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Antibody response and persistence in volunteers following immunization with varying dosages of a trivalent surface antigen influenza virus vaccine

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SUMMARY

The serum antibody responses and 50% protective levels (PL50) of antibody were determined, using the SRH test, at one and twelve months post-vaccination in a group of student volunteers immunized with one of three dosages of a trivalent surface-antigen influenza virus vaccine, or with placebo.

It was found that, for the H3, H1 and B haemagglutinin components present in the vaccine, a dose of 6 μg HA elicited high serum antibody responses at one month post-immunization. High mean antibody levels and a high incidence of volunteers with PL_{50} values of antibody against each of the HA components of the vaccine remained in the volunteer group twelve months later. The results are discussed in relation to the vaccine dosage used and the nature of the population immunized.

INTRODUCTION

The present-day highly purified vaccines for use against influenza contain predominantly the surface haemagglutinin (HA) and neuraminidase (NA) proteins of the virus, obtained from inactivated, purified whole virus particles after disruption with detergents such as Triton N 101 (Brady & Furminger, 1976). These vaccines are normally trivalent and include surface proteins from current A/H3N2, A/H1N1 and B influenza viruses. They are standardized according to the amount of HA present (Wood *et al.* 1977) and, as used commercially in the United Kingdom, normally contain 9–10 μg HA of each of the three component virus antigens per dose.

In recent years workers have reported on the duration of serum haemagglutination-inhibiting (HI) antibodies induced by inactivated influenza virus vaccines (Kunz *et al.* 1978; Potter *et al.* 1980; Lerman, Wright & Patil, 1980; Cate *et al.* 1983; Clark *et al.* 1983). These studies have shown that either the mean titre of serum HI antibody, or the incidence of volunteers having protective levels of such antibody, remain relatively stable in adults six to thirteen months following immunization with subunit influenza virus vaccine (Noble *et al.* 1977; Kunz *et al.* 1978; Cate *et al.* 1983; Clark *et al.* 1983). In children these measures of antibody

level and protection appear markedly reduced at similar times following immunization (Noble *et al.* 1977; Potter *et al.* 1980; Lerman, Wright & Patil, 1980).

In the present study, using single radial haemolysis, SRH (Oxford, Yetts & Schild, 1982; Goodeve, Jennings & Potter, 1983) the response to immunization of a group of student volunteers given varying doses of a trivalent subunit influenza virus vaccine was investigated. The changes in mean antibody titre and protective antibody levels at one and twelve months following immunization to all three antigens present in the vaccine were determined.

MATERIALS AND METHODS

Virus vaccines

A commercially available, subunit, influenza virus vaccine, manufactured by Glaxo Operations (U.K.) Ltd, Speke, Liverpool, was used. This was prepared by the treatment of concentrated, purified egg-grown viruses with Triton N 101 (Brady & Furminger, 1976). Purified HA and NA preparations from three virus strains, A/Bangkok/1/79 (H3N2), A/Brazil/11/78 (H1N1) and B/Lyon/1847/79, were included in the vaccine. Three vaccine formulations were used, one containing 24 μg each of A/Bangkok HA/0·5 ml, A/Brazil HA/0·5 ml and B/Lyon HA/0·5 ml, a total concentration of 72 μg HA/0·5 ml. The other formulations contained 12 and 6 μg HA/0·5 ml of each virus, total concentrations of 36 and 18 μg HA/0·5 ml respectively. A placebo (0·85% saline) was also available.

Volunteers and study design

First-year students from the 1982 intake at the University of Sheffield volunteered to take part in the study. The design of the study and the handling of the blood samples were essentially similar to that described elsewhere (Potter *et al.* 1977; Jennings *et al.* 1981, 1984). Inoculation of vaccine or placebo was by the deep subcutaneous route in 0·5 ml volumes under double-blind conditions.

Blood samples were collected from each volunteer immediately before, and four weeks following immunization, and again, eleven to twelve months following immunization (autumn 1983). Reactions to inoculation were assessed using a questionnaire (Jennings *et al.* 1978).

Single radial haemolysis

SRH tests were carried out as described previously (Goodeve, Jennings & Potter, 1983; Jennings *et al.* 1984; Al-Khayatt, Jennings & Potter, 1984). Essentially, gels were prepared according to the method of Oxford, Yetts & Schild (1982). Sheep erythrocytes were sensitized for use in SRH, with three virus strains, either X 73 (H3N2), a recombinant of A/Bangkok/1/79 (H3N2) and A/PR/8/34 (H1N1), NIB 7 (H1N1), a recombinant of A/Brazil/11/78 (H1N1) and A/PR/8/34 (H1N1), bearing A/Brazil surface proteins, or B/Lyon/1847/79. All virus strains were kindly supplied by Dr J. S. Oxford, National Institute for Biological Standardisation and Control, Hampstead, London.

