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Safety of and Serum Antibody Response to Cold-Recombinant Influenza A and Inactivated Trivalent Influenza Virus Vaccines in Older Adults with Chronic Diseases

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Forty 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 morbidity or decrement in pulmonary function occurred postvaccination. Two (15%) recipients of CR37 virus and twelve (44%) recipients of CR48 virus became infected with vaccine virus, as indicated by a fourfold rise in serum hemagglutination inhibition antibody titer; a fourfold rise in serum immunoglobulin G (IgG) or IgA antibody titer, indicated by enzyme-linked immunosorbent assay; isolation of vaccine virus from nasal washings; or all of these. Within 1 year after cold-recombinant vaccine virus vaccination, 18 vaccinees received inactivated trivalent influenza virus vaccine parenterally. Of the vaccinees, 13 (72%) developed a fourfold rise in serum antibody titer to H1N1 antigen and 16 (89%) developed a fourfold rise in serum antibody titer to H3N2 antigen. We conclude that administration of these cold-recombinant vaccine viruses to older adults with chronic diseases was safe, but that serum antibody response rates were lower than those achieved with subsequently administered inactivated influenza virus vaccine given parenterally. However, the higher seroconversion rates attained by using the inactivated trivalent influenza virus vaccine do not necessarily mean that it is more efficacious in preventing infection or severe illness or both due to natural wild-type influenza A virus.

Before each respiratory disease season, it is recommended that populations at high risk for complications resulting from influenza virus infection be immunized against the prevalent strains of influenza viruses (11, 12). The purpose of this immunization is to reduce mortality and morbidity from influenza illness and pneumonia (either primary viral or secondary bacterial pneumonia). Among those recommended to receive influenza vaccine are persons over the age of 65 years and patients with chronic lung, heart, or metabolic disease. Despite a documented need for influenza immunization in these populations, compliance may be low and the efficacy of inactivated influenza virus vaccine may be lower in the elderly than in younger persons (26). The fourfold rate of serologic response to inactivated virus parenteral vaccines has ranged from 70 to 90%, and in certain elderly subpopulations the response rate has been as low as 21% (4, 8, 9, 16–18, 21, 26, 28, 31). Previous influenza virus vaccination appears to decrease the rate of serologic response to subsequent vaccination as well (8).

Intranasally administered live-attenuated, cold-recombinant (CR) influenza A virus vaccines offer a potentially more desirable and effective approach to immunization against influenza A virus infection. CR virus vaccines do not require parenteral injection and offer the theoretical advantage of stimulating secretory immunoglobulin A (IgA) antibody production at the natural portal of entry (31). Neutralizing IgA antibody and, to a lesser extent, IgG antibody are present in nasal wash specimens taken from volunteers who are resistant to challenge with influenza A. The anti-hemagglutinin serum IgA antibody level may correlate with the production of secretory IgA in the respiratory tract, perhaps due to the escape of locally produced IgA antibody from the nasal mucosa into the systemic circulation (10, 24). Clements et al. (14) reported that protection against wild-type virus challenge in young adults was greater after vaccination with influenza A/Washington/897/80 (H3N2) (CR48) virus than after parenteral inactivated trivalent influenza virus vaccine. The CR virus vaccine also reduced the titer of wild-type virus shed in respiratory secretions compared with the inactivated virus vaccine.

Worsening of chronic airways disease is a potential hazard with widespread use of live-attenuated virus vaccine. This has not, however, been clinically apparent in previously reported series in which persons with chronic bronchitis, asthma, or chronic obstructive pulmonary disease (COPD) were vaccinated with other live-attenuated or CR virus strains (1, 19, 22). To further investigate influenza A virus vaccination in high-risk adults, we studied the safety of and serum antibody responses to intranasally administered CR influenza A virus vaccines. Subsequently, many adults have undergone vaccination with parenterally administered, inactivated trivalent influenza virus vaccine; we report their responses to inactivated virus vaccine after live-attenuated virus vaccination.

MATERIALS AND METHODS

Vaccines. The 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 (15). The resulting CR H1N1 vaccine (CR37, lot no. E167) had a titer of $10^{7.4}$ 50% tissue culture infective doses ($TCID_{50}$s) per ml, and the CR H3N2 vaccine (CR48, lot no. E179) had a titer of 10^{8} $TCID_{50}$s 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 hemagglutinin (HA) and neuraminidase were derived from

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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.) $TCID_{50}$s 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) $TCID_{50}$s per 0.5-ml dose.

