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Haemagglutination-inhibiting Antibody Response to and Efficacy of Inactivated Hong Kong Influenza Vaccine

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In a field study involving some 3000 soldiers in Japan, various doses from 0.2 ml to 1.0 ml of formol-killed monovalent Hong Kong influenza vaccine were administered. Before vaccination 92% of the subjects had haemagglutination-inhibiting antibody titres of <1 : 16; one month after vaccination, only from 1% to 57% (depending on the dose and the soldiers' camp involved) had so low an antibody level. In one vaccinated camp an epidemic of Hong Kong influenza broke out in January–March 1969. In a unit in which 80% of the men had been vaccinated, there was an infection rate of 16% among the vaccinated, of 26% among the unvaccinated living in the same barracks and of 63% among the unvaccinated living in separate barracks; these figures suggest that vaccination of at least 80% of a population group will afford some protection to the unvaccinated remainder of the group against an epidemic of influenza provided the vaccinated and the unvaccinated are living in close association.

Since the Asian influenza epidemic, the use of inactivated A2 influenza vaccine has been investigated in Japan (Fukumi et al., 1960) and it is now generally considered effective in preventing the disease. Influenza vaccination is now carried out routinely in Japan among more than 20 million schoolchildren and other persons (mainly those in group or institutional work) to prevent them from becoming involved in influenza epidemics.

We conducted field trials with monovalent Hong Kong influenza vaccine during the winter of 1968–69, and the results will be described here with emphasis on the following 3 points: (1) haemagglutination-inhibiting (HI) antibody response to vaccination with various doses, (2) the dose of vaccine required to protect from infection, and (3) the circulating antibody level necessary to protect from infection.

MATERIALS AND METHODS

Vaccine

The Hong Kong-type influenza virus A2/Aichi/2/68 was used in the preparation of a formol-killed monovalent vaccine containing 300 chicken-cell agglutinating (CCA) units per ml. A commercial bivalent vaccine which happened also to be used in the field study was prepared from the same virus strain, together with strains B/Tokyo/7/66 and B/Tokyo/1/67; it contained 200 CCA/ml of Hong Kong virus and 50 CCA/ml of each B virus, totalling 300 CCA/ml of virus.

Vaccinated population people and dosage

About 3000 soldiers of the Japanese Ground Self-Defence Force (GSDF) in 5 camps were inoculated subcutaneously with various doses (0.2 ml, 0.25 ml, 0.5 ml and 1.0 ml as single doses, and 1 ml in 2 separate 0.5-ml doses) of Hong Kong influenza vaccine. They were mostly 20–35 years old.

Blood samples were taken and vaccinations were carried out in October 1968. Blood-sampling was repeated 4 or 5 weeks later in all camps and also 3 months later in Camp C, and yet again in Camp A after an epidemic due to Hong Kong virus, i.e., at the beginning of May 1969.

Serological methods

Haemagglutination-inhibiting antibody titres against the strain A2/Aichi/2/68 were measured simultaneously for paired sera by the routine techniques; the titres shown represent the final dilution.

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HI ANTIBODY RESPONSES TO HONG KO

HI titre [b]	0.2-ml dose		0.25-ml dose		0.5-ml dose								
	Camp A		Camp B		Camp A		Camp B		Camp C [a]			Camp D	
	30 Oct. 68 (pre)	30 Nov. 68 (1 month post)	1 Nov. 68 (pre)	9 Dec. 68 (1 month post)	30 Oct. 68 (pre)	30 Nov. 68 (1 month post)	1 Nov. 68 (pre)	9 Dec. 68 (1 month post)	15 Oct. 68 (pre)	19 Nov. 68 (1 month post)	19 Jan. 69 (3 months post)	16 Oct. 68 (pre)	14 Nov. 68
<16	46	22	95	26	90	11	89	16	58	24	8	92	6
16	4	3	2	17	5	12	6	12	1	8	5	2	5
32	2	9	1	9	5	16	5	21		2	5	3	18
64		6		7	1	15		6		4	6	1	27
128		3		4		14		7		1	3		18
256		2				4		7		1	3		13
512						6				2	2		5
Total	52	45	98	63	101	78	100	69	59	42	32	98	92
Percentage with titre <16	88	49	97	41	89	14	89	23	98	57	25	94	7
Geometric mean of titres ≥16	1 : 52.0		1 : 29.8		1 : 64.0		1 : 45.2		1 : 45.2		1 : 64.0	1 : 84.4	

[a] The ages of the vaccinated soldiers ranged in general from 19 years to 49 years, but in Camp E the range was 17–18 years and for the 0.5 ml dose in Camp C was 45–64 years.

