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A comparison of monovalent Hong Kong influenza virus vaccine with vaccines containing only pre-1968 Asian strains in adult volunteers

A report to the Medical Research Council Committee on Influenza and other Respiratory Virus Vaccines*

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SUMMARY

A total of 1601 adult industrial workers were vaccinated with either monovalent inactivated vaccine of the Hong Kong strain of influenza A virus, or with polyvalent vaccine containing only pre-1968 Asian viruses. Serological investigations on a random sample of volunteers showed that 53/56 (95%) given Hong Kong vaccine developed a significant rise in specific haemagglutination-inhibiting antibody; final titres were 1/48 or greater in 39 (70%) and the GMT (geometric mean titre) was 96·5. After polyvalent Asian vaccine, 40/67 (60%) also produced antibody against Hong Kong virus, but only 21 (31%) had final titres of 1/48 or above, and the GMT rose only to 14·1. An intranasal spray of the Hong Kong vaccine in addition to injected Asian vaccine gave no additional increase in antibody.

Each type of vaccine stimulated a recall of pre-existing antibody against Asian viruses. The possible significance of heterologous responses to the two vaccines is discussed.

The incidence of clinical influenza in the trial population was sporadic, and the infection rates were too low to allow any accurate estimate of the protective efficiency of the two vaccines.

INTRODUCTION

In the summer of 1968 the epidemic of influenza which began in Hong Kong spread rapidly through populations in which protective antibodies against Asian (A 2) strains of influenza virus were already widely distributed. The causative virus

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isolated from outbreaks in many parts of the world, including Britain, was an influenza type A strain with marked antigenic differences from Asian or any other foregoing epidemic subtype. In laboratory tests with animal antisera, the haemagglutinin (HA) of the Hong Kong virus and Asian viruses showed little cross-reactivity, but the viral enzyme neuraminidase was antigenically similar in both kinds of virus. Hence, Hong Kong (HK) strains have been regarded as an extreme variation within the A 2 family of viruses rather than a new subtype A 3 (Coleman *et al.* 1968). However, all available evidence from previous epidemics and from influenza vaccine trials (reviewed by Hobson, 1967) suggests that, although antibody directed against the viral HA is correlated with immunity to infection, haemagglutination-inhibiting (HI) antibody against one subtype of influenza A confers no protection against strains with different HA antigens. Thus it was urgently necessary to determine whether an entirely new vaccine formulation containing HK strains would be essential to limit future epidemic risks, or whether already available commercial vaccines containing A 2 strains isolated in 1964–6 could be relied upon to stimulate antibody effective against HK virus. In human adult populations with a broad previous experience of the various influenza A subtypes, it seemed possible that A 2 vaccines might invoke a wider range of immunity than in experimental animals.

Accordingly, the Medical Research Council initiated a series of clinical trials in which the serological responses of adult volunteers to either commercial polyvalent (CP) vaccine of A2+B viruses or monovalent HK vaccine could be compared. and their protective effect against infection be evaluated in a double-blind procedure. In the present trial of this series, 1601 adult volunteers from the management and industrial personnel of the Mond Division of Imperial Chemical Industries Ltd. were given one or other of the vaccines by intramuscular injection; approximately half of the volunteers given CP vaccine also received an intranasal spray of inactivated HK virus to test the possibility that local stimulation by antigen might induce a higher degree of protection against infection than does parenteral inoculation (Waldman *et al.* 1968).

The effectiveness of each vaccine in producing or enhancing circulating HA antibody against each type of virus was estimated in approximately 11% of the population. Clinical histories were taken from all volunteers absent through sickness over the period 18 November 1968 to 30 April 1969, and each respiratory illness was classified, before the double-blind vaccine code was broken, into the various clinical syndromes characterized by Stuart-Harris (1965). In addition, all sickness absence due to respiratory disease in the four main factories from which the trial population was drawn, with a total payroll of 3580, was recorded from week 45 of 1968 (2 weeks before vaccination of the trial group) to week 20 of 1969.

