Antibody Correlates and Predictors of Immunity to Naturally Occurring Influenza in Humans and the Importance of Antibody to the Neuraminidase

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Background. Serum antibody to the hemagglutinin (HA) of influenza viruses is a correlate and predictor of immunity to influenza in humans; the relative values of other correlates are uncertain.

Methods. Serum and nasal secretions (NS) were collected in fall and spring of 2009–2011 from healthy adults who were monitored for acute respiratory illness (ARI). Serum samples were tested for hemagglutination-inhibition (HAI) antibody increase and secretions for virus if ill; enrollment sera were also tested for neuraminidase-inhibiting (NI) antibody and NS for neutralizing (neut), NI, immunoglobulin A (IgA), and immunoglobulin G (IgG) anti-HA antibody.

Results. Serum anti-HA and anti-neuraminidase (NA) antibody titers to 2009(H1N1) pandemic influenza virus (pH1N1) correlated with titers in NS (including IgA and IgG antibody). Increasing anti-HA and anti-NA titers in serum and NS tests all correlated with reducing infection and infection-associated illness. Multivariate analyses indicated serum HAI and NI each independently predicted immunity to infection and infection-associated illness. Only serum NI independently predicted reduced illness among infected subjects.

Conclusions. Increasing anti-HA and NA antibody in serum and secretions correlated with reducing pH1N1 influenza virus infection and illness in healthy young adults. Both anti-HA and anti-NA antibody are independent predictors of immunity to influenza; ensuring induction of both by vaccination is desirable.

Keywords. influenza; hemagglutinin; neuraminidase; antibody; immunity.

Considerable information is available on immune responses and correlates of immunity to influenza virus infections in animal models, and much is available for human influenza. However, significant deficiencies remain in defining the role of the correlates of immunity to epidemic and pandemic influenza in humans. Studies of influenza in mice have established serum antibodies to the virion surface glycoproteins hemagglutinin (anti-HA) and neuraminidase (anti-NA) and various cell-mediated immune (CMI) mechanisms as correlates of immunity to influenza. These animal model studies have indicated the primary role for anti-HA antibody is prevention of infection; for anti-NA antibody, anti-M2 antibody, and CMI mechanisms, it is in reducing the intensity and duration of infection that reduces the severity and duration of disease [1–6]. Additionally, a role for anti-HA antibody in respiratory secretions was demonstrated in mice decades ago [7].

Anti-HA antibody in serum is established as both a correlate and predictor of immunity to influenza virus...
infection and infection-associated illness in humans; the correlation between increasing serum anti-HA antibody titer and reducing occurrence of influenza has been repeatedly demonstrated [8]. Moreover, available data indicate this antibody is not just a correlate but is a major mediator of prevention of infection [8, 9]. The absence of this antibody to new emerging subtypes of type A influenza is considered to be the primary reason they cause pandemic influenza. Serum anti-NA was demonstrated as a correlate of immunity to influenza in humans years ago, and anti-HA antibody in nasal secretions has correlated with protection against influenza in intranasally challenged volunteers [10, 11]. Studies of the respiratory tract of humans have shown that IgA antibody produced locally is the dominant immunoglobulin in nasal secretions, whereas IgG derived from serum predominates in lower respiratory secretions [11].

Although some of these modalities for immunity to influenza in humans have been shown to convey immunity using vaccines and human challenge systems, their relative significance in naturally occurring influenza in humans has not been elucidated. The emergence of a new influenza A (H1N1) virus that spread worldwide and was designated as a pandemic virus (pH1N1) provided an opportunity to determine the relative importance of various immune mechanisms for conveying protection against naturally occurring influenza infection and illness in humans [12]. Findings in this report indicate that anti-NA antibody is an independent predictor of immunity to naturally occurring influenza in the presence of anti-HA antibody.

