The influenza virus: antigenic composition and immune response

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Summary

The architecture and chemical composition of the influenza virus particle is described with particular reference to the protein constituents and their genetic control. The dominant role in infection of the surface proteins – haemagglutinins and neuraminidases – acting as antigens and undergoing variation in time known as antigenic drift and shift is explained. The immuno-diffusion technique has illuminated the inter-relationships of the haemagglutinins of influenza A viruses recovered over long periods of time. The H₀ and H₁ haemagglutinins are now regarded as a single sub-type with H₂ and H₃ representing the haemagglutinins of the 1957 and 1968 sub-types. Animal influenza viruses of pigs, horses and birds are described. A relation to human influenza strains has been shown to exist in certain instances as is the capacity of some human strains to pass to the animal kingdom.

Introduction

The chemical and antigenic composition of the influenza virus is probably more fully understood than that of any other virus which infects man. Although the influenza A virus was first isolated from man over 45 years ago, much of the detailed information on its structure and composition, which is essential for the full understanding of its immunological properties, has been obtained over the past 10 years by the application of the techniques of modern molecular virology. However, one cannot possibly overlook the importance to the study of influenza of the pioneering work on the immunological and epidemiological properties of the virus and its clinical manifestations, to which Sir Charles Stuart-Harris, in whose honour this series of papers in the *Postgraduate Medical Journal* is devoted, has contributed in such a significant way. It is something of a paradox that although major successes have been achieved in the control by vaccines of many other viral diseases of man, for which knowledge of the chemical composition and antigenic structure of the causative virus is less well developed than for influenza, attempts at the control of influenza are rewarded by only limited success. The reasons for this are the high degree of antigenic variability and

capricious epidemiological behaviour of the causative virus and perhaps also the nature of the immune response in man to influenza antigens.

Antigenic composition of the virus

Studies of the single-stranded, negative-strand, RNA genome of the influenza A virus have revealed the existence of 8 species of RNA molecules (gene segments) in the virus (McGeoch, Fellner and Newton, 1976; Palese and Schulman, 1976a). Each gene segment provides the code for one of the 8 virus specific proteins synthesized in infected cells. Seven of these proteins are integrated into influenza virus particles. Table 1 summarizes the protein components of influenza virus particles and infected cells.

Three internal components of the virus P1, P2 and P3 of relatively high mol. wt (81–94 000 daltons) are present in only small amounts in the virus particle. They have been associated with RNA transcriptase and gene transcription. The antigenic character of these proteins is not known, since they have not been purified in adequate amounts to enable their detailed characterization. The 4 other proteins present in the influenza virus particle are of established antigenicity. The nucleoprotein (NP) and the matrix protein (M) are major internal antigens of the virus. The haemagglutinin (HA) and neuraminidase (NA) are the 2 surface antigens of the virus. A more detailed description of these antigens of the virus follows below. A non-structural, virus-coded protein (NS₁), present in large quantities in infected cells during virus replication, is not incorporated into the virus particle and its antigenic characteristics are at present not known.

(a) The nucleoprotein (NP) antigen

The NP which has a mol. wt of 55–60 000 daltons is closely associated with the RNA genome of the virus in the form of RNA-protein complexes (Pons *et al.*, 1969). The antigen is type specific, forming the basis for the classification of influenza viruses into types A, B and C (WHO, 1971). Antibody to NP is a frequent component of the antibody response to natural influenza infections in man and

TABLE 1. Virus-specific polypeptide and antigens of the influenza virus* particle and infected cells

Polypeptide	Polypeptide (mol. wt.)	Approx no. of molecules/virus particle	Assignment to structural and antigenic properties of virus
P-1	94 000-84 000	< 50	Minor internal proteins of the virus: unknown antigenicity, associated with RNA polymerase activity of virus
P-2		< 50	
P-3		< 50	
NA	60 000	~ 200	Envelope glycoprotein, neuraminidase antigen. Antigenically variable
NP	53 000	~ 1000	Internal protein, ribonucleoprotein antigen. Some degree of antigenic variability. Associated with RNA
HA { HA-1 HA-2†	58 000	~ 1000	Envelope glycoproteins, components of haemagglutinin antigen. Heavy (HA-1) and light (HA-2) chains linked by disulphide bonds. Antigenically variable
	28 000	~ 1000	
MP	25 000	~ 3000	Internal protein, matrix protein antigen. Antigenically stable. Situated beneath lipid membrane of virus
NS ₁	23 000	not present in virus particle	Non-structural protein present in infected cells. Antigenicity unknown

* The values stated were determined for A/Hong Kong/68 (H₃N₂) virus, X-31 strain (Skehel and Schild, 1971).

