Recipients of Vaccine against the 1976 “Swine Flu” Have Enhanced Neutralization Responses to the 2009 Novel H1N1 Influenza Virus

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Background. The world is facing a novel H1N1 influenza pandemic. A pandemic scare with a similar influenza virus in 1976 resulted in the vaccination of nearly 45 million persons. We hypothesized that prior receipt of the 1976 “swine flu” vaccine would enhance immune responses to the 2009 novel H1N1 influenza strain.

Methods. A prospective, volunteer sample of employees aged ≥55 years at a children’s cancer hospital in August 2009 was assessed for antibody responses to the 2009 pandemic H1N1 influenza virus and the 2008–2009 seasonal H1N1 influenza virus.

Results. Antibody responses by hemagglutination-inhibition assay were high against both the seasonal influenza virus (89.7% had a titer considered seroprotective) and pandemic H1N1 influenza virus (88.8% had a seroprotective titer). These antibodies were effective at neutralizing the seasonal H1N1 influenza virus in 68.1% of participants (titer ≥40), but only 18.1% had detectable neutralizing titers against the pandemic H1N1 influenza virus. Of 116 participants, 46 (39.7%) received the 1976 “swine flu” vaccine. Receipt of this vaccine significantly enhanced neutralization responses; 8 (17.4%) of 46 vaccine recipients had titers ≥160, compared with only 3 (4.3%) of 70 who did not receive the vaccine (P = .018 by χ² test).

Conclusions. In this cohort, persons aged ≥55 years had evidence of robust immunity to the 2008–2009 seasonal H1N1 influenza virus. These antibodies were cross-reactive but nonneutralizing against the 2009 pandemic H1N1 influenza strain. Receipt of a vaccine to a related virus significantly enhanced the neutralization capacity of these responses, suggesting homologous vaccination against the 2009 pandemic H1N1 influenza virus would have a similar effect.

The world is facing a new influenza pandemic for the first time in >40 years [1]. A triple reassortant influenza virus of the H1N1 subtype emerged from an animal reservoir in early 2009 and has spread worldwide. This strain’s H1 hemagglutinin (HA), the surface protein against which the majority of our neutralizing antibody responses are directed, is derived from the “classic” swine lineage [2]. These “classic” H1N1 influenza viruses are endemic in pigs and are derived from a progenitor strain that entered the swine population in 1918, the same virus that caused a human pandemic resulting in >40 million deaths [3].

The epidemiology of this nascent pandemic has been different than that of recent seasonal epidemics, with the majority of cases and hospitalizations being identified in children and young adults. Severe illness has frequently been seen in this age demographic among persons with no underlying chronic medical conditions [4, 5]. However, unlike for seasonal influenza, in which the majority of hospitalizations and deaths are among the elderly [6], <5% of hospitalizations for the pandemic H1N1 influenza have involved persons aged ≥65 years, primarily among those with underlying chronic medical conditions [7]. The reason for this relative sparing of elderly persons is unclear but is likely related to cross-reactive antibody responses that provide some measure of immunity [8]. Whether this cross-reactive antibody is from prior infection with a specific, related virus or whether it is the result of the accumulation of
exposures to unrelated viruses that share epitopes with the pandemic H1N1 influenza [9] is not known at this time.

In 1976, an influenza outbreak of an H1N1 influenza virus of the “classic” swine lineage caused a pandemic scare. More than 200 military recruits at Fort Dix, New Jersey, were infected, but the virus did not spread beyond the military base [10]. Fears about a repeat of the disastrous 1918 pandemic, however, prompted a rapid and massive immunization campaign, resulting in the vaccination of 45 million persons, nearly a quarter of the population of the United States [11]. Phylogenetic analysis of the hemagglutinin of H1N1 influenza viruses that have spread among humans in the past century demonstrates that the hemagglutinins of viruses that circulated in humans in the 1930s and 1940s are more closely related to the 2009 H1N1 influenza pandemic strain than are recent seasonal strains, suggesting that exposure to these viruses in early childhood might provide some cross-protective immunity and might help explain the age distribution (Figure 1). However, the A/New Jersey/76 influenza strain that caused the Fort Dix outbreak is the most closely related human virus to the 2009 H1N1 influenza pandemic strain. Thus, receipt of vaccine in 1976 might provide some current benefit to vaccinees exposed to the 2009 pandemic H1N1 influenza. We undertook this study to define the influenza specific antibody response in older persons and to determine whether receipt of the 1976 “swine flu” vaccine influences those responses.