The three strains were used as egg-grown, allantoic fluid virus pools for erythrocyte sensitization, which was carried out using 12000 haemagglutinating units (HAU) of B/Lyon, 24000 HAU of X 73 or 10240 HAU of NIB 7, per ml of 10% sheep erythrocytes. Wells of 3 mm diameter were cut into 1% agarose

(Indubiose A 37, Uniscience Ltd, London) gels, contained in 10×10 mm^2 Petri dishes, and filled with 5 μl of test or control, undiluted serum, previously inactivated at 56 °C for 30 min. After incubation at 37 °C for 18 h, the diameters of any haemolytic zones, including the well, were measured using a Transidyne Calibrating Viewer (Transidyne General Corporation, Ann Arbor, Michigan, USA) and zone areas of haemolysis calculated.

Increases in zone areas of haemolysis of $\geqslant 50\%$ between pre- and post-immunization sera from the same individual, tested on the same immunoplate, were taken to represent significant increases in antibody against A/H3N2 or A/H1N1 viruses (Jennings *et al.* 1984; Al-Khayatt, Jennings & Potter, 1984). For influenza B, the equivalent increase in haemolytic zone areas was $\geqslant 45\%$ (Goodeve, Jennings & Potter, 1983). Protective levels (PL50) of antibody have been reported to correlate with SRH zone areas of $\geqslant 25$ mm^2 for A/H3N2 and A/H1N1 viruses (Jennings *et al.* 1984; Al-Khayatt, Jennings & Potter, 1984), and with SRH zone areas of $\geqslant 45$ mm^2 for influenza B (Goodeve, Jennings & Potter, 1983).

Statistical methods

These were carried out using either the paired t test or the χ^2 test.

RESULTS

Short-term responses to immunization

Incidence of reactions and antibody rises

The incidence of both local and systemic reactions, and local pain, 24 h after immunization, is shown in Table 1. All reactions were mild and transient. The greatest incidence of both local reactions and local pain was reported for the vaccine containing the highest HA concentration. No significant systemic reaction was reported.

The incidence of volunteers showing significant antibody responses as determined by SRH, at four weeks following immunization to each HA component present in the vaccine, is also shown in Table 1. For A/Bangkok, 97% of volunteers receiving the highest, and 94% of volunteers who received the lowest vaccine dosage responded with significant antibody rises, while 84% of volunteers given 12 μg of A/Bangkok HA responded significantly. Immunization at all dosage levels also induced good SRH antibody responses to both the A/Brazil and B/Lyon HA components in the vaccine. For all three HA components in the vaccine, there was no significant difference between the dosage level of vaccine administered and the incidence of significant antibody responses as determined by SRH.

Volunteers receiving placebo showed no antibody increase to A/Brazil and A/Bangkok, but five increases to B/Lyon were observed in this group, all in volunteers with pre-immunization antibody ranging from areas of < 15 to 19 mm^2 in the SRH test.

Mean antibody increases and protective levels of antibody

Immunization elicited high serum antibody levels at four weeks to all three HA components in the vaccine, with respect to both mean titres in the immunized group and the percentage of volunteers with antibody at PL50 values (Table 2).

For A/Bangkok, mean antibody levels, measurable by SRH, in the volunteer

Table 1. *Reactions and antibody responses of volunteers following immunization with varying doses of trivalent surface antigen influenza virus vaccine*

Vaccine dose* (μg HA)	Number of volunteers	Percentage incidence of			Numbers/total (%) volunteers showing significant SRH responses to†		
		Local reactions	Local pain	Systemic reactions	A/Bangkok/79	A/Brazil/78	B/Lyon/79
24	34	21·9	44·8	1·0	31/32 (97)‡	27/29 (93)	23/29 (79)
12	33	9·1	39·4	Nil	26/31 (84)	23/30 (77)	25/30 (83)
6	33	12·1	26·3	Nil	30/32 (94)	22/31 (71)	22/31 (71)
Nil (placebo)	32	Nil	2·9	Nil	0/32 (−)	0/32 (−)	5/32 (16)

* Each vaccine dose contained 24, 12 or 6 μg HA of each of the three strains present.

† Increases in zone area of haemolysis between pre-and post-immunization sera $\geqslant 50\%$ for A/Bangkok/79 and A/Brazil/78, and $\geqslant 45\%$ for B/Lyon/79, were taken as significant.