† HA-1 and HA-2 are synthesized as a single HA polypeptide which is later cleaved to HA-1 and HA-2 in egg-grown virus.

animals (Schild, Oxford and Virelizier, 1976) and its detection is widely used for diagnostic purposes. Early studies using complement-fixation tests and immuno-double-diffusion (IDD) suggested that the NP components of influenza A viruses of human, animal and avian origin were antigenically identical (Schild and Pereira, 1969; Schild, 1972). However, more recent studies (Oxford *et al.*, 1978a; Schild, Oxford and Newman, 1978) have shown that the NP antigens of certain influenza A viruses can be distinguished. These studies involved the use of IDD tests with potent antisera to chemically purified NP antigens. It was found that whilst the NP antigens of all viruses of influenza A Hong Kong (H₃N₂) subtype isolated from 1968 to 1978 were alike, these could be clearly distinguished from the NP of A/PR8/34 (H₃N₂) virus by the production of 'spurs' between the precipitin reactions (Fig. 1a). The NP antigens of swine influenza A virus, A/swine/Iowa/15/30 (Hsw₁N₁) and an avian virus, A/duck/Ukraine/63 (Hav₇Neq₂) were also readily distinguished from the NP antigen of human Hong Kong (H₃N₂) viruses. These studies confirmed the lack of antigenic cross-reactions between the NP antigens of influenza A and B viruses. Further work on the NP antigens of influenza A virus is in progress but the recent findings (G. C. Schild, unpublished) indicate that a slow but progressive antigenic 'drift' occurred in the NP antigens of the human influenza A viruses prevalent from 1933 to 1978. The biological and immunological implications of these findings require further study. There is no evidence at present that antibody to NP antigen exerts a protective effect against influenza A virus infection in experimental influenza in mice (Virelizier, Oxford and Schild, 1976; Oxford and Schild, 1976). However, it is of interest that NP antigen has been demonstrated on

the surface of cells infected with influenza A virus (Virelizier *et al.*, 1977) and this antigen might be available for participation in antibody or cell-mediated immunological events. Indeed, T-lymphocytes, cytotoxic for influenza A virus infected target cells can be detected in the spleens of mice immunized with purified NP antigens (F. Ennis and G. C. Schild, unpublished).

(b) *The matrix (M) protein antigen*

The M protein is the most abundant component of the influenza virus particle and is situated just beneath the outer lipid envelope of the virus. Of the protein components of the virus particle the M protein has the lowest mol. wt (25 000 daltons). Like the NP, the M protein is of type-specific antigenic specificity (Schild, 1972). All influenza A viruses possess antigenically common M antigens which are distinct from those of influenza B viruses. No convincing evidence of antigenic variability of M protein has been reported. Identification of M protein by IDD tests is used as a routine method for typing of influenza virus isolates (Dowdle *et al.*, 1974a).

Antibody to M protein does not induce protection against influenza-infected mice (Virelizier *et al.*, 1976; Oxford and Schild, 1976). However, it has been shown (Webster and Hinshaw, 1977) that in mice immunized with influenza A, M protein 'cleared' influenza A virus more rapidly from their lungs but they developed pneumonia that was at least as severe as in non-immunized control mice. Biddison, Doherty and Webster (1977) and Braciale (1977) have demonstrated M protein on the surface of infected cells and it has been shown that mice immunized with purified M antigen from influenza

FIG. 1. Immuno-double-diffusion reaction of influenza antigens and antibodies. The figure illustrates immuno-double-diffusion reactions in agar gel (Schild, 1972) of antisera to the NP and HA antigens of influenza A virus.

Antigens: Throughout Fig. 1 (a—d), wells marked A34, A68, A73, A77 (H_3N_2), A77 (H_1N_1), D/U/63 and B73 contained influenza virus antigens A/PR8/34 (H_0N_1), A/Hong Kong/68 (H_3N_2), A/Port Chalmers/73 (H_3N_2), A/Texas/1/77 (H_3N_2), A/USSR/77 (H_1N_1), A/duck/Ukraine/63 (Hav, Neq₂) and B/Hong Kong/8/73 viruses.

