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Age-related response to 1000 CCA unit zonally purified, inactivated influenza vaccines in volunteers in the U.S.A.

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Summary

Response to 1000 CCA unit Aichi/68 and Japan/57 influenza vaccines was studied in 687 volunteers from ages 6–101 in the summer of 1971. The vaccines, prepared by zonal ultracentrifugation, were well tolerated in all age-groups. Antibody responses were comparable with each vaccine and were strongly influenced by age of the volunteer. Persons born since 1940 (age 31 and under) had a much more impressive response as determined by both overall geometric mean titre rise and % with ⩾ four-fold increase in titre than persons born before 1940. The most reasonable explanation for this phenomenon seems to be the greater prior exposure of the younger age-groups to the strains in the vaccines. It is concluded that more attention needs to be given in the future to assessing vaccine potency in the age-groups for which the maximum protection is desired, namely, the elderly.

Introduction

Until recently, the major determinant of virus concentration in influenza vaccines has been an acceptably low side-reaction rate. Zonal ultracentrifugation has liberated us from this bondage. Now, concentration can be almost exclusively determined by the degree of antibody response that is desired. It was shown over 20 years ago that vaccines containing 1000 chick-cell-agglutinating (CCA) units or more of a single virus strain were superior to lower concentrations but the 60–80% febrile reaction rate in adults precluded their use (Salk, 1948). The ratio of haemagglutinating activity to protein concentration suggests that the zonal method provides a ten- to twenty-fold purer product than the Sharples process. Mostow *et al.* (1969) have shown that vaccine containing 3000 CCA units of the zonally purified product can be well tolerated by children as well as adults.

The present study conducted in the summer of 1971 examines primarily the response of persons over a wide age range to 1000 CCA unit monovalent vaccines prepared against the Asian and Hong Kong pandemic prototype strains. Some guidelines for selecting vaccine virus concentration based on potency in man are suggested by the results.

Materials and methods

Vaccines

All vaccines in this study were produced for investigational use by the Biological Laboratories of the National Drug Company and prepared by the zonal ultracentrifugation method. Monovalent vaccines were supplied in the following concentrations as measured by CCA units (specially determined by the Division of Biologics Standards) and protein concentration.

Vaccine	CCA units/ml	Protein conc. (μg/ml)
Aichi/2/68 (H3N2)	1714	165
Japan/305/57 (H2N2)	1685	150
A/FM/1/47 (H1N1)	571	137
A/PR/8/34 (H0N1)	909	175

The dosage was adjusted by altering the total volume of vaccine given subcutaneously to contain 1000 CCA units per dose with the exception of the FM1 vaccine in which a 1ml dose (571 CCA units) was given.

Study population

Healthy volunteers were recruited from three sources. For the age group 6–19, volunteers came from a church-related children's home. Persons 20–65 were employees of an Atlanta-based firm, and those 65 and over were from a county nursing home. All members of the study population were from the Atlanta, Georgia, Metropolitan Area and so would be expected to reflect recent and past influenza experience in that part of the world. Only in the nursing home was there a yearly influenza immunization programme so that the pre-immunization sera should largely reflect natural experience.

Immunization procedure

All immunizations were conducted in the months of July–September 1971 at a time when there were no natural occurring influenza infections. All volunteers in each age-group had pre-immunization

antibody levels determined on the initial serum specimen obtained 4–10 days before immunization. The haemagglutination-inhibition (HI) test was performed using A/Hong Kong/8/68 (H3N2) and A/Japan/305/57 (H2N2) antigens with all sera; in addition, for the 20–31 age-group the A/FM/1/47 (H1N1) antigen was used, for the 32–43 age-group the A/PR/8/34 (H0N1) antigen was used, and for the 44–59 age-group A/Swine/1976/31 antigen was used. The vaccine groups for each age-group were then assigned on a randomly stratified basis using the baseline antibody levels so that, as nearly as possible, the pre-immunization level against each influenza A antigen was the same within each age-group. All adults who received a vaccine not containing A/Aichi/2/68 were offered an injection of 1971 commercial bivalent influenza vaccine at the time of the post-immunization bleeding which was obtained 3 weeks after immunization.