**METHODS**

**Study Design**

The study was conducted at Texas A&M University, College Station, Texas, during 2009–2011. Healthy university students, staff, and community residents between 18 and 49 years of age were invited to enroll during September of 2009 and 2010 to be followed for acute respiratory illness (ARI) through the subsequent influenza season. The protocol and informed consent were approved by the Baylor College of Medicine and Texas A&M University institutional review boards, and all subjects provided written informed consent. The clinical methods have been described for the 2009–2010 study year; methods were similar for the 2010–2011 year [13]. In brief, a medical history was taken to ensure good health, blood and nasal wash specimens were obtained, and subjects were given thermometers and guidelines to record illness and call the study site to be seen within 48 hours of ARI onset. To identify the exposure period, surveillance of ARI was conducted at the University health center. Because of early onset of epidemic influenza, surveillance of study subjects was conducted continuously during 2009–2010 from enrollment to termination in April 2010 by a coordinator and physician except for 4 days of the Thanksgiving holiday and 4 weeks of the Christmas holiday. Similar methods were applied for the 2010–2011 influenza season except that surveillance of subjects was conducted mid-January to mid-April 2011 because surveillance at the health center did not detect influenza virus infections during the fall of 2010.

**Antibody Titers**

Serum specimens obtained each year at enrollment, acute and convalescent visits for illness, and a terminal spring visit were tested simultaneously using hemagglutination-inhibition (HAI) antibody tests as described, except concentrations of reagents were adjusted to permit a starting dilution of 1:4 [14, 15]. Nasal secretion (NS) specimens were supernates from nasal washes with 8 mL of Ringer solution that had been homogenized and centrifuged. NS specimens collected at enrollment each year were tested in neutralization (neut) tests as described except fresh serum was not added; the neut test is a 5-day procedure with a wash and media replacement step 16 hours after transfer of antigen and antibody dilutions so as to provide specificity for anti-HA antibody only [15, 16]. The antigen for the HAI and neut assays was a locally obtained pandemic A (H1N1) virus (A/Baylor/09). Serum and NS specimens collected at enrollment were also tested for neuraminidase-inhibition (NI) antibody using virus-like-particles (VLPs) as antigen that contained only the neuraminidase (NA) protein of pH1N1 virus and were prepared as described elsewhere [17, 18]. The NI titer was the dilution providing 50% inhibition of enzyme activity in a lectin-based procedure described earlier by us and similar to the Lambré procedure but using VLPs as the NA antigen source [19, 20]. Assays for IgA and IgG anti-HA antibody were performed on NS specimens collected at enrollment in enzyme-linked immunosorbent assays (ELISA) using recombinant A/Calif/04/09 (H1N1) hemagglutinin (HA) obtained from BEI Resources as described elsewhere [15].

**Virus Detection**

A combined 8 mL nasal wash and throat swab specimen collected from ill subjects at the initial and day 2 visit was tested in tissue cultures and reverse-transcriptase polymerase chain reaction (RT-PCR) tests for influenza A and pH1N1 2009–2010 and for influenza A, (pH1N1), (H3N2) and B in RT-PCR tests only in 2010–2011 as described elsewhere [13, 21].

**Infection and Illness**

Influenza virus infection was defined as a significant serum antibody increase between enrollment (fall, 2009 or 2010) and spring the following year or either culture or RT-PCR positive specimen from ill subjects; only ill subjects were tested for virus and all culture/RT-PCR positive subjects had an antibody increase. Illness was defined as an influenza virus infection-associated acute respiratory illness with fever (≥100°F) or
causing the subject to miss class or activities for at least 1 day. All illnesses occurring during active surveillance were seen and characterized by a single study physician (R. B. C.). Illnesses during holiday periods were recorded in a diary and reviewed and characterized by the study physician (R. B. C.) about 21 days after onset. All illnesses used in the analysis were associated with influenza virus infection. All 88 ill subjects in the analysis had a significant serum HAI antibody increase to influenza virus, and all but one of those seen in the clinic were RT-PCR positive. Sixty-five other illnesses were negative for influenza virus infection; 43 were caused by another virus, and 22 were virus-negative. Immunity was defined as absence of influenza virus infection or influenza virus infection-associated illness.

Statistics
Sample size estimates were based on an anticipated influenza illness attack rate of 15%–40%; about 150 infections and 100 influenza illnesses were expected and would permit analysis of immune correlates. Because the H1N1 pandemic was mild in the population, the study was repeated in 2010 to obtain a larger number of influenza cases and to allow for influenza vaccinations.

Correlation analyses used Spearman rank correlation tests; median titer comparisons used Mann Whitney U tests; protective effects were evaluated using $\chi^2$ for trend and univariate and multivariate logistic regression tests. Significance $P$ values were adjusted for multiple comparisons using the Holm-Bonferroni method [22].

