Prior H1N1 Influenza Infection and Susceptibility of Cleveland Family Study Participants during the H2N2 Pandemic of 1957: An Experiment of Nature

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(See the editorial commentary by Kilbourne, on pages 7–8.)

During a pandemic, influenza vaccines that rely on neutralizing antibodies to protect against matched viruses might not be available early enough. Much broader (heterosubtypic) immune protection is seen in animals. Do humans also have cross-subtype immunity? To investigate this issue, archival records from the Cleveland Family Study, which was conducted before and during the 1957 pandemic (during which a shift from subtype H1N1 to H2N2 occurred), were analyzed. Only 5.6% of the adults who had had symptomatic influenza A in earlier study years developed influenza during the pandemic, despite living in households with participants who had influenza. In contrast, 55.2% of the children who had had symptomatic influenza A contracted it again. These findings suggest an impact of accumulated heterosubtypic immunity during a pandemic. Such immunity, as well as its implications for vaccination, should be further investigated.

Influenza vaccination is problematic, because the virus evolves rapidly and antigens differ between virus strains. In animals, exposure to influenza A virus of one subtype can induce immunity that is protective against challenge with other subtypes (termed “heterosubtypic immunity” or “cross-protection”). Vaccination with conserved antigens can also induce this kind of immunity, which does not completely prevent infection but limits viral replication and accelerates clearance. Heterosubtypic immunity reduces viral titers and prevents mortality in mice (reviewed in [1]) and chickens [2]; it also reduces titers of shed virus during nonlethal infection of ferrets [3] and pigs [4]. The protection conferred can be long lasting.

Can immunity to conserved antigens provide a basis for human vaccination? Human immunity to influenza A virus is often considered to be subtype specific [5], as would be expected for neutralizing antibody (hereafter, “influenza” will be used in this report to refer to influenza A, except for the mention of influenza B in table 1). There is some evidence of human heterosubtypic immunity [7], but it is considered to be weak and has not been investigated thoroughly. In a clinical study of live attenuated vaccines, prior infection did not interfere with infection by virus of a differing subtype [8]. Although this was interpreted as indicating a lack of cross-protection in humans, the participants were children <3 years old.

In animals, live influenza virus infection generally induces more potent immune cross-protection than do vaccines. Thus, evidence of such immunity in humans could potentially be found in the impact that infection with one subtype would have on subsequent susceptibility to infection with another subtype. Most informative would be a pandemic in which both hemagglutinin (HA) and neuraminidase shifted. This occurred in 1957, with a shift from H1N1 to H2N2. One Russian study, by Slepushkin, suggested that recent infection conferred partial protection during the 1957 pandemic [9], but the study was based on symptom reporting, not laboratory-confirmed influenza.

If laboratory confirmations of influenza in the same population before and during a pandemic were available, with data individually identified to link histories and outcomes, it would be possible to look for evidence of cross-protection. Such data were collected in the Cleveland Family Study, but they were not examined at the time for evidence of cross-protection (W. S. Jordan, personal communication); animal studies of heterosubtypic immunity had not yet been conducted (they began in the 1960s [10]). To evaluate the possible impact of heterosubtypic immunity during a pandemic, I conducted a study using archival records from the Cleveland Family Study.

Participants, materials, and methods. Individual archival case records were examined at the Stanley A. Ferguson Archives of University Hospitals of Cleveland (UHC), under a protocol approved by the Food and Drug Administration Research Involving Human Subjects Committee and the Institutional Review
The design of the Cleveland Family Study called for intensive active surveillance, with mothers keeping diaries of any symptoms for each family member and reporting new illnesses by telephone at onset. Relevant to influenza ascertainment, a physician examined anyone with respiratory symptoms and obtained pharyngeal specimens for virus isolation.

For the present analysis, entry and dropout dates were found and used to identify participating families who were monitored throughout the period from 1950 to 1957, to avoid false negatives. Of the 42 families meeting this criterion, only 2 were untouched by influenza in all years. (Three children in included families dropped out before 1957 and so were excluded.) Within this monitored population, participants with culture-proven influenza in 1950, 1951, or 1953 were further examined for this monitored population, participants with culture-proven influenza in those years (table 3 in bold).

