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Immunity to attenuated influenza virus WRL 105 infection induced by heterologous, inactivated influenza A virus vaccines

Report to the Medical Research Council Committee on Influenza and other Respiratory Virus Vaccines

By C. W. POTTER and R. JENNINGS

Department of Virology, University of Sheffield Medical School, Beech Hill Road, Sheffield S10 2RX

K. NICHOLSON and D. A. J. TYRRELL

Division of Communicable Diseases, Medical Research Council, Clinical Research Centre, Watford Road, Harrow

and K. G. DICKINSON

Student Health Centre, University of Birmingham, Elms Road, North Campus, Birmingham B15 2SE

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SUMMARY

Groups of student volunteers were immunized with one of five different inactivated influenza virus vaccines. The concentration of virus in the various vaccines differed by both the international unitage test and by the concentration of haemagglutinin, as measured by the single radial diffusion test; the results of the two methods of standardization showed no correlation. The serum HI response to immunization was variable; volunteers given A/England/72 showed a 16·6-fold increase in homologous serum antibody titre whilst volunteers given A/Hong Kong/68 vaccine showed a 4·2-fold increase. The variable response of volunteers to immunization could not be explained by the varied concentration of virus in the vaccines, as measured by either test, the titres of serum HI antibody present before immunization, or a combination of these two factors.

The ability to infect volunteers with WRL 105 virus 4 weeks after immunization with heterologous, inactivated virus vaccine was directly related to the degree of cross-reactivity between the haemagglutinins of this vaccine virus and WRL 105 virus. Thus, the greatest number of infections by the challenge virus were seen in volunteers given A/Hong Kong/68 vaccine, less were observed in volunteers given A/England/72 vaccine, and least were found in groups given A/Port Chalmers/73 or A/Scotland/74 vaccine. However, compared with the incidence of infection in volunteers given B/Hong Kong/73 vaccine, all the heterologous influenza A vaccine gave some immunity to challenge infection.

INTRODUCTION

Immunization with inactivated influenza virus vaccines during interpandemic periods has been shown to induce immunity against infection by homologous virus in 60–90% of vaccines (Beare, Hobson, Reed & Tyrrell, 1968; Foy *et al.* 1971; Foy, Cooney & McMahan, 1973; Hobson, 1975); the results varied according to the nature and dose of vaccine, the amount of antibody present before immunization, whether natural or attenuated virus challenge was used, and the methods used to assess infection by the challenge virus (Hobson, 1975). In addition, inactivated virus vaccines have been reported to induce immunity against heterologous influenza virus infections. Thus, several studies have reported that immunization with inactivated A/Hong Kong/68 vaccine gave protection against infection by influenza virus A/England/72 (Pereira *et al.* 1972; Stiver, Graves, Eickhoff & Meiklejohn, 1973; Haigh & Howell, 1973; Hoskins *et al.* 1973; Ruben, Johnston & Streiff, 1974; Potter, Jennings, McLaren & Clark, 1975*b*). This finding was probably due to the cross-reactivity of the haemagglutinins of the vaccine and infecting viruses (Schild *et al.* 1974).

The frequency of significant antigenic drift of influenza A (H3N2) viruses (Schild *et al.* 1974) has required that vaccine be prepared from a new virus variant each year. However, this requirement may not be necessary when the old vaccine virus can induce protective titres of cross-reacting antibody against a recent epidemic strain; the degree of cross-reactivity required between vaccine virus and infecting virus to be effective for cross-immunity is not known. In the present study, volunteers were inoculated with one of five inactivated influenza virus vaccines; 4 weeks later all the volunteers were challenged with attenuated influenza virus WRL 105 (Morris, Freestone, Stealey & Oliver, 1975). The serum HI antibody response of the volunteers to both the vaccine virus and to the challenge virus was measured, together with the antibody response to heterologous influenza viruses. Infection by the challenge virus was then related to the antibody response to immunization, since the serum HI antibody titre has been reported to correlate directly with immunity to challenge infection (Meiklejohn, Kempe, Tahalman & Lennette, 1952; Hobson, Beare & Ward-Gardner, 1972).

MATERIALS AND METHODS

Influenza Viruses

Influenza viruses A/Scotland/74 (H3N2), A/Port Chalmers/73 (H3N2), A/England/72 (H3N2), A/Hong Kong/68 (H3N2), A/Victoria/75 (H3N2) and B/Hong Kong/73 were obtained from Dr G. C. Schild, National Institute of Medical Research, Mill Hill, London. Virus pools were prepared from the allantoic fluids of 10-day embryonated eggs inoculated with 0·2 ml of a $10^{-2\cdot0}$ dilution of seed virus. After 48 h incubation at 33 °C, the allantoic fluids were harvested and stored at −80 °C.

