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Antigenicity of Licensed Whole Virion and Subvirion Influenza Vaccines in "High Risk" Persons (39298)

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Since 1973, both inactivated whole virus and subvirus vaccines have been available for the prevention of influenza in man. Recently, we had the opportunity to compare the immunizing capacity of a vaccine representative of each type in persons considered to be a risk of pneumonia after infection with influenza virus. The recognition that type A influenza viruses possess cross-reacting and type-specific envelope antigens (1-4) and that these antigenic determinants can exist on the same hemagglutinin subunit (5) allowed for the further assessment of the specificity of the serum antibody response to these two vaccines. This report describes the results of these evaluations.

Materials and methods. Vaccine and study group. Commercially available inactivated whole virus (Eli Lilly and Co.) and subvirion (Wyeth Laboratories, Inc.) vaccines containing the 1974-1975 formula were used. Each 0.5 ml contained 700 chicken cell agglutination (CCA) units of a strain antigenically representative of A/Port Chalmers/1/73 and 500 CCA units of a strain antigenically representative of B/Hong Kong/5/72.

The study group included 85 males or females with advanced age and/or with chronic pulmonary, neurologic, or cardiac disease. The individuals ranged in age from 21 to 94 years, and the mean age of the group was 64. Admission to the study was based on a willingness to participate, absence of a recent respiratory illness, and a need for vaccination. Participants were assigned by random allocation to receive a single 0.5-ml dose of either whole virus or subvirion vaccine. Serum specimens collected prior to and approximately 60 days after vaccination were used for antibody assays.

Immunologic procedures. Serum antihemagglutinin antibody (AHAb) titers (expressed as the reciprocal of the serum dilution) were determined by the conventional microtiter hemagglutination-inhibition method (6) using H3N2 virus strains representative of A/Port Chalmers/73, A/England/72, A/Hong Kong/68, B/Hong Kong/72 virus (high passage), and antigenic hybrids possessing the hemagglutinin of either A/Port Chalmers/73, A/England/72, A/Hong Kong/68 and the neuraminidase of A/equine 1/Prague/56 virus.

Serum antineuraminidase antibody (ANAb) assays were performed by the enzyme-inhibition tests (7). These titers are also expressed as the reciprocal of the serum dilution. An antigenic hybrid possessing the neuraminidase of A/Port Chalmers/73 and the hemagglutinin of A/equine 1 virus was used as a source of enzyme.

Heterologous serum AHAb was selectively removed from specimens by precipitation with formalin inactivated zonal centrifuged A/Hong Kong/68 whole virus containing 50,000 hemagglutination units/ml by a previously described method (5).

Agar-gel double immunodiffusion tests were performed as previously described (8) using immunodiffusion plates purchased from Hyland.

Results. Homologous serum AHAb and ANAb responses. The AHAb responses to the vaccine antigens in each vaccine group are shown in Tables I and II. Results are presented according to the titer of prevaccination antibody. The distribution of initial antibody titers in both groups was similar. Only 17 individuals in the study group had antibody titers of 40 or greater to the type A component and 6 had that level to type B. Administration of whole virion vaccine was

TABLE I. SERUM HEMAGGLUTINATION-INHIBITING ANTIBODY RESPONSES TO A/PORT CHALMERS/73 (H3N2) INFLUENZA VIRUS.

Type of vaccine	Prevaccination antibody titer[a]	Number of persons	Incidence of significant rise (≥4-fold)		Geometric mean titer[b]
			Number	Percentage	
Whole virion	<10[c]	16	10	63	38 (<10-640)[d]
	10-20	22	15	68	85 (10-1280)
	40-80	8	3	38	113 (40-320)
Subvirion	<10	13	10	77	38 (<10-320)
	10-20	17	13	76	50 (10-1280)
	40-160	9	3	33	180 (80-320)

[a] A/Port Chalmers/73 (H3N2) used as test virus.
[b] For geometric mean calculations, a titer of <1:10 was classified as 1:5.
[c] Titers expressed as reciprocal of serum dilution.
[d] Numbers in parentheses indicate range of titers.