‡ From a small number of volunteers, insufficient serum was obtained to enable tests against all three virus strains to be carried out.

Table 2. *Antibody responses and protective antibody levels in volunteers following immunization with varying doses of trivalent surface antigen influenza virus vaccine*

Vaccine dose* (μg HA)	Number of volunteers	Mean SRH zone area (mm²) (*a*) pre-immunization and (*b*) post-immunization and (*c*) the percentage with antibody at PL50								
		A/Bangkok/79			A/Brazil/78			B/Lyon/79		
		(*a*)	(*b*)	(*c*)	(*a*)	(*b*)	(*c*)	(*a*)	(*b*)	(*c*)
24	32	11·6	85·6	91·4	21·9	122·5	91·3	29·7	116·8	95·7
12	31	14·1	88·4	84·2	28·6	124·5	89·3	23·9	96·6	89·3
6	32	14·6	103·3	100·0	30·2	104·3	83·9	28·2	91·8	80·7
Nil (placebo)	32	12·0	10·2	6·1	28·0	27·9	37·5	26·7	38·9	31·3

* Each vaccine dose contained 24, 12 or 6 g HA of each of the three strains present.

groups at four weeks post-immunization were not significantly different from one another, irrespective of the vaccine dosage given. Similarly, the percentage of volunteers with serum PL50 values of SRH antibody was high following immunization, with 91% of volunteers who received 24 μg A/Bangkok HA, 84% of volunteers receiving 12 μg HA and all volunteers given 6 μg HA, showing antibody zones $\geqslant 25$ mm².

High post-immunization mean SRH antibody levels were also observed against A/Brazil, irrespective of vaccine dosage administered (Table 2). Mean SRH zone areas at least four times greater than the PL50 value were recorded at all dosages four weeks after immunization, and between 84 and 91% of the volunteers tested possessed serum SRH antibody zones $\geqslant 25$ mm². Mean SRH zone antibody areas against B/Lyon of 92–117 mm² were observed in volunteer groups immunized with the various dosage levels at four weeks, and between 81 and 96% of volunteers in these groups showed SRH antibody zones $\geqslant 45$ mm². Raised, but not significantly increased mean zone areas of antibody to B/Lyon, and incidence of volunteers with B/Lyon SRH antibody zones $\geqslant 45$ mm² post-immunization, were noted in the placebo group.

Changes in mean antibody levels and protective levels of antibody twelve months after immunization

Blood specimens were not available from every volunteer twelve months following immunization. Table 3 shows both the mean SRH antibody zone areas in each volunteer group and the incidence of volunteers with SRH antibody values $\geqslant$ PL50, at both one and twelve months following immunization, for those individuals from whom serum specimens were obtained.

Mean zone areas of SRH antibody to all three virus strains had decreased significantly at twelve months compared to their values one month following immunization at all dosage levels, and this decrease was greatest for A/Bangkok antibody. Furthermore, except for the placebo group, there was a significantly greater number of decreases in antibody to A/Bangkok, as compared to either A/Brazil or B/Lyon, over this period, at all dosage levels (data not shown).

Table 3. *Persistence of SRH antibody in volunteers one and twelve months after immunization with trivalent surface antigen vaccine*

Vaccine dose* (μg HA)	Number of volunteers	A/Bangkok/79				A/Brazil/78				B/Lyon/79			
		Mean SRH zone area (mm²) at		Number (%) with antibody at PL50 at		Mean SRH zone area (mm²) at		Number (%) with antibody at PL50 at		Mean SRH zone area (mm²) at		Number (%) with antibody at PL50 at	
		1 month	12 months	1 month	12 months	1 month	12 months	1 month	12 months	1 month	12 months	1 month	12 months
24	17	99·8	53·3	15 (88)	13 (77)	122·3	98·3	15 (88)	17 (100)	116·6	92·2	16 (94)	14 (82)
12	26	110·9	56·6	23 (89)	22 (85)	122·8	93·5	23 (88)	24 (92)	96·4	81·3	23 (88)	22 (85)
6	26	116·8	72·0	26 (100)	24 (92)	108·8	89·3	22 (85)	22 (85)	96·5	83·6	22 (85)	19 (73)
Nil (placebo)	26	12·8	10·6	3 (12)	2 (8)	29·5	30·1	9 (35)	10 (39)	41·2	34·4	8 (31)	6 (23)

* Each vaccine dose contained 24, 12 or 6 μg HA of each of the three strains present.

