Superiority of Live Attenuated Compared with Inactivated Influenza A Virus Vaccines in Older, Chronically Ill Adults*

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Forty-eight older adults with chronic diseases were vaccinated intranasally with live attenuated influenza A/Korea/1/82 (H3N2), CR59 virus. Forty-two (88 percent) CR59 virus recipients became infected with vaccine virus without adverse effects or change in mean pulmonary function even among the 29 infected recipients with moderate to severe chronic obstructive pulmonary disease. Among control groups who received either monovalent or trivalent inactivated flu virus vaccines intramuscularly, the rates of fourfold rises in serum antibody titer to hemagglutinin (HA) were not different from the rate following CR59 virus inoculation. However, CR59 virus was superior to inactivated virus vaccine at stimulating secretory antibody to HA. Vaccinees age 65 years and older were more likely to shed CR59 virus in nasal secretion than were younger vaccinees, but antibody responses were not different. CR59 virus vaccine was safe and immunogenic in this population and more often induced a nasal wash IgA antibody response than the inactivated vaccine.

(CHEST 1991; 100:977-84)

CR = cold-recombinant; ELISA = enzyme-linked immunosorbent assay; FE25-75% = forced expiratory flow; HA = hemagglutinin; HAI = hemagglutination inhibition

During recent influenza epidemics in the United States, influenza resulted in an average of 20,000 excess deaths per year. However, influenza immunization levels among US adults at risk for influenza virus-related morbidity and mortality are low. Intra-nasal live attenuated influenza A virus vaccines offer the advantages of a convenient and easily accepted route of administration and a broader immune response including stimulation of nasal secretory antibodies.

Previously, we reported the safety and immunogenicity of two live attenuated, cold-recombinant influenza A viruses in an adult population with a mean age of 58±1±2 years and with chronic underlying diseases, including chronic obstructive pulmonary disease (COPD) of mild severity on average. We extend these observations in 48 volunteers with more severe illnesses and compare the results with two control groups who received inactivated virus vaccines.

MATERIALS AND METHODS

Vaccines

The cold-recombinant (CR), live attenuated influenza A/Korea/1/82 (H3N2) virus vaccine was derived from cold-adapted influenza A/Ann Arbor/6/60 virus by H. F. Maassab using methods previously described. The resulting CR virus recombinant (CR-59), lot No. E-204 and E-223) contained six genes that code for internal proteins from the donor cold-adapted strain, and genes that code for hemagglutinin (HA) and neuraminidase derived from influenza A/Korea/1/82 (H3N2) virus. In addition to being cold adapted, the vaccine virus was temperature-sensitive (restrictive temperature, 39°C). The inoculum of H3N2, CR59 vaccine virus was 108 for lot No. E-204 and 10^8 for lot No. E-223 (undiluted as provided by the National Institute of Allergy and Infectious Diseases, Bethesda) plaque forming units per 0.5 ml intranasal dose.

The inactivated trivalent influenza virus vaccines used were the commercially available zonal purified preparations for the 1986-1987 influenza season (including influenza A/Chile/1/83 [H1N1] and influenza A/Mississippi/1/85 [H3N2]), the 1987-1988 influenza season (including influenza A/Taiwan/1/86 [H1N1] and influenza A/Leningrad/369/66 [H3N2]), and the 1988-1989 influenza season (including influenza A/Taiwan/1/86 [H1N1] and influenza A/Sichuan/287/83 [H3N2]) containing 15 μg of each HA per dose. The inactivated monovalent influenza A/Taiwan/1/86 (H1N1) subvirion vaccine (Connaught Laboratories, Inc, Swiftwater, PA, lot No. 4624) contained 15 μg of H1 HA per dose. Monovalent influenza A (H3N2) subvirion vaccine would have been preferable, but it was not available from the manufacturer. The inactivated virus vaccines were given as a 0.5-ml deltoid intramuscular injection.

Clinical Studies and Selection of Patients

Volunteers were recruited from a Veterans Affairs population of older, chronically ill adults who were ambulatory and not institutionalized. The age range of persons accepted into the study to receive CR59 virus was 43 to 75 years and the median age was 63 years. The age range of persons accepted into the study to receive the inactivated monovalent virus vaccine was 60 to 73 years and the median age was 67 years. Inclusionary criteria were the presence of moderate to severe COPD or other chronic medical illness. Volunteers were not excluded if there was preexisting antibody to H1 and H3 HA or a history of prior parenteral trivalent inactivated influenza virus vaccination in a previous influenza virus season at least nine months prior to entry into our study. None had previously been vaccinated with a live attenuated, cold-recombinant influenza
A virus vaccine.