MATERIALS AND METHODS

Volunteers

A total of 1601 adult workers of both sexes, with an age range of 16–64 years, volunteered to enter the trial and were allocated to one of three groups by random

numbers. The age and sex distribution, and the scatter of volunteers through the various factory sites, were similar in each group.

Vaccines

Formalin-inactivated influenza virus vaccines were obtained from British Drug Houses Ltd. CP, the polyvalent vaccine (Admune aqueous), contained, per 1·0 ml. dose,

	HA units
A2/Eng/12/64	3000
A2/Eng/76/66	6000
B/Eng/5/66	3000
B/Swiss/265/67	3000

HK vaccine (Admune Mono 68) contained 7000 HA units of A2/Eng/344/68 per 1·0 ml. dose.

Individual doses of the vaccines for intramuscular injection (i.m.) and pools of HK vaccine or sterile normal saline for intranasal instillation (i.n.) by fine hand-spray set to deliver metered doses of 1·0 ml. were prepared and letter-coded in another laboratory, and a double-blind procedure was maintained throughout the trial.

Each volunteer received both an injection and an intranasal spray as follows:

Group	Intranasal	I.m.
A	Saline	CP
B	HK	CP
C	Saline	HK

Serological procedures

Paired samples of sera were taken on the day of vaccination and 14 days later from 183 volunteers previously selected by random numbers. There were no significant differences in the number selected from groups A, B and C, in the age and sex distribution in these groups or in the distribution of prevaccine antibody titres against HK or earlier A2 viruses.

The procedure for HI tests differed from the standard W.H.O. method (Report, 1953) only in certain details; sera, pretreated with cholera filtrate (Philips Duphar) were diluted in M/50 phosphate buffered saline pH 7·6 and incubated with 4 HA units of non-inactivated virus for 1 hr. at room temperature before adding a 1% suspension of human group O Rh-negative erythrocytes.

Preliminary HI tests with A2/Eng/12/64 and A2/Eng/76/66 gave identical titres with either human and immune rabbit sera. Hence, only the former strain was used in the main titrations shown in the text.

The original seed cultures of A2/Eng/12/64 (A64 virus) and A2/Eng/344/68 (HK virus) from which the trial vaccines had been prepared were kindly provided by Dr D. C. Breeze, Evans Medical Ltd., Speke, Liverpool. Eleven-day chick embryos were inoculated with 10^4 egg infective doses (EID 50) of seed virus, and infected allantoic fluids were harvested after 48 hr. incubation at 36° C. A single working pool of each strain was used throughout.

Rabbit antisera were prepared by six serial intravenous injections of seed cultures of the test viruses at 3 to 4-day intervals. Neutralization tests were performed by incubating serial dilutions of antisera with 10^3 EID 50 of virus for 1 hr. at room temperature before inoculating each mixture into 4–6 eleven-day chick embryos. Spot HA tests on each egg were made after 48 hr. incubation at 36° C.

Clinical assessment

All persons with sickness absence were seen by one of us (C.P.C.) and the type of illness was categorized as indicated above. Where possible, paired sera were obtained in the acute stage of the illness and 14 days later. Throat swabs were taken for attempted virus isolation by courtesy of Dr J. O'H. Tobin, Public Health Laboratory, Manchester.

RESULTS

Serological investigations

Titrations with specific animal antisera

Hyperimmune rabbit sera prepared against A 64 and HK viruses were titrated with each virus in HI tests and in neutralization tests in chick embryos. The results (Table 1) confirm the marked antigenic differences between these strains: less than 5% cross-reactivity was shown by either antiserum against the heterologous virus.

Table 1. *The activity of specific animal antisera on A2/Eng/12/64 (A64 virus) and A2/Eng/344/68 (HK virus)*

Rabbit antiserum	HI titres		Neutralization titres	
	HK virus	A 64 virus	HK virus	A 64 virus
Anti HK	3072	96	2560	100
Anti A 64	96	3072	30	3200

Titrations with human sera

Despite the specific behaviour of HK and A 64 viruses with animal antisera, rises in HI antibody titre were detected by both agents in many volunteers, apparently regardless of the schedule of vaccination. The serological data presented below have therefore been analysed to determine the frequency and possible significance of cross-reactions induced by the two vaccines.