Monovalent, inactivated virus vaccines were prepared from influenza viruses A/Scotland/74, A/Port Chalmers/73, A/England/72, A/Hong Kong/68 and B/Hong

Kong/73 by Evans Biologicals Ltd. The vaccines were standardized to contain 400 international units (i.u.) per 0·5 ml.

Attenuated influenza virus WRL 105 (H3N2), a recombinant of influenza A/Finland/74 (H3N2) and A/Okuda/57 (H2N2), was kindly supplied by Dr D. S. Freestone, Wellcome Research Laboratories, Beckenham, Kent; the properties of this vaccine virus have been described previously (Morris *et al.* 1975; Moffat, Stealey, Freestone & MacDonald, 1976; Freestone *et al.* 1976). The vaccine virus was reconstituted from freeze-dried ampoules with sterile distilled water at 4 °C and was used within 1 h of preparation. Each volunteer was inoculated with 0·5 ml of virus containing $10^{7\cdot0}$ EID 50, as described previously (Potter *et al.* 1975*a*).

Experimental design

A total of 138 students at the Universities of Birmingham and Sheffield volunteered for the study; all were healthy with no known allergy to eggs or egg products. A blood sample was obtained from each volunteer, after which they were inoculated subcutaneously with 400 i.u. of one of the five influenza vaccines in random order. Four weeks after immunization, a second blood sample was collected and each volunteer was inoculated with attenuated influenza virus WRL 105. The virus was given by intranasal drops to volunteers lying on a couch with the head hyperextended; after inoculation the volunteers remained horizontal for a minute. Throat swabs, taken into medium '199' containing 2·0% bovine serum albumin and antibiotics, were obtained 3 days after virus inoculation and stored at −80 °C, and a further blood sample was obtained 18 days later.

Virus isolation

Throat swabs were thawed from −80 °C, after 3–4 weeks storage, and 0·2 ml of the containing medium inoculated by the allantoic route into each of two 10-day embryonated eggs. The eggs were incubated at 33 °C for 48 h, after which time the allantoic fluids were tested for virus by haemagglutination with fowl cells.

Serological tests

Haemagglutination-inhibition (HI) tests. These were carried out by a modification of the microtitre method (Sever, 1962), as described previously (Potter *et al.* 1975*a*). Before test, the sera were treated with cholera filtrate (Burroughs Wellcome Ltd.) for 18 h at 37 °C, and subsequently heated for 30 min at 56 °C.

Neuraminidase-inhibition (NI) tests. NI antibody tests were performed by an automated method, based on the Standard World Health Organization technique (Aymard-Henry *et al.* 1973), as described previously (Bevan, Furminger & Smith, 1975). The source of neuraminidase for all the antibody titration was purified influenza virus A/Scotland/74 disrupted with 1·0% Triton (Bevan *et al.* 1975).

Complement-fixation (CF) tests. These tests were carried out by the method of Bradstreet & Taylor (1962) using 2·5 MHD 50 of guinea-pig complement and A/Port Chalmers/73 virus soluble antigen extracted from the chorio-allantoic membranes of virus-infected eggs. The tests were incubated overnight at 4 °C before the addition of sensitized sheep cells, and the antibody titres were taken as the highest serum dilution which gave 75% or more fixation of complement.

RESULTS

Standardization of virus vaccines

All the inactivated vaccines used in the present study were originally standardized to contain 400 i.u./0·5 ml. Several months later the vaccine concentrations were re-tested, and were found to give results in good agreement with the initial tests. The results are shown in Table 1. On retesting, the concentration of the five vaccines varied from 850 i.u./0·5 ml to 222 i.u./0·5 ml; thus, the concentrations were within approximately 2-fold of the original estimation. Comparison of these results with the haemagglutinin (HA) concentration, as measured by the single radial diffusion tests (Schild, Wood & Newman, 1975) gave results which did not correlate with i.u. content. Thus, the B/Hong Kong/73 vaccine which had the highest concentration of virus by the second i.u. test, contained the lowest concentration of HA by the single radial diffusion tests, and the A/Port Chalmers/73 vaccine which had the lowest content of antigen by the i.u. test had the highest concentration of HA by the single radial diffusion test (Table 1).