**METHODS**

**Study design and participants.** Employees of St Jude Children’s Research Hospital and their spouses were eligible for recruitment if they were aged ≥ 55 years. Of 250 randomly selected employees contacted for this study, 110 (44%) elected to participate, and 6 spouses volunteered. The mean age of those contacted who did not volunteer to participate (59.6 years) was not different from that of participants. This study population was chosen partly for convenience and partly because the 1976 “swine flu” vaccine was administered to hospital employees as part of a clinical trial in 1976, and many of these vaccinated persons still work at the hospital. Enrollment took place in late July and early August 2009, prior to the widespread circulation of the 2009 pandemic H1N1 influenza virus in Memphis. Demographic characteristics and elements of history, including age, sex, history of chronic medical conditions predisposing to influenza hospitalization as defined by the Advisory Committee on Immunization Practices [13], and receipt of influenza vaccine in 2008 (seasonal vaccine) or 1976 (“swine flu” vaccine) were collected in a standardized manner under an institutional review board–approved protocol. Discard serum samples collected anonymously at Le Bonheur Children’s Medical Center during a 2001 study of seroresponses were used as controls because these children could not have been exposed to any of the viruses studied.

**Ascertainment of outcomes.** The main outcomes to be studied were hemagglutination-inhibition (HAI) and micro-neutralization (MN) titers stratified by age and by prior receipt of the 1976 “swine flu” vaccine. Serum samples were treated with receptor destroying enzyme (Accurate Chemical & Scientific) and were heat inactivated prior to analysis for influenza-specific antibody using a standard HAI titer assay [14]. For determination of HAI titers, individual H1N1 influenza virus stocks expressing hemagglutinin from A/Brisbane/59/07 or A/California/7/09 strains were adjusted to 4 hemagglutinin units and were incubated with diluted serum samples for 1 h at 4°C. Chicken red blood cells (0.5%) were added to the plates, and HAI titers, reported as the reciprocal of the final serum dilution that inhibits hemagglutination, were recorded 30 min later.

For determination of MN titers, serum samples diluted in infection media were incubated with individual viral stocks (2000 doses infectious for 50% of tissue culture wells per mL [2000 TCID₅₀ mL⁻¹]) expressing hemagglutinins from A/Brisbane/59/07 or A/California/7/09 for 2 h. Confluent Madin Darby canine kidney (MDCK) monolayers (3 × 10⁴ cells/mL) were rinsed with phosphate-buffered saline and were exposed
Table 1. Geometric Mean Titers (GMTs) to Influenza, According to Age Group and Assay

<table>
<thead>
<tr>
<th>Age group, years</th>
<th>GMT to 2008–2009 seasonal H1N1 influenza (95% CI)</th>
<th>GMT to 2009 pandemic H1N1 influenza (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–18 (n = 20)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAI</td>
<td>9.3 (8.2–10.4)</td>
<td>5a</td>
<td>.015b</td>
</tr>
<tr>
<td>MN</td>
<td>9.3 (8.1–10.5)</td>
<td>5.9 (4.9–7.0)</td>
<td>.27</td>
</tr>
<tr>
<td>55–59 (n = 62)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAI</td>
<td>70.0 (57.5–82.4)</td>
<td>54.1 (41.7–66.4)</td>
<td>.35</td>
</tr>
<tr>
<td>MN</td>
<td>84.1 (69.3–98.8)</td>
<td>12.8 (–1.3 to 26.9)</td>
<td>.001b</td>
</tr>
<tr>
<td>60–64 (n = 36)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAI</td>
<td>80.0 (67.6–92.4)</td>
<td>54.4 (41.7–67.2)</td>
<td>.081</td>
</tr>
<tr>
<td>MN</td>
<td>59.9 (45.0–74.9)</td>
<td>12.6 (–2.6 to 27.8)</td>
<td>&lt;.001b</td>
</tr>
<tr>
<td>≥65 (n = 18)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAI</td>
<td>86.4 (72.2–100.6)</td>
<td>63.5 (48.5–66.4)</td>
<td>.21</td>
</tr>
<tr>
<td>MN</td>
<td>54.4 (36.4–72.5)</td>
<td>14.1 (–3.0 to 31.3)</td>
<td>.005b</td>
</tr>
</tbody>
</table>