Antisera: The well marked anti-NP contained immune goat serum to purified NP antigen derived from A/Port Chalmers/73 (H_3N_2) virus (Schild, Oxford and Newman, 1978) and that marked anti-HA contained goat serum to purified HA antigen of A/Hong Kong/68 (H_3N_2) virus. Wells marked CR(68) and SS(68) contained cross-reactive and strain-specific antibodies derived from immune goat serum to the HA of A/Hong Kong/68 (H_3N_2) virus as described by Schild, Oxford and Virelizier, 1976.

(a) The reactions of antiserum to purified A/Port Chalmers/73 NP. It is seen that the two H_3N_2 viruses gave reactions of immunological identity, indicating that they contain antigenically common NP, whilst for A/PR8/34 virus and A/duck/Ukraine/6 virus 'spur' formation indicated antigenic differences between their NP antigens and those of the H_3N_2 viruses.

(b) The reactions of antiserum to purified A/Hong Kong/68 HA. 'Spur' formation indicates antigenic differences (antigenic 'drift') between the HA antigens of the homologous virus and that of A/Port Chalmers/73 and A/Texas/1/77. In contrast A/USSR/77 (H_1N_1) virus gave no reaction indicating that its HA antigen was antigenically completely unrelated to that of H_3N_2 viruses.

(c) and (d) The reactions of SS and CR antibodies to A/HK/68. With CR antibody A/Hong Kong/68 and A/Port Chalmers/73 viruses gave a common reaction whilst in contrast the SS antibody reacted with the homologous virus only.

A virus, developed type-specific cytotoxic T-lymphocyte responses for infected target cells (F. Ennis and G. C. Schild, unpublished).

It is possible that M, or NP, antigens exposed on the surface membrane of influenza virus-infected cells contribute to damaging immunopathological processes mediated by T-lymphocytes. In this context it is of interest that although antibody to M protein is in general rarely detected in human sera, it has been found in the sera of some patients recovering from severe influenza A infections (Mostow *et al.*, 1975; Beare, Cretescu and Schild, 1978) and, much more rarely, in sera of those with infections of mild or moderate severity.

(c) *The surface antigens of the virus, haemagglutinin (HA) and neuraminidase (NA)*

The biological activities, haemagglutination and neuraminidase, of the influenza virus particle reside on separate glycoprotein subunits which form a covering of surface projection on the surface of the virus particles (Laver and Valentine, 1969). The detailed physical and chemical structure of the surface antigens has been reviewed extensively elsewhere (Kilbourne, 1975; Stuart-Harris and Schild, 1976) and needs little description here. The HA is quantitatively the major surface glycoprotein (Skehel and Schild, 1971; White, 1974). It is also the most important surface antigen, and anti-HA antibody has direct neutralizing activity on virus infectivity (Laver and Kilbourne, 1966). The enzyme, neuraminidase, is also antigenic but antibody to NA does not efficiently neutralize virus infectivity. It does, however, inhibit the release of newly formed virus particles from infected cells (Webster and Laver, 1967; Dowdle, Downie and Laver, 1974b).

Antibody to HA and NA are frequent components of the immune response to influenza (Schild and Dowdle, 1975; Schild *et al.*, 1976) and there is an extensive body of evidence that the presence in human sera of antibody to HA and, to a lesser extent, antibody to NA, correlate with protection from influenza infection in man (Stuart-Harris and Schild, 1976). In certain epidemiological situations, antibody to NA may, however, be more closely correlated with protection in man than antibody to HA. This was the case for A/Hong Kong/68 (H_3N_2) virus in 1968 when antibody to the NA, but not to the HA, of the new strain was frequently present in the sera of individuals who had been infected with the formerly prevalent Asian (H_2N_2) virus (Slepuškin *et al.*, 1971). Protection can be a direct manifestation of the presence of circulating antibody to HA, as was clearly demonstrated by Virelizier (1975) who found that mice were fully protected by passive administration of specific antibody to homologous

virus HA antigen even when the immune responses of the recipient mice were inhibited by treatment with immunosuppressive drugs.