Table 1. *Standardization of virus vaccine*

	Virus concentration in vaccine		
Vaccine virus	i.u./0·5 ml 1st testing	i.u./0·5 ml 2nd testing	μg HA (per 0·5 ml)
A/Scotland/74	400	380	16·3
A/Port Chalmers/73	400	222	35·2
A/England/72	400	599	32·9
A/Hong Kong/68	400	395	17·1
B/Hong Kong/73	400	850	11·3

Homologous antibody response to immunization

The serum HI and NI response to immunization with the various inactivated influenza vaccines is shown in Table 2. The greatest serum HI antibody response was found in volunteers given A/England/72 vaccine, where the geometric mean titres increased 16·6-fold to 812·0, and the lowest response was in volunteers given A/Hong Kong/68 vaccine where the gmt increased 4·2-fold. The antibody response to immunization with influenza virus A/Scotland/74 vaccine was similar to that obtained for the A/England/72 vaccine, whilst the response to A/Port Chalmers/73 vaccine was similar to that obtained for A/Hong Kong/68 vaccine (Table 2). The differences in the response to immunization with the various influenza virus vaccines may have been due to the different titres of pre-immunization antibody to the vaccine virus. Thus, the mean titre of serum HI antibody to A/England/72 virus before immunization was 48·8 for volunteers given this vaccine, whereas the mean pre-immunization titre of volunteers given A/Scotland/74 was 15 to this virus. However, a comparison of the antibody responses to immunization with the pre-immunization antibody titres showed no clear correlation. In addition, no clear relation was seen between the serum HI antibody response to immunization and the different concentrations of virus in the vaccines, by either international

Table 2. *Serum HI and NI antibody responses of volunteers to immunization with inactivated influenza virus vaccines*

		Serum HI antibody response			Serum NI antibody response		
Inactivated vaccine given (400 i.u.)	No. tested	No. 4-fold increase (%)	Pre-immune titre, gmt	Post-immune titre, gmt (fold increase)	No. 4-fold increase (%)	Pre-immune titre, gmt	Post-immune titre, gmt (fold increase)
A/Scotland/74	27	22 (81)	15·0	238·8 (15·9)	16 (59)	19·3	71·6 (3·7)
A/Port Chalmers/73	25	14 (56)	39·1	256·3 (6·6)	16 (64)	16·5	96·9 (5·9)
A/England/72	26	24 (92)	48·8	812·0 (16·6)	6 (23)	12·0	26·3 (2·2)
A/Hong Kong/68	25	11 (44)	81·2	340·0 (4·2)	3 (12)	12·6	18·3 (1·5)
B/Hong Kong/73	31	18 (58)	6·1	28·3 (4·6)	– (0)	14·7	14·5 (1·0)

Table 3. *Serum HI antibody response of volunteers to A/Scotland/74 virus following immunization with various inactivated influenza virus vaccines*

		Serum HI antibody response to A/Scotland/74 virus						
		HI titre post-immune						
Vaccine given (400 i.u.)	No. tested	< 20	20–40	60–120	> 120	No. 4-fold increase (%)	Pre-immune gmt	Post-immune gmt (fold increase)
A/Scotland/74	27	1	1	3	22	22 (81)	15·0	239 (15·9)
A/Port Chalmers/73	25	2	2	10	11	13 (52)	20·7	119 (5·7)
A/England/72	26	4	7	7	8	15 (58)	10·8	63 (5·8)
A/Hong Kong/68	25	14	2	5	4	4 (16)	11·6	25 (2·1)
B/Hong Kong/73	31	19	5	6	1	— (0)	14·7	15 (1·0)

units or HA content (Table 1), or by a combination of vaccine concentration and the pre-immune serum HI antibody titre (Tables 1 and 2).

The changes in serum antibody titre to the neuraminidase component of the vaccines was determined using neuraminidase from influenza virus A/Scotland/74; an identical neuraminidase is present in A/Port Chalmers/73 virus, but the A/England/72 virus neuraminidase is distinguishable whilst the A/Hong Kong/68 neuraminidase is even less related to the A/Scotland/74 enzyme (Stuart-Harris & Schild, 1976). Comparison of the serum antibody response to the neuraminidase antigen of the vaccines must take account of the degree of heterogeneity between the vaccine virus neuraminidase and the enzyme used in the NI antibody tests. The results are shown in Table 2. The greatest NI antibody response was observed in volunteers given the A/Port Chalmers/73 vaccine, where the antibody titre for the group increased 5·9-fold to a gmt of 96·9; this was expected since homologous neuraminidase was used for the antibody assay. The response of volunteers given A/Scotland/74 vaccine was to increase the NI antibody titre 3·7-fold to a gmt of 71·6. Since homologous neuraminidase was used in the assay of NI antibody in volunteers given A/Scotland/74 vaccine and A/Port Chalmers/73 vaccine, a comparison can be made for these two groups; thus, A/Port Chalmers vaccine induced a better NI antibody response than A/Scotland vaccine, whilst the antibody response to the HA component of the vaccines showed the reverse (Table 1).

