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Antibody responses to vaccination with WRL 105 strain live influenza vaccine in previously vaccinated and unvaccinated volunteers

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SUMMARY

Intranasal vaccination with a single 0·5 ml dose of $10^{7.0}$ EID 50 WRL 105 strain live influenza vaccine elicited four-fold or greater increases in circulating homotypic haemagglutinating inhibiting (HAI) antibody in 60 (64·5 %) of 93 volunteers, or in 58 (74·4 %) of 78 volunteers with HAI antibody titres before vaccination of $\leqslant 1/20$. In comparison, in a group of volunteers vaccinated 9 months previously re-vaccination elicited antibody responses in only 4 (6·9 %) of 58 volunteers, or in 3 (14·3 %) of 21 volunteers with antibody titres before vaccination of $\leqslant 1/20$.

Titres of vaccine-induced antibody and antibody resulting from earlier natural infection appeared to fall slowly and at equivalent rates over a 9 month period.

INTRODUCTION

Immunity to influenza appears to be a complex and at present ill-defined mosaic of various local and circulating antibodies and cellular immune factors. The assessment of immunity to influenza is complicated by the presence of the two main epidemiological types (A and B) and the propensity of each type to undergo periodic changes in their surface antigens. Although no serological test shows exactly whether or not an individual is immune to influenza, the presence of homotypic haemagglutinating inhibiting (HAI) antibody at a titre of 1/40 or more has been found generally to be associated with protection against infection (Meiklejohn *et al.* 1952; Hobson, Beare & Ward-Gardner, 1972). Furthermore, the presence of circulating HAI antibody appears to be a better indicator of protection against infection than circulating anti-neuraminidase or neutralizing antibodies or local nasal antibodies (Freestone *et al.* 1972).

The percentage and height of antibody responses elicited by vaccination with

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WRL 105 strain live influenza vaccine administered intranasally as drops were found to be remarkably similar in five geographically separate studies with populations of boys aged 14 to 18 years, young adults and factory and laboratory employees. Four-fold or greater antibody responses were obtained in 70–80% of subjects with homotypic HAI antibody titres before vaccination of $\leqslant 1/20$ (Moffat *et al.* 1976; Morris *et al.* 1975; Freestone *et al.* 1976; Evans *et al.* 1976; Stealey, McCahon & Freestone, 1976). The proportion of subjects in whom vaccination elicited fourfold or greater antibody responses was inversely related to the titre of homotypic HAI antibody present before vaccination.

The study described here was undertaken to compare antibody responses and clinical reactions elicited by intranasal vaccination with the WRL 105 strain in a group of subjects not previously vaccinated with this strain, with those of a group of subjects who had been vaccinated with the same strain nine months earlier (Evans *et al.* 1976) and were now revaccinated. Persistence of vaccine-induced antibody was also studied in the latter group by comparing HAI antibody titres three weeks and nine months after vaccination.

METHOD

A total of 198 adult employees aged 18 to 61 years of the Organics Division, ICI, Manchester offered to take part in this study. Informed consent was obtained. Three (1·5%) were excluded because of current steroid therapy or histories of chronic bronchitis. Seventy-eight subjects had been vaccinated intranasally in January 1975 nine months earlier with either one or two doses of WRL 105 strain live influenza vaccine (Group A), while the other 117 subjects had not received live influenza vaccine (Group B). These groups were similar in regard to age and sex. Previous histories of natural influenza were somewhat more frequent in the unvaccinated group (Table 1). Blood samples were collected immediately before and 3 weeks after vaccination, and a diary record of clinical reactions was recorded by volunteers for the first 7 days after vaccination.

Vaccine and method of administration

WRL 105 strain live influenza vaccine is a recombinant of attenuated 280th egg passage A/Okuda/57 (H2N2) and unattenuated A/Finland/4/74 (H3N2) strains. The preparation of this recombinant and the clinical assessment of its immunogenicity, transmissibility and reactivity have been described elsewhere (Stealey *et al.* 1976; Morris *et al.* 1975; Moffat *et al.* 1976). The vaccine was administered intranasally as drops to give $10^{7.0}$ EID50 in an 0·5 ml volume (0·25 ml vaccine being administered to each nostril).

Serological testing

Haemagglutinating inhibiting (HAI) antibody titrations were performed by the micro-method of Takatzy (1955), as modified by Sever (1962), using 0·025 ml volumes, 8 haemagglutinating units of virus (WRL 105) and 0·6% chicken erythrocytes. Serum, virus and erythrocytes were all diluted in isotonic saline.

