NOTES

Use of Live Cold-Adapted Influenza A H1N1 and H3N2 Virus Vaccines in Seropositive Adults

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To investigate the immunogenicity and protective efficacy of cold-adapted influenza vaccine in individuals with underlying immunity to influenza A virus, we administered cold-adapted H1N1 and H3N2 vaccines to adults with prevaccination serum hemagglutination inhibition antibody titers of 1:16 or more and challenged them 1 month afterwards with homologous wild-type influenza A virus. Both cold-adapted vaccines were immunogenic in seropositive adults. In addition, individuals receiving cold-adapted vaccines had lower rates of virus shedding and illness following challenge with wild-type influenza virus than did unvaccinated seropositive volunteers.

Cold-adapted influenza A viruses generated by genetic reassortment between a wild-type influenza A virus and the cold-adapted influenza A/Ann Arbor/6/60 virus are currently being evaluated as live attenuated influenza A vaccines. Such vaccines are safe and immunogenic in seronegative children and young adults (1, 2, 5, 7, 9, 10, 12, 15) and induce resistance to challenge with homologous wild-type influenza A viruses in such individuals at least equal to that induced by currently available inactivated influenza vaccines (3, 4). Individuals with various levels of preexisting immunity to influenza A virus will ultimately need to be included in any strategy to use these vaccines, but the presence of antibodies induced by prior natural infection with influenza A virus may dampen the immune response to the cold-adapted virus vaccine (6, 8). To determine the extent to which prior natural immunity might influence the immunogenicity of attenuated influenza A virus vaccines, we administered H3N2 and H1N1 cold-adapted reassortant influenza A viruses to adults with serum hemagglutination inhibition (HI) antibody titers of 1:16 or more and measured their immune responses and subsequent resistance to challenge with homologous wild-type influenza A virus.

The wild-type and cold-adapted influenza A/California/10/78 (H1N1) and A/Bethesda/1/85 (H3N2) viruses used in this study were obtained from Flow Laboratories, Inc., McLean, Va. These viruses were cloned and safety tested as described previously (12, 13). Commercially available inactivated subvirion vaccine contained 15 µg each of the B/Ann Arbor/1/86, A/Chile/1/83 (H1N1), and A/Mississippi/1/85 (H3N2) hemagglutinin (HA) antigens per 0.5-ml dose (Wyeth Laboratories, Marietta, Pa.).

Two studies were performed. In the first study, conducted in April 1985, 10 healthy young adults with serum HI antibody titers of 1:16 or more were administered $10^{7.5}$ 50% tissue culture infective doses (TCID₅₀) of the cold-adapted influenza A/California/78 (H1N1) virus (lot E-162) intranasally. One month after vaccination, these volunteers and an

additional group of unvaccinated seronegative (serum HI titer, 1:4 or less) and seropositive (serum HI titer, 1:16 or more) volunteers were admitted to an isolation facility and challenged intranasally with $10^{4.5}$ TCID₅₀ of the wild-type influenza A/California/78 virus (lot E-167), a dose which had infected 100% of seronegative adults in a previous experiment (12). In the second study, conducted in August 1987, 22 adults with serum HI antibody titers of 1:16 or more to the A/Mississippi/85 virus were randomly assigned to receive (i) intranasal vaccination with $10^{7.2}$ TCID₅₀ of the A/Bethesda/85 cold-adapted reassortant virus (lot E-259), (ii) intramuscular vaccination with trivalent inactivated vaccine containing 15 µg of A/Mississippi/85 antigen, or (iii) placebo in a double-blind fashion. One month after vaccination, these volunteers and an additional group of five volunteers with serum HI antibody titers of 1:4 or less were challenged with $10^{4.5}$ TCID₅₀ of the wild-type A/Bethesda/85 virus (lot E-241) intranasally.

