CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, Sept. 1996, p. 511–516 Vol. 3, No. 5
1071-412X/96/$04.00+0

Neuraminidase-Specific Antibody Responses to Inactivated Influenza Virus Vaccine in Young and Elderly Adults

DOUGLAS C. POWERS,[1,2]* EDWIN D. KILBOURNE,[3] AND BERT E. JOHANSSON[3]

Geriatric Research, Education and Clinical Center, St. Louis Veterans Administration Medical Center,[1] and Department of Internal Medicine and Center for Vaccine Development, St. Louis University,[2] St. Louis, Missouri, and Department of Microbiology and Immunology, New York Medical College, Valhalla, New York[3]

Received 14 March 1996/Returned for modification 30 April 1996/Accepted 20 May 1996

Little information is available on the potential role of antibody to influenza virus neuraminidase (NA) in vaccine-induced immunity. In the present study, serologic responses to the $N1_{Texas/91}$ and $N2_{Beijing/92}$ NA components of trivalent inactivated influenza virus vaccine were measured by NA inhibition (NI) and enzyme-linked immunosorbent assay (ELISA), and the results for adults aged 18 to 45 (young) or ≥65 (elderly) years were compared. The two age groups had comparable rates (32 to 50%) of NI response. In contrast, ELISA immunoglobulin G (IgG) antibody responses to N1 and N2 NAs occurred in 70 to 71 and 67 to 83%, respectively, of young subjects but in only 3 to 18 and 18 to 35%, respectively, of elderly subjects. Prevaccination mean ELISA IgG and IgA NA antibody titers were generally lower for the young adults than they were for the elderly, whereas the corresponding NI titers were comparable. In young adults, plaque size-reducing NA antibody increases were positively associated with ELISA but not with NI antibody increases. There were no apparent age-related differences in the immunoglobulin isotype distribution of the anti-NA response, with IgG being the dominant class and IgG1 the dominant subclass of serum antibody. Anti-hemagglutinin antibody responses to $H1_{Texas/91}$ and $H3_{Beijing/92}$ were greater in magnitude and frequency than the corresponding NA-specific responses to $N1_{Texas/91}$ and $N2_{Beijing/92}$ when measured by hemagglutination inhibition and NI, respectively, but not when measured by ELISA. The discordance between NI and ELISA for measurement of NA-specific vaccine responses may reflect the relative insensitivity of NI in discriminating differences when initial antibody titers are low.

Immunity to influenza virus is mediated principally by antibodies directed against the hemagglutinin (HA) and neuraminidase (NA) surface glycoproteins. Functionally distinct roles have been attributed to the humoral responses elicited by these two viral proteins. Antibody against HA generally neutralizes viral infectivity, presumably by interfering either with virus attachment to sialic acid receptors on the host cell surface or with the subsequent process of fusion between viral and endosomal membranes (20, 42). The prophylactic role of anti-HA antibody in conferring resistance to influenza virus infection is well documented (31, 40). In contrast, antibody to NA is infection permissive but limits the extent of disease by inhibiting the release of progeny viruses from infected cells (11, 12, 24, 25). Serum anti-NA titers are thus inversely related to the severity of clinical illness as well as the quantity and duration of viral shedding among infected persons (3, 26, 28, 30). Optimal protection against epidemic influenza illness is probably afforded by a combination of antibodies against both HA and NA glycoproteins of the circulating strain.

Inactivated influenza virus vaccines are recommended for annual immunization of targeted populations at high risk of serious medical complications following influenza infection (10). Currently available whole-virus or subvirion vaccines contain both HA and NA but are standardized according to the antigenic content of HA only. Immune responses following influenza virus vaccination have most commonly been assessed by using the hemagglutination inhibition (HAI) assay, which detects antibodies to HA and has been validated as a surrogate measure of vaccine efficacy (6, 8). NA-specific responses to vaccination have been less well characterized than HA-specific responses, in part because measurement of anti-NA antibodies has traditionally relied upon the NA inhibition (NI) assay, which is more cumbersome to perform than HAI. Serologic analysis by enzyme-linked immunosorbent assay (ELISA) using purified NA antigen may be more suitable than NI for screening large numbers of clinical specimens and can be more easily modified to assess the immunoglobulin (Ig) heavy-chain isotype profile of the antibody which is present. With the hope of finding reasons for the occasionally poor protection provided the elderly by influenza virus vaccines (2, 5, 27), this study was undertaken to compare NA-specific serum antibody responses to influenza virus vaccination measured by ELISA with those measured by NI in both young and elderly adults, as well as to assess the Ig class and subclass distribution of the anti-NA response.

