Local and Systemic Antibody Responses in High-Risk Adults Given Live-Attenuated and Inactivated Influenza A Virus Vaccines

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Forty seropositive older adults with chronic diseases were vaccinated intranasally with either influenza A/California/10/78 (H1N1) (CR37) or influenza A/Washington/897/80 (H3N2) (CR48) virus. No clinically significant decrements in pulmonary function occurred postvaccination. Eight (62%) recipients of CR37 virus and 16 (59%) recipients of CR48 virus became infected with vaccine virus, as indicated by a fourfold rise in nasal wash immunoglobulin G (IgG) or IgA antibody titer, a fourfold rise in serum antibody titer, isolation of vaccine virus from nasal washings, or all of these. Within 2 years after cold-recombinant virus vaccination, 29 vaccinees received trivalent inactivated influenza virus vaccine parenterally. After inactivated virus vaccination, 23 (79%) vaccinees developed a fourfold rise in nasal wash or serum antibody titer to H1 antigen and 24 (83%) developed a fourfold rise in nasal wash or serum antibody titer to H3 antigen. Significantly more cold-recombinant virus vaccinees developed a fourfold rise in nasal wash IgA antibody to H1 or H3 hemagglutinin compared with inactivated virus vaccinees (17 [43%] versus 9 [17%], $P = 0.01$). We conclude that these cold-recombinant virus vaccines are safe and immunogenic in seropositive older high-risk adults and more often induced a nasal wash IgA antibody response than the inactivated virus vaccine.

Protection against influenza A virus illness in high-risk populations is a goal only partially attained with currently available inactivated influenza virus vaccines (10, 11, 32). While antibody response is higher in younger populations, in certain elderly populations the rate of serum antibody response after inactivated trivalent virus vaccination has been as low as 21% (4, 8, 9, 19-22, 26, 32, 36, 39). In addition, stimulation of secretory or cell-mediated immunity may be of equal or greater importance than that of the systemic humoral response in determining immunity to influenza A virus (13, 15, 17, 23). Stimulation of secretory immunoglobulin A (IgA) antibody production is thought to occur at low rates with parenteral influenza vaccines but is rarely measured, and only limited data are available in one study involving elderly adults (41). An approach to vaccination against influenza A virus infection which stimulates both local and systemic antibody responses may be preferable. Studies in seronegative children and young adults demonstrate that live-attenuated, cold-adapted reassortants of cold-adapted influenza A/Ann Arbor/6/60 virus effectively achieve this goal (5, 6, 13, 16, 17, 29). Although the immune response to influenza A virus is complex, protection from live virus challenge in children and young adults has been associated with secretory IgA antibody to hemagglutinin (HA), local neutralizing antibodies, serum IgG to HA and serum hemagglutination inhibition (HAI) antibody to the challenge virus strain (13, 14, 17, 23). A live-attenuated virus was more effective than an inactivated virus vaccine in an efficacy trial (13).

Only limited information regarding serum antibody responses and none regarding secretory antibody responses to live-attenuated virus vaccines in high-risk older adults has been published. This population generally has had previous experience with circulating wild-type influenza A virus strains and also possibly with inactivated virus vaccines. We have previously reported higher serum antibody response

rates in this population to inactivated virus vaccine compared with live-attenuated viruses (21). A more complete comparison must include the secretory antibody response to these vaccines. In the current study, we report the nasal wash antibody responses to intranasally administered live-attenuated, cold-adapted reassortant influenza A viruses in high-risk adults. Most of these adults subsequently underwent vaccination with parenterally administered trivalent inactivated influenza virus vaccine. We report their nasal wash antibody responses to inactivated virus vaccine administered 5 to 24 months after live-attenuated virus vaccine.

