Immunity to Influenza in Older Adults with Chronic Obstructive Pulmonary Disease

Geoffrey J. Gorse, Theresa Z. O’Connor, Frances K. Newman, Mahendra D. Mandava, Paul M. Mendelman, Janet Wittes, and Peter N. Peduzzi

Background. Chronically ill older adults constitute a population vulnerable for complications associated with influenza. Study of their immunity to influenza virus may help design better strategies to stimulate protective immune responses.

Methods. Immunogenicity of influenza vaccines and immune protection from natural influenza were assessed in older adults with chronic obstructive pulmonary disease as part of a vaccine efficacy trial. Subjects received either trivalent inactivated influenza virus vaccine (TVV) intramuscularly and trivalent live cold-adapted influenza virus vaccine (CAIV-T) intranasally or TVV and placebo (P).

Results. In the subsets of study subjects assessed, serum hemagglutination inhibition (HAI) and nasal-wash antihemagglutinin (HA) immunoglobulin (Ig) A and IgG antibody levels and anti–influenza virus CD8+ cytotoxic T lymphocyte activity increased after immunization. Mean postimmunization nasal-wash IgA antibody levels to influenza A H3/HA and B HA were statistically higher in the group than in the TVV + CAIV group. Postimmunization serum HAI and nasal-wash IgA antibodies to influenza A/H3N2 and B viruses were associated with a reduced relative risk for natural influenza infection.

Conclusions. TVV + CAIV appeared more immunogenic than TVV + P, but the observed difference may be clinically unimportant. Anti-influenza serum and nasal-wash antibodies were associated with immune protection.

Serum and nasal antibodies and major histocompatibility complex HLA class I–restricted CD8+ cytotoxic T lymphocytes (CTLs) against influenza A virus may be associated with protection from influenza A virus infection and illness [1, 2]. In children and healthy adults, serum hemagglutination inhibition (HAI) antibodies and IgG and IgA antibodies to hemagglutinin (HA) in respiratory secretions correlate with protection from infection [1–7]. In healthy young adults, anti–influenza A virus CTLs correlate with reduced shedding of wild-type (wt) influenza A virus [8]. Whereas antibodies against HA and neuraminidase (NA) are subtype specific [1, 2, 9, 10], anti–influenza A virus CTLs can be cross-reactive, recognizing heterologous influenza A viruses but not influenza B virus [1, 2, 8, 11–15]. Cross-reactive CTLs may explain, in part, why children vaccinated with 1 strain of cold-adapted influenza A (H3N2) virus were protected against a variant A (H3N2) strain [16]. Inactivated virus vaccine is a better inducer of serum antibody to HA than of mucosal antibodies and CTLs [3, 10, 14, 17, 18].

Serum neutralizing and IgG antibodies to the HA and NA of influenza B virus have correlated with resistance to viral replication and protection against influenza B virus infection, but nasal-wash IgA antibodies to HA were not well correlated with protection from experimental challenge with influenza B virus in young adults [6, 19, 20]. Anti–influenza B virus CTLs are not well characterized.

Immunologic responses to cold-adapted influenza virus (CAIV) vaccines mimic those induced by wt vi-
virus, although preexisting immunity blunts serum antibody responses to CAIV [14, 18, 21–23]. In chronically ill older adults, cold-adapted influenza A virus vaccines induced higher levels of nasal-wash anti-HA IgA antibody than did cold-adapted influenza B virus vaccine [24]. Lower rates of mucosal IgA antibody responses to cold-adapted influenza A virus vaccines have been reported in healthy elderly persons, compared with children and healthy young adults [25–28]. CAIV has stimulated anti–influenza A virus CTLs in both healthy young adults and chronically ill older adults better than has inactivated virus vaccine [14, 23, 29]. Monovalent CAIV alone or bivalent CAIV coadministered with inactivated virus vaccine, given to patients with chronic obstructive pulmonary disease (COPD) and elderly nursing home residents, better induced anti–influenza A virus CTLs and antibodies, in nasal secretions, whereas coadministration of inactivated virus vaccine maintained higher systemic antibody responses than did CAIV alone [14, 15, 26, 28, 30–33]. Our clinical trial that assessed the added efficacy of trivalent CAIV when coadministered with inactivated virus vaccine to chronically ill older adults with COPD [34] provided us the opportunity to learn more about vaccine immunogenicity and immune protection from influenza virus.