MATERIALS AND METHODS

Clinical specimens and vaccines. Serum specimens used for these analyses were available from three separate investigations that had been previously conducted at St. Louis University (34, 36, 39). In each protocol, subjects had been immunized with a standard 0.5-ml intramuscular dose of trivalent subvirion vaccine for the 1993 to 1994 season which contained 15 μg each of the HAs from influenza A/Texas/36/91 (H1N1), A/Beijing/32/92 (H3N2), and B/Panama/45/90 viruses (either Fluzone, Connaught Laboratories, Swiftwater, Pa., or Flushield, Wyeth-Ayerst, Philadelphia, Pa.). Serum specimens had been obtained on the day of vaccination and 3 to 4 weeks later. Volunteers were excluded from enrollment in these studies if they had a history of either chronic underlying disease or use of medication known to be associated with immune suppression. Young and elderly adult subjects were aged 18 to 45 (mean ± standard deviation, 27 ± 8) or ≥65 (mean ± standard deviation, 76 ± 7) years, respectively.

NA antigens. The influenza A viruses used for the preparation of purified NA antigens were high-yield X-113 and X-118 reassortants containing HA and NA genes derived from the A/Texas/36/91 (H1N1) and A/Beijing/32/92 (H3N2) strains, respectively. Purified N1 and N2 NAs were obtained by passing octyl-

* Corresponding author. Mailing address: GRECC (11G-JB), St. Louis VAMC, St. Louis, MO 63125. Phone: (314) 894-6510. Fax: (314) 894-6614. Electronic mail address: powersdc@sluvca.slu.edu.

glucoside-disrupted virus through a DEAE-Sephadex A-50 column as previously described (12).

Serology. NI tests were performed in duplicate using purified $N1_{Texas/91}$ and $N2_{Beijing/92}$ NA antigens and an initial serum dilution of 1:4 (24). NA-specific IgG, IgA, and IgG subclass antibodies were measured by a previously described ELISA (12), with minor modifications. The sequence of reagents from the solid phase outward consisted of (i) purified NA antigen; (ii) serum specimen; (iii) alkaline phosphatase-conjugated anti-human IgG, IgA, IgG1, IgG2, IgG3, or IgG4; and (iv) *p*-nitrophenyl phosphate disodium substrate. The ELISA titer was expressed as the highest serum dilution at which the optical density of the antigen-containing well was at least twice that of the corresponding control well without antigen. Plaque size reduction (PSR) was assayed as previously described (11, 12), using X-118 virus-infected MDCK cells with incorporation of antiserum dilutions in agar overlays. Specimens were tested in duplicate. For titration of antibodies reactive with NA, the number and size of all individual plaques were scored for all serum dilutions. The endpoint was determined as the serum titer associated with 50% reduction of mean plaque size. HAI antibodies to influenza A/Texas/36/91 (H1N1) and A/Beijing/32/92 (H3N2) whole-virus antigens were measured by a standard microtiter assay (17), using an initial serum dilution of 1:4. HA-specific IgG antibodies were measured by ELISA as described above, using baculovirus-expressed purified recombinant HA (MicroGeneSys, Inc., Meriden, Conn.) from influenza A/Texas/36/91 (H1N1) or A/Beijing/32/92 (H3N2) virus as the coating antigen.

Statistical analyses. Reciprocal antibody titers were logarithmically transformed for statistical analysis. A significant antibody response was defined as a fourfold or greater rise in titer between prevaccination and postvaccination specimens. Differences between groups were analyzed by using the Fisher's exact test to compare proportions of vaccinees with significant antibody responses and the Mann-Whitney U test to compare reciprocal $\log_2$ antibody titers.

RESULTS

Age-related differences in NA-specific antibody responses to vaccination. The results of NA-specific antibody assays are summarized in Table 1. For each of the two influenza A virus NA antigens contained within the vaccines, the data are stratified into four groups according to the vaccine manufacturer and the age of the recipients. Neither of these variables (i.e., vaccine manufacturer or subject age) had a significant effect on NI antibody responses to vaccination. In the case of both $N1_{Texas/91}$ and $N2_{Beijing/92}$ antigens, all groups mounted two- to fourfold increases in the mean titers of NI antibody and demonstrated comparable rates (32 to 50%) of NI response. In contrast, there was a consistent and statistically significant effect of age on the rate of NA-specific responses measured by ELISA. Fourfold or greater increases in titers of IgG antibody to N1 and N2 NAs occurred in 70 to 71 and 67 to 83%, respectively, of young subjects but in only 3 to 18% ($P < 0.0005$) and 18 to 35% ($P < 0.002$), respectively, of elderly subjects, as determined by ELISA. A similar trend was apparent when ELISA IgA NA responses for the two age groups were compared, although the frequency and magnitude of serum IgA antibody titer rises following vaccination were lower than the corresponding IgG responses. These age-related differences in rates of ELISA NA-specific antibody responses to vaccination may reflect the fact that the mean ELISA antibody titers at baseline were generally lower for the young adults than they were for the elderly. For example, younger persons had prevaccination mean ELISA IgG N1 NA titers that were 2- to 10-fold lower than those of the elderly despite having corresponding NI titers that were almost equivalent.

