Antibody Response in Man to Hong Kong Influenza Following 1967 Formula Influenza Vaccine in Adjuvant 65 (33911)

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Previous reports by our group (1–11) described the development and clinical evaluation of a new immunologic adjuvant, called adjuvant 65, which has been applied extensively to killed influenza virus vaccine. The adjuvant preparation consists of a water-in-peanut oil emulsion of the aqueous vaccine, employing mannide monooleate (Arlacel A) as emulsifier and aluminum monostearate as stabilizer. The outstanding attributes of the adjuvant 65 formulation are the greatly enhanced antibody response, the long duration of such response which lasts for years, and the safety and freedom from untoward reaction as revealed in long-term tests in man and in animals. A further remarkable attribute of the adjuvant has been the broadening of antibody response to afford immunity against antigenically different virus strains, not present in the vaccine, as well as against the homologous viruses. This was demonstrated in previous experiments (5) for variants of Asian influenza (A2) virus which have appeared since the first occurrence of the subtype in 1957, and for influenza B viruses.

A number of lots of a new highly purified polyvalent influenza virus vaccine (8) were tested in man by our group during January and February of 1968. These vaccines were composed of the 1967 influenza strain formula and were evaluated both in aqueous and adjuvant 65 formulation. The late summer of 1968 (12) saw the emergence of the new and radically different Hong Kong influenza virus and the opportunity was provided to measure the extent to which the old formula vaccine would evoke antibody against Hong Kong virus. The expected failure of the 1967 formula aqueous vaccine to stimulate significant antibody against Hong Kong virus was clearly shown. Remarkably, however, more than 50% of the recipients of the same vaccine in adjuvant developed antibody against the Hong Kong virus. The present report summarizes the antibody responses to homologous Asian and heterologous Hong Kong viruses following use of these vaccines and, in addition, provides information concerning the occurrence and distribution of influenza antibody in persons of various ages, prior to appearance of the new virus.

TABLE I. Influenza Virus Antigen Content of the Vaccines.

Influenza virus strain	CCA units of influenza virus/dose		
	Adjuvant 65		Aqueous
	600 CCA[a]	300 CCA	(600 CCA)
A/PR8/34	100	50	100
A1/AA/1/57	100	50	100
A2/Japan/170/62	100	50	100
A2/Taiwan/1/64	100	50	100
B/Mass/3/66	200	100	200

[a] Chick cell hemagglutinating units (a measure of influenza viral antigen content).

Materials and Methods. Vaccines. The compositions of the polyvalent vaccines used in the study are shown in Table I. The aqueous vaccine was in 1.0-ml volume and the 600 and 300 CCA unit adjuvant 65 vaccines were in 0.5- and 0.25-ml volumes, respectively. The adjuvant vaccines were prefilled in disposable syringes.

Study plan. The study was carried out at Pennhurst State School which is located in Pennsylvania. The age range of the recipients of the vaccine was from 7 to 75 years with a mean of 38.5 years. The first dose of vaccine was given between December 11, 1967 and January 12, 1968; the second dose between January 8 and February 9, 1968. Nonimmunized persons were followed for control pur-

pose and it was shown that natural influenza did not occur in the institution during the study period.

The subjects were injected with 1 or 2 doses of vaccine in the regimens shown in the text. Two doses, when given, were administered 1 month apart. Aqueous vaccines were administered subcutaneously and adjuvant type vaccines were injected intramuscularly. Blood samples for serologic determinations were taken immediately prior to vaccination and again 1, 2, and 4 months later.

Serologic testing. Antibody responses were measured by the hemagglutination-inhibition technique described previously (8). The findings presented in this report are limited to those which were obtained in tests with the homologous A2/Taiwan/1/64 and B/Mass/3/66 viruses which were present in the vaccine, and with the heterologous A2/Hong Kong/8/68 strain which was obtained from Dr. W. R. Dowdle of the National Communicable Disease Center.