Antigenic variation and the origins of new pandemic viruses

One of the most important features of the HA and NA antigens of influenza A viruses is their high degree of antigenic variability. From time to time 'new' antigenic subtypes of influenza A virus bearing novel HA or NA antigens appear in man (Table 2). When this occurs, the previously prevalent influenza A virus characteristically ceases to circulate. The term antigenic 'shift' has been used to describe the emergence of new influenza A viruses, and such an event, when it occurred in 1918, 1957 and 1968, was accompanied by the onset of influenza pandemics. In between antigenic 'shifts', minor but progressive antigenic modification occurs in the HA and NA antigens of the prevalent influenza A viruses. The term antigenic 'drift' has been used to describe this type of antigenic variation which is due to the selection of antigenic mutants during transmission of the virus in a partially immune population. Thus the HA and NA antigens of A/Texas/77 (H_3N_2) virus, the currently prevalent virus of the Hong Kong (H_3N_2) subtype, show considerable differences from those of the prototype A/Hong Kong/68 (H_3N_2) virus which first appeared in man in 1968 – although both viruses can be clearly identified by IDD (Fig. 1b) and other tests, as belonging to the same antigenic subtype, H_3N_2 .

There is circumstantial evidence (Stuart-Harris and Schild, 1976) that the causative virus of the 1918 pandemics was antigenically closely akin to the 'swine', Hsw $_1N_1$, influenza A virus which has been epidemic in pigs in the U.S.A. for many years and probably since 1918. Between 1918 and 1956 the HA and NA antigens of the prevalent viruses underwent progressive antigenic 'drift' (Table 2). Although the viruses prevalent from 1933 to 1946 and 1947 to 1957 have been designated H_0N_1 and H_1N_1 (WHO, 1971) they have been shown to be antigenically related (Schild, 1970), and with Hsw $_1N_1$ virus should be regarded as a single subtype. In this respect it seems desirable to update the current system of nomenclature of influenza viruses so as to include these viruses within a single subtype designation. In 1957, the Asian (H_2N_2) virus appeared; it was first isolated in China followed by rapid international spread. The HA and NA molecules of the Asian virus were antigenically completely different from those of the H_1N_1 virus. The Asian virus was prevalent until 1967, and between 1957 and 1967 underwent considerable antigenic 'drift' until in 1968 it was replaced by the newly emerged Hong Kong (H_3N_2) virus. The HA antigen of the Hong

TABLE 2. Prevalence of antigenic subtypes of influenza A virus in man, 1918-1978

Antigenic designation: haemagglutinin (H) and neuraminidase (N) antigen subtype	Period of prevalence	Type of antigenic variation*	Probable mechanism of origin of new virus
Hsw ₁ N ₁ † H ₀ N ₁ H ₁ N ₁	1918-1957	Shift progressive antigenic drift	Unknown, but Hsw ₁ N ₁ virus was probably epidemic in pigs in the USA as early as 1918
H ₂ N ₂	1957-1968	Shift progressive antigenic drift	Genetic recombination between former human H ₁ N ₁ virus and animal influenza A virus from animal or avian sources with surface antigens H ₂ N ₂ . Genes for H ₂ N ₂ transferred to new pandemic virus
H ₃ N ₂	1968 and still prevalent	Shift progressive antigenic drift	Genetic recombination between former human H ₂ N ₂ virus and an influenza A virus from animal or avian sources with surface antigens H ₃ N ₂ . Gene for H ₃ transferred to new pandemic virus
Hsw ₁ N ₁	1976	reappearance	Limited spread of swine Hsw ₁ N ₁ virus from pigs to military recruits in U.S.A.
H ₁ N ₁	1977 and still prevalent	reappearance of 1950 H ₁ N ₁ virus	Derived from 1950 H ₁ N ₁ virus preserved in nature by unknown mechanism

* The appearance of new viruses with completely new H and N antigens as in 1957 and 1968, has been termed antigenic 'shift', whilst minor, progressive antigenic changes are termed antigenic 'drift'.

† Although Hsw₁N₁, H₀N₁ and H₁N₁ viruses have different designations they contain related H and N antigens and were derived one from another antigenic 'drift'.

Kong virus was distinct from that of the Asian virus but its NA antigen was antigenically close to the NA of the Asian viruses isolated in 1967 (Coleman *et al.*, 1968). Thus, the emergence of the Hong Kong virus involved antigenic 'shift' in the HA, but not the NA antigen. There is much circumstantial evidence (Webster and Laver, 1975; Kaplan and Webster, 1977) to support the concept that the Asian virus arose as a result of genetic recombination (Table 2) between the prevalent human H₁N₁ influenza A viruses and another influenza A virus, possibly of animal or avian origin, bearing surface antigens H₂N₂. Similarly, the Hong Kong virus possibly arose as a result of recombination between the Asian (H₂N₂) virus and a virus from animal or avian sources bearing surface antigens H₃N₂. The recent studies of Scholtissek *et al.* (1977, 1978, and personal communication) using cRNA-vRNA hybridization techniques for the analysis of the gene segments of influenza A virus strains provide strong support for this concept of the origin of the Asian and Hong Kong viruses.