Discordance between NI and ELISA for measurement of vaccine NA response. Young adults invariably had higher and more frequent rises in titer of ELISA IgG NA antibody than of NI antibody. The converse was true for the elderly in the case of N1 NA responses, whereas N2 NA-specific ELISA IgG and NI responses were comparable in this age group. Contingency analysis was used to determine whether those persons who mounted a significant (i.e., fourfold or greater) increase in NI titer following vaccination were the same individuals who demonstrated a significant rise by ELISA. We pooled the data for all subjects within each age group because serologic responses

TABLE 1. Serum NA-specific antibody responses to the influenza A virus N1 and N2 components of licensed trivalent subvirion vaccines for 1993 to 1994 in young and elderly adult subjects

Antigen	Vaccine manufacturer	Age group (no. tested)	NI antibody			ELISA IgG NA antibody			ELISA IgA NA antibody		
			Titer[a]		% of subjects with ≥4-fold titer rise	Titer		% of subjects with ≥4-fold titer rise	Titer		% of subjects with ≥4-fold titer rise
			Pre	Post		Pre	Post		Pre	Post	
$N1_{Texas/91}$	Connaught	Young (23)	2.7 ± 0.2	4.0 ± 0.4	35	6.7 ± 0.2	8.5 ± 0.3[b]	70[c]	5.9 ± 0.3	6.7 ± 0.3[d]	17
		Elderly (63)	3.0 ± 0.2	4.3 ± 0.2	35	10.0 ± 0.1	10.4 ± 0.2	3	7.4 ± 0.2	7.8 ± 0.2	5
	Wyeth-Ayerst	Young (24)	2.4 ± 0.2	3.6 ± 0.3	33	8.2 ± 0.3	10.2 ± 0.2	71[c]	5.6 ± 0.2	6.7 ± 0.3[e]	25
		Elderly (22)	2.2 ± 0.1	3.2 ± 0.3	32	9.1 ± 0.3	9.7 ± 0.3	18	7.6 ± 0.3	8.3 ± 0.3	18
$N2_{Beijing/92}$	Connaught	Young (23)	2.2 ± 0.1	3.8 ± 0.4	43	11.1 ± 0.2	14.3 ± 0.3	83[c]	6.4 ± 0.4	9.0 ± 0.6	70[c]
		Elderly (63)	2.6 ± 0.1	3.8 ± 0.2	35	12.4 ± 0.1	13.6 ± 0.2	35	8.2 ± 0.3	9.2 ± 0.3	24
	Wyeth-Ayerst	Young (24)	2.6 ± 0.2	4.4 ± 0.3	50	12.6 ± 0.2	14.8 ± 0.3[b]	67[f]	7.6 ± 0.6	9.4 ± 0.6	50
		Elderly (22)	2.3 ± 0.2	3.4 ± 0.4	32	12.0 ± 0.2	13.1 ± 0.3	18	8.4 ± 0.4	9.7 ± 0.5	36

[a] Antibody titers are expressed as mean reciprocal $\log_2$ ± standard error of the mean (SEM). Pre and Post, prevaccination and postvaccination, respectively.
[b] $P < 0.0001$ in comparison with the respective value for elderly subjects (Mann-Whitney U test).
[c] $P < 0.0005$ in comparison with the respective value for elderly subjects (Fisher's exact test).
[d] $P < 0.01$ in comparison with the respective value for elderly subjects (Mann-Whitney U test).
[e] $P < 0.005$ in comparison with the respective value for elderly subjects (Mann-Whitney U test).
[f] $P < 0.001$ in comparison with the respective value for elderly subjects (Fisher's exact test).

TABLE 2. Concordance between NI and ELISA IgG NA antibody responses[a] following influenza virus vaccination in young and elderly adults

Age group	No. tested[b]	N1-specific antibody			N2-specific antibody		
		NI response	No. with the indicated ELISA response		NI response	No. with the indicated ELISA response	
			Positive	Negative		Positive	Negative
Young	47	Positive	8	8	Positive	18	4
		Negative	25	6	Negative	17	8
Elderly	85	Positive	4	25	Positive	9	20
		Negative	2	54	Negative	17	39

[a] For each assay, a response to vaccination was defined as a fourfold or greater rise in antibody titer.
[b] Each group includes all individuals within the respective age category shown in Table 1.

to the Connaught and Wyeth-Ayerst vaccines were approximately equivalent (with the single exception of the ELISA IgG N1 NA response rate of elderly subjects to the Connaught [3%] versus the Wyeth-Ayerst [18%] vaccine [$P = 0.036$, Fisher's exact test]). A total of 41 young and 31 elderly vaccine recipients demonstrated a fourfold or greater rise in N1 NA-specific antibody titer by either NI or ELISA (Table 2). Of these N1 responders, only eight (20%) young and four (13%) elderly subjects demonstrated a significant increase in titer by both assays. Twenty-five (61%) of the young N1 responders, compared with only two (6%) of the elderly ($P < 0.0001$), had a vaccine response determined by ELISA but not by NI (i.e., were $NI^-/ELISA^+$). Conversely, 8 (20%) of the young N1 responders, compared with 25 (81%) of the elderly ($P < 0.0001$), responded only by NI (i.e., were $NI^+/ELISA^-$). Similar trends were apparent when the results of N2 NA-specific assays were examined. Among the N2 responders, an $NI^-/ELISA^+$ pattern of response occurred in 44% of young and 37% of elderly subjects, whereas 4 (10%) of the young and 20 (43%) of the elderly ($P < 0.001$) were $NI^+/ELISA^-$.