Antibody determination

All sera were stored at – 20°C until tested. Sera were treated by the method of Burnet & Stone (1947) with receptor-destroying enzyme (RDE) of *Vibrio cholerae* to remove non-specific serum inhibitors. Haemagglutination-inhibition (HI) tests (Davenport & Minuse, 1964) were performed with 4 haemagglutinating units of antigen and 0·5% rooster cells using the microtitre method of Sever (1962) and titres recorded per 0·025 ml serum dilution in 0·1 ml final volume. Egg allantoic fluid antigens used were A/Hong Kong/8/68 (H3N2) and prototype strains of A/Japan/305/57 (H2N2), A/FM/1/47 (H1N1), A/PR/8/34 (H1N1), A/Swine/1976/31. In addition, the haemagglutinin-specific recombinant strains, A/Hong Kong/8/68 (H3) – NWS/33 (N1), and A/Japan/305/57 (H2) – NWS/33 (N1) received as HKe and 305e from Dr J. L. Schulman and Dr E. D. Kilbourne, Mount Sinai School of Medicine, New York, were used. The same sample of inactivated serum was tested with all antigens, and sera obtained from the same individual at different times were tested with an antigen in the same HI test in duplicate. All HI tests were conducted over a 3 month period using the cells from three roosters. Volunteers receiving different vaccines within each age-group were included in each test so as to allow for comparability between vaccine groups. High and low titre positive controls and negative controls were used for each antigen to provide assurance that day-to-day variation did not preclude comparison between age-groups for each vaccine given. Geometric mean titres were calculated using logarithms to the base 2 (HI titre of <8 equal to 2), and are expressed as reciprocal serum titres.

Results

Vaccine reactions

Vaccine reactions were systematically obtained for all volunteers below age 70. They were similar for all vaccines and are summarized in Table 1. There was a uniformly low rate of febrile reactions ranging from 2 to 6%. The overall reaction rate was highest in the 10–19 age-group (46%) and lowest in the 32–69 age-group (13%). Local pain formed the largest single category of reaction. Perhaps the best indication of the mildness of the reactions was the 90% plus acceptance rate at the time of the post-immunization bleeding for an additional injection of standard vaccine for those who did not receive the vaccine containing the Aichi/68 antigen. The very low rate of reactions in the older age-groups is especially encouraging.

Baseline antibody profile

Previous influenza A experience for each of the age-groups as reflected by the pre-immunization sera is summarized in Table 2. Division of the volunteers into the specified age-groups was accomplished by graphing of the baseline titres according to individual years of birth and selecting the 'cut-off' for each primary influenza infection group by the last year showing a distinct prevalence of seropositive individuals.

The antibody prevalence figures for Japan/57 yields serological confirmation that virtually the entire population had been exposed to the Asian virus with the highest geometric mean titre (GMT) of 89 coming, as expected, in the 1950–61 cohort. A similar GMT is also seen in the 1940–49 cohort but then drops off considerably to the range of 30 in older age-groups despite maintaining a high antibody

TABLE 1. Monovalent influenza A vaccine reaction summary (1000 CCA units zonal purified)

Age group	Total	Total no. with reactions	No. with specific reactions			
			Local pain	Induration	Fever	Other
32–69	196	26 (13%)	12 (6%)	8	6 (3%)	10
20–31	136	53 (39%)	37 (27%)	22	3 (2%)	14
10–19	138	64 (46%)	60 (43%)	34	8 (6%)	0
6–9	50	15 (30%)	14 (28%)	9	1 (2%)	
Total	520	158 (30%)	123 (24%)	73	18 (3%)	24

TABLE 2. Initial geometric mean titres and prevalence of HI antibodies to major influenza A viruses in sera from 687 persons in summer 1971

Birth date	Primary infection with	No. of subjects	Influenza A antigens									
			Aichi/68 H3N2		Japan/57 H2N2		FM/1/47 H1N1		PR/8/34 H0N1		A/Swine/31 HswIN1	
			GMT	%	GMT	%	GMT	%	GMT	%	GMT	%
1962–65	HK	50	*16*	*60*	16	80	< 8	2	< 8	0	< 8	0
1950–61	Asian	173	41	80	*89*	*99*	< 8	13	< 8	22	< 8	2
1940–49	FM1	101	21	66	81	100	*23*	*90*	< 8	29	< 8	11
1928–39	PR8	86	20	69	36	91	16	93	*24*	*98*	< 8	31
1921–27	?	53	21	64	35	87	12	75	20	85	14	75
1906–20	Swine	66	16	59	23	83	13	83	11	73	*46*	*98*
1892–1905	?	75	35	91	23	89	13	83	11	68	42	92
1878–91	HK-like	60	*83*	*100*	17	80	9	65	9	58	17	77
1870–77	Asian-like	23	107	100	*26*	*87*	10	70	16	78	8	70

frequency throughout. The high prevalence of Asian antibodies in the 1962–65 cohort is doubtless because a last major Asian epidemic occurred less than a year before the Hong Kong epidemic of 1968–69.

It is readily apparent that human experience with the Hong Kong virus is much less extensive than that with Asian until one reaches the 1892–1905 cohort. The 1962–65 group had been insufficiently exposed to reflect all of the characteristics expected of the Hong Kong cohort with only a 60% HI antibody prevalence and a GMT of 16. The highest prevalence of Hong Kong antibodies before 1892–1905 is noted in the 1950–61 cohort, the 80% prevalence resulting in a GMT of 41. The previously documented period of Hong Kong-like virus prevalence is reflected here by the cohorts before 1892 having a 100% prevalence of Aichi/68 antibodies with the highest observed GMTs, 83 in the 1878–91 and 107 in the 1870–77 cohort.