RESULTS

Study Population
The characteristics of the study populations in 2009–2010, 2010–2011, and the 2 years combined are shown in Table 1. Six hundred fifteen subjects were enrolled in 2009, and 995 in 2010 for a total of 1610 subjects. Fifty-seven percent were male, 43% were female; 67% were white, 25% Asian, and 8% black or biracial. Complete data for analyses were available on 93.7% of subjects for the 2 years. To focus immunity considerations on naturally acquired immunity, subjects who were vaccinated with either seasonal or pH1N1 vaccine in 2009 or seasonal (which contained pH1N1 virus) vaccine in 2010 were removed from the analyses. Antibody to A/Brisbane/59/07 (H1N1) (in 2009 seasonal vaccine) had been shown to protect against pH1N1 infection [13]. Subjects who gave a history of having seasonal vaccine within the previous 2 years (23% for 2009–2010) were compared to those not having received vaccine. The pH1N1 HAI titer distribution and influenza infection percentages in each antibody group were generally identical supporting the preexisting pH1N1 antibody as having been acquired by prior natural A/H1N1 infection (data not shown). Testing of sera obtained from the same population in studies in the fall 2008 and spring 2009 before appearance of pH1N1 indicated about 20% had cross-reacting antibody ($\geq 1:8$) to pH1N1. A comparison of these data to enrollment antibody data suggested 10%–15% of the population had been infected with pH1N1 during the summer of 2009 when the virus was known to have been circulating in Texas [13]. Final analyses were conducted on 1109 subjects (68.9%) of the enrolled population.

Influenza viruses detected in the study population and college clinic during the 2009–2010 year were all pH1N1 virus, and all A (H1N1) viruses in 2010–2011 were pH1N1. Influenza A (H3N2) and influenza B virus infections were also detected in 2010–2011; however, because of the small number, the analysis is confined to pH1N1 virus infections and illnesses. As shown, 24.3% of the study population were infected with pH1N1 influenza virus in 2009–2010 and 17.5% in 2010–2011 for a total of 226 of 1109 (20.4%) subjects; 88 (7.9%) influenza virus infection-associated illnesses were identified.

Antibody Correlations
Antibody titer correlations were evaluated on enrollment serum and NS for anti-HA and NA antibody. HAI and NI titers in serum correlated with neut and NI titers in NS, respectively. Additionally, titers of the 2 types of antibody in serum and in NS correlated with each other, and the titer of anti-HA antibody in serum correlated with the titer of NA antibody in NS as did the titer of NA antibody in serum and

Table 1. Naturally Occurring Influenza in the Study Population 2009–2010 and 2010–2011

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Enrolled</td>
<td>615</td>
<td>995</td>
<td>1610</td>
</tr>
<tr>
<td>Complete data</td>
<td>579 (94.1)</td>
<td>930 (93.5)</td>
<td>1509 (93.7)</td>
</tr>
<tr>
<td>Vaccinated$^a$</td>
<td>106 (17.2)</td>
<td>294 (29.5)</td>
<td>400 (24.8)</td>
</tr>
<tr>
<td>Analyzed (no vaccination)</td>
<td>473 (76.9)</td>
<td>636 (63.9)</td>
<td>1109 (68.9)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Infection</th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A (H1N1)$^b$</td>
<td>115/473 (24.3)</td>
<td>111/636 (17.5)</td>
<td>226/1109 (20.4)</td>
</tr>
<tr>
<td>A (H3N2)$^c$</td>
<td>0/23636 (3.6)</td>
<td>(… )</td>
<td>(… )</td>
</tr>
<tr>
<td>B$^c$</td>
<td>0/28636 (4.1)</td>
<td>(… )</td>
<td>(… )</td>
</tr>
</tbody>
</table>

Data are no. (%) unless otherwise indicated.

$^a$ Seasonal or pandemic 2009 (H1N1) vaccine.

$^b$ A (H1N1) = Pandemic 2009 H1N1 virus.

$^c$ A (H3N2) and B viruses not further defined. Epidemic viruses in Texas were A (H3N2) = A/Perth/09-like viruses and B=B/Brisbane/08-like viruses.
HA antibody (neut) in NS. Although some $r$ values were low, the correlations were significant for each study year and for the 2 years combined (Spearman Rank Test; $P < .001$ for each [see Supplementary Table 1 for $r$ values and titer ranges]). Correlations for ELISA titers of IgA and IgG antibody to the HA in NS were available for the 2009–2010 year. The serum HAI titers correlated with both ng and percent specific IgA and IgG antibody to HA in NS; NS neut titers also correlated with IgA and IgG antibody and IgA and IgG antibody correlated with each other ($P < .001$ for each; Supplementary Table 1). Total IgA and IgG in NS did not correlate with any of the virus-specific antibody measurements.

