Antigenic memory to influenza A viruses in man determined by monovalent vaccines

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Summary
This study was conducted to test the limits of the doctrine of 'original antigenic sin' in influenza A. The design included use of zonal purified 1000 CCA (chick cell agglutinating) units monovalent vaccines consisting of H<sub>N1</sub>, H<sub>N2</sub>, H<sub>N3</sub> and H<sub>N4</sub>. Age cohorts with different primary influenza A infections were established for the 687 volunteers. The vaccines administered to each age cohort were selected to test the responsiveness of original antigenic sin antibody to homologous and heterologous challenge. Anamnestic responses were demonstrated with Hsw<sub>N1</sub>, H<sub>N2</sub>, and H<sub>N3</sub>, and with H<sub>N2</sub> and H<sub>N3</sub> but not between the groups. The synthesis of these findings is that there are 2 original antigenic sins – 2 families of influenza A viruses.

Introduction
Monovalent vaccines administered to volunteers in selected age groups in the mid-1950s established the haemagglutinin relationships among the then known human influenza A viruses – Hsw<sub>N1</sub>, H<sub>N2</sub>, H<sub>N3</sub>, and H<sub>N4</sub> (Davenport and Hennessy, 1956; Jensen et al., 1956; Davenport, Hennessy and Francis, 1957). This study reaffirmed the 'doctrine of original antigenic sin' as the most adequate explanation for the observed phenomenon of anamnestic response in previously acquired antibodies and, most especially, in antibodies to the initial influenza A virus infection of childhood (Francis, Davenport and Hennessy, 1953; Francis, 1955). A similar study has not been designed since the emergence of H<sub>N2</sub> in 1957 and H<sub>N3</sub> in 1968. Special impetus for such an investigation was the observation in both 1957 and 1968 that infection and immunization with H<sub>N2</sub> or H<sub>N3</sub> produced less than the predicted anamnestic response in H<sub>N1</sub> antibody (Hilleman et al., 1958; Marine, Workman and Webster, 1969; Suto and Morita, 1969). Also, since 1968 the haemagglutinin interrelationships between H<sub>N2</sub> and H<sub>N3</sub> have become clarified and established (Dowdle et al., 1972).

The vaccine study was designed and executed in the summer of 1971 to replicate in part the study of the mid-1950s by Davenport and Hennessy (1956), and to extend the observations to include vaccines with the H<sub>N2</sub> and H<sub>N3</sub> haemagglutinins. The results confirm the original observations, but also establish the lack of anamnestic antibody response and haemagglutinin relationship between the influenza A viruses circulating between 1918 and 1957 and those circulating between 1957 and 1977.

Materials and methods
Detailed description of the vaccines used, study population, immunization procedure and antibody determinations were included in a previous paper and will be summarized only briefly here (Marine and Thomas, 1973).

Each volunteer received 1000 CCA (chick cell agglutinating) units of zonal purified vaccine except those receiving the FM<sub>1</sub> vaccine (H<sub>N1</sub>) who received 571 CCA units. The Biological Laboratories of the National Drug Company prepared the vaccines.

The 687 volunteers came from 3 population groups in the Atlanta, Georgia, metropolitan area so that the entire spectrum from the age of 6 to 101 years could be included.

The vaccine study was conducted in July–September, 1971, at a time when there were no naturally occurring influenza infections. Preimmunization antibody level was determined and used for stratified random assignment of volunteers to each vaccine group.

Prototype viruses were used to determine antibody response, as well as haemagglutinin-specific recombinant strains, H<sub>N2</sub>, and H<sub>N3</sub>, received as HK<sub>N</sub>305e from Dr J. L. Schulman and Dr E. D. Kilbourne, Mount Sinai School of Medicine. The same sample of RDE*-inactivated serum was tested with all antigens, and sera obtained from the same individual at different times were tested in duplicate with an antigen in the same microtitre HI test. Response to vaccines are reported in 2 ways, geometric mean (GM) titre and percentage rise, 2-fold

* RDE = receptor-destroying enzyme.
and 4-fold. High and low titre positive controls and negative controls were used for each antigen to provide assurance that day-to-day variation did not preclude comparison between age groups for each vaccine given. Testing in duplicate allowed for an analysis of within-test variability for each antigen used. No duplicate test showed more than a 2-fold difference, and the proportion that showed as much as a 2-fold difference ranged from 16 to 24% depending on the antigen. When a 2-fold difference in titre occurred, the antibody level was recorded as the geometric mean titre.