PATIENTS AND METHODS

Vaccines and Placebo

Trivalent inactivated influenza virus vaccine (TVV) for the 1998–1999 season, containing 15 μg of each of the influenza A/Beijing/262/95–like (H1N1), influenza A/Sydney/5/97–like (H3N2), and influenza B/Harbin/07/94 (B/Beijing/184/93–like) HA antigens (trivalent, purified surface antigen vaccine, types A and B, 1998–1999 formula; Fluvirin; Evans Medical Limited) in each 0.5-mL dose, was used. Trivalent, live cold-adapted influenza A and B virus vaccine (CAIV-T; MedImmune Vaccines) contained \( \sim 10^7 \) TCID\(_50\)/0.5 mL of each of 3 virus strains, corresponding antigenically to the strains included in TVV for the 1998–1999 season, in egg allantoic fluid with sucrose-phosphate-glutamate (SPG) stabilizer; placebo was egg allantoic fluid with SPG.

Study Design

Volunteers aged \( \geq 50 \) years who met spirometric criteria for COPD were recruited at 20 Department of Veterans Affairs (VA) Medical Centers and underwent clinical evaluation, as described elsewhere [34]. Informed consent was obtained from study subjects. Human experimentation guidelines of the US Department of Health and Human Services and those of the authors’ institutions were followed in the conduct of clinical research. Before immunization, blood was obtained from a convenience subset of individuals (\( n = 61 \)) enrolled at the St. Louis site for use in serum antibody and cytotoxic T lymphocyte (CTL) assays, and nasal washings were obtained from 400 subjects participating across the 20 study centers for use in antibody assays. TVV was administered intramuscularly in the deltoid to all subjects. On the same day, they were randomly assigned (in a 1:1 ratio) to receive CAIV-T (TC group, \( n = 1107 \)) or placebo (TP group, \( n = 1108 \)) intranasally as a large-particle aerosol (0.25 mL/nostril). Subjects and study personnel were blinded to group assignment. Subjects returned 3–4 weeks after immunization, for a clinical evaluation and collection of serum and nasal washings for use in antibody assays (\( n = 2215 \)) and blood for use in CTL assays (\( n = 61 \)).

From November 1998 through April 1999, study personnel conducted active surveillance for acute respiratory illnesses so that clinical evaluation could be performed and nasal and oropharyngeal (NOP) swabs, for use in virus cultures, and serum, for use in antibody assays, could be obtained. Three to four weeks after the acute illness, serum was obtained for use in antibody assays. Laboratory-documented influenza-caused illness (LDI) was the sudden onset of respiratory illness with a positive NOP swab culture for influenza A or B virus and/or a 4-fold increase in serum antibody titer to influenza A or B virus [34].

Laboratory Assessment

Serum HAI antibody assays. HAI antibody end-point titers were determined in a microtiter assay (Centers for Disease Control and Prevention) [35, 36]. Serum samples without detectable antibody at a dilution of 1:4 were assigned a reciprocal titer of 2.

Nasal-wash anti-HA antibody assays. After sonication, nasal-wash specimens were concentrated, as described elsewhere [32]. Anti–influenza virus HA nasal-wash IgA and IgG antibody titers were determined by use of an end-point ELISA and purified HA homologous to the vaccine strains (viruses were grown at DynCorp [L. Potash]; HA was extracted and enriched at Aviron [H. Mehta]) [30, 32]. The nasal-wash anti-HA IgA and IgG antibody titers were adjusted to total IgA or IgG antibody concentrations of 500 mg/L or 100 mg/L, on the basis of the total IgA and IgG antibody concentrations in each specimen, by ELISA, as described elsewhere [32]. Analyses excluded specimens with a total IgA antibody concentration <3 mg/L or a total IgG concentration <2 mg/L, because the correction for total antibody would have resulted in artificially high anti-HA antibody titers.