**Antibody Titer Relation to Infection and Illness**

**Median Titer**
The median antibody titer for enrollment serum HAI and NI, NS neut and NI, and NS IgA and IgG antibody were compared for those infected with pH1N1 to those not infected and those infected and ill to those not ill for each study year and the 2 years combined. The median values year 2 were higher than those of year 1, but the combined year comparisons were unaffected when a year effect was considered. In each comparison, the median titer for those infected and those infected and ill was significantly lower than for those not infected or not ill (Mann Whitney $U$, 2009–2010, all $P \leq .01$ except for NS neut for infection vs not infected [$P = .026$]; 2010–2011 and 2009–2011, all $P < .001$) (data not shown).

**Frequencies of Infection and Illness**
Infections and infection-associated illness frequencies were lower as preexposure serum and NS anti-HA and anti-NA antibody titers increased for the 2009–2010, 2010–2011 years, and the 2009–2011 combined years (Table 2). Similarly, reductions were seen for IgA and IgG antibody in NS for 2009–2010 for infection and illness, but only IgG for infection-associated illness was significant after correcting for multiple comparisons ($P = .004$; Table 3).

Table 2. Frequency of Influenza A/2009 (H1N1) Infections and Illnesses in Relation to Serum and Nasal Secretion Anti-hemagglutinin and Anti-neuraminidase Antibody in the Analyzed Population at Enrollment

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Year</th>
<th>Anti-HA Titera</th>
<th>No.</th>
<th>No. (%) Infected and Ill</th>
<th>Anti-NA Titerb</th>
<th>No.</th>
<th>No. (%) Infected and Ill</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>2009–2010</td>
<td>≤2</td>
<td>177</td>
<td>54 (30.5)</td>
<td>&lt;6.5</td>
<td>147</td>
<td>45 (30.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.5–3</td>
<td>147</td>
<td>48 (32.7)</td>
<td>20 (13.6)</td>
<td>167</td>
<td>45 (26.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥3</td>
<td>149</td>
<td>13 (8.7)</td>
<td>3 (2.0)</td>
<td>159</td>
<td>25 (15.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\chi^2$ (P Value)</td>
<td></td>
<td></td>
<td>19.6 &lt;0.001</td>
<td>12.4 &lt;0.002</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2010–2011</td>
<td>≤2</td>
<td>122</td>
<td>49 (40.2)</td>
<td>&lt;6.5</td>
<td>164</td>
<td>46 (28.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.5–3</td>
<td>139</td>
<td>35 (25.2)</td>
<td>10 (7.2)</td>
<td>100</td>
<td>25 (25.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥3</td>
<td>366</td>
<td>18 (4.9)</td>
<td>4 (1.1)</td>
<td>372</td>
<td>40 (10.8)</td>
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<tr>
<td></td>
<td></td>
<td>$\chi^2$ (P Value)</td>
<td></td>
<td></td>
<td>93.3 &lt;0.001</td>
<td>60.1 &lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2009–2011 Combined</td>
<td>≤2</td>
<td>299</td>
<td>103 (34.4)</td>
<td>&lt;6.5</td>
<td>311</td>
<td>91 (29.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.5–3</td>
<td>286</td>
<td>83 (29.0)</td>
<td>30 (10.5)</td>
<td>267</td>
<td>70 (26.2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥3</td>
<td>515</td>
<td>31 (6.0)</td>
<td>7 (1.4)</td>
<td>531</td>
<td>65 (12.2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\chi^2$ (P Value)</td>
<td></td>
<td></td>
<td>107.5 &lt;0.001</td>
<td>66.1 &lt;0.001</td>
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<tr>
<td></td>
<td>2010–2011</td>
<td>≤1</td>
<td>311</td>
<td>82 (26.4)</td>
<td>&lt;1</td>
<td>311</td>
<td>82 (26.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1–1.5</td>
<td>103</td>
<td>22 (21.4)</td>
<td>4 (3.9)</td>
<td>68</td>
<td>24 (35.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥2</td>
<td>55</td>
<td>7 (12.7)</td>
<td>2 (3.6)</td>
<td>87</td>
<td>6 (6.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\chi^2$ (P Value)</td>
<td></td>
<td></td>
<td>5.1 0.079</td>
<td>7.5 0.023</td>
<td></td>
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<tr>
<td></td>
<td>2010–2011</td>
<td>≤1</td>
<td>333</td>
<td>85 (25.5)</td>
<td>&lt;1</td>
<td>332</td>
<td>82 (24.7)</td>
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<tr>
<td></td>
<td></td>
<td>1–1.5</td>
<td>165</td>
<td>21 (12.7)</td>
<td>3 (1.8)</td>
<td>111</td>
<td>12 (10.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥2</td>
<td>134</td>
<td>5 (3.7)</td>
<td>2 (1.5)</td>
<td>187</td>
<td>17 (9.1)</td>
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<tr>
<td></td>
<td></td>
<td>$\chi^2$ (P Value)</td>
<td></td>
<td></td>
<td>34.7 &lt;0.001</td>
<td>17.4 &lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2009–2011 Combined</td>
<td>≤1</td>
<td>644</td>
<td>167 (25.9)</td>
<td>&lt;1</td>
<td>640</td>
<td>163 (25.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1–1.5</td>
<td>268</td>
<td>43 (16.0)</td>
<td>7 (2.6)</td>
<td>182</td>
<td>37 (20.3)</td>
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<tr>
<td></td>
<td></td>
<td>≥2</td>
<td>189</td>
<td>12 (6.3)</td>
<td>4 (2.1)</td>
<td>274</td>
<td>23 (8.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\chi^2$ (P Value)</td>
<td></td>
<td></td>
<td>38.5 &lt;0.001</td>
<td>25.9 &lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