PSR. The lack of concordance between NI and ELISA for detection of vaccine responses suggested that the two assays may be measuring different antibodies. To investigate whether antibodies detected by NI and ELISA can be distinguished on the basis of functional (i.e., infection-limiting) characteristics, $N2_{Beijing/92}$ NA-specific PSR was measured in paired serum specimens from a subset of individuals who demonstrated either an $NI^+/ELISA^-$ or an $NI^-/ELISA^+$ profile of response to vaccination. Among young adults, PSR increased at least twofold in three of four individuals who responded only by ELISA but in zero of three who responded only by NI (Table 3). Elderly adults, on the other hand, showed a modest rate of PSR response (20 to 29%) to vaccination regardless of whether they were $NI^-/ELISA^+$ or $NI^+/ELISA^-$. As expected, individuals of either age group who had fourfold or greater rises in titers of both NI and ELISA antibody were also generally found to have increases in PSR (data not shown).

IgG subclass distribution of NA-specific antibodies. IgG subclass-specific antibody titers in a subset of postvaccination serum specimens selected on the basis of having similar titers of total IgG NA antibody were measured by ELISA. Serum anti-NA antibodies were predominantly of the IgG1 subclass, with a minor contribution by IgG3 (Table 4). Using a conventional positive-to-negative ratio of 2:1 to discriminate optical density measurements for antigen-coated wells from those for control wells, we were unable to detect antibodies of the IgG2 or IgG4 subclass in any of the specimens, even at serum dilutions as low as 1:4. There were no apparent age-related differences between the IgG subclass distributions of antibodies to N1 and N2 NA.

HA-specific antibody responses to vaccination. Serum specimens were tested for HA-specific antibodies by HAI and ELISA. Vaccination induced higher and more frequent HAI antibody titer rises in young adults than in elderly subjects (Table 5), unlike the NI response, which was not appreciably influenced by age (Table 1). Immunization elicited better HAI than NI responses overall, except for the poor responses to both $H1_{Texas/91}$ and $N1_{Texas/91}$ antigens that occurred in elderly recipients of the Connaught vaccine. ELISA IgG HA responses, on the other hand, were approximately equivalent to ELISA IgG NA responses with regard to age-related differences in mean antibody titers at baseline, mean titer rises following immunization, and fourfold or greater response rates.

DISCUSSION

Despite the fact that currently available inactivated influenza virus vaccines contain both HA and NA, relatively little

TABLE 3. Relationship between N2 NA-specific PSR and either NI or ELISA IgG NA antibody response following immunization with subvirion influenza virus vaccine in young and elderly adults

Antibody response profile	Age group (no. tested)	NI antibody			ELISA IgG NA antibody			PSR		
		Titer[a]		% of subjects with ≥4-fold titer rise	Titer		% of subjects with ≥4-fold titer rise	Titer		% of subjects with ≥2-fold titer rise
		Pre	Post		Pre	Post		Pre	Post	
$NI^+/ELISA^-$	Young (3)	2.0 ± 0.0	5.2 ± 0.2	100	12.6 ± 0.8	13.6 ± 0.8	0	11.0 ± 0.1	11.1 ± 0.1	0
	Elderly (7)	2.0 ± 0.0	5.2 ± 0.3	100	12.7 ± 0.2	13.2 ± 0.3	0	11.3 ± 0.3	11.9 ± 0.3	29
$NI^-/ELISA^+$	Young (4)	3.3 ± 0.9	3.4 ± 1.0	0	12.1 ± 0.9	15.3 ± 1.1	100	10.5 ± 0.6	11.9 ± 0.5	75
	Elderly (5)	2.6 ± 0.6	2.7 ± 0.7	0	11.9 ± 0.4	15.5 ± 0.7	100	11.1 ± 0.3	11.7 ± 0.4	20

[a] Titers are expressed as mean reciprocal $\log_2$ ± SEM. Pre and Post, prevaccination and postvaccination, respectively.