The protocol for clinical studies was approved by the Human Subjects Committee of the Huntington Veterans Affairs Medical Center, Huntington, WV, where the study was conducted. Volunteers gave written informed consent. The study was conducted in the summer and fall seasons of 1985, 1986, 1987, and 1988 prior to the influenza season. Volunteers were either intranasally inoculated with live attenuated influenza A/Korea (H3N2) CR59 virus vaccine as previously described or intramuscularly injected with monovalent inactivated influenza A/Taiwan/L86 (H1N1) virus vaccine. A subgroup of CR59 virus vaccinees who later received commercially available trivalent inactivated influenza virus vaccine consented to measurements of antibody responses to this vaccination and are included as an additional control group.

The CR59 virus vaccine and the monovalent inactivated H1N1 vaccine recipients underwent history and physical examination prior to vaccination and were clinically examined at 7, 14, and 28 days after vaccination. The CR59 virus vaccinees also were clinically examined daily during the first four days after vaccination. Vaccinees were assessed at these times for respiratory illness, including history and examination, and vital signs were measured. Also, standard spirometry was performed in the clinical pulmonary function testing (PFT) laboratory before and one to two weeks after vaccination with CR59 virus vaccine or monovalent inactivated influenza A/Taiwan subvirion vaccine with a computerized series 2500 Pulmonary Function Laboratory (Gould, Dayton, Ohio) using previously described methods. The forced expiratory volume in 1 s (FEV1) and forced vital capacity (FVC) were determined and the degree of obstruction to airflow was categorized on the basis of the FEV1/FVC (percent). The forced expiratory flow (FEF25-75%) was determined at the same time and is reported as a possible measure of changes in small airways obstruction.

Nasal wash specimens obtained before and daily for four days after vaccination with CR59 virus vaccine were inoculated onto tissue culture cells for virus isolation as previously described.

Serologic Tests

Nasal wash specimens (20 ml of L-15 medium) were obtained before, and at 7 and 28 days after administration of CR59 (H3N2) virus vaccine and monovalent inactivated influenza A/Taiwan subvirion vaccine, and before and at 28 days after administration of the trivalent inactivated virus vaccine. Serum specimens were obtained from each vaccinee before and 28 days after vaccination.

Hemagglutination inhibition (HAI) antibodies in serum were measured by using whole virus homologous to the vaccine strains in a standard microtiter assay, although the H3N2 virus used in the assay of HAI antibody titers before and after the trivalent inactivated virus vaccine was influenza A/Sichuan/987 (H3N2). This strain is antigenically related to the A/Mississippi and A/Leningrad (H3N2) strains present in the trivalent virus vaccine used in this study.

The CR59 virus vaccine was grown in the allantoic cavity of eggs, and the HA was extracted and purified by previously described methods. Purified influenza A/Taiwan/H1N1 virus HA and Sichuan/H3N2 virus HA were provided to us by Connaught Laboratories, Inc. An end point enzyme-linked immunosorbent assay (ELISA) was employed as previously described to determine the anti-influenza virus HA serum and nasal wash immunoglobulin titer and immunoglobulin isotype using the homologous HA for the CR59 virus vaccinee group, and using the Taiwan/H1N1 virus HA and Sichuan/H3N2 virus HA in the case of specimens obtained before and after administration of the inactivated virus vaccine.

In addition, the nasal wash IgA anti-HA titers were corrected to a total secretory IgA concentration of 100 mg/L and nasal wash IgG anti-HA titers were corrected to a total IgG concentration of 100 mg/L. The total secretory IgA concentration and total IgG concentration in each concentrated nasal wash specimen was determined by ELISA. The serum of reagents from solid phase out consisted of (1) rabbit anti-human immunoglobulins IgG, IgA, and IgM (Cappel, West Chester, PA); (2) nasal wash specimen; (3) goat anti-human IgA conjugated with alkaline phosphatase (Cappel, West Chester, PA) or goat anti-human IgG conjugated with alkaline phosphatase (Cappel, West Chester, PA); and (4) p-nitrophenyl phosphate disodium substrate. The standard curves of known secretory IgA concentration were determined using serial dilutions of human secretory IgA (Jackson Immunoresearch Laboratories, Inc, Avondale, PA) and nasal wash specimen in the ELISA. The standard curves of known IgG concentration were determined using serial dilutions of human IgG (Jackson Immunoresearch Laboratories, Inc, Avondale, PA) instead of nasal wash specimen in the ELISA.