a Serum anti HA-hemagglutination-inhibiting antibody (HAI) log2; nasal secretions anti HA-neutralizing antibody, log2.

b Anti-NA in serum and NS = neuraminidase-inhibiting antibody (NI), log2.

c $\chi^2$ for trend; bold = significant after correction for multiple comparisons.

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Logistic Regression Analyses

Results of univariate and multivariate analyses of anti-HA and anti-NA antibody in serum and NS as predictors of immunity to infection and infection-associated illness are shown in Table 4. Univariate analyses indicated both HAI and NI in serum and NI in NS were significant predictors of immunity each year and for the 2009–2011 combined years. This was also true for IgA and IgG antibody for infection and infection-associated illness (2009–2010; \( P < .01 \) for each; data not shown). Multivariate analyses indicated that serum HAI was a significant independent predictor of immunity to infection for each study year and the 2009–2011 combined years as well as for infection-associated illness for 2010–2011 and the combined years. Serum NI was an independent predictor of immunity to infection for the combined years and for infection-associated illness for the 2010–2011 and the 2009–2011 combined years. Multivariate analyses did not show NS neut or NI antibody as...
predicators of immunity to either infection or infection-associated illness for 2009–2010, 2010–2011, or the combined 2009–2011 years. Serum NI antibody was a significant predictor for a lower illness frequency among infected subjects when both study years were combined (2009–2011, \( P = .005 \)). Median prevalece antibody titers were significantly higher year 2, but considering year as an interaction variable did not affect predictors in the multivariate analyses. Neither IgA nor IgG anti-HA antibody in NS was an independent predictor of immunity for 2009–2010 (data not shown).

**DISCUSSION**

The present study focused on antibodies as correlates and predictors of immunity to naturally occurring influenza virus infections and infection-associated illnesses that were acquired by healthy young adults from prior influenza virus infections. Knowing that the antibodies were acquired from prior natural infections was made possible by (1) removing from analysis all subjects who received vaccines during the study periods, (2) having data from prior studies in the same population that showed presence of cross-reacting antibody to pH1N1 in approximately 20% of the population before appearance of pH1N1, and (3) finding an identical titer distribution and infection rate in 23% for 2009–2010 with a history of vaccinations in the past and in 77% with no history of prior vaccinations.

A major finding of the study is that, regardless of test method, antibody to the pH1N1 influenza virus HA and the NA in serum and in nasal secretions all significantly correlate with immunity to naturally occurring pH1N1 influenza virus infection and illness in humans. It is reasonable for all the antibody evaluations to exhibit this finding since they were shown to correlate significantly with each other regardless of antibody specificity or location. These findings also seem reasonable since influenza is an infection of the respiratory tract passages to which healthy individuals respond with immune responses to the virus HA and NA antigens in both respiratory secretions and serum [8, 11, 13, 23–25]. So correlations of the various antibody titers with each other and of the titers of each to resistance to influenza infection and infection-associated illness is to be expected.