TABLE 4. IgG subclass distribution of serum NA-specific antibodies following immunization of young and elderly adults with trivalent subvirion influenza virus vaccine

Antigen	Age group (no. tested)	Postvaccination ELISA NA antibody titer (reciprocal mean $\log_2 \pm$ SEM)				
		IgG	IgG1	IgG2	IgG3	IgG4
$N1_{Texas/91}$	Young (11)	10.4 ± 0.3	8.8 ± 0.3	<2.0	4.6 ± 0.6	<2.0
	Elderly (6)	10.3 ± 0.6	8.3 ± 0.4	<2.0	5.2 ± 0.5	<2.0
$N2_{Beijing/92}$	Young (12)	14.1 ± 0.3	12.6 ± 0.3	<2.0	5.0 ± 1.0	<2.0
	Elderly (12)	14.1 ± 0.3	12.5 ± 0.4	<2.0	5.6 ± 1.1	<2.0

attention has been given to the potential contribution of NA-specific responses to influenza virus vaccine-induced immunity. Antibody to NA is nonneutralizing and infection permissive but capable of inducing protection by reducing viral replication to below a pathogenic threshold (25). Clinical studies have demonstrated that preexisting antibodies to influenza virus NA are associated with the amelioration of the severity of illness following natural exposure or artificial challenge with either wild-type or attenuated influenza viruses (3, 26, 28, 30). NA-specific immunity may be particularly relevant for older adults, since this population is known to incur a disproportionately high rate of serious medical complications despite experiencing low rates of infection during influenza epidemics (33). There is little published information regarding the ability of inactivated influenza virus vaccines to elicit NA-specific antibodies, particularly in the elderly (4, 15, 29). Furthermore, anti-NA responses to vaccination in older adults have been measured exclusively by NI. The present study characterized NA-specific serologic responses of young and elderly adults to licensed subvirion vaccines by using both NI and ELISA. Our data reveal that the two assays can yield discordant results and that their relative sensitivity for detecting vaccine responses may be differentially affected by age.

Given that both H1N1 and H3N2 subtypes of influenza A virus have been in circulation for the past 2 or 3 decades, and given that primary influenza virus infection in humans tends to occur during childhood, it can be assumed that most current-day adults are immunologically primed to mount secondary responses upon reexposure to either N1 or N2 antigen. The 30 to 50% rates of serum NI antibody response to the N1 and N2 NAs of the component strains that were observed among our subjects are comparable to those that have been previously reported following administration of conventional inactivated-virus vaccine to immunologically primed adults (4, 15, 16, 19, 21, 22, 29). As in this study, other investigators have found no significant effect of age on NI responses to vaccination apart from the influence of immunologic priming engendered by prior natural exposure to heterologous NA antigens (18).

The present data show that ELISA was more sensitive than NI for detecting NA-specific antibody responses to vaccination in young adults. This finding contrasts with a recently published clinical trial comparing serologic responses of young adults following immunization with conventional subvirion or purified NA vaccines containing $N2_{Beijing/92}$, in which there was generally good agreement between the results of ELISA and NI (23). Differences in the vaccines and assay antigens used may account for the contrasting results between these investigations. On the other hand, the discordance between the two serologic methods shown here may reflect the relative insensitivity of NI for discriminating differences between levels of antibody when present at low initial titers. The young adults clearly had lower prevaccination titers of ELISA NA-specific

TABLE 5. Serum HA-specific antibody responses to the influenza A virus H1 and H3 components of licensed trivalent subvirion vaccines for 1993 to 1994 in young and elderly adult subjects

Antigen	Vaccine manufacturer	Age group (no. tested)	HAI antibody			ELISA IgG HA antibody		
			Titer[a]		% of subjects with ≥4-fold titer rise	Titer		% of subjects with ≥4-fold titer rise
			Pre	Post		Pre	Post	
$H1_{Texas/91}$	Connaught	Young (23)	6.2 ± 0.4	11.0 ± 0.3[b]	91[c]	9.5 ± 0.4	12.2 ± 0.3	74[c]
		Elderly (63)	7.0 ± 0.2	7.5 ± 0.2	10	11.7 ± 0.1	12.1 ± 0.1	3
	Wyeth-Ayerst	Young (24)	8.1 ± 0.4	12.3 ± 0.3[b]	83	11.1 ± 0.4	13.8 ± 0.3	63[d]
		Elderly (22)	7.5 ± 0.5	9.9 ± 0.4	73	12.7 ± 0.3	13.6 ± 0.3	14
$H3_{Beijing/92}$	Connaught	Young (23)	3.9 ± 0.4	9.4 ± 0.5[e]	100[f]	8.3 ± 0.4	12.3 ± 0.4	91[c]
		Elderly (63)	4.7 ± 0.3	8.1 ± 0.3	76	10.4 ± 0.2	11.6 ± 0.2	37
	Wyeth-Ayerst	Young (24)	6.1 ± 0.4	10.0 ± 0.4[g]	75	12.0 ± 0.2	13.9 ± 0.3	63[h]
		Elderly (22)	6.0 ± 0.4	8.2 ± 0.4	59	12.7 ± 0.2	13.8 ± 0.3	32

[a] Antibody titers are expressed as mean reciprocal $\log_2 \pm$ SEM. Pre and Post, prevaccination and postvaccination, respectively.
[b] $P < 0.0001$ in comparison with the respective value for elderly subjects (Mann-Whitney U test).
[c] $P < 0.0001$ in comparison with the respective value for elderly subjects (Fisher's exact test).
[d] $P < 0.001$ in comparison with the respective value for elderly subjects (Fisher's exact test).
[e] $P < 0.02$ in comparison with the respective value for elderly subjects (Mann-Whitney U test).
[f] $P < 0.01$ in comparison with the respective value for elderly subjects (Fisher's exact test).
[g] $P < 0.005$ in comparison with the respective value for elderly subjects (Mann-Whitney U test).
[h] $P < 0.05$ in comparison with the respective value for elderly subjects (Fisher's exact test).

antibody than their elderly counterparts that were not reflected by differences in NI titer. It has similarly been shown that ELISA using purified HA antigen is superior to HAI at distinguishing differences in response with low initial antibody titers (35).