MATERIALS AND METHODS

Vaccines. The cold-recombinant (CR) influenza A/California/10/78 (H1N1) virus and influenza A/Washington/897/80 (H3N2) virus vaccines were derived from cold-adapted influenza A/Ann Arbor/6/60 virus by H. F. Maassab (University of Michigan) by methods previously described (18). The resulting CR H1N1 vaccine (CR37, lot no. E167) had a titer of $10^{7.4}$ 50% tissue culture infective doses (TCID₅₀) per ml, and the CR H3N2 vaccine (CR48, lot no. E179) had a titer of 10^8 TCID₅₀ per ml in Madin-Darby canine kidney cells. The vaccine viruses contain six genes that code for internal proteins from the donor cold-adapted strain, and the genes that code for HA and neuraminidase were derived from influenza A/California/10/78 (H1N1) virus and influenza A/Washington/897/80 (H3N2) virus, respectively. In addition to being cold adapted, the vaccine viruses were temperature sensitive (restrictive temperature, 39°C). Dilution of vaccine virus was made in Leibowitz medium (L-15) without pH indicator and resulted in an inoculum of H1N1 vaccine virus of either $10^{6.1}$ (10-fold dilution) or $10^{7.1}$ (undiluted as provided by the National Institute of Allergy and Infectious Diseases, Bethesda, Md.) TCID₅₀ and of an inoculum of H3N2 vaccine virus of either $10^{6.7}$ (10-fold dilution) or $10^{7.7}$ (undiluted as provided by the National Institute of Allergy and Infectious Diseases) TCID₅₀ per 0.5-ml dose.

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The zonal-purified whole-virion inactivated trivalent influenza virus vaccine available for the 1984 to 1985 influenza season (influenza A/Chile/1/83 [H1N1], influenza A/Philippines/2/82 [H3N2], and influenza B/USSR/83 [Connaught Laboratories, Swiftwater, Pa.]) was administered parenterally.

Selection of patients. Volunteers were ambulatory and not institutionalized. Initial groups of live-attenuated virus vaccinees did not have a history of pulmonary disease severe enough to require pharmacologic therapy. After it was clear that these initial volunteers did not experience clinically detectable lower respiratory symptoms and signs, persons with various degrees of chronic obstructive pulmonary disease (COPD) and other chronic diseases were identified. Because CR37 (H1N1) virus was not shed in nasal secretions of the initial volunteer groups, subsequent vaccinees with chronic diseases received CR48 (H3N2) virus. Volunteers were not excluded because of preexisting antibody to H1 and H3 HA or prior parenteral inactivated influenza virus vaccination; most older adults in the general population have had significant prior experience with influenza viruses.

Clinical studies. The protocol for clinical studies was approved by the Institutional Review Boards of the Huntington Veterans Administration Medical Center and Marshall University. After informed consent was obtained, volunteers were vaccinated with either CR influenza A/California/10/78 (H1N1) (CR37) or influenza A/Washington/89/80 (H3N2) (CR48) virus vaccines as previously described (21). The history of previous vaccination with inactivated trivalent influenza virus vaccine allowed us to analyze retrospectively the influence of inactivated virus vaccine on subsequent live-attenuated virus vaccination. Many volunteers subsequently received inactivated virus vaccine within 5 to 24 months after they received live-attenuated virus vaccine.

Standard spirometry was performed in the clinical pulmonary function testing laboratory before and 10 to 14 days after vaccination with the CR48 (H3N2) influenza A virus vaccine in 21 individuals with a Series 5000 Pulmo-Lab spirometer (Gould, Dayton, Ohio) by previously described methods (21). Spirometry was not performed in patients who received CR37 (H1N1) virus and inactivated virus vaccine. The forced expiratory volume (FEV₁) and forced vital capacity (FVC) were determined (2, 3), and the degree of obstruction to airflow was categorized on the basis of the FEV₁/FVC (percent).

Isolation of viruses. Nasal wash specimens were obtained before and daily for 4 days after vaccination with CR virus vaccine and were inoculated onto tissue culture cells as previously described (7, 21).

Serological tests. Nasal wash specimens (10 ml of L-15 medium) were done before and at 14 and 28 days after administration of CR37 (H1N1) and CR48 (H3N2) virus vaccines. To increase the volume of nasal wash specimen returned, nasal washes with 20 ml of L-15 medium were done before and at 28 days after administration of the inactivated virus vaccine. Serum specimens were obtained from each vaccinee before and 28 days after administration of CR37 (H1N1), CR48 (H3N2), and the inactivated trivalent influenza virus vaccine.