TABLE II. SERUM HEMAGGLUTINATION-INHIBITING ANTIBODY RESPONSES TO B/HONG KONG/72 INFLUENZA VIRUS.

Type of vaccine	Prevaccination antibody titer	Number of persons	Incidence of significant rise (≥4-fold)		Geometric mean titer[a]
			Number	Percentage	
Whole virion	<10[b]	29	13	44	16.1 (<10-167)[c]
	10-20	15	6	40	29.0 (10-320)
	40	2	2	50	– (80-640)
Subvirion	<10	18	7	39	14.1 (<10-160)
	10-20	17	9	53	36.9 (10-320)
	40	4	0	0	– (40-80)

[a] For geometric mean calculations, a titer of <1:10 was classified as 1:5.
[b] Titers expressed as reciprocal of serum dilution.
[c] Numbers in parentheses indicate range of titers.

followed by a fourfold or greater rise in antibody titer to A/Port Chalmers and B/Hong Kong in 28 (61%) and 21 (46%) persons, respectively. These frequencies were not significantly different from those that were observed after immunization with the subvirion vaccine (65 and 41%, respectively). Moreover, in each vaccine group, the seroconversion rates were comparable among individuals who lacked prevaccination antibody, among those with initial titers of 10 to 20, and among those with ≥40.

The geometric mean postvaccination antibody titers to each vaccine strain among the whole virion group were similar to those in the subvirion group (in each case, $P > .05$) as were the mean titers for each of the vaccine subgroups.

At the time of vaccination, approximately one-third of the study population lacked serum ANAb (<4) to the A/Port Chalmers enzyme. An analysis of antibody responses was made among 44 individuals who lacked or had low levels (<10 to 20) of serum ANAb to type A virus prior to vaccination. The results are presented in Table III. The mean prevaccination levels of ANAb for the whole virus and subvirion were 39 and 32, respectively. Seroconversion rates based on a fourfold or greater rise in titer for each vaccine groups were similar. On the other hand, the mean postvaccination antibody titer among the whole virion vaccinated group was significantly higher than that observed for the subvirion group ($P < .05$).

Specificity of the type A AHAb response. To further characterize the serum AHAb responses, sera from 48 of 54 individuals who exhibited a rise in antibody titer to A/Port Chalmers were assayed for antibody responses to other variants within the H3N2 antigenic subtype, the A/Hong Kong, and A/England viruses. The results are shown in

Table IV. Response frequencies to both test antigens in each vaccine groups were similar. A high proportion of persons, 34 of 48, exhibited a response to at least one of the variants, and approximately half demonstrated a response to both variants. Of the 51 heterologous antibody responses observed in both vaccine groups, 31 or 61% of these occurred in the absence of detectable prevaccination antibody to the test virus. Although not shown, heterologous antibody responses occurred in 5 of 26 individuals in the absence of homologous response.

The high frequency of heterologous antibody responses prompted an absorption study to determine whether response to the common determinant shared by variants of the H3 era accounted for the finding. Twenty-three postvaccination sera with high titers of antibody from individuals representing each vaccine group were absorbed with intact A/Hong Kong virus. As shown in Table V, this absorption removed all detectable antihemagglutinin antibody to Hong Kong virus. Complete absorption of antibody to A/Port Chalmers by the A/Hong Kong virus was interpreted as an antibody response to the common antigenic determinant alone, and this response to A/Port Chalmers vaccine occurred in 19 of the 23 individuals, 10 of whom received whole virion vaccine and 9 who received the subvirion vaccine. Antibody to A/Port Chalmers persisting after absorption with A/Hong Kong virus was interpreted as indicating a response to the type-specific antigen of the vaccine virus. Sera from two individuals in each vaccine group yielded this evidence for a type-specific antibody response, although the reduced antibody titers to A/Port Chalmers after absorption with A/Hong Kong virus indicated that each of these four individuals had also responded to the cross-reacting determinant.