Limitation of data. The data relating to natural occurrence of influenza antibody in the sera of persons prior to vaccination included the entire study group who received the 5 different lots of vaccine or who were held as unvaccinated controls. The data concerning antibody responses to vaccination relate to the combined experiences in that portion of the total population who received vaccine lots 13 and 14 of aqueous or adjuvant vaccine.

Other details of the clinical and laboratory testing program are given in the earlier report (8).

Results. Asian, Hong Kong, and influenza B antibody in sera of normal persons. An analysis was made of the occurrence of Asian, Hong Kong, and influenza B antibody in the sera of normal persons, according to age, prior to receiving vaccine, which was before the emergence of Hong Kong influenza virus. The findings shown in Fig. 1 revealed the expected high percentage of persons with antibody against Asian influenza and influenza B viruses. By contrast, the population was remarkably devoid of antibody against the Hong Kong virus, as would be expected for

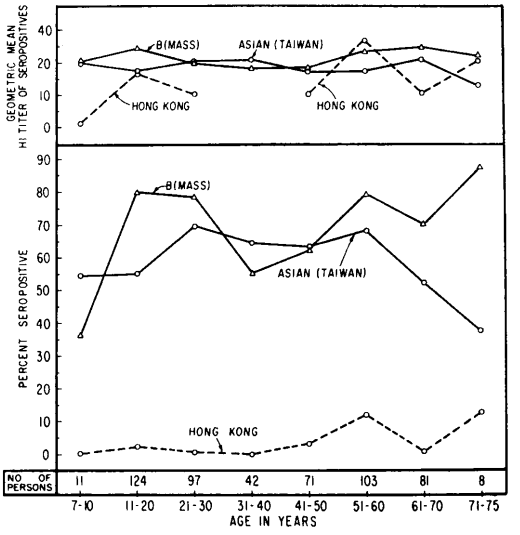


FIG. 1. Distribution, according to age, of HI antibody against Asian, Hong Kong, and B influenza viruses among population prior to vaccination (1967 and 1968).

new virus of such marked antigenic difference and for which the population had no prior experience. It is of interest, however, that detectable antibody against Hong Kong virus was present in a small proportion of persons of widely different ages and that the chance for occurrence of such antibody increased with age. Such finding was consistent with the development, with increasing age, of a broad composite immunity to influenza viruses. The height of the naturally occurring Hong Kong antibody in those particular persons who were seropositive was not markedly different on the average, from that for Asian or B influenza viruses.

Antibody response to Hong Kong influenza virus among persons given 1967 formula influenza vaccine in aqueous or adjuvant 65 formulation. Figure 2 shows the geometric mean HI antibody response against homologous Asian and heterologous Hong Kong virus, according to time following vaccination with 1 or 2 doses of aqueous or with adjuvant influenza vaccine. The adjuvant contained an amount of viral antigen equal to or half that present in the aqueous vaccine. The extreme potentiation of antibody response to the homologous Asian virus by adjuvant compared with aqueous vaccine was clearly

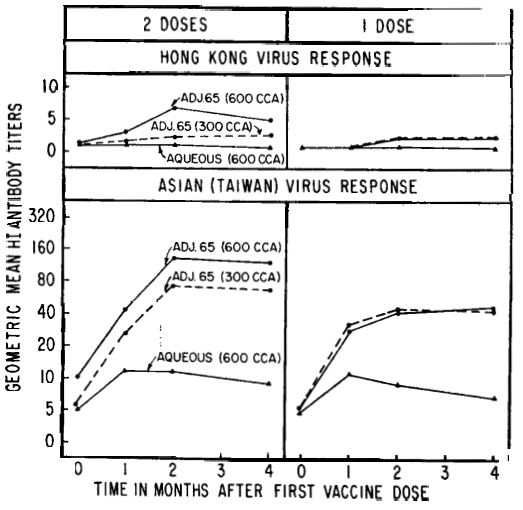


FIG. 2. Geometric mean homologous (Asian-Taiwan) and heterologous (Hong Kong) antibody responses to 1967 formula influenza vaccine in adjuvant or aqueous formulation.