The technique of the antibody absorption studies was that previously described (Marine et al., 1969). Each serum antibody titre was adjusted so that the 50/50 serum/virus mixture had an end point of 1 : 2 and was defined as containing 2 HI antibody units.

**Results**

**Primary infection age cohorts**

Previous influenza A experience for each of the age groups as reflected by pre-immunization sera is summarized in Fig. 1. Division of the volunteers into the specified age groups was accomplished by graphing of the baseline titres according to individual years of birth and selecting the ‘cut-off’ for each primary influenza infection group by the last year showing distinct prevalence of seropositive individuals. Persons were at least 5 years of age to be included in the age cohort for each influenza A virus. The selection of the dates for each cohort would be expected to reflect circulation of influenza A viruses in the south-eastern United States at the time and should not be generalized.

According to the doctrine of ‘original antigenic sin’, antibodies to the initial influenza A infection are uniquely sensitive to anamnestic response by subsequent influenza A infection or immunization. The design of the study was focused on the relative responsiveness of this original antigenic sin antibody for each age cohort group in Fig. 1 to H₀, H₁, H₂, and H₃ vaccines. Vaccines selected for each age group were selected based on this study design and the knowledge of prior influenza A experience (Table 1). H₂ and H₃ vaccines were administered to each age cohort to test for anamnestic responses and to document extent of homologous response in the corresponding age cohort. In the H₀ and H₁ age cohorts the respective monovalent vaccine was used to establish the degree of responsiveness of the antibody to homologous stimulation. In the H₁ age cohort, H₀ vaccine was used also to test for anamnestic response, while in the H₂ age cohort

![Graph](http://pmj.bmj.com/)

Fig. 1. Prevalence of HI antibodies to major influenza A virus in sera from 687 persons, 1971. The ▲ indicates the year of first occurrence of the respective influenza A virus.
both \( H_3 \) and \( H_1 \) vaccines were used to test for anamnestic response. In the \( H_3 \) age cohort, \( H_2 \) and \( H_3 \) vaccines were administered to examine further the relationship of these haemagglutinins.

<table>
<thead>
<tr>
<th>Birth dates</th>
<th>Primary infection with</th>
<th>( H_3N_1 )</th>
<th>( H_2N_1 )</th>
<th>( H_2N_2 )</th>
<th>( H_3N_2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1906–1920</td>
<td>( Hsw_1N_1 )</td>
<td>—</td>
<td>—</td>
<td>32</td>
<td>34</td>
</tr>
<tr>
<td>1928–1939</td>
<td>( H_2N_1 )</td>
<td>29</td>
<td>—</td>
<td>28</td>
<td>29</td>
</tr>
<tr>
<td>1940–1949</td>
<td>( H_3N_1 )</td>
<td>20</td>
<td>28</td>
<td>29</td>
<td>24</td>
</tr>
<tr>
<td>1950–1961</td>
<td>( H_2N_2 )</td>
<td>46</td>
<td>42</td>
<td>42</td>
<td>43</td>
</tr>
<tr>
<td>1962–1965</td>
<td>( H_3N_2 )</td>
<td>—</td>
<td>—</td>
<td>24</td>
<td>26</td>
</tr>
</tbody>
</table>

**Table 1. Persons receiving monovalent influenza A vaccines by birthdate and primary infection cohort**

**Monovalent vaccine potency**

The age-related nature of the response to these vaccines has been reported previously (Marine and Thomas, 1973). Figure 2 summarizes and underscores the potency of these vaccines in the 6–43-year age group. Two-fold or greater homologous antibody response was noted in 89–99\%, and 4-fold or greater antibody response occurred in 51–89\%.