NOTE. CI, confidence interval; HAI, hemagglutination-inhibition assay; MN, microneutralization assay.

a All titers were below the limit of detection (10).
b Statistically significant difference in titer (P < .05), by the Mann-Whitney rank-sum test.

to serum-virus mixtures for 18 h. Inoculum was removed, and cells were incubated for 18 h in infection media supplemented with 2 μg/mL TPCK-trypsin. Cells were fixed with 80% acetone, and influenza virus nucleoprotein was detected using monoclonal antibodies to nucleoprotein (Millipore Fisher; catalog number MAB8251) at a dilution of 1:2000 as described elsewhere [15]. MN titers are reported as the reciprocal of the final dilution that neutralizes virus to a neutralization end point defined as described elsewhere [8, 16].

Statistical analyses. Data from all participants were included in the analyses. Geometric mean titers were calculated for both hemagglutinin and MN. In cases in which all titers were below the level of detection (<1:10), a value of “5” was used for comparisons. For hemagglutinin, a geometric mean titer of ≥40 was considered “protective”; for MN, a titer of ≥160 was used as a correlate of seroprotection [8]. Categorical variables were compared using χ² tests with Yates correction, and the Mann-Whitney U test was used for continuous variables. When relevant, 95% confidence intervals were computed. P values < .05 were considered statistically significant. SigmaStat for Windows (SysStat Software, version 3.11) was used for all statistical analyses.

RESULTS

Subjects. This volunteer sample of 116 persons was composed of 81 women (69.8%) and 35 men and had a mean age of 60.1 years (range, 55–73 years). Chronic medical conditions predisposing to complications of influenza were present in 44 subjects (37.9%). Because the sample was derived mainly from employees of a children’s cancer hospital, this was a highly vaccinated population, with 106 (94.1%) of subjects having received the 2008–2009 seasonal vaccine the prior year, and 46 (39.7%) having received the 1976 “swine flu” vaccine during the national campaign in 1976.

Antibody responses to seasonal and pandemic H1N1 influenza viruses. The participants had high levels of influenza-specific antibody by HAI assay against the 2008–2009 seasonal H1N1 influenza strain (Table 1). The geometric mean titer trended up with increasing age, and 89.7% of participants had a titer ≥40, which is considered to be seroprotective (Figure 2A). This was specific to this older population and was not an artifact of the assay used, because the children included as control subjects had negligible antibody titers, with only 3 reaching seroprotective titers. Similar levels of antibody were detected against the novel pandemic H1N1 influenza strain, with 88.8% achieving a seroprotective titer (Figure 2A). No differences in any age group could be determined between the HAI responses to seasonal and pandemic H1N1 influenza strains by comparison of the geometric mean titers (Table 1). The control serum samples from children had no detectable

Figure 2. Antibody titers by hemagglutination-inhibition (HAI) (A) and microneutralization (MN) (B) methods against the 2008–2009 seasonal H1N1 and the 2009 pandemic H1N1 influenza viruses, stratified by patient age in years. The dotted line in panel A represents the breakpoint for presumed seroprotection.
Table 2. Characteristics of Study Population by 1976 Vaccination Status

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Recipients of 1976 vaccine (n = 46)</th>
<th>Nonrecipients of 1976 vaccine (n = 70)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years ± SD</td>
<td>60.8 ± 3.8</td>
<td>59.7 ± 5.5</td>
<td>.56</td>
</tr>
<tr>
<td>Female</td>
<td>27 (58.7)</td>
<td>52 (74.3)</td>
<td>.08</td>
</tr>
<tr>
<td>Chronic medical condition</td>
<td>17 (37.0)</td>
<td>27 (38.6)</td>
<td>.86</td>
</tr>
<tr>
<td>Received 2008 seasonal influenza vaccine</td>
<td>45 (97.8)</td>
<td>61 (87.1)</td>
<td>.044*</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. (%) of individuals, unless indicated otherwise. SD, standard deviation. 
*P < .05 by χ² test.

antibody to the pandemic H1N1 influenza. Because the study was conducted prior to the availability of the monovalent H1N1 influenza vaccine or widespread circulation of the pandemic H1N1 influenza in Memphis, the immune responses to the 2009 H1N1 influenza likely represent cross-reactive antibody responses from prior infection or vaccination.

