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Resistance of Adults to Challenge with Influenza A Wild-Type Virus after Receiving Live or Inactivated Virus Vaccine

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The efficacy of live attenuated cold-adapted (*ca*) reassortant influenza A H3N2 and H1N1 virus vaccines against experimental challenge with homologous wild-type virus 7 months after vaccination was compared with that of licensed inactivated virus vaccine in 106 seronegative (hemagglutination-inhibiting antibody titer ≤1:8) college students. The live attenuated virus vaccines induced as much resistance against illness as did the inactivated vaccine. Vaccine efficacy, measured by reduction in febrile or systemic illness in vaccines, compared with that in controls was 100% for *ca* H3N2 vaccine, 84% for inactivated H3N2 vaccine, 79% for *ca* H1N1 vaccine, and 67% for inactivated H1N1 vaccine. Less protection was conferred against upper respiratory tract illness; there was 50 and 77% protection in *ca* and inactivated H3N2 vaccinees, respectively, but there was no protection in *ca* or inactivated H1N1 vaccinees. The duration, but not the magnitude, of H1N1 wild-type virus shedding in both *ca* and inactivated vaccinees was significantly reduced compared with controls. In contrast, a significant reduction in the duration and magnitude of H3N2 virus shedding was observed in *ca* vaccinees but not in inactivated vaccines. After wild-type virus challenge, live *ca* virus vaccinees demonstrated resistance at least as great 7 months postvaccination as did inactivated virus vaccinees. These observations indicate that live virus vaccines may be a satisfactory alternative to inactivated vaccines for healthy persons.

Currently licensed, parenterally administered, inactivated influenza vaccines induce partial and short-lived immunity to influenza A virus disease (7). For this reason there is interest in developing new vaccines that will provide more solid and longer-lasting immunity. One strategy that is under investigation involves the development of a live attenuated influenza A vaccine that would stimulate both local and systemic antibody responses comparable with those induced by natural infection and hence would provide effective and durable protection against influenza illness. Cold-adapted (*ca*) reassortant influenza A viruses administered intranasally have been shown to induce hemagglutinin (HA) antibodies effectively in both serum and nasal compartments (7a, 10). Moreover, *ca* reassortant viruses which derived their HA and neuraminidase glycoprotein genes from epidemic wild-type virus and the remainder of their RNA segments from the A/Ann Arbor/6/60 (H2N2) *ca* donor virus have been shown to be safe, infectious, immunogenic, nontransmissible, and phenotypically stable even in fully susceptible children and seronegative adults (1, 3, 7a, 8, 9, 14).

Clearly, the protective efficacy of a new candidate influenza vaccine must be compared with conventional inactivated vaccine to determine whether the former offers advantages over the latter. We had previously demonstrated that a single intranasal dose of live attenuated A/Washington/897/80 (H3N2) *ca* virus vaccine given to adults 1 to 2 months before challenge was more effective in inducing resistance to infection with homologous wild-type virus than was licensed inactivated subvirion vaccine (4). The present challenge study was conducted to compare the efficacy of live attenuated H1N1 and H3N2 *ca* reassortant viruses with that of licensed inactivated vaccine 7 months after vaccination.

(This study was presented in part at the Second Annual Meeting of the American Society of Virology, Madison, Wis., July 23, 1984, and at the UCLA Symposia on Molecular and Cellular Biology, Keystone, Colo., April 22, 1985.)