HAI antibodies in serum were measured by using whole virus homologous to the two CR virus vaccine strains in a standard microtiter assay (42).

Each CR vaccine virus strain was grown in the allantoic cavity of eggs, and the HA was extracted and purified by previously described methods (33). A one-step enzyme-linked immunosorbent assay (ELISA) was used to determine

the anti-influenza virus HA serum and nasal wash immunoglobulin titer and immunoglobulin isotype (21, 29, 30). The ELISA titers were calculated by a conventional positive-over-negative method in which the endpoint was the highest dilution that gave a positive-over-negative ratio of ≥ 2 . The ELISA nasal wash antibody titers were not corrected to a specific IgA concentration, because a proportion of the specimens did not have IgA detectable by the standard radial immunodiffusion assay (Technicon Instruments, South Plainfield, N.J.). IgA antibody titer to HA did not correlate with IgA concentration in the nasal wash specimens. The nasal wash value is thus not a corrected titer, but represents the titration endpoint for antibody present in 100 μ l of a nasal wash specimen concentrated to 1-ml volume (approximately 5- to 10-fold) in Aquacide (Calbiochem-Behring, La Jolla, Calif.). Precedent for this approach to nasal wash antibody titer determination has been previously reported (23, 29). Laboratory evidence of response to vaccination was defined by any of the following criteria: isolation of live-attenuated vaccine virus from a nasal wash specimen; a fourfold or greater rise of nasal wash IgA or IgG antibody titer to HA; or a fourfold or greater rise of serum HAI or serum IgG or IgA antibody titer to HA.

Statistical methods. Statistical tests included Fisher's exact test or chi-square analysis with the Yates' correction for two-by-two comparisons. Student's *t* test was used to compare the mean numerical measurements of the vaccine populations. Reciprocal antibody titers pre- and postvaccination were converted to log₂, and mean values were compared by the Wilcoxon signed ranks nonparametric test. Reciprocal geometric mean antibody titers are reported in the tables.

RESULTS

Characteristics of vaccine study populations. Thirteen volunteers (mean age, 55.2 ± 5.3 years) received CR37 (H1N1) virus intranasally. The initial eight persons were vaccinated with $10^{6.1}$ TCID₅₀. Because virus was not shed in nasal washings from these individuals and because no adverse effects were noted, five additional volunteers were vaccinated with undiluted virus preparation ($10^{7.1}$ TCID₅₀). Twenty-seven volunteers (mean age, 59.4 ± 8.2 years) subsequently received CR48 (H3N2) virus intranasally with $10^{6.7}$ TCID₅₀ (1 volunteer) or with $10^{7.7}$ TCID₅₀ (26 volunteers) per dose. A total of 10 of the CR37 (H1N1) vaccinees and 19 of the CR48 (H3N2) vaccinees (29 volunteers, mean age, 59.2 ± 7.6 years) received the inactivated trivalent influenza virus vaccine parenterally 5 to 24 months after receiving the CR virus vaccination. Nasal wash specimens were obtained from 27 of the 29 inactivated virus vaccinees.

The mean age was not significantly different among the vaccinee groups. Underlying diseases included COPD in 24 vaccinees, gastrointestinal diseases (usually cirrhosis or peptic ulcer disease) in 12, hypertension in 10, cardiovascular disease in 6, neoplastic disease in 2, diabetes mellitus in 2, and restrictive pulmonary disease in 1 vaccinee.

Postvaccination follow-up for possible CR vaccine virus-related complications. Twenty-one CR48 (H3N2) vaccinees underwent pulmonary function testing (Table 1). Of the 12 infected vaccinees tested, 1 experienced a worsening in FEV₁/FVC from one category to another and 5 experienced an improvement. A larger proportion of vaccinees who became infected with CR48 (H3N2) virus experienced an improvement or no change in FEV₁ postvaccination (10 of 12 infected vaccinees) compared with those vaccinees who did

TABLE 1. Comparison of the degree of obstructive lung disease measured prevaccination with results measured 10 to 14 days postvaccination among 21 older adults given CR48 (H3N2) virus vaccine^a

Prevaccination assessment of pulmonary function (FEV ₁ /FVC) (%)	No. of patients with the indicated FEV ₁ /FVC (%) postvaccination (no. infected with vaccine virus) ^b			
	≥70	60-69	45-59	<45
≥70	5 (2)	0	0	0
60-69	3 (3)	3 (2)	2 (1)	0
45-59	1 (1)	0	4 (2)	0
<45	0	0	1 (1)	2

^a Obstructive lung disease is defined by the FEV₁/FVC (percent) as follows: ≥70, normal; 60 to 69, mild obstruction to airflow; 45 to 59, moderate obstruction; <45, severe obstruction.