The zonal-purified whole virion, inactivated trivalent influenza virus vaccine available for the 1984–1985 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. They were recruited from outpatient clinics and were 43 to 73 years of age. Initial groups of live-attenuated virus vaccinees did not have a history of pulmonary disease which was 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 COPD and other chronic diseases were identified by screening the pulmonary function laboratory logbook and outpatient clinic records for recruitment in subsequent studies. Because CR37 (H1N1) virus was not shed in nasal secretions of the initial volunteer groups, subsequent vaccinees with chronic diseases received CR48 (H3N2) virus. All potential vaccinees underwent a complete history, physical examination, and screening laboratory procedures that included assessing levels of antibody to H1 and H3 HA, urinalysis, complete and differential blood cell counts, alanine amino and gamma glutamyltransferase level determinations, and chest roentgenograms. Pulmonary function testing (PFT) that consisted of basic spirometry performed in the clinical PFT laboratory using a Series 5000 Pulmo-Lab (Gould, Dayton, Ohio) was done before and after live-attenuated virus vaccination in vaccinees with a history of pulmonary disease and other chronic diseases in the subsequent studies. Spirometry was not performed before and after parenteral administration of inactivated virus vaccine. Volunteers were not selected on the basis of prevaccination level of antibody to H1 and H3 HA or on the basis of parenteral inactivated influenza virus vaccination before a previous influenza season; most older adults in the general population have had significant prior experience with influenza viruses. Criteria used to exclude potential volunteers included the following: life-threatening cardiac dysrhythmias, concomitant antineoplastic chemotherapy, an absolute neutrophil count of <1,000 cells per mm^3, clinically unstable COPD or cardiovascular disease, and a known allergy to eggs, neomycin, or amphotericin B.

Clinical studies. The protocol for clinical studies was approved by the Institutional Review Boards of the Huntington Veterans Administration Medical Center and of Marshall University. After informed consent was obtained, volunteers were placed in the supine position, and the vaccine was administered intranasally in a total volume of 0.5 ml (0.25 ml in each nostril). Volunteers (see Results section for numbers of volunteers in each group) were vaccinated with either CR influenza A/California/10/78 (H1N1) (CR37) or influenza A/Washington/897/80 (H3N2) (CR48) virus vaccines. The frequency of previous vaccination with inactivated trivalent influenza virus vaccine in previous years allowed us to retrospectively analyze the influence of inactivated virus vaccine on subsequent live-attenuated virus vaccination. Many volunteers received inactivated virus vaccine within 5 to 12 months after they received live-attenuated virus vaccine. This allowed us to prospectively evaluate the influence of live-attenuated virus vaccine on subsequent antibody response to inactivated virus vaccine.

Recipients of CR influenza A virus vaccines were observed daily for symptoms and signs of adverse reactions related to infection with vaccine virus (e.g., runny nose, sore throat, cough, earache, red eyes, fever, congestion, increased shortness of breath, and auscultatory pulmonary changes) on days 1 to 5 and on days 7, 14, and 28 postvaccination.

Standard spirometry was performed in the clinical PFT laboratory pre- and 10 to 14 days postvaccination with the CR48 (H3N2) influenza A virus vaccine in 21 individuals but not with the CR37 (H1N1) virus vaccinees. The forced expiratory volume (FEV_1), forced vital capacity (FVC), and FEV_1/FVC (percent) were determined (2, 3). The degree of obstruction to airflow was categorized on the basis of the FEV_1/FVC (percent), and an FEV_1/FVC of $\geq$70% was defined as normal. Mild obstruction was defined as an FEV_1/FVC of 60 to 69%, moderate obstruction was defined as 45 to 59%, and severe obstruction was defined as an FEV_1/FVC of 45%. Several spirometric trials were done, and the best effort was reported. When the trials were inconsistent, the test was discarded and repeated.

