

A comparison of live and inactivated influenza A (H1N1) virus vaccines

2. Long-term immunity

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SUMMARY

Groups of volunteers were immunized with one of three influenza virus vaccines, and the resistance to challenge infection with attenuated influenza A (H1N1) virus was measured 8 months later. The vaccines were aqueous subunit influenza A/USSR/77 (H1N1) vaccine, aqueous subunit influenza B/Hong Kong/73 vaccine, or attenuated influenza virus A (H1N1) vaccine. The B virus vaccine was included as a control to assess the incidence of natural A virus infection during the study period. A proportion of the B virus vaccinees had pre-existing A (H1N1) virus antibody and were used to study the immunity conferred by natural infection to the live virus challenge. The serum antibody responses were measured at 1 and 8 months after immunization. The results showed that all the vaccines induced serum HI antibody in a proportion of the volunteers; however, after 1 month, higher titres of serum antibody were found in volunteers given inactivated A vaccine than in those given live attenuated A virus vaccine. Eight months post-immunization the titres of serum antibody in volunteers given inactivated vaccine had declined significantly, but there were no changes in the antibody titres of those given live virus vaccine. The incidence of infection by the challenge virus at 8 months post-immunization was directly related to the serum antibody titres 1 month post-immunization; no evidence was obtained to suggest that those given live virus vaccine had a more solid immunity than those given inactivated vaccine.

INTRODUCTION

Comparative studies of attenuated and inactivated influenza vaccines have suggested that the former may induce a better immunity against challenge virus infection (Beare *et al.* 1968; Freestone *et al.* 1972). However, in these studies challenge virus infection was given 3–4 weeks after immunization, and there are no data that show there is a difference beyond this time. The more solid immunity reported for immunization with attenuated vaccines may be due to induction of local antibody synthesis, since local antibody has been shown to contribute to immunity (Potter *et al.* 1975; Potter & Oxford, 1979) and immunization with attenuated vaccines induced higher titres of local antibody than inactivated vaccines (Waldman *et al.* 1968; Kasal *et al.* 1969). However, the local antibody response to influenza virus infection is short-lived, and the immunity associated with attenuated influenza virus vaccines may also be short-lived. To test this thesis, a comparative study of inactivated and live attenuated influenza virus vaccines was carried out in which the challenge virus infection was given eight months after immunization.

We report the results of a study in which volunteers were immunized with either aqueous subunit influenza A (H1N1) or B virus vaccines; or with a live attenuated influenza A virus vaccine. The B virus vaccine acted as a control but also conferred the advantage of a degree of immunity to current influenza virus infections. The serum HI antibody responses were determined 1 month and 8 months after immunization. Volunteers were then inoculated intranasally with a challenge live attenuated influenza A (H1N1) virus. The incidence of challenge virus infection was determined serologically 1 month later.

MATERIALS AND METHODS

Influenza virus vaccines

Monovalent, aqueous subunit influenza virus A/USSR/77 (H1N1) and B/Hong Kong/73 virus vaccines were supplied by Glaxo Operations, Speke; these were surface antigen vaccines prepared from Triton 101-treated virus particles (Brady & Furminger, 1978), and were not adsorbed by alhydrogel. Both vaccines contained 200 International Units (i.u.) of haemagglutinin (HA) per 0.5 ml, which corresponded to approximately 10 µg of HA per 0.5 ml inoculation volume.

Attenuated influenza virus A (H1N1) clone 144-B was obtained from Dr B. K. Murphy, N.I.H., Bethesda, M.D., USA: This virus strain was a recombinant of influenza virus ts/H2N2 and A/USSR/77 (H1N1) and has been shown to be attenuated in volunteers (Van Voorthuizen, Jens & Saes, 1981). The virus was supplied in ampoules containing $10^{7.0}$ egg-infectious doses (EID₅₀) per 0.5 ml, which was used to inoculate volunteers.

Experimental design

One hundred and twenty-eight students from the Universities of Sheffield and Birmingham aged 18–20 years volunteered to take part in the study; all were in good health, and had no known allergy to eggs. The volunteers were divided into four groups based on pre-existing haemagglutination-inhibiting (HI) antibody to influenza virus A/USSR/77 (H1N1). Three groups contained individuals the

majority of whom had no significant HI antibody to A/USSR/77 (H1N1) virus and a fourth group contained volunteers with relatively high titres of serum HI antibody to influenza A/USSR/77 (H1N1) virus. In the low antibody groups, group 1 were immunized intranasally with 0.5 ml of attenuated influenza A virus (Potter *et al.* 1977); group 2 were each immunized deep subcutaneously with 0.5 ml of inactivated subunit influenza A/USSR/77 (H1N1) vaccine; volunteers in the third and fourth groups, the latter of which was composed of vaccinees who had relatively high titres of antibody to A/USSR/77 virus, were immunized subcutaneously with 0.5 ml of influenza B vaccine.

