Postgraduate Medical Journal (February 1979) **55**, 98–108.

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

WILLIAM M. MARINE
M.D., M.P.H.

JAMES E. THOMAS

Department of Preventive Medicine and Community Health, Emory University School of Medicine, Atlanta, Georgia, U.S.A.

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_0N_1, H_1N_1, H_2N_2 and H_3N_2. 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_1N_1, H_0N_1, and H_1N_1 and with H_2N_2 and H_3N_2 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_1N_1, H_0N_1, and H_1N_1 (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_2N_2 in 1957 and H_3N_2 in 1968. Special impetus for such an investigation was the observation in both 1957 and 1968 that infection and immunization with H_2N_2 or H_3N_2 produced less than the predicted anamnestic response in H_1N_1 antibody (Hilleman *et al.*, 1958; Marine, Workman and Webster, 1969; Suto and Morita, 1969). Also, since 1968 the haemagglutinin interrelationships between H_2 and H_3 have become clarified and established (Dowdle *et al.*, 1972).

Correspondence: William M. Marine, Professor and Chairman, Department of Preventive Medicine and Comprehensive Health Care, University of Colorado Medical Center, Box C-245, Denver, Colorado 80262.

This 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_2 and H_3 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_1 vaccine (H_1N_1) 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. Pre-immunization 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_3N_1, and H_2N_1, received as HKe and 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.

0032-5473/79/0200-0098 $02.00

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 H_0, H_1, H_2, and H_3 vaccines. Vaccines selected for each age group were selected based on this study design and the knowledge of prior influenza A experience (Table 1). H_2 and H_3 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 H_0 and H_1 age cohorts the respective monovalent vaccine was used to establish the degree of responsiveness of the antibody to homologous stimulation. In the H_1 age cohort, H_0 vaccine was used also to test for anamnestic response, while in the H_2 age cohort

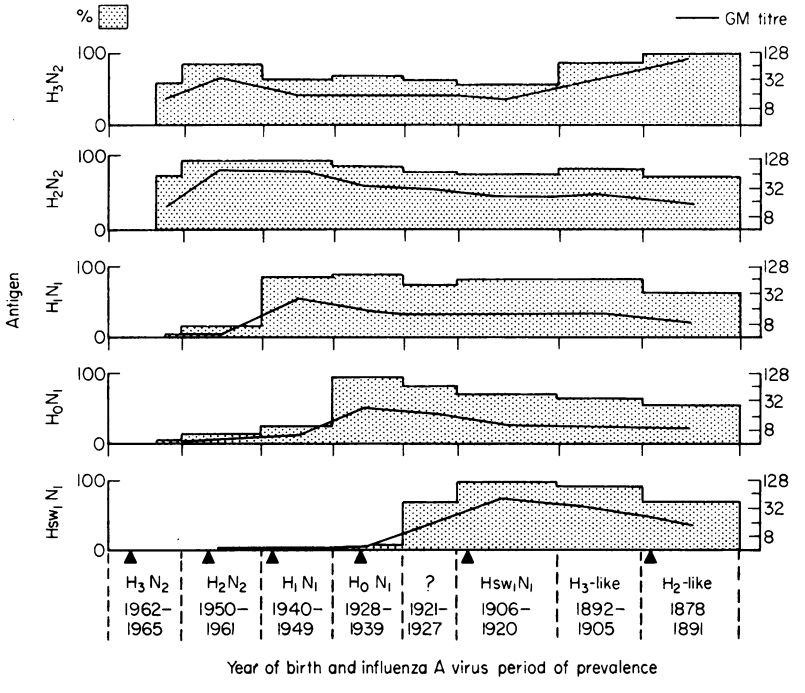

FIG. 1. Prevalence of HI antibodies to major influenza A virus in sera from 687 persons, 1971. The ▲ indicates the year of first occurrence of the respective influenza A virus.

both H_0 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 1. Persons receiving monovalent influenza A vaccines by birthdate and primary infection cohort

Birth dates	Primary infection with	Monovalent vaccine type H_0N_1	H_1N_1	H_2N_2	H_3N_2
1906–1920	Hsw_1N_1	—	—	32	34
1928–1939	H_0N_1	29	—	28	29
1940–1949	H_1N_1	20	28	29	24
1950–1961	H_2N_2	46	42	42	43
1962–1965	H_3N_2	—	—	24	26

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%.

