Prior Infections With Seasonal Influenza A/H1N1 Virus Reduced the Illness Severity and Epidemic Intensity of Pandemic H1N1 Influenza in Healthy Adults

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Background. A new influenza A/H1N1 (pH1N1) virus emerged in April 2009, proceeded to spread worldwide, and was designated as an influenza pandemic. A/H1N1 viruses had circulated in 1918–1957 and 1977–2009 and were in the annual vaccine during 1977–2009.

Methods. Serum antibody to the pH1N1 and seasonal A/H1N1 viruses was measured in 579 healthy adults at enrollment (fall 2009) and after surveillance for illness (spring 2010). Subjects reporting with moderate to severe acute respiratory illness had illness and virus quantitation for 1 week; evaluations for missed illnesses were conducted over holiday periods and at the spring 2010 visit.

Results. After excluding 66 subjects who received pH1N1 vaccine, 513 remained. Seventy-seven had reported with moderate to severe illnesses; 31 were infected with pH1N1 virus, and 30 with a rhinovirus. Determining etiology from clinical findings was not possible, but fever and prominent myalgias favored influenza and prominent rhinorrhea favored rhinovirus. Tests of fall and spring antibody indicated pH1N1 infection of 23% had occurred, with the rate decreasing with increasing anti-pH1N1 antibody; a similar pattern was seen for influenza-associated illness. A reducing frequency of pH1N1 infections was also seen with increasing antibody to the recent seasonal A/H1N1 virus (A/Brisbane/59/07). Preexisting antibody to pH1N1 virus, responses to a single vaccine dose, a low infection-to-illness ratio, and a short duration of illness and virus shedding among those with influenza indicated presence of considerable preexisting immunity to pH1N1 in the population.

Conclusions. The 2009 A/H1N1 epidemic among healthy adults was relatively mild, most likely because of immunity from prior infections with A/H1N1 viruses.
period April 2009 to April 2010 are 60.8 million cases, 274,304 hospitalizations, and 12,469 deaths, less than some seasonal epidemics but substantial in children [6].

The hemagglutinin (HA) of the pH1N1 virus is a swine virus HA similar to the HA of viruses isolated from swine in North America in recent years and related to influenza A/H1N1 viruses that have circulated in swine since first detected in 1930 [7]. Antigenically related influenza A/H1N1 viruses circulated in humans from 1918 to 1956 and reappeared in 1977 as the “Russian flu” [8]. At that time, persons ≥25 years of age were “primed” for H1 antigens from prior A/H1N1 virus infections; they experienced very little clinical influenza during the 1977–1978 A/H1N1 virus epidemic [9]. Because A/H1N1 viruses had caused infections and influenza as well as having been included in the annual influenza vaccine since 1977, it seemed likely that large numbers of people would have a degree of immunity to the pH1N1 virus, with increasing susceptibility likely to increase with decreasing age. However, no specific age could be designated for full susceptibility as was possible in 1977. It seemed likely that young adults would have a high degree of susceptibility. A serological survey for specific antibody conducted by the Centers for Disease Control and Prevention had indicated very little pH1N1 antibody in them [10].

To assess the clinical and epidemiological impact of pH1N1 infections and to identify immunologic factors correlating with infections and illnesses, we conducted a prospective study of influenza in a young adult population. Here we describe the infections and illnesses in this population with the pH1N1 virus, assess the role of prior seasonal H1N1 infections in occurrences of infection and illness with the pH1N1 virus, and discuss the similarity of the pH1N1 experience with that of the “Russian” H1N1 influenza that emerged in 1977.