Serum and nasal wash specimens collected prior to and 1 month after vaccination and a third serum sample collected 1 month after challenge were used for the determination of immunoglobulin G (IgG) and IgA antibody responses to the viral HA by HI and an enzyme immunoassay (EIA) (11). The EIA was performed as previously described for the determination of IgG responses to the influenza virus HA (11). Briefly, 96-well polystyrene plates (Immulon; Dynatech Laboratories, Inc., Alexandria, Va.) were coated with partially purified HA of influenza A/Chile/83 (H1N1) or A/Mississippi/85 (H3N2) virus (WHO Collaborating Center on Influenza), followed in sequential order by serial dilutions of the serum or nasal wash to be tested, alkaline phosphatase-labeled goat anti-human IgG or IgA (Tago, Burlingame, Calif.), and orthoparadiazine substrate. The titer of antibody present was defined as the highest dilution giving a positive/negative ratio of >2 or an optical density of >0.10. Nasal wash titers represent the level of antibody present after approximately 10-fold concentration by evaporation in dialysis tubing. Significant antibody responses in the EIA were determined by the area method (11). For serum and nasal wash IgA determinations, an area difference of >50

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TABLE 1. Response of seropositive young adults to vaccination with inactivated or live cold-adapted influenza A virus vaccines

Vaccine	No. of subjects tested	Reciprocal log ₂ geometric mean antibody titer $\pm$ SE in ^a :											
		Serum HI test			Serum HA IgG EIA			Serum HA IgA EIA			Nasal HA IgA EIA		
		Pre	Post	%	Pre	Post	%	Pre	Post	%	Pre	Post	%
Cold-adapted A/California/78	10	5.4 $\pm$ 0.2	5.5 $\pm$ 0.2	0 ^b	10.9 $\pm$ 0.1	11.3 $\pm$ 0.2	30 ^c	8.2 $\pm$ 0.3	8.7 $\pm$ 0.3	60	3.2 $\pm$ 0.5	4.6 $\pm$ 0.4	70
Cold-adapted A/Bethesda/85	8	4.9 $\pm$ 0.3	5.0 $\pm$ 0.3	12	11.0 $\pm$ 0.3	11.0 $\pm$ 0.3	12 ^d	8.4 $\pm$ 0.2	9.5 $\pm$ 0.4	25	4.0 $\pm$ 0.5	4.8 $\pm$ 0.2	50
Inactivated A/Mississippi/85	7	5.5 $\pm$ 0.3	6.2 $\pm$ 0.3	43	11.3 $\pm$ 0.5	14.0 $\pm$ 0.7	86	8.2 $\pm$ 0.8	9.9 $\pm$ 0.6	29	4.6 $\pm$ 0.3	5.2 $\pm$ 0.4	57

^a Percentages represent the percentages of volunteers exhibiting a fourfold titer rise (HI test) or significant area increase (EIAs).

^b $P = 0.051$ as compared with those receiving inactivated vaccine; Fisher exact test.

^c $P < 0.01$ as compared with those receiving inactivated vaccine.

^d $P < 0.05$ as compared with those receiving inactivated vaccine.

units was considered significant after preliminary experiments determined that this value was greater than 2 standard deviations from that obtained after 15 determinations on four samples representing initial optical density values from 0.10 to 1.50 for each HA antigen used.

Volunteers were examined twice daily for 7 days following challenge with wild-type influenza A virus (14). Volunteers experiencing cough, sore throat, stuffy nose, or myalgia on two or more occasions were considered ill. Individuals with an oral temperature of $\geq 38^\circ\text{C}$ were considered febrile, and individuals with fever were also considered ill. Nasal wash specimens for isolation and titration of influenza A virus were obtained prior to challenge and daily for 7 days afterward and inoculated freshly onto Madin-Darby canine kidney (MDCK) cells. The titer of virus in samples from which virus was recovered was determined by standard methods.