Anti-influenza CTL assays. Peripheral blood mononuclear leukocytes (PBLs) were separated by Ficoll-Paque (Pharmacia) density gradient centrifugation, as described elsewhere [37], by use of heparinized whole blood obtained from 61 subjects before and after immunization. In vitro–stimulated memory anti–influenza A and B virus CTLs were measured after either CD8\(^+\) cell or sham depletion, by use of immunomagnetic beads coated with anti–human CD8 or sheep anti–mouse Ig antibody (Dynal), in a \( ^{32} \text{Cr} \) sodium chromate–
release cytotoxicity assay using fresh PBLs, as described elsewhere [14, 15]. The percentage of specific lysis of autologous phytohemagglutinin-P–stimulated PBL target cells, either infected with influenza virus or sham-infected with allantoic fluid, was calculated as described elsewhere [14, 15]. The net percentage of specific lysis was the percentage of specific lysis of allantoic fluid–treated autologous target cells subtracted from that of the influenza virus–infected targets, at the same E:T ratio (ratio of effector cells:target cells). The net percentage of specific lysis of $\geq 10\%$ was considered to be positive for antiviral CTL activity [8]. CTL responders had a postimmunization increase in the net percentage of specific lysis of $\geq 10\%$, compared with preimmunization, for any 1 E:T ratio, because the change was large enough to reduce the likelihood that it was due to interassay variability and was at least 50% more than mean preimmunization levels.

**Statistical Analysis**

Reciprocal antibody titers were logarithmically transformed, and mean values were compared. For CTL assays, mean net percentage of specific lysis for each E:T ratio and vaccine group was determined at each study time point and was compared. For continuous variables, differences between and paired data within vaccine groups were tested by nonparametric methods (Wilcoxon rank sum and signed rank tests). For categorical data within vaccine groups were tested by nonparametric methods (Wilcoxon rank sum and signed rank tests). For categorical data within vaccine groups were tested by nonparametric methods (Wilcoxon rank sum and signed rank tests).

**RESULTS**

**Immunogenicity**

**Serum antibody responses.** Mean serum log, reciprocal anti-influenza HAI antibody titers increased significantly for each virus strain, within each vaccine group (pre- and postimmunization mean $\pm$ SD, TC group [$n = 29$]: $3.28 \pm 1.62$ and $6.14 \pm 0.96$ [A/H1N1], $5.28 \pm 1.65$ and $7.38 \pm 1.97$ [A/H3N2], and $4.76 \pm 1.55$ and $6.38 \pm 1.72$ [B]; TP group [$n = 32$]: $3.28 \pm 1.84$ and $5.84 \pm 1.94$ [A/H1N1], $5.44 \pm 1.41$ and $7.28 \pm 1.82$ [A/H3N2], and $5.06 \pm 1.97$ and $6.56 \pm 1.44$ [B]; $P < 0.001$, for all pre- vs. postimmunization comparisons, Wilcoxon signed rank test; no significant differences between vaccine groups at either time point, Wilcoxon rank sum test). The proportions of subjects experiencing a 4-fold increase in serum anti-influenza HAI antibody titer after immunization did not differ significantly ($x^2$ test) between vaccine groups (number with 4-fold increase [%]/number tested, TC group: $19 \pm 65.5\%$/$29$ [A/H1N1], $14 \pm 48.3\%$/$29$ [A/H3N2], and $11 \pm 37.9\%$/$29$ [B]; TP group: $19 \pm 59.4\%$/$32$ [A/H1N1], $17 \pm 53.1\%$/$32$ [A/H3N2], and $11 \pm 34.4\%$/$32$ [B]). Mean log$_e$ reciprocal HAI antibody titers measured 3–4 weeks after immunization in all available serum samples ($n = 2101$) were not significantly different (Wilcoxon rank sum test) between vaccine groups (mean $\pm$ SD, TC group: $5.48 \pm 2.30$ [A/H1N1], $6.93 \pm 2.23$ [A/H3N2], and $6.50 \pm 1.74$ [B]; TP group: $5.52 \pm 2.34$ [A/H1N1], $6.99 \pm 2.31$ [A/H3N2], and $6.54 \pm 1.74$ [B]).