The production of HK antibody

Prevaccination sera from 54/183 (29%) volunteers reacted with HK virus; almost all HI titres were at 1/6 only, and were equally distributed amongst the 3 trial groups (Table 2). There was no apparent correlation between the presence of prevaccine HK antibody and prevaccine titres of A 64 antibody.

After vaccination 131/183 (71%) showed a 4-fold or greater rise in HK antibody, i.e. immune responses were not confined to those given homologous HK vaccine.

Table 2. *HI antibody titres against HK influenza virus* (A2/*Eng*/344/68) *after vaccination with homologous* (*HK*) *and heterologous* (*CP*) *influenza A virus vaccines*

Group	Vaccine schedule		No. of persons in group	No. with pre-vaccine antibody	No. with ⩾ 4-fold rise in antibody	No. with final titre ⩾ 48	Geom. mean titres	
	IN	IM					Pre-vaccine	Post-vaccine
A	Saline	CP	67	18 (27%)	40 (60%)	21 (31%)	1·8	14·1
B	HK	CP	60	19 (31%)	38 (63%)	23 (38%)	2·1	21·2
C	Saline	HK	56	17 (30%)	53 (95%)	39 (70%)	2·0	96·5

In this and subsequent tables: HI = haemagglutination-inhibition; IN = intranasal; IM = intramuscular; CP vaccine = A2/Eng/12/64 (A 64) and A2/Eng/76/66; HK vaccine = A2/Eng/344/68 (HK)

Table 3. *HI antibody titres against influenza virus A2/Eng/*12/64 *after vaccination with heterologous* (*HK*) *and homologous* (*CP*) *influenza A virus vaccines*

Group	Vaccine schedule		No. of persons in group	No. with pre-vaccine antibody	No. with ⩾ 4-fold rise in antibody	No. with final titre ⩾ 48	Geom. mean titres	
	IN	IM					Pre-vaccine	Post-vaccine
A	Saline	CP	67	60 (90%)	59 (88%)	63 (94%)	17·4	220·0
B	HK	CP	60	58 (97%)	50 (83%)	55 (92%)	23·1	215·6
C	Saline	HK	56	51 (91%)	31 (55%)	47 (84%)	24·0	108·5

Table 4. *The effect of CP and/or HK vaccines in producing homologous or heterologous HI antibody responses in individual volunteers*

Group	Vaccine schedule		No. of volunteers with ⩾ 4-fold rise in antibody after vaccine				
	IN	IM	Both HK + A 64	HK only	A 64 only	Neither	Totals
A	Saline	CP	35	5	24	3	67
B	HK	CP	33	5	17	5	60
C	Saline	HK	31	22	0	3	56
Totals			99	32	41	11	183

However, the greatest response was found in group C, where injected HK vaccine induced a 48-fold rise in geometric mean titre (GMT) overall, and the conversion rate to titres of 1/48 or greater was 70%. In group B the intranasal spray of HK vaccine given in addition to injected CP vaccine gave results little or no better than in group A given CP vaccine alone. In both groups less HK antibody was induced than in group C, e.g. the GMT rose 7·7-fold in group A and 10-fold in group B, and the conversion rate to titres of 1/48 or above was only 31% for group A and 38% for group B.

The production of A 64 antibody

Antibody against A 64 virus was found before vaccination in 169/183 (92%) volunteers (Table 3). Individual titres varied from 1/6 to 1/384 but the GMT did not differ significantly from group to group.

The recall of antibody to the Asian strain by CP vaccine was greater than by heterologous HK vaccine. In groups A and B the GMT increased by 12-fold and 9-fold respectively and more than 80% of volunteers showed a 4-fold or greater rise in titre. On the other hand, the GMT of group C increased only by 4·5-fold and only 55% showed a 4-fold or more increase in titre.