Serum antibody response to influenza virus A/Scotland/74

The serum HI antibody response of volunteers to influenza virus A/Scotland/74 after immunization with the different inactivated influenza virus (H3N2) vaccines is shown in Table 3. The titres of serum HI antibody before immunization were very similar in the five groups; the geometric mean titre (gmt) varied from 10·8 for volunteers given A/England/72 vaccine to 20·7 for those given A/Port Chalmers/73 vaccine. In addition, the distribution of serum HI antibody titres to A/Scotland/74 before immunization was similar in all five groups of vaccinees, and showed the same range of titres as shown after immunization for vaccinees given B/Hong Kong/73 (Table 3). The post-immunization titres of antibody to A/Scotland/74 virus varied considerably for the five vaccine groups. The best heterologous HI antibody response was found in the group immunized with influenza virus A/Port Chalmers/73, and the worst was with A/Hong Kong/68; the haemagglutinins of these viruses are respectively, the most and least cross-reactive haemagglutinins with A/Scotland/74 virus used in the present study (Schild *et al.* 1974).

The distribution of serum HI antibody titres to influenza virus A/Scotland/74 after immunization with the various vaccines is also shown in Table 3. The antibody titres are placed in four groups to correlate with the titres which have been reported to give immunity to challenge virus infection (Meiklejohn *et al.* 1952; Hobson *et al.* 1972); these studies have shown that subjects with serum HI antibody titres of $\geqslant 40$ exhibited $\geqslant 50\%$ immunity to virus infection, whilst titres below this level gave $< 50\%$ immunity. Of 27 volunteers given A/Scotland vaccine only two (7%) had serum HI antibody titres of $\leqslant 40$ to the homologous virus after immunization, and therefore may be presumed to be susceptible to the

Table 4. *Changes in serum HI antibody titre to heterologous influenza virus following immunization with various influenza A vaccines*

Vaccine given (400 i.u.)	No. tested	Serum HI antibody response (gmt) to influenza virus				
		A/Vic/75	A/Scot/74	A/PC/73	A/Eng/72	A/HK/68
A/Scot/74	27	8·8–22·3	**15·0–238·8**	26·9–148·7	32·7–201·2	48·8–170·0
A/PC/73	25	20·1–46·6	20·7–118·6	**39·1–256·3**	119·2–423·8	92·4–176·8
A/Eng/72	26	9·9–26·2	10·8–63·1	15·1–119·9	**48·8–812·0**	42·4–270·5
A/HK/68	25	9·8–13·7	11·6–24·7	22·1–55·1	39·4–115·7	**81·2–340·0**

Table 5. *Infection by WRL-105 virus in volunteers previously immunized with various inactivated influenza vaccines*

Vaccine given	No. tested	HI antibody titre*		Infection with WRL 105		Total (%)
		< 20–30	> 40	Virus isolation	Significant HI/CF antibody response	
A/Scotland/74	27	2	25	1	—	1 (4)
A/Port Chalmers/73	25	2	23	—	1	1 (4)
A/England/72	26	8	18	1	2	2 (8)
A/Hong Kong/68	25	16	9	3	5	7 (28)
B/Hong/Kong/73	31	24	7	4	14	15 (48)

* Serum HI antibody to influenza virus A/Finland/74 after immunization and before challenge infection with WRL 105.

challenge virus; this virus was antigenically very similar to A/Scotland/74 virus. The results for the volunteers in the other groups were 4 of 25 (16%), 11 of 26 (42%) and 16 of 25 (64%) for volunteers given inactivated influenza vaccines A/Port Chalmers/73, A/England/72 and A/Hong Kong/68, respectively.