Statistical Methods

Statistical tests included Fisher's exact test or x² analysis with the Yates' correction for two-by-two comparisons. Reciprocal antibody titers before and after vaccination were converted to log₁₀, and mean values were compared by the Wilcoxon signed ranks nonparametric test. Mean values are reported with the standard error of the mean. A greater than 15 percent change in FEV₁/FVC (percent) and greater than 25 percent change in FEF₂₅₋₇₅ percent from prevaccination levels were considered to possibly exceed normal variations.

RESULTS

Clinical Characteristics of the Study Populations

CR59 Vaccine Recipients: Forty-eight volunteers (mean age, 62.1 ± 1.0 years) were enrolled and examined. Pulmonary function tests were performed before vaccination in 44 volunteers and demonstrated obstruction to airflow in 37 volunteers (Table 1). In addition to COPD, 33 (89 percent) of these 37 volunteers had other significant clinical characteristics (13 [35 percent] with hypertension; 11 [30 percent] aged ≥65 years; 11 [30 percent] with cardiovascular disease; ten [27 percent] with gastric ulcer, gastritis, and/or gastric resection; nine [24 percent] recently treated with prednisone; seven [19 percent] with a history of cancer of the lung, stomach, breast, colon, larynx, and lymphoreticular system; five [14 percent]...
with neurologic diseases; and three (8.1 percent) with renal dysfunction, diabetes mellitus, and/or anemia). Of the seven volunteers with normal prevaccination lung function (mean age, 62.4 ± 3.1 years), four were aged ≥65 years; three had a history of alcohol abuse and hypertension; two had symptoms of allergic rhinitis; and one each had hepatic cirrhosis, bronchitis, history of a cerebrovascular accident, or previous gastrectomy. Of the four volunteers in whom PFTs could not be performed, one was aged ≥65 years; two had ≥60 pack-year cigarette smoking histories; and one each had cardiovascular disease, seizure disorder, Alzheimer’s disease, hemicolecctomy for colon cancer, or hypertension.

Monovalent Inactivated Virus Vaccine Recipient Control Group: Nine volunteers (mean age, 66.1 ± 1.2 years) who had not received any cold-recombinant virus vaccine were included as a comparison group and received inactivated influenza A/Taiwan/1/86 (H1N1) subvirion vaccine. Six volunteers were aged ≥65 years, four had COPD (one mild, one severe, and two were unable to cooperate with PFT), three had diabetes mellitus, four had coronary artery disease, two had hypertension, two had neurologic diseases (Parkinson’s disease and cerebrovascular accident), and one had a history of duodenal ulcer and gastrointestinal surgery.

Trivalent Inactivated Virus Vaccine Recipient Control Group: Twenty-five of the chronically ill CR59 virus vaccinees (mean age at the time of inactivated virus vaccination, 64.9 ± 1.1 years) who were available for follow-up and agreed to receive trivalent inactivated influenza virus vaccine prior to an influenza season at least six months following inoculation with CR59 virus were included as an additional comparison group. Twelve volunteers received the vaccine prepared for use during the 1986-1987 influenza season, nine received the 1987-1988 influenza season preparation, and four received the 1988-1989 influenza season preparation.

Safety of CR59 Virus Vaccine

Forty-four (92 percent) CR59 recipients developed no clinical signs or symptoms of respiratory virus infection following vaccination. Two volunteers reported rhinitis during the first seven days after vaccination with CR59 virus. One volunteer reported slightly increased shortness of breath on the seventh day after vaccination; however, his postvaccination lung function was better than prevaccination (prevaccination: FEV1/FVC = 23 percent, FEF25-75% = 0.19 L/s; 14 days postvaccination: FEV1/FVC = 29 percent, FEF25-75% = 0.35 L/s). All three of these volunteers were infected with the vaccine virus as indicated by antibody response, but none shed vaccine virus in nasal secretions. One volunteer who had no evidence for vaccine virus infection was hospitalized two days after vaccination for presumed bacterial pneumonia. No volunteer experienced fever during the first seven days after vaccination other than the volunteer who developed pneumonia.