Eight months following immunization, each volunteer was bled and then inoculated intranasally with $10^{7.0}$ EID₅₀ of the attenuated challenge influenza virus A (H1N1) clone 144-b. Serum was assayed for HI antibody against both influenza A/USSR/77 (H1N1) and B/Hong Kong/73 viruses 1 month and 8 months after immunization. This also served to assess the incidence of natural infections by the two viruses during the period of study. A final blood specimen was obtained from each volunteer 1 month following challenge virus infection, and titrated for HI antibody against influenza A/USSR/77 (H1N1) virus; a fourfold or greater increase in serum HI antibody titre following virus immunization was taken as proof of infection by the challenge virus.

Haemagglutination-inhibiting (HI) antibody titres

All serum specimens from the volunteers were coded and forwarded to the National Institute for Biological Standardization and Control, and titrated for HI antibody, using standard techniques following treatment with cholera filtrate (Phillips-Duphar-B.V.) for 18 h at 37 °C and subsequently by incubation at 56 °C for 1 h (W.H.O., 1953). Reference sera were included in each assay.

RESULTS

Reactions to immunization

Specific measurements of the incidence of reactions to immunization with the inactivated or attenuated vaccines were not made in the present study, since previous studies in similar volunteer groups had shown the vaccines to induce only a low incidence of mild reactions (Clark *et al.* 1983). However, volunteers were asked to report any reactions; no reports were made.

Serum HI antibody response to immunization

(1) *Inactivated influenza A/USSR/77 (H1N1) vaccine*

The distribution of serum HI antibody titres prior to and 1 month following immunization is shown in Tables 1 and 2. Thirteen (33%) of the 39 volunteers had HI antibody titres to influenza A/USSR/77 of ≥ 40 prior to immunization, compared to 30 (77%) following immunization, and this titre of antibody has been shown to correlate with 50% immunity to challenge virus infection (Hobson, Beare & Ward-Gardner, 1972; Potter & Oxford, 1979). The geometric mean titre (g.m.t.) increased from 23.5 to 232.5 over this period and 31 volunteers (80%) showed a fourfold rise in titre (Table 2). This group did not show a rise in serum HI antibody to influenza B/Hong Kong/73 virus (Table 2).

Table 1. Serum HI antibody response in volunteers to A/USSR/77 (H1N1) and to B/Hong Kong/73 viruses 1 month after immunization

Vaccine given	No. of volunteers	Immunization status	Serum HI antibody titres to A/USSR/77 virus				Serum HI antibody titres to B/Hong Kong/73 virus					
			< 10	10-20	30-40	60-120	≥ 160	< 10	10-20	30-40	60-120	≥ 160
Inactivated A (200 i.u.)	(39)	Pre	13	4	9	10	3	23	5	7	3	1
		Post	8	1	—	2	28	23	4	8	4	—
Inactivated B group 1* (200 i.u.)	(29)	Pre	17	3	9	—	—	15	3	3	5	3
		Post	17	3	9	—	—	2	2	3	5	17
Inactivated B group 2* (200 i.u.)	(29)	Pre	—	—	—	21	8	21	2	1	5	—
		Post	—	—	2	19	8	2	2	7	2	16
Live A (10 ⁷ EID ₅₀)	(31)	Pre	13	6	7	2	3	21	2	6	1	1
		Post	9	1	3	7	11	21	3	5	2	—

* Group 1 comprises subjects with low titres of pre-immune HI antibody to influenza virus A/USSR/77; group 2, subjects with relatively high titres of serum HI antibody to influenza virus A/USSR/77.