The independence of immune responses to CP and HK vaccines

The results shown in Tables 2 and 3 make it unlikely that A 64 and HK viruses were merely inducing or measuring the same antibody with differing efficiency. Further evaluation of individual responses (Table 4) confirmed that A 64 and HK antibodies were independently variable. Thus, in group C, given only HK vaccine, 22/56 (27%) showed a 4-fold or greater increase only in HK antibody, and none had an increase in A 64 antibody alone. In groups A and B, on the other hand, CP vaccine produced an isolated increase in HK antibody in only 10/127 (7·9%) volunteers, whereas A 64 antibody alone increased by 4-fold or more in 41/127 (32%). Even in the 99 volunteers who developed a simultaneous increase in both antibodies there appeared to be some specificity of response, i.e. there was no consistent relationship between the height of the HK and A 64 antibody responses. HK antibody increased by more than 16-fold in 25/35 (65%) of group C, but only in 22/64 (34%) in groups A and B.

The effect of neuraminidase antigen in HI tests

The viral neuraminidase of the HK strain is antigenically similar to that of the foregoing Asian strains (Coleman *et al.* 1968), and thus both types of vaccine used in the present trial induced, in addition to the HI antibody responses already described, a common enzyme-inhibiting antibody in each group of volunteers (G. C. Schild, personal communication).

It thus seemed possible that the haemagglutination-inhibition observed when HK virus was titrated against sera from volunteers given heterologous CP vaccine might not be the result of cross-reacting antibodies against the HA antigen, but attributable to uptake by the virus particles of common anti-neuraminidase antibody, with steric hindrance to haemagglutination.

Accordingly, pairs of sera from groups A and C which showed a high degree of cross-reactivity in HI tests were re-titrated against a genetic recombinant virus A/3/1/A (kindly provided by Dr G. C. Schild, World Influenza Centre, London) which contains only the neuraminidase antigen of HK virus, but the HA antigen of fowl plague virus. None of 20 serum pairs from group A volunteers who had