Neutralizing antibodies against the seasonal H1N1 influenza, as measured by the MN assay, were detected at similar levels as were hemagglutinating antibodies detected in the HAI assay (Figure 2B). Geometric mean titers did not differ significantly as measured by HAI versus MN for any age group (P = .549 for age 55–59 years, P = .272 for age 60–64 years, and P = .103 for age ≥65 years). However, MN responses to the pandemic H1N1 influenza were significantly lower than corresponding titers to the seasonal H1N1 influenza (Figure 2 and Table 1) and were also significantly lower than titers measured by HAI for all age groups (P < .001 for age 55–59 years, P < .001 for age 60–64 years, and P = .001 for age ≥65 years). Overall, 68.1% of participants had MN titers ≥1:40 to the seasonal H1N1 influenza strain, compared with only 18.1% to the pandemic H1N1 influenza strain (P < .001). We conclude from these data that persons aged >55 years have high levels of influenza-specific antibodies that can recognize both seasonal and pandemic H1N1 influenza strains, but these responses differ in quality, since they are poorly neutralizing against the 2009 pandemic H1N1 influenza strain.

**Effect of vaccination against the 1976 “swine flu.”** We next assessed the effect of vaccination in 1976 against A/New Jersey/76 (H1N1) influenza on both total virus-specific antibody responses as well as neutralizing responses. Among this volunteer sample, 46 (37.9%) of 116 subjects received the “swine flu” vaccine in 1976. Recipients did not differ in age, sex, or frequency of chronic medical conditions from those who did not receive this vaccine (Table 2). However, recipients were more likely to have been vaccinated in 2008 with the seasonal vaccine. Despite this higher rate of vaccine uptake in 2008, antibody levels against the seasonal H1N1 influenza strain contained in the vaccine did not differ between the groups by HAI assay (geometric mean titer of recipients, 81.2 [95% confidence interval (CI), 68.8–93.6]; geometric mean titer of nonrecipients, 71.7 [95% CI, 59.7–83.8]; P = .482). Similarly, the percentage of participants reaching a titer of 40 or 160 by MN assay did not differ by 1976 vaccination status (Figure 3A).

When responses against the 2009 H1N1 influenza were assessed, they were similar by HAI between recipients of the 1976 vaccine and those who did not receive vaccine (geometric mean titer of recipients 59.2 [95% CI, 46.5–71.9]; geometric mean titer of nonrecipients, 53.3 [95% CI, 41.2–65.4]; P = .924). However, neutralizing antibody levels against the pandemic H1N1 influenza were higher by MN assay in recipients of the 1976 vaccine, with 11 of 46 achieving a titer ≥40, compared

**Figure 3.** Microneutralization titers against the 2008–2009 seasonal H1N1 and the 2009 pandemic H1N1 influenzaviruses, stratified by receipt of the “swine flu” vaccine in 1976. Bars represent serum samples achieving titers of ≥40 and serum samples achieving titers ≥160. An asterisk (*) indicates a significant difference (P < .05) compared with the subjects who did not receive the 1976 “swine flu” vaccine.
with 11 of 70 who did not receive vaccine, and 8 of 46 recipients achieved a titer ≥160, compared with 3 of 70 nonrecipients (Figure 3B). Only the comparison of titers at ≥160 was statistically significant ($P = .018$). We conclude from these data that receipt of the 1976 “swine flu” vaccine results in enhanced neutralization responses against the 2009 pandemic H1N1 influenza strain.

**DISCUSSION**

In this study, we have demonstrated that a cohort of older adults has a significant amount of antibody that cross-reacts with the 2009 pandemic H1N1 influenza strain. However, these antibodies are generally nonneutralizing. Vaccination against a related virus, A/New Jersey/1976, enhanced these neutralizing responses. Since neutralizing responses were very low in adults who did not receive the “swine flu” vaccine in 1976 (geometric mean titer, 11.3; 95% CI, −1.9 to 24.4; only 3 of 70 subjects had a titer ≥160), it is unlikely that virus neutralization accounts for the low clinical attack rate and relatively low hospitalization rate observed among elderly persons without chronic medical conditions. Indeed, the epidemiology of pandemic H1N1 influenza from many countries where the 1976 “swine flu” vaccine was never used is similar to that in the United States [17]. However, our data suggest that vaccination against a homologous or even closely related strain is likely to significantly boost neutralizing responses to the pandemic H1N1 influenza. The limited data published so far on vaccination with the monovalent H1N1 influenza vaccine show good responses by HAI assay among elderly persons [18, 19], but only limited data on neutralizing responses have been reported [20].