Among all 44 volunteers who received CR59 virus vaccine and underwent PFTs, the mean FEV1/FVC (percent) before vaccination was 48.0 ± 2.4 and one to two weeks after vaccination was 48.0 ± 2.5. Also, the mean FEV1/FVC (percent) did not change at one to two weeks after vaccination compared with before vaccination within any of the volunteer groups categorized by the severity of their prevaccination obstructive lung disease. Of the 39 volunteers who had prevaccination and postvaccination PFTs performed and were infected by CR59 virus, six experienced improvement (three with >15 percent change) and eight had worsening (three with >15 percent change) in obstruction to airflow from one category to another (Table 1).

Among all 44 volunteers who received CR59 virus vaccine and underwent PFTs, the mean FEF25-75% before vaccination was 0.64 ± 0.08 and one to two weeks after vaccination was 0.69 ± 0.09. Also, the mean FEF25-75% did not change at one to two weeks after vaccination compared with before vaccination within any of the volunteer groups categorized by the severity of their prevaccination obstructive lung disease. Of the 39 volunteers who had prevaccination and postvaccination PFTs performed and were infected by CR59 virus, 11 experienced a rise in FEF25-75% >25 percent above the prevaccination value, and six experienced a decrease in FEF25-75% >25 percent below the prevaccination value. Rhinitis occurred in one vaccinee with a rise and in one with a decrease in FEF25-75%. Otherwise, no changes in clinical signs and symptoms were associated with a change in FEF25-75%.

The two monovalent inactivated virus vaccine recipients with obstruction to airflow documented on prevaccination spirometry experienced no significant changes in FEV1/FVC (percent) and FEF25-75% one to two weeks after vaccination.

Nasal Shedding of CR59 Virus

Among the ten (21 percent) volunteers who shed CR59 virus in nasal washings, the mean duration of shedding was 1.9 ± 0.2 days (range, one to three days). The highest titer shed was 10^6.5 TCID50/ml, which was observed in two vaccinees. Volunteers aged 65 years or older were more likely to shed virus compared with younger volunteers (Table 2).

Of the ten volunteers who shed vaccine virus, three had a prevaccination HAI reciprocal serum antibody titer ≤16, two did not develop a fourfold rise in serum or nasal wash antibody titers to H3 HA, four developed a fourfold rise in both serum and nasal wash antibody
Table 2 — Number of Volunteers with Vaccine Virus Shed from Nasal Washings and with Fourfold Antibody Titer Rises in Serum and Nasal Washings to H3 Hemagglutinin 14* and/or 28 Days After Vaccination with Live Attenuated Influenza A/Korea (H3N2), CR59 Virus and Number of Volunteers with Fourfold Antibody Titer Rises to H1 Hemagglutinin After Vaccination with Monovalent Inactivated Influenza A/Taiwan/1/86 (H1N1) Virus Vaccine

<table>
<thead>
<tr>
<th>Vaccine Group</th>
<th>No. of Volunteers with Antibody Response (%)</th>
<th>Total No. with Response (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Volunteers with Antibody Titer Rises</td>
<td>Serum</td>
</tr>
<tr>
<td></td>
<td>n (%)</td>
<td>HAI</td>
</tr>
<tr>
<td>Live virus CR59 All</td>
<td>48</td>
<td>10 (21)</td>
</tr>
<tr>
<td>&lt;65 yr</td>
<td>32</td>
<td>3 (9)**</td>
</tr>
<tr>
<td>≥65 yr</td>
<td>16</td>
<td>7 (44)**</td>
</tr>
</tbody>
</table>

* Nasal wash antibody titers were measured at 14 and 28 days after vaccination.
† Number of volunteers with a fourfold rise in serum HAI, serum IgG, serum IgA, nasal wash IgG, and/or nasal wash IgA antibody titers and/or nasal wash shedding of CR59 virus. Number of volunteers with a fourfold rise in serum and/or nasal wash antibody to the parenteral monovalent inactivated virus vaccine.
‡ Proportion with fourfold serum IgG antibody rise higher than HAI antibody rise, p<0.01.
§ Proportion with fourfold serum HAI antibody rise higher after inactivated virus vaccine than after CR59, p<0.001.
¶ Proportion with fourfold nasal wash IgA antibody rise higher than serum IgA antibody rise, p<0.05.
# Proportion with fourfold nasal wash IgA antibody rise higher than IgG antibody rise, p<0.05.
** Rate of virus shedding higher in ≥65 year age group than in younger group, p<0.05.

Titers to H3 HA, two developed only a fourfold rise in serum antibody titer to H3 HA, and two developed only a fourfold rise in nasal wash antibody titer to H3 HA.