Reappearance of swine Hsw₁N₁ influenza in man in 1976

In 1976 a limited outbreak of infection due to Hsw₁N₁ virus occurred in a military camp in New Jersey, U.S.A. (WHO, 1976). Antigenic and genetic characterization of the virus, A/New Jersey/56

(Hsw₁N₁), isolated from military recruits, supported the view that the human infections occurred as a result of spread of virus from infected pigs to man (Kendal, Noble and Dowdle, 1977; Hinshaw *et al.*, 1978; Palese and Schulman, 1976b). These studies provided the only definitive evidence for a direct role of animal influenza A viruses in human influenza.

Reappearance of H₁N₁ influenza virus in 1977

Less readily interpreted was the sudden reappearance of H₁N₁ influenza virus in man in 1977. In May 1977 influenza A viruses were isolated from young persons in the Northern region of the People's Republic of China. The virus spread rapidly, and by early autumn influenza had reached epidemic proportions in China. The epidemic had a number of notable features. Firstly, the causative influenza A virus was identified as belonging to the H₁N₁ subtype of influenza which was prevalent 20 to 30 years previously from 1947 to 1957, until its abrupt disappearance in 1957 when it was replaced by the newly emerged Asian (H₂N₂) influenza virus. Secondly, the outbreak appeared to affect only young persons, in particular school children and others under the age of 23 years, but in these groups it had an apparently high attack rate.

The H₁N₁ virus was detected in the Philippines in June 1977, and by November had spread to the

U.S.S.R., Hong Kong and Singapore. Spread in the U.S.S.R. was from the far East to the West; the first outbreak was noted amongst naval cadets in the region of Khabarovsk, and outbreaks in Moscow and Leningrad were identified some weeks later. The virus appeared to spread with great efficiency around the world, for by January it had been detected in many European countries including the U.K., in several Asian countries and in the U.S.A. In March 1978 it was detected in Australia and by July 1978 had been isolated in several South American countries. The great similarities in the epidemiological reports from different countries of high attack rates among children and young adults were striking. Nowhere were significant numbers of infections reported to occur in older persons, and no country reported increased mortality rates associated with this virus.

The 'new' H_1N_1 viruses were compared antigenically with historic H_1N_1 viruses, isolated from 1947 to 1957, using conventional antigenic analyses of the virus surface antigen molecules, HA and NA. The results of these tests indicated a close antigenic resemblance between the haemagglutinin and neuraminidase antigens of the 'new' H_1N_1 strains and those of a virus isolated in the U.S.A. in 1950, A/Fort Warren/50 (H_1N_1) (WHO, 1978).

Further comparisons of the 'new' and 'old' H_1N_1 viruses were performed using methods to analyse the genetic composition of the virus. Nakajima, Desselberger and Palese (1978), using oligonucleotide mapping techniques of virus RNA, found evidence of a high degree of similarity between the genomes of the A/Fort Warren/50 and the 1977 H_1N_1 viruses. The conclusion from these studies was that all 8 gene segments of the 1977 H_1N_1 virus were similar to those of the A/Fort Warren/50 strain.

The important question arising from these findings is how the 1950 virus survived, genetically unchanged, for a period of 27 years. There are a number of alternative possibilities to consider. Firstly, the H_1N_1 virus might have been preserved in the frozen state under natural conditions or in a laboratory. The strain may have 'escaped' during laboratory work after preservation in a laboratory deep freeze but virologists from China and the U.S.S.R. have stated that H_1N_1 viruses were not available in any laboratory in the areas where the outbreaks were first noted. A second possibility is that the virus has existed for 27 years in an animal or bird reservoir, perhaps in China, without undergoing genetic change. However, antigenic variation and genetic heterogeneity occurs amongst influenza A viruses in animals and birds and it would seem unlikely that persistence of the virus over many years in an animal or avian species in infective form would be consistent with the high degree of conservation

of the genome indicated by the serological and molecular biological studies. Moreover, despite intensive studies of ecology of influenza in animals and birds in several countries, H_1N_1 viruses have not been detected so far.