Serum HI antibody response to heterologous influenza A viruses

Serum specimens from all the volunteers were tested for HI antibody to five influenza A viruses. The results are shown in Table 4; the data from volunteers given influenza B/Hong Kong/73 vaccine are not included, since the antibody titres of these volunteers to influenza A viruses was not significantly changed by immunization. The results show that the post-immunization HI antibody titre (gmt) to influenza virus A/Scotland/74, A/Port Chalmers/73, A/England/72 and A/Hong Kong/68 was greatest for the volunteers given homologous vaccine, and the heterologous antibody response was directly related to the degree of cross-reactivity between the test and vaccine viruses. These results are presented in the form of fold-increases in serum HI antibody titre (gmt) in Fig. 1; again, the magnitude of the serum HI antibody response to immunization was directly related to the degree of cross-reactivity between the vaccine and the test virus.

Response of volunteers to challenge infection

Four weeks after immunization, all the volunteers were inoculated intranasally with $10^{7\cdot0}$ EID 50 of attenuated influenza virus WRL 105 in an 0·5 ml volume. Infection by the challenge virus was determined by either virus recovery from throat swabs taken 3 days after infection, or by a 4-fold or greater increase in serum HI antibody response to A/Finland/74 virus; rises in antibody were determined by both the HI and CF test, and a 4-fold or greater increase in antibody by either test was taken as proof of virus infection (Potter *et al.* 1977). The results are shown in Table 5. Evidence of infection was obtained for one volunteer previously immunized with A/Scotland/74 and for one volunteer immunized with A/Port Chalmers/73 vaccine. Thus, evidence of infection by WRL 105 virus was approximately the same for volunteers given A/Scotland/74 or A/Port Chalmers/73 vaccines (Table 5). For volunteers given A/England/72, A/Hong Kong/68 and B/Hong Kong/73, evidence of virus infection was obtained in 2 of 25 (8%), 7 of 25 (28%) and 15 of 24 (48%) volunteers, respectively. Compared with the results obtained for volunteers given B/Hong Kong/73 vaccine, which served as a control group, measurable immunity to challenge infection with WRL 105 virus was found after immunization with all the influenza A vaccines tested. However, the degree of immunity was variable; the most solid immunity was induced by influenza A/Scotland/74 and A/Port Chalmers/73 vaccines, and the least significant results were found for volunteers immunized with A/Hong Kong/68 vaccine (Table 5).

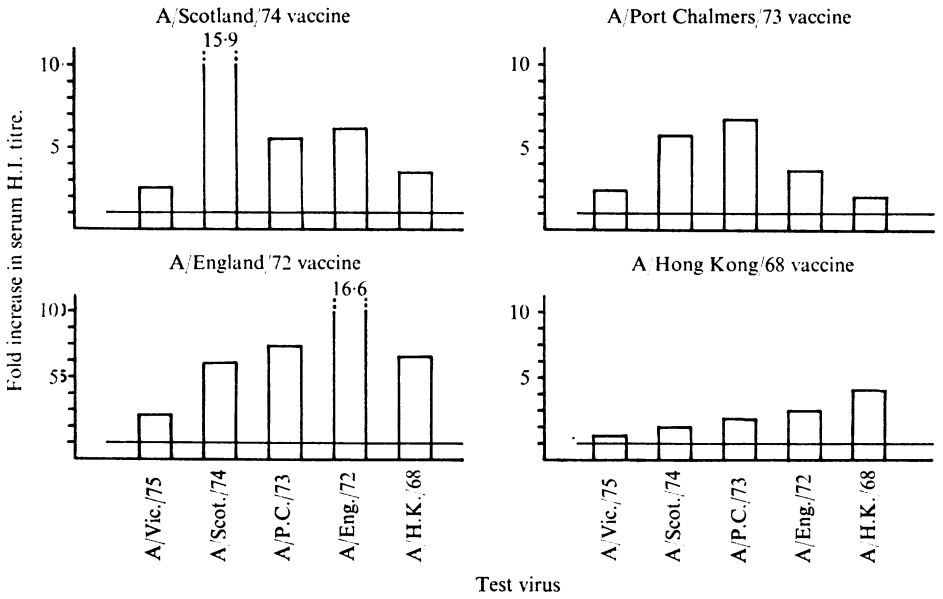

Fig. 1. Heterologous serum HI antibody response to immunization.