Isolation of viruses. Nasal wash specimens (5 ml of veal infusion broth in each nostril) were obtained before vaccination with CR virus vaccine and on days 1 to 5 and on day 7 postvaccination. Specimens were transported on wet ice to the laboratory for inoculation onto tissue culture cells. A portion of each specimen was frozen at −70°C, and a portion was inoculated onto primary rhesus monkey kidney or Madin-Darby canine kidney cells with trypsin overlay and onto MRC-5 cells and incubated at 34°C for 14 days. Virus isolates were identified as previously described (7). When influenza A virus was isolated, it was reinoculated onto rhesus monkey kidney or Madin-Darby canine kidney cells and incubated at 39°C to assess the presence of revertant virus, i.e., virus that had regained the ability to replicate at 39°C. In addition, when influenza A virus was isolated, a portion of the frozen clinical specimen was thawed and inoculated onto tissue culture cells in serial 10-fold dilutions from an undiluted state to a 1:10,000 dilution to quantitate viral shedding.

Serological tests. Serum specimens were obtained from each vaccinee before and 28 days after administration of CR37 (H1N1), CR48 (H3N2), or the inactivated trivalent influenza virus vaccine. These specimens were stored at −20°C until testing for antibody to H1N1 or H3N2.

Hemagglutination inhibition (HAI) antibodies were measured by using whole virus homologous to the two CR virus vaccine strains in a standard microtiter assay (32).

Each CR vaccine virus strain was grown in the allantoic cavity of eggs, and the HA was extracted and purified by previously described methods (27), including nonionic detergent solubilization, density gradient centrifugation, and affinity chromatography. A one-step enzyme-linked immunosorbent assay (ELISA) previously described by Murphy et al. (24, 25) was used to detect anti-influenza virus serum immunoglobulin titer and immunoglobulin isotype. The sequence of reagents from the solid phase onward consisted of

purified HA, human serum (in serial 1:4 dilutions), and a goat anti-human immunoglobulin serum (anti-IgG or anti-IgA) conjugated with alkaline phosphatase (Miles Scientific, Div. Miles Laboratories, Inc., Naperville, Ill.) and substrate (*p*-nitrophenyl phosphate disodium; Sigma Chemical Co., St. Louis, Mo.). The yellow chromogen produced by cleavage of the substrate was measured by A_{405} on a multichannel spectrophotometer (Dynatech Industries, Inc., McLean, Va.). 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.

Statistical methods. Statistical tests were performed on an Apple IIE computer using the Stats Plus program. 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. The Mann-Whitney test was used to compare the fold increase in reciprocal antibody titer to vaccination with CR37 (H1N1) versus inactivated virus vaccine H1N1 antigen and with CR48 (H3N2) versus inactivated virus vaccine H3N2 antigen. The Mann-Whitney test also was used to compare prevaccination reciprocal antibody titers of patients who demonstrated a fourfold seroresponse to vaccination with titers of nonseroresponders.

RESULTS

Characteristics of the vaccine study populations. A total of 13 volunteers received CR37 (H1N1) virus intranasally. The initial eight persons were vaccinated with $10^{6.1}$ $TCID_{50}$s. Because virus was not shed in nasal washings from these individuals and because no adverse effects were noted, five other volunteers were vaccinated with the undiluted virus preparation ($10^{7.1}$ $TCID_{50}$s). A total of 27 volunteers subsequently received CR48 (H3N2) virus intranasally. Initially, one volunteer was vaccinated with $10^{6.7}$ $TCID_{50}$s. When virus was not shed in nasal washings of this volunteer or in those of the CR37 (H1N1) volunteers and when no adverse effects were noted, 26 persons were vaccinated with $10^{7.7}$ $TCID_{50}$s per dose (undiluted preparation). Ten of the CR37 (H1N1) vaccinees and eight of the CR48 (H3N2) vaccinees received the inactivated trivalent influenza virus vaccine parenterally 5 to 12 months after receiving the CR virus vaccination.

The mean age was not significantly different among the vaccinee groups (Table 1). 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. We obtained each vaccinee's history of influenza virus vaccination before his or her enrollment in the current study. Four of the CR37 (H1N1) vaccinees had received inactivated influenza virus vaccine at some time in previous years, one had not, and this information was not known for the remaining eight vaccinees. Of the CR48 (H3N2) vaccinees, 14 had received inactivated influenza virus vaccine at some time in previous years, 11 had not, and this information was not known for the remaining 2 vaccinees.