![Fig. 2. Homologous response to 1000 CCA monovalent vaccines ages 6–43 years.](http://pmj.bmj.com/)

**Hsw\(_1N_1\) age cohort**

The influenza A antibody profile in volunteers born between 1906 and 1920 fulfills the criterion for \( Hsw_1N_1 \)'s being the initial influenza A infection inasmuch as 98\% have \( Hsw_1N_1 \) antibody, and the GM titre of 46 to \( Hsw_1N_1 \) represents the highest antibody level to any influenza A antibody measured (Fig. 1). \( H_2 \) and \( H_3 \) vaccines produced good homologous responses, but evidence for anamnestic response of \( Hsw_1 \) antibody is absent (Fig. 3). No significant change in GM titre to \( Hsw_1 \) occurred, and only one volunteer in each group experienced a 4-fold rise in titre.

**H3N1 age cohort**

Volunteers born between 1928 and 1939 were distinctive in having the greatest prevalence (98\%) and highest GM titre to \( H_3N_1 \) (24). However, this age cohort had a slightly higher baseline titre to \( H_2N_3 \) (36) (Fig. 1). \( H_3 \), \( H_2 \), and \( H_3 \) vaccines were administered to this age cohort (Fig. 4). \( H_0 \) vaccine produced a striking homologous antibody response and impressive anamnestic responses in \( Hsw_1 \) and \( H_1 \) antibodies as well – 55\% 2-fold rise in \( Hsw_1 \) and 70\% 2-fold rise in \( H_1 \). Contrary to no change in \( H_3 \) and \( H_1 \) antibody levels occurred, and only 10\% showed a 2-fold rise in titre. Likewise, \( H_2 \) and \( H_3 \) vaccines, while producing excellent homologous responses, effected virtually no anamnestic response in \( H_0 \) or \( H_1 \) antibody. Thus, in this age group, we see a distinct dissociation of anamnestic responsiveness between the \( Hsw_1 \), \( H_0 \), and \( H_1 \) group, and the \( H_2 \) and \( H_3 \) group.

**H1N1 age cohort**

Volunteers in the 1940–1949 birthdates group had the highest GM titre (23) and a 90\% prevalence of antibody to \( H_1N_1 \). However, the GM titre to \( H_1N_1 \) (23) was considerably lower than that to \( H_2N_2 \) (81). This antibody profile in itself suggests a dissociation from the pattern of anamnestic stimulation of initial influenza A antibody. \( H_2 \), \( H_3 \), and \( H_3 \) vaccine administered to this group demonstrated a response similar to the \( H_3N_1 \) cohort-impressive anamnestic
response of H1N1 antibody following H6N1 vaccine but no response following H3 and H8 vaccines (Fig. 5). The anamnestic response in H1N1 after H3N1 was a GM titre rise from 36 to 128, while the homologous response in H1N1 after H1N1 vaccine was a GM titre rise from 27 to 208.

**H3N2 age cohort**

The antibody profile in the 1950–1961 birthdate cohort is distinctive for having the peak GM titre (89) and a 99% prevalence of antibody to H3N2. H6, H1, and H3 vaccines in this group further documented the patterns observed with the H3N1 and H1N1 cohorts. Here, however, the original antigenic sin antibody-H3N2 was boosted only slightly better by H3N2 vaccine than by H6 or H1 (Fig. 6). This boost was shown also when the antigen in the test for H3 antibody was haemagglutinin-specific-H3N1. The prevalence of 2-fold anamnestic responses to H3 after H6 or H1 was in the range of 25%, while after H3 vaccine it was in the range of 50%. This age cohort, then, demonstrates the greatest degree of anamnestic responsiveness between the 2 influenza family groups.

**H3N3 age cohort**

The 1962–1965 age group was only 6–9 years of age in 1971 and thus had relatively low titres to any influenza A viruses. Nevertheless, it is the age group available that had greatest exposure in H3H2 virus and has its highest GM titre to H3N2. Figure 7 documents with both H3 and H6 vaccine that strong anamnestic responses occur to the other virus due to the haemagglutinin relationship since a similar pattern of response is observed when haemagglutinin-specific recombinants are used as antigens H3N1 and H6N1.