The humoral immune response of seropositive volunteers to immunization with cold-adapted H1N1 and H3N2 virus vaccines and inactivated trivalent vaccine is shown in Table 1. All three vaccines were immunogenic in this group of seropositive volunteers. Overall, 90% of those receiving the cold-adapted A/California/78, 50% of those receiving the cold-adapted A/Bethesda/85, and 86% of those receiving the inactivated A/Mississippi/85 vaccines had a response in at least one of the tests used. Inactivated vaccine was more effective at inducing a serum antibody response in this group than were the cold-adapted vaccines. Both the frequency of significant responses and the postvaccine geometric mean titers of serum HI antibody and HA IgG antibody were highest in those receiving the inactivated vaccine. The rates of serum HA IgA and nasal secretion HA IgA responses were slightly higher in those receiving the cold-adapted A/California/78 vaccine, although these differences were not statistically significant. The mean titer of nasal IgA following vaccination was similar in all three groups. Although the prevaccination nasal IgA titers were lowest in the group which demonstrated the best nasal response to vaccine, there was no apparent relationship within each group between prevaccination serum or nasal IgA titer and response to vaccine (data not shown).

One month following vaccination, volunteers were admitted to an isolation facility and challenged with $10^{4.5}$ TCID₅₀ of the homologous wild-type influenza A virus. The response of volunteers to challenge with the influenza A/California/78 (H1N1) wild-type virus is shown in Table 2. All eight unvaccinated volunteers with prechallenge serum HI titers of 1:4 or less shed moderate levels of virus in their nasal secretions. Two volunteers in this group experienced mild

influenzalike illness with fever and/or systemic symptoms. These results were similar to those of a previous study of the wild-type A/California/78 virus in seronegative adults (14), suggesting that the challenge virus was fully virulent. Unvaccinated volunteers with serum HI antibody titers of 1:16 or more were protected from challenge when compared with seronegative volunteers. A smaller proportion of unvaccinated seropositive than seronegative volunteers were infected with the challenge virus, and the duration and level of virus shedding among infected individuals were significantly lower in the unvaccinated seropositive than seronegative group ($P < 0.05$ for both).

Seropositive volunteers who received the cold-adapted A/California/78 vaccine prior to challenge were protected from subsequent infection with the wild-type virus when compared with unvaccinated seropositive controls. None of the vaccinated volunteers had virus recoverable from their nasal secretions, as compared with four of the unvaccinated seropositive volunteers. In addition, only one vaccinated volunteer had any evidence of infection by the wild-type virus, as compared with 7 of 23 unvaccinated seropositive volunteers ($P = 0.17$).

The response of those challenged with the A/Bethesda/85 (H3N2) wild-type virus is also shown in Table 2. All of the seronegative volunteers were infected by the wild-type virus, and 80% shed detectable virus in their nasal secretions in moderate levels. In addition, two of five of these volunteers experienced mild influenzalike symptoms following challenge, similar to the response seen in previous studies (13). Relatively high rates of infection, detected primarily by the EIA seroresponse, were also seen in the three groups of seropositive volunteers. However, all three groups of seropositive volunteers had lower rates of virus shedding and illness than did seronegative volunteers. Individuals receiving the cold-adapted A/Bethesda/85 vaccine had the lowest rates of virus shedding following challenge, but rates were also low in unvaccinated seropositive volunteers.

When the results from both wild-type virus challenges were combined, a total of 0 of 18 seropositive volunteers who received a cold-adapted influenza A vaccine shed virus following wild-type influenza A virus challenge, as compared with a total of 6 of 30 unvaccinated seropositive volunteers ($P = 0.07$; Fisher exact test).

In this study, both the cold-adapted A/California/78 (H1N1) and cold-adapted A/Bethesda/85 (H3N2) virus vaccines were immunogenic in adults with prevaccination serum HI antibody titers of 1:16 or more. Measurable immune responses were seen in more than half of the recipients. The most frequent response seen following vaccination of sero-

TABLE 2. Protective efficacy of cold-adapted influenza A/California/78 (H1N1) or A/Bethesda/85 (H3N2) vaccines against challenge with homologous wild-type influenza A virus in seropositive (serum HI titer, 1:16 or more) adults