A third possibility is that the genome of virus has been preserved in stable form by latent infection in human or animal cells and subsequently reactivated. It is conceivable that complementary DNA copies of influenza RNA molecules might become integrated in cell DNA and carried along during cell replication until reactivated by an unknown mechanism, but as yet there is no convincing experimental proof that the influenza virus genome can persist in this way. Clearly several other possibilities exist including persistence of the virus genome in other organisms which have not yet been considered and evaluated as potential hosts for influenza virus. When new pandemic influenza A viruses have emerged in the recent years, as in 1957 and 1968, they appear to have done so in China which is also the most probable place of reappearance of H_1N_1 virus in 1977. It is therefore tempting to seek mechanisms for the biological 'storage' of human influenza viruses which would be of particular relevance to that country (Kaplan and Webster, 1977).

The immunology of haemagglutinin

The HA molecule is antigenically complex and bears at least 2 sets of antigenic determinants. One set of determinants is antigenically 'common' within a single subtype of HA and the antibody corresponding to these determinants reacts broadly with HA antigens of all members of the subtype irrespective of antigenic 'drift' (Virelizier *et al.*, 1974a; Laver, Downie and Webster, 1974; Schild *et al.*, 1976). This population of anti-HA antibody molecules has been termed 'cross-reactive' (CR). In addition, a further population of anti-HA antibody is relatively strain-specific (SS), reacting only with the HA antigens of the homologous or very closely related virus strains but not with the HA of antigenic variants showing considerable antigenic 'drift' from the homologous virus (see Fig. 1c and d).

In mouse protection studies Virelizier (1975) found that CR antibody molecules were only weakly protective against influenza infection when passively administered to mice whilst SS antibodies conferred complete protection. Other studies (Schild *et al.*, 1976; L. Haaheim and G. C. Schild, unpublished) have shown that CR and SS antibodies differed considerably in their capacity to neutralize virus infectivity (Table 3). In these investigations, IgG molecules of CR specificity were found to be 10–1000-fold less effective in virus neutralizing activity than was strain-specific IgG. It was shown by

TABLE 3. Biological properties of antibody to strain-specific and cross-reactive determinants of the HA molecules of A/Hong Kong/68 (H₃N₂) virus and its 1973 variant, A/Port Chalmers/1/73 (H₃N₂)

Specificity of antibody	Antibody elicited by	Biological reactivity of antibody							
		Tests with A/HK/1/68 virus				Tests with A/PC/1/73 virus			
		HI	VN	SRDT +	$\frac{VN}{SRDT}$	HI	VN	SRDT	$\frac{VN}{SRDT}$
Strain specific	A/HK/1/68 HA	5.4	5.8	57	10 ^{4.0}	<1.0	<1.0	0	—
	A/PC/1/73 HA	<1.0	<1.0	0	—	5.2	5.5	31	10 ^{4.0}
Cross reactive	A/HK/1/68 HA	3.2	2.8	43	10 ^{1.2}	2.9	2.6	45	10 ^{0.0}
	A/PC/1/73 HA	2.9	2.5	28	10 ^{1.1}	3.2	2.9	26	10 ^{1.5}

HI = haemagglutination inhibition titres expressed as log₁₀.

VN = virus neutralization titres in eggs versus 100 egg-infective doses 50% of virus, titres expressed as log₁₀.

SRDT = zone annulus area of reaction (mm²) in single-radial-diffusion tests (Schild, Aymard-Henry and Pereira, 1972). A measure of the specific activity of serum in virus neutralization tests is given by the ratio VN/SRDT.

Antibody preparations obtained as described by Schild, Oxford and Virelizier, 1976.

Virelizier *et al.* (1974a) that antibody formation in mice to both CR and SS determinants was thymus-dependent requiring the helper effect of T-lymphocytes. However, the thymus dependence of SS antibody production was more pronounced than that of the CR population, since production of CR, but not of SS antibody, could be obtained after repeated immunization of thymectomized, X-irradiated, bone-marrow reconstituted (TXBM) mice. These studies indicated an indirect role of T-lymphocytes in antibody response to influenza virus HA. In contrast to the thymus dependence of antibody formation, the development of antigenic memory for HA antigens in mice was shown to be thymus-independent (Virelizier *et al.*, 1974a; Virelizier, Allison and Schild, 1974b). T-lymphocytes, at least in mice, appear to be essential in providing a 'triggering' mechanism to enable the transformation of primed HA antigen-primed B-lymphocytes into antibody secreting cells.