Analyses of data for each epidemic year are shown separately, but each year constituted a new study in the population and its antibody distributions at enrollment. Although all antibodies measured were not clearly significant for reducing infection and infection-associated illness each year, the patterns of reductions were similar; moreover, controlling for a year effect did not affect significance testing results, supporting combining the 2 years for analysis. The combined 2009–2011 years with the increased sample size indicated significance for all measured antibodies in the \( \chi^2 \) for trend and univariate analyses. Thus, anti-HA and anti-NA antibody in serum and secretions are each a correlate of immunity to influenza and influenza-associated illness in humans.

To assess for independent contributions of the antibodies and locations to immunity to influenza, multivariate analyses were performed. Those analyses confirmed serum antibody to the HA protein as a correlate of immunity and indicated it is an independent predictor of immunity to influenza virus infection, a finding consistent with available evidence that serum anti-HA antibody is actually a major mediator of immunity to influenza virus infection in humans [8, 9, 11]. Anti-HA antibody titers (HAI) in serum correlated with those in NS (neut), a finding reported previously, but antibody to the HA in NS was not an independent predictor of immunity to infection and illness [11, 26]. Antibody in serum will be primarily IgG while that in nasal secretions could be primarily IgA developed in local mucosal tissues although IgG that is primarily systemic in origin will be present [11]. To clarify, we performed ELISA assays for IgA and IgG antibody to the HA of pH1N1 virus on NS specimens for the 2009–2010 year. Both IgA and IgG anti-HA antibody in NS correlated with immunity to infection and illness but neither independently predicted immunity that year. To our knowledge, however, the separate correlation of NS IgA and IgG antibodies to the HA of influenza virus with immunity to naturally occurring influenza has not been reported previously. While some of the anti-HA immune modalities were not shown to independently predict immunity to influenza, it is likely that they contribute to immunity and that serum HAI titer is a surrogate for their contribution to immunity.

The finding in the present study for a significance of NI antibody in naturally occurring influenza has not to our knowledge been reported previously. NI antibody titers in serum and in NS correlated with immunity to both influenza infection and infection-associated illness; moreover, in multivariate analyses, serum NI was shown to be a predictor of immunity to both infection and to infection-associated illness that is independent of serum HAI antibody. Additionally, the titer of serum NI antibody was the only clearly independent predictor of immunity to illness among those who were infected. This is of interest as NI antibody has long been proposed as conveying immunity to illness among infected persons by reducing the magnitude of the infection [10].

The present study was limited to the study of influenza A (H1N1) infections causing the annual epidemic the 2 years of study. However, the findings should apply to other influenza A virus infections and possibly influenza B infections in humans. Serum anti-HA antibody is established as a correlate and predictor of immunity to different naturally occurring influenza A (H1N1), A (H2N2), A (H3N2), and B virus infections [11, 24]. Serum anti-NA antibody has been shown to predict immunity to infection and illness from an influenza A (H3N2) virus in challenged volunteers [27]. Anti-HA and anti-NA antibody in NS have also been shown to correlate
with immunity to influenza infection or illness in persons challenged intranasally with influenza A and B viruses [11, 28]. The present study extends these findings to naturally-occurring influenza and adds a new value for NI antibody as an independent predictor of immunity when HAI antibody is present and also has predicted immunity. This is of interest because the NA protein generally exhibits a slower rate of antigenic variation than the HA so that immunity induced could be of longer duration [29]. It is also of interest because the N1 of A/California/09 virus has been shown to induce immunity to influenza A(H5N1) in animals [30]. Ensuring the induction of NI antibody should increase the value of influenza vaccines.

Limitations of the present study are restriction to one age group, one virus, and one location. However, the findings were similar for each year of study despite 2 different populations in 2 different study years with minimal subject overlap, and performance of antibody assays a year apart. Also, the 2 epidemic years each proved to be mild and infection-associated illnesses were less than the estimate of a need for 100 illnesses. Nevertheless, the findings on immunity to influenza infections and infection-associated illnesses were significant and likely to be true for other influenza viruses and other populations.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyrighted. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Note

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