RESULTS

HI antibody response to Hong Kong influenza vaccine

Paired sera obtained from about half of the subjects before and 1 month after inoculation were examined for their HI antibody titres; the results are shown in Table 1. The prevaccination titres were <1 : 16 in 92%; 1 : 16 in 3.8%; 1 : 32 in 3.6%; 1 : 64 in 0.5% and none ≥1 : 128.

One month after vaccination, titres of <1 : 16 were seen in from 1% of subjects (Camps D and E, 1.0 ml in 2 0.5-ml doses) to 57% (Camp C, 0.5 ml).

In Camp D (recruits), where the HI titres turned out to be extremely high, 102 paired sera from unvaccinated persons, which were drawn at the same time as the sera from the vaccinated groups (i.e., at the time of the 1-month post-vaccination bleeding), were measured for their HI titres. There were 22 individuals whose HI titres showed 4-fold rises. This was serological evidence that more or less sporadic Hong Kong influenza infections occurred within 1 month after vaccination and that high HI titres in this camp were due to a combination of vaccination and subsequent infection with Hong Kong virus. In Camp C, where the percentages of the subjects with a HI titre of <1 : 16 1 month after inoculation were found to be reduced 3 months after inoculation, there was serological evidence of infection (4-fold increase in antibody level) among 10 of 36 persons with respiratory illnesses and from one of them Hong Kong virus was isolated during this period.

Efficacy of the Hong Kong virus vaccination

An epidemic due to Hong Kong virus occurred from January through March 1969 in Camp A in Tokyo, containing approximately 2000 GSDF soldiers. In a unit of 980 men, of whom 90% were living on post, 80% had been vaccinated on 30 October 1968—139 with 0.2 ml of the Hong Kong vaccine, 288 with 0.5 ml, and 241 with 1.0 ml in a single dose.

The paired sera taken from 45 persons in the group given 0.2 ml and from 78 and 88 respectively in the groups given 0.5 ml and 1.0 ml were examined for

LUENZA VACCINE IN VARIOUS DOSES [a]

1 ml in 2 doses of 0.5 ml each									1.0 ml in single dose									Prevaccination total (15 Oct. to 1 Nov. 68)	
Camp C			Camp D		Camp E [a]		Camp A		Camp B		Camp C			Camp D					
15 Oct. 68 (pre)	15 Nov. 68 (1 month post)	19 Jan. 69 (3 months post)	15 Oct. 68 (pre)	14 Nov. 68 (1 month post)	9 Oct. 68 (pre)	8 Nov. 68 (1 month post)	30 Oct. 68 (pre)	30 Nov. 68 (1 month post)	1 Nov. 68 (pre)	9 Dec. 68 (1 month post)	15 Oct. 68 (pre)	19 Nov. 68 (1 month post)	19 Jan. 69 (3 months post)	16 Oct. 68 (pre)	14 Nov. 68 (1 month post)			No.	%
129	19	4	185	2	88	1	92	6	91	10	41	8	5	99	7			1 195	92
3	20	15	8	3	7	4	6	7	2	9			1	4	3			50	3.8
3	23	30	10	24	6	13	4	26	4	17	1	7	5	4	10			48	3.6
	16	23	2	52	1	34	2	13		19		6	4		28			7	0.5
	13	8		64		33		16		9		6	5		22			0	0
	8	6		37		14		15		5		2	4		9			0	0
	1			17		1		5		2		2	1		19			0	0
135	100	86	205	199	102	100	104	88	97	71	42	31	25	107	98			1 300	
96	19	5	90	1	86	1	88	7	94	14	98	26	20	93	7				92
1 : 48.5		1 : 45.2	1 : 111.4		1 : 84.4		1 : 78.8		1 : 55.7		1 : 84.4		1 : 84.4	1 : 119.4					

[b] No HI titre of 1 : 1024 was recorded.

their HI titres before and 1 month after inoculation, as shown in Table 1.