The two patterns of antibody response defined by the absorption studies, to common antigen along and to common plus type-specific antigens, were further confirmed by double immunodiffusion assays. Figure 1 illustrates the reactivity of two postvaccination sera exhibiting these patterns of response with purified hemagglutinin subunits of A/Hong Kong and A/Port Chalmers. Reaction of A/Port Chalmers subunits against each of the specimens results in the formation of a precipitin line that was continuous with a line produced by the hemagglutinin of A/Hong Kong indicating the presence of the common antibody. In Fig. 1B, the formation of a spur demonstrates the presence of antibody produced to the type-specific antigenic determinant of the vaccine hemagglutinin in addition to the cross-reacting antibody.

Discussion. The present report has described the serologic responsiveness of

TABLE III. RELATIVE FREQUENCY AND LEVEL OF SERUM ANTINEURAMINIDASE ANTIBODY RESPONSE TO INFLUENZA TYPE A/PORT CHALMERS/73 (H3N2) AMONG "HIGH RISK" PERSONS AFTER VACCINATION.

Vaccine group	Number of persons	Incidence of significant rise		Geometric mean titer
		Number	Percentage	
Whole virion	22	12	55	293
Subvirion	22	11	50	92

TABLE IV. HETEROLOGOUS SERUM ANTIHEMAGGLUTININ ANTIBODY RESPONSES AMONG PERSONS WITH A HOMOLOGOUS RESPONSE FOLLOWING VACCINATION.

Vaccine	Number of persons tested	Number of persons with a rise to the indicated test virus[a]		Total number of persons with a heterologous response
		A/Hong Kong/68[b]	A/England/72[b]	
Whole virion	23	13	11	16
Subvirion	25	13	14	18

[a] Includes individuals with a ≥ fourfold rise in titer.

[b] Antigenic hybrids possessing the indicated hemagglutinin and equine-1 neuraminidase were used as test antigens.

TABLE V. INFLUENZA VIRUS ANTIBODY TITERS IN POSTVACCINATION SERA FOLLOWING ABSORPTION WITH A/HONG KONG/68 (H3N2) VIRUS.

Vaccine group	Pattern of antibody response	Number of persons with a similar pattern of response	Test antigen[a]	Geometric mean hemagglutination-inhibition antibody titer[b]	
				Before absorption	After absorption
Whole virion	Common and specific	2	A/PC	240	32
			A/HK	60	<8
	Common	10	A/PC	149	<8
			A/HK	75	<8
Subvirion	Common and specific	2	A/PC	120	16
			A/HK	50	<8
	Common	9	A/PC	80	<8
			A/HK	100	<8

[a] Antigenic hybrids possessing the indicated hemagglutinin and equine-1 neuraminidase were used as test antigens.

[b] Expressed as the reciprocal of the serum dilution.