^b Infection defined by isolation of live-attenuated vaccine virus, fourfold or greater rise of nasal wash IgA or IgG antibody titer to HA, or fourfold or greater rise of serum HAI or serum IgG or IgA antibody titer to HA.

not become infected with vaccine virus (one of nine noninfected vaccinees) ($P < 0.001$, Fisher's exact test, two-tailed). Means of FEV₁ and FEV₁/FVC for various vaccinee groups before and after vaccination with CR48 (H3N2) have been published previously (21). The mean FEV₁/FVC for all vaccinee groups was 60 to 63%, borderline between moderate and mild airflow obstruction.

Infection as indicated by nasal shedding of CR virus. None of the volunteers vaccinated with CR37 (H1N1) virus shed virus in nasal washings. Two CR48 (H3N2) virus vaccinees shed vaccine virus on day 2 or 3 after inoculation with CR vaccine. One of these two persons also developed a fourfold rise in ELISA serum IgG antibody titer; neither had a fourfold rise in nasal wash antibody titer.

Nasal wash antibody response to CR virus or inactivated trivalent virus vaccine administered within 2 years after CR virus vaccination. The proportion of vaccinees with a fourfold rise in IgA antibody to H3 antigen after CR48 virus vaccination was significantly higher than after the inactivated virus vaccination (Table 2). The proportion of vaccinees who developed a fourfold rise in IgA antibody to H1 antigen was not significantly higher after CR37 administra-

tion than after the inactivated virus vaccination. However, among all CR virus vaccinees, a larger proportion developed a fourfold rise in nasal wash IgA antibody to H1 or H3 HA compared with inactivated virus vaccinees (17 of 40 versus 9 of 54, $P = 0.01$). The proportion of CR37 (H1N1) and CR48 (H3N2) virus vaccinees who developed a fourfold nasal IgA secretory antibody response to HA 14 or 28 days postvaccination was not different from the proportion who developed an IgG anti-HA rise in nasal wash specimens (Table 2). The mean reciprocal IgA antibody titer to H1 antigen rose 14 days postvaccination compared with prevaccination levels. The mean reciprocal IgA and IgG antibody titers to H3 antigen both rose significantly at 14 and 28 days postvaccination compared with prevaccination levels (Table 3).

The proportion of vaccinees who developed a fourfold IgA or IgG antibody response to H1 HA or to H3 HA 28 days after the inactivated virus vaccine administration was not significantly different from that after CR37 or CR48 virus vaccination except in the case of IgA antibody response to H3 HA as noted above (Table 2). The mean reciprocal IgA and IgG antibody titers to H1 antigen and the mean reciprocal IgG antibody titer to H3 antigen all rose at 28 days postvaccination with the inactivated virus (Table 3).

Overall local and systemic response to CR virus or inactivated trivalent virus vaccine administered within 2 years after CR virus vaccination. A significantly larger proportion of CR37 (H1N1) but not CR48 (H3N2) virus vaccinees developed a fourfold rise in nasal wash antibody than developed a serum antibody response (Table 2). All persons who developed a serum antibody response to the H1 antigen of CR37 also developed a nasal wash antibody response. One person shed CR48 (H3N2) virus postvaccination, but did not exhibit a nasal wash or serum humoral response. The other person who shed CR48 (H3N2) virus developed a serum antibody response, but no rise in nasal wash antibody. Three CR48 (H3N2) virus vaccinees developed a serum antibody response without a concomitant nasal wash response. The overall proportion of CR virus vaccinees who developed a nasal wash antibody response, serum antibody response, or shed CR virus was not significantly different when the two CR virus groups were compared (Table 2).