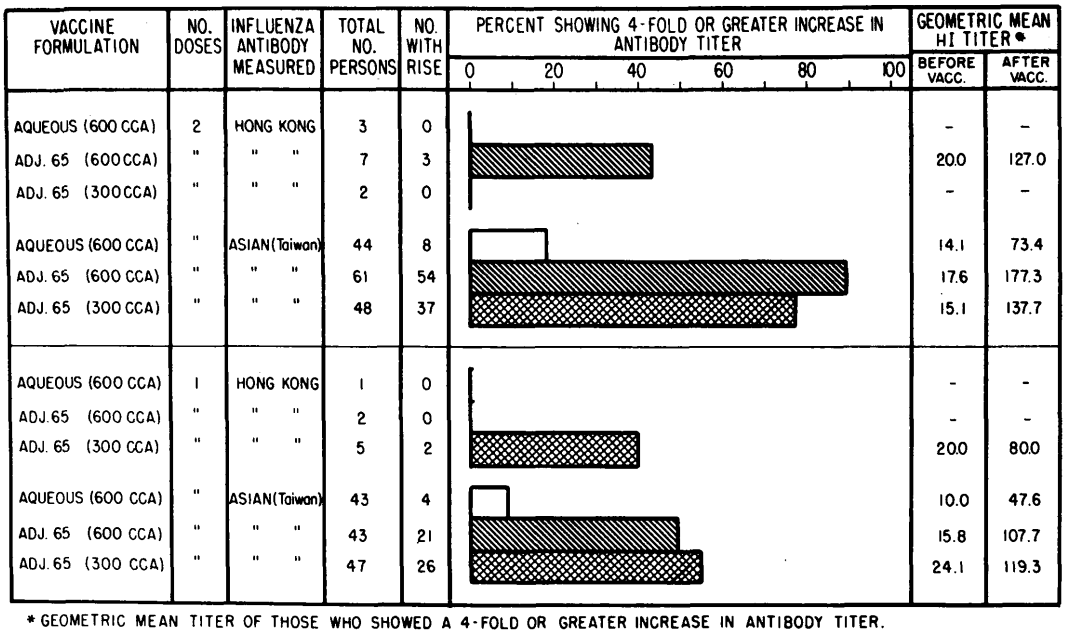
shown. Two doses of vaccine were more effective than a single dose, and a single dose of adjuvant vaccine was far more effective than 2 doses of aqueous vaccine. Very little antibody against Hong Kong virus developed following administration of the aqueous vaccine. By contrast, there was considerable antibody response against the heterologous Hong Kong virus in persons given the vaccine in adjuvant 65, especially when 2 doses of the 600 CCA unit vaccine were given.

Further analysis of the data was made in terms of development of detectable antibody against the heterologous Hong Kong and homologous Asian virus in persons who were seronegative to these agents prior to vaccination. As shown in Fig. 3, the seroconversion rates in persons given aqueous vaccine were generally poor and this was especially true for antibodies against the Hong Kong strain. Only 40–60% of these persons developed antibody against homologous Asian virus following 1 or 2 doses of aqueous vaccine and the response to Hong Kong virus was negligible. By contrast, an excess of 93% of persons who received adjuvant vaccine in 1 or 2 doses at either strength responded serologically to the homologous Asian virus. Fifty-five percent of persons developed antibody against the Hong Kong virus following 2 doses of 600 CCA unit adjuvant vaccine. The responses to Hong Kong virus were less