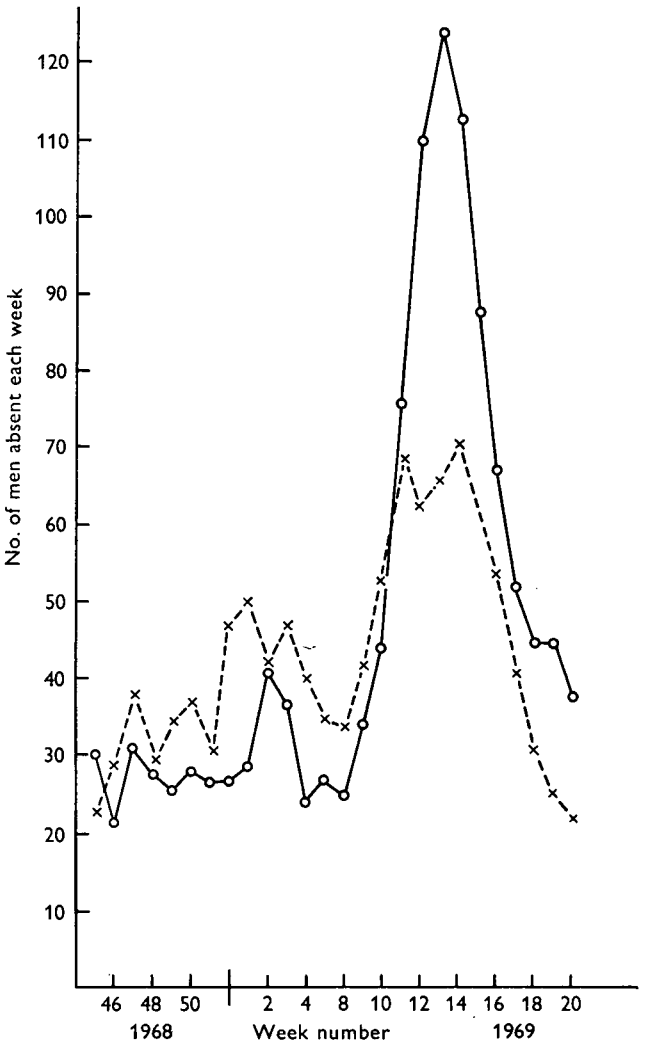

Fig. 1. Sickness absence caused by respiratory infections in the general factory population from which volunteers for influenza vaccination trial were drawn. Combined figures for four main factory sites only, for week 45, 1968, to week 20, 1969. Number on payroll, 3580. O—O, Upper respiratory tract infections; × – – ×, lower respiratory tract infections.

shown a 4- to 8-fold increase in HI titres against the heterologous HK virus gave any detectable HI with A/3/1/A virus. Similarly none of 25 serum pairs of group C volunteers who had shown 4- to 8-fold increases in HI titres to the heterologous A 64 virus showed any HI activity against the A/3/1/A recombinant.

It thus seems improbable that the heterologous antibody responses to the vaccines demonstrable in HI tests were the result of interference by common anti-neuraminidase antibody.

Clinical investigations

There were no untoward effects of the vaccination procedures. In the general factory population (Fig. 1) the incidence of both upper and lower respiratory tract infections began to rise after week 8 of 1969, and the total peak incidence was 190/3580 (5%) in week 13. This did not fall to the 8th week level again until week 18, 2 weeks before the conclusion of the trial. These weekly total figures include all types of respiratory disease, including exacerbations of chronic lower respiratory tract disabilities, and influenza was not the predominant clinical syndrome in any week. However, illnesses clinically diagnosed as influenza occurred sporadically between weeks 8 and 20 of 1969; in week 11 two strains of influenza virus antigenically identical with HK virus were isolated from men reporting sick, and in both cases a 4-fold or greater rise in HK antibody occurred over the courses of their illness. In a further 9/20 other persons with acute respiratory disease, paired sera also showed an increase in HK antibody; the first of these was in week 9 and the last in week 18, 1969. None of these confirmed cases of influenza was a member of the trial groups.

Table 5. *Sickness absence caused by respiratory infections in volunteers inoculated with HK or CP influenza vaccines (week* 45, 1968 *to week* 20, 1969)

Trial group	No. in group	No. of men with respiratory illness	Working days lost	No. of men with 'clinical influenza'	Working days lost by 'influenza'
A	545	82	481	16	88
B	491	80	494	14	77
C	508	77	473	8	52

Table 6. *The incidence of clinical influenza in weeks* 11–14, 1969, *in volunteers previously inoculated with HK or CP influenza vaccines*

Trial group	No. in group	No. of men ill	Sickness rate (%)	Mean duration of illness (days)
A	545	6	1·1	7·7
B	491	7	1·4	5·0
C	508	3	0·59	4·7

Respiratory illness amongst the volunteers (Table 5) followed the pattern of the general factory population. The incidence of respiratory illness throughout the winter was investigated in 1544 of the original 1601 volunteers; the remainder left employment or their work took them to other Divisions of the Company before the completion of the trial period. No schedule of vaccination had any significant effect on the total number of men with respiratory illnesses or on the mean duration (6 days). Illnesses clinically diagnosed as influenza occurred in only 38/1544 (2·5 %) volunteers; 16 of these were in group A given only CP vaccine

and 8 in group C given only HK vaccine, i.e. an influenzal rate of 2·9% with a mean duration of illness of 5·5 days in group A, and a rate of 1·7% with a duration of 6·5 days in group C.

A dilution effect, by non-specific 'influenzal' illnesses not caused by influenza A viruses, could possibly have obscured the true picture in these data covering the whole winter. Hence, the clinical influenza attack rate was calculated for each trial group over the period weeks 11–14 1969 when the presence of HK in the general factory population had been proven (Table 6). With such low rates of influenzal illness statistical evaluation would have little meaning, and the slight apparent advantage of HK vaccine to group C in both the short- and long-term survey may be merely adventitious.