TABLE 2. Number of patients with fourfold serum or nasal wash antibody responses or both to H1 and H3 antigens after vaccination with monovalent live-attenuated, cold-recombinant and inactivated influenza virus vaccines

Vaccine type	No. of patients with fourfold antibody rise/no. tested (%)							No. of patients with any antibody response ^a and/or nasal wash shedding of CR virus/no. tested (%)
	Serum antibody				Nasal wash antibody			
	HAI ^b	IgG ^c	IgA ^c	Any ^d	IgG ^c	IgA ^c	Either	
Live virus								
CR37 (H1)	1/13 (8) ^e	0/13 (0) ^e	1/13 (8)	2/13 (15) ^{e,f}	7/13 (54)	5/13 (38)	8/13 (62) ^f	8/13 (62)
CR48 (H3)	3/27 (11) ^e	9/27 (33) ^e	7/27 (26)	11/27 (41) ^e	10/27 (37)	12/27 (44) ^h	12/27 (44)	16/27 (59) ⁱ
Inactivated trivalent virus								
H1 antigen	15/29 (52) ^e	17/29 (59) ^e	8/29 (28)	21/29 (72) ^e	13/27 (48)	6/27 (22)	13/27 (48)	23/29 (79)
H3 antigen	15/29 (52) ^e	21/29 (72) ^e	11/28 (39)	23/29 (79) ^{e,g}	9/27 (33)	3/27 (11) ^h	9/27 (33) ^g	24/29 (83) ⁱ

^a Response = fourfold rise in serum HAI, serum IgG, serum IgA, nasal wash IgG, and/or nasal wash IgA antibody titer.

^b HAI antibody to H1 or H3 HA.

^c Antibody determined by ELISA to H1 or H3 HA.

^d HAI, IgG, and/or IgA antibody.

^e $P < 0.02$, when comparing serum antibody responses in each category indicated for CR37 versus inactivated virus H1 and for CR48 versus inactivated virus H3.

^f $P = 0.04$.

^g $P < 0.01$.

^h $P < 0.02$.

ⁱ $0.1 > P > 0.05$.

TABLE 3. Secretory antibody in the upper respiratory tract as indicated by reciprocal geometric mean IgA and IgG nasal wash antibody titers to HA^a

Vaccine type immunoglobulin determined	Reciprocal geometric mean ELISA antibody titer					
	Immunoglobulin type-specific ELISA antibody titer to H1 antigen at the indicated times			Immunoglobulin type-specific ELISA antibody titer to H3 antigen at the indicated times		
	Prevaccination	Postvaccination		Prevaccination	Postvaccination	
		14 days	28 days		14 days	28 days
Live virus						
CR37 (H1N1)						
IgA	2.1	4.2 ^b	4.2			
IgG	2.6	6.5	5.2			
CR48 (H3N2)						
IgA				1.8	3.4 ^c	4.7 ^c
IgG				3.9	7.4 ^b	9.3 ^c
Inactivated trivalent influenza virus ^d						
IgA	8.6		13.0 ^b	2.9		4.2
IgG	6.0		18.6 ^c	4.3		10.6 ^c

^a Specimens were collected before and after vaccination with live-attenuated, cold-recombinant CR37 (H1N1) and CR48 (H3N2) and inactivated influenza virus vaccines.

^b $P < 0.05$, comparing the postvaccination mean antibody titer with the prevaccination level.

^c $P < 0.01$, comparing the postvaccination mean antibody titer with the prevaccination level.

^d The volume of L-15 used for nasal washes was doubled for those specimens obtained before and after the inactivated virus vaccine compared with the volume used before and after CR virus vaccines.