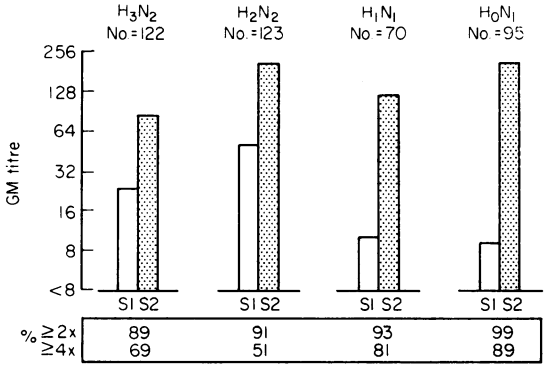

FIG. 2. Homologous response to 1000 CCA monovalent vaccines ages 6–43 years.

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.

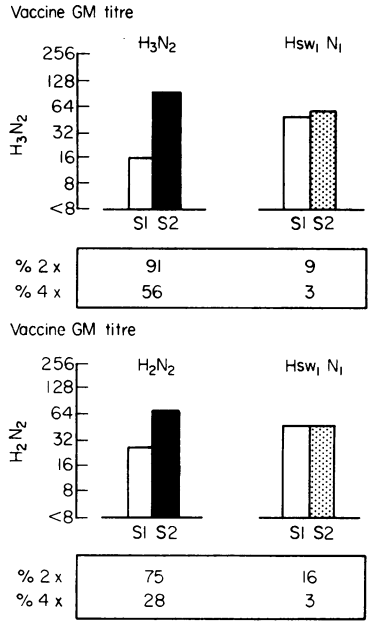

FIG. 3. Hsw_1N_1 age cohort (persons born 1906–1920) HI response to H_3N_2 and H_2N_2 vaccines in 1971.

H_0N_1 age cohort

Volunteers born between 1928 and 1939 were distinctive in having the greatest prevalence (98%) and highest GM titre to H_0N_1 (24). However, this age cohort had a slightly higher baseline titre to H_2N_2 (36) (Fig. 1). H_0, 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. In contrast no change in H_2 and H_3 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.

H_1N_1 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_0, H_2, and H_3 vaccine administered to this group demonstrated a response similar to the H_0N_1 cohort-impressive anamnestic

response of H_1N_1 antibody following H_0N_1 vaccine but no response following H_2 and H_3 vaccines (Fig. 5). The anamnestic response in H_1N_1 after H_0N_1 was a GM titre rise from 36 to 128, while the homologous response in H_1N_1 after H_1N_1 vaccine was a GM titre rise from 27 to 208.

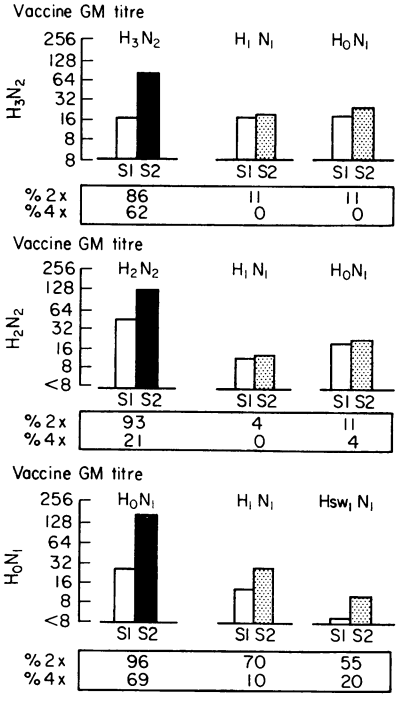

FIG. 4. H_0N_1 age cohort (persons born 1928–1939) HI response to H_3N_2, H_2N_2 and H_0N_1 vaccines in 1971.

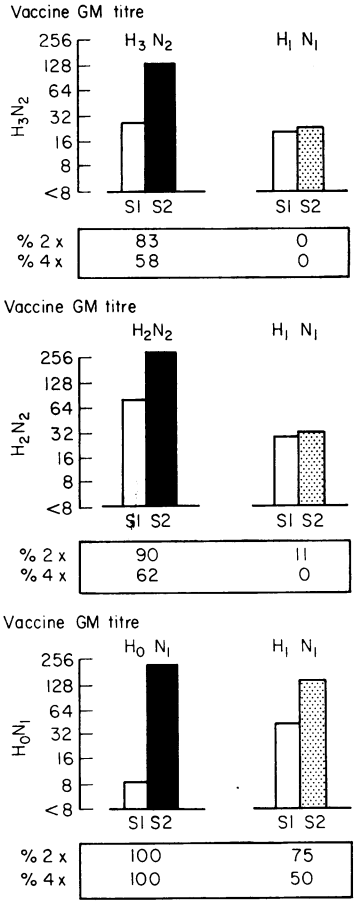

FIG. 5. H_1N_1 age cohort (persons born 1940–1949) HI response to H_3N_2, H_2N_2, and H_0N_1 vaccines in 1971.