However, with respect to each dose, mean SRH antibody zone areas to all three virus antigens remained markedly higher than their respective PL50 levels, and the percentage of individuals with SRH antibody zone areas $\geqslant 25$ mm^2 against A/Bangkok HA, or $\geqslant 45$ mm^2 against B/Lyon HA showed no significant reduction (Table 3).

With respect to A/Brazil and the two highest dosages of vaccine used, the incidence of volunteers having SRH antibody zone areas $\geqslant 25$ mm^2 had actually increased (although not significantly) at twelve months, compared to the incidence at one month post-immunization. From individual paired sera it appeared that an H1N1, A/Brazil-like virus had been active amongst the volunteer group during the intervening period, as five significant increases in antibody to A/Brazil, measurable by SRH, were detected; two in volunteers who received placebo, two who received the 24 μg dosage and one who received the 12 μg dosage. All five increases were in volunteers whose SRH antibody zone areas were less than the PL50 value four weeks after immunization.

DISCUSSION

The results in the present paper show that an increased antigenic mass of influenza virus HA, above the 9–10 μg present in commercially available subunit influenza virus vaccines, did not elicit significantly higher antibody levels, determined by SRH, than the dose containing 6 μg HA. Moreover the lowest amount of HA used, 6 μg, induced a high incidence of PL50 values of antibody in volunteers, and this incidence remained high at twelve months post-immunization.

The vaccines used in these studies were trivalent, administered as a single dose, and the above findings apply to all three vaccine components. It is clear that no advantage is to be gained by increasing HA dosages above that recommended for commercial use. Indeed, in times of antigenic drift as at present, with respect to A/H3N2, A/H1N1 and B influenza viruses, the lowest vaccine dose used elicited mean SRH antibody zone areas and PL50 values at levels commensurate with protection (Goodeve, Jennings & Potter, 1983; Jennings *et al.* 1984; Al-Khayatt, Jennings & Potter, 1984), against all three virus strains at both one and twelve months post-immunization. Furthermore, when sera obtained twelve months after immunization from volunteers who had received 6 μg HA of A/Bangkok were tested for antibody using a heterologous A/H3N2 strain, A/Philippines/82, a mean zone area of SRH antibody of 74·7 mm^2 was found, and 92 % of the volunteers had A/Philippines SRH antibody zone areas above the PL50 value (unpublished observations). Although the reactions in the present study were mild and transient, a greater incidence of local reactions and pain were reported by volunteers who received the highest vaccine dose. Lower vaccine dosage would further reduce such reactions.

Previous studies in this laboratory and elsewhere (Ruben & Jackson, 1972; Goodeve *et al.* 1983) have shown that antibody responses to split or subunit vaccines may reach a plateau, or even decrease, with increasing dose. Similar findings have been reported for inactivated, whole influenza A/New Jersey/76 (H1N1) vaccine (Pandemic Working Group, 1977), although no such plateau effect was observed in a volunteer study using an adjuvanted, subunit influenza virus vaccine (Potter *et al.* 1977).

The studies described here indicate that dosages of 6 μg HA will induce high antibody responses in primed populations (Parkman *et al.* 1976). However, during periods of antigenic shift, and in unprimed populations such as children, subunit vaccines are less effective (Jennings *et al.* 1981). The persistence of high mean levels of antibody, and of high numbers of volunteers with antibody above the PL50 value at twelve months post-immunization in the present study, is thus related to the background experience of those individuals with influenza virus strains. Other workers have found antibody levels following immunization to remain stable in adults over periods ranging from five to thirteen months (Kunz *et al.* 1978; Cate *et al.* 1983), while in children after similar time periods following immunization marked reductions in antibody levels have been noted (Zavadova *et al.* 1972; Potter *et al.* 1980; Lerman, Wright & Patil, 1980), suggesting that the lowest dosage of subunit vaccine used in the present study may not induce the persistence of antibody seen in adults.

A minor qualification with respect to the results in the present study is that there was evidence of influenza B virus activity at the commencement of the study, and this may have modified the antibody levels to B/Lyon seen one month following immunization. There was also evidence of H1N1 virus activity in the volunteer group during the period between collection of the one- and twelve-month blood specimens, which may have had some influence on the high antibody levels to this virus seen twelve months post-immunization. The infections noted at these times for both B/Lyon and A/Brazil occurred in volunteers who had SRH antibody below the PL50 values predicted by ourselves in previous studies (Goodeve, Jennings & Potter, 1983; Jennings *et al.* 1984; Al-Khayatt, Jennings & Potter, 1984).

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