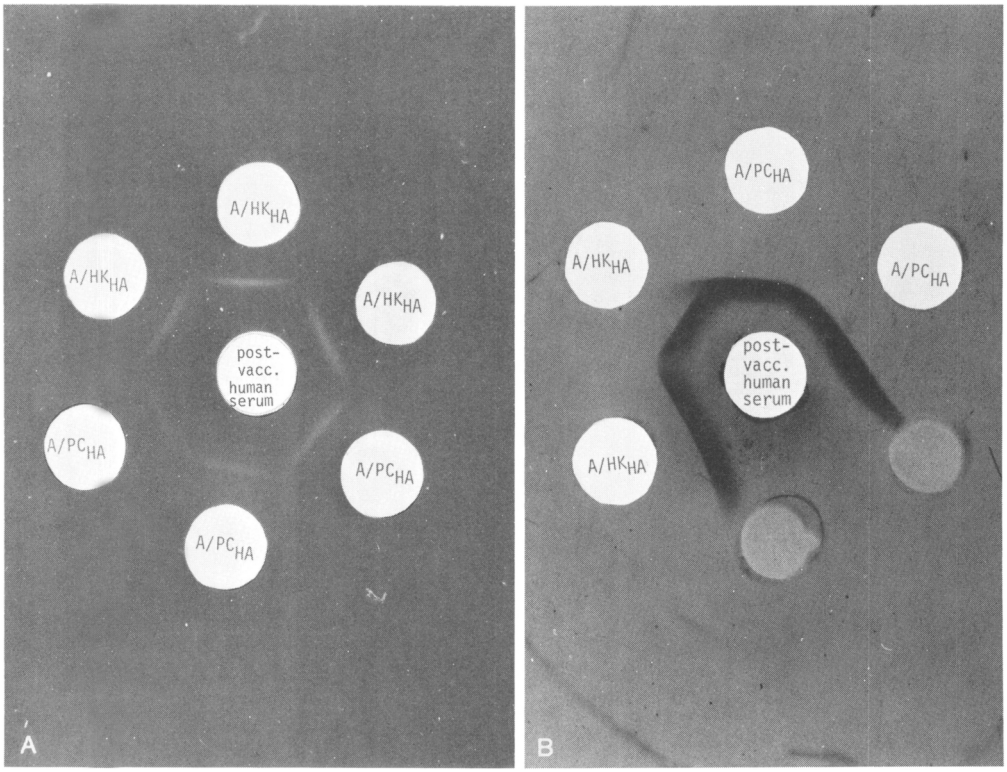

FIG. 1. Precipitation bands obtained with postvaccination serum specimens by agar-gel double diffusion technique. Ten microliters of sera were reacted against 10 μl of purified hemagglutinin subunits of A/Port Chalmers and A/Hong Kong (1 mg/ml). Figure 1A (common antibody only) was photographed without further treatment, and the plate in Fig. 1B (common and specific antibodies) was stained with Coomassie brilliant blue.

"high risk" individuals to licensed influenza virus vaccines prepared by two different production processes. There were no significant differences in the incidence and magnitude of serum AHAb response to the vaccine antigens. Although the mean titer of the ANAb responses was significantly higher among persons administered the

whole virion vaccine, the level of response resulting from vaccination with the subvirion product was relatively high.

The overall seroconversion rates of about 70% for the type A component and about 40% for the type B component among persons with lower initial antibody titers is less than desired. This seroconversion rate is comparable to that reported recently by others for similar vaccine preparations in this type of population (9, 10). The combined results suggest that little more antibody response can be expected in such individuals after use of currently available vaccines according to current recommendations.

Studies by Mostow *et al.* in a retirement community with a purified A/Hong Kong vaccine containing 3000 CCA units revealed a seroconversion rate of 88%, suggesting it may be possible to improve seroconversion rates with a further increase in antigenic content (11). However, in the Mostow *et al.* study, the reaction rate also increased with the increase in antigen content. An alternative approach might be to alter schedule. In this regard, earlier studies in a comparable population showed that nasopharyngeal vaccination in conjunction with parenteral vaccine resulted in a higher frequency of conversions and higher titer of serum antibody than did a single parenteral dose of vaccine (12).

The present study has also provided information, by means of absorption with heterologous virus, on the specificity of the type A AHAb response following vaccination. While immunization uniformly induced an antibody directed toward the common antigenic determinant shared by A/Hong Kong and A/Port Chalmers viruses in all individuals tested, antibody to the type-specific determinant of the vaccine antigen was produced, but in a small number of individuals. Although the relative significance of common and type-specific antibody for protection against disease in man has not yet been defined, it seems likely that antibody to the type determinant in addition to the common antigen would augment host resistance to infection. Serum antibody to each determinant has been reported to be capable of reducing infectivity of virus *in vitro* (5). The evaluation of the significance of each antibody in man is needed.

This study has identified two possible reasons for limitation in the protective effect of current influenza vaccines. Based on the known correlation between serum antibody and resistance in influenza, the frequency and magnitude of the serum antibody response is less than desirable. Moreover, the antibody response is primarily directed toward the antigen on the hemagglutinin common to all subtype variants rather than to type-specific antigens of the new variant. As further information is collected, it may be necessary to reevaluate the present approach to vaccination for influenza.

Summary. The frequency and magnitude of serum antibody response to type A and B influenza viruses induced by whole virion and subvirion vaccines were essentially comparable. Immunization was followed in vaccinated individuals by an antihemagglutinin antibody response to the common antigenic determinant shared by the type A H3N2 viruses. Relatively few individuals developed antibody to the type-specific determinant.

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