**Nasal-wash antibody responses.** The mean nasal-wash IgA antibody titers to all 3 HA antigens increased after immunization and, after immunization, were higher to A/H3 and B in the TC group than in the TP group (table 1). After immunization, mean IgG antibody titers increased to all 3 HA antigens in the TC group and to the A/H1 and H3 HA antigens in the TP group (table 1). Among all nasal-wash specimens obtained 3–4 weeks after immunization (1908/2053 specimens had total IgA antibody concentration above the minimum cut-off), mean log, reciprocal IgA antibody titers to A/H3 and B HA, but not to A/H1, were higher in the TC group than in the TP group (mean $\pm$ SD, TC and TP groups: $7.12 \pm 1.79$ and $6.93 \pm 1.72$ [A/H1] [$P = 0.05$], $7.55 \pm 1.77$ and $7.12 \pm 1.73$ [A/H3N2] [$P = 0.0001$], and $6.70 \pm 1.65$ and $6.33 \pm 1.59$ [B] [$P = 0.0001$, Wilcoxon rank sum test].

**Anti-influenza CTL assays.** Effector cells stimulated with influenza A (H3N2 or H1N1) exhibited CTL activity against both homologous and heterologous influenza A viruses, demonstrating cross-reactivity. There was influenza virus type specificity, since influenza A virus–stimulated effector cells did not lyse influenza B virus–infected target cells, and influenza B virus–stimulated effector cells did not lyse influenza A virus–infected target cells. CD8$^+$ cell depletion of bulk effector cells reduced the net percentage of specific lysis of influenza virus–infected target cells, indicating that CTL activity was mediated by CD8$^+$ cells.

The means of net percentage of specific lysis of influenza A (H1N1) virus–infected target cells by influenza A (H1N1 and H3N2) virus–stimulated bulk effector cells, at the 50:1 E:T ratio, were significantly higher after immunization than before immunization, in the TC group (pre- and postimmunization mean $\pm$ SD, $20.9\% \pm 14.1\%$ and $26.2\% \pm 17.1\%$ [A/H1N1 effector cells] and $15.9\% \pm 13.5\%$ and $22.5\% \pm 15.6\%$ [A/H3N2 effector cells]; $P = 0.05$ for both comparisons, Wilcoxon signed rank test). The TP group had a significant postimmunization increase in mean net percentage of specific lysis of influenza A (H3N2) virus–infected target cells by influenza A (H3N2) virus–stimulated bulk effector cells, at both E:T ratios (mean $\pm$ SD for the 50:1 and 25:1 ratios, preimmunization: $10.7\% \pm$
Table 1. Nasal-wash IgA and IgG antibody titers to influenza A virus–purified H1 and H3 hemagglutinins (HAs) and influenza B virus HA, before and after immunization.

<table>
<thead>
<tr>
<th>Vaccine group, no. of subjects, a influenza virus HA</th>
<th>Nasal-wash IgA antibody to HA</th>
<th>Dental No. (%) of subjects with 4-fold increase in postimmunization antibody titer</th>
<th>Dental No. (%) of subjects with 4-fold increase in postimmunization antibody titer</th>
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<td></td>
<td>Log2 reciprocal antibody titer</td>
<td>Before immunization 3–4 weeks after immunization</td>
<td>Before immunization 3–4 weeks after immunization</td>
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<tr>
<td>TVV + CAIV-T (175 for IgA, 105 for IgG)</td>
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<tr>
<td>A/H1</td>
<td>6.40 ± 1.77 7.08 ± 1.82b</td>
<td>29 (16.6) 0.68 ± 1.60</td>
<td>5.41 ± 1.30 6.20 ± 1.32c 22 (21.0) 0.80 ± 1.48</td>
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<tr>
<td>A/H3</td>
<td>6.83 ± 1.80 7.54 ± 1.83b,c,d</td>
<td>36 (20.6) 0.71 ± 1.86</td>
<td>5.32 ± 1.29 6.03 ± 1.41c 15 (14.3) 0.71 ± 1.64</td>
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<tr>
<td>B</td>
<td>6.24 ± 1.74c 6.86 ± 1.74b,c,d</td>
<td>31 (17.7) 0.62 ± 1.49</td>
<td>5.21 ± 1.38 5.71 ± 1.28c 11 (10.5) 0.50 ± 1.22</td>
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<tr>
<td>TVV + P (182 for IgA, 104 for IgG)</td>
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<tr>
<td>A/H1</td>
<td>6.40 ± 1.84 7.01 ± 1.82b</td>
<td>37 (20.3) 0.61 ± 1.81</td>
<td>5.49 ± 1.27 6.13 ± 1.48c 22 (21.2) 0.63 ± 1.66</td>
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<tr>
<td>A/H3</td>
<td>6.75 ± 1.90 7.22 ± 1.85b</td>
<td>31 (17.0) 0.47 ± 1.62</td>
<td>5.42 ± 1.35 5.93 ± 1.49c 15 (14.4) 0.51 ± 1.47</td>
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<tr>
<td>B</td>
<td>5.84 ± 1.60 6.38 ± 1.72b</td>
<td>33 (18.1) 0.53 ± 1.57</td>
<td>5.34 ± 1.25 5.60 ± 1.37 9 (8.7) 0.26 ± 1.53</td>
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**NOTE.** Unless otherwise noted, data are mean ± SD. Nasal-wash specimens were obtained before and after immunization, from 400 subjects, and were assayed for antibodies. CAIV-T, trivalent, live cold-adapted influenza A and B virus vaccine; TVV, inactivated influenza virus vaccine; TVV + CAIV-T, TC group; TVV + P, TP group.