H_2N_2 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 H_2N_2. H_0, H_1, and H_3 vaccines in this group further documented the patterns observed with the H_0N_1 and H_1N_1 cohorts. Here, however, the original antigenic sin antibody-H_2N_2 was boosted only slightly better by H_3N_2 vaccine than by H_0 or H_1 (Fig. 6). This boost was shown also when the antigen in the test for H_2 antibody was haemagglutinin-specific-H_2N_1. The prevalence of 2-fold anamnestic responses to H_2 after H_0 or H_1 was in the range of 25%, while after H_3 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.

H_3N_2 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 H_3H_2 virus and has its highest GM titre to H_3N_2. Figure 7 documents with both H_2 and H_3 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 H_2N_1 and H_3N_1.

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 H_2 and H_3 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 N_2 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 H_2 antibodies after H_3 vaccine, H_3N_1 removed H_2 antibody from 10 of 11 sera tested, 2 or more antibody units in 8 of the sera. Likewise, following the anamnestic response in H_3 antibodies after H_2 vaccine, H_2N_1 removed 2 or more antibody units of H_3 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).

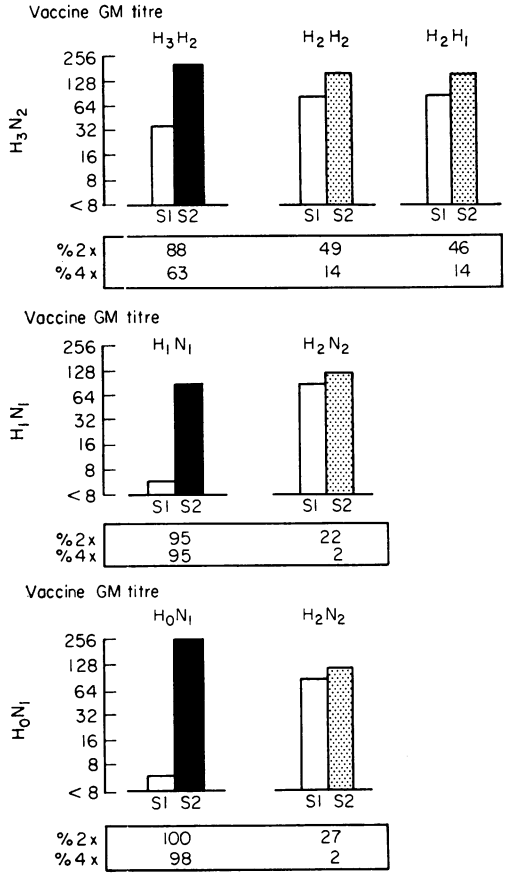

FIG. 6. H_2N_2 age cohort (persons born 1950–1961) HI response to H_3N_2, H_1N_1 and H_0N_1 vaccines in 1971.

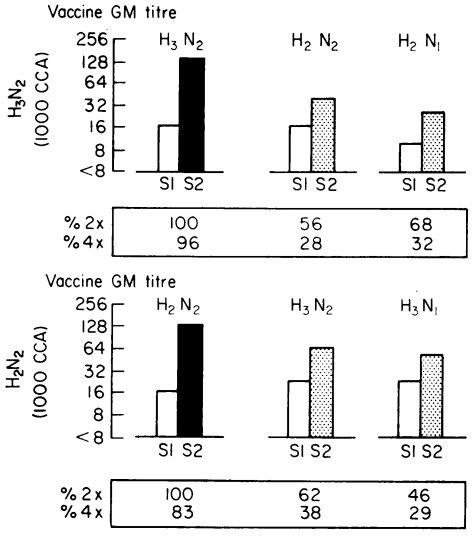

FIG. 7. H_3N_2 age cohort (persons born 1962–1965) HI response to H_3N_2 and H_2N_2 vaccines in 1971.