Serum and Nasal Wash Antibody Responses to CR59 Virus

Overall, 42 (88 percent) were infected with CR59 virus considering serum and nasal wash fourfold antibody titer rises and nasal wash shedding of vaccine virus (Table 2). Rates of fourfold rises in serum and nasal wash antibody titers to H3 HA were not significantly different among volunteers with a prevaccination serum reciprocal HAI antibody titer ≥16 compared with volunteers with a prevaccination titer ≥32. The overall rate of infection in the group with a prevaccination serum reciprocal HAI antibody titer ≥16 was not different from that in the group with a titer ≥32 (nine [90 percent] of ten volunteers vs 33 [87 percent] of 38 volunteers, respectively).

Significantly more vaccinees developed a fourfold rise in serum IgG antibody to H3 HA than a fourfold rise in HAI antibody titer (p<0.01). This is consistent with the higher sensitivity of the ELISA antibody assay. The proportion of all vaccinees with a fourfold nasal wash IgA antibody titer rise was significantly higher than the proportion with a serum IgA antibody titer rise (Table 2). Thirteen vaccinees experienced a fourfold nasal wash antibody titer rise without an accompanying fourfold serum antibody titer rise. Nineteen vaccinees experienced a fourfold serum antibody titer rise with an accompanying fourfold nasal wash antibody rise, eight had a fourfold rise in serum antibody titer without a fourfold rise in nasal wash antibody titer, and two shed virus in nasal washings without a fourfold rise in antibody titer.

Postvaccination mean serum HAI, IgG, and IgA and mean nasal wash IgA reciprocal antibody titers to H3 HA were significantly higher compared with respective prevaccination mean reciprocal antibody titers (Table 3).

Twenty-one (44 percent) of the CR59 virus vaccinees reported having received trivalent influenza virus vaccine in the two years prior to enrollment in this study; in each case, at least nine months had elapsed prior to enrollment in this study. History of trivalent vaccine was not associated with protection from infection with CR59 virus (Table 4).

Serum and Nasal Wash Antibody Responses to Monovalent Inactivated Influenza A/Taiwan (H1N1) Virus Vaccine

Seven (78 percent) of the nine monovalent inactivated virus vaccinees developed a fourfold serum antibody titer rise after vaccination (Table 2). When postvaccination mean serum and nasal wash reciprocal antibody titers to H1 HA were compared with prevaccination levels, a statistically significant increase was achieved only in the case of the mean reciprocal HAI antibody titer to H1N1 virus (Table 3).

Serum and Nasal Wash Antibody Responses to Trivalent Inactivated Influenza A Virus Vaccine

The proportions of vaccinees with a fourfold serum antibody titer response to H3 and H1 HA were not different statistically (Table 5). Volunteers more often
developed a fourfold serum antibody rise than nasal wash antibody rise to H3 and H1 HA.

Postvaccination mean serum HAI, IgG, and IgA reciprocal antibody titers to H3 and to H1 HA were significantly higher than respective prevaccination titers. No significant changes occurred in mean reciprocal nasal wash antibody titers to H3 or to H1 HA as a result of vaccination (Table 6).

Comparison of Antibody Responses to the Live Attenuated (CR59) and Inactivated Influenza A Virus Vaccines

The proportions of volunteers who developed fourfold nasal wash antibody titer rises to H3 HA after CR59 virus vaccination were higher than those following inactivated virus vaccine to either H1 or H3 HA. These differences achieved statistical significance when the rate of fourfold nasal wash IgG and/or IgA antibody titer rises following CR59 virus vaccination is compared with the rate following trivalent inactivated virus vaccination (CR59 vs trivalent inactivated virus: H3 HA, 32 [67 percent] vs H3 HA, seven [28 percent], p<0.01; H3 HA, 32 [67 percent] vs H1 HA, five [22 percent], p<0.01). Comparisons of only fourfold nasal wash IgA antibody titer rises after vaccination also demonstrate significantly higher rates for CR59 vaccinees compared with inactivated virus vaccinees (CR59 vs trivalent inactivated virus: H3 HA, 29 [60 percent] vs H3 HA five [20 percent], p<0.01; H3 HA, 29 [60 percent] vs H1 HA, two [9 percent], p<0.001; CR59 vs monovalent inactivated virus: H3 HA, 29 [60 percent] vs H1 HA, one [11 percent], p<0.05). The rates of fourfold changes in nasal wash IgG and/or IgA antibody titer to H3 HA were higher in the CR59 virus vaccinees younger than age 65 years compared with the trivalent inactivated virus vaccinees younger than age 65 years to H3 HA (23 [72 percent] vs three [23 percent], p<0.01) and to H1 HA (23 [72 percent] vs three [25 percent], p<0.01). The rates in fourfold changes in nasal wash IgA antibody titer to H3 HA were higher in the CR59 virus vaccinees younger than age 65 years compared with the trivalent inactivated virus vaccinees younger than age 65 years to H3 HA (20 [63 percent] vs three

