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Systemic and Local Antibody Responses in Elderly Subjects Given Live or Inactivated Influenza A Virus Vaccines

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Intranasal live attenuated cold-adapted (*ca*) influenza A/Kawasaki/9/86 (H1N1) reassortant virus and parenteral inactivated influenza A/Taiwan/1/86 (H1N1) virus were given alone or in combination to 80 ambulatory elderly subjects. An enzyme-linked immunosorbent assay was used to measure hemagglutinin-specific (HA) antibodies in serum and nasal wash specimens collected before vaccination and 1 and 3 months later. Serum immunoglobulin G (IgG) and nasal wash IgA HA responses were elicited in 56 and 20%, respectively, of 25 inactivated-virus vaccinees and in 67 and 48%, respectively, of 27 recipients of both vaccines but in only 36 and 25%, respectively, of 28 vaccinees given live virus alone. Inactivated virus, administered alone or with live virus vaccine, induced higher titers of serum antibody than did the live virus alone. In contrast, nasal IgA HA antibody was elicited more often and in greater quantity by the vaccine combination than by either vaccine alone. Despite these differences, the peak titers of local antibody mounted by each group of vaccinees were similar. By 3 months postvaccination, serum IgG and nasal IgA HA antibody titers remained elevated above prevaccination levels in 50 and 17%, respectively, of the inactivated-virus vaccinees and in 46 and 23%, respectively, of recipients of both vaccines but in only 19 and 7%, respectively, of the live-virus vaccinees. The finding that live *ca* influenza A virus induced short-lived local and systemic antibodies, if confirmed, suggests that live virus vaccination may not be a suitable alternative or adjunct to inactivated virus vaccination for the elderly.

Influenza epidemics have been consistently associated with increased rates of severe illness and mortality in elderly populations (5). Persons aged 65 years or more have therefore been targeted along with other high-risk groups for annual influenza immunization (21). Parenterally administered inactivated influenza virus vaccines have generally demonstrated limited efficacy in preventing illness among institutionalized elderly populations, and they may confer less protection to older adults than to younger persons (3, 6, 14, 19, 20, 33, 37). For this reason, attention has been directed to the development of new approaches to influenza immunization that will provide better protection against serious illness in the elderly.

Resistance to influenza virus infection in children and young adults has been found to correlate with several immune parameters, including the presence of hemagglutinin-specific (HA) antibodies in both serum and nasal secretions (11, 22). Thus, influenza immunization that stimulates both systemic and local antibody responses may be optimal. Parenteral inactivated influenza virus vaccines induce antibody to HA primarily in the serum, but they are less effective at stimulating the production of local (secretory) immunoglobulin A (IgA) HA antibody (10, 12). On the other hand, intranasally administered, live attenuated cold-adapted (*ca*) influenza A reassortant virus vaccines elicit secretory IgA HA antibody responses in nasal secretions of children and young adults, in addition to IgG and IgA HA antibodies in serum (12, 28). In older adults, however, *ca* influenza A virus vaccines have been shown to be substantially less effective than subsequently administered inactivated virus vaccine at inducing serum antibody (17). Moreover, only one previously published study has reported secretory antibody responses in older adults after live virus vaccination (18).

This investigation was undertaken to evaluate the immunogenicity of live attenuated *ca* influenza A virus in elderly adults and to determine whether the frequency, magnitude, and duration of secretory and serum antibody responses in this population were greater after simultaneous immunization with live and inactivated virus vaccines than with either vaccine alone.

MATERIALS AND METHODS

Vaccines. Two antigenically similar, monovalent influenza A (H1N1) virus vaccines were used in this study: a live attenuated *ca* A/Kawasaki/9/86 reassortant virus and a commercial, inactivated influenza A/Taiwan/1/86 subvirion vaccine. The live attenuated *ca* influenza A virus (CR 125, lot E-263) possessed the six transferable RNA segments from the attenuated influenza A/Ann Arbor/6/60 (H2N2) *ca* donor and the remaining two genes which encode the HA and neuraminidase glycoproteins from the influenza A/Kawasaki/9/86 wild-type virus. The production, characterization, and safety testing of *ca* reassortant viruses have been described previously (30). The virus suspension was grown in the allantoic cavity of specific-pathogen-free eggs and tested for the presence of adventitious agents by Louis Potash (Flow Laboratories, McLean, Va.). A $10^{7.5}$ 50%

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tissue culture infective dose of the live virus vaccine was administered intranasally in a total volume of 0.5 ml (0.25 ml per nostril). The inactivated subvirion vaccine was ether extracted and contained 15 μg of HA from influenza A/Taiwan/1/86 (H1N1) virus per 0.5-ml dose (Wyeth Laboratories, Marietta, Pa.). The inactivated virus vaccine was injected intramuscularly into the deltoid region.

