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Responses of volunteers to inactivated influenza virus vaccines

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SUMMARY

Three different types of bivalent influenza virus vaccine, a whole virus, an aqueous-surface-antigen vaccine and an adsorbed-surface-antigen vaccine were tested at three dosage levels in volunteers primed with respect to only one of the haemagglutinin antigens present in the vaccines.

The local and systemic reactions to all three vaccine types were mild in nature and, following first immunization, the aqueous-surface-antigen vaccine was the least reactogenic. The serum haemagglutination-inhibiting antibody response to the A/Victoria/75 component of the vaccines, to which the volunteer population was primed, was greatest following immunization with the aqueous-surface-antigen vaccine; the greatest antibody response to the A/New Jersey/76 component of the vaccines was observed following immunization with whole virus vaccine.

INTRODUCTION

In the past few years, several studies have been carried out comparing purified, inactivated whole influenza virus vaccines with vaccines prepared from influenza virus surface antigens (Ruben & Jackson, 1972; Barry *et al.* 1976; Jennings *et al.* 1978). Studies of these surface antigen materials in animals have shown them to be of relatively low antigenicity (Barry, Staton & Mayner, 1974; Jennings *et al.* 1975; McLaren *et al.* 1977) but their immunogenicity in man may depend on the degree of priming of the population to the surface antigens in the vaccine (Parkman *et al.* 1976; Wright *et al.* 1976). This has important implications for immunization

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against influenza, and it has been reported that a single dose of subunit vaccine, is less effective in promoting HI antibody in an unprimed population than an equivalent dose of whole virus vaccine (Parkman *et al.* 1976; Welty *et al.* 1977); however, in primed populations, sub-unit vaccines appear at least as effective as the conventional whole virus vaccine (Nicholson *et al.* 1979). As a result of these observations, it has been suggested that non-primed subjects should receive two doses of split sub-unit vaccine in order to stimulate satisfactory serum antibody levels (Gross & Ennis, 1977; Eastwood *et al.* 1979). In addition, the serum HI antibody response of non-primed individuals to immunization with a single dose of whole virus vaccine may not always induce a satisfactory antibody response (Pandemic Working Group of the Medical Research Council, 1977; Nicholson *et al.* 1979) and similarly two doses of such vaccine may be required to induce acceptable levels of immunity (Gross & Ennis, 1977; Eastwood *et al.* 1979). The adsorption of purified haemagglutinin and neuraminidase surface antigens on to aluminium hydroxide gel (Brady, Furminger & Stones, 1976) results in an increase in the antigenicity of these glycoproteins for animals (Jennings *et al.* 1975) and the adsorbed-surface-antigen vaccine has been shown in clinical studies to induce acceptable serum haemagglutination-inhibiting and neuraminidase-inhibiting antibody levels (Potter *et al.* 1977). However, comparisons of the adsorbed-surface-antigen vaccine with equivalent whole virus vaccines were not carried out in these studies and the volunteer populations were primed with respect to the antigens used in the vaccine.

In the present study, three different types of bivalent influenza virus vaccine, a whole virus vaccine, an aqueous-surface-antigen vaccine and an adsorbed-surface-antigen vaccine, were tested at three dosage levels, in volunteer populations primed with respect to only one of the haemagglutinin antigens present in the vaccines. The reactions and antibody responses following two doses of these vaccines are reported.

MATERIALS AND METHODS

Viruses

Influenza virus A/New Jersey/8/76 (HSw1N1) was obtained from Dr J. J. Skehel, National Institute for Medical Research, Mill Hill, London; influenza virus RIT4050 (H3N2) a recombinant of influenza A/Victoria/3/75 (H3N2) and A/PR/8/34 (H0N1) viruses, was obtained from Dr C. Huygelen, Recherche et Industrie Therapeutique, Rixensaart, Belgium. For use in HI tests, pools of these viruses were prepared from seed virus stocks by the allantoic inoculation of 10-day-old embryonated hen's eggs. After incubation at 33 °C for 48 h, the virus-infected allantoic fluids were harvested and stored at −80 °C.