In the present study, it is also possible that the lack of agreement between NI and ELISA may be related to qualitative differences between the antibodies detected by these two assays. Among a small subset of young adults who achieved fourfold or greater rises in antibody titers in only one serologic test (i.e., who were either $NI^+/ELISA^-$ or $NI^-/ELISA^+$), PSR was enhanced exclusively in persons with an ELISA response. This intriguing observation needs to be verified with larger sample sizes but suggests that there may be functional differences between NA antibodies measured by NI and by ELISA and that the latter assay may give a more physiologically relevant in vitro measure of NA-specific immunity.

NA-specific antibodies in postvaccination serum specimens were found to have an Ig isotype profile similar to that which has previously been reported for anti-HA responses, with IgG being the dominant class and IgG1 the major subclass (1, 9, 41). Earlier studies have also documented, as we did, modest titers of IgG3 antibody following influenza virus vaccination but little or no IgG2 or IgG4 antibody (9, 32, 37, 41). These findings are consistent with the observation that soluble peptides preferentially induce IgG1 and IgG3 responses in humans (7). Since IgG subclasses vary considerably with respect to physiologic function (38), we speculated that the observed discrepancies between total IgG antibody levels measured by ELISA versus functional activity measured by NI might be related to age-associated differences in the IgG isotype profile of NA-specific antibodies. No such age effect was apparent, however, when the data from a subset of specimens were compared (Table 4).

The administration of inactivated influenza virus vaccine to immunologically primed subjects has repeatedly been shown to induce better HAI than NI responses to the corresponding HA and NA antigens of the component strain(s) (16, 19, 21, 22). This observation has been explained on the basis of intermolecular HA-NA antigenic competition that occurs when the two glycoproteins are presented as structurally associated antigens on intact or disrupted viral particles (13, 14). In a host that has been previously immunized with an antigenically similar HA, a whole-virus or subvirion vaccine will thus induce a skewed response favoring the HA as the major viral glycoprotein. In the present study, HA-specific responses to $H1_{Texas/91}$ and $H3_{Beijing/92}$ were greater in magnitude and frequency than the corresponding NA-specific responses to $N1_{Texas/91}$ and $N2_{Beijing/92}$ when measured by HAI and NI, respectively, but not when measured by ELISA. The similarity between HA- and NA-specific ELISA responses was unexpected and difficult to reconcile with the concept of HA-NA antigenic competition favoring an anti-HA response.

To our knowledge, this is the first published report of a study in which HA- and NA-specific responses to influenza virus vaccination in adult subjects have both been measured by ELISA. Additional studies are warranted to confirm the present observations, which suggest the possibility that the skewing of vaccine responses heretofore attributed to HA-NA antigenic competition may be due, in part, to relative differences in the sensitivities of HAI and NI for detection of antibodies. On the other hand, priming of this population with N2 NA has been going on since 1957, and priming with N1 NA has taken place since 1918 or 1947. With respect to young adults, HA priming is relatively recent, dating from 1968 in the case of H3 and 1977 in the case of H1. HA antibodies, even measured by ELISA, can thus be viewed as relatively more abundant than those induced by NA, even after more-protracted exposure to the latter antigen.

ACKNOWLEDGMENTS

Funding for this work was provided by Public Health Service grant AG-11876 (D.C.P.) from the NIA and contract AI-05051 from the NIAID.

We thank Mary Manning, Bernardo Grajower, and Barbara Pokorny for technical assistance in the laboratory.