TABLE 1. Numbers and ages of individuals who received each vaccine

Vaccine	No. of vaccinees	Mean age (yr)	Age range (yr)
CR37 (H1N1)	13	55.2 ± 5.3	45–63
CR48 (H3N2)	27	59.4 ± 8.2	43–73
Either CR	40	58.1 ± 7.7	43–73
Inactivated virus	18[a]	58.4 ± 8.4	43–73

[a] Of these vaccinees, 10 had received CR37 (H1N1) virus and 8 had received CR48 (H3N2) virus before receiving the inactivated virus vaccine.

TABLE 2. PFTs done prevaccination and 10 to 14 days postvaccination in 21 older adults given CR48 (H3N2) virus vaccine[a]

Prevaccination PFT (FEV_1/FVC) result (%)	No. of patients with the following postvaccination PFT results (no. infected with vaccine virus)			
	≥70	60–69	45–59	<45
≥70	5 (1)	0	0	0
60–69	3 (3)	3 (1)	2 (1)	0
45–59	1 (1)	0	4 (2)	0
<45	0	0	1 (1)	2

[a] PFT results are as follows: ≥70, normal; 60 to 69, mild obstruction to airflow; 45 to 59, moderate obstruction; <45, severe obstruction.

Postvaccination follow-up for possible CR vaccine virus-related complications. Morbidity during the first 7 days postvaccination occurred in five CR virus vaccinees, who developed upper-respiratory-tract symptoms (e.g., rhinitis and sore throat). Only one of these five persons had become infected with the vaccine virus. Conjunctivitis developed in two CR vaccinees, neither of whom had become infected with the vaccine virus; viral cultures of conjunctival swab specimens did not yield a virus. One CR virus vaccinee required hospitalization for a supraventricular tachycardia. He had not become infected with vaccine virus and had a history of supraventricular arrhythmias predating vaccination. A CR48 (H3N2) vaccinee who had developed a fourfold rise in IgG antibody titer and who shed vaccine virus on days 2 and 3 postvaccination developed an influenzalike clinical illness 10 months after live-attenuated virus vaccination, and wild-type influenza A virus H3N2 was isolated from a nasal swab culture at that time.

None of the vaccinees developed increased dyspnea postvaccination. Of the 21 CR48 (H3N2) vaccinees who underwent PFT, 2 experienced a worsening in FEV_1/FVC from one category of airflow obstruction to another and 5 experienced an improvement in FEV_1/FVC from one category to another (Table 2). Of the 10 infected vaccinees, 1 experienced a worsening in FEV_1/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 FEV_1 postvaccination (9 of 10 infected vaccinees) compared with those vaccinees who did not become infected with vaccine virus (2 of 11 noninfected vaccinees) ($P = 0.0016$, Fisher's exact test, one-tailed). Differences in means of FEV_1 and FEV_1/FVC for various vaccinee groups before and after vaccination with CR48 (H3N2) were not significant (Table 3). The mean FEV_1/FVC for all vaccinee groups was 60 to 63%, borderline between moderate and mild airflow obstruction; the mean values for the group of vaccinees over 65 years of age were somewhat lower but not significantly so compared with those of other groups (P was not significant).

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 ($10^{1.0}$ $TCID_{50}$s/ml at 34°C incubation, no viral growth at 39°C incubation) on day 2 or 3 after inoculation with CR vaccine or on both days. One of these two

TABLE 3. Mean FEV_1 and FEV_1/FVC (%) (± standard deviation) for patients before and after vaccination with CR48 (H3N2) virus[a]

Patient group	No. of patients	FEV_1 (liters)		ΔFEV_1[b] (%)	FEV_1/FVC (%)		$\Delta FEV_1/FVC$[c] (%)
		Pre-vaccination	Post-vaccination		Pre-vaccination	Post-vaccination	
All CR48 (H3N2) virus vaccinees tested	21	1.84 ± 0.71	1.85 ± 0.74	0.01 ± 0.22	61.6 ± 12.5	62.0 ± 13.4	0.4 ± 6.2
≥65 Yrs old	7	1.52 ± 0.40	1.52 ± 0.52	0.004 ± 0.21	60.1 ± 9.5	60.1 ± 13.0	0.0 ± 5.7
<65 Yrs old	14	2.01 ± 0.76	2.02 ± 0.77	0.01 ± 0.22	62.4 ± 13.8	62.9 ± 13.6	0.6 ± 6.4
Infected[d] with CR48 (H3N2) virus	10	1.71 ± 0.53	1.87 ± 0.60	0.16 ± 0.14	60.2 ± 10.8	63.2 ± 10.2	3.0 ± 5.9
Not infected with CR48 (H3N2) virus	11	1.96 ± 0.82	1.84 ± 0.84	−0.13 ± 0.18	62.9 ± 13.8	60.9 ± 15.7	−2.0 ± 5.5