NOTE. Data are no. (%) of participants and represent all participants in the indicated study years, not just the subset analyzed in table 3. Four case-report forms for 1956 were also found. These data would have been excluded from the analysis shown in table 3 in any case, because the participants entered the study too late to be monitored in all relevant years. Anecdotally, the 1 adult among them did not contract influenza in 1957, whereas 2 of the 3 children among them did; this is consistent with the other findings of the present analysis.

Table 1. Influenza virus infections, as measured by virus isolation.

<table>
<thead>
<tr>
<th>Study year</th>
<th>Adults Total</th>
<th>Adults Positive</th>
<th>Adults Negative</th>
<th>Children Total</th>
<th>Children Positive</th>
<th>Children Negative</th>
<th>P²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1950</td>
<td>114</td>
<td>5 (4.4)</td>
<td>109 (95.6)</td>
<td>143</td>
<td>11 (7.7)</td>
<td>132 (92.3)</td>
<td>.407</td>
</tr>
<tr>
<td>1951</td>
<td>108</td>
<td>14 (13.0)</td>
<td>94 (87.0)</td>
<td>137</td>
<td>16 (11.7)</td>
<td>121 (88.3)</td>
<td>.914</td>
</tr>
<tr>
<td>1953</td>
<td>136</td>
<td>7 (5.1)</td>
<td>129 (94.9)</td>
<td>189</td>
<td>15 (7.9)</td>
<td>174 (92.1)</td>
<td>.445</td>
</tr>
<tr>
<td>1957</td>
<td>119</td>
<td>22 (18.5)</td>
<td>97 (81.5)</td>
<td>189</td>
<td>104 (55.0)</td>
<td>85 (45.0)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%) of participants and represent all participants in the indicated study years, not just the subset analyzed in table 3. Four case-report forms for 1956 were also found. These data would have been excluded from the analysis shown in table 3 in any case, because the participants entered the study too late to be monitored in all relevant years. Anecdotally, the 1 adult among them did not contract influenza in 1957, whereas 2 of the 3 children among them did; this is consistent with the other findings of the present analysis.

Two influenza B viruses, 1 isolated from an individual specimen in 1950 and 1 in 1952, are mentioned in Jordan et al. [6]. (Note that influenza B is not otherwise discussed in the present article; “influenza” elsewhere refers to influenza A.)

By the x² test (no. of virus isolation–positive and –negative adults vs. no. of virus isolation–positive and –negative children).

Table 2. Completeness of individual case records.

<table>
<thead>
<tr>
<th>Study year</th>
<th>Participants from whom virus was isolated, no. [reference]</th>
<th>Case-report forms found in the archives, no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1950</td>
<td>18 [6]</td>
<td>16</td>
</tr>
</tbody>
</table>

NOTE. According to Jordan et al. [6, 11], 1958, 1961, 1953, and 1957 were the only influenza epidemic years during the study. They mention that influenza virus was isolated in 1949 but do not indicate how many isolations there were, and no case-report forms for 1949 were found. Data for 1950–1953 (shown in table 4 of Jordan et al. [6]) and for 1957 (shown in tables 5 and 6 of Jordan et al. [11]) indicate the no. of persons from whom influenza virus was isolated, not the no. of cases of influenza. One isolate for 1950 was from a throat swab from an asymptomatic person; thus, a case-report form would not be expected. The original report [11, p. 197] mentions that clinical data were recorded for 125 cases of Asian influenza.
face. Although many of the children who had had influenza in earlier study years were infected again in 1957 (16/29 [55.2%]), the adults with a history of influenza almost never were (1/18 [5.6%]). With respect to the apparent protective effect of prior infection, the adults differed dramatically from the children ($P = 0.002, \chi^2$ test).