**Antibody absorption studies**

The present study complements the extensive antibody absorption studies of Morita, Suto and Ishida (1972) which led to their conclusion that there are 2 major groups of human influenza A viruses. However, their antibody absorption studies with H3 and H8 viruses did not use haemagglutinin-specific recombinants nor did those in a previous
report (Marine et al., 1969). Consequently, some doubt may persist that the common N₂ neuraminidase may have been responsible for the absorption results. Table 2 shows the results of antibody absorption with heterologous haemagglutinin-specific recombinants. Following the anamnestic response in H₂ antibodies after H₃ vaccine, H₂N₁ removed H₂ antibody from 10 of 11 sera tested, 2 or more antibody units in 8 of the sera. Likewise, following the anamnestic response in H₃ antibodies after H₂ vaccine, H₂N₁ removed 2 or more antibody units of H₃ antibody from all 9 sera tested. That doubly-absorbable antibody occurred in each serum was further demonstrated by use of the haemagglutinin-specific recombinant with a haemagglutinin that was not homologous with the vaccine used. Heterologous removal could be demonstrated in over half of the sera tested (Table 2).

![Vaccine GM titre](image1)


![Vaccine GM titre](image2)


### Table 2. Absorption of H₃ and H₂ HI antibodies with heterologous haemagglutinin specific recombinants from selected human sera following 1000 CCA units of H₂N₂ and H₃N₂ vaccine

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Geometric mean HI titre</th>
<th>Absorption of S₂ sera with haemagglutinin-specific-recombinant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H₂N₂</td>
<td>H₂N₂</td>
</tr>
<tr>
<td></td>
<td>S₁ S₂</td>
<td>S₁ S₂</td>
</tr>
<tr>
<td>H₂N₂</td>
<td>10 193</td>
<td>25 175</td>
</tr>
<tr>
<td>1000 CCA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H₂N₂</td>
<td>31 189</td>
<td>26 377</td>
</tr>
<tr>
<td>1000 CCA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Discussion

It is proposed that these immunization studies taken together with a number of already published reports lead to the conclusion that there are 2 original antigenic sins to influenza A viruses. Leichtenstern (1896) first proposed the concept of families of influenza. Masurel and Mulder (1962) reinterpreted this hypothesis to mean that there are two 'eras' of influenza A viruses. Salk (1952) and Davenport, and Hennessy (1958) proposed the concept of recycling of influenza A viruses. The demonstration of recycling in the same sequence for H4 and H3 viruses by Masurel and Marine (1973) led them to repeat the earlier prediction by Masurel (1968) that swine influenza would recur. Now there has been a pandemic recurrence of H3N2 in the form of A/USSR/77. Therefore, a previous pandemic strain of influenza A virus has for the first time been reisolated in humans. Thus, we are observing the recycling of a family of influenza A viruses absent from man since 1957, just as the isolation of H3N2 in 1957 heralded the recycling of a family of influenza A viruses that dominated the world from 1889 to 1918.

Sero-epidemiological studies of influenza have yielded great insights into its epidemiology (Shope, 1936; Francis et al., 1953; Mulder and Masurel, 1958; Davenport and Hennessy, 1958; Masurel and Mulder, 1962; Schild and Stuart-Harris, 1965; Masurel, 1969; Marine and Workman, 1969). The profile of antibodies in Fig. 1 continues to show the unique identification of age cohorts with specific influenza A viruses – a reaffirmation of the doctrine of original antigenic sin. However, by the time of the present study, certain inconsistencies could be identified with the concept that there is an anamnestic response in original antigenic sin antibody following all subsequent influenza A virus infections. Only in the Hsw1N1 and H2N2 age cohorts are all the conditions met, namely highest prevalence and highest GM titre in original antigenic sin antibody. This finding for the swine age cohort further supports the circumstantial evidence that Hsw1N1 was responsible for the 1918 pandemic (Shope, 1936; Stuart-Harris, 1970). For both the H2N1 and H1N1 age cohorts, the H3N2 GM titre was highest, suggesting that anamnestic response in the H2 and H1 antibody had not occurred following H3 and H2 infection. The fact that both H2 and H1 had followed Hsw1 could explain the very high Hsw1 titre.