Vaccines

All virus vaccines were prepared by Glaxo Operations (U.K.) Ltd, Speke, Liverpool, and were prepared from influenza viruses A/New Jersey/8/76 and A/Victoria/3/75. Three types of vaccine were used; a whole virus vaccine (WV)

an aqueous-surface-antigen vaccine (AqSA) and an adsorbed-surface-antigen vaccine (AdSA). The WV vaccine was prepared from egg-grown virus purified by continuous flow ultracentrifugation on a sucrose gradient and inactivated with β-propiolactone. The AqSA vaccine was prepared from WV by disruption with Triton N101, and separation and purification of the surface antigens by centrifugation on sucrose gradients (Brady & Furminger, 1976). The AdSA vaccine was prepared by adsorption of purified subunits to aluminium hydroxide gel (Brady & Furminger, 1976). Three dose levels of each vaccine type were prepared with nominal potencies of 400, 100 and 25 international units (i.u.) of both component virus haemagglutinins per 0·5 ml dose; the two lower dosage levels were prepared by four-fold dilution steps from the highest one. All vaccine samples were coded for use, and the study carried out under double-blind conditions.

Vaccine potency

Vaccines were assayed for potency at the National Institute for Biological Standardization and Control, Hampstead, London, and the concentration of both virus haemagglutinins contained in each vaccine and at each dose level are shown in Table 1. The assays were carried out by single radial diffusion (SRD), as described by Wood *et al.* (1977), and show that the highest dose of each vaccine, nominally 400 i.u. of each HA antigen, contained approximately twice as much A/New Jersey/76 haemagglutinin as that of A/Victoria/75. Furthermore, the lowest dose of the WV and AqSA vaccines apparently contained similar amounts of A/New Jersey/76 haemagglutinin to that of the intermediate vaccine dose, nominally containing four times the amount of this antigen. However, the SRD test is unreliable for the measurement of low levels of haemagglutinin (Wood, personal communication). The highest vaccine dose in these studies, 400 i.u., is twice the normal dosage for WV and four times that for sub-unit vaccines.

Volunteer groups and study design

A total of 344 individuals of both sexes ranging from 17 to 60 years of age volunteered for the study. The majority, 322, were recruited from staff at Central Electricity Generating Board centres at Birmingham and Gloucester, and the remainder were students at the University of Birmingham. Volunteers allergic to egg or egg protein were excluded from the study. Each volunteer was test bled and then inoculated with 400, 100 or 25 i.u. of one of the three vaccines in an 0·5 ml volume by deep subcutaneous injection. The vaccines were given by random allocation. The reactions to immunization were assessed 24 h later from questionnaires as described previously (Jennings *et al.* 1978). The volunteers were bled a second time four weeks later to determine their serological responses to the first dose of the vaccine. At the same time each group of volunteers was divided into two further groups, and one of these sub-groups received a further inoculation with 400 i.u. of the same vaccine type as that given previously; volunteers in the other sub-group were given 25 i.u. of that same vaccine type. Reactions to second immunization were again assessed at 24 h post-immunization, and a final blood sample collected four weeks later.

Table 1. *Concentration of haemagglutinin protein in whole, aqueous-surface antigen and adsorbed-surface antigen A/Victoria/75: A/New Jersey/76 influenza virus vaccines*

	μgHA/0·5 ml		μgHA/0·5 ml		μgHA/0·5 ml	
Vaccine type	A/New Jersey/76	A/Victoria/75	A/New Jersey/76	A/Victoria/75	A/New Jersey/76	A/Victoria/75
Whole virus	14·0	7·7	3·0	2·0	2·7	1·6
Aqueous-surface antigen	15·0	7·1	4·2	3·2	1·0	1·0
Adsorbed-surface antigen	14·0	8·7	3·1	2·6	3·0	0·8
Nominal potency (i.u./0·5 ml)	400		100		25	

Serological investigations

Haemagglutination-inhibiting (HI) tests were carried out using the microtitre method as described previously (Potter *et al.* 1975). Before testing, serum specimens were treated with cholera filtrate (Burroughs Wellcome Ltd) for 18 h, and subsequently heated at 56 °C for 60 min. In all HI tests, eight (50%) haemagglutinating units were used as antigen.