Table 2. Serum HI antibody responses 1 and 8 months after immunization and before challenge virus infection

Vaccine given (no. of volunteers)	Serum HI antibody titres for influenza viruses													
	A/USSR/77 (H1N1)						B/Hong Kong/73							
	1 month post immunization			8 months post immunization			1 month post immunization			8 months post immunization				
	No. rises of ≥ 4 -fold	No. titres ≥ 40	Change in g.m.t.	No. (%)	No. (%)	No. titres ≥ 40	No. rises of ≥ 4 -fold	No. (%)	No. (%)	No. rises of ≥ 4 -fold	No. titres ≥ 40	Change in g.m.t.	No. (%)	No. (%)
Inactivated A (39)	31 (80)	30 (77)	23.5-232.5	29 (74)	141.8	0	0	(—)	6 (15)	11.0-10.9	5 (13)	10.7	5 (13)	10.7
Inactivated B group 1* (29)	0	5 (17)	10.3-10.6	6 (21)	10.4	22	22	(76)	22 (76)	17.2-167.2	23	(79)	23 (79)	121.9
Inactivated B group 2* (29)	0	29 (100)	107.4-95.8	27 (93)	81.8	26	26	(90)	23 (79)	9.5-125.6	18	(62)	18 (62)	76.6
Live A (31)	17 (55)	18 (58)	16.6-50.0	19 (61)	49.3	0	0	(—)	6 (19)	9.7-9.5	4	(13)	4 (13)	9.5

* As Table 1.

(2) *Inactivated influenza B/Hong Kong/73 virus vaccine*

The 29 volunteers with low or undetectable serum HI antibody titres to influenza A/USSR/77 virus who were immunized with influenza B vaccine, showed no increases in serum HI antibody to A/USSR/77 virus (Table 1). Considerable increases in serum HI antibody to the homologous influenza B virus were detected: thus, only 8 volunteers (28%) had titres of ≥ 40 prior to immunization compared with 22 (76%) following immunization (Table 1 and 2). In addition, 22 (76%) showed a ≥ 4 -fold rise in serum HI antibody titre to influenza B/Hong Kong/73 virus and the g.m.t. increased from 17.2 to 167.2.

The 29 volunteers in the second (Group II) B vaccine group who had relatively high titres of serum HI antibody to influenza A/USSR/77 prior to immunization with influenza B vaccine also showed a considerable HI antibody response to the B virus vaccine. Thus, 5 (17%) had serum HI antibody to B/Hong Kong/73 virus at titres of ≥ 40 prior to immunization compared with 23 (79%) after immunization with a g.m.t. change of 9.5–125.6 (Table 1 and 2). None of these volunteers showed changes of any significance in serum HI antibody titres to influenza A/USSR/77 virus (Table 1 and 2), although the g.m.t. fell slightly from 107.4 to 95.8 following immunization (Table 2).

(3) *Live attenuated influenza A (H1N1) virus vaccine*

Of the 31 volunteers immunized with attenuated influenza A (H1N1) virus vaccine, 5 (16%) had serum HI antibody titres of ≥ 40 to influenza virus A/USSR/77 prior to immunization compared with 18 (58%) following immunization (Table 1 and 2). In addition, 55% showed ≥ 4 -fold rise in HI antibody titre with a g.m.t. increase from 16.6 to 50.0 (Table 2). No changes of any significance in serum HI antibody titre to influenza virus B/Hong Kong/73 were detected in these volunteers (Table 2 and 3).

(4) *Decline in antibody titres following immunization*

Serum specimens were collected again 8 months after immunization prior to challenge virus infection, and titrated for serum HI antibody. The changes in antibody titres to influenza viruses A/USSR/77 and B/Hong Kong/73 which occurred during this interval are shown in Table 2. In the volunteer group given inactivated influenza A virus vaccine, 30 (77%) had serum HI antibody titres of ≥ 40 at 1 month post-immunization, and 29 (74%) had titres of ≥ 40 8 months after immunization. However, during this time, the g.m.t. fell from 232.5 to 141.8. No changes of any significance in serum HI antibody titres to influenza B/Hong Kong/73 virus were seen in this group during the same period. For group 1 volunteers given influenza B vaccine, 22 (76%) had HI antibody titres of ≥ 40 to influenza B/Hong Kong/73 virus 1 month following immunization, and 23 (79%) had similar antibody titres 7 months later. Again, although the number of volunteers with protective levels of antibody did not fall during this time, the g.m.t. fell from 167.2 to 121.9 (Table 2). Similar results were recorded for volunteers who had relatively high titres of HI antibody to influenza virus A/USSR/77 (H1N1) prior to immunization with influenza B vaccine (group 2): 23 (79%) had serum HI antibody titres to the B virus of ≥ 40 1 month after immunization and 18 (62%)

had similar antibody titres after 8 months; during this period the g.m.t. fell from 125.6 to 76.6 (Table 2).