[a] Data are shown for all patients who underwent PFT.
[b] Mean of pre- minus postvaccination FEV_1 (± standard deviation) for each vaccinee group pre- and postvaccination.
[c] Mean of pre- minus postvaccination FEV_1/FVC (± standard deviation) (%) for each vaccinee group pre- and postvaccination.
[d] Includes all vaccinees with evidence of infection with the CR48 (H3N2) vaccine virus, including nasal shedding of vaccine virus, fourfold rise in serum IgG or IgA titer, fourfold rise in HAI antibody titer, or all of these.

persons also developed a fourfold rise in ELISA serum IgG antibody titer; the other did not have a fourfold rise in any serum antibody measured.

Serologic response to CR virus or inactivated trivalent virus vaccine administered within 1 year after CR virus vaccination. Significantly fewer CR37 (H1N1) virus vaccinees manifested fourfold increases in HAI antibody to H1 HA compared with inactivated virus vaccinees (Table 4; $P = 0.05$, Fisher's exact test, one-tailed). The one CR37 (H1N1) virus vaccinee who developed HAI antibody had received the higher dose of CR37, $10^{7.1}$ $TCID_{50}$s. Significantly fewer CR48 (H3N2) virus vaccinees manifested fourfold HAI antibody increases to H3 HA (all seroresponders received the higher dose, $10^{7.7}$ $TCID_{50}$s) compared with inactivated virus vaccinees (Table 4; $\chi^2 = 6.5$, $P < 0.05$). Mean fold increases in HAI antibody titer to H1 and H3 HA were significantly greater among inactivated virus vaccine recipients than among CR37 or CR48 virus vaccinees (Table 5; Mann-Whitney test, $z = -2.1$, $P = 0.03$ and $z = -2.2$., $P = 0.03$, respectively).

As measured by IgG ELISA, significantly fewer CR37 (H1N1) virus vaccinees manifested a fourfold antibody response to H1 than did inactivated virus vaccinees (Table 4; $P < 0.001$, Fisher's exact test, one-tailed), and the mean fold increase in IgG antibody to H1 was significantly higher after inactivated trivalent influenza virus vaccine than after live-attenuated virus vaccine (Table 5; Mann-Whitney test, $z = -2.6$, $P = 0.011$). Although a larger proportion of vaccinees seroresponded to CR48 virus than to CR37 virus, CR48 (H3N2) virus vaccinees manifested fourfold increases in IgG antibody (all seroresponders received the $10^{7.7}$ $TCID_{50}$s of virus) significantly less often than did recipients of inactivated virus vaccine (Table 4; $\chi^2 = 8.9$, $P < 0.01$). The mean fold increase in IgG antibody to H3 HA was significantly greater after inactivated virus vaccine (Table 5; Mann-Whitney test, $z = -2.5$, $P = 0.01$).

As measured by IgA ELISA, 1 (inoculum of $10^{6.1}$ $TCID_{50}$s) of 13 CR37 (H1N1) virus vaccinees manifested a serum IgA antibody response to H1. The proportion of CR48 (H3N2) virus vaccinees manifesting a fourfold serum IgA antibody response (all seroresponders received $10^{7.7}$ $TCID_{50}$s of virus) was not significantly different from that of inactivated virus vaccinees (Table 4). The mean fold increases in serum IgA antibody to H1 and H3 HA were not significantly different among the vaccine groups (Table 5).

A significant positive association between a fourfold rise in IgA and IgG titers occurred in recipients of CR48 (H3N2) virus. Of 7 vaccinees with a fourfold serum IgA antibody titer rise, 5 also had a rise in IgG antibody titer; of 20 without a fourfold serum IgA antibody titer rise, only 4 had a rise in IgG antibody titer ($P = 0.023$, Fisher's exact test, one-tailed). One of the CR37 (H1N1) virus and two of the CR48 (H3N2) virus vaccinees developed fourfold rises in IgA antibody titer without an accompanying rise in either HAI or IgG antibody titer or isolation of virus from nasal wash specimen. None of the inactivated virus vaccine recipients developed a fourfold rise in serum IgA antibody titer without a concomitant rise in serum IgG antibody titer.

