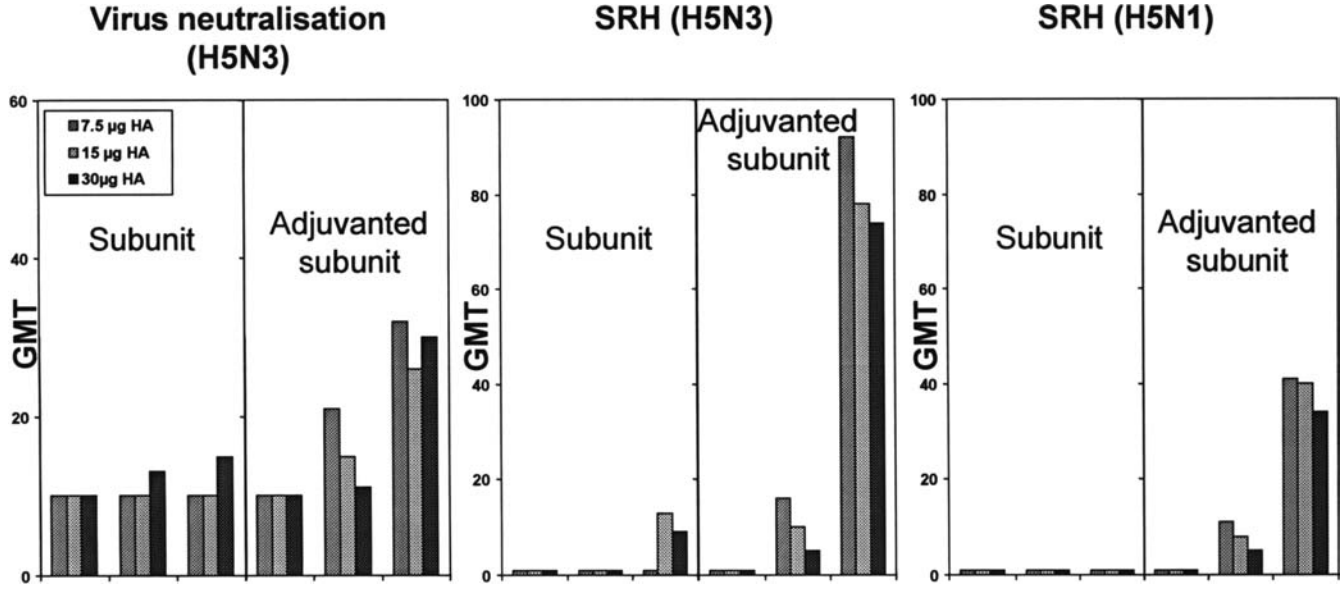


Fig. 2 Immunogenicity of A/Duck/Singapore/97 (H5N3) subunit and MF59-adjuvanted subunit vaccine. Virus neutralisation and single radial haemolysis (*SRH*) antibody against A/Duck/Singapore/97 (H5N3) virus and SRH antibody against A/Hong Kong/1073/99 (H5N1) virus are indicated. Responses after one vaccine dose are indicated at day 21 and after two vaccine doses at day 42 (*GMT* geometric mean antibody titres) (data from [18])

antibody titres were significantly lower than those induced by the adjuvanted vaccine (Fig. 2). When SRH antibody titres were measured using the Hong Kong H5N1 virus, they were about 50% reduced when compared with A/Duck/Sing/97 antibody. This may be a reflection on antigenic differences between the vaccine virus and the H5N1 virus. It was intriguing that the highest dose (30 μg HA) of the adjuvanted vaccine induced less antibody than that of the lowest dose (7.5 μg HA). Nicholson et al. [18] proposed that the relative quantities of MF59 adjuvant and antigen could affect vaccine immunogenicity, which is a subject for further investigation. There were no significant differences in vaccine tolerability between the MF59 vaccine and the conventional subunit vaccine. There are no accepted clinical correlates of protection for the VN test, but protective levels of HI and SRH antibody have already been determined [2, 5, 8], and there are CPMP licensing criteria for the results of HI and SRH tests. When the SRH antibody stimulated by A/Duck/Sing/97 vaccine was tested for compliance with CPMP criteria, the conventional subunit vaccine failed to meet licensing standards, even after two doses of 30 μg HA. However, two 15-μg HA doses of adjuvanted vaccine met all three CPMP criteria (Fig. 3). Indeed the results indicated that two 7.5-μg HA doses of adjuvanted vaccine were also satisfactory [18].