Neuraminidase-inhibiting (NI) antibody tests were carried out by an automated modification (Bevan, Furminger & Smith, 1975) of the standard World Health Organization method (Aymard-Henry *et al.* 1973), using the X48 influenza strain (HEqui-1 N2), a recombinant of A/Victoria/75 and A/Equi/63 viruses, as the source of neuraminidase.

RESULTS

Reactions to Immunization

Figure 1 illustrates the reactions, both local and systemic, reported by volunteers 24 h following first and second immunization with WV, AqSA or AdSA vaccine at all three dose levels. All reactions were mild, and systemic reactions following all three types of vaccines were rare. Local reactions following first immunization with either of the two lower doses of the WV or AdSA vaccines were reported by only 4–12% of the volunteers, significantly less than those recorded following the highest dose of these vaccines (Fig. 1*a*). No differences in reaction rates were

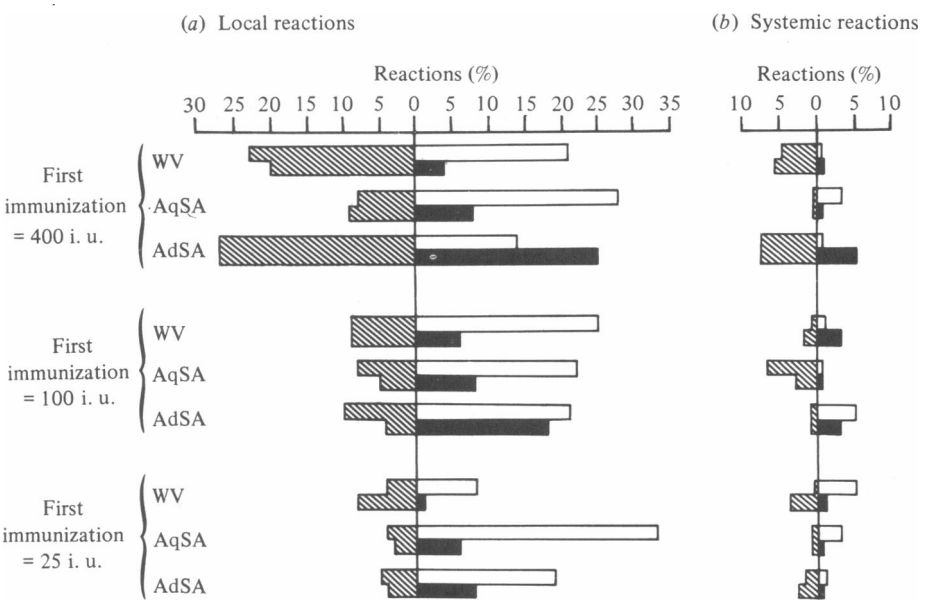

Fig. 1. Reactions of volunteers following first and second immunization with either WV, AqSA or AdSA influenza virus vaccines. ▨, Reactions following first immunization; □, reactions following second immunization with 400 i.u. of vaccine; ■, reactions following second immunization with 25 i.u. of vaccine.

observed between the WV and AdSA vaccines at any dosage level after first immunization but the incidence of local reactions following the highest dose of AqSA vaccine were significantly less than those reported after immunization with either WV or AdSA vaccines.

The highest incidence of local reactions following second immunization were generally reported after inoculation with the 400 i.u. dose, irrespective of the initial dose of vaccine administered (Fig. 1*a*). Furthermore, with the exception of persons receiving 400 i.u. of either WV or AdSA vaccine at both first and second immunization, volunteers receiving the higher dose of any vaccine at second immunization reported a greater incidence of local reactions than were seen following initial immunization. This was especially noticeable in volunteers receiving the AqSA vaccine, who reported the lowest incidence of reactions on first immunization. In general, individuals receiving 25 i.u. of WV or AqSA vaccines on second immunization, reported either the same or fewer reactions than experienced after first immunization, irrespective of the initial dose of vaccine received. However, volunteers given either of the two doses of the AdSA vaccine on first immunization, reported a greater incidence of reactions to 25 i.u. of this vaccine after second immunization.