MATERIALS AND METHODS

Viruses and vaccines. The A/Washington/897/80 (H3N2) and A/California/10/78 (H1N1) wild-type viruses (provided by H. W. Kim, Children's Hospital, National Medical Center, Washington, D.C., and by A. P. Kendal, Centers for Disease Control, Atlanta, Ga., respectively) were antigenically similar to A/Bangkok/1/79 (H3N2) and A/Brazil/11/78 (H1N1) viruses, respectively. The viruses were cloned and safety tested as described previously (3, 4, 9). A $10^{6.0}$ 50% tissue culture infective dose ($TCID_{50}$) of the A/Washington/897/80 (H3N2) virus (lot E174) or $10^{4.0}$ $TCID_{50}$ of the A/California/10/78 (H1N1) virus (lot E162) was administered intranasally to vaccinees. Unvaccinated volunteers served as controls. Data obtained from unvaccinated seronegative volunteers who were challenged with the same dose of A/Washington/897/80 or A/California/10/78 wild-type virus previously (3, 4, 9) were combined with data from concurrent controls in the present study. The same suspension of wild-type virus was used in both the previous and present studies to ensure comparability of the challenge inoculum.

The live attenuated A/Washington/897/80 (H3N2) and A/California/10/78 (H1N1) *ca* reassortant viruses each derived their six internal RNA segments from the attenuated A/Ann Arbor/6/60 (H2N2) *ca* donor, whereas the two remaining genes (i.e., those that code for viral surface HA and neuraminidase glycoproteins) were derived from their respective wild-type influenza A virus parents. The commercial inactivated vaccine was ether extracted and contained 15 μg each of A/Brazil/11/78 (H1N1), A/Bangkok/1/79

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TABLE 1. Resistance of vaccinees to challenge with wild-type influenza A (H3N2) virus 7 months after vaccination

Vaccine group (no. challenged)	Response to challenge with wild-type virus[a]							
	Virus shedding				% With indicated illness			
	% Infected[b]	% Shedding	Avg duration (days ± SD)[c]	Peak Mean (log_{10} titer/ml ± SD)[c]	Febrile, systemic, or both	Upper respiratory tract	Lower respiratory tract	Any illness
Live H3N2 (16)	69[d]	69	2.5 ± 1.5[f]	1.6 ± 0.8[g]	0[h]	13	0	13[j]
Inactivated (16)	69[e]	69	3.2 ± 1.8	2.2 ± 1.3	6[i]	6	0	6[k]
None (27)	93[d,e]	81	4.3 ± 2.0[f]	3.8 ± 1.6[g]	37[h,i]	26	11	44[j,k]

[a] Differences between proportions or means not footnoted were not statistically significant.
[b] Virus recovery or a significant serum HAI antibody response or both signified infection.
[c] Data from each infected volunteer were used for calculations.
[d] $P = 0.05$.
[e] $P = 0.05$.
[f] $P < 0.01$.
[g] $P < 0.001$.
[h] $P < 0.003$.
[i] $P < 0.04$.
[j] $P < 0.05$.
[k] $P < 0.02$.

(H3N2), and B/Singapore/222/79 HA per 0.5-ml dose (Fluogen; Parke, Davis & Co., Morris Plains, N.J.).

Clinical studies. The study protocols were approved by the Clinical Research Subpanel of the National Institute of Allergy and Infectious Diseases, the Human Volunteer Research Committee at the University of Maryland, and the Committee on Human Investigation at the University of Rochester. Healthy volunteers who were 18 to 35 years old, lacked a history of influenza vaccination, and had a serum hemagglutination-inhibiting (HAI) antibody titer of 1:8 or less to either A/Washington/897/80 (H3N2) or A/California/10/78 (H1N1) virus were recruited from college students and community members in Maryland or Rochester. Each volunteer gave written, informed consent.