By any criterion of infection, i.e., fourfold rise in serum HAI, IgG, IgA antibody titer, isolation of vaccine virus from nasal washings, or all of these, 2 (15%) of the 13 CR37 (H1N1) virus vaccinees and 12 (44%) of the 27 CR48 (H3N2) virus vaccinees became infected with vaccine virus. This

TABLE 4. Numbers of patients with fourfold antibody responses to CR or inactivated influenza virus vaccine

Vaccine type/antibody measured	No. of patients with fourfold antibody rise/no. tested	
	H1 antigen	H3 antigen
CR37 (H1N1)		
HAI	1/13[a]	
IgG	0/13[b,c]	
IgA	1/13	
Any of the above	2/13[d]	
CR48 (H3N2)		
HAI		3/27[e]
IgG		9/27[c,f]
IgA		7/27
Any of the above		11/27[g]
Inactivated virus		
HAI	7/18[a]	9/18[e]
IgG	11/18[b]	15/18[f]
IgA	3/18	4/17
Any of the above	13/18[d]	16/18[g]

[a] $P = 0.05$; Fisher's exact test, one-tailed.
[b] $P < 0.001$; Fisher's exact test, one-tailed.
[c] $P = 0.017$; Fisher's exact test, one-tailed.
[d] $P = 0.002$; Fisher's exact test, one-tailed.
[e] $\chi^2 = 6.5$; $P < 0.05$ with the Yates correction.
[f] $\chi^2 = 8.9$; $P < 0.01$ with the Yates correction.
[g] $\chi^2 = 8.52$; $P = 0.003$ with the Yates correction.

TABLE 5. Serum HAI antibody and serum IgG and IgA antibody responses (ELISA) to CR or inactivated influenza virus vaccine

Vaccine type/antibody measured	H1 antigen			H3 antigen		
	Reciprocal antibody titer (geometric mean)		Mean fold increase (± SD)	Reciprocal antibody titer (geometric mean)		Mean fold increase (± SD)
	Prevaccination	Postvaccination		Prevaccination	Postvaccination	
CR37 (H1N1) virus						
HAI	8.0	11.0	1.5 ± 0.9[a]	NT[b]	NT	
IgG	3,294	3,294	1.0 ± 0[c]	NT	NT	
IgA	45	64	5.8 ± 17.5	NT	NT	
CR48 (H3N2) virus						
HAI	NT	NT		35.5	44.7	1.7 ± 1.5[d]
IgG	NT	NT		1,089	2,116	5.0 ± 13.6[e]
IgA	NT	NT		39	56	1.9 ± 1.8
Inactivated trivalent influenza virus						
HAI	10.5	27.4	3.8 ± 3.9[a]	32.0	71.8	2.8 ± 1.9[d]
IgG	3,995	10,000	3.3 ± 3.5[c,f]	1,011	4,660	7.6 ± 8.3[e,f]
IgA	111	147	2.2 ± 3.6	42	79	4.5 ± 8.0

[a] Mann-Whitney test, $z = -2.1$, $P = 0.03$.
[b] NT, Not tested.
[c] Mann-Whitney test, $z = -2.6$, $P = 0.011$.
[d] Mann-Whitney test, $z = -2.2$, $P = 0.03$.
[e] Mann-Whitney test, $z = -2.5$, $P = 0.01$.
[f] Mann-Whitney test, $z = -1.7$, $P = 0.08$.

may be a low estimate of the proportion of infected individuals, because additional vaccinees may have developed isolated rises in nasal wash secretory IgA antibody titer without other evidence of vaccine virus infection. Fourfold rises in serum HAI, IgG, or IgA antibody titer or in all three among the 18 recipients of the inactivated influenza virus vaccine were more frequent to both H1 ($P = 0.002$, Fisher's exact test, one-tailed) and H3 HA ($\chi^2 = 8.52$, $P = 0.003$) than they were among the CR virus vaccinees (Table 4).