Table 3—Serum and Nasal Wash Antibody Mean Reciprocal Log Antbody Titers to H1 and H3 Hemagglutinins Before and After Vaccination with Live Attenuated Influenza A/Korea (H3N2) CR59 Virus (48 Volunteers) and with Monovalent Inactivated Influenza Virus Vaccine (Nine Volunteers)

<table>
<thead>
<tr>
<th>Antibody Determined</th>
<th>Before</th>
<th>14 Day</th>
<th>28 Day</th>
<th>Before</th>
<th>14 Day</th>
<th>28 Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAI</td>
<td>4.67±0.41</td>
<td>6.78±0.52</td>
<td>5.69±0.21</td>
<td>6.17±0.22†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>11.21±0.35</td>
<td>12.10±0.40</td>
<td>8.02±0.27</td>
<td>9.13±0.26‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgA</td>
<td>8.10±0.40</td>
<td>9.43±0.82</td>
<td>8.19±0.34</td>
<td>9.36±0.29‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nasal wash</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>4.63±0.44</td>
<td>5.56±0.71</td>
<td>5.48±0.57</td>
<td>6.00±0.31</td>
<td>6.27±0.42</td>
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</tr>
<tr>
<td>IgA</td>
<td>11.90±0.69</td>
<td>11.99±0.64</td>
<td>11.89±0.69</td>
<td>9.56±0.45§</td>
<td>10.14±0.47§</td>
<td></td>
</tr>
</tbody>
</table>

*Influenza A/Taiwan/586 (H1N1) virus vaccine.
†Postvaccination value higher than the corresponding prevaccination value, p<0.05.
‡Postvaccination value higher than the corresponding prevaccination value, p<0.01.
§Postvaccination value higher than the corresponding prevaccination value, p<0.001.

Table 4—Evidence for Infection with Influenza A/Korea(H3N2) CR59 Virus Vaccine Correlated with Receipt of Trivalent Inactivated Influenza Virus Vaccine within Two Years Prior to Administration of CR59 Virus

<table>
<thead>
<tr>
<th>Trivalent Inactivated Vaccine in the 2 Years Prior to CR59 Virus Administration?</th>
<th>Total Vaccines</th>
<th>CR59 Virus Nasal Shedding</th>
<th>Any Serum Antibody to H3 HA</th>
<th>Any Nasal Wash Antibody to H3 HA by any Fourfold Rise</th>
<th>Infected with CR59 by any Fourfold Rise Measure</th>
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</thead>
<tbody>
<tr>
<td>Yes</td>
<td>21</td>
<td>4 (19)</td>
<td>10 (48)</td>
<td>16 (76)</td>
<td>19 (90)</td>
</tr>
<tr>
<td>No</td>
<td>27</td>
<td>6 (22)</td>
<td>17 (63)</td>
<td>16 (59)</td>
<td>23 (85)</td>
</tr>
</tbody>
</table>

Chest 100/4 October, 1991
Table 5—Number of Volunteers with Fourfold Antibody Titer Rises in Serum and Nasal Washings to H1 and H3 Hemagglutinin (HA) 28 Days after Vaccination with Trivalent Inactivated Virus Vaccines