In the group of 31 who received live influenza A (H1N1) virus vaccine, 18 (58%) had serum HI titres of ≥ 40 1 month after immunization, and 19 (61%) had similar titres 8 months after immunization. No decline in serum HI antibody titre to the vaccine virus was observed in this group 8 months, post-immunization the g.m.t. was 50.0, and 8 months later the g.m.t. was 49.3, (Table 2).

The absence of significant changes in serum HI antibody titres to influenza B virus in volunteers given the influenza A vaccine, and to influenza A (H1N1) in those given the influenza B vaccine indicated that no natural infection by these viruses occurred in the volunteers during the study period.

Incidence of challenge infection following immunization

A previous study (Clark *et al.* 1983) showed that short term immunity to challenge with live homologous virus correlated with the HI antibody response to immunization. Therefore, in this long-term study, volunteers were divided into six groups based on their antibody responses 1 month after immunization and were then analysed by their resistance to live challenge virus infection. The six groups were: 1 and 2, volunteers given inactivated influenza A vaccine divided into responders who showed ≥ 4 -fold rise in serum HI antibody following immunization, and non-responders who showed no significant antibody response, groups 3 and 4, volunteers given attenuated influenza A (H1N1) vaccine divided into those who were infected as evidenced by a ≥ 4 -fold rise in serum HI antibody, and those who were not infected and did not produce a significant antibody response, groups 5 and 6, volunteers with low titres or with relatively high titres of serum HI antibody titre to influenza A/USSR/77 (H1N1) virus respectively and both received inactivated B vaccine.

The incidence of live challenge virus infection in these six groups as evidenced by a ≥ 4 -fold rise in HI antibody is shown in Table 3. Following immunization with inactivated influenza A vaccine, of the eight volunteers who failed to respond, four were infected by the challenge virus; none of this group had detectable serum HI antibody to influenza A/USSR/77 virus at the time of virus challenge (Table 3). Following immunization with attenuated influenza A (H1N1) virus, seven of 14 volunteers who failed to produce significant serum HI antibody were infected by the challenge virus. Thus, the incidence of infection was the same for non-responders to both inactivated and live virus vaccines.

A comparison of volunteers who responded to inactivated or attenuated A vaccine showed very similar results. Thus, of 31 volunteers given inactivated vaccine, two (6%) were infected by the challenge virus; and of the 17 volunteers infected by the attenuated virus vaccine, one (6%) was infected by challenge (Table 3).

Analysis using a chi-squared test for differences in proportions confirmed that there was no significant difference between the inactive and live influenza virus vaccines in either the proportions of responders or in the proportions of non-responders who are infected by challenge ($P > 0.1$). However, there was a highly significant difference between the responders and non-responders in the proportions becoming infected by challenge ($P < 0.01$). In addition, the proportions of

Table 3. Incidence of infection as assessed by a subsequent fourfold or greater HI antibody rise following challenge with live attenuated influenza A (H1N1) vaccine

Vaccine group	No. of volunteers	Serum HI antibody titres against influenza A/USSR/77 (H1N1) virus prior to challenge 8 months post immunization					Total infections (%)
		< 10	10-20	30-40	60-120	≥ 160	
Inactivated A (Responders)†	31	1/1 ⁺	0/1	1/1	0/2	0/26	2 (6)
Inactivated A (non-responders)†	8	4/8	—	—	—	—	4 (50)
Live A (infected)‡	17	—	1/2	0/1	0/7	0/7	1 (6)
Live A (Non-infected)‡	14	5/8	—	2/2	—	0/4	7 (50)
Inactivated B (group 1)*	29	9/18	1/2	7/9	—	—	17 (59)
Inactivated B (group 2)*	29	—	—	2/4	4/20	0/5	6 (21)
Total	128	19/35	2/5	12/17	4/29	0/42	37 (29)
Percent infected		(54%)	(40%)	(71%)	(14%)	(—%)	

* See footnote to Table 1.

† Number infected with challenge/number volunteers.

‡ Defined on the basis of the response one month after immunization.

responders to the vaccines is significantly larger for the inactive than for the live vaccine ($P < 0.05$). These results indicate the same level of protection for both vaccines at 8 months after immunization, provided that the individual responded to the vaccine.