Serological response to immunization

(*a*) *Serum HI antibody responses to whole virus* (*WV*) *vaccine*

The serum HI antibody response of volunteers to A/Victoria/75 and A/New Jersey/76 following both first and second dose of WV vaccine is shown in Table 2. Following a single dose of 400 i.u. of this vaccine, 75 % of persons had HI antibody levels to A/New Jersey/76 of $\geqslant$ 40, the geometric mean titre (gmt) for this group of volunteers increasing from a pre-immunization level of 6·9 to 87·2 four weeks later. However, a second dose of either 400 or 25 i.u. of WV vaccine induced only a small further increase in antibody titres to this virus. Initial immunization with lower doses of WV vaccine, were even less effective in eliciting serum HI antibody and the response of volunteers to a second dose of either 400 or 25 i.u. of this vaccine induced only small further increases in antibody titre.

Volunteers, receiving either 400 or 100 i.u. of WV vaccine on first immunization responded equally well to the A/Victoria/75 component of the vaccine. However, in both these groups of volunteers, a second dose of 400 i.u. of the same vaccine failed to elicit any significant booster effect; in contrast, individuals receiving 25 i.u. of the WV vaccine on second immunization, showed a marked further increase in HI antibody titres, the greatest percentage of individuals with titres of $\geqslant$ 40, and the highest gmt, being observed in volunteers receiving 400, followed by 25 i.u. of this vaccine. The serum HI antibody response to A/Victoria/75 virus in volunteers initially inoculated with 25 i.u. of WV vaccine was relatively low following both first and second immunization, irrespective of the vaccine dose given on the second occasion.

Table 2. *Serum HI antibody response of volunteers following one and two doses of bivalent A/Victoria/75: A/New Jersey/76 whole virus vaccine*

Vaccine dose (i.u.)			Percentage of vaccinees with HI titres shown to							
			A/New Jersey/76				A/Victoria/75			
First immunization	Second immunization	Immunization status of volunteers	No. tested	< 10	⩾ 40	Geometric mean titre	No. tested	< 10	⩾ 40	Geometric mean titre
400	—	Pre-imm.	41	80	5	6·9	41	63	10	8·9
	—	Post-1st imm.	41	5	75	87·2	41	7	68	62·2
	400	Post-2nd imm.	21	0	81	100·9	21	5	57	46·3
	25	Post-2nd imm.	20	5	75	92·7	20	0	80	92·7
100	—	Pre-imm.	41	78	10	8·1	41	54	15	12·0
	—	Post-1st imm.	41	59	29	15·6	41	0	56	56·9
	400	Post-2nd imm.	21	43	29	18·6	21	0	62	52·4
	25	Post-2nd imm.	20	50	30	15·3	20	0	70	73·6
25	—	Pre-imm.	40	78	10	7·8	40	78	10	12·8
	—	Post-1st imm.	40	40	33	17·6	40	40	33	21·4
	400	Post-2nd imm.	21	16	47	34·2	21	0	50	37·4
	25	Post-2nd imm.	19	32	32	17·7	19	16	16	23·5

Table 3. *Serum HI antibody response of volunteers following one and two doses of bivalent A/Victoria/75: A/New Jersey/76 aqueous-surface-antigen vaccine*

Vaccine dose (i.u.)			Percentage of vaccinees with HI titres shown to							
			A/New Jersey/76				A/Victoria/75			
First immunization	Second immunization	Immunization status of volunteers	No. tested	< 10	⩾ 40	Geometric mean titre	No. tested	< 10	⩾ 40	Geometric mean titre
400	—	Pre-imm.	41	80	12	8·0	41	63	20	11·0
	—	Post-1st imm.	41	22	51	53·8	41	2	76	96·4
	400	Post-2nd imm.	21	10	62	74·1	21	0	90	93·1
	25	Post-2nd imm.	20	33	50	42·0	20	0	89	164·8
100	—	Pre-imm.	39	77	13	8·2	39	64	10	9·2
	—	Post-1st imm.	39	31	46	31·8	39	5	69	80·0
	400	Post-2nd imm.	20	21	53	38·4	20	5	74	80·1
	25	Post-2nd imm.	19	26	53	37·6	19	0	84	87·2
25	—	Pre-imm.	41	76	15	9·0	40	40	20	13·0
	—	Post-1st imm.	41	24	46	36·1	40	10	63	54·2
	400	Post-2nd imm.	20	5	65	64·3	20	0	84	99·0
	25	Post-2nd imm.	20	25	40	26·5	20	10	65	48·3