Volunteers seronegative for the H1N1 virus were randomly assigned to receive either two 0.5-ml doses of inactivated virus vaccine subcutaneously 1 month apart or one dose ($10^{7.8}$ $TCID_{50}$) of A/California/10/78 *ca* reassortant virus administered intranasally (5). (A two-dose regimen of inactivated virus vaccine was the recommended immunization practice for individuals susceptible to H1N1 virus.) Volunteers who were H3N2 seronegative were randomly assigned to receive one dose of inactivated virus vaccine (0.5 ml) subcutaneously or $10^{7.5}$ $TCID_{50}$ of A/Washington/897/80 *ca* reassortant virus intranasally. Vaccines were administered in October, 1982. The demonstration of safety and immunogenicity of the live virus vaccines and the procedures used in this outpatient study were described previously (3, 9, 12). Vaccinees who developed a significant increase in titer of serum or nasal wash antibody between 1 and 6 months postvaccination were considered to have undergone natural influenza A virus infection and were thus excluded from participation in the challenge study. At 7 months after vaccination, vaccinated volunteers together with unvaccinated seronegative volunteers (controls) were isolated in a dormitory-like setting in Baltimore or Rochester for 3 days before and 7 to 10 days after intranasal administration of wild-type virus. Oral temperatures were recorded four times a day, and the volunteers were examined twice a day by investigators who did not know the vaccination status of the volunteers.

Definitions of influenza illness. A volunteer was considered ill if fever (oral temperature ≥37.8°C), symptoms, or physical findings consistent with influenza developed within 5 days after inoculation. Illnesses were categorized by the following criteria: systemic illness, the occurrence of myalgia alone or with chills or sweats; upper respiratory tract illness, rhinorrhea or pharyngitis on 2 consecutive days; and lower respiratory tract illness, a persistent cough on 2 or more consecutive days.

Laboratory studies. Nasal-wash specimens for isolation of wild-type virus were collected before challenge and daily for 10 days afterward. The virologic methods have been previously described (12). Selected nasal-wash specimens were inoculated into tissue cultures that support the growth of rhinoviruses, enteroviruses, respiratory syncytial virus, and parainfluenza viruses. Volunteers from whom an adventitious virus was recovered before or up to 5 days after inoculation of influenza A virus were excluded from analysis.

Serum and nasal-wash specimens were collected from vaccinees before and after vaccination and from all volunteers before and 4 weeks after challenge for measurement of antibodies as previously described (10, 11). All serum specimens were also tested by HAI assay with two antigens: a reassortant virus possessing A/Texas/1/77 H3-HA and equine-1 neuraminidase and the A/Washington/897/80 (H3N2) virus or for those in the H1N1 study, two subvirion H1N1 vaccines, A/Brazil/11/78 and A/USSR/92/77. In the challenge study, virus recovery or a fourfold or greater rise in serum HAI antibody titer or both signified infection.

Statistical analysis. Student's *t* test, the chi-square test with Yates' correction, and Fisher's exact test were performed where appropriate. The reduction in rate of illness in vaccinees (the efficacy rate) was calculated as follows: (rate of illness in placebo control group − rate in live or inactivated virus vaccine group) × 100/rate in placebo control group.

RESULTS

Response of vaccinees to challenge with H3N2 wild-type virus. The immune responses to vaccination with the live and inactivated viruses are detailed elsewhere (5). At 7 months after vaccination, volunteers were challenged with homologous wild-type virus to determine whether or not the vaccine had induced resistance. Occurrence of infection and illness and level of virus shedding for live and inactivated virus vaccinees and unvaccinated controls challenged with A/Washington/897/80 (H3N2) wild-type virus are presented in Table 1.

TABLE 2. Resistance of vaccinees to challenge with wild-type influenza A (H1N1) virus 7 months after vaccination

Vaccine group (no. challenged)	Response to challenge with wild-type virus[a]							
	Virus shedding				% With indicated illness			
	% Infected[b]	% Shedding	Avg duration (days ± SD)[c]	Peak mean (log_{10} titer/ml ± SD)[c]	Febrile, systemic, or both	Upper respiratory tract	Lower respiratory tract	Any illness
Live H1N1 (14)	50	43	4.4 ± 2.4[d]	2.4 ± 1.6	7	29	0	29
Inactivated (18)	67	67	4.7 ± 1.4[e]	3.1 ± 1.4	11	28	1	33
None (15)	73	73	6.8 ± 1.5[de]	3.8 ± 1.6	33	20	13	40

[a] Differences between proportions or means not footnoted were not statistically significant.
[b] Virus recovery or a significant serum HAI antibody response or both signified infection.
[c] Data from each infected volunteer were used for calculations.
[d] $P < 0.04$.
[e] $P < 0.004$.

