Reactogenicity and immunogenicity of a surface-antigen-adsorbed influenza virus vaccine in children

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SUMMARY An influenza virus vaccine containing the purified surface haemagglutinin and neuraminidase antigens of A/Victoria/75 and B/Hong Kong/73 viruses adsorbed to an aluminium hydroxide gel was assessed for reactogenicity and immunogenicity in children aged 4 to 11 years, since there is no influenza virus vaccine available for this age group. Significant serum haemagglutination-inhibiting antibody responses to the A/Victoria/75 and B/Hong Kong/73 haemagglutinin antigens present in the vaccine were observed in 47% and 35%, respectively, of the children vaccinated, with a single dose. The vaccine induced no significant local or systemic reactions.

Although the routine immunisation of children against influenza is not obligatory, influenza A viruses are an important cause of illness in infants and young children (Fox and Kilbourne, 1973) and can cause serious morbidity and even death in children of any age with chronic heart, respiratory, and other underlying disease (Phillips *et al.*, 1973; Glezen, 1977). Unfortunately, conventional, inactivated whole influenza virus vaccines are reactogenic in infants and young children (Marine and Stuart-Harris, 1976; Wright *et al.*, 1976), and this has prohibited their use in this cohort of the population. Studies comparing whole, inactivated influenza virus vaccines with split or the more highly purified subunit influenza virus vaccines in adults have shown the latter to be less reactogenic (Brandon *et al.*, 1967; Barry *et al.*, 1976). It is, therefore, considered possible that these vaccines may be more acceptable for children.

In the present study, an assessment of the reactogenicity and immunogenicity of a bivalent influenza virus vaccine containing only the haemagglutinin and neuraminidase surface antigens of A/Victoria/75 and B/Hong Kong/73 influenza viruses was carried out in young children. The surface antigens of these

viruses were adsorbed to aluminium hydroxide gel. A similar, surface-antigen-adsorbed vaccine has been shown to induce few reactions and good serum antibody responses in adults (Potter *et al.*, 1975, 1977).

Material and methods

VACCINE

The adsorbed, surface-antigen influenza virus vaccine, Fluvirin, manufactured by Evans Biologicals for Duncan Flockhart Ltd, was used in the present study. The vaccine was prepared from egg-grown virus and purified by zonal centrifugation; the surface antigens of the intact virus particle, the haemagglutinin (HA) and neuraminidase (N), were separated from unwanted core material by centrifugation through a sucrose gradient containing Triton N 101 and then adsorbed on to an aluminium hydroxide gel (Brady and Furminger, 1976). Before adsorption on to the gel, rocket immunoelectrophoresis (Oxford and Schild, 1978), carried out at the National Institute of Biological Standards and Control, showed that the vaccine contained 8.4 µg HA of A/Victoria/3/75 (H3N2) and 12.7 µg HA of B/Hong Kong/8/73 per dose; 10 µg HA is approximately equivalent to 200 international units (IU). When the HA concentration of the virus was tested after adsorption to aluminium hydroxide gel and just before inoculation, the

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vaccine was found to contain only 2.9 μg HA of A/Victoria/3/75 and 2.0 μg HA of B/Hong Kong/8/73 per dose. However, these latter figures may be low since some of the HA antigen has probably remained attached to the gel, and antigen thus bound would be unmeasurable by rocket immunoelectrophoresis.

STUDY GROUP

Children from 2 to 11 (median 7) years of age attending the outpatient clinic at the Children's Hospital, Sheffield were recruited after written consent had been obtained from informed parents. Only one child was under 4 years, and only three were over 9 years of age. Children with a history of convulsions or allergy to eggs, and those receiving immunosuppressive or corticosteroid therapy, were excluded. Altogether 49 children completed the study, including 13 asthmatics, seven diabetics, and one child with cystic fibrosis. The remainder were healthy children who were mainly siblings of patients attending the hospital. Each child received a single, 0.5 ml, dose of vaccine by the intramuscular route immediately after collection of a venous blood sample. Three days after immunisation the parents were contacted by telephone and carefully questioned as to the nature of any local or systemic reactions. Four to six weeks later a second blood sample was collected from each child.

SEROLOGICAL STUDIES

Paired sera were tested for haemagglutination-inhibiting (HI) antibodies using 8 haemagglutinating units of virus and employing the microtitre technique (Sever, 1962).

The sera were pretreated with cholera filtrate to remove non-specific inhibitors and tested for HI antibodies to the homologous A/Victoria/75 and B/Hong Kong/73 viruses.

Assays for neuraminidase-inhibiting (NI) antibodies were carried out by an automated modification (Bevan *et al.*, 1975) of the standard World Health Organisation methods (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

CLINICAL REACTIONS

The clinical reactions attributable to the vaccine were mild, and the findings are shown in Table 1. Of 49 children, 18 (37%) developed mild local reactions consisting of slight soreness and aching at the inoculation site at some time during the three days after immunisation. Two of these children also had local swellings, two had redness, and one a bruise at the inoculation site. The incidence of systemic reactions was also low in the present study, and only one child, a 7-year-old diabetic girl, with normally stable diabetes, had a systemic reaction apparently associated with immunisation. This child showed raised levels of sugar and ketones in the urine for two days after immunisation but no other effects. In addition, one boy suffered an asthmatic attack 36 hours after immunisation. Although temperatures were not recorded, there were no reports of fever after immunisation in any of the children.