Table 1. *Age, sex and history of natural influenza of volunteers*

	Group A	Group B
Number of volunteers	78	117
Number female	11 (14·1 %)	19(16·2 %)
Age – average (range)	38·9 (21–59 yrs)	41·2 (18–61 yrs)
History of natural influenza during or since 1973	15 (19·2 %)	36 (30·8 %)

Before testing, sera were treated with receptor-destroying enzyme (RDE; cholera filtrate) to eliminate non-specific inhibitors. After incubation overnight at 37 °C RDE-serum mixtures were heated for 30 min at 56 °C to inactivate the RDE.

RESULTS

Antibody responses to vaccination

Paired pre- and post-vaccination sera were obtained from 58 (74·4 %) of the volunteers vaccinated nine months previously in January 1975 (Group A) and 93 (79·5 %) of those not vaccinated before with the WRL 105 strain (Group B). The HAI antibody titres of the two groups before vaccination in November 1975 were dissimilar as expected (geometric mean titres Group A – 27; Group B – 7). Twenty-one (36·2 %) of 58 subjects in Group A and 78 (83·9 %) of 93 subjects in Group B had titres of $\leqslant 1/20$.

In subjects in Group B with HAI antibody titres of $\leqslant 1/20$, vaccination elicited four-fold or greater antibody responses in 58 (74·4 %) volunteers. In comparison in the previously vaccinated subjects in Group A with antibody titres of $\leqslant 1/20$, revaccination elicited a four-fold or greater antibody response in only 3 (14·3 %) (Table 2).

The previously vaccinated group may be divided into three sub-groups: those with HAI antibody titres of $\leqslant 1/20$ who showed a four-fold or greater antibody response to vaccination in January 1975 (Group A1 – 21 volunteers); those who had HAI antibody titres of $\leqslant 1/20$ before vaccination in January 1975 but who failed to show a four-fold or greater antibody response to vaccination (Group A2 – 20 subjects); and those with HAI antibody titres of $\geqslant 1/40$ before vaccination (Group A3 – 17 subjects). Revaccination elicited a four-fold or greater antibody response in one volunteer in Group A1 and 2 volunteers in Group A2. However, no great differences were found between these groups in the geometric mean antibody responses elicited by revaccination (Table 3).

Persistence of antibody for nine months after vaccination

Sera were collected 3 weeks and 9 months after vaccination in January 1975 from 21 subjects with initial HAI antibody titres of $\leqslant 1/20$ in whom vaccination elicited a four-fold or greater antibody response (sub-group A1). Results of titration of these sera in parallel for homotypic HAI antibody are shown in Fig. 1 and Table 3. In general, antibody persisted well. Only two subjects showed four-fold

Table 2. *HAI antibody responses to vaccination*

Antibody titre† before vaccination	Fourfold or greater antibody response (No. responding/No. in group)	
	Group A	Group B
< 10	3/4–75·0 %	36/38–94·7 %
10	0/5–0*	13/23–56·2 %*
20	0/12–0**	9/17–52·9 %**
	3/21 14·3 %***	58/78*** 74·4 %
40	1/22–4·5 %	1/7–14·3 %
80	0/13–0	1/4–25·0 %
> 80	0/2–0	0/4–0
Total	4/58–6·9 %***	60/93–64·5 %***

† Expressed as reciprocals.

Fisher's exact probability test: * $P < 0{\cdot}05$, ** $P < 0{\cdot}005$, *** $P < 0{\cdot}0005$.

Table 3. *Geometric mean HAI antibody titres following vaccination*

Group	Pre-vaccination January 1975	Post-vaccination February 1975	Pre-vaccination November 1975	Post-vaccination December 1975
A1 (n = 21)	4	64	40	43
A2 (n = 20)	5	8	12	25
A3 (n = 17)	68	71	45	58
B (n = 93)	—	—	7	50

Group A1 – volunteers with HAI antibody titres of $\leqslant 1/20$ before vaccination in January 1975 and who responded to vaccination with a four-fold or greater increase in titre.

Group A2 – volunteers with HAI antibody titres of $\leqslant 1/20$ before vaccination in January 1975 who did not respond serologically to vaccination.

Group A3 – volunteers with HAI antibody titres of $\geqslant 1/40$ before vaccination in January 1975.

or greater falls in antibody titre during the 9 month period. One subject lost antibody from a titre of 1/640 following vaccination to a titre of 1/10 immediately before re-vaccination 9 months later and then responded with a four-fold antibody response. The limited fall in geometric mean antibody titre over the 9 month period of follow-up in those serologically responding to primary vaccination (Group A1) is similar to the decline in titre seen in those volunteers with antibody titres of $\geqslant 1/40$ before, and in whom vaccination in January 1975 elicited no antibody response (Group A3). Such antibody was presumably a result of earlier natural infection.