a The no. shown contained total IgA and IgG antibody concentrations greater than the respective minimum cutoffs.

b P < .001; mean log reciprocal IgA antibody titers higher after immunization than before immunization, for each respective influenza virus HA in each vaccine group (Wilcoxon signed rank test).

c P = .0001, for TC group; P = .001 for TP group; mean log reciprocal IgG antibody titers higher after immunization than before immunization, for the indicated HA antigens (Wilcoxon signed rank test).

d P = .05, for influenza A/H3; P < .01 for influenza B HA; postimmunization mean log reciprocal IgA antibody titers higher for TC group than for TP group (Wilcoxon rank sum test).

e P < .05, preimmunization mean log reciprocal IgA antibody titer to influenza B HA for TC group higher than for TP group (Wilcoxon rank sum test).
11.8% and 8.1% ± 8.6%; postimmunization: 16.5% ± 12.6% and 12.4% ± 10.5%; P < .05 for both comparisons, Wilcoxon signed rank test) and by influenza A (H1N1) virus–stimulated bulk effector cells, at only the 25:1 E:T ratio (mean ± SD, preimmunization: 6.6% ± 7.4%; postimmunization: 10.1% ± 10.2%; P < .01, Wilcoxon signed rank test). The means of net percentage of specific lysis of influenza B virus–infected target cells by influenza B virus–stimulated bulk effector cells were significantly higher after immunization in the TC group, compared with the TP group, at both E:T ratios (mean ± SD for the 50:1 and 25:1 ratios, TC group: 8.4% ± 6.2% and 7.1% ± 5.9%; TP group: 5.1% ± 6.7% and 3.7% ± 5.7%; P = .01 for both comparisons, Wilcoxon rank sum test). The proportions of subjects after immunization with a net percentage of specific lysis of at least 10% against influenza B virus–infected target cells by influenza B virus–stimulated effector cells were significantly greater in the TC group than in the TP group, at both E:T ratios (proportions for 50:1 and 25:1 E:T ratios, TC group: 13 [44.8%] and 8 [27.6%] of 29 subjects; TP group: 4 [12.9%] of 31 subjects and 2 [6.9%] of 29 subjects; P < .01 for 50:1 cell ratio and P < .05 for 25:1 cell ratio, χ² test). For the 5 effector and target cell combinations, there were consistently higher proportions of CTL responders at both E:T ratios concomitantly in the TC group than in the TP group (table 2).

**Immune Correlates of Protection**

Higher postimmunization serum and nasal-wash antibody titers against influenza A (H3N2) and B viruses were associated with lower rates of virus-specific LDI (figure 1). Of the subjects with LDI due to influenza A (H3N2) virus, 70 (90.9%) of 77 had a serum log, reciprocal anti–influenza A (H3N2) HA antibody titer of ≤7, and 64 (91.4%) of 70 had a nasal-wash log, reciprocal anti-A/H3 HA antibody titer of ≤9. Of the subjects with LDI due to influenza B virus, 28 (90.3%) of 31 had a serum log, reciprocal anti–influenza B HA antibody titer of ≤7, and 26 (92.9%) of 28 had a nasal-wash log, reciprocal anti-B HA antibody titer of ≤9.