Live H3N2 virus vaccine completely protected vaccinees against febrile systemic influenza illness (0 of 16 versus 10 of 27 controls, $P < 0.003$) and partially prevented upper respiratory tract symptoms. Febrile or systemic illness also occurred less often among inactivated vaccine recipients (1 of 16) than among unvaccinated controls (10 of 27, $P < 0.04$); the single ill vaccinee also had rhinorrhea. Both vaccines prevented infection and virus shedding in about one-third of the vaccinees who were challenged. The 11 live virus vaccinees infected with wild-type virus shed significantly less virus and for a briefer period than did 25 infected, unvaccinated controls. In contrast, the 11 infected, inactivated vaccine recipients and the infected, unvaccinated controls did not differ significantly in the quantity of virus shed and duration of virus shedding.

Response of vaccinees to challenge with H1N1 wild-type virus. Live H1N1 (one dose) and inactivated virus (two doses) vaccines both provided partial protection against febrile or systemic illness, but the difference in frequencies of these illnesses in vaccinees and unvaccinated controls was not statistically significant (Table 2). Neither vaccine was protective against upper respiratory illness. There was a tendency toward a lower frequency of infection and virus shedding after challenge of live virus vaccinees than was observed in unvaccinated controls, but the differences were not significant ($P < 0.18$ and $P < 0.10$, respectively). Among infected volunteers the duration of shedding was shorter for both the live and inactivated virus vaccinees than it was for the controls ($P < 0.04$ and $P < 0.004$, respectively).

Comparison of resistance conferred by H3N2 virus vaccines 2 and 7 months after vaccination. At 1 to 2 months after vaccination with live *ca* virus vaccination, 81% of the vaccinees resisted infection by wild-type H3N2 virus challenge (4), whereas after 7 months only 31% of vaccinees resisted infection (Table 3).

DISCUSSION

Previous studies indicated that immunization of seronegative adults with live virus vaccine administered intranasally provided more complete resistance against homologous wild-type influenza A (H3N2) virus challenge 1 to 2 months after vaccination than did parenteral vaccination with inactivated virus vaccine (4). In the present study, we compared the protection against homologous wild-type virus challenge conferred by H3N2 and H1N1 live virus vaccines with that conferred by inactivated subvirion vaccine 7 months after vaccination. Despite the fact that the live virus vaccines developed serum HA antibody responses less often or to lower titers or both than did inactivated virus vaccinees (5), live virus vaccines provided as much protection against illness as did inactivated virus vaccine.

After challenge with wild-type virus 7 months after vaccination, live H3N2 virus vaccinees were completely resistant to febrile or systemic illness. Inactivated virus vaccinees also exhibited significant resistance, i.e., 84% reduction in febrile or systemic disease compared with unvaccinated controls. Similar protective efficacy of H3N2 influenza A vaccine was observed previously when other volunteers were challenged within 2 months after vaccination (4). Likewise, the reduction of febrile disease observed among inactivated H3N2 virus vaccinees in our study was comparable with that reported from field trials in which vaccine was given 3 to 14 months before the epidemic and the vaccine strain resembled the epidemic strain (range, 68 to 80%) (6, 13). This suggests that the conditions of experimental challenge in our study approximated those that occur naturally. Importantly, the duration and magnitude of virus shedding in the infected live virus vaccinees was significantly less than that in the unvaccinated controls, but this was not true for the inactivated virus vaccinees. This finding is consistent with a previous observation of markedly reduced virus shedding in live virus vaccinees challenged 1 to 2 months after vaccination (4). These observations suggested that the live H3N2 virus vaccine provided slightly greater resistance than did the inactivated virus vaccine.