<table>
<thead>
<tr>
<th>Antibody Compartiment</th>
<th>Age Group</th>
<th>H1 Antigen</th>
<th>H3 Antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>HA IgC IgA Any</td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>25 all</td>
<td>3 (12) 8 (32) 9 (36)†</td>
<td>14 (56)†</td>
</tr>
<tr>
<td></td>
<td>13 &lt;65</td>
<td>2 (15) 5 (38) 4 (31)</td>
<td>7 (54)</td>
</tr>
<tr>
<td></td>
<td>12 ≥65</td>
<td>1 (8) 3 (23) 5 (42)†</td>
<td>7 (58)</td>
</tr>
<tr>
<td>Nasal wash</td>
<td>25 all</td>
<td>4 (17)† 2 (9)† 5 (22)†</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>13 &lt;65</td>
<td>2 (17)[†] 2 (17)[†] 3 (25)[†]</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>12 ≥65</td>
<td>2 (15)[†] 0 (0)[†] 2 (15)[†]</td>
<td>---</td>
</tr>
<tr>
<td>Serum and/or Nasal Wash</td>
<td>25 all</td>
<td>3 (12) 11 (44) 10 (40) 16 (64)</td>
<td>9 (36) 14 (56) 15 (60) 19 (76)</td>
</tr>
<tr>
<td></td>
<td>13 &lt;65</td>
<td>2 (15) 6 (46) 5 (38) 8 (62)</td>
<td>4 (31) 7 (54) 9 (69) 9 (89)</td>
</tr>
<tr>
<td></td>
<td>12 ≥65</td>
<td>1 (8) 5 (42) 5 (42) 8 (67)</td>
<td>5 (42) 7 (55) 6 (60) 10 (83)</td>
</tr>
</tbody>
</table>

*Twelve vaccinees received influenza A/Chile/1/83 (H1N1) and A/Mississippi/1/85 (H3N2) viruses, nine received influenza A/Taiwan/1/86 (H1N1) and A/Leningrad/360/86 (H3N2), and four received influenza A/Taiwan/1/86 (H1N1) and A/Sichuan/2/87 (H3N2) viruses.

†Proportion of volunteers with a fourfold serum antibody titer rise higher than with a nasal wash antibody titer rise, respectively for volunteer subgroup and antibody isotype, p<0.05.

§Specimens from 24 volunteers tested in this category.

||Specimens from 11 volunteers tested in this category.

*Specimens from 33 volunteers tested in this category.

| [23 percent], p<0.05 and to H1 HA (20 [63 percent] vs two [17 percent], p<0.001), as well as in the CR59 virus vaccinees age 65 years and older to H3 HA compared with trivalent virus vaccinees age 65 years and older to H1 HA (nine [56 percent] vs zero [0 percent], p<0.01).

There were significantly more vaccinees who developed a fourfold serum HAI antibody titer rise to H1 HA following monovalent inactivated virus vaccination than to H3 HA following CR59 virus administration (seven [78 percent] vs nine [19 percent], p<0.001). However, the proportions of volunteers who developed fourfold serum HAI antibody titer rises to H3 HA after CR59 virus vaccination were not different from the corresponding proportions to H3 HA and to H1 HA following trivalent inactivated virus vaccination.

Table 6—Serum and Nasal Wash Antibody Mean Reciprocal Log, Antibody Titer to H1 and H3 Hemagglutinins Before and After Vaccination with Trivalent Inactivated Influenza Virus Vaccines (25 Volunteers)

<table>
<thead>
<tr>
<th>Antibody Determined</th>
<th>H1 Antigen</th>
<th>H3 Antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>28 Days After</td>
</tr>
<tr>
<td>Serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAI</td>
<td>5.48 ± 0.21</td>
<td>6.00 ± 0.20†</td>
</tr>
<tr>
<td>IgG</td>
<td>10.32 ± 0.31</td>
<td>10.88 ± 0.37†</td>
</tr>
<tr>
<td>IgA</td>
<td>8.96 ± 0.44</td>
<td>9.92 ± 0.48‡</td>
</tr>
<tr>
<td>Nasal wash</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>6.99 ± 0.59</td>
<td>6.54 ± 0.58</td>
</tr>
<tr>
<td>IgA</td>
<td>8.89 ± 0.60</td>
<td>8.76 ± 0.61</td>
</tr>
</tbody>
</table>

*Twelve vaccinees received influenza A/Chile/1/83 (H1N1) and A/Mississippi/1/85 (H3N2), nine received influenza A/Taiwan/1/86 (H1N1) and A/Leningrad/360/86 (H3N2), and four received influenza A/Taiwan/1/86 (H1N1) and A/Sichuan/2/87 (H3N2) virus vaccines.

†Postvaccination value higher than the corresponding prevaccination value, p<0.05.

‡Postvaccination value higher than the corresponding prevaccination value, p<0.01.