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 seroresponse to vaccination. Prevaccination HAI antibody titer did not appear to influence HAI antibody response to live-attenuated virus vaccine; reciprocal geometric mean HAI titers of nonseroresponders compared with those of seroresponders to each of the live-attenuated virus vaccines were not significantly different. However, prevaccination reciprocal geometric mean HAI titer to H3 among the nonseroresponders who received inactivated virus vaccine was significantly higher than among seroresponders (47.0 versus 21.8; $z = 2.56$, $P = 0.01$, Mann-Whitney test). Among CR48 virus vaccine recipients, the prevaccination reciprocal IgG ELISA geometric mean titer of nonseroresponders was higher than that of seroresponders (2,011 versus 319; $z = 2.37$, $P = 0.017$). Among the inactivated virus vaccine recipients, high ELISA IgG antibody was correlated with absence of ELISA antibody response (nonseroresponders versus seroresponders to H1 antigen, 7,071 versus 2,530, $z = 2.22$, $P = 0.025$; H3 antigen, 7,113 versus 475, $z = 2.55$, $P = 0.011$, Mann-Whitney test). No significant differences were found in prevaccination reciprocal geometric mean IgA ELISA titers comparing the nonseroresponders and seroresponders.

No significant differences occurred in prevaccination reciprocal geometric mean HAI, IgG, or IgA antibody titers between nonseroresponders to CR37 (H1N1) virus and nonseroresponders to the H1 antigen of the inactivated virus vaccine or between nonseroresponders to CR48 (H3N2) virus and the H3 antigen of inactivated virus vaccine. In addition, there were no significant differences by the same measures among seroresponders to CR48 (H3N2) virus and the H3 antigen of the inactivated virus vaccine.

Effect of history of influenza virus vaccination on seroresponse to CR48 (H3N2) virus vaccine. Of the 27 CR48 (H3N2) virus vaccine recipients, 25 were able to give a vaccine history that was believed to be reliable before this study. Fourteen recipients had been vaccinated in a previous year with inactivated influenza virus vaccine, and 11 had never been vaccinated for influenza. Of the 14 vaccine recipients with a history of inactivated virus vaccination, 5 became infected with the live-attenuated CR48 (H3N2) vaccine virus, and 9 did not. This was not significantly different from the 7 of the 11 who became infected with vaccine virus and gave a history of no previous influenza vaccination.

Effect of CR virus on subsequent seroresponse to inactivated virus vaccine. Infection with the CR vaccine viruses may have primed the response to subsequent inactivated virus vaccine administration. Of the eight persons who did not become infected with CR37 (H1N1) virus who subsequently received the inactivated virus vaccine, five had a seroresponse to both H1 and H3 antigens and three responded only to the H3 antigen. Of the two persons who became infected with CR37 (H1N1) virus and subsequently received inactivated virus vaccine, both developed a seroresponse to both the H1 and H3 antigens. Of the four persons who did not become infected with CR48 (H3N2) virus and who subsequently received inactivated virus vaccine, two seroresponded to both H1 and H3 antigen and two did not serorespond to either antigen. Of the four persons who became infected with CR48 (H3N2) virus and who subsequently received inactivated virus vaccine, all seroresponded to both H1 and H3 antigens. In summary, all 6 persons who became infected with CR virus and 7 of 12 persons who did not become infected with CR virus had a seroresponse to subsequent inactivated virus vaccine homologous antigen ($P = 0.09$, Fisher's exact test, one-tailed).

DISCUSSION

The two CR influenza A virus vaccines used in this study were safe when administered intranasally to older adults with chronic diseases. Significant changes in PFTs did not occur after administration of these vaccines. Our safety data confirm previous reports by the Medical Research Council Advisory Group (1, 22) (PFT done at 7 to 21 days postvaccination) that live-attenuated influenza virus vaccines do not acutely change the pulmonary function of adults with chronic obstructive airways disease. Kava and Laitinen (19) have also found no change in specific airway conductance among patients with asthma who received live-attenuated influenza A virus vaccines.

Although transient spirometric changes in the first 10 days postvaccination are not ruled out by our data, testing PFTs at 10 to 14 days postvaccination would probably reveal spirometric changes as a result of influenza A virus infection in persons with COPD. Smith et al. (30) demonstrated reductions in FEV_1 and FVC lasting for up to 6 months after natural influenza A virus infection in patients with COPD. They did not comment on whether decrements in pulmonary function were more severe in the first 1 to 2 weeks after the onset of clinical signs and symptoms of infection, however. It might be expected that infection with live-attenuated influenza A virus is less likely to cause pulmonary function decrements, since experimental wild-type influenza A virus infection does not result in abnormalities among healthy adults, whereas natural infection does (20). When detected, the changes occurred at both 3 and 10 days postvaccination. The same studies have not been done in patients with underlying COPD.