Of the volunteers with relatively low levels of serum HI antibody to influenza virus A (H1N1) and given inactivated B vaccine, 17 (59%) were infected by the challenge virus; in contrast, of the 29 volunteers with relatively high titres of serum HI antibody to influenza A (H1N1) from natural influenza virus infections, 6 (21%) were infected by the challenge virus. Thus, naturally-acquired serum HI antibody gave relative protection. The results for these two groups show that 10 of 20 (50%) volunteers with HI antibody titres of ≤ 20 were infected by the challenge virus, whilst nine of 13 (71%) with HI antibody titres of 30-40 were infected.

DISCUSSION

The results of the present study show that immunization of volunteers with inactivated influenza A (H1N1) subunit vaccine produced a good serum HI antibody response in the majority of volunteers: of the volunteers who produced a ≥ 4 -fold increase in antibody titre (80%), significant protection against challenge virus infection was found 8 months after immunization. The incidence of challenge virus infection 8 months later in these volunteers was directly related to the titre of serum HI antibody, as was observed in an earlier study when the challenge was

given 1 month after immunization (Clark *et al.* 1983). However, there was a considerable fall in individual titres of serum HI antibody in the 8 months following immunization, and this may have implications concerning the efficacy of inactivated influenza vaccines in the long term. In contrast, although volunteers immunized with the attenuated virus had lower titres of HI antibody they showed no decline in titre between 1 and 8 months, post-immunization. The same incidence of infection by the challenge virus at 8 months, was observed in volunteers given live or inactivated virus provided that they responded to the immunization. In the control group where the antibody was presumably acquired by natural infection prior to the study, titres of HI antibody did not decline during the observation period; this may be due to the strong antigenic stimulus of a natural infection. The decline in serum HI antibody titres in volunteers given inactivated vaccine may be due to the higher titres seen in these volunteers following immunization; but it is more likely that serum HI antibody acquired as a result of live virus infection may persist longer than antibody induced with inactivated virus vaccines.

In all groups, the incidence of infection by the challenge virus was related to the titres of homologous serum HI antibody 1 month after immunization; this was seen for volunteers given inactivated or attenuated virus vaccine, or with antibody induced by natural infection. Although the numbers of volunteers in the study was small, the results support the observation that a serum HI antibody titre of approximately 30–40 corresponded to 50% immunity (Meikeljohn *et al.* 1952; Hobson *et al.* 1972; Potter & Oxford, 1979). In this respect the inactivated vaccine induced considerably more seroconversions than the live vaccine which offers advantages for inactivated vaccines, although this might be in the relative short-term. The finding of a lower incidence of infection by challenge virus in volunteers with no demonstrable serum HI antibody as compared to those with low titres of antibody has been reported previously (Hobson *et al.* 1972): no explanation for this observation has been presented, but it may indicate the existence of individuals with an innate resistance to influenza virus infection.

The results of the present study do not support the observation that immunization with attenuated virus vaccine induced a more solid immunity than seen following inoculation with inactivated vaccines, as reported in other studies (Beare *et al.* 1968; Freestone *et al.* 1972). In these studies the challenge virus infection was given at 3–4 weeks, post-infection, whereas in the present study the challenge infection was given at 8 months after virus infection. However, Clark *et al.* (1983) were unable to show that live vaccines induced a more solid immunity in the short-term. In studies using an early challenge infection, it is suggested that local neutralizing antibody could have been responsible for the differences, since this antibody is induced to higher titre following live virus infection than following immunization with killed virus (Waldman *et al.* 1968), and this local antibody contributes to immunity (Potter *et al.* 1975; Potter & Oxford, 1979). No measurements of local antibody production were made in the present study; however, the results of previous studies have shown that the production of local antibody is ephemeral, and antibody was not detectable after 3 months, post-inoculation (Murphy *et al.* 1973; Kasel *et al.* 1969; Potter & Oxford, 1979), which would suggest that local antibody was not present at the time of virus challenge in the present long-term

study. The more solid immunity reported for live influenza virus vaccines by some workers may be only a short-term feature demonstrable when the challenge virus was given 1 month after immunization, and may be due to the presence of local antibody. In this study, when the challenge virus infection was given later, at a time when local antibody production would have ceased, no distinction between the immunity induced by attenuated and inactivated influenza virus vaccines could be demonstrated. However, resistance to challenge virus infection was directly related to the rise in serum antibody following immunization and this was better induced by the inactivated rather than the live vaccines.

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