Our study differs in several important ways from previously published data on pre-existing immune responses to the 2009 pandemic H1N1 influenza and the effect of 1976 “swine flu” vaccination [8, 21]. The current study was prospective and measured antibody responses in persons in 2009, whereas the only other study that examined responses to the 1976 vaccine used stored serum samples from prior studies conducted in prior years, primarily influenza vaccine studies [8]. Immunity to the 1976 “swine flu” vaccine was thus assessed immediately after vaccination against the virus, rather than now, after >30 years have passed. In that study, Hancock et al [8] found that >30% of serum samples from persons born in the 1940s showed neutralizing responses of ≥40 to the 2009 pandemic H1N1 influenza, and the 1976 “swine flu” vaccine boosted these responses, such that >60% had an MN titer against the 2009 H1N1 influenza of ≥160. In our population, only 18.1% of the overall cohort had MN titers ≥40 against pandemic H1N1 influenza, and of the subgroup who received the 1976 vaccine, only 17.4% retained MN titers ≥160 to the present day. Two other studies that reported neutralization titers to the 2009 pandemic H1N1 influenza showed either no responses (retrospective study of stored serum samples from April 2009 obtained from hospital employees and patients) [21] or responses similar to ours (prevaccination titers in a monovalent H1N1 influenza vaccine study) [20] but neither assessed the impact of the 1976 vaccine.

A major difference from previous studies appears to be that our population was highly vaccinated; overall, 91.4% received the seasonal vaccine in 2008–2009, and most of the cohort has had repeated annual immunization because of their status as health care workers [22]. Thus, overall HAI titers against seasonal H1N1 influenza were very high in comparison with other published data [8, 20, 21], and cross-reactive responses against the pandemic H1N1 influenza were similar to those against the seasonal H1N1 influenza. Hancock et al [8] showed very low postvaccination geometric mean titers against the 2009 H1N1 influenza (geometric mean titer by HAI, 10–11; 95% CI, 7–14) in their cohorts of older adults (aged >60 years) receiving recent seasonal influenza vaccines and saw predicted seroprotective responses (≥40) in only 12%–13% of subjects. Greenberg et al [20] saw low prevaccination baseline geometric mean titers against the 2009 H1N1 influenza (geometric mean titer of 15.0 and 13.8 by HAI in 2 vaccine groups; 95% CIs, 11.4–19.6 and 8.4–14.3, respectively) in their cohort of older adults (50–64 years), and only 27.4% and 13.8% in the 2 groups had predicted seroprotective responses. Thus, their populations, which were selected for inclusion in vaccine trials and had low baseline titers to influenza virus, are likely different from our population, who are routinely immunized annually and in whom higher geometric mean titers and more frequent seroprotective responses were identified.

This study has several important limitations that must be considered to best understand the data. First, this was a highly vaccinated population, so it probably represents the upper end of the spectrum in terms of antibody responses. This is partly because of the study design, which was a convenience sampling of employees, and is partly because of a likely selection bias, in that persons who volunteered were aware that we were studying responses to the 1976 “swine flu” vaccine and nearly 40% of all participants had received the vaccine previously. We did not attempt to assess other groups that might be more representative of the general population, because we were focused on the central hypothesis that the 1976 vaccine would enhance responses to the current 2009 pandemic strain. Therefore, generalization of these results to other groups should be done cautiously. Finally, the assays in use are subject to some variation between laboratories, although we attempted to minimize the effect of this by using the same methods and definitions as those used in the previously reported study [8].

In summary, we present the first prospective data analyzing antibody responses to the 2009 pandemic H1N1 influenza virus
in relation to the 1976 “swine flu” vaccine. Our findings are notable in that little neutralizing activity was seen in our highly vaccinated cohort of older adults despite high titers of cross-reactive antibody by HAI assay. Prior receipt of the 1976 vaccine, however, enhanced these neutralizing responses. These results suggest that neutralizing immunity against the 2009 pandemic H1N1 influenza strain is unlikely to account for the low morbidity seen among elderly persons during the current pandemic, and the results of vaccine trials that report only HAI data need to be interpreted cautiously.

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