VACCINE FORMULATION	NO. DOSES	INFLUENZA ANTIBODY MEASURED	NO. PERSONS SERONEG	NO. RESPOND	PERCENT DEVELOPING ANTIBODY 0 20 40 60 80 100	GEOMETRIC MEAN HI TITER * BEFORE VACC.	AFTER VACC.
AQUEOUS (600 CCA)	2	HONG KONG	71	1		<10	20.0
ADJ. 65 (600 CCA)	"	" "	67	37		<10	28.0
ADJ. 65 (300 CCA)	"	" "	74	14		<10	26.9
AQUEOUS (600 CCA)	"	ASIAN(Taiwan)	32	19		<10	16.7
ADJ. 65 (600 CCA)	"	" "	17	17		<10	98.1
ADJ. 65 (300 CCA)	"	" "	30	30		<10	49.3
AQUEOUS (600 CCA)	1	HONG KONG	72	5		<10	20.0
ADJ. 65 (600 CCA)		" "	69	12		<10	17.8
ADJ. 65 (300 CCA)	"	" "	72	11		<10	20.0
AQUEOUS (600 CCA)		ASIAN (Taiwan	35	14		<10	19.0
ADJ. 65 (600 CCA)		" "	30	28		<10	36.2
ADJ. 65 (300 CCA)		" "	30	28		<10	31.2

* GEOMETRIC MEAN TITER OF THOSE WHO RESPONDED TO VACCINATION.

FIG. 3. Homologous (Asian-Taiwan) and heterologous (Hong Kong) antibody responses among initially seronegative persons given 1 or 2 doses of aqueous or adjuvant 65 formulated influenza vaccine as measured 2 months after the first dose of vaccine.



* GEOMETRIC MEAN TITER OF THOSE WHO SHOWED A 4-FOLD OR GREATER INCREASE IN ANTIBODY TITER.

FIG. 4. Homologous (Asian-Taiwan) and heterologous (Hong Kong) antibody increases among initially seropositive persons given 1 or 2 doses of aqueous or adjuvant 65 formulated influenza vaccine as measured 2 months after the first dose of vaccine.

when 1 rather than 2 doses was given and when the amount of virus in the vaccine was 300 rather than 600 CCA units. The degree of antibody response, as indicated by the geometric mean titer, reflected the same general findings as did the seroconversion rates.

The similar analysis of the findings in relation to 4-fold or greater increase in amount of antibody in persons who had antibody prior to vaccination is presented in Fig. 4. Between 49 and 89% of persons given adjuvant vaccine containing 300 or 600 CCA units and given in 1 or 2 doses showed 4-fold or greater increase in antibody against homologous Asian virus while only 9–18% of persons given aqueous vaccine showed this change. The number of persons with Hong Kong antibody prior to vaccination were too small to provide meaningful data.

The extent of the responses to the heterologous Hong Kong virus is shown further in Table II which presents the individual titer values against Hong Kong virus in persons given 2 doses of 600 CCA unit aqueous or adjuvant vaccine. Only 1 of 71 persons (1.4%) developed detectable antibody against Hong Kong virus following aqueous vaccine. By contrast, 37 of 67 persons, or 55%, developed antibody against Hong Kong virus after adjuvant vaccine. The titer values following adjuvant ranged from 1:10 to 1:160.

TABLE II. Distribution of Antibody Titers against Hong Kong Virus among Initially Seronegative Persons 2 Months Following Administration of the First of 2 Doses of Aqueous or Adjuvant 65 Formulated Vaccine.

Antibody titer	No. of persons with titer following: Aqueous vaccine (600 CCA)	Adjuvant 65 vaccine (600 CCA)
<10	70	30
10	0	5
20	1	17
40	0	9
80	0	4
160	0	2
No. responding/total	1/71	37/67
% responding	1.4	55.2

Discussion. The new Hong Kong influenza virus which emerged during mid-July of 1968

(12–14) showed major antigenic difference from the Asian influenza virus which has been dominant since 1957. The striking deficiency of antibody in the human population against the Hong Kong virus noted in this and in previous studies (13, 14) was consistent with the rapid spread of the agent throughout the world. The alteration in antigenic form of Hong Kong virus did not represent the creation *de novo* of a new virus with no relationship to the past. There was, instead, a remote sharing of antigens with Asian influenza virus (13, 14).