A larger proportion of inactivated virus vaccine recipients developed a fourfold rise in nasal wash IgG antibody titer to H1 or H3 HA or both than developed an IgA antibody titer rise (22 of 54 versus 9 of 54, $P = 0.01$). A larger proportion of inactivated virus vaccinees developed a fourfold rise in serum antibody to H3 antigen than developed a rise in nasal wash antibody (Table 2). The inactivated virus vaccinees developed significantly higher fourfold serum HAI and IgG antibody response rates to H1 antigen and H3 antigen than did CR37 and CR48 virus vaccinees, respectively (Table 2). When all criteria for response to vaccination are considered (vaccine virus shedding, nasal wash antibody, and serum antibody), there was no significant difference in the proportions of CR37 virus vaccinees and inactivated virus vaccinees who developed a response to H1 antigen and no difference in the response rates of the inactivated virus vaccinees compared with CR48 virus vaccinees to H3 antigen (Table 2).

Seroresponse as a function of prevaccination antibody titer. Because prevaccination antibody titer may have influenced response to the vaccines, we compared reciprocal prevaccination antibody titers as a function of subsequent response to live virus vaccination. Neither mean prevaccination nasal wash titers (IgA and IgG anti-HA) nor mean serum antibody titers (HAI and IgA and IgG anti-HA) differed significantly when responders to the live-attenuated vaccines were compared with nonresponders.

In contrast, among volunteers given inactivated vaccine, prevaccination reciprocal geometric mean serum HAI titer to H1 antigen was significantly lower among seroresponders (7.8 versus 18.0; $P = 0.02$). In addition, the same was true for prevaccination reciprocal geometric mean IgG antibody titer to H1 antigen in these two groups (2,951 versus 6,310; $P = 0.04$). No significant differences occurred in reciprocal geometric mean serum IgA antibody titer to H1 antigen of nonresponders versus responders to the inactivated virus vaccine. No differences in prevaccination reciprocal geometric mean serum HAI, IgG antibody, or IgA antibody to H3 antigen occurred when nonresponders and responders to inactivated virus vaccine were compared.

Effect of history of influenza virus vaccination on seroresponse to CR48 (H3N2) virus vaccine. Of the 27 CR48 (H3N2) virus vaccinees, 26 had medical records or were able to give a vaccine history that was believed to be reliable. Fifteen recipients had been vaccinated in a previous year with inactivated influenza virus vaccine, and 11 had never been vaccinated for influenza. Of the 15 vaccinees with a history of inactivated virus vaccination, 6 became infected with the CR48 (H3N2) virus and 9 did not. This was not significantly less than the 9 who became infected with CR48 virus of the 11 vaccinees who gave a history of no previous influenza virus vaccination ($P = 0.072$, Fisher's exact test, two-tailed). Not all the patients received the inactivated vaccine within 6 to 12 months before vaccination with the CR virus.

Effect of CR virus on subsequent response to inactivated virus vaccine. Infection with the CR virus vaccines did not alter the rate of the response to subsequent inactivated virus vaccine administration. All three persons who did not become infected with CR37 (H1N1) virus who subsequently received the inactivated virus vaccine had an antibody response to both H1 and H3 antigens. Of the seven persons who became infected with CR37 (H1N1) virus and subsequently received inactivated virus vaccine, six developed an antibody response to both the H1 and H3 antigens and one developed an antibody response to only the H3 antigen. Of the nine persons who did not become infected with CR48 (H3N2) virus and who subsequently received inactivated virus vaccine, five had an antibody response to both H1 and H3 antigen, two did not respond to either antigen, and two responded to only the H3 antigen. Of the 10 persons who became infected with CR48 (H3N2) virus and who subsequently received inactivated virus vaccine, 7 had an antibody response to both H1 and H3 antigens, 1 did not respond to either antigen, and 2 responded to only the H1 antigen. In summary, 13 (76.5%) of 17 persons who became infected with CR virus and 7 (58.3%) of 12 persons who did not become infected with CR virus had an antibody response to subsequent inactivated virus vaccine homologous antigen (P is not significant).