(*b*) *Serum HI antibody responses to aqueous surface antigen* (*AqSA*) *vaccine*

These results are shown in Table 3. The serum antibody responses of volunteers to 400 i.u. of the A/New Jersey/76 component of the AqSA vaccine were lower following both first and second immunization, than seen following WV vaccine; thus, two doses of 400 i.u. of WV vaccine induced titres of $\geqslant 40$ in 81% of individuals, and a mean titre of 100·9 (Table 2), but a similar regimen of AqSA vaccine elicited final serum HI titres of $\geqslant$ 40 in 62% of the volunteers and a final gmt of 74·1 (Table 3). Volunteers given vaccine doses of 400 i.u., followed by 25 i.u. of AqSA vaccine responded less well than individuals given similar doses of WV vaccine, and also less well than volunteers inoculated with two doses of 400 i.u. of AqSA vaccine. However, subjects given either 100 or 25 i.u. of AqSA vaccine on first immunization showed a greater antibody response than individuals receiving equivalent doses of WV vaccine. Furthermore, the second dose of 400 i.u. of AqSA vaccine in individuals receiving an initial dose of 25 i.u. of this vaccine, elicited a marked booster response, but the final mean titre of serum HI antibody in this group of volunteers was lower than that seen in volunteers given two doses of 400 i.u. of this vaccine (Table 3). No other dosage regimen in volunteers given 100 or 25 i.u. of this vaccine on first immunization, induced any significant boosting of the A/New Jersey/76 antibody levels.

Table 3 also shows that the A/Victoria/75 component of the AqSA vaccine, following both initial and second immunization, irrespective of the dosages given, induced a greater serum HI antibody response, in terms of both incidence of volunteers with titres $\geqslant$ 40 and gmt, than that seen after immunization with the WV vaccine. A slight booster effect on A/Victoria/75 antibody titres was observed following second immunization with the AqSA vaccine in volunteers receiving either an initial dose of 400 followed by 25 i.u., or the reverse.

(*c*) *Serum antibody responses to adsorbed-surface-antigen* (*AdSA*) *vaccine*

The serum HI antibody responses of volunteers to immunization with the AdSA vaccines are shown in Table 4. Volunteers receiving 400 i.u. of AdSA vaccine on first inoculation were of a younger age-group than all other volunteers, and their response to the A/New Jersey/76 component of the vaccine was poor. However, following second immunization with 400 i.u. of this vaccine, the percentage of volunteers with serum HI antibody titres of $\geqslant 40$ increased to 45% and the gmt to 49·9. A second dose of 25 i.u. of AdSA vaccine had no booster effect. Volunteers receiving 100 or 25 i.u. of AdSA vaccine on first immunization, developed relatively high serum HI antibody titres to A/New Jersey/76, and following a second dose of 400 i.u., these volunteers had final mean antibody titres of 60·5 and 87·0 respectively. Volunteers inoculated with 25 i.u. of AdSA vaccine after an initial dose of 100 or 25 i.u. showed no booster response. However, the final mean titres and the percentage of volunteers with titres $\geqslant$ 40, were higher than those seen in volunteers given 400 i.u. of this vaccine at first immunization. The serum HI antibody levels to A/New Jersey/76 virus following both first and second immunization in volunteers given 400 i.u. of AdSA vaccine at first im-

Table 4. *Serum HI antibody response of volunteers following one and two doses of bivalent A/Victoria/75: A/New Jersey/76 adsorbed-surface-antigen vaccine*