The pattern of the other major influenza A antibodies shows the highest prevalence of respective antibodies associated with the FM1, PR8, and Swine virus cohorts; however, their geometric mean titres are quite low, lower in each instance than to Japan/57 except for the Swine virus period. In the 1906–20 Swine cohort the Swine GMT was twice the Japan/57 GMT, 46 compared with 23. Returning to the Aichi/68 column, we see that in the summer of 1971 more than 1/4 of the population born since 1905 had no detectable antigenic experience as measured by the HI test with this virus. This is entirely as expected since we had had only one severe HK epidemic, 1968–69, with a milder outbreak in 1969–70, and essentially no influenza A activity in 1970–71. This, then, gave us the opportunity to look at the primary response to the 1000 CCA unit Aichi vaccine. The response of those without detectable HI antibody was similar in the various age-groups and is summarized for these fifty-nine vaccinees in Table 3. The ⩾ four-fold seroconversion rate was excellent—85%; however, the final geometric mean titre was quite low—31. The heterologous responses in Japan/57 antibody are also nicely documented here with both the parent and haemagglutinin-specific recombinant showing greater than two-fold rises with final GMTs above that to Aichi virus which is the expected anamnestic response (Dowdle *et al.*, 1972). The low antibody level is consistent also with our results using 400 CCA unit Aichi vaccine in 1968 which also included simultaneous infection in a number of vaccinees and thus probably represents about all one can expect from initial exposure to the Hong Kong antigen (Marine, Workman & Webster, 1969).

Response to Japan/57 and Aichi/68 vaccines for those with HI antibody

In the major focus of the discussion we examine the response to Japan/57 and Aichi/68 vaccines among persons who had evidence of prior experience to the virus in the respective vaccine as determined by detachable HI antibody in the pre-immunization serum (Table 4). The age groups with similar response were further combined so that we have four major ranges represented here: 6–9, 10–31, 32–79, and 80–101. We have represented the response to each vaccine in the following ways: the GMT to S1 and S2, fold-rise in GMT, and the percentage with four-fold or greater rises.

TABLE 3. Response to 1000 CCA unit Aichi/68 zonal purified vaccine in fifty-nine persons with no initial homologous antibody, 1971

Antigen	Geometric mean titre		% with ⩾four-fold rise
	S1	S2	
Homologous:			
Aichi/68 H3N2	<8	31	85·0
Heterologous:			
Japan/57 H2N2	17	43	36·0
Japan/57 (H2)-NWS/33 (N1)	18	44	

TABLE 4. Homologous HI antibody response to 1000 CCA unit monovalent zonal purified inactivated vaccines by age, 1971

Birth date (age)	Antigen: Japan/57 (H2N2) 239 persons with initial antibody					Antigen: Aichi(68 (H3N2) 198 persons with initial antibody				
	No. of subjects	Geometric mean titre			% with ⩾four-fold rise	No. of subjects	Geometric mean titre			% with ⩾four-fold rise
		S1	S2	Fold-rise			S1	S2	Fold-rise	
1962–65 (6–9)	19	21	162	7·7	84·0	16	36	278	7·7	100·0
1940–61 (10–31)	71	82	309	3·8	52·0	54	53	221	4·2	54·0
1892–1939 (32–79)	113	38	98	2·6	30·0	88	38	119	3·1	39·0
1870–91 (80–101)	36	27	70	2·6	33·0	40	83	176	2·1	18·0

When measured in terms of fold-rises in titre and percentage with four-fold or greater rise, the 6–9 age-group clearly shows the best response; however, the S2 GMT in the 10–31 age-group was as high as the 6–9 with the Aichi vaccine and actually two-fold higher than the 6–9 with the Japan/57 vaccine. To some extent the higher S1 GMT in the 10–31 age-group, especially in the Japan/57 vaccine group, would explain the relative decrease in both GMT fold-rise and percentage with four-fold response. Certainly both these age-groups showed exceedingly good responses. On the other hand in the 32–79 age-group, there was much lower responsiveness as evaluated by *all* parameters. High S1 GMT cannot explain these results with either vaccine. Considerably less than a four-fold GMT response to each vaccine is seen with the final GMT only reaching the neighbourhood of 100 and only 30–40% showing a four-fold or greater rise in titre. In the 80 and over age-group, the response to Japan/57 vaccine differs from that to Aichi/68. With Japan/57 vaccine the low degree of response mimics that observed in the 32–79 age-group. With Aichi/68 vaccine, the considerably higher GMT of the baseline sera makes interpretation difficult. The final level of GMT reached was intermediate between 32–79 and 31 and under age-groups. We demonstrated in 1968 the unique responsiveness of this age-group to Aichi vaccine as an indication of prior antigenic experience with a Hong Kong-like virus (Marine & Workman, 1969).