MATERIALS AND METHODS

Study Design

The study was conducted at Texas A&M University, College Station. Healthy persons ages 18 to 49 at the college and in the community were invited to enroll to be followed for acute respiratory illness (ARI) through the influenza season. The protocol and informed consent were approved by the Baylor College of Medicine and Texas A&M University institutional review boards before the study began. After subjects provided consent, a medical history was taken to ensure good health, and baseline specimens were obtained. Surveillance for influenza began during the September 2009 enrollment period because pH1N1 as a cause of influenza was identified in the population during enrollment. Subjects were given thermometers and instructions to call within 48 hours of onset for any ARI. Except for 4 days of the Thanksgiving holiday period and 4 weeks of the Christmas holiday period, a coordinator and physician were available every day to see patients. Those persons presenting within 48 hours of onset with a new ARI with fever or that caused them to miss school, work, or social activities were enrolled for evaluation. Specimens were obtained and medical care was provided, including the antiviral zanamivir if indicated. Ill persons were seen 2, 4, and 6 days later for repeat evaluation, specimen collections, and medical care and 21 days later for collection of convalescent specimens. For illnesses occurring during the Christmas holiday, subjects obtained an oral temperature and completed a symptom and medication diary for 7 days; a physician reviewed the diary and blood was obtained on return to College Station about 21 days after the illness onset. Surveillance for influenza was terminated after 5.5 months; all subjects were asked to return for specimen collection and to provide a medical and ARI history.

Illnesses

The criteria for illness enrollment are those categorizing an illness as moderate or severe (Table 1). A study physician obtained an oral temperature, completed a symptom survey, and performed a respiratory system examination at each illness visit. Each symptom or physical finding was graded as mild, moderate, or severe using a 1–3 scale.

A retrospective survey for subjects with moderate to severe ARI who had not reported to the study site was conducted at the final visit. Based on symptom complexes, a reported ARI was classified by a study physician as probable influenza, possible influenza, or not influenza before testing of fall and spring sera for evidence of pH1N1 infection.

Laboratory Assays

Serology

Serum specimens obtained at enrollment, acute and convalescent visits for illnesses, and the terminal visit were tested simultaneously using hemagglutination-inhibition (HAI) antibody tests following previously described methods [11, 12]. Virus antigens were a locally obtained pH1N1 virus (A/Baylor/09) and the most recently prevalent seasonal A/H1N1 virus (A/Brisbane/59/07).

Virus Infections

A combined 8-mL nasal wash and throat swab specimen was collected at each illness visit. Specimens were tested for all respiratory viruses in tissue cultures; influenza-positive specimens were titered in 96-well plates for quantity of virus. For quantitation, plates were incubated for 5 days and endpoints were determined by hemagglutination. All specimens were also tested by reverse-transcriptase polymerase chain reaction (RT-PCR) for respiratory viruses including influenza A, pH1N1 influenza, influenza B, picornavirus/rhinovirus, respiratory syncytial virus, human metapneumovirus, parainfluenza viruses, coronaviruses, and adenoviruses, as described elsewhere [13–19].
Statistical Methods
RXC contingency and χ² for trend tests were used for frequency comparisons and the Mann–Whitney U test and Kruskal–Wallis test for comparisons of means.

RESULTS

Study Population
When the study began, Texas A&M University had 48,702 students and College Station had a population of approximately 95,000. During September 2009, 615 healthy adults were enrolled in our study; 578 (95%) were between the ages of 18 and 30; 362 (59%) were male and 253 (41%) were female. Three hundred ninety-four (64%) were white, 158 (26%) Asian, 69 (11%) Hispanic, 14 (2%) black, and the remainder multiracial or not reported. Spring 2010 follow-up information and specimens were obtained from 579 (94%) subjects. Sixty-six subjects had obtained vaccination with the 2009 pH1N1 vaccine and 50 of the 66 (76%) had developed a significant antibody response between fall and spring. None of the 66 reported ill with a pH1N1 infection. Thirty-eight subjects had received only seasonal inactivated vaccine and 3 (7.9%) reported ill with a pH1N1 infection. The pH1N1 vaccinees were excluded; the final population for analysis was 513.

