

**Immunity to challenge in volunteers
vaccinated with an inactivated current or earlier strain
of influenza A(H3N2)**

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SUMMARY

Volunteers were inoculated with vaccine made from the 30c mutant, A/Port Chalmers/73 or B/Hong Kong/8/73. Preliminary experiments showed that the 30c strain was antigenically quite close to A/HK/8/68. Volunteers given 30c developed haemagglutination inhibiting antibodies against the 'current' 1973 serotypes (as well as to the vaccine virus) but the titres were less than those after the A/PC/73 vaccine. Volunteers were then challenged with a live attenuated virus, WRL 105, with A/Finland/4/74 antigens, by intranasal inoculation. The rates of infection were 43% after B/Hong Kong/8/73, 20% after 30c and 5% after A/PC/73. This indicated that the 30c gave some protection but that the vaccine prepared from the current strain gave more.

INTRODUCTION

In spite of many years of experience and many technical improvements there are still a number of uncertainties in the effects produced by influenza vaccines (Stuart-Harris & Schild, 1976). There is evidence that at the time of major antigenic shift vaccines made with 'obsolete' strains do not protect and this was seen when the Hong Kong (H3N2) virus (HK) appeared (Mostow *et al.* 1969; Tyrrell, Buckland, Rubenstein & Sharpe, 1970). In periods of antigenic drift vaccines made against earlier strains may give protection (Stiver, Graves, Eickhoff & Meiklejohn, 1973), for instance three annual doses of HK vaccine protected schoolboys against the A/Eng/42/72 serotype (Hoskins *et al.*, 1973). However at present it is not

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possible to define the degree of antigenic difference between two strains which is compatible with effective protection.

As a contribution to the solution of this problem we have recently studied the protective effect of a rather unusual laboratory modified strain. This was a mutant, 30c, selected by serial passage of H3N2 Hong Kong serotype in the presence of antiserum – it possessed a haemagglutinin, which was said to resemble the A Eng/42/72 serotype (Fazekas de St Groth & Hannoun, 1973; Hannoun, 1973). This was compared with an influenza B vaccine and one prepared with the prevalent serotype of influenza A (A/Port Chalmers/73).

Protection was detected by challenging all the volunteers with a live attenuated virus of the serotype A/Finland/4/74 which circulated in the general population during the winter in which these experiments were done. The results were compared with those of the serum antibody responses to vaccination to determine to what extent these were satisfactory indicators of the degree of protection shown.

MATERIALS AND METHODS

Inactivated vaccines

Monovalent, whole virus A/Port Chalmers/1/73 (H3N2) and B/Hong Kong/8/73 vaccines were prepared by Evans Medical, UK. For the A/Port Chalmers/73 preparation the production strain was a high yielding recombinant, MRC11, obtained from the 1973 parent and A/PR8/34 (HON1) virus. Vaccines were prepared by standard procedures involving inactivation by β -propiolactone and purification by zonal ultracentrifugation. The A/Port Chalmers/73 vaccine contained 400 i.u./dose by haemagglutination assays (Westwood, Woodward & Perkins, 1971). B/Hong Kong/8/73 vaccine contained 300 i.u./dose. The 30c vaccine was a gift from Dr C. Hannoun of the Pasteur Institute, Paris, and was a whole virus vaccine inactivated by formalin and contained 450 i.u./dose.

Volunteers

Students from the University of Sheffield and staff of the Wellcome Research Laboratories, Berkhamsted volunteered to be vaccinated, to donate specimens, and to complete a standard record of symptoms after the dose of live vaccine. The live vaccine was given three weeks after the inactivated vaccine and blood was collected at each vaccination and three weeks after the live vaccination.

Antibody measurements

Serum specimens were titrated by a standard haemagglutination inhibition (HI) test using 4 units of haemagglutinin and chicken red cells. Before testing, the sera were treated with cholera filtrate. Neuraminidase inhibition (NI) tests were performed as described by Aymard-Henry *et al.* (1973).