Group	Vaccine administered	Wild-type virus challenge	No. of volunteers	Reciprocal mean log ₂ prechallenge serum HI titer $\pm$ SE	% Infected ^a	Virus shedding (nasal wash)			% with indicated symptom(s)		
						% Shedding	Mean duration (days $\pm$ SE) ^b	Mean peak titer (log ₁₀ TCID ₅₀ /ml $\pm$ SE) ^b	Fever, systemic, or both ^c	Upper or lower respiratory tract or both ^d	Any illness
Seropositive	Cold-adapted A/California/78	Wild-type A/California/78	10	5.9 $\pm$ 0.2	10 ^e	0 ^f	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0	0	0 ^f
	None		23	4.8 $\pm$ 0.2	30 ^e	17 ^e	1.1 $\pm$ 0.6	1.1 $\pm$ 0.2	0	22	22
Seronegative	None		8	1.6 $\pm$ 0.2	100	100	3.6 $\pm$ 0.6	2.4 $\pm$ 0.4	25	37	50
	Cold-adapted A/Bethesda/85	Wild-type A/Bethesda/85	8	5.0 $\pm$ 0.3	75	0 ^f	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	12	12	12
Seropositive	Inactivated A/Mississippi/85		7	6.2 $\pm$ 0.3	43	14 ^f	0.7 $\pm$ 0.7	0.8 $\pm$ 0.3	28	14	28
	None		7	4.1 $\pm$ 0.5	71	28	2.2 $\pm$ 1.4	1.5 $\pm$ 0.6	14	14	14
Seronegative	None		5	2.0 $\pm$ 0.3	100	80	3.0 $\pm$ 1.0	2.4 $\pm$ 0.6	40	40	40

^a Virus shedding, seroresponse, or both.^b In volunteers with virus shedding.^c Temperature of greater than 38°C, myalgia, or malaise.^d Cough, sore throat, or stuffy nose.^e $P < 0.01$ as compared with seronegative volunteers receiving a corresponding wild-type virus challenge.^f $P < 0.05$ as compared with seronegative volunteers receiving a corresponding wild-type virus challenge.

positive adults with the cold-adapted influenza viruses in this study was an increase in nasal secretion HA IgA.

The rates of immune response to the cold-adapted A/Bethesda/85 (H3N2) vaccine in this study were comparable to those seen previously when the cold-adapted A/Washington/80 (H3N2) vaccine was administered to seropositive volunteers (6). In that study, 33% of young adults with preimmunization serum HI titers of 1:8 or more had a nasal HA IgA antibody response, and 71% achieved a postvaccination nasal HA IgA titer of 1:64 or greater (6). A somewhat higher rate of nasal IgA response was seen following the H3N2 vaccine in the current study, but the ultimate titer achieved was slightly lower. These differences could conceivably be accounted for by differences in the techniques used for measurement of antibody in the two studies.

In this study, the frequency of both serum and nasal antibody responses was greater following vaccination with the A/California/78 (H1N1) vaccine than with the A/Bethesda/85 (H3N2) vaccine, possibly reflecting a different underlying level of immunity of the volunteers to these two viruses or an inherent difference in the immunogenicity of the two virus vaccines. More frequent immune responses to another cold-adapted H1N1 virus, A/Taiwan/86, than to the cold-adapted H3N2 A/Bethesda/85 virus were also seen in an inactivated vaccine also induced nasal secretion HA IgA antibody in most volunteers, and the geometric mean nasal IgA titer was similar in all three vaccinated groups.

Intranasal vaccination of seropositive volunteers with the cold-adapted H1N1 and H3N2 vaccines provided protection against virus shedding and influenzalike illness following challenge with homologous wild-type influenza A virus when compared with the results in unvaccinated seropositive volunteers. In addition, the protection afforded by the cold-adapted vaccines appeared to be at least equal to that provided by the inactivated vaccine. The rates of virus shedding and illness in the unvaccinated seropositive volunteers were too low to allow statistically significant conclusions to be drawn. However, the results suggest that the live attenuated H1N1 and H3N2 influenza reassortant virus vaccines may provide protection against influenza virus shedding and virus-induced illness even in seropositive individuals. Further studies to determine the protective efficacy of such vaccines against natural infection in other seropositive groups such as the elderly are warranted.

