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Response of Seronegative and Seropositive Adult Volunteers to Live Attenuated Cold-Adapted Reassortant Influenza A Virus Vaccine

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The infectivity and immunogenicity of live attenuated A/Washington/897/80 cold-adapted reassortant virus vaccine was evaluated in seronegative (hemagglutination inhibition titer, ≤1:4) and seropositive (hemagglutination inhibition titer, >1:4) adult volunteers. The vaccine was efficient in infecting seronegative volunteers (94%). Moreover, 51% of seropositive vaccinees were infected by the virus. After live virus vaccination, greater than 83% of both seronegative and seropositive vaccinees achieved a level of nasal wash antibody previously associated with resistance to infection with influenza A virus. These findings indicate that both seronegative and seropositive vaccinees can benefit from live virus vaccination.

Attenuated cold-adapted (*ca*) reassortant influenza A viruses have emerged as promising vaccine candidates. A wild-type influenza A virus isolate can be rapidly and reliably attenuated by the transfer of the six "internal genes" (i.e., genes coding for nonsurface proteins) from the A/Ann Arbor/6/60 (H2N2) *ca* donor to the wild-type virus. Studies have demonstrated that these *ca* reassortant viruses fulfill all of the desirable properties of a live virus vaccine: attenuation, infectivity for seronegative children and adults, phenotypic stability, lack of transmissibility, and induction of resistance to infection with wild-type virus (2–4, 10, 13).

Despite these encouraging observations, there has been concern that a live attenuated vaccine may not be sufficiently antigenic to immunize an entire community composed of individuals with various levels of antibody. This concern has been based in part on the observation that live attenuated viruses may not infect the respiratory tract of individuals with preexisting serum antibodies (1, 5, 7, 8, 12). Heretofore, the infectivity and immunogenicity of the *ca* virus vaccine had been studied mainly in adults and children who initially had a low or undetectable titer (≤1:8) of serum hemagglutination inhibition (HAI) antibody (2–4, 10, 13). To evaluate the infectivity of an attenuated *ca* reassortant virus in persons with serum HAI antibody induced by prior natural infection, we inoculated HAI-seropositive adults with *ca* reassortant A/Washington/897/80 (H3N2) virus and compared their responses with those of seronegative adults. The data were stratified and analyzed by the preimmunization HAI antibody titers of the vaccinees.

Study protocols were approved by the Clinical Research Subpanel of the National Institute of Allergy and Infectious Diseases and the Human Volunteer Research Committee at the University of Maryland, Baltimore. The preparation, genetic characterization, and safety testing of the influenza A/Washington/897/80 *ca* reassortant virus, as well as the immunological and virological methods and procedures for the outpatient studies, have been reported previously (2, 4). Healthy volunteers between the ages of 18 and 35 years who had no history of influenza vaccination were recruited from among students and employees of the University of Maryland. After giving written informed consent, each volunteer received $10^{7.5}$ 50% tissue culture infective doses (0.5 ml) of the *ca* reassortant virus vaccine intranasally. This was equivalent to 70 50% human infectious doses for HAI-seronegative adult volunteers (2). Serum and nasal wash specimens were collected before and 1 month after vaccination for antibody determinations. Nasal wash specimens for virus isolation were obtained daily for 4 days after inoculation.

Since the A/Washington/897/80 antigen was closely related to A/Bangkok/1/79 and A/Texas/1/77 antigens (i.e., the difference in serological results with each antigen was no greater than one tube dilution), these antigens were used in the immunological assays. All prevaccination serum specimens were tested at the same time by HAI assay with influenza A/Washington/897/80 (H3N2) and A/Bangkok/1/79 (H3N2) detergent-disrupted virus as antigens. Those volunteers who had a preimmunization serum HAI antibody titer of ≤1:4 to A/Washington/80 virus were designated as seronegative, and those with a higher titer were designated as seropositive. Paired prevaccination and postvaccination sera were tested by HAI assay with a reassortant virus containing influenza A/Texas/77 hemagglutinin (HA) and equine-1 neuraminidase (NA) as antigen because of its greater sensitivity in detecting serum antibody than a reassortant containing the influenza A/Bangkok/79 HA and equine-1 NA. These serum pairs were also tested for NA-inhibiting antibody with an influenza A reassortant virus possessing the HA of equine-1 and the NA of A/Bangkok/79. Paired prevaccination and postvaccination sera and nasal wash specimens were tested for immunoglobulin G (IgG) and IgA HA antibody, respectively, by enzyme-linked immunosorbent assay (ELISA) with purified A/Bangkok/79 HA (11). A volunteer was considered infected with the vaccine virus if viral shedding or a fourfold-or-greater rise in titer of serum HAI, ELISA IgG HA or NA-inhibiting antibody, or nasal wash ELISA IgG HA antibody was detected.