Discussion

The main general conclusion we draw from these data is that a markedly better response is observed to both Japan/57 and Aichi/68 vaccines in persons born since 1940 than in persons born before 1940. What is the explanation for this rather striking difference?

One explanation might be that since the original antigenic sin of the 32–79 age-group was neither Japan/57 nor Aichi/68 perhaps the homologous response was attenuated due to heterologous response in original antigenic sin antibody. For example the PR8 cohort had PR8 antibody anamnestic response and the Swine cohort responded with Swine antibody. This explanation was easily put in doubt since no such boosting of antibodies to the other major influenza A viruses was noted—except of course that already mentioned of Aichi/68 stimulating Japan/57 antibody anamnestically.

A second explanation might interpret the response of the older age-groups as being abnormally low and cause one to wonder if some 'ageing' of the antibody-producing process is responsible. If this were true the process starts at a relatively young age, a phenomenon that has not been found with other antigens in man.

A third explanation which includes epidemiological considerations is the one that seems most plausible to us and is as follows: Persons born since 1940 were in their youth and consequently were most heavily exposed to both Japan/57 and Aichi/68 infection during their periods of prevalence. This undoubtedly included repeated exposures to Japan/57 and a residual reflection of this is the high baseline antibody levels against both viruses, 82 to Japan/57 and 53 to Aichi/68. It is these age-groups then who have had the most opportunity for stimulation of antibody-producing cells and might be expected to respond to antigenic stimulation by these viruses most vigorously. A more representative expression of each cohort's prior influenza experience is the overall GMT against each antigen as shown in Table 2. The distinctively high GMT in both the 1940–49 and 1950–61 cohorts to Japan/57 and in the 1950–61 cohort to Aichi/68 supports the suggestion of intensive prior exposure to these particular influenza antigens.

One implication of this line of reasoning is examined by comparing the response of the 1928–39 cohort, ages 32–43, to PR8, Asian, and Aichi vaccine in Table 5. We would expect the PR8 response to be the most pronounced due to its being the original

TABLE 5. Comparison of homologous HI antibody responses to 1000 CCA unit monovalent vaccines (1971), 1928–39 birth date cohort

Vaccine	No. subjects with initial antibody	Homologous geometric mean titre			% with ⩾four-fold rise
		S1	S2	Fold-rise	
PR/8/34 H0N1	29	26	184	7·1	66·0
Japan/57 H2N2	28	46	132	2·9	21·0
Aichi/68 H3N2	21	24	83	3·4	48·0

antigenic sin of this group, and so it was with a 7·1-fold GMT rise in this group compared to the three-fold range to the other vaccines and a 66% incidence of ⩾ four-fold rise compared to 21% for Japan/57 and 48% for Aichi/68.

The superior response to both vaccines in the 6–9 age-group cannot be explained by proposing multiple previous exposures to influenza virus. It is clear that this cohort's initial exposure to both Japan/57 and Aichi/68 antigens was at a time when the thymus was actively producing uncommitted lymphocytes. The secondary stimulus by the vaccines was also presented exclusively in this age-group at a time when the thymus was still active, and this combination of factors may be responsible for the extraordinary antibody response.

Whether one accepts these explanations of the findings, the central implication of the data is clear—one cannot expect antigenic response to influenza vaccine in one age-group to be mirrored by that in others. There has been a general tendency in the past to decide on dosage of vaccine based on studies in young adults and children. These data point to the necessity to determine potency in the age-group one is trying to protect. Since it is the elderly group for whom the toll of influenza includes mortality as well as morbidity, more attention needs to be paid to this age-group in determining potency of influenza vaccines.

The level at which HI antibody produces a protective effect varies with the antigen involved. Recent studies by Hobson *et al.* (1972) suggest that HI titres of 24 or above give good protection to Aichi/68 virus challenge. Even if an HI titre of 24 is all that is required, the observed response of fifty-nine persons with no detectable HI titre to 1000 CCA unit vaccine Aichi/68 would provide protective levels in approximately one-half, since the final GMT was 31. Assuming these findings can be confirmed, one has to question whether inactivated whole virus vaccine can produce a sufficient primary antigenic stimulus to yield sufficiently high levels for protection. One might consider giving a second injection of vaccine but we were unable to find data in the influenza literature that demonstrate a good booster effect to the second dose of vaccine except with a 7 month interval (Hennessy & Davenport, 1961). On the other hand we can be quite confident that inactivated vaccine can produce excellent levels of humoral antibody when the person has had prior exposure to the strain in question. Thus for primary immunizattion at the time of emergence of a new variant, we must consider use of adjuvant vaccines and live vaccines.

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