Clinical studies. Study protocols were approved by the Clinical Research Subpanel of the National Institute of Allergy and Infectious Diseases and the Joint Committee on Clinical Investigation of the Johns Hopkins Medical Institutions. Ambulatory volunteers aged 65 years or more were recruited from senior centers in southeast Baltimore County. Potential vaccinees had a history taken and a physical examination, and the following laboratory tests were done: measurement of serum hemagglutination inhibition (HAI) antibody titer, complete blood cell count, SMA-12 biochemistry panel, and hepatitis B surface antigen. Persons were excluded from the study if they had markedly abnormal results of any of the screening laboratory tests, had clinically unstable chronic obstructive pulmonary or cardiovascular disease, had end-stage renal failure, were taking immunosuppressive or antineoplastic medication, or were allergic to influenza vaccine, eggs, neomycin, amphotericin B, erythromycin, or amantadine. Those who participated in these studies gave written, informed consent.

To control for preimmunization antibody status, subjects were stratified according to their screening serum HAI antibody levels into low (≤1:16)- or high (≥1:32)-titer groups. Persons within each group were then randomized to receive one of the three following vaccine combinations in a blinded manner: (i) intranasal *ca* reassortant virus and intramuscular saline (placebo), (ii) intranasal saline (placebo) and intramuscular inactivated virus, or (iii) intranasal *ca* reassortant virus and intramuscular inactivated virus. These vaccines were administered between July and August of 1987. At the conclusion of the study between October and November of 1987, all subjects were offered the commercially available, trivalent inactivated influenza vaccine recommended for the 1987 to 1988 season.

All vaccine recipients took and recorded their temperature four times daily for 4 days after immunization. During this period, they were questioned by telephone daily about the development of any symptoms; reported symptoms were confirmed by a physician. A volunteer was considered ill if he or she developed any of the following findings within 4 days after vaccination: oral temperature of ≥37.8°C (fever), myalgia alone or with chills or sweats (systemic illness), rhinitis or pharyngitis on two or more consecutive days (upper respiratory illness), a persistent cough or dyspnea on 2 or more consecutive days (lower respiratory illness), or local reactions at the injection site. Influenza A wild-type viruses were not detected in the community during the course of this study.

Laboratory studies. Serum and nasal wash specimens were collected prior to vaccination and 1 and 3 months afterwards. Nasal wash specimens were obtained by inserting a thin plastic catheter attached to a syringe into each nostril, flushing the nasal cavity with 10 ml of Ringer lactate solution, and collecting the effluent. The method for concentrating nasal wash specimens for antibody determination has been described elsewhere (27).

Serum HAI antibody was measured in screening blood specimens by standard techniques using influenza A/Kawasaki/9/86 (H1N1) virus as antigen. An enzyme-linked immunosorbent assay (ELISA) previously described (28, 29) was used to measure serum IgG and serum and nasal wash IgA antibodies. The antigen used in the ELISA was purified HA of the influenza A/Kawasaki/9/86 (H1N1) virus (provided by Mark H. Snyder, Laboratory of Infectious Disease, National Institute of Allergy and Infectious Diseases, Bethesda, Md.). The sequence of reagents from solid phase outward consisted of (i) influenza A/Kawasaki/9/86 (H1) purified HA, (ii) human serum or nasal wash specimen, (iii) rabbit anti-human IgG or IgA, (iv) goat anti-rabbit IgG conjugated with alkaline phosphatase, and (v) *p*-nitrophenyl phosphate disodium substrate. The ELISA titer was expressed as the highest dilution in which the optical density of the antigen-containing well was at least twice the optical density of the respective control well lacking antigen. The ELISA nasal wash anti-HA IgA titers were corrected to a total IgA concentration of 100 mg/ml, as described previously (36). A significant antibody response was defined as a fourfold (or greater) increase between prevaccination and postvaccination ELISA antibody titers.

Statistical analysis. Data were analyzed for statistical significance by using the two-tailed Student *t* test for comparing means and the Fisher exact test or chi-square test for comparing proportions (35).