The term 'original antigenic sin' has been used (Davenport, Hennessy and Francis, 1953) to describe the phenomenon which occurs when sequential infection of a host occurs with 2 different but antigenically related strains of influenza A virus. The anti-HA antibodies produced as a result of the second ('booster') infection react more readily with the virus encountered in the first ('priming') infection than with the 'booster' virus which stimulated the response. Analysis of this system in mice (Virelizier *et al.*, 1974b) showed that when mice were 'primed' by immunization with the HA antigen of one virus (A/PR8/34 (H₃N₁)) and 'boosted' with antigenically variant HA from A/FM1/47 (H₃N₁) virus, the secondary antibody response involved an increase of CR antibodies, reacting with the HA antigens of both virus strains. In addition, SS antibodies directed against strain-specific HA determinants of the primary virus (A/PR8/34) were stimulated by

the booster injection. This was a paradoxical immune response since SS antigenic determinants of the priming virus were not present in the 'boosting' antigen (Virelizier *et al.*, 1974a). Further studies (Virelizier *et al.*, 1974b) indicated that the secondary response was dependent upon B-lymphocytes.

Since CR and SS antibodies differ markedly in their protective properties as described above, this paradoxical 'recall' phenomenon may have important implications for immunity to influenza in man. In the interpandemic periods individuals are sequentially infected with successive variants of influenza A virus which arise as a result of antigenic 'drift'. The 'priming' effect of infection with the first encountered member of a series of antigenic variants may 'condition' the immunological system so that when later variants are encountered, the antibody response includes production of anti-HA antibody of CR specificity and, in addition, SS antibody directed against the SS determinants to the HA of the first strain. This latter antibody would be redundant in its protective effect. Evidence that this situation actually occurs during natural influenza infection in man has been recently obtained (Oxford, Schild and Jennings, 1978b). In this study persons who were naturally infected with A/Port Chalmers/73 (H₃N₂) virus in 1974, and would be expected to have been antigenically primed by previous infections with A/Hong Kong/68 (H₃N₂) virus, were studied. As a result of the A/Port Chalmers/73 infection they developed CR antibody, reacting equally well with the HA antigens of the 1974 and 1968 viruses, and SS antibody directed against the A/Hong Kong/68 virus but they rarely produced SS antibody to the A/Port Chalmers/73 virus.

Similar studies were also performed to establish the nature of the anti-HA antibody response following immunization of human adults with inactivated A/Port Chalmers/73 vaccines in 1974 (Schild *et al.*,

TABLE 4. Development of cross-reactive (CR) anti-H₃ antibodies in human vaccinees receiving inactivated A/Port Chalmers/73 vaccine

Vaccine dose	No. of vaccinees tested	No. and percentage of vaccinees with CR antibody detectable				* Mean zone area of CR antibody reaction (mm ²)	
		Pre-vaccine		Post-vaccine		Pre-vaccine	Post-vaccine
		No.	(%)	No.	(%)		
100 i.u.	44	23	(52)	39	(89)	2.7	4.8
400 i.u.	39	22	(56)	35	(91)	2.9	6.6
1600 i.u.	40	22	(55)	37	(93)	2.1	9.5
All doses	123	67	(54)	111	(90)	2.5	6.9

* Antibody detected by single-radial-haemolysis as described by Schild, Pereira and Chakraverty (1975).

1977). In this case the antibody elicited by vaccination included production of CR antibodies in most recipients (Table 4). In addition, many individuals developed SS antibody (Table 5) but this was more frequently directed against the SS determinant of A/Hong Kong/68 HA than against the HA of the vaccine virus. The specificity of the antibody response was independent of vaccine dose and this situation was not greatly changed when the vaccinees were given a second dose of A/Port Chalmers/73 vaccine one year later. Very few subjects developed SS antibodies against both A/Hong Kong/68 and A/Port Chalmers/73 viruses, suggesting that ability to produce SS antibody against one strain had to some extent a 'blocking' effect on development of SS antibody to the other. These findings have been extended to studies of antibody responses in man to inactivated vaccines containing a further antigenic variant of H₃N₂ virus A/Victoria/3/75 in 1976 (Oxford *et al.*, 1978b; A. Hanson and G. C. Schild, unpublished). In these studies CR antibody was again the most common type of antibody produced and SS antibody to the HA of the vaccine virus was produced infrequently. Hanson and Schild detected the production of CR antibody in 88% of vaccinees, but SS antibody for the vaccine strain was detected in only 35% of vaccinees. Although the HA antigens of A/Hong Kong/68 and A/Victoria/3/75 are distantly related, vaccination stimulated acquisition or a rise in level of SS antibody to the HA of A/Hong Kong/68 virus in 16% of vaccinees whilst 75% possessed SS antibody to this virus at levels which were unaffected by immunization.