TABLE 2. Absorption of H_3 and H_2 HI antibodies with heterologous haemagglutinin specific recombinants from selected human sera following 1000 CCA units of H_3N_2 and H_2N_2 vaccine

	Geometric mean HI titre					Absorption of S2 sera with haemagglutinin-specific-recombinant			
						H_2 antibody removal by H_3N_1		H_3 antibody removal by H_2N_1	
	H_3N_2		H_2N_2		No. of subjects	No. of HI antibody units		No. of HI antibody units	
Vaccine	S1	S2	S1	S2		1 or more	2 or more	1 or more	2 or more
H_3N_2 1000 CCA	10	193	25	175	11	10	8	6	3
H_2N_2 1000 CCA	31	189	26	377	9	5	2	9	9

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 H_2 and H_3 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 H_1N_1 in the form of A/USSR/77. Therefore, a previous pandemic strain of influenza A virus has for the first time been re-isolated 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 H_2N_2 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 Hsw_1N_1 and H_2N_2 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 Hsw_1N_1 was responsible for the 1918 pandemic (Shope, 1936; Stuart-Harris, 1970). For both the H_0N_1 and H_1N_1 age cohorts, the H_2N_2 GM titre was highest, suggesting that anamnestic response in the H_0 and H_1 antibody had not occurred following H_2 and H_3 infection. The fact that both H_0 and H_1 had followed Hsw_1 could explain the very high Hsw_1 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 Hsw_1N_1 age cohort, neither H_2 nor H_3 vaccine stimulated Hsw_1N_1 antibodies (Fig. 3). The authors were unable to obtain swine vaccine for this study to test the response of H_2 and H_3 antibodies following Hsw_1N_1 vaccine. Noble *et al.* (1977) recently reported the experience with A/New Jersey/76 (Hsw_1N_1) vaccine in 1976–77 and found only slight heterologous response in H_0N_1 and H_1N_1 antibodies compared with pronounced heterologous response in H_0N_1 and H_1N_1 antibodies especially in the age groups that had initial exposure to those influenza A viruses.

In the H_0N_1 age cohort, H_0 vaccine stimulated H_1N_1 and Hsw_1N_1 antibodies, while H_2 and H_3 vaccines failed to stimulate Hsw_1, H_0, or H_1 antibodies (Fig. 4). In the H_1N_1 age cohort, there was marked response in H_1N_1 antibody after H_0 vaccine with no response following H_2 and H_3 vaccine (Fig. 5). In the H_2N_2 age cohort, good response in H_2 antibody followed H_3 vaccine, with only slight response after H_0 and H_1 vaccine (Fig. 6). Finally in the H_3N_2 age cohort the strong interrelationship between H_2 and H_3 was further emphasized (Fig. 7). It is important to note, also, that the H_2 and H_3 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 Hsw_1N_1, H_0N_1, and H_1N_1 while in the second family are H_2N_2 and H_3N_2. 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 H_2 and H_3 pandemics (Masurel and Marine, 1973). Thus, the current facts relevant to recycling of influenza A viruses are as follows:

(1) An H_2-like virus was almost certainly responsible for the 1889–90 pandemic which was distantly related to H_2N_2 but distinct from H_3N_2. H_2N_2 was the pandemic strain of 1957–58.

(2) An H_3-like virus was responsible for the 1900–01 pandemic and was more closely related to H_3N_2, but Fedson *et al.* (1972) have shown that the earlier H_3 virus contained a neuraminidase antigen similar to the equine (Neq_2) virus.

(3) For a precise sequence of recycling, another member of the H_2 and H_3 family was expected to occur followed by Hsw_1. Instead, what has happened is the emergence of A/USSR/77 (H_1N_1) to produce

pandemic disease without as yet replacing the H_3N_2 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.

Acknowledgments

The authors wish to thank Drs Gordon Meiklejohn and Walter R. Dowdle for their critical review of the manuscript.

This study was supported in part by Public Health Service general research grant FR 05364 from the Division of Research Facilities and Resources; by Public Health Service Infectious Disease training grant 5 T01 AI00394; and by Dr Marine's Milbank Faculty Fellowship.

References

DAVENPORT, F.M. & HENNESSY, A.V. (1956) A serologic recapitulation of past experiences with influenza A; antibody response to monovalent vaccine. *Journal of Experimental Medicine*, **104**, 85.

DAVENPORT, F.M. & HENNESSY, A.V. (1958) The clinical epidemiology of Asian influenza. *Annals of Internal Medicine*, **49**, 493.

DAVENPORT, F.M., HENNESSY, A.V. & FRANCIS JR, T. (1957) Influence of primary antigenic experience upon the development of a broad immunity to influenza. *Transactions of the Association of American Physicians*, **70**, 81.

DOWDLE, W.R., MARINE, W.M., COLEMAN, M.T. & KNEZ, V. (1972) Haemagglutinin relationships of Hong Kong (H_3) and Asian (H_2) influenza strains delineated by antigen-specific recombinants. *Journal of General Virology*, **16**, 127.

DOWDLE, W.R. & MILLAR, J.D. (1978) Swine influenza: lessons learned. *Medical Clinics of North America*, **62**, 1047.