Vaccine dose (i.u.)			Percentage of vaccinees with HI titres shown to							
			A/New Jersey/76				A/Victoria/75			
First immunization	Second immunization	Immunization status of volunteers	No. tested	< 10	⩾ 40	Geometric mean titre	No. tested	< 10	⩾ 40	Geometric mean titre
400	—	Pre-imm.	21	90	5	6·1	21	52	19	13·2
	—	Post-1st imm.	21	62	24	12·3	21	0	76	92·1
	400	Post-2nd imm.	11	0	45	49·9	11	0	91	108·0
	25	Post-2nd imm.	10	30	20	12·8	10	0	70	72·2
100	—	Pre-imm.	40	55	25	12·3	40	28	25	17·1
	—	Post-1st imm.	40	20	60	43·5	40	5	53	47·2
	400	Post-2nd imm.	20	10	70	60·5	20	10	45	51·0
	25	Post-2nd imm.	20	10	45	37·1	20	0	60	48·9
25	—	Pre-imm.	40	40	40	20·8	40	25	33	21·6
	—	Post-1st imm.	40	20	65	50·9	40	8	65	52·3
	400	Post-2nd imm.	20	5	75	87·0	20	0	85	81·7
	25	Post-2nd imm.	20	11	58	43·5	20	5	58	53·1

munization and either 400 or 25 i.u. at second immunization were low (Table 4) compared to antibody levels observed in volunteers given similar dosages of WV or AqSA vaccines (Tables 2 and 3). However, this finding was reversed after both first and second immunization in volunteers initially receiving 100 or 25 i.u. and subsequently given either 400 or 25 i.u., of AdSA vaccine.

The response of volunteers to the A/Victoria/75 virus component of the AdSA vaccine is also shown in Table 4. Following initial immunization with 400 i.u. of this vaccine, serum HI antibody levels to A/Victoria/75 were greater than those seen after first immunization with an equivalent dose of WV vaccine, but similar to those observed following 400 i.u. of AqSA vaccine. Two doses of 400 i.u. of AdSA vaccine induced serum HI antibody titres of $\geqslant 40$ to A/Victoria/75 in 91% of vaccinees and a gmt level of 108·0, and these final serum HI antibody levels to A/Victoria/75 were similar to those observed in volunteers receiving the same dosage regimen of the AqSA vaccine, and markedly better than those induced by equivalent doses of WV vaccine. Volunteers inoculated with lower doses of AdSA vaccine on first immunization responded less well at second immunization than individuals receiving 400 i.u. of this vaccine on first immunization. This finding parallels the results obtained with both WV and AqSA vaccines.

(d) NI antibody responses to A/Victoria/75

The results in Table 5 show the serum antibody response of volunteers to A/Victoria/75 neuraminidase antigen following two doses of WV, AqSA or AdSA vaccines, and it can be seen that, compared to the HI antibody responses, the NI antibody responses were relatively low. In general, the lowest geometric mean titres and incidence of seroconversions were observed following the two doses of WV vaccine, and even those volunteers receiving two doses of 400 i.u. of this vaccine showed a post-immunization gmt of only 12·1. The greatest responses in terms of seroconversions and gmt levels of NI antibody were seen following inoculation of the volunteers with the AqSA vaccine, particularly in those volunteers given 400 i.u. of this vaccine at either first or second inoculation.

Serum HI antibody responses of volunteers according to age

The age spectrum of the volunteers who took part in the study included older persons who had experienced infection with influenza A (HSw1N1) viruses in the past, and younger individuals with no such previous experience. Thus, it was important to determine the serum antibody responses of volunteers to influenza A/New Jersey/76 virus with reference to prior priming of these individuals. Volunteers given the highest dose of each vaccine were divided into three groups with respect to age; subjects aged < 24 years, those aged 24 to 44 years and individuals aged 45 years or older. The results of the serum HI antibody responses of the volunteers to both A/New Jersey/76 and A/Victoria/75 antigens are shown in Table 6. The serum HI antibody response of volunteers to A/New Jersey/76 haemagglutinin was lowest, for all types of vaccine, in the youngest age-group; this was most noticeable in volunteers receiving AdSA or AqSA vaccines, where the gmt following immunization increased by 1·6- and 1·9-fold respectively, and

Table 5. *Serum NI antibody responses of volunteers to A/Victoria/75 following immunization with two doses of various influenza virus vaccines*