RESULTS

Study population. Eighty volunteers aged 65 to 83 years were enrolled in the study; 28 were immunized with live *ca* virus vaccine alone, 25 were immunized with inactivated virus vaccine alone, and 27 were immunized with both vaccines. The mean age (plus or minus standard deviation) of the volunteers in each vaccine group was the same (71 ± 5 years). Forty-seven subjects were female, and the gender ratio in the vaccine groups was roughly equivalent. Approximately two-thirds of the subjects in each group had a prevaccination HAI antibody titer of ≤1:16.

Safety. The live and inactivated virus vaccines were well tolerated. Only three subjects experienced illness during the 4-day postvaccination period. One who received live *ca* virus alone developed rhinitis; another, administered both vaccines, had pharyngitis; a third person, given inactivated virus vaccine alone, had fever (39.4°C) and a local reaction at the injection site.

Lack of effect of prevaccination antibody on serum and nasal wash antibody responses. Because preexisting antibody levels can influence the immunologic responses to vaccination, the subjects were stratified prior to vaccination on the basis of their screening HAI antibody titer. Within each vaccine group, the frequency and magnitude of serum IgG HA, serum IgA HA, and nasal wash IgA HA antibody responses were comparable between vaccinees with prevaccination HAI titers of ≤1:8, ≤1:16, or ≥1:32, respectively (data not shown). For this reason, data for all persons within each vaccine group were subsequently pooled and the data for each vaccine group were compared without stratification by prevaccination HAI status.

Serum and nasal wash antibody responses 1 month after vaccination. All seroconversions were detected in the serum specimens collected 1 month after vaccination. The live virus vaccine was least effective at inducing serum IgG antibody to HA in elderly volunteers (Table 1). The magnitude of rise in serum IgG HA titers was significantly greater in the groups that received the inactivated vaccine ($P < 0.05$) or both live and inactivated virus vaccines ($P < 0.01$) than in the group given only the live virus vaccine. Those subjects who received both vaccines mounted a fourfold rise in serum

TABLE 1. HA antibody responses in serum and nasal wash specimens of elderly persons immunized with live attenuated *ca* reassortant or inactivated influenza A (H1N1) virus vaccines given alone or in combination

Vaccine group (no. of patients)	Serum IgG HA antibody response at specified time[a]							Nasal wash IgA HA antibody response at specified time[a]							
	Anti-HA titer (reciprocal mean $\log_2$)			Rise in anti-HA titer (mean $\log_2$)[b] postvaccination		% Postvaccination with antibody titer elevated fourfold above prevaccination level[c]		Anti-HA titer (reciprocal mean $\log_2$)			Rise in anti-HA titer (mean $\log_2$) postvaccination		% Postvaccination with antibody titer elevated fourfold above prevaccination level		% Postvaccination with any antibody titer elevated fourfold above prevaccination level	
	Before vaccination	Postvaccination		1 mo	3 mo	1 mo	3 mo	Before vaccination	Postvaccination		1 mo	3 mo	1 mo	3 mo	1 mo	3 mo
		1 mo	3 mo						1 mo	3 mo						
Live (28)	10.2	10.9	10.4	0.7[d,e]	0.2[f,g]	36[h]	19[i,j]	7.4	8.6	7.4	1.2[k]	0.0	25	7	57	22[l,m]
Inactivated (25)	10.3	12.2	11.6	1.9[d]	1.3[f]	56	50[i]	8.4	8.8	8.3	0.4[n]	−0.1	20[g,o]	17	64	55[l]
Combined (27)	9.5	11.6	10.7	2.1[e]	1.2[g]	67[h]	46[j]	7.0	9.1	7.8	2.1[k,n]	0.8	48[j,o]	23	75	63[m]

[a] Standard deviations of the means were similar; they ranged from 0.3 to 0.5 $\log_2$.
[b] Statistically significant differences between the magnitude of antibody responses of designated vaccine groups (two-tailed Student *t* test).
[c] Statistically significant differences between the percentages of antibody responses of designated vaccine groups (chi-square test).
[d] $P < 0.05$. See footnote b.
[e] $P < 0.01$. See footnote b.
[f] $P < 0.05$. See footnote b.
[g] $P < 0.05$. See footnote b.
[h] $P < 0.05$. See footnote c.
[i] $P < 0.05$. See footnote c.
[j] $P < 0.05$. See footnote c.
[k] $P < 0.05$. See footnote b.
[l] $P < 0.05$. See footnote c.
[m] $P < 0.005$. See footnote c.
[n] $P < 0.005$. See footnote b.
[o] $P < 0.05$. See footnote c.