Parenteral administration of inactivated trivalent influenza virus vaccine 5 to 12 months after CR virus vaccination resulted in significantly higher rates of fourfold serum antibody increases compared with the rates of fourfold antibody increases after the intranasal administration of CR vaccine viruses. It is possible that the response rates to the inactivated virus vaccine were enhanced by the patients' exposure to the CR vaccine virus within the previous year. However, 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, 16–18, 21, 26, 28, 29, 31). Alternatively, it is likely that the preexisting experience with other influenza A virus serotypes prevented infection with CR virus but did not prevent a serologic response to the inactivated virus vaccine.

As in seronegative children (5, 6), the CR48 (H3N2) vaccine virus was more infectious in our older adult population than was the CR37 (H1N1) vaccine virus. This may reflect an actual difference in the infectivity of the two viruses. No definite explanation for this difference is available, since the infectivity of these two vaccine viruses is determined theoretically by the six genes provided by the common parent virus strain (influenza A/Ann Arbor/6/60).

Most previous studies of older, seropositive adults report the serologic response to influenza A vaccine in terms of the HAI antibody titer. Our trivalent virus vaccine recipients demonstrated an HAI response rate comparable to that seen in other series (9, 21). However, the IgG antibody (ELISA) response to vaccination demonstrated a higher proportion of fourfold rises in these same vacinees. It is not known whether an ELISA-determined IgG antibody titer rise corresponds directly to subsequent protection from wild-type virus challenge. The CR48 (H3N2) virus vaccinee who developed a natural influenza A virus infection 10 months postvaccination had low ELISA IgG and IgA antibody titers to H3 antigen at 28 days postvaccination (1:80 and 1:20, respectively). Although this IgG antibody titer represented a fourfold rise compared with prevaccination, the level attained was still low in comparison with pre- and postvaccination antibody titers of other CR48 recipients.

As expected, the rates of serologic response to the two CR virus strains in this study were lower than those in studies of seronegative children or adults. Belshe et al. (5, 6) reported vaccine virus infection rates of 60 to 79% after vaccination with CR37 (H1N1) virus and up to 100% after CR48 (H3N2) virus vaccination of seronegative children. Approximately one-half of seropositive children became infected with CR37 or CR48 virus (6). Clements et al. (14) reported an HAI rise in 94% of young adults who were seronegative prior to vaccination with commercial inactivated trivalent influenza virus vaccine, compared with a 44% response rate among seronegative young adults who were vaccinated with CR48 (H3N2) virus ($10^{7.5}$ $TCID_{50}$s). 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 was 88 to 96% (13, 14, 23). The Medical Research Council Advisory Group (22) reported a 31% HAI serologic response rate to CR48 (H3N2) virus (10^7 50% egg infective doses) among previously seropositive adults with chronic bronchitis. This is a higher response rate than was found in our CR48 (H3N2) virus recipients, as measured by HAI response, but similar to the 33% rate of ELISA serum IgG fourfold rises in our patients.

Rates of fourfold rises in serum IgA antibody titer were not significantly different among the vaccine groups. Three of our CR virus recipients but none of our inactivated virus vaccine recipients developed fourfold rises in serum IgA antibody titer without accompanying changes in serum HAI or IgG antibody titer. Additional CR vaccinees may have become infected, and they demonstrated this only by rises in local secretory IgA antibody titer. Murphy et al. (24) did not detect a serum IgA response in all young children who developed a nasal wash secretory IgA antibody response after becoming infected with cold-adapted influenza A virus vaccines. Clements et al. (14) 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%). For these reasons, determination of nasal wash IgA antibody levels in our patients will provide further important information regarding the local immune response to CR virus vaccine and may increase the total number of infected vaccinees detected.

The higher seroconversion rates attained by using the inactivated trivalent influenza virus vaccine do not necessarily mean that it is more efficacious in preventing infection or severe illness or both due to natural wild-type influenza A virus. Differences in the stimulation of secretory humoral immunity and of cellular immunity (e.g., cytotoxic T cells and enhanced lymphokine production) produced by the various vaccines may be more important determinants of protection against natural challenge and recovery from infection. Furthermore, 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 immune to naturally acquired influenza A virus, 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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