SERUM ANTIBODY RESPONSES AFTER IMMUNISATION

The serum HI antibody responses of the volunteers to the bivalent A/Victoria/75 and B/Hong Kong/73 influenza virus vaccine are shown in Table 2. A total of 23 (47%) of the 49 children responded to immunisation with four-fold rises in serum HI antibody titre to influenza A/Victoria/75. The geometric mean titre (gmt) of this antibody rose from 17.2 pre-immunisation to 67.4 four to six weeks after immunisation, an increase of 3.9-fold. In addition, the number of children with serum HI antibody levels of $\geq 1:40$ to A/Victoria/75 rose from 14 (29%) to 29 (59%) after immunisation.

The serum HI antibody response to the B/Hong Kong/73 component of the vaccine is also shown in Table 2. Seventeen (35%) of the 49 children responded with ≥ 4 -fold serum HI antibody increases to this virus, and the gmt rose from 5.9 to 15.7, an increase of 2.7-fold.

The serum NI antibody response of the children to immunisation with the A/Victoria/75:B/Hong Kong/73 influenza virus vaccine is also presented in Table 2. Owing to the small volume of serum avail-

Table 1 Incidence of clinical reactions in children after immunisation with influenza A/Victoria/3/75: B/Hong Kong/8/73 surface-antigen-adsorbed vaccine

No. of children	No. (%) with local reactions lasting:			Total with local reactions		Systemic reactions	
	1 day	2 days	3-5 days	No.	%	No.	%
49	5 (10.20)	9 (18.37)	4 (8.16)	18	36.73	1	2.04

Table 2 Serum HI and NI antibody responses of children after immunisation with bivalent surface-antigen-adsorbed influenza virus vaccine

	Response to A/Victoria/75		Response to B/Hong Kong/73	
	HI antibodies	NI antibodies	HI antibodies	NI antibodies
No. of serum pairs tested	49	31	49	38
No. (%) with $\geq$ 4-fold antibody increase post-immunisation	23 (47)	10 (32)	17 (35)	17 (45)
No. (%) with titres < 1:10 before and after immunisation	19 (39) - 3 (6)	15 (48) - 6 (19)	42 (86) - 22 (45)	36 (95) - 16 (42)
No. (%) with titres $\geq$ 1:40 before and after immunisation	14 (29) - 29 (59)	3 (10) - 15 (48)	0 (-) - 16 (33)	0 (-) - 18 (47)
GMT of antibody before and after immunisation (fold increase)	17.2 - 67.4 (3.9)	13.5 - 30.5 (2.3)	5.9 - 15.7 (2.7)	2.5 - 17.9 (7.1)

able in some of the specimens, sera from all the children who took part in the study were not tested, but the results do show that 10 (32%) of the 31 volunteers tested responded with $\geq$ 4-fold serum NI antibody increases to A/Victoria/75 after immunisation. In addition, 17 (45%) of the 38 children whose sera were tested for B/Hong Kong/73 NI antibody showed evidence of significant antibody rises to the neuraminidase antigen of this virus.

Discussion

Several workers have reported adverse local and systemic reactions after the use of conventional, inactivated, whole virus vaccines in children (Parkman *et al.*, 1976; Wright *et al.*, 1976; Schevill and Marks, 1977) and particularly in infants (Marine and Stuart-Harris, 1976; Wright *et al.*, 1976). The present results suggest that the A/Victoria/75:B/Hong Kong/73 surface-antigen-adsorbed vaccine used in the present study is both safe and acceptable in children aged 4 years or older. The local systemic reactions after vaccine administrations were mild, and this is in accordance with the findings in adults receiving surface-antigen-adsorbed vaccine (Potter *et al.*, 1977). This may well apply generally to subunit and split influenza virus vaccines (Davenport *et al.*, 1964; Phillips *et al.*, 1973). Recent studies in both infants and children (Gross *et al.*, 1977) showed that a split-product A/Port Chalmers/73:B/Hong Kong/73 influenza virus vaccine produced markedly fewer systemic reactions than a conventional whole virus vaccine given at an equivalent dose. The present studies did not extend to infants, and the reactogenicity of surface-antigen-adsorbed vaccine in this group has yet to be determined.

The serological response of adults to surface-antigen-adsorbed influenza virus vaccine was found to be good (Potter *et al.*, 1977) but the results reported here suggest that the response of children may be lower. Only 47% of the children receiving

immunisation showed significant HI antibody increases to the A/Victoria/75 component of the vaccine, and only 35% responded similarly to the B/Hong Kong/73 component. However, the percentage of children with serum HI antibody titres to influenza A/Victoria/75 of $\geq$ 1:40, the postulated protective level against homologous virus infection (Hobson *et al.*, 1972), increased from 29 to 59 after immunisation. Other workers have shown that children respond less well than adults to subunit or split influenza vaccines (Parkman *et al.*, 1976), and young persons unprimed with the swine influenza virus respond poorly to split A/New Jersey/76 virus vaccine (Gross and Ennis, 1977). The influenza B/Hong Kong/73 component of split virus vaccine has been shown to be a poor immunogen in children under 10 years of age (Gross *et al.*, 1977), and the results of the present study are in agreement with these findings.

The poor response of children is almost certainly due to their relative lack of prior exposure to influenza viruses, and this view is supported by the similarly poor response of young adults even to whole virus vaccines containing antigens to which they were unprimed. For this reason it seems probable that a second, booster dose of subunit influenza vaccine will be required to afford adequate protection in a population which has not experienced the relevant influenza virus strains.

The serological response of children in the present study to the A/Victoria/75 and B/Hong Kong/73 neuraminidase antigens was similar to that seen for the HA antigens, and less than half of the subjects tested showed significant serum NI antibody responses. However, the gmt of serum NI antibody was increased in the group as a whole, and high levels of this antibody may contribute to protection against influenza (Monto and Kendal, 1973).

In conclusion, these studies show that the surface-antigen-adsorbed vaccine used is of low reactogenicity in children 4 to 11 years of age and induces significant serum HI and NI antibody responses.

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