Vaccine dose (i.u.)		Response to WV vaccine			Response to AqSA vaccine			Response to AdSA vaccine		
First imm.	Second imm.	No. pos.*/ total	% Pos.	gmt Pre-post 2nd immunization	No. pos.*/ total	% Pos.	gmt Pre-post 2nd immunization	No. pos.*/ total	% Pos.	gmt Pre-post 2nd immunization
400	400	3/19	15·8	6·7–12·1	9/21	42·9	3·8–11·0	2/11	18·2	6·6–19·8
	25	8/18	44·4	3·5–10·0	5/18	27·8	4·8–11·9	0/9	—	22·6–39·4
100	400	1/21	4·8	5·6–7·5	6/19	31·6	8·5–22·3	6/20	30·0	4·4–11·2
	25	2/19	10·5	8·3–10·8	6/18	33·3	5·7–13·6	3/18	16·7	5·7–12·0
25	400	1/19	5·3	4·5–6·2	10/21	47·6	7·9–24·9	4/16	25·0	7·2–17·6
	25	1/19	5·3	4·7–5·6	3/20	15·0	6·4–14·0	2/19	10·5	8·4–11·2

gmt = geometric mean titre.
* ⩾ 4-fold rise in NI antibody.

Table 6. *Serum HI antibody responses to A/Victoria/75 and A/New Jersey/76 according to age of volunteers following inoculation with the highest dose of various bivalent influenza virus vaccines*

Vaccine type	Age of volunteers (years)	Number tested	Serum HI antibody responses to A/New Jersey/76				Serum HI responses to A/Victoria/75			
			No. pos.*	% Pos.	gmt Pre-post immunization	Fold increase	No. Pos.	% Pos.	gmt Pre-post immunization	Fold increase
Whole virus (WV)	<24	6	3	50	5·0–28·0	5·6	3	50	6·0–55·0	9·2
	24–44	25	23	92	6·1–102·7	6·8	15	60	9·7–70·8	7·3
	⩾45	10	7	70	11·5–112·6	9·8	6	60	12·8–48·8	3·8
Aqueous surface antigen (AqSA)	<24	6	0	—	5·0- 7·8	1·6	3	50	10·2- 31·0	3·0
	24–44	21	13	62	7·0–46·3	6·6	14	67	9·4–107·8	11·5
	⩾45	13	9	69	13·8–135·8	9·8	8	62	13·3–97·3	7·3
Adsorbed surface antigen (AdSA)	<24	15	3	20	5·0–9·4	1·9	10	67	19·4–137·2	7·1
	24–44	3	0	—	15·9–20·0	1·3	2	67	5·0–28·8	5·8
	⩾45	2	1	50	7·1–69·3	9·8	1	50	5·0–60·0	12·0

gmt = geometric mean titre.
* ⩾ 4-fold antibody increase.

least apparent for volunteers given the WV vaccine where the gmt increase was 5·6-fold. In contrast, volunteers aged ⩾ 45 years responded well to all three types of vaccine and each vaccine induced increases of 9·8-fold in the gmt of antibody to A/New Jersey/76 in this age-group.

DISCUSSION

The results of several studies comparing inactivated whole influenza virus vaccines with split or subunit vaccines indicate that the reactogenicity of the latter vaccine is significantly reduced compared to the former (Brandon *et al.* 1967, Jennings *et al.* 1978), and the findings in the present study confirm this. Thus, although the reactions to all three vaccine types were of a low-grade nature, the highest dose of the AqSA vaccine induced, on first immunization, significantly less of these reactions than equivalent doses of WV or AdSA vaccines, and this represents an advantage of aqueous sub-unit vaccines over either whole or adsorbed vaccines in terms of their reactogenicity. However, following second immunization with the higher dose of vaccine used in the present study, the incidence of local reactions was greatest following the AqSA vaccine in those volunteers given either 400 or 25 i.u. of this vaccine initially. Furthermore, the incidence of local reactions following second immunization with any of the vaccines used in the present study was generally greater, although still mild in nature, than those reported after first immunization. Similar observations have been reported by other workers (Howells, Evans & Vesselinova-Jenkins, 1973; Wright, Dolin & La Montaigne, 1976; Welty *et al.* 1977). The reasons for these observations are not clear, although reactions to influenza virus immunization may depend partly on the levels of circulating antibody prior to immunization (Parkman *et al.* 1976; Pandemic Working Group of the Medical Research Council, 1977). Thus, the marginal increases in serum HI antibody seen following the second, high doses of vaccine in the present study, were offset by the increased reactogenicity observed. It is of interest to note that the incidence of local reactions to the AdSA vaccine on second immunization, in comparison with either WV or AqSA vaccine, was less dependent on antigen concentration, suggesting that the aluminium hydroxide gel carrier, present in this vaccine in similar amounts irrespective of antigen quantity, may be contributing to the observed reactions.