REFERENCES

1. **Clements, M. L., and B. R. Murphy.** 1986. Development and persistence of local and systemic antibody responses in adults given live attenuated or inactivated influenza A virus vaccine. J. Clin. Microbiol. **23:**66–72.
2. **Coles, F. B., G. J. Balzano, and D. L. Morse.** 1992. An outbreak of influenza A (H3N2) in a well immunized nursing home population. J. Am. Geriatr. Soc. **40:**589–592.
3. **Couch, R. B., J. A. Kasel, J. L. Gerin, J. L. Schulman, and E. D. Kilbourne.** 1974. Induction of partial immunity to influenza by a neuraminidase-specific vaccine. J. Infect. Dis. **129:**411–420.
4. **Fedson, D. S., R. V. Fulk, M. A. Huber, M. A. Reisberg, and J. A. Kasel.** 1971. Antineuraminidase antibody response in serum and nasal secretions following intranasal or subcutaneous inactivated A2/Hong Kong/68 influenza virus vaccine. J. Immunol. **107:**730–737.
5. **Feery, B. J., M. G. Evered, and E. Morrison.** 1979. Different protection rates in various groups of volunteers given subunit influenza virus vaccine in 1976. J. Infect. Dis. **139:**237–241.
6. **Gross, P. A., F. A. Ennis, P. F. Gaerlan, L. J. Denson, C. R. Denning, and D. Schiffman.** 1977. A controlled double-blind comparison of the reactogenicity, immunogenicity, and protective efficacy of whole-virus and split-product influenza vaccines in children. J. Infect. Dis. **136:**623–632.
7. **Hammarström, L., and C. I. Edvard Smith.** 1986. IgG subclass changes in response to vaccination. Monogr. Allergy **19:**241–252.
8. **Hobson, D., R. C. Curry, A. S. Beare, and A. Ward-Gardner.** 1973. Hemagglutination inhibition antibody titers as a measure of protection against influenza in man. Dev. Biol. Stand. **20:**164–168.
9. **Hocart, M. J., J. S. Mackenzie, and G. A. Stewart.** 1990. Serum IgG subclass responses of humans to inactivated and live influenza A vaccines compared to natural infections with influenza A. J. Med. Virol. **30:**92–96.
10. **Immunization Practices Advisory Committee.** 1994. Prevention and control of influenza, part I. Vaccines. Morbid. Mortal. Weekly Rep. **43:**1–13.
11. **Jahiel, R. I., and E. D. Kilbourne.** 1966. Reduction in plaque size and reduction in plaque number as differing indices of influenza virus-antibody reactions. J. Bacteriol. **92:**1521–1534.
12. **Johansson, B. E., D. J. Bucher, and E. D. Kilbourne.** 1989. Purified influenza virus hemagglutinin and neuraminidase are equivalent in stimulation of antibody response but induce contrasting types of immunity to infection. J. Virol. **63:**1239–1246.
13. **Johansson, B. E., and E. D. Kilbourne.** 1993. Dissociation of influenza virus hemagglutinin and neuraminidase eliminates their intravirionic antigenic competition. J. Virol. **67:**5721–5723.
14. **Johansson, B. E., T. M. Moran, and E. D. Kilbourne.** 1987. Antigen-presenting B cells and T_H cells cooperatively mediate intravirionic antigenic competition between influenza A virus surface glycoproteins. Proc. Natl. Acad. Sci. USA **84:**6869–6873.
15. **Kasel, J. A., R. B. Couch, H. R. Six, and V. Knight.** 1976. Antigenicity of licensed whole virion and subvirion influenza vaccines in "high risk" persons. Proc. Soc. Exp. Biol. Med. **151:**742–747.
16. **Kendal, A. P., F. M. Bozeman, and F. A. Ennis.** 1980. Further studies of the neuraminidase content of inactivated influenza vaccines and the neuraminidase antibody responses after vaccination of immunologically primed and unprimed populations. Infect. Immun. **29:**966–971.
17. **Kendal, A. P., and W. R. Dowdle.** 1986. Influenza virus, p. 515–520. *In* N. R. Rose, H. Friedman, and J. L. Fahey (ed.), Manual of clinical laboratory immunology, 3rd ed. American Society for Microbiology, Washington, D.C.
18. **Kendal, A. P., E. Minuse, H. F. Maassab, A. V. Hennessy, and F. M. Davenport.** 1973. Influenza neuraminidase antibody patterns of man. Am. J. Epidemiol. **98:**96–103.
19. **Kendal, A. P., G. R. Noble, and W. R. Dowdle.** 1977. Neuraminidase content of influenza vaccines and neuraminidase antibody responses after vaccination of immunologically primed and unprimed populations. J. Infect. Dis. **136:**S415–S424.
20. **Kida, H., R. G. Webster, and R. Yanagawa.** 1983. Inhibition of virus-induced hemolysis with monoclonal antibodies to different antigenic areas on the hemagglutinin molecule of A/Seal/Massachusetts/1/80 (H7N7) influenza virus. Arch. Virol. **76:**91–99.
21. **Kilbourne, E. D.** 1976. Comparative efficacy of neuraminidase-specific and conventional influenza virus vaccines in induction of antibody to neuramin-