Exposure was widespread during such an intense outbreak, but did the adults with apparent protection escape exposure simply by chance? Of these 17 adults, all lived with schoolchildren, and 12 lived in households with 1 or more participants with culture-proven influenza during the pandemic. Anecdotally, the sole adult with apparent failure of protection lived in a household in which both adults and all 4 children became infected.

Data were also compared for participants who did not have influenza in earlier study years (shown in the rows of table 3 not in boldface). The adults who had not been infected in earlier study years were ~3-fold more susceptible than those who had been infected (16.7% vs. 5.6%), which is in agreement with Slepushkin’s symptom reporting–based findings [9]. Among children, the 2 groups were similar (52.0% vs. 55.2%). The trend observed in adults is not statistically significant, but the total number of study adults with influenza in 1957 was low, which could in itself reflect immunity. It is likely that none of the study adults were truly naive to influenza; no data on either infections before 1950 or subclinical infections are available.

Some participants (both adults and children) were given a vaccine of unknown efficacy late during the pandemic, and, despite an extensive search, records indicating which participants received vaccine were not found. Analysis of the expected versus the observed number of cases, done at the time of the original study on the basis of person-days of exposure [11], showed that a reduction of only 7 cases could be attributed to vaccination. Even if it is assumed that all 7 cases occurred in adults in the group who were apparently protected and, therefore, are removed, the difference in the effect of prior infection between adults and children remains highly significant ($P = 0.012$, Fisher’s exact test). Note also that, among the total Cleveland Family Study population, 24% of vaccinated adults had influenza in 1957, whereas 18.5% of unvaccinated adults did [11]. Thus, vaccination cannot account for the findings of the present analysis.

Could the lower susceptibility of adults be attributed to pre-existing antibodies to H2N2? On the basis of serological testing of elderly adults, the virus that circulated in humans in 1889–1890 was suggested to have been H2N2 [12], but more-recent evidence points to H3N2 [13, 14]. Regardless, all of the parents in the Cleveland Family Study were too young for the 1889–1890 outbreak; the oldest adult was born in 1905 [15]. Also, participant serum samples collected before the pandemic were tested and were found to react with the 1957 H2N2 virus either marginally or, in almost all cases, not at all [11].

With regard to the possibility that influenza was transmitted to adults by family members, to search for clues, the sequence and timing of cases in 1957 was examined within each monitored family. Among 10 families, 11 cases occurred in adults after other cases had occurred in the family. For 10 of these cases, the dates of onset were 1–6 days after the onset of a prior case in the family; for 1 case, the onset interval was 9 days. Only 1 adult had the first case in a family.

In the present analysis, the children with prior influenza who contracted it again in 1957 and the children with prior influenza in earlier study years were

<table>
<thead>
<tr>
<th>Age group, prior-infection status</th>
<th>Influenza in 1957</th>
<th>No influenza in 1957</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No influenza in earlier study years</td>
<td>11 (16.7)</td>
<td>55 (83.3)</td>
</tr>
<tr>
<td>Influenza in earlier study years</td>
<td>1 (5.6)</td>
<td>17 (94.4)</td>
</tr>
<tr>
<td>Children</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No influenza in earlier study years</td>
<td>39 (52.0)</td>
<td>36 (48.0)</td>
</tr>
<tr>
<td>Influenza in earlier study years</td>
<td>16 (55.2)</td>
<td>13 (44.8)</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. (%) of participants. Data in the rows not in boldface show participants who were positive for virus isolation in a study year before 1957 (1950, 1951, or 1953); adults differed from children with respect to the effect of prior symptomatic influenza on outcome in 1957 ($P = 0.002, \chi^2$ test). Data in the rows not in boldface represent participants from whom virus was not isolated in any earlier study year. The adults who had not been infected in earlier study years were ~3-fold more susceptible than those who had been infected, but the difference is not significant by Fisher’s exact test. The ages of the children ranged from <1 to 11 years in 1950 (mean age, 4.6 years); the ages of adults ranged from 28 to 37 years in 1960 (mean age, 32 years). Only those participants who were monitored from 1950 to 1957 are included in the analysis. If participants who were born or entered the study after 1950, had influenza in 1951 or 1953, and then were monitored through 1957 are included, the difference is even more pronounced (influenza vs. no influenza in 1957, 19 vs. 13 for children and 1 vs. 19 for adults).
za who did not were compared in additional ways. The mean number of siblings was not significantly different between the 2 groups. There was a trend toward children who did not contract influenza again in 1957 being slightly older than those who did, but this difference was not statistically significant.