The question might be raised, as in 1957, whether the principal antigen (or antigens) of the Hong Kong virus was a new component arising through genetic mutation or whether it represented the re-emergence into prominence of a minor component present in earlier influenza viruses. Mulder (15) demonstrated the presence of antibody against Asian virus in sera from persons 80–90 years of age and suggested that the influenza pandemic of 1957 might have been related to re-emergence of a virus which caused the pandemic of 1889–90. Data obtained by Hilleman *et al.* (16), however, demonstrated the presence of antibody against Asian influenza virus in young adults who were born many years after the 1889–90 event. Further, there appeared to be increase in occurrence of such antibody with increase in age. It was apparent, therefore, that the presence of Asian antibody depended more upon experience with a composite of influenza viruses which contained antigens also present in the Asian virus than with Asian virus itself. The same general finding of occurrence of antibody against Hong Kong virus was shown in the present studies. Hong Kong antibody was found in persons as young as 13 years (titer 1:20) and there was a general overall increase in such antibody with age. It appeared, therefore, that the composite type antibody resulting from repeated experience with influenza viruses was active against Hong Kong virus as it had been against Asian virus. Thus, occurrence of Hong Kong antibody in persons of comparatively young age made it unlikely that its presence was due to prior experience with a Hong Kong-like influenza virus of an earlier era.

The present outstanding deficiencies of influenza vaccine are the relatively short duration of the immunity which follows use of the aqueous vaccine and the continuing need for change of the strain formula of the vaccine to make it contemporary with the viruses circulating in the population. Strains of greatest antigenic difference generally spread most rapidly in the population. As a consequence, the vaccine is least efficacious when it is needed most and when there is least amount of time to prepare vaccine prior to the major occurrence of the epidemic.

This problem might be reduced significantly by incorporating the acqueous vaccine in adjuvant, thereby decreasing the amount of antigen required per dose and stretching the vaccine supply in time of critical need. Of greater importance, the antigenic response to vaccine could be considerably broadened. It was shown previously (5) that the broadening of antibody response against heterologous viruses within a particular subtype, e.g., Asian influenza viruses, may be sufficient to reduce the importance of the minor antigenic changes which tend to occur at 2–3-year intervals within influenza A subtypes. Surprisingly, as shown in the present study, this broadening of response may be extensive enough to potentiate an immunologic overlap between old and new viruses of very different form. It was shown in the present study, for example, that the 1968 formula vaccine given in 2 doses (600 CCA amounts) in adjuvant 65 sufficed to stimulate antibody against Hong Kong virus in 55% of recipients. Such antibody engendered by old formula vaccine in adjuvant 65 might have provided substantial immunity against the morbidity and mortality from Hong Kong virus in a portion of the population at a time when Hong Kong vaccine was not yet available or was still in short supply.

Summary. The development of adjuvant 65 has added a new dimension to the prospects for the control of influenza by vaccine. Previous studies with the adjuvant revealed heightened antibody response of long dura-

tion, employing a reduced amount of antigen, and broadening of the antigenic coverage between heterologous strains within a single subtype, e.g., Asian. The present study revealed a broadening of antigenic coverage to cross the barriers of major antigenic change as revealed by the stimulation of antibody against Hong Kong virus in 55% of 67 persons given 2 doses of 1967 formula polyvalent influenza vaccine in adjuvant. Such antibody might have provided substantial protection against the morbidity and mortality of Hong Kong virus before the new vaccine was available and might also have contributed to limitation of spread of the virus. Only 1 of 71 persons given the corresponding aqueous vaccine developed such antibody. There was a striking deficiency in antibody against Hong Kong virus in the sera of persons collected prior to the emergence of the virus. However, antibody was occasionally seen in persons as young as 13 years and there was generally an increase in seropositive persons with age. This appeared to be related to development of composite immunity against influenza virus strains by repeated experience with these agents rather than to previous experience with an antigenically similar virus.

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