Epidemic Pattern
The number of persons reporting to the University Health Center with an ARI by week for the year after classes commenced is shown in Figure 1. All health center visits are coded; codes used for possible influenza were International Classification of Diseases, Ninth Revision codes that correlate with proven influenza [20]. Also shown is the number of specimens that tested positive for influenza A in rapid diagnostic tests at the clinic or in RT-PCR tests from subjects reporting an ARI. As shown, the pH1N1 epidemic was ongoing among students during the enrollment period (weeks 37–39); no other influenza virus was detected during surveillance. The epidemic peaked in a 4-week period in late September and early October (weeks 37–40); illnesses caused by pH1N1 virus continued at a low level throughout the remaining surveillance period.

Study Illnesses
Seventy-six subjects with 77 illnesses reported to the study site with a moderate or severe ARI during the surveillance period. Sixty-three (82%) of these illnesses yielded a virus; 24 were pH1N1 influenza virus, 22 rhinovirus, 7 both pH1N1 and rhinovirus, 1 coronavirus, 1 human respiratory syncytial virus (hRSV), 1 adenovirus, and 1 human metapneumovirus (hMPV). Table 1 compares the illnesses associated with influenza virus and rhinovirus infections.

Table 1. Comparison of Illnesses Associated With Influenza Virus and Rhinovirus Infections

<table>
<thead>
<tr>
<th>Variable</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 5</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>I and R</td>
<td>R</td>
<td>I</td>
</tr>
<tr>
<td>No. w/ fever</td>
<td>20/24 (83%)</td>
<td>6/7 (86%)</td>
<td>6/23 (26%)</td>
<td>1</td>
</tr>
<tr>
<td>Mean score</td>
<td>1.46</td>
<td>1.72</td>
<td>1.26</td>
<td>0.42</td>
</tr>
<tr>
<td>Headache</td>
<td>1.79</td>
<td>1.86</td>
<td>1.04</td>
<td>1.29</td>
</tr>
<tr>
<td>Myalgias</td>
<td>2.04</td>
<td>2.00</td>
<td>2.00</td>
<td>1.29</td>
</tr>
<tr>
<td>Malaise</td>
<td>0.96</td>
<td>1.00</td>
<td>1.97</td>
<td>1.08</td>
</tr>
<tr>
<td>Rhinorrhea</td>
<td>1.13</td>
<td>1.29</td>
<td>1.65</td>
<td>1.21</td>
</tr>
<tr>
<td>Sore throat</td>
<td>1.83</td>
<td>1.86</td>
<td>1.43</td>
<td>1.63</td>
</tr>
<tr>
<td>Mean virus titer</td>
<td>40,000</td>
<td>...</td>
<td>80</td>
<td>...</td>
</tr>
</tbody>
</table>

Abbreviations: I, influenza; R, rhinovirus.

a Day of illness evaluation. Day 1 was <48 hours after onset.
b Symptoms and signs scored as 0 = absent, 1 = mild, 2 = moderate, 3 = severe. For headache, myalgias, malaise: 1 = present, 2 = impairs activity, 3 = prevents activity. For rhinorrhea: 1 = present, 2 = frequent nose blowing, 3 = continuous rhinorrhea. For sore throat: 1 = present, 2 = discomfort with erythema, 3 = severe throat discomfort, erythema, and edema. For cough: 1 = present, 2 = frequent day and night, 3 = nearly continuous with paroxysms.
c The median tissue culture infective dose per milliliter of influenza virus in nasal wash/throat swab specimens; 29 of 30 isolation-positive subjects.
rhinovirus, 1 rhinovirus and HKU1 coronavirus, 4 coronavirus (2 HKU1, 1 229E, 1 NL63), 2 enterovirus, 1 respiratory syncytial virus, and 2 herpes simplex virus, and 14 were negative. During the peak of pH1N1 infections, less than half of the illnesses yielded pH1N1. Mean quantity of infectious influenza virus in respiratory secretion samples of 29 subjects on visit days 1, 3, 5, and 7 is shown in Table 1. Mean virus titer at presentation was 40 000 (10^{4.6}) median tissue culture infective dose (TCID_{50})/mL of specimen. The mean titer was only 80 TCID_{50} 2 days later and <10 at the day 5 and day 7 visits. Eighteen of these subjects were given zanamivir as treatment (day 1 or day 2 of illness) and 11 were not (physician decision). Mean titers at presentation were higher for those treated (10^{5.3} vs 10^{4.0}), but the means were not significantly different (Mann–Whitney U test). Titers were low at subsequent visits and did not differ; similarly, the means between day 1 and day 3 for those treated and not treated did not differ.