With regard to cumulative effects, only 1 participant (an adult) had 2 symptomatic cases of influenza during 1950–1953. This likely was a result of the similarity of HAs during a short H1N1 period. Even children were apparently protected against repeat infections in the prepandemic years by antibody to H1N1. Other infections that might have contributed to a cumulative effect in adults would have occurred before study initiation.

**Discussion.** The age difference in influenza frequency in 1957 was noted by the Cleveland Family Study investigators, who suggested that “unknown factors … apart from possession of antibody” influenced rates of infection [11, p. 210]. Given the findings of animal studies that have been conducted since 1965, the unknown factors could be immunity to viral epitopes that are conserved among subtypes. Possibilities include immune protection mediated by T cells [2, 16] and/or mucosal antibody [17]. Immunological maturation and/or a need for multiple exposures to establish a sufficiently potent immunological response and sufficient immunological memory could explain the age difference. The adults had decades to accumulate exposures, some of them resulting in asymptomatic infection.

Other explanations can be considered for the difference between adults and children. School outbreaks are common, and the high exposure of children has been proposed as an explanation for their high rates of illness, including in 1957. Indeed, children are often the vectors that introduce influenza into a household, and, among the overall adult population, many do not come into contact with children acting as vectors. However, without exception the adults in the Cleveland Family Study lived in households with school-age children, and, in 1957, most lived in households with participants who had culture-proven influenza. Thus, the exposure of the adults in the study population was considerable.

Transmission of influenza within families is demonstrated by several lines of evidence. Vaccination of children reduces cases in their parents [18], and treatment of 1 family member with antiviral drugs reduces additional cases in the family [19]. In a study of viral sequences, HA1 domain sequences of the HA gene differed among families in a community but were identical within each family [20]. In the monitored Cleveland Family Study population, the high attack rate in children 0–4 years old, who would not have been in school during the 1950s, suggests exposure by school-age siblings, and almost all cases in adults occurred several days after a case in another family member.

Do human immune responses to influenza include mediators that have the potential to provide heterosubtypic immunity? Human CD4+ and CD8+ T cells have been found to recognize epitopes of several conserved proteins [21]. Human memory T cells have been found to react with antigens from avian and swine viruses, including H5N1 [22]. Because the humans who provided the T cells for these studies had been exposed to circulating H1N1 or H3N2, these broad cross-reactivities are encouraging. Also, antibody to the conserved antigen M2 has been found in serum from some convalescent individuals [23].

Would a vaccine based on heterosubtypic immunity be useful? It would if cross-protection lasted for a few years, as is suggested by the greater susceptibility of study adults whose last infection preceded the study years—especially for a disease that is currently managed via annual vaccination. Furthermore, if children were to require 2 doses for initial priming, then that is no less practical than the 2-dose recommendation for children that is in place for current influenza vaccines.

These historical data alone cannot prove the existence of cross-protection in humans or establish its mechanism, but the findings of the present analysis suggest that it plays a role. No larger data set that is from a pandemic and that has comparably detailed and individually linked records is available for analysis. The importance of the present analysis is to highlight the outcomes in this unusually well-documented population. The findings are consistent with the existence of human heterosubtypic immunity and call for further investigation. If such immunity is confirmed in humans, new vaccines that are designed to optimize its induction will be valuable. Clinical studies to evaluate its potential in the control of influenza could use contemporary analytical methods and end points rather than traditional serological testing. Such vaccines could be used off the shelf as a first line of defense early during an epidemic or pandemic to reduce morbidity and mortality, complementing efforts in surveillance and the time-consuming preparation of strain-matched vaccines.

**Acknowledgments**

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**References**