The efficacy of live H3N2 virus vaccine decreased demonstrably between 2 and 7 months after vaccination. It has been observed that the level of local immunoglobulin A antibody induced by live virus vaccination remains elevated for 6 months after vaccination, but there is a statistically

TABLE 3. Comparison of resistance of vaccinees to challenge with wild-type influenza A (H3N2) virus 1 to 2 months[a] and 7 months after vaccination

Vaccine group (no.)	% Vaccinees and symptoms at indicated mo postvaccination					
	Infection		Fever or systemic illness		Upper respiratory tract illness	
	1–2	7	1–2	7	1–2	7
Live H3N2 (16, 16)	19	69[b]	0	0	0	13
Inactivated (16, 16)	63	69	13	6	6	6
None (24, 27)	96	93	38	37	29	26

[a] Data from reference 4.
[b] Statistically significant difference in proportion infected between 1 to 2 months and 7 months after vaccination, $P < 0.006$.

significant diminution in the level of such antibody with time (5). In adult vaccinees who received a live *ca* H3N2 or H1N1 virus vaccine, the nasal-wash immunoglobulin A HA antibody titers induced by vaccine virus infection had decreased about threefold by 6 months (5). The present findings demonstrating increased susceptibility to infection and upper respiratory tract illness 7 months after vaccination suggest that this degree of decrease of local immunoglobulin A antibody is significant.

A single intranasal dose ($10^{7.8}$ $TCID_{50}$) of live H1N1 virus vaccine afforded as much protection against febrile or systemic illness 7 months after vaccination as did two parenteral doses of inactivated virus vaccine. However, the efficacy of the live H1N1 and inactivated H1N1 virus vaccines was not as great as that of the H3N2 virus vaccines. Compared with unvaccinated subjects, live H1N1 virus vaccinees had 77% reduction in febrile or systemic illness, and inactivated virus vaccinees had a 67% reduction. A previous study showed that intranasal immunization with one dose of live *ca* reassortant A/Hong Kong/123/77 (H1N1) virus provided the same level of protection (82%) against febrile or systemic illness 1 to 3 months after vaccination (2). Vaccination with either H1N1 virus was also less effective than vaccination with H3N2 virus vaccines in preventing upper respiratory tract illness. Again, this was probably because of waning immunity, particularly local antibody stimulated by live virus vaccines (5). Shedding of wild-type virus after challenge with H1N1 virus was comparable in live and inactivated H1N1 virus vaccinees. The duration of shedding, but not the quantity shed, was significantly reduced compared with controls. This finding differed from that in the H3N2 wild-type virus challenge study in which H3N2 live virus vaccinees shed significantly less virus for a shorter duration than did controls, whereas shedding by the inactivated H3N2 virus vaccinees was not significantly different from controls. Differences between prevaccination immune status of H1N1 and H3N2 vaccinees may, in part, explain this discrepancy. Presumably, the initially H3N2-seronegative volunteers had been infected previously with H3N2 viruses, whereas the majority of the initially H1N1-seronegative volunteers had not been previously infected with an H1N1 virus and thus had not been immunologically primed against this virus. The greater susceptibility to illness and deterioration in resistance to virus replication in H1N1 vaccinees within 7 months is consistent with the lack of prior experience with H1N1 influenza A viruses.

Both the previous (4) and present experimental wild-type challenge studies demonstrated that, in comparison with inactivated virus vaccine, live H3N2 *ca* virus vaccine induced significantly greater resistance to wild-type virus infection 1 to 2 months after vaccination and the H1N1 and H3N2 *ca* virus vaccines induced comparable or only slightly greater resistance 7 months postvaccination. These observations indicate that the live virus vaccines may be a satisfactory alternative to inactivated vaccine for use in healthy persons. To achieve long-lasting protective immunity, it may be necessary to administer live virus vaccines with newly formulated adjuvants, to administer booster doses of vaccine, or to revaccinate annually. Studies to address these questions are in progress.

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