IgG HA antibody significantly more often than did those who received the live virus vaccine alone ($P < 0.05$). The inactivated vaccine also elicited IgG HA seroresponses more frequently than did the live *ca* virus, but the differences did not reach statistical significance.

Serum IgA HA responses among the elderly vaccinees were meager (data not shown). On average, the titers of serum IgA antibody to HA in each vaccine group increased less than twofold by 1 month after vaccination. Fourfold rises in antibody titer were detected in only 10 subjects: 5 recipients of the inactivated vaccine and 5 who were administered both the live and inactivated vaccines. Only 4 of these 10 volunteers mounted a fourfold rise in nasal wash IgA HA antibody.

For reasons that are not known, the prevaccination titer of nasal IgA HA antibody in the inactivated-vaccine group was twice as high as that detected in the prevaccination nasal washes of the other two vaccine groups. Despite the differences in prevaccination local antibody titers, the nasal wash IgA HA antibody levels achieved 1 month postvaccination were similar for each of the vaccine groups. The combination of live and inactivated vaccines elicited nasal wash IgA HA antibody responses significantly more often ($P < 0.05$) than did the inactivated vaccine given alone, and the magnitude of rise was greater ($P < 0.005$) (Table 1). The live-virus vaccinees mounted a higher, but not a statistically different, rise in nasal IgA HA antibody titer than did the inactivated- vaccine recipients. Moreover, the percentages of live- and inactivated-virus vaccinees who had significant nasal antibody responses to HA were similar.

Serum and nasal wash antibodies at 3 months postvaccination. The levels of serum IgG HA and nasal wash IgA HA antibodies declined in each group of vaccinees by 3 months after immunization (Table 1). The fall in both serum and nasal wash antibody titers was greatest among the live-virus vaccinees, most of whose postvaccination antibody titers had returned to baseline levels by 3 months. Serum IgG HA antibody titers remained elevated fourfold above prevaccination levels more frequently in subjects who received inactivated virus vaccine alone ($P < 0.05$) or both vaccines ($P < 0.05$) than in those who were given live virus vaccine alone. Overall, subjects immunized with the inactivated vaccine or with both vaccines sustained a level of systemic or local antibody at least fourfold above prevaccination levels significantly more often than did subjects immunized with the live virus vaccine ($P < 0.05$ and $P < 0.005$, respectively).

DISCUSSION

This study characterized the systemic and local antibody responses of ambulatory elderly persons to immunization with live attenuated *ca* influenza A/Kawasaki/86 (H1N1) reassortant virus administered intranasally, inactivated influenza A/Taiwan/86 (H1N1) subvirion vaccine administered parenterally, and both vaccines given simultaneously. The live attenuated *ca* influenza A virus vaccine used in this study was well tolerated. This finding corroborates earlier reports documenting the safety of live attenuated influenza virus vaccination among older adults (1, 2, 17, 31). However, the live virus vaccine did not effectively induce antibodies in the serum and nasal compartments when administered alone. Furthermore, when the magnitude and duration of immune responses to the combination of both vaccines were compared with those to the inactivated vaccine, little increase in immunogenicity was achieved by supplementing the inactivated virus vaccine with the live virus vaccine.

Elderly persons are likely to have accumulated a wide repertoire of antibodies from repeated infections with influ-

enza A viruses. We therefore attempted to control for the potential influence of preexisting humoral antibody by stratifying the subjects in our study into groups according to their prevaccination HAI levels before vaccination. We found that the subjects with lower levels of serum HAI antibody had antibody responses to vaccination similar to those with higher titers. This suggested that other immune factors, such as cell-mediated immunity or secretory antibody, may be partly responsible for resistance to infection with live virus vaccine or for interference with the production of antibody responses to inactivated virus vaccine. About half of the elderly subjects in our study mounted a fourfold rise in serum IgG HA antibody to the inactivated influenza A virus vaccine, but only one-third of the live virus vaccine recipients achieved a seroresponse. These low to moderate rates of seroconversion were similar to those reported previously for noninstitutionalized older persons after vaccination with inactivated or live influenza A virus vaccines (2, 8, 9, 15, 17, 23, 25). As noted in this and other studies, rates of serum IgG HA antibody responses of older adults to live *ca* influenza A H1N1 reassortant viruses were generally lower (36 to 60%) than those in published reports with seronegative young adults and children (65 to 100%) (7, 12, 15, 17, 26, 28, 30, 34). Also, we and others (17) found that the majority of older adults failed to mount fourfold rises in serum IgA HA antibody titer to vaccination with live or inactivated influenza A virus vaccines. Conversely, in other studies the majority (87 to 100%) of seronegative young adults given live or inactivated vaccines developed significant rises in serum IgA HA antibody (12, 13, 26, 34).