Clinical reactions

Upper respiratory and systemic reactions were recorded after vaccination in November 1975 less frequently in those who had been previously vaccinated than in the previously unvaccinated group although the differences did not achieve statistical significance (Table 4).

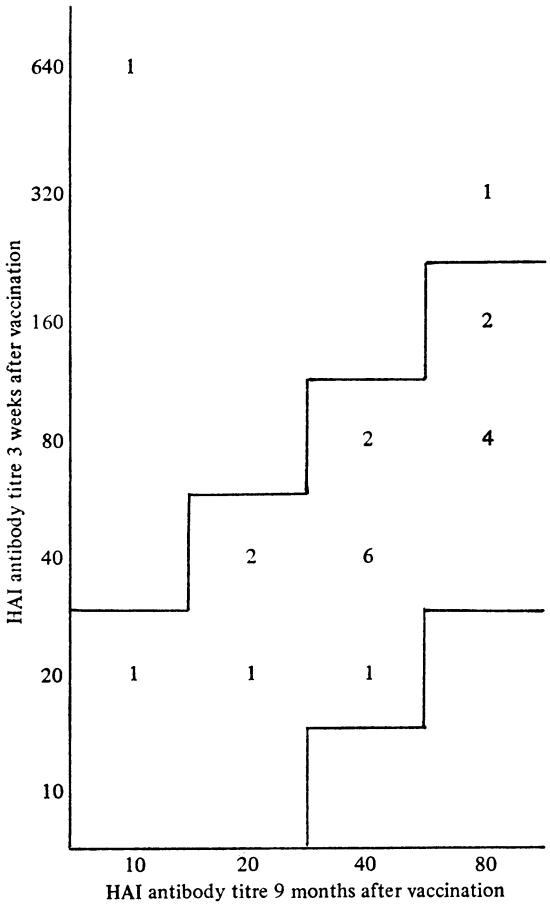

Fig. 1. HAI antibody titre persistence. GMT 3 weeks after vaccination = 64. GMT 9 months after vaccination = 40.

Table 4. *Clinical reactions recorded following vaccination*

	Group A	Group B
Total number per group	78	117
Number returning reaction forms	72	105
Number recording symptoms	35 (48·6%)	61 (58·1%)
Analgesics taken	3 (8·6%)	12 (19·7%)
Local symptoms*		
Mild	19 (26·4%)	32 (30·5%)
Moderate	2 (2·8%)	8 (7·6%)
Severe	0	2 (1·9%)
Lasting ⩾ 4 days	8 (11·1%)	12 (11·4%)
General symptoms†		
Mild	5 (6·9%)	12 (11·4%)
Moderate	4 (5·6%)	9 (8·6%)
Severe	1 (1·4%)	1 (1·0%)
Lasting ⩾ 4 days	0	2 (1·9%)
Negligible symptoms	11 (15·3%)	13 (12·4%)

* Symptoms relating to the upper respiratory tract (nasal obstruction, nasal discharge or sore throat).

† Headache, fever or myalgia.

DISCUSSION

The serological responses obtained with the WRL 105 strain in the previously unvaccinated group are similar to those of earlier studies. The strain elicited antibody responses in 74.4% of those with low titres of antibody before vaccination and did not cause an unacceptable level of clinical reactions. In contrast, antibody responses were elicited in only 14·3% of those vaccinated 9 months previously who had equivalent low antibody titres before the re-vaccination procedure. Since all subjects in the study were vaccinated at the same time and in a similar manner the low percentage of antibody responses in those previously vaccinated cannot be explained by factors known to affect the rate of antibody responses after intranasal administration of vaccine such as poor vaccination technique, use of vaccine of too low a titre or the presence of overt nasal infections. The study shows that reliance on the titre of circulating homotypic HA antibody as an index of susceptibility to infection may be misleading. Since our study population did not regularly receive inactivated influenza vaccine, circulating HAI antibody present before vaccination in those previously unvaccinated (Group B) must have originated from earlier natural infection, probably with heterotypic strains. Thus it appears that low titres of antibody which is mainly homotypic in origin may be associated with a greater degree of protection than equivalent titres of mainly heterotypic antibody. Clearly it would be possible inadvertently to recruit volunteer populations with low homotypic HAI antibody titres who are not open to infection and for whom, therefore, a new candidate vaccine strain appeared over-attenuated. Indeed, this may provide some explanation of earlier unpublished paradoxical results in which strains previously shown to be infective in one test population have almost completely failed to infect another (I. B. Hillary; K. G. Nicholson, pers. comms.). The initial screening of a new candidate vaccine strain on small numbers of volunteers may therefore sometimes give misleading results and supports the attempt to predict the degree of attenuation of strains in the laboratory or in animal models (Mostow & Tyrrell, 1972; Fenton, Jennings & Potter, 1977).

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