The data in Table 1 summarize the immunological and virological responses of 70 volunteers to influenza A/Washington/80 *ca* reassortant virus in relation to their preimmunization level of serum HAI antibody. The results indicate that the susceptibility of adults to infection with the vaccine virus is inversely related to their preexisting immune status. The live virus vaccine infected almost all (94%) of the 35 seronegative volunteers and 51% of the 35 seropositive volunteers. In other tests not shown in Table 1, a significant rise in serum HAI antibody titer to influenza A/Texas/77

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TABLE 1. Responses of 70 adult volunteers who possessed various preimmunization levels of serum HAI antibody to *ca* reassortant influenza A/Washington/897/80 virus vaccine

Prevaccination HAI geometric mean antibody titer to the indicated virus:		No. receiving vaccine	% Infected	% Who shed virus	Reciprocal geometric mean antibody titer (% of vaccinees with significant antibody rise) by the following test:					
					Serum				Nasal wash ELISA HA IgA	
					ELISA HA IgG		NA-inhibiting			
A/Washington	A/Bangkok				Before vaccination	After vaccination	Before vaccination	After vaccination	Before vaccination	After vaccination
<1:4	1:9	12	100	42	955	2,702 (75)	2	6 (50)	10	143 (75)
1:4	1:24	23	91	0	1,722	6,081 (77)	5	16 (61)	27	145 (68)
1:8	1:39	20	65	5	2,896	5,405 (40)	13	19 (5)	31	85 (35)
>1:8[a]	1:106	15	33	7	7,643	11,037 (27)	31	42 (7)	58	125 (33)

[a] HAI titers in this group ranged from 1:16 to 1:32 with a geometric mean titer of 1:18.

virus was detected in 75, 52, 40, and 13% of the volunteers with prevaccination HAI antibody titers of <1:4, 1:4, 1:8, and >1:8, respectively. In 12 of the 70 vaccinees, the only evidence of infection was a fourfold-or-greater rise in titer of serum HAI antibody (2 vaccinees), serum ELISA IgG HA antibody (4 vaccinees), or nasal wash ELISA IgA HA antibody (6 vaccinees). Importantly, the serum IgG anti-HA ELISA and the nasal wash IgA anti-HA ELISA were both more sensitive than the HAI or serum NA-inhibiting test for detection of infection with vaccine virus.

Clearly, however, serum HAI antibody serves as an index of previous natural infection. In our study, volunteers with higher levels of HAI antibody before vaccination also tended to have higher levels of other influenza-specific antibodies in baseline serum and in nasal wash specimens. Our data indicated that natural infection produced higher levels of serum antibodies than those that were evoked in seronegative volunteers by live virus vaccination. Conversely, vaccination induced a higher mean titer of virus-specific local IgA HA antibody in each group of vaccinees than that which was present before vaccination. In another study, we observed that an increased level (titer, ≥1:64) of nasal wash IgA antibodies correlated with resistance to infection with wild-type virus (M. L. Clements, R. F. Betts, and B. R. Murphy, manuscript in preparation). In the present study, we found that this level of local IgA antibody was induced by live virus vaccination in the majority of seronegative as well as seropositive vaccinees (Table 2). Thus, the net effect of the live virus vaccine was to increase local secretory IgA HA antibody levels in both groups of vaccinees to a level that is associated with resistance to infection.

The level of HAI antibody response in serum has been used to assess the immunogenicity of live as well as inactivated influenza vaccines (1, 7–9). In a recent study (3), however, we showed that immunity conferred by infection with live *ca* virus vaccine protected humans against wild-type virus infection without regard to level of serum antibody induced. Liew and co-workers (6) confirmed this finding in animal studies. Their data suggested that specific secretory IgA in the respiratory tract may be the most important immunological mediator of protection induced by influenza virus infection. Thus, evaluation of this type of antibody response is important when assessing the immunogenicity and efficacy of a live influenza virus vaccine. Importantly, in the present study, both seronegative and seropositive vaccinees with low levels of nasal wash IgA HA antibody before vaccination were infected with the vaccine virus and mounted a vigorous local antibody response. Those who failed to mount a nasal antibody response usually possessed a high local level of specific antibody before vaccination. These results suggest that certain seropositive as well as all seronegative volunteers will benefit from live virus vaccination.

TABLE 2. Local HA-specific IgA antibody responses of seronegative and seropositive adults to intranasal vaccination with *ca* influenza A/Washington/987/80 virus

Prevaccination HAI antibody status of volunteers (*n*)	No. (%) of volunteers with nasal wash IgA HA antibody titer (log_2) of ≥1:6		ELISA HA IgA mean titer (reciprocal)[a]	
	Before vaccination	After vaccination	Before vaccination	After vaccination
Seronegative (34)	7 (21)	28 (82)[b]	4.2 ± 2.1	6.6 ± 1.9
Seropositive (35)	13 (37)	25 (71)[b]	5.3 ± 1.9[c]	7.2 ± 2.2

[a] Log_2 titer plus or minus the standard deviation.
[b] $P < 0.01$ versus before vaccination proportion by chi-square analysis with Yates correction.
[c] $P < 0.04$ versus seronegative mean by the two-tailed Student's *t* test.

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