A comparison of illnesses among subjects infected with pH1N1 influenza, a rhinovirus, or both is shown in Table 1. At presentation, fever (≥100°F) was more common among subjects with influenza (RXC contingency, P < .001). For mean symptom severity scores, myalgia scores were greater for influenza; rhinorrhea scores were greater for rhinovirus infections (P = .01 and <.01, respectively; Mann–Whitney U test). Myalgias and rhinorrhea were also different among the 3 groups (P = .02 and P = .01, respectively, Kruskal–Wallis test). Rhinorrhea persisted as a major symptom for rhinovirus-infected subjects for the 7 days of observation.

**Correlations With pH1N1 Antibody**

Occurrences of pH1N1 influenza infections and illnesses in relation to serum HAI antibody titer at enrollment are shown in Table 2. One hundred twenty-two (23.0%) subjects exhibited a significant antibody response to pH1N1 between enrollment (September 2009) and spring follow-up (March 2010). One hundred eighty-eight (37%) had serum antibody (≥1:8) to pH1N1 virus at enrollment. There was an inverse correlation between baseline serum antibody titer to pH1N1 virus and occurrence of pH1N1 infection during the surveillance period ($\chi^2$ for trend, P < .001). Thirty-one symptomatic influenza infections among enrolled subjects were detected, 30 by virus and serologic tests and 1 by serologic tests only. The apparent inverse correlation for infection and illness among those enrolled with ARI was not significant. Forty-five retrospectively identified ARIs were considered moderate to severe; pH1N1 infection was detected in 14 of 19 (74%) subjects with probable influenza, 4 of 9 (44%) subjects with possible influenza, and 2 of 17 (12%) subjects not designated to have clinical influenza (the clinical diagnosis for the 2 was a severe cold). When the retrospectively identified moderate to severe ARIs with a significant antibody response were included, the inverse correlation between baseline titer and frequency of pH1N1 infection and illness was significant ($\chi^2$ for trend, P = .01).

**Relation of Seasonal H1N1 to pH1N1**

Two studies of seasonal inactivated influenza vaccine indicating the antigenic relationship of seasonal H1N1 and pH1N1 viruses had been conducted in the population in the year preceding the 2009–2010 study, 1 among males only (September 2008) and the other (March 2009) in a mixed population similar to that of the present study. In the 2008 study, 60% had seasonal H1N1 antibody (≥1:8) and 71% (confidence interval [CI], 63%–79%) developed a response to the seasonal vaccine virus; 17% had pH1N1 antibody and 19% (CI, 13%–27%) developed a response to pH1N1 virus. For the spring 2009 study, 70% had seasonal antibody and 62% (CI, 54%–70%) developed antibody to seasonal virus; 24% had pH1N1 antibody and 17% (CI, 12%–24%) developed a response to pH1N1 virus. Forty-four of 48 antibody responses (92%) to pH1N1 were in subjects with prevaccination titers of <1:8.

The frequency of pH1N1 infections among participants in relation to their serum antibody titer to the recent seasonal H1N1 virus decreased with increasing baseline seasonal

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**Table 2. Frequency of 2009 H1N1 Infections and Illnesses in Relation to Baseline Serum Hemagglutination-Inhibiting Antibody Titer to Pandemic 2009 H1N1 Virus**