TABLE 4. Summary of studies reporting serum and nasal wash antibody responses to live-attenuated, cold-recombinant influenza virus vaccines and inactivated influenza virus vaccines

Reference	Seronegative before vaccination?	Vaccine type		Ages of vaccinees (yr)	No. of patients with fourfold antibody rise to HA/no. tested (%)				
		Live virus subtype	Parenteral inactivated virus subtype		Serum antibody to HA measured			Nasal wash antibody to HA measured	
					HAI ^a	IgG ^b	IgA ^c	IgG	IgA
6	Yes	H1N1 CR37		1–6	32/45 (71)	33/45 (73) ^d			
6	No	H3N2 CR48		1–6	16/46 (35)	25/46 (54) ^d			
23	No	H3N2 CR48		2–7	14/47 (30)	21/47 (45)	18/47 (38)	29/43 (67)	26/43 (60)
29	Yes	H3N2 CR29 H1N1 CR35		1.5–4.5		17/17 (100)	13/17 (76)	9/17 (53)	14/17 (82)
35	Some		H1N1	16–25	79/132 (60)				
35	Some		H1N1	≥26	92/185 (50)				
35	Some		H3N2	16–83	149/260 (57)				
43	Yes	H1N1 CR35		18–27	3/17 (18) ^e			1/17 (6)	7/17 (41)
43	No	H1N1 CR35		18–27	13/18 (72) ^e			9/18 (50)	13/18 (72)
43	Yes		H1N1	18–27	18/24 (75) ^e			8/24 (33)	3/24 (13)
43	No		H1N1	18–27	8/12 (67) ^e			6/12 (50)	2/12 (18)
12	Yes	H3N2 ^f CR48		Young adults					54/115 (47)
28	Yes	H1N1 ^g CR37		Young adults		41/58 (71) ^h			40/58 (69)
16	Yes	H1N1 CR37		18–35		15/23 (65)	20/23 (87)	14/23 (61)	20/23 (87)
16	Yes	H3N2 CR48		18–35		24/31 (77)	25/31 (81)	18/31 (58)	25/31 (81)
16	Yes		H1N1	18–35		20/20 (100)	18/20 (90)	19/20 (95)	6/20 (30)
16	Yes		H3N2	18–35		35/35 (100)	35/35 (100)	33/35 (94)	15/35 (44)
27	Some	H3N2 RIT4199		Adults (COPD)	24/48 (50)				
27	No	H3N2 CR48		Adults (COPD)	9/29 (31)				
9	No		H1N1	67–97	18/43 (42)				
9	No		H3N2	67–97	22/43 (51)				
41	No		H3N2	≥60	1/8 (13)				6/11 (55)

^a HAI antibody to HA of vaccine.^b IgG antibody to HA of vaccine.^c IgA antibody to HA of vaccine.^d Antibody to HA of the vaccine by ELISA, but immunoglobulin isotype not determined.^e Serum neutralizing antibody to HA of vaccine used.^f Dose varied from 10^{5.5} to 10^{7.5} TCID₅₀; 77 (67%) volunteers were infected of 115 tested by serum and nasal wash antibody responses and by virus shedding.^g Dose varied from 10^{5.8} to 10^{7.8} TCID₅₀; 47 (81%) volunteers were infected of 58 tested by serum and nasal wash antibody responses and by virus shedding.^h Percentage with any antibody rise by HAI, neuraminidase inhibition, or ELISA.

DISCUSSION

The two CR influenza A virus vaccines used in this study were safe when administered intranasally to older adults with chronic diseases. Significant decrements in pulmonary function tests did not occur after administration of these vaccines. Although the occurrence of transient spirometric changes during the first 10 days postvaccination are not ruled out by our data, testing pulmonary functions at 10 to 14 days postvaccination would probably reveal spirometric changes as a result of influenza A virus infection in persons with COPD (21, 25, 38). Our safety data confirm previous reports by two groups (1, 24, 27) that live-attenuated influenza A virus vaccines did not acutely change the pulmonary function of adults with chronic obstructive airway disease nor the specific airway conductance among patients with asthma.