Little is known about the ability of older adults to mount a local IgA HA antibody response to live attenuated *ca* influenza A reassortant virus vaccines. Gorse and colleagues measured IgA HA antibody to *ca* influenza A/California/78 virus in nasal washes of older adults by using an ELISA similar to the one used in our study, although they did not adjust the level of nasal wash HA antibody to a standard concentration of IgA (18). They detected nasal IgA HA antibody in 38% of their subjects who received live virus vaccine but in only 22% of those who received inactivated influenza vaccine. Among the elderly subjects in our study, only 25% of live-virus vaccinees and 20% of inactivated-virus vaccinees attained a fourfold rise in nasal IgA HA antibody. The slightly higher local antibody response rate observed in the former study may have been due to the different methods of quantification of antibody in nasal washes, the inherent differences in immunogenicity of the live virus vaccine derived from different wild-type viruses, or the younger age of their subjects (mean age, 58, versus 71 years in this study). Clearly, intranasal immunization with live *ca* influenza A vaccine in both studies elicited local IgA HA antibody less often in older adults than observed in other studies with seronegative young adults and children (7, 10, 11, 22, 28, 34, 39). The blunted local antibody response in older adults may be due, in part, to reduced susceptibility to infection with live attenuated influenza virus because of cumulative immunity resulting from repeated previous infections with wild-type influenza A viruses. It is also possible that changes in nasal epithelium may occur as a person ages and that these may alter the ability of the mucosal cells to produce secretory IgA HA antibody. Fulk and co-workers (16) found that children and young adults, but not older adults, were able to produce IgA neutralizing antibody in nasal washes after intranasal application of inactivated influenza vaccine. Taking a different approach, Waldman and colleagues immunized young and elderly volunteers with inactivated influenza vaccine given orally (38). They found that the frequencies of nasal antibody responses were similar for the two age groups but that the level of IgA antibody attained in the saliva decreased with increasing age. Further study is needed to determine the effect of age on antibody-producing cells in the mucosa of the respiratory tract.

To our knowledge, this is the first report of the simultaneous administration of live and inactivated influenza virus vaccines. Whereas serum antibody responses to inactivated and both vaccines were comparable, local antibody production was more frequent and of greater magnitude with the vaccine combination than with inactivated vaccine alone. However, these results should be interpreted with caution since the group who received the inactivated vaccine had higher prevaccination titers of nasal IgA HA antibodies which could have altered their ability to mount responses. Despite the higher rate of local antibody production induced by the vaccine combination, the mean peak titers of nasal antibody achieved were virtually the same for the two groups. Thus, the differences observed between the subjects given inactivated vaccine alone or both vaccines may not be of biologic importance. Moreover, less than 25% of the elderly subjects who received live, inactivated, or both vaccines maintained elevated nasal wash IgA HA antibodies during the subsequent 3 months. These findings, if confirmed, suggest that the contribution, if any, of live attenuated *ca* influenza vaccine to local immune responses induced by inactivated influenza virus vaccine is very short lived in the elderly.

Information regarding the duration of antibody responses to influenza vaccination in elderly populations is limited. Two studies demonstrated no differences in the rate of decline of serum HAI antibody titers between younger and older subjects after immunization with inactivated influenza vaccine (25, 32). However, we had previously found in seronegative young adults that serum IgG HA antibody induced by live or inactivated influenza A virus vaccines remains elevated for at least 6 months after vaccination (12). Some investigators reported that the levels of HAI antibody in sera of institutionalized or ambulatory elderly subjects decline between 3 and 6 months after immunization with inactivated influenza vaccine (4, 9, 24, 25). Moreover, the present study showed that the levels of both serum IgG and nasal wash IgG HA antibodies declined in all three groups of vaccinees between 1 and 3 months after immunization. Additional studies are required to determine whether the persistence of immune responses induced by influenza vaccines is a function of age.

If verified, our findings suggesting a short duration of systemic and local antibody responses have obvious implications with regard to the scheduling of vaccination for the elderly so that they can derive maximum protective immunity against influenza. If live *ca* influenza reassortant viruses are ineffective at stimulating the production of systemic and local immune responses for a long enough period to provide durable protection for at least one influenza season, it is unlikely that they can be employed for vaccination of elderly persons, either as an alternate or an adjunct to inactivated influenza vaccines. Additional studies are required to determine the role, if any, of live influenza virus vaccines for the elderly.

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