Results from 1999

A few months after the H5N1 infections in Hong Kong, human infections caused by a further avian influenza virus subtype H9N2 were detected in China and Hong Kong [7, 20]. Although the H9N2 viruses were non-pathogenic, they caused clinical illness in man, they were genetically related to the H5N1 virus and similar viruses had widespread distribution in poultry [1]. H9N2 viruses thus posed a pandemic threat and efforts to develop vaccines received high priority. Experimental H9N2 vaccines were developed in the UK and the USA and were shown to stimulate protective immune responses in mice [13, 14]. Clinical trial lots of whole virus and subunit H9N2 vaccine were subsequently produced and after being administered to volunteers in a Phase I study, the results are awaiting evaluation.

Conclusions

There are some important conclusions that can be drawn from the H1N1 and H5N3 vaccine trials:

- In unprimed populations, whole virus vaccines were more immunogenic than split or subunit vaccines (H1N1 trials).
- To induce a satisfactory immune response in an unprimed population, high antigen concentrations were needed (more than 50 μg HA in H1N1 trials, more than 30 μg HA in H5N3 trial)
- Two doses of 15 μg HA for an H1N1 vaccine were sufficiently immunogenic in unprimed populations, but even 30 μg HA of the H5N3 subunit vaccine was weakly immunogenic.
- An MF59 adjuvant significantly improved the immunogenicity of the H5N3 subunit vaccine so that two 7.5-μg HA doses were satisfactory.

Thus, from the available evidence, it appears that H5N3 vaccines were less immunogenic than those produced from H1N1 viruses. Poor immunogenicity in man has also been demonstrated for a recombinant H5 HA vaccine produced in baculoviruses [24] and for an inactivated H5N1 vaccine produced by reverse genetics (S. Itamura, NIID, Japan, personal communication). Whether these results are specific to the use of the H5 HA subtype or whether they are due to avian virus HA

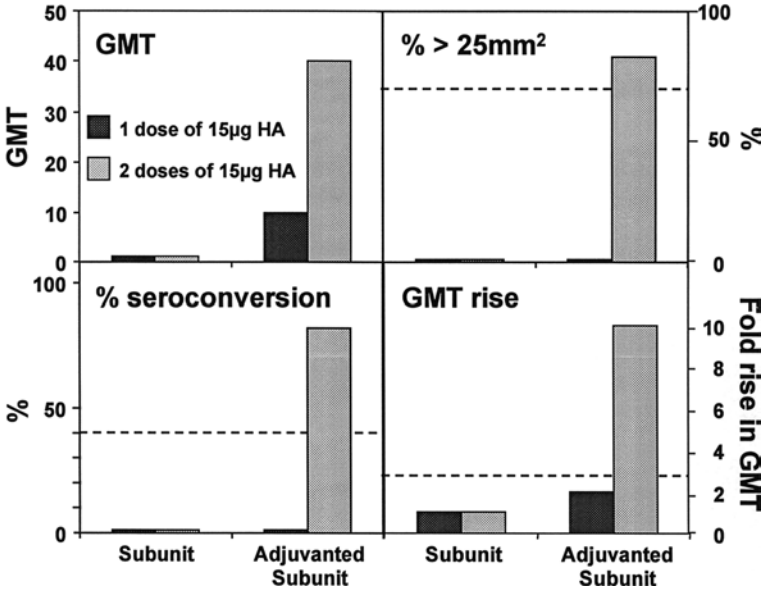


Fig. 3 Compliance with CPMP criteria of SRH data from A/Duck/Singapore/97 (H5N3) vaccine clinical trial. SRH antibody to A/Hong Kong/1073/99 (H5N1) virus is indicated. The criteria of the CPMP for incidence (%) of SRH antibody above 25 mm^2, % seroconversion and rise of SRH geometric mean titres are indicated (*dashed line*) (data from [18]).

being generally less immunogenic than those of human viruses is open to speculation. Further clinical studies of vaccines prepared from other influenza HA subtypes are needed to answer these questions.

The results with the MF59 adjuvant are particularly encouraging and suggest that the use of adjuvants and antigen delivery systems should be further evaluated in relationship to pandemic situations.

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