A multivariate Cox regression proportional hazards model showed that serum HA1 and nasal-wash anti-HA IgA antibody titers to influenza A (H3N2) virus were both associated with a reduction in the risk of LDI due to influenza A (H3N2) virus (table 3). When both serum HA1 and nasal-wash anti-HA IgA antibody titers to influenza B were included in models together, only serum HA1 antibody was significantly associated with a reduced risk of LDI due to influenza B virus (table 3).

**DISCUSSION**

The TC regimen appeared to be statistically more immunogenic than TP, with respect to induction of nasal-wash anti–influenza A H3 and B HA IgA antibodies, by comparing postimmunization mean antibody titers between vaccine groups; however, the differences were <2-fold in magnitude and of little clinical significance. Our findings of modestly higher nasal-wash IgA antibody response to TC, compared with TP, corroborate reports of enhanced immunogenicity in some, but not all, previous studies of the combination vaccine approach in seropositive adults [15, 25, 26, 28, 32, 33, 40, 41]. The TC regimen induced higher levels of anti–influenza virus CD8+ CTLs against influenza B virus. The proportions of TC recipients with any postimmunization increase in CD8+ CTLs also suggested better stimulation of anti-influenza CTLs by TC than by TP.

Coadministration of CAIV-T with TVV did not enhance the serum antibody response to TVV, and the rates of at least 4-fold increases in these antibodies, although lower than those reported in children and young adults, were similar to those observed in other studies of chronically ill older adults [5, 17, 24, 28, 33, 41–43]. Preimmunization antibody titer inversely correlated with serum HA1 antibody response, and serum antibody response rates decreased on repeated immunization with

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<th>Table 2. Subjects categorized as being anti–influenza virus–specific cytotoxic T lymphocyte (CTL) responders.</th>
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<tr>
<td><strong>Influenza virus–stimulated effecter cell, infected autologous target cell</strong></td>
</tr>
<tr>
<td><strong>A/H1N1, A/H1N1</strong></td>
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<tr>
<td><strong>A/H1N1, A/H3N2</strong></td>
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<td><strong>A/H3N2, A/H3N2</strong></td>
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<tr>
<td><strong>A/H3N2, A/H1N1</strong></td>
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<tr>
<td><strong>B, B</strong></td>
</tr>
<tr>
<td><strong>Any combination</strong>&lt;sup&gt;b&lt;/sup&gt;</td>
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</table>

**NOTE.** CAIV-T, trivalent, live cold-adapted influenza A and B virus vaccine; E:T, ratio of effector cells:target cells; P, intranasal placebo; TVV, inactivated influenza virus vaccine.

<sup>a</sup> A CTL responder had an increase in net percentage of specific lysis of at least 10% after immunization, compared with before immunization, at 1 E:T ratio. “Both 50:1 and 25:1 E:T” means that there was a 10% increase in net percentage of specific lysis concomitantly at both E:T ratios in the same subject after immunization, compared with before immunization. There were 29 recipients of TVV + CAIV-T and 32 recipients of TVV + P assessed for anti–influenza virus CTLs. The denominators differ from these numbers in some combinations because of missing values, usually for the 25:1 E:T ratio. The 50:1 E:T ratio was included preferentially in an assay whenever there were inadequate numbers of effector cells to measure both the 50:1 and 25:1 E:T ratios. Subjects who were CTL responders at both the 50:1 and 25:1 E:T ratios, for the same influenza virus–stimulated effector and influenza virus–infected target cell combination, likely had a biologically more significant response than did those who had a CTL response at only 1 of the E:T ratios, for a particular effector and target cell combination.

<sup>b</sup> Any combination of effector and target cells refers to subjects who were CTL responders for ≥1 influenza A effector and A target and influenza B effector and B target combination.