In the same camp, one unvaccinated group—Control (1)—lived in a different barrack building from the vaccinated soldiers, and another unvaccinated group—Control (2)—lived in the same barracks as the vaccinated soldiers. Sera were drawn from all members of this unit at the beginning of May 1969 for HI titration.

From the time of vaccination to May a number of soldiers left the camp and there were consequently fewer sera from the vaccinated and control groups for examination after the epidemic. The frequency distribution of HI titres in the 162 post-epidemic sera of vaccinated persons and in the control sera in May 1962 is shown in Table 2.

There is a smaller proportion of HI titres $<1:16$ in the vaccinated groups than in either control group, and the geometric means of the HI titres in the vaccinated groups are about half as high as those in the control groups, except for persons vaccinated with 0.2 ml.

These findings are taken to indicate that the HI titres of those who have received a satisfactory dose of vaccine will not be influenced by an epidemic and may be expected to be lower than those of naturally infected persons.

The mean HI titre for Control (2) is lower than that for Control (1), but we are unable to furnish a satisfactory explanation for this—particularly in view of the infectivity rates now to be discussed—unless it is simply due to the small number of subjects and of infected persons in the control groups.

Only 134 pairs of sera from vaccinated persons taken in the pre- and post-epidemic periods are available, owing to movements out of Camp A and other absences at the time of the second blood-drawing. As shown in Table 3, the over-all infectivity rate (as defined below) was 16% in the vaccinated groups (7% for persons given 1.0 ml in a single dose, 13% for those given 0.5 ml and 43% for those given 0.2 ml), as against 26% in the unvaccinated Control (2) and 63% in Control (1).

The infectivity rate in the unvaccinated groups was calculated as follows: (*a*) 46 paired sera of the 90

TABLE 2

FREQUENCY DISTRIBUTION OF POST-EPIDEMIC HI TITRES AMONG PERSONS GIVEN HONG KONG INFLUENZA VACCINE AND CONTROL GROUPS (MAY 1969, CAMP A) [a]

Vaccine dose	No. of sera examined	Post-epidemic HI titre									Percentage with titre <1 : 16	Geometric mean of titres ≥1 : 16
		<16	16	32	64	128	256	512	1 024	2 048		
1.0 ml	65	10	8	15	13	10	8		1		15	1 : 64.0
0.5 ml	66	9	12	11	13	8	5	7		1	14	1 : 73.5
0.2 ml	31	5	3	2	2	7	6	6			16	1 : 137.1
Control (1) [b]	90	22	5	5	7	11	17	11	11	1	24	1 : 193.9
Control (2) [c]	74	37	6	7	6	8	5	4	1		50	1 : 84.4

[a] The epidemic, due to Hong Kong-type virus, occurred in January–March 1969 in Camp A.
[b] Control (1) = unvaccinated group living in different barrack building from vaccinated group.
[c] Control (2) = unvaccinated group living in same barracks as vaccinated group.

in Control (1) were measured for their HI titres and 35 of these (76%) showed a 4-fold rise; (*b*) 44 of the 90 sera in Control (1) and all 74 sera in Control (2) were tested for their HI titres only once—after the epidemic—and those ≥1 : 128 were considered to indicate infection because there were very few HI titres ≥1 : 32 in the pre-epidemic period (as shown in Table 1).

Twenty-two paired Control (1) sera showed HI titres ≥1 : 128; thus 57 (22+35) of the 90 (63%) were considered to have proved infection.

During the same period there was an epidemic of B virus influenza in this camp; the infectivity rate among the vaccinated group was 14% (16 of 116 paired sera showed 4-fold rises of HI titre against B virus) and that among Control (1) was also 14% (6 of 43 paired sera).