The above findings indicated that natural infection and immunization may often induce antibody responses which are 'outdated' in terms of immunity to the virus strain inducing them and may, in part, explain the epidemiological success of influenza and also the incomplete success of attempts at the prophylaxis of influenza by inactivated vaccines. In the sphere of vaccination it is important to determine if the efficacy of influenza vaccines

could be improved if new methods of presentation of influenza HA antigens were devised which ensured a higher efficiency of stimulation of homologous SS antibodies.

The role of cell-mediated immune factors in protection against influenza is at present unknown. Immunization of mice with influenza results in the development of cytotoxic T-lymphocytes which are able to destroy 'target' cells infected with influenza virus. The studies of Doherty, Effros and Benninck (1977) indicated that the T-lymphocytes were of wide specificity reacting with target cells infected with any influenza A virus, but not with influenza B. In contrast, Ennis, Martin and Verbenitz (1977) found evidence of cytotoxic 'T'-lymphocytes which were HA subtype specific. These findings may reflect differences in the nature of the influenza antigens exposed on the surfaces of the various target cells used in the studies. Whether such T-cell responses to HA antigens exist in man and, if so, whether they would be beneficial, contributing to protection, or disadvantageous, contributing to immunopathological phenomena, requires investigation. Certainly there are a number of instances where protection in man or experimental animals cannot be readily attributed to circulating antibody to the HA or NA. Firstly, mice previously infected with one subtype of influenza A virus show partial immunity to challenge with another influenza A subtype bearing entirely different surface antigens (Schulman and Kilbourne, 1965; Werner, 1966). Secondly, in some studies, live influenza vaccines have appeared to confer significant protection against homologous or closely related influenza A viruses even when levels of circulating antibody to HA or NA were low or undetectable. Thirdly, the H₁N₁ virus when it reappeared in 1977 did not infect persons over the age of 23 years or so. Older individuals appear to have been protected because of infection with H₁N₁ viruses some 20-30 years previously, but in many such older individuals antibody to the HA or NA antigens of the 1977 H₁N₁ are absent or at

TABLE 5. Development of strain-specific (SS) anti-H₃ antibody in sera* of human vaccinees receiving inactivated A/Port Chalmers/73 vaccine

Vaccine dose	No. of vaccinees tested	Number and percentage of vaccinees with SS antibody of stated strain specificity†											
		SS _{HK} †			SS _{PC} †			SS _{HK} only			SS _{PC} only		
		Pre-vac.		Post-vac.	Pre-vac.		Post-vac.	Pre-vac.		Post-vac.	Pre-vac.		Post-vac.
		No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
100 i.u.	45	6 (13)	30 (67)	1 (2)	10 (22)	6 (13)	29 (64)	1 (2)	9 (20)	0	1 (2)		
400 i.u.	40	6 (15)	28 (70)	0	9 (23)	6 (15)	27 (68)	0	8 (20)	0	1 (2)		
1600 i.u.	42	5 (12)	26 (62)	2 (5)	14 (33)	5 (12)	24 (57)	2 (5)	12 (27)	0	2 (5)		
All doses	127	17 (13)	84 (66)	3 (2)	33 (26)	17 (13)	80 (63)	3 (2)	29 (23)	0	4 (3)		

* Antibody assays by single-radial-haemolysis tests on adsorbed sera as described by Schild, Pereira and Chakraverty (1975).

† SS_{HK} represents antibody directed against strain-specific antigenic determinants of the HA of A/Hong Kong/68/H₃N₂.SS_{PC} represents antibody directed against strain-specific antigenic determinants of the HA of A/Port Chalmers/73 (H₃N₂), the vaccine virus.

levels which are undetectable by conventional techniques. Thus it seems possible that mechanisms, other than the presence of circulating antibody to HA or NA, may contribute to immunity to influenza infections. Whether immunological memory phenomena, such as that described for antibody production in mice by Virelizier *et al.* (1974b), or cellular immune processes (Doherty *et al.*, 1977; Ennis *et al.*, 1977), contribute to such unexplained protection remains to be established.

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