FEDSON, E.S., HUBER, M.A., KASEL, J,A. & WEBSTER, R.G. (1972) Presence of A/equine/2 hemagglutinin and neuraminidase antibodies in man. *Proceedings of the Society for Experimental Biology and Medicine*, **139**, 125.

FRANCIS JR, T. (1953) Influenza: the newe acquayantance. *Annals of Internal Medicine*, **39**, 203.

FRANCIS JR, T. (1955) The current status of the control of influenza. *Annals of Internal Medicine*, **43**, 534.

FRANCIS JR, T., DAVENPORT, F.M., & HENNESSY, A.V. (1953) A serologic recapitulation of human infection with different strains of influenza virus. *Transactions of the Association of American Physicians*, **66**, 231.

HILLEMAN, M.R., FLATLEY, F.J., ANDERSON, S.A. & LUECKNIG, M.L. (1958) Antibody response in volunteers to Asian influenza vaccine. *Journal of the American Medical Association*, **166**, 1134.

JENSEN, K.E., DAVENPORT, F.M., HENNESSY, A.J. & FRANCIS JR, T. (1956) Characterization of influenza antibodies by serum absorption. *Journal of Experimental Medicine*, **104**, 199.

LEICHTENSTERN, O. (1896) Influenza. In: *Specielle Pathologie und Therapie* (Ed. by Nothnagel, H.), vol IV, part 2, p. 22. Vienna.

MARINE, W.M. & THOMAS, J.E. (1973) Age-related response to 1000 CCA units zonally purified, inactivated influenza vaccines in volunteers in the U.S.A. *Postgraduate Medical Journal*, **49**, 164.

MARINE, W.M. & WORKMAN, W.M. (1969) Hong Kong influenza immunologic recapitulation. *American Journal of Epidemiology*, **90**, 406.

MARINE, W.M., WORKMAN, W.M. & WEBSTER, R.G. (1969) Immunological interrelationships of Hong Kong, Asian and Equi-2 influenza viruses in man. *Bulletin of the World Health Organization*, **41**, 475.

MASUREL, N. (1968) Prevention of influenza A by immunization. *Maandschrift voor Kindergeheeskunde*. Amsterdam, **36**, 207.

MASUREL, N. (1969) Relation between Hong Kong virus and former human A2 isolates and the A/Equi2 virus in human sera collected before 1957. *Lancet*, **i**, 907.

MASUREL, N. & MARINE, W.M. (1973) Recycling of Asian and Hong Kong influenza A virus hemagglutinins in man. *American Journal of Epidemiology*, **97**, 44.

MASUREL, N. & MULDER, J. (1962) Studies on the content of haemagglutination-inhibiting antibody for swine influenza virus A. *Verhandelingen Instituut voor preventieve geneeskunde*. Leiden, **52**, 1.

MORITA, M., SUTO, T. & ISHIDA, N. (1972) Antigenic memory in man in response to sequential infections with influenza A viruses. *Journal of Infectious Diseases*, **126**, 61.

MULDER, J. & MASUREL, N. (1948) Pre-epidemic antibody against 1957 strain of Asiatic influenza in serum of older people living in The Netherlands. *Lancet*, **i**, 810.

NOBLE, G.R., KAYE, H.S., KENDAL, A.P. & DOWDLE, W.R. (1977) Age-related heterologous antibody response to influenza virus vaccination. *Journal of Infectious Diseases*, **136** (Suppl.), S686.

SALK, J.E. (1952) An interpretation of the significance of influenza virus variation for the development of an effective vaccine. *Bulletin of the New York Academy of Medicine*, **28**, 748.

SCHILD, G.C. & STUART-HARRIS, C.H. (1965) Serological epidemiological studies with influenza A viruses. *Journal of Hygiene*, **63**, 379.

SCHOENBAUM, S.C., COLEMAN, M.T., DOWDLE, W.R. & MOSTOW, S.R. (1976) Epidemiology of influenza in the elderly: evidence of recycling. *American Journal of Epidemiology*, **103**, 166.

SHOPE, R.E. (1936) The incidence of neutralizing antibodies for swine influenza virus in the sera of human beings of different ages. *Journal of Experimental Medicine*, **63**, 669.

STUART-HARRIS, C.H. (1970) Virus of the 1918 epidemic. *Nature*. London, **225**, 850.

SUTO, T. & MORITA, M. (1969) An epidemic of Hong Kong influenza in a dormitory of student nurses. *Report of the Akita Central Hospital of Medicine*, **6**, 78.