Table 6. *Relationship of serum HI antibody to A/Finland/74 virus and infection by WRL 105 virus*

	Titres of serum HI antibody to A/Finland/74 virus						
	< 10	10–20	30–40	60–80	120–160	⩾240	Total
No. sera	22	25	10	16	31	30	134
No. virus infections	11	12	1	1	—	1	26
% infections	50	48	10	6·25	—	3·3	19·4

Relation of serum HI antibody to infection with WRL 105 virus

As a result of immunization with the various inactivated influenza A vaccines, serum HI antibody to A/Finland/74 was induced by all the influenza A vaccines used; however, the titres of antibody produced were related to the degree of cross-reactivity between the vaccine virus and A/Finland/74 virus. The relation between post-immunization titres of antibody to A/Finland/74 virus and infection by WRL 105 virus is shown in Table 6. Infection with WRL 105 virus was directly related to the serum HI antibody titre to the challenge virus. Thus, 23 of 47 (50%) of volunteers with serum HI antibody titres of $\leqslant 20$, 2 of 26 (7·7%) volunteers with titres of 30–80 and 1 of 61 (1·6%) of volunteers with titres of > 80 were infected with WRL 105 virus.

DISCUSSION

Significant antigenic drift of influenza H3N2 viruses has occurred regularly during the past few years (Schild *et al.* 1974), and this has meant that influenza virus vaccines have had to be changed yearly to include the current infecting virus strains. Because influenza viruses have shown rapid antigenic drift, immunization has been carried out in some years with virus strains which were antigenically distinct from the current infecting virus. However, the results of several studies have shown that one heterologous vaccine induced significant immunity; thus, immunization with inactivated A/Hong Kong/68 vaccine conferred significant immunity against subsequent infection by A/England/72 virus (Pereira *et al.* 1972; Stiver *et al.* 1973; Ruben *et al.* 1974). The present study was carried out to investigate further the extent of immunity induced by heterologous inactivated influenza virus vaccines. In addition, all the published examples of heterologous immunity to influenza virus describe immunity to A/England/72 infection after immunization with A/Hong Kong/68 vaccine; this observation could relate to these two viruses only, and not find parallels with other influenza virus strains.

The response of volunteers to the different influenza A vaccines given varied widely. The best serum HI antibody response was to A/England/72 vaccine where the gmt increased 16·6–fold, and the worst was for volunteers given A/Hong Kong/68 vaccine which induced a 4·2-fold increase in serum antibody. The other results fell between these two extremes. When the concentration of virus in the vaccines was re-determined using the measurement of international units, the vaccines were found to be of similar strength. However, measurements of the concentration of HA using the single radial diffusion method (Schild *et al.* 1975) indicated that the vaccines differed in antigen content, and that there were large differences between the results obtained in the two tests. It is not possible from the present results to comment on which method of standardization is the best, since the immune response of volunteers given the different vaccines did not correlate with differences in antigen content of the vaccines as measured by either test. In addition, the antibody response to immunization did not correlate with the amounts of pre-immunization antibody, either directly or with reference to the varying concentrations of antigen in the vaccines. This suggests that the antigenic potency of influenza virus haemagglutinin may vary from strain to strain, and this conclusion is supported by the results of animal studies (Potter & Jennings, 1976).

Although the vaccines varied in potency, the results indicate that the heterologous HI antibody response to immunization was directly related to the degree of cross-reactivity between the vaccine virus and the virus antigen used in the HI test. Specifically, the serum HI antibody titres for A/Scotland/74 were greatest for the homologous virus vaccine, less for A/Port Chalmers/73 and A/England/72 vaccines and least for A/Hong Kong/68 vaccine. The results of challenge infection showed that immunity to WRL 105 virus, which resembles most closely A/Scotland/74 virus, was greatest after immunization with A/Scotland/74 and A/Port Chalmers/73 vaccine, less after A/England/72 vaccine and least after A/Hong Kong vaccine. Thus, immunity to challenge infection was directly related to the titre

of serum HI antibody to the challenge virus. In addition, the relation between immunity to challenge infection and the titre of homologous serum HI antibody was apparent for both homologous and heterologous virus vaccines.

The present results indicate that immunization with inactivated A/Port Chalmers/73 or A/Scotland/74 virus vaccines induced protection against influenza by WRL 105 virus. This result, together with the published reports of immunity to A/England/72 infection after immunization with A/Hong Kong/68 vaccine, indicate that immunity can be accomplished by immunization with heterologous, cross-reacting virus vaccine. However, heterologous vaccines do not induce antibody to the same titre as homologous virus vaccine, and it would be expected, therefore, that the immunity induced by the former would be shorter-lived. Since the influenza A (H3N2) viruses have shown antigenic drift each year, the heterologous immunity could persist long enough. Thus, A/Port Chalmers/73 vaccine could have been as effective as homologous virus vaccine for immunization against A/Scotland/74 infection, and the shorter duration of immunity would not have been a practical problem since the A/Victoria/75 virus caused epidemic infection the following year.

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