Live vaccine

The virus used was the recombinant WRL105 (Morris, Freestone, Stealey & Oliver, 1975). Two pools were available, one being used at Sheffield and the other at Berkhamsted. Three weeks after vaccination the challenge virus was given by

Table 1. Cross-reactions of 30c virus with A/Hong Kong/68 (H3N2) and its natural variants in HI tests

	Haemagglutination-inhibition titres using post-infection ferret sera against				
	A/HK/68	A/Eng/72	A/PC/73	A/Fin/74	30c*
A/Hong Kong/8/68	3840	2560	320	160	1280
A/England/42/72	1280	7680	2560	1280	1280
A/Port Chalmers/1/73	320	960	2560	1280	120
A/Finland/4/74	160	320	1280	2560	80
30c	2560	2560	320	160	960

* Titres shown are mean titres of two ferret sera which gave closely similar HI reactions.

Table 2. Antigenic comparisons of neuraminidase of 30c with A/Hong Kong/68 (H3N2) virus and its natural variants

Source of neuraminidase	Neuraminidase-inhibition titres using immune rabbit serum against		
	Purified N2 (1968) neuraminidase derived from A/Hong Kong/1/68	Purified recombinant virus A/equine/Prague/56 (Heq1)-A/Eng/42/72 (N2)	Purified recombinant virus A/equine/Prague/56 (Heq1)-A/Port Chalmers/73 (N2)
A/Hong Kong/1/68	1250	160	20
A/England/42/72	450	640	50
A/Port Chalmers/1/73	14	100	200
A/England/929/73	13	100	200
30c	1400	120	20

drops intranasally and the dose in both places was $10^{7.0}$ EID₅₀ in 0.5 ml volume per volunteer. Infection was detected (a) by attempting virus isolation in embryonated eggs from a throat swab which was collected 3 days after the drops were given, and (b) by HI tests against the challenge virus – a fourfold rise was regarded as significant.

RESULTS

The haemagglutinin and neuraminidase antigens of 30c were compared with those of A/Hong Kong/68 and its natural variants which appeared up to 1974 by means of HI tests with post-infection ferret serum (Table 1) and by NI tests with hyperimmune rabbit sera to purified neuraminidase of purified virus antigens (Table 2).

The conclusion from the HI tests was that 30c was very close to A/Hong Kong/68 and A/England/42/72 and reacted relatively poorly with viruses isolated in or after 1973. The neuraminidase of 30c was antigenically close to that of A/Hong Kong/68 virus and did not show the antigenic 'drift' which occurred in nature as in A/England/42/72 and A/Port Chalmers/73 viruses (Table 2).

In addition to the data shown in Table 1 ferret antiserum to 30c was tested in HI tests with a collection of 22 influenza strains isolated during 1973 in several

Table 3. *Serological responses to three vaccines measured by haemagglutination-inhibition*

Vaccine given	Geometric mean titres against indicated antigen		
	A/Hanover/63/73*	30c	B/Hong Kong/8/73
A/Port Chalmers/73	31/217† 7 × ‡	12/96 8 ×	11/12 1.1 ×
30c	39/100 2.6 ×	15/92 6.1 ×	13/11 0.85 ×
B/Hong Kong/8/73	36/46 1.3 ×	18/25 1.4 ×	11/55 5 ×

* This serotype is antigenically close to A/Port Chalmers/1/73 and A/Finland/4/74.

† Before vaccination/after vaccination.

‡ Rise in mean titre.

countries (Australia, Central African Republic, France, New Zealand, UK). Although these strains were antigenically closely related to A/Port Chalmers/73 they showed some evidence of antigenic 'drift' away from it. Ferret serum to 30c (homologous HI titre 960) gave HI titres in the range 40 to 120 with these strains. Thus the findings did not support the claim (Hannoun 1973) that 30c virus is a prospective vaccine strain which would show close antigenic affinity to future influenza virus variants.

The antigenic relations between 30c and naturally occurring variants of A/Hong Kong/68 virus were also examined by immuno-double-diffusion (IDD) and single radial-diffusion (SRD) using methods as described by Schild *et al.* (1974). IDD tests indicated that the haemagglutinin antigen of 30c was different from that of the prototype A/Hong Kong/68 virus but not identical with natural variants isolated in 1972, 1973 and 1974. In SRD tests the haemagglutinin and neuraminidase antigens of 30c were indistinguishable from those of A/HK/68.