<table>
<thead>
<tr>
<th>Baseline 2009 pH1N1 Titer</th>
<th>No. Subjects</th>
<th>No. Infecteda</th>
<th>Prospectivec</th>
<th>Retrospectivea</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;8</td>
<td>325</td>
<td>99 (30.5%)</td>
<td>24</td>
<td>18</td>
<td>42 (12.9%)</td>
</tr>
<tr>
<td>8–16</td>
<td>100</td>
<td>18 (18.0%)</td>
<td>5</td>
<td>2</td>
<td>7 (7.0%)</td>
</tr>
<tr>
<td>24–48</td>
<td>88</td>
<td>3 (3.4%)</td>
<td>2</td>
<td>0</td>
<td>2 (2.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>513</td>
<td>122 (23.0%)</td>
<td>31</td>
<td>20</td>
<td>51 (9.9%)</td>
</tr>
</tbody>
</table>

a Illness = moderate to severe (≥2 on a 0–3 scale).
b As determined by serum antibody increases (≥4-fold increase).
c Prospective diagnosis; 30 virus positive, 31 with antibody rise.
d Retrospective diagnosis; moderate to severe acute respiratory illness with antibody increase.
Table 3. Frequency of 2009 H1N1 Infections in Relation to Baseline Serum Antibody Titer to Seasonal H1N1 Virus

<table>
<thead>
<tr>
<th>Baseline Seasonal H1N1 Titer&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. Subjects</th>
<th>No. 2009 H1N1 Infections&lt;sup&gt;b&lt;/sup&gt;</th>
<th>% 2009 H1N1 Infections&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;8</td>
<td>145</td>
<td>48</td>
<td>33.1</td>
</tr>
<tr>
<td>8–16</td>
<td>121</td>
<td>23</td>
<td>19.0</td>
</tr>
<tr>
<td>24–48</td>
<td>164</td>
<td>32</td>
<td>22.6</td>
</tr>
<tr>
<td>&gt;64</td>
<td>81</td>
<td>13</td>
<td>16.0</td>
</tr>
</tbody>
</table>

Preexposure seasonal virus antibody absent (<8) = 33.1% infected with 2009 H1N1; preexposure seasonal virus antibody present (>8) = 18.6% infected with 2009 H1N1, 44% decrease.

<sup>a</sup> Seasonal virus = A/Brisbane/59 (H1N1) virus.

<sup>b</sup> As determined by serum hemagglutination-inhibiting antibody responses to 2009 H1N1 virus.

antibody titer (Table 3; χ² for trend, P = .02). A 44% reduction in occurrence of infection with pH1N1 influenza virus was noted among those with seasonal antibody at baseline. A reduction in frequencies of subjects with moderate to severe illness among those infected was not statistically significant (data not shown).

DISCUSSION

This prospective study of 2009 pandemic influenza A infections and illnesses in a university community detected an infection frequency of 23%, with a moderate to severe ARI frequency of 9.6%. The epidemic peaked in late September–early October 2009, but infections occurred for months thereafter. Most participants were 18–30 years of age, and yet 37% had serum antibody to the pH1N1 virus at enrollment. A similar population at the same site 6 months earlier, preceding the spread of pH1N1 in the United States, exhibited an antibody frequency of 24%, suggesting that about 13% of the population was infected with the pH1N1 virus during the interval. There had been reports of outbreaks of pH1N1 influenza during that period in the United States and in Texas. Serum antibody responses to pH1N1 virus among young adults in the same population who were given seasonal inactivated vaccine containing A/Brisbane/59/07 (H1N1) virus before the appearance of pH1N1 suggests that seasonal vaccine might contribute cross-reacting antibody and priming to pH1N1 virus. However, only 10% of the study population reported prior seasonal vaccination. Consequently, prior A/H1N1 infections in combination with some pH1N1 infections preceding enrollment and not vaccinations will have induced the high frequency of preexisting pH1N1 antibody at enrollment.

Although rapid worldwide spread suggested high transmissibility, the pH1N1 viruses failed to become dominant as the cause of ARI in our population during the period of maximal occurrence. Rhinoviruses, known to be common causes of ARI in college populations in early fall, were as prominent as pH1N1 viruses as a cause of illness, and some subjects were infected with both simultaneously [21, 22]. Comparison of influenza and rhinovirus illnesses indicated that presentation with fever and prominent myalgias increased the likelihood that influenza virus infection induced the illness, while prominence of rhinorrhea increased the likelihood of a rhinovirus infection. However, it was not possible to make a designation of etiology based on clinical findings only.