**Discussion**

This study extends our previous experience with influenza A virus vaccines in older chronically ill adults to a population with more severe underlying COPD and other chronic diseases, using vaccines with virus strains different from those in previous studies.\

The CR59 virus recipients tolerated intranasal live virus infection without significant change in obstruction to airflow measured by spirometry. Previous literature suggests that significant changes in pulmonary function following wild-type influenza A virus acute infection can be detectable for up to three months in patients with COPD. The high percentage of volunteers manifesting infection with CR59 virus without changes in PFTs or other clinical findings indicates the safety of this CR virus strain, and corroborates and expands safety data after administration...
Infectivity of the CR59 virus was high in this patient population regardless of preexisting serum antibody levels or old age. The fourfold rise in mean nasal wash IgA antibody titer to HA following CR59 virus vaccination is in sharp contrast to the lack of stimulation by inactivated virus vaccines of secretory IgA antibody levels.

Vaccinees who shed CR59 virus in nasal secretions did so in low titer and for short periods. Many of those who did not shed virus as measured by our culture technique were still able to mount an antibody response to levels of virus replication in the nasal mucosa that we were not able to detect. This indicates encouraging immunogenicity for this vaccine and an ability to boost the underlying anti-influenza A virus immunity possessed by the study volunteers due to previous experience with influenza A viruses prior to participation in this study.

The rates of seroresponse to inactivated virus vaccine in our study are similar to those reported in other studies of older adults. Our results suggest that administration of CR virus at least six months prior to parenteral inactivated virus vaccine did not enhance the seroresponse rate to the H3 HA component of the inactivated virus vaccine in this population. Enhancement of response has been reported in elderly patients when the inactivated virus was given simultaneously with the CR virus. Our results confirm the better stimulation of mucosal immunity by nasal infection with CR viruses compared with parenteral inactivated virus vaccination observed in young adults and children.

Possible reasons for higher rates of infectivity with CR59 virus in this study compared with other CR virus strains that have been tested in older adults with chronic illnesses and in healthy elderly patients may include the following. The CR59 virus is an H3N2 subtype and may have been more virulent than an H1N1 subtype in our population that was born in an H1N1 influenza A virus era. We previously noted a higher rate of serum antibody response with live attenuated influenza A/Washington/897/80 (H3N2) CR48 virus compared with live attenuated influenza A/California/10/78 (H1N1) CR37 virus in older adult population. The patient population which received CR59 virus in the current study had more severe obstruction to airflow than that which received the CR37 virus (no history of clinical COPD) and the CR48 virus (mean FEV/FVC, 61.6 percent). The MRC study used CR48 virus, did not measure serum ELISA antibody or nasal wash antibody responses to HA, and reported a fourfold antibody response in 31 percent of volunteers by the less sensitive HA1 method. Powers et al did not perform PFTs in conjunction with their study, but our CR48 and CR59 virus recipients had more significant chronic health problems than did their healthy elderly subjects. Infectivity rates of the portion of our CR59 virus vaccinees who were age 65 years and older were not different from our younger vaccinees, although virus shedding was more frequent in the elderly population. The use of an H1N1 subtype virus vaccine in the healthy elderly population studied by Powers et al which was born in an H1N1 era may explain in part their results, and rates of serum and nasal wash IgA antibody rises with their live attenuated CR125 (H1N1) strain are more similar to those we reported with the CR37 (H1N1) virus. Besides host factors, another consideration is that the CR59 virus was simply more immunogenic than the other CR strains that have been tested in older adults in prior studies.

Whether infection with CR59 virus by any of the measures included in our analysis would correlate with protection from subsequent wild-type virus challenge at least in the first few months following vaccination in this population was not determined in this study. However, stimulation of mucosal immunity with CR viruses has in other studies and populations resulted in higher protective efficacy rates compared with parenteral inactivated virus vaccine. CR viruses were at least as protective as inactivated virus vaccine when administered to previously seropositive young adults who were subsequently challenged with wild-type virus.

We conclude that at least some live attenuated CR influenza A virus vaccines can infect most older adults with underlying chronic diseases and can stimulate mucosal immunity better than inactivated virus vaccines in this population. Intranasal inoculation of these viruses appears safe even in patients with severe pulmonary obstruction. These live attenuated CR viruses may infect larger proportions of older populations with chronic diseases than the healthy elderly. Further study is needed to determine whether CR virus vaccination induces an immune response that is protective in this older adult population and whether those older individuals who do not exhibit signs of CR virus infection by antibody titer changes or virus shedding are immune to subsequent wild-type virus infection.

ACKNOWLEDGMENTS: We thank Diane Tominson, Betty Burk, Frances Newman, Lisa Henseler, Anne Foster, Beverly Fofahl, and Julie Bartram for technical assistance, and June Bricker for secretarial assistance.

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