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LITERATURE CITED

1. Belshe, R. B., and L. P. Van Voris. 1984. Cold-recombinant influenza A/California/10/78 (H1N1) virus vaccine (CR-37) in seronegative children: infectivity and efficacy against investigational challenge. *J. Infect. Dis.* **140**:735-740.
2. Clements, M. L., R. F. Betts, H. F. Maassab, and B. R. Murphy. 1984. Dose response of influenza A/Washington/89/78 (H3N2) cold-adapted reassortant virus in adult volunteers. *J. Infect. Dis.* **149**:814.
3. Clements, M. L., R. F. Betts, and B. R. Murphy. 1984. Advantage of live attenuated cold-adapted influenza A virus over inactivated vaccine for A/Washington/80 (H3N2) wild-type virus infection. *Lancet* **i**:705-708.
4. Clements, M. L., R. F. Betts, E. L. Tierney, and B. R. Murphy. 1986. Resistance of adults to challenge with influenza A wild-type virus after receiving live or inactivated virus vaccine. *J.*

- Clin. Microbiol. **23**:73-76.
5. **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.
 6. **Clements, M. L., E. L. Tierney, and B. R. Murphy.** 1985. Response of seronegative and seropositive adult volunteers to live attenuated cold-adapted reassortant influenza A virus vaccine. *J. Clin. Microbiol.* **21**:997-999.
 7. **Feldman, S., P. F. Wright, R. G. Webster, P. K. Roberson, J. Mahoney, J. Thompson, M. Doolittle, L. Lott, P. Johnson, and R. C. Christoph.** 1985. Use of influenza A virus vaccine in seronegative children: live cold-adapted versus inactivated whole virus. *J. Infect. Dis.* **152**:1212-1218.
 8. **Gorse, G. J., R. B. Belshe, and N. J. Munn.** 1986. Safety of and serum antibody response to cold-recombinant influenza A and inactivated trivalent influenza virus vaccines in older adults with chronic diseases. *J. Clin. Microbiol.* **24**:336-342.
 9. **Johnson, P. R., S. Feldman, J. M. Thompson, J. D. Mahoney, and P. F. Wright.** 1985. Comparison of long-term systemic and secretory antibody responses in children given live, attenuated, or inactivated influenza A vaccine. *J. Med. Virol.* **17**:325-335.
 10. **Johnson, P. R., S. Feldman, J. M. Thompson, J. D. Mahoney, and P. F. Wright.** 1986. Immunity to influenza A virus infection in young children: a comparison of natural infection, live cold-adapted vaccine, and inactivated vaccine. *J. Infect. Dis.* **154**:121-127.
 11. **Madore, H. P., R. C. Reichman, and R. Dolin.** 1983. Serum antibody responses in naturally occurring influenza A virus infection determined by enzyme-linked immunosorbent assay, hemagglutination inhibition, and complement fixation. *J. Clin. Microbiol.* **18**:1345-1350.
 12. **Murphy, B. R., M. L. Clements, H. P. Madore, J. Steinberg, S. O'Donnell, R. Betts, D. Demico, R. C. Reichman, R. Dolin, and H. F. Maassab.** 1984. Dose response of cold-adapted, reassortant influenza A/California/10/78 virus. *J. Infect. Dis.* **149**:816.
 13. **Sears, S. D., M. L. Clements, R. F. Betts, H. F. Maassab, B. R. Murphy, and M. H. Snyder.** 1988. Comparison of live, attenuated H1N1 and H3N2 cold-adapted and avian-human influenza A reassortant viruses and inactivated virus vaccine in adults. *J. Infect. Dis.* **158**:1209-1219.
 14. **Treanor, J. J., R. F. Betts, S. E. Erb, F. K. Roth, and R. Dolin.** 1987. Intranasally administered interferon as prophylaxis against experimentally induced influenza A virus infection in humans. *J. Infect. Dis.* **156**:379-383.
 15. **Wright, P. F., N. Okabe, K. T. McKee, H. F. Maassab, and D. T. Karzon.** 1982. Cold-adapted recombinant influenza A virus vaccines in seronegative young children. *J. Infect. Dis.* **146**: 71-79.