These findings are interpreted as indicating that both the vaccinated and the unvaccinated groups have the same risk of exposure and that consequently the Hong Kong influenza vaccine was definitely effective (as measured by serological tests).

TABLE 3

EFFECTIVENESS OF HONG KONG INFLUENZA VACCINATION (MAY 1969, CAMP A)

Vaccine dose	No. of paired sera	Infectivity (≥4-fold titre rise)					
		No.	Rate (%)	Protection ratio in relation to:		P of protection ratio in relation to:	
				Control (1) [a]	Control (2) [b]	Control (1) [a]	Control (2) [b]
1.0 ml	55	4	7	9.0	3.7	<0.001	<0.05
0.5 ml	56	7	13	4.8	2.0	<0.001	>0.05
Subtotal	111	11	10	6.3	2.6	<0.001	<0.01
0.2 ml	23	10	43	1.5	0.6	>0.05	>0.05
Total	134	21	16	3.9	1.6	<0.001	>0.05
Control (1) [a]	90	57	63			<0.001	
Control (2) [b]	74	19	26				

Control (1) = unvaccinated group living in different barrack building from vaccinated group.
Control (2) = unvaccinated group living in same barracks vaccinated group.

TABLE 4

CORRELATION BETWEEN PRE- AND POST-EPIDEMIC HI TITRES (CAMP A)

Post-epidemic sera		24	23	28	28	25	19	13	1	1	162	
	Total	23	17	21	24	21	15	12	1	0	134	245
Pre-epidemic HI titres (30 Nov. 1968)	1 024											
	512					2	4	3	1		10	13
	256				1	3	6	2			12	23
	128			8	11	6					25	34
	64	3	3	4	6	3	1				20	41
	32	7	10	7	4	4	1	3			36	60
	16	6	4	2			1	3			16	29
	<16	7			2	3	2	1			15	45
		<16	16	32	64	128	256	512	1 024	2 048	Total	Sera showing change in positivity between bleeds
		Post-epidemic HI titres (6 May 1969)										

Critical HI antibody titres

In Camp A, 21 sera showed a 4-fold rise of HI titre against Hong Kong virus among 134 paired sera taken 1 month after vaccination and again after the Hong Kong influenza epidemic (Table 4); 20 of these 21 had had HI titres of $\leq 1:32$, and only 1 had had 1 : 64 (none having $\geq 1:128$) before the epidemic.

In an epidemic due to Hong Kong virus that also broke out in Camp C in January 1969, 19 of 106 paired sera drawn 1 month after vaccination and again after the epidemic showed 4-fold rises in HI titre; 12 of these 19 had an HI titre of $<1:16$, 4 of 1 : 16, 2 of 1 : 32 and only 1 of 1 : 64 (none $\geq 1:128$). These findings from Camp C are taken as indicating that an HI titre of 1 : 64 is the critical level for protection from influenza infection.

Clinical incidence rate

On the other hand, there was no significant difference in the clinical incidence rate (based on soldiers treated for respiratory illness) in Camp A between the vaccinated and unvaccinated groups, the rates being 53% in the vaccinated, 51% in Control (1) and 58% in Control (2).

Infectivity rate in another unit

In another unit of about 1200 men in Camp A, half of them living in the camp and the remainder outside it, about one-third were inoculated in December 1968 with 2 doses of commercial vaccine in a dose of 0.3 ml. Early in June 1969 sera were taken from almost all of these soldiers. The infectivity rate in the vaccinated group could not be ascertained because sera had not been drawn before the epidemic; however, the frequency distribution of HI titres in the vaccinated and unvaccinated is shown in Table 5.

There were fewer people with a HI titre of $<1:16$ among the vaccinated than among the unvaccinated, and the geometric mean of the HI titres in the vaccinated is about half as high as that in the unvaccinated. This finding appears to indicate that the commercial vaccine protected against infection with Hong Kong virus, as was mentioned earlier when considering the experimental results among those given monovalent Hong Kong virus vaccine.