The immunization studies by age cohort objectively demonstrate that anamnestic response occurs within the family but not between families. It is the consistency of the findings that is most convincing. In the Hsw1N1 age cohort, neither H2 nor H3 vaccine stimulated Hsw1N1 antibodies (Fig. 3). The authors were unable to obtain swine vaccine for this study to test the response of H3 and H8 antibodies following Hsw1N1 vaccine. Noble et al. (1977) recently reported the experience with A/New Jersey/76 (Hsw1N1) vaccine in 1976–77 and found only slight heterologous response in H2N1 and H1N1 antibodies compared with pronounced heterologous response in H3N1 and H2N1 antibodies especially in the age groups that had initial exposure to those influenza A viruses.

In the H2N1 age cohort, H0 vaccine stimulated H1N1 and Hsw1N1 antibodies, while H2 and H3 vaccines failed to stimulate Hsw1, H0, or H1 antibodies (Fig. 4). In the H1N1 age cohort, there was marked response in H1N1 antibody after H0 vaccine with no response following H2 and H3 vaccine (Fig. 5). In the H3N2 age cohort, good response in H2 antibody followed H2 vaccine, with only slight response after H8 and H1 vaccine (Fig. 6). Finally in the H3N2 age cohort the strong interrelationship between H2 and H3 was further emphasized (Fig. 7). It is important to note, also, that the H2 and H3 interrelationships remain when haemagglutinin-specific recombinants are used. These interrelationships are further documented with the antibody absorption studies using haemagglutinin-specific recombinants (Table 2).

These immunization and special antibody absorption studies complement the antibody absorption work of Morita et al. (1972) and add credence to their conclusion that there are 2 major groups of influenza A viruses in man. The demonstration of a limit to anamnestic response with influenza A viruses fits with the hypothesis that there are 2 families of influenza A viruses. In the first family are Hsw1N1, H4N1, and H1N1 while in the second family are H2N2 and H3N2. Thus the distinctive features of the 2 families are different neuraminidases and original antigenic sin operative within the family, but not between families. The evidence presented of no boosting between families would support the thesis that 'antibody erosion' explains differences in timing of earlier H8 and H3 pandemics (Masurel and Marine, 1973). Thus, the current facts relevant to recycling of influenza A viruses are as follows:

1. An H4-like virus was almost certainly responsible for the 1889–90 pandemic which was distantly related to H3N2 but distinct from H2N2. H3N2 was the pandemic strain of 1957–58.

2. An H3-like virus was responsible for the 1900–01 pandemic and was more closely related to H5N2, but Fedson et al. (1972) have shown that the earlier H3 virus contained a neuraminidase antigen similar to the equine (Neq) virus.

3. For a precise sequence of recycling, another member of the H2 and H3 family was expected to occur followed by Hsw1. Instead, what has happened is the emergence of A/USSR/77 (H1N1) to produce...
pandemic disease without as yet replacing the H2N2 strains. Consequently, the authors’ experience is that recycling is not exact.

Some have concluded from these facts that if there is a reappearance of old strains, it is likely to be a random one (Schoenbaum et al., 1976; Dowdle and Millar, 1978.) The authors would suggest that the entire literature regarding original antigenic sin in influenza A is compelling evidence for strict limits on this randomness within families. In addition, they propose that evidence to date speaks strongly for 2 original antigenic sins – 2 families, one, or more, of whose members has caused human disease in 2 separate periods during the last 90 years. It is not understood how one gets from one family to the next, and the authors cannot be sure that there are not more families of influenza A to come. However, consideration should be given to ‘priming’ persons to one member of each family of influenza A as a foundation for rapid and high order protection against future strains of pandemic influenza that may arise from these families. Francis (1953) long ago advocated this approach to influenza control.

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