DISCUSSION

Monovalent HK vaccine, composed of inactivated A2/Eng/344/68 virus, was more efficient in producing serum HI antibody against the new epidemic strain than was CP vaccine containing A2/Eng/76/66 and A2/Eng/12/64 viruses, the only preparation generally available in autumn 1968. Of those injected with HK vaccine, 95% showed a 4-fold or greater rise in HK antibody titre, and the mean post-vaccine titre in this group C was 96·5. In contrast, although CP vaccine injected alone in group A gave a 4-fold rise in HK antibody in 60% of volunteers, most of their post-vaccine titres were low, and the mean titre of the whole group was only 14·1. As far as specific serum antibody was concerned, the intranasal spray of HK vaccine given to group B achieved nothing. The possibility of induction of nasal antibody, particularly IgA, by this procedure was not investigated, and the clinical attack rates of influenza in all groups were too low for any protective advantage of such local immune stimulation to be inferred.

The apparent cross-immunization against HK virus by vaccines containing A 64 is somewhat surprising in view of the apparently distinct antigenic constitution of the haemagglutinin of these strains in laboratory tests with animal antisera. However, this heterologous response was considerably less than the homologous response to HK vaccine, as indicated above, and may not have the same significance as far as immunity to HK infection is concerned. Most of the volunteers were over 35 years of age, with a wide range of antibodies against foregoing epidemic strains of influenza A. It seems probable that with such an immunological background the antibody response would be less narrowly specific than in laboratory animals not previously exposed to influenzal antigens, and that the serum reactions against HK virus induced by CP vaccine may represent 'poor fit' heterologous immunoglobulins with kinetics of neutralization quite different from those of true homologous immunoglobulins induced by HK vaccine. The HI test gives no true measure of 'goodness of fit' or avidity of antibody to HA antigen, and the clinical rates of infection in the present trial were too low for the protective quality of HK antibodies in groups A and C to be compared.

The enhancement of heterologous A 2 antibodies by HK vaccine is in agreement with many earlier experiences. Newly emerging antigenic variants may recall antibody induced by earlier epidemic strains which have been experienced

by human populations, either because the new strain continues to carry small amounts of antigens predominant in earlier strains, or by mal-recognition of a new stimulus by previously committed clones of immunocytes.

Although monovalent HK vaccine may thus help to maintain a pre-existing immunity against A 2 viruses, it is doubtful if it would induce A 2 antibodies in those lacking previous experience. In the present trial, 12 volunteers were without prevaccine antibody to Asian viruses; 6/7 developed such antibody after CP vaccine, but 0/5 after HK vaccine.

Preliminary observations on sera from the present trial with a sensitive enzyme-inhibition test have shown (G. C. Schild, personal communication) that anti-neuraminidase antibody was produced by both types of vaccine. The significance of such antibody in reducing the spread of influenza virus infection has been suggested in animal experiments (Schulman, Khakpour & Kilbourne, 1968) but the low attack rates in the present trial did not allow this aspect to be investigated further.

It is clear therefore from the present trial that an inactivated polyvalent vaccine containing only pre-1968 strains of Asian viruses can no longer be relied upon to produce uniformly distributed high levels of serum HI antibody against current epidemic strains of influenza virus. In the past it has always appeared that such vaccines (i.e. which fail to induce specific HI antibody) would fail to protect in the field (Hobson, 1967). A monovalent vaccine of HK virus, however, did stimulate high titres of specific HI antibody in almost all volunteers and would seem to be the essential basis of future vaccine formulations over the next few years, unless another new antigenic variant of influenza should emerge. If, however, Hong Kong and Asian strains should continue to coexist a polyvalent vaccine of both types of virus would probably be necessary to maintain a sufficient breadth of immunity.

We are grateful to the staff and volunteers in Mond Division, I.C.I. Ltd., for their wholehearted co-operation in making this trial possible, and it is a pleasure to thank members of the Medical Department, Mond Division, and of the Department of Medical Microbiology, University of Liverpool, for their skilful organization of the vaccination sessions.

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