Response to vaccine

The results in the 68 volunteers from Sheffield and the 64 volunteers from Berkhamsted were similar and have therefore been combined for tabulation. The distribution of antibody titres in the experimental groups were similar (Table 3). There was no stimulation of HI antibodies to influenza A viruses by B vaccine or *vice versa*, but there was some stimulation of heterologous HI antibodies by the A vaccines. Furthermore while the 1973 strain stimulated good antibodies against the homologous and the earlier type, the 30c strain induced worse responses against the 1973 serotype than against the homologous virus. Both the mean titres and the rises in titres indicated that the A/PC/73 was likely to give better immunity than 30c against the current serotype. They did not however indicate whether this amount of immune response would give any measurable amount of protection. The sera were also tested by single radial diffusion and similar antibody responses were observed.

Challenge with live virus

The virus did not infect all volunteers given the B/HK/8/73 vaccine since they were unselected and a number possessed homologous antibody before they were inoculated. However, as judged both by virus isolation and antibody response,

Table 4. *Inoculation of volunteers with WLR 105: laboratory results*

Vaccine given	Proportion of volunteers with indicated result		
	Virus isolated	Antibody rise	infected†
A/Port Chalmers/73	0/40*	2/36	2/41 (5%)
30c	1/46	9/47	10/49 (20%)
B/Hong Kong/8/73	8/42	16/39	18/42 (43%)

* No. positive/no. tested.

† Virus isolated or antibody rise or both.

Note that a few volunteers failed to provide serum samples and a few virus isolations were technically unsatisfactory so the numbers do not exactly correspond.

Table 5. *Inoculation of volunteers with WRL 105: clinical results*

Vaccine given	Upper respiratory symptoms within 4 days after vaccine	Constitutional symptoms
A/Port Chalmers/73	4/36	17/36
30c	11/44	16/44
B/Hong Kong/8/73	8/39	19/39

the 30c vaccine produced some immunity to challenge and the A/PC/73 vaccine produced even more (Table 4).

The virus produced mild symptoms in a proportion of volunteers but they were difficult to differentiate from the high background level of respiratory complaints in the present study. Thus, for example 76% of the volunteers recorded some clinical findings. These are summarized in Table 5. The volunteers who had received A/PC/73 vaccine had a lower incidence of upper respiratory symptoms than the two other groups. However, this is of doubtful significance since further analysis showed that in the whole group 23% of the 30 infected subjects had such symptoms while 18% of those who were uninfected did so – clearly relatively few of the symptoms were induced by the virus.

It was concluded that the homologous virus vaccine had given good protection against infection but that the 30c vaccine containing an 'earlier' haemagglutinin and a still 'earlier' neuraminidase had given some protection also.

DISCUSSION

The haemagglutinin and neuraminidase of 30c were only distantly related to the antigens of the '73 and '74 serotypes when examined by means of animal immune sera. Nevertheless the 30c induced antibody in man which reacted with these strains and gave significant protection against one of them. This supports the view that the circulation of antibody which can be detected by HI tests provides a useful index of protection against that strain (Hobson, Curry, Beare & Ward-Gardener, 1972). However it would be desirable to characterize antibodies further by methods such as immunodiffusion (Laver, Downie & Webster, 1974; Virelizier, Postlethwaite, Schild & Allison, 1974) to see whether they are 'cross-reacting' or

not and by separating antibodies of high and low avidity (Lecomte & Tyrrell, 1976). Furthermore protection experiments should be repeated using vaccines containing naturally occurring serotypes as well as those produced by laboratory manipulation. The duration of immunity should be studied also – this heterologous immunity might last only a short period. Although the 30c vaccine did not achieve its objective, namely of acting as a ‘senior’ strain and conferring good immunity against a range of future antigen variants, its use did contribute to knowledge of the protective effect of ‘outdated’ influenza vaccine strains.

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