There was no difference in the infectivity rate (calculated as mentioned above) between the unvaccinated control group and the unvaccinated individuals working with the vaccinated subjects. This finding is ascribed to the relatively small number of

TABLE 5

FREQUENCY DISTRIBUTION OF POST-EPIDEMIC HI TITRES AMONG PERSONS GIVEN COMMERCIAL VACCINE AND AN UNVACCINATED GROUP [a]

Subjects	No. of sera examined	Post-epidemic HI titre									Percentage with titre <1 : 16	Geometric mean of titres ≥1 : 16
		<16	16	32	64	128	256	512	1024	2048		
Vaccinated	157	49	21	29	21	16	14	5	2		31	1 : 64.0
Unvaccinated [a]	240	108	19	13	16	18	34	19	12	1	45	1 : 137.1

[a] The unvaccinated were working with the vaccinated.

TABLE 6

INFECTIVITY AMONG UNVACCINATED PERSONS LIVING IN OR OUTSIDE CAMP A, JUNE 1969

Subjects		No. examined	Infectivity (≥4-fold titre rise)	
			No.	Rate (%)
Unvaccinated control group	No. living in camp	116	40	34
	No. living outside camp	93	30	32
	Total	209	70	33
Unvaccinated people working with the vaccinated	No. living in camp	123	50	41
	No. living outside camp	117	34	29
	Total	240	84	35
Total unvaccinated	No. living in camp	239	90	38
	No. living outside camp	210	64	30
	Total	449	154	34

subjects living in the camp and the small percentage of vaccinated individuals.

The infectivity rate was 38% among the unvaccinated living in the camp and 30% among the unvaccinated living outside (Table 6).

DISCUSSION

One of the objects of the field trials was to determine whether the HI antibody response to Hong Kong virus vaccine was similar to that to the earlier Asian virus vaccine. In Japan, the Asian virus vaccine became available after the first wave of Asian influenza in 1957, and Ground Self-Defence Force soldiers were inoculated with this vaccine before the second wave. It was found in field trials that the HI antibody response to Asian virus vaccine was very good among those with prevaccination titres ≥1 : 16, but not among those with a prevaccination titre of <1 : 16. The response of the latter was worse than that of those infected with Asian virus in the first wave (Fukumi et al., 1960). The HI antibody response of the 92% with HI antibody titres of <1 : 16 to Hong Kong virus vaccine was similar to that with the same titre to Asian virus vaccine and, again, worse than that of those infected with the Hong Kong virus.

These findings are attributed to the fact that the antigenic shift of Hong Kong virus away from the classical A2 was similar in degree to the shift of the now classical A2 virus, when it first appeared, away from the strains of the A1 influenza era (Fukumi et al., 1968).

As we have seen (Table 3), the infectivity rate in

Camp A was 7% and the protection ratio 9 for subjects given 1.0 ml in a single dose of the Hong Kong virus vaccine while for those receiving 0.2 ml the rate was 43% and the protection ratio 1.5. The infectivity rate was 26% for the unvaccinated group —Control (2)— who lived in the same barracks as the vaccinated soldiers in Camp A, but 63% for Control (1), the group living in different barracks. This suggests that vaccination does protect the unvaccinated from an epidemic spread of influenza provided the latter are living among a group 80% of whom have been vaccinated.

We have a lot of data (not included here) obtained during the period between the appearance of Asian influenza and 1962 from which we estimate that 90% of those persons with an HI titre of 1 : 64 are protected from influenza, whether the titres are due to infection or to vaccination (Sonoguchi, 1969).

In conclusion, we consider proven and further confirmed by the above findings, particularly those In Camps A and C, the theory which we have repeatedly stated on the basis of our studies of the Asian influenza epidemics (Fukumi et al., 1960; Sonoguchi, 1969)—namely, that an HI titre of 1 : 64 in the circulating blood represents the critical level for protection from influenza infection.

REFERENCES

Fukumi, H. et al. (1960) [*Epidemiological record of Asian influenza in Japan*], Tokyo, Public Health Association, pp. 307-397 (in Japanese)

Fukumi, H. et al. (1968) *Jap. med. J.*, No. 2328, pp. 19-26 (in Japanese)

Sonoguchi, T. (1969) *Jap. med. J.*, No. 2335, pp. 21-28 (in Japanese)