idase in humans. J. Infect. Dis. **134:**384–394.
22. **Kilbourne, E. D., C. P. Cerini, M. W. Khan, J. W. Mitchell, Jr., and P. L. Ogra.** 1987. Immunologic response to the influenza virus neuraminidase is influenced by prior experience with the associated viral hemagglutinin. I. Studies in human vaccinees. J. Immunol. **138:**3010–3013.
23. **Kilbourne, E. D., R. B. Couch, J. A. Kasel, W. A. Keitel, T. R. Cate, J. H. Quarles, B. Grajower, B. A. Pokorny, and B. E. Johansson.** 1995. Purified influenza A virus N2 neuraminidase vaccine is immunogenic and non-toxic in humans. Vaccine **13:**1799–1803.
24. **Kilbourne, E. D., W. G. Laver, J. L. Schulman, and R. G. Webster.** 1968. Antiviral activity of antiserum specific for an influenza virus neuraminidase following influenza. I. In vitro effects. J. Virol. **2:**281–288.
25. **Kilbourne, E. D., P. Palese, and J. L. Schulman.** 1975. Inhibition of viral neuraminidase as a new approach to the prevention of influenza, p. 99–113. *In* M. Pollard (ed.), Perspectives in virology, vol. 9. Academic Press, New York.
26. **Monto, A. S., and A. P. Kendal.** 1973. Effect of neuraminidase antibody on Hong Kong influenza. Lancet **i:**623–625.
27. **Morens, D. M., and V. M. Rash.** 1995. Lessons from a nursing home outbreak of influenza A. Infect. Control Hosp. Epidemiol. **16:**275–280.
28. **Murphy, B. R., J. A. Kasel, and R. M. Chanock.** 1972. Association of serum antineuraminidase antibody with resistance to influenza in man. N. Engl. J. Med. **286:**1329–1332.
29. **Odelin, M. F., B. Pozzetto, M. Aymard, M. Defayolle, and J. Jolly-Million.** 1993. Role of influenza vaccination in the elderly during an epidemic of A/H1N1 virus in 1988–1989: clinical and serological data. Gerontology **39:** 109–116.
30. **Ogra, P. L., T. Chow, K. R. Beutner, E. Rubi, J. Strussenberg, S. DeMello, and C. Rizzone.** 1977. Clinical and immunologic evaluation of neuraminidase-specific influenza A virus vaccine in humans. J. Infect. Dis. **135:**499–506.
31. **Potter, C. W., and J. S. Oxford.** 1979. Determinants of immunity to influenza infection in man. Br. Med. Bull. **35:**69–75.
32. **Powers, D. C.** 1994. Effect of age on serum immunoglobulin G subclass antibody responses to inactivated influenza virus vaccine. J. Med. Virol. **43:**57–61.
33. **Powers, D. C.** 1994. Influenza vaccination strategies in the elderly. Aging Immunol. Infect. Dis. **5:**47–54.
34. **Powers, D. C., P. J. Hanscome, and P. J. F. Pietrobon.** 1995. In previously immunized elderly adults inactivated influenza A (H1N1) virus vaccines induce poor antibody responses that are not enhanced by liposome adjuvant. Vaccine **13:**1330–1335.
35. **Powers, D. C., B. R. Murphy, L. F. Fries, W. H. Adler, and M. L. Clements.** 1992. Reduced infectivity of cold-adapted influenza A H1N1 viruses in the elderly: correlation with serum and local antibodies. J. Am. Geriatr. Soc. **40:**163–167.
36. **Powers, D. C., G. E. Smith, E. L. Anderson, D. J. Kennedy, C. S. Hackett, B. E. Wilkinson, F. Volvovitz, R. B. Belshe, and J. J. Treanor.** 1995. Influenza A virus vaccines containing purified recombinant H3 hemagglutinin are well-tolerated and induce protective immune responses in healthy adults. J. Infect. Dis. **171:**1595–1599.
37. **Remarque, E. J., W. C. A. van Beek, G. J. Ligthart, R. J. A. Borst, L. Nagelkerken, A. M. Palache, M. J. W. Sprenger, and N. Masurel.** 1993. Improvement of the immunoglobulin subclass response to influenza vaccine in elderly nursing-home residents by the use of high-dose vaccines. Vaccine **11:**649–654.
38. **Spiegelberg, H. L.** 1974. Biological activities of immunoglobulins of different classes and subclasses. Adv. Immunol. **19:**259–294.
39. **Treanor, J. J., R. F. Betts, G. E. Smith, E. L. Anderson, C. S. Hackett, B. E. Wilkinson, R. B. Belshe, and D. C. Powers.** 1996. Evaluation of a recombinant hemagglutinin expressed in insect cells as an influenza vaccine in young and elderly adults. J. Infect. Dis. **173:**1467–1470.
40. **Virelizier, J.** 1975. Host defense against influenza virus: the role of anti-hemagglutinin antibody. J. Immunol. **115:**434–439.
41. **Wagner, D. K., M. L. Clements, C. R. Reimer, M. Snyder, D. L. Nelson, and B. R. Murphy.** 1987. Analysis of immunoglobulin G antibody responses after administration of live and inactivated influenza A vaccine indicates that nasal wash immunoglobulin G is a transudate from serum. J. Clin. Microbiol. **25:**559–562.
42. **Yoden, S., H. Kida, M. Kawabara, R. Yanagawa, and R. G. Webster.** 1986. Spin labeling of influenza virus hemagglutinin permits analysis of the conformational change at low pH and its inhibition by antibody. Virus Res. **4:**251–261.