Following first immunization, the serum HI antibody response of volunteers to the highest dose of A/Victoria/75 in either the AqSA or AdSA vaccines was higher than the response to an equivalent amount of WV vaccine. Thus, at a time of antigenic drift, when the population is already primed by prior infection with viruses of a similar serotype, a single immunization with either of these vaccines will induce a good antibody response. However, since the AqSA vaccine induced the lowest reaction rate on first immunization, this is the most acceptable to use in these circumstances. In the present study, a single dose of 400 i.u. of AqSA vaccine induced a gmt of 96, and antibody titre levels of ⩾ 40 in 90% of the volunteers and these levels of antibody are commensurate with protection (Hobson

et al. 1972; Gross, 1977), and suggest that two doses of this vaccine would be superfluous in a primed population.

The serum HI antibody response of volunteers to the A/New Jersey/76 haemagglutinin was greatest in those receiving WV, as compared to AqSA or AdSA vaccine. The greatest response was elicited following the highest dose of this vaccine, and further slight increases in antibody elicited by a second dose of either 400 i.u. or 25 i.u. of this vaccine. Any two dose regimen of AqSA or AdSA vaccines did not induce equivalent HI antibody levels to A/New Jersey/76 to those seen following WV vaccine. These findings are in agreement with previous studies (Parkman *et al.* 1976; Nicholson *et al.* 1979), and suggest that in times of antigenic shift a whole virus vaccine must be used in order to induce an acceptable degree of immunity. However, the antibody induced by WV vaccines in unprimed populations has been reported to be of short duration even when two doses are administered (Wright *et al.* 1980; Potter *et al.* 1980).

As in previous studies (Pandemic Working Group of the Medical Research Council, 1977; Nicholson *et al.* 1979), the age of the volunteers at the time of immunization influenced their response to the A/New Jersey/76 component of the vaccines in the present study; thus, volunteers aged < 24 at the commencement of the study were unprimed for the antigens of this virus, and responded markedly less well to the A/New Jersey/76 component in all the vaccines than did those volunteers aged 45 or more, who were likely to have been exposed to the surface antigens of this virus at some time in the past (Parkman *et al.* 1976). However, the greatest response to the A/New Jersey/76 antigen in the youngest age-group was seen in those individuals receiving WV vaccines; in volunteers aged $\geqslant 45$ years, the WV and AqSA vaccines proved equally effective.

Although the efficacy of influenza virus vaccines is mainly judged by their ability to induce circulating HI antibody, some protection may also be provided by NI antibody (Monto & Kendal, 1973). However, in the present study, none of the vaccines, at any dosage level, induced either a high incidence of fourfold NI antibody rises, or a high gmt following immunization.

The findings reported in this study clearly show that in a population primed by previous infection with the antigen to be used, a single dose of AqSA vaccine induced at least as good antibody responses and significantly less reactions than either WV or AdSA vaccines. In contrast, in unprimed populations, the vaccine of choice is WV, but two doses of such a vaccine may be needed to elicit adequate levels of serum HI antibody. However, the relatively high incidence of reactions observed following this type of vaccine, particularly in children (Marine & Stuart-Harris, 1976; Wright *et al.* 1976), may preclude its use in favour of the more purified and less reactogenic subunit vaccines. Furthermore, if two doses of any of these vaccines are used, then the initial inoculation should be carried out using high rather than low concentrations of virus antigen.

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