Resistance to 2009 H1N1 influenza infection and illness in our study conformed to the well-documented inverse correlation with increasing preexposure serum anti-HA antibody. The significance of an antigenic relationship between H1N1 viruses was shown in a similar inverse correlation with the titer of antibody to the most recently preceding seasonal A/H1N1 virus. Mean titers in our HAI tests for those >25 years of age in 1977 and those 18–40 years in spring 2009 were both 1:6; titers >1:40 were detected in 20% of persons in our 1977 tests and >1:32 in 8% in 2009 tests [23]. Despite the relatively low antibody titers in 1977, substantial immunity to A/H1N1 illness was seen in adults. The present study also found evidence of a substantial degree of immunity despite the fact that about two-thirds of the population lacked detectable serum HAI antibody to the pH1N1 virus [9]. Presence of heterologous immunity in the absence of detectable serum HAI antibody to the epidemic virus has been shown previously [24]. Additional evidence of preexisting immunity to pH1N1 virus in our study was indicated by the relatively low illness-to-infection ratio, the short duration of fever, the rapid disappearance of virus in respiratory secretions, and the antibody responses to a single dose of the 2009 H1N1 vaccine. The fact that immunity to pH1N1 is conveyed by prior seasonal A/H1N1 infection has been reported for animal model infections and suggested for humans [25–27].

Three new and distinct introductions of influenza A/H1N1 viruses that caused widespread influenza occurred in 1918, 1977, and 2009 [1, 2, 8, 28]. In each instance, the viruses displayed a high capability for transmissibility and infectivity. The new strain of influenza A/H1N1, identified in the Soviet Union in 1977 (A/USSR/77 [H1N1]), proceeded to spread worldwide, with high infection rates among susceptibles that were comparable to those for the A/H2N2 and A/H3N2 pandemics of 1957 and 1968, respectively [29, 30]. Since then, antigenic variants of the 1977 H1N1 virus have continued as causes of human infections and illnesses. In the spring of 2009, pH1N1 virus emerged and spread rapidly worldwide. Thus, the A/H1N1 influenza viruses that emerged in 1918, 1977, and 2009 demonstrated a high capacity for transmissibility among humans. The 1918 H1N1 viruses caused high frequencies of severe influenza, a pattern also seen for the A/H2N2 and A/H3N2 virus pandemics [28–32]. However, this was not clear for the
1977 A/H1N1 viruses; although high ratios of symptomatic to asymptomatic infections were reported in some outbreaks, other reports described low illness rates among susceptible groups [9, 29, 33, 34]. In 1977, antibody prevalence, vaccine responses, and subsequent surveillance data indicated a high level of immunity among those >25 years of age despite relatively low levels of antibody; they were likely exposed to A/H1N1 viruses before the viruses disappeared in 1957 [9, 35, 36]. The 2009 A/H1N1 infections caused concern for a pandemic with high frequencies of severe disease that would cause high hospitalization and death rates. This concept did not adequately consider the high level of preexisting experience with A/H1N1 viruses in human populations. Based on prior experience with A/H1N1 viruses in 1976–1977, a considerable degree of immunity was expected for pH1N1 viruses among those >55 years of age and against the swine HA among those >85 years [35, 36]. There was uncertainty as to which age groups would be fully susceptible to the 2009 virus, with accompanying high illness rates, because circulation of A/H1N1 viruses had occurred over the 32 years since they were reintroduced in 1977.

This study has indicated that a high level of immunity existed among adults 18 years and older despite relatively low levels of antibody. The 2009 pandemic influenza experience indicated that the full susceptibility with high illness rates among healthy persons was only in children [37, 38]. Overall, the human experience with influenza A/H1N1 viruses over the 93-year interval since 1918 has considerably increased our knowledge of influenza and influenza immunity.

Notes

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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