As expected, the rates of serum antibody response to the two CR virus strains in this study were lower than those in studies of seronegative children (71 to 100%) and seronegative adults (18 to 87%) (Table 4). The rates of fourfold nasal wash IgA antibody titer rises in our CR vaccinees were lower than those reported in either seronegative or seropositive (41 to 87%) children and young adults who received CR vaccine (Table 4). However, the rate of fourfold rises in nasal wash IgA antibody titer to H1 and H3 HA combined was significantly higher among our CR vaccinees than among our inactivated virus vaccinees. Belshe et al. (5, 6) reported infection rates of 60 to 79% after vaccination with CR37 (H1N1) virus and up to 100% after CR48 (H3N2) virus vaccination in seronegative children. Approximately one half of seropositive children became infected with CR37 or CR48 virus (although nasal wash antibodies were not measured) (6). Clements et al. (13) reported an HAI antibody rise in 94% in seronegative young adults vaccinated with inactivated trivalent influenza virus vaccine, compared with a 44% response rate with CR48 (H3N2) virus ($10^{7.5}$ TCID₅₀). If serum antibody, nasal wash antibody, and virus shedding are all considered, the percentage of seronegative young adults infected with CR37 (H1N1) virus and with CR48 (H3N2) virus administered at doses of $10^{7.5}$ to $10^{7.8}$ TCID₅₀ was 85 to 96% (12, 13, 28). This is higher than the overall response in our patients of 59 to 62%.

Clements et al. (13) found a higher rate of nasal wash secretory IgA antibody rises among seronegative young adults who received intranasal CR48 (H3N2) virus compared with the response after parenteral administration of inactivated virus vaccine (69 versus 31%). Clements and Murphy (16) and Zahradnik et al. (43) also observed better local IgA antibody to HA responses to live-attenuated virus vaccines than to inactivated virus vaccine. The inactivated virus vaccine more often stimulated a nasal wash IgG response. The pattern of nasal wash antibody responses in our patients was similar. The nasal wash IgG antibody response to inactivated virus vaccine may be due to passive diffusion from the intravascular space (29, 31, 40).

Parenteral administration of inactivated trivalent influenza virus vaccine 5 to 24 months after CR virus vaccination resulted in significantly more serum antibody responses compared with those occurring after the intranasal administration of CR viruses. Although it is possible that the response rates to the inactivated virus vaccine were altered by the exposure of the patients to the CR virus within the previous 2 years, our data suggest otherwise. The rates of seroresponse to inactivated virus vaccine in our patients were similar to those reported in the literature for this type of noninstitutionalized older adult (4, 8, 9, 19, 20, 22, 26, 32,

35–37, 39), and repeated vaccination with inactivated influenza A virus vaccine does not result in a significant increase in the proportion of individuals with protective levels of serum antibody or in the proportion of individuals with fourfold serum antibody rises (34). Exposure to CR virus would not be expected to be the factor determining the rates of serological response to inactivated virus vaccine in these patients with previous influenza virus experience.

In our study, previous inactivated virus vaccine may have interfered with response to the live-attenuated virus vaccine, although our data did not achieve statistical significance. Evaluation of a larger number of volunteers might have shown that the inactivated virus vaccine imparts some degree of persistent immunity against challenge with live virus.

The higher rates of nasal wash antibody response to the CR virus vaccines support the contention that these vaccines may be more efficacious than parenterally administered inactivated virus vaccines. Clements et al. (15) observed that live-attenuated virus vaccine infection-induced and inactivated virus vaccine-induced immunity may involve different compartments of the immune system. Infection-induced resistance to wild-type virus infection correlated with local IgA antibody to HA (15); live-attenuated virus vaccines stimulate the production of this local IgA antibody to HA. In another study, however, Clements et al. (13) found no significant correlation between the level of nasal wash IgA antibody to HA before challenge with wild-type virus and the magnitude and duration of virus shedding after challenge. Clearly, factors additional to the local antibody response (e.g., cytotoxic T cells and enhanced lymphokine production) may play a role in protection against natural challenge and recovery from influenza A virus infection. Response to live-attenuated influenza A virus vaccine may separate vaccinees with previous influenza A virus experience into two groups, those who are susceptible to wild-type influenza A virus infection and those who are already immune to natural challenge. Perhaps protection from infection by CR virus vaccination correlates with protection from naturally acquired wild-type influenza A virus. If infection caused by CR virus vaccine induces protection against wild-type influenza A virus and if adults who fail to become infected after CR virus vaccination are also immune to naturally acquired influenza A virus infection, CR virus vaccine may have superior efficacy when compared with inactivated virus vaccine. Our safety and antigenicity tests provide the foundation for further studies with these promising live-attenuated influenza virus vaccines.

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