<sup>c</sup> P = .06; proportion in the TVV + CAIV-T (TC) group borderline higher than in the TVV + P (TP) group (χ² test), for any combination of effector and target cells.
Figure 1. Distributions of titer-specific rates of laboratory-documented influenza-caused illness (LDI)/100 subjects, shown for all subjects and for each immunization group, by anti–influenza virus log₂ reciprocal antibody titers measured at 3–4 weeks after immunization. The titer-specific rates are shown by columns. Lines located at a zero titer–specific rate of disease indicate that there were subjects with that measured antibody titer but no cases of LDI for that titer. No line at the zero titer–specific rate of disease level or column indicates that there were no subjects with that measured antibody titer. In panels A and B, the 3 column and/or line sets are centered over each serum log₂ reciprocal antibody titer integer to which they refer. In panels C and D, the 3 column and/or line sets are centered between 2 consecutive nasal-wash log₂ reciprocal antibody titer integers and correspond to all persons with measured nasal-wash log₂ reciprocal antibody titers falling into the range greater than the lower integer, up to and including the next higher integer. Also, a probability curve from logistic regression analysis is shown for all subjects in each panel. Unadjusted probability curves are shown, because adjustment for age, percentage of predicted forced expiratory volume in 1 s (FEV₁), and chronic lung disease severity index did not materially change the curves. Chronic lung disease severity index was calculated as described elsewhere [39]. CAIV-T, trivalent, live cold-adapted influenza A and B virus vaccine; TVV, inactivated influenza virus vaccine. A, Titer-specific rate of LDI due to influenza A (H3N2) virus, by serum log₂ reciprocal anti–influenza A (H3N2) hemagglutination (HA) inhibition (HAI) antibody titer. B, Titer-specific rate of LDI due to influenza B virus, by serum log₂ reciprocal anti–influenza B HAI antibody titer. C, Titer-specific rate of LDI due to influenza A (H3N2) virus, by nasal-wash log₂ reciprocal anti–influenza A/H3 HA antibody titer. D, Titer-specific rate of LDI due to influenza B virus, by nasal-wash log₂ reciprocal anti–influenza B HA antibody titer.

TVV [17, 27, 28, 41, 44]. Repeated immunization may still improve protective efficacy against influenza, despite blunted antibody responses [18, 44].

The modest differences in immunogenicity observed here between the 2 immunization regimens may indicate that immune memory from previous experience with influenza viruses in older seropositive patients provided some level of protection against infection with the CAIV-T strains, thereby limiting their immunogenicity. Those already immune to the vaccine virus strains may be a group of patients with preexisting immunity to the wt influenza virus strains expected to circulate that season. Patients without preexisting immunity to the CAIV-T
strains would likely represent those who contributed most to the increased immunogenicity of TC, compared with TP, in the present study. If the patients who are susceptible to the CAIV-T strains could be identified in advance, they might then be targeted for immunization with both TVV and CAIV-T, whereas the others would still benefit from TVV. In a year in which either a viral antigenic shift or a major antigenic drift is possible, resulting in the use of vaccine virus strains unlike those representative of vaccines or wt strains circulating in previous years, more vaccine recipients may respond immunologically to the CAIV-T vaccine component, making it a potentially more effective addition to TVV in those years.

The present study is the first large clinical trial of chronically ill older adults to suggest that nasal-wash anti-HA IgA antibody is associated with resistance to influenza. Other studies have shown this association in children and healthy young adults, either against natural infection or experimental challenge with wt or live CAIVs [3, 5, 7, 16, 45]. The correlation between serum HAI antibody and resistance to natural influenza virus infection in the present study is consistent with findings of previous studies of pediatric, young adult, and elderly populations [7, 17, 41, 43, 46–48]. Breakthrough infections in subjects with high antibody levels may reflect the multifactorial nature of immune protection against influenza or differences in the degree of exposure to circulating wt strains. Also, the breakthrough infections may have occurred after the initially higher protective antibody levels measured 3–4 weeks after immunization decayed later in the influenza season. However, our analysis of associations between antibody levels and protection from LDI must be interpreted cautiously, because patients without an LDI comprised one population that was exposed to influenza virus and not infected and another that was never exposed. Everyone with LDI was exposed to influenza virus.

In summary, the combination vaccine regimen showed possible advantages over TVV alone, in terms of anti-influenza nasal-wash IgA antibody stimulation and CTL induction, which may be of little clinical significance. This is consistent with the lack of statistically significant added efficacy of protection against LDI, for TC over TP [34]. However, our results suggest reasons that TC could provide added protection against influenza, compared with TVV alone, at least in certain patients susceptible to infection with CAIV-T virus strains, through better stimulation of mucosal and cellular responses.

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Immunity to Influenza Virus