J. Hyg., Camb. (1970), **68**, 497
Printed in Great Britain

Changes in the antibody status of a population following epidemic infection by influenza virus A2/Hong Kong/1/68

By S. J. MACHIN, C. W. POTTER and J. S. OXFORD
Department of Medical Microbiology and Virus Research Laboratory, Lodge Moor Hospital, The University of Sheffield

(*Received* 24 *April* 1970)

SUMMARY

The haemagglutinin of influenza virus A2/Hong Kong/1/68 was shown to be markedly different from that of previously isolated A2 virus strains. No haemagglutination-inhibiting (HI) antibody to A2/Hong Kong/1/68 virus was detected in serum specimens collected in 1966 from persons aged 60 years or less. In contrast, HI antibody tests with 270 sera collected in 1968 indicated that 9·6% had demonstrable HI antibody at low titres, and 35·2% of 454 postepidemic (1969) sera had demonstrable HI antibody at relatively high titres. Most sera from persons aged 80 years and more collected in 1968 and 1969 had demonstrable HI antibody to influenza virus A2/Hong Kong/1/68. No HI antibody to the Hong Kong virus was detected in pre-epidemic sera from children aged 6 months to 3 years, whereas 32% of postepidemic sera had HI antibody. The acquisition of HI antibody to A2/Hong Kong/1/68 was not accompanied by an increase in the incidence or titres of HI antibody to heterotypic A2 influenza viruses. For sera from children aged 4–11 years, an increase of HI titre to heterotypic A2 influenza was found.

INTRODUCTION

Although a number of studies have reported the distribution in the population of antibody to influenza viruses, and the relationship of these findings to the sequential appearance of the different families of influenza A virus (Davenport, Hennessy & Francis, 1953; Schild & Stuart-Harris, 1965), little attention has been given to the effects of minor antigenic variations occurring in interpandemic periods. Because of extensive cross-reactions between influenza A viruses of the same family, interpretation of serological data is difficult. Zhdanov (1967) suggested that population studies of serum antibody to interpandemic influenza A viruses may allow prediction of the antigenic nature of future epidemic strains. Thus, a high incidence of antibody would indicate an immune population while the absence of antibody might indicate the likelihood of the virus, or closely related virus, giving rise to an epidemic in the future. Schild & Stuart-Harris (1967) suggested that such studies were more likely to give definitive information if sera from children were used, because as a result of their shorter time of exposure to infection, the pattern of influenza antibody is more restricted in children than in adults.

The arrival of the Hong Kong variant of A2 influenza virus in the United Kingdom during 1968 allowed a serological study of the epidemiology of this pandemic virus in the population. The incidence of haemagglutination-inhibiting antibody to influenza virus A2/Hong Kong/1/68 was estimated in sera collected in 1966, 1968 and 1969 from both children and adults; the largest number were collected shortly before and after the outbreak in 1968 and early 1969. Sera were also tested for HI antibody to four other representative influenza A2 viruses.

METHODS AND MATERIALS

Viruses

Influenza viruses A2/Singapore/1/57 and A2/England/12/64 were the same as used in a previous study (Schild & Stuart-Harris, 1967). Influenza viruses A2/England/10/67; A2/Tokyo/1/67; A2/Hong Kong/1/68 and a recombinant strain containing Fowl Plague haemagglutinin and A2/Hong Kong/1/68 neuraminidase (FPV/A2HK) were obtained from Dr G. C. Schild, World Influenza Centre, Mill Hill, London. Virus pools were prepared in 10-day embryonated eggs by allantoic inoculation of 10^{-3} dilution of seed virus. After 48 hr. incubation at 35° C., allantoic fluids were harvested and stored at − 80° C.

Serum specimens

Sera were obtained in the periods of May–June 1966, September–October 1968 and May–July 1969, from blood donors, women attending antenatal clinics, and from specimens submitted for Wasserman tests. Seventy-nine sera were obtained in August–November 1968, and 75 in May–June 1969 from children admitted to Sheffield Children's Hospital for treatment of accidents and burns, and from specimens submitted for antistreptolysin 'O' testing. All human sera were from persons living in the Sheffield Hospital region. They were stored at − 20° C.

Antisera were obtained by inoculating adult ferrets intranasally with 0·5 ml. of live, egg-grown influenza virus. Ferrets were bled 3–4 weeks after virus infection.

Haemagglutination inhibition (HI) tests

HI tests were carried out in Perspex plates (W.H.O., 1953). Before test, sera were incubated for 18 hr. at 37° C. with five volumes of cholera filtrate (N. V. Philips Duphar, Amsterdam) and subsequently heated at 56° C. for 1 hr. Twofold dilutions of serum in 0·2 ml. volumes were mixed with an equal volume of virus containing eight haemagglutinating units (50 % end-point). After 10 min. incubation at room temperature, 0·2 ml. of fowl erythrocytes (0·5 % suspension in phosphate-buffered saline, pH 7·4) were added, and the HI titre read from the pattern of haemagglutination produced after the cells had settled at room temperature. Fowl erythrocytes were obtained from a single fowl bled at weekly intervals.

RESULTS

Comparison of A2 influenza viruses by HI tests

Table 1 shows the HI antibody titres of five ferret antisera tested against homologous and heterologous A2 influenza viruses. The results indicate that influenza

virus A2/Hong Kong/1/68 represents a large antigenic deviation from previously isolated A2 virus strains. Ferret antisera prepared against heterologous A2 influenza viruses showed only low titres of haemagglutination-inhibiting antibody when tested against A2/Hong Kong/1/68 virus. Similarly, the A2/Hong Kong/1/68 ferret antiserum had only low titres of HI antibody against the four heterologous A2 influenza viruses. Influenza viruses A2/England/12/64 and A2/England/10/67 appeared to be closely related antigenically, while the virus strain A2/Tokyo/1/67 was recognizably distinct from the former two virus strains. All four influenza viruses isolated from 1964 to 1968 were distinct from the prototype influenza virus A2/Singapore/1/57.

Table 1. *Haemagglutination inhibition titres of ferret antiserum against homologous and heterologous A2 influenza viruses*

	Ferret antisera against influenza virus				
Influenza viruses	A2/Sing/1/57	A2/Eng/12/64	A2/Eng/10/67	A2/Tokyo/1/67	A2/HK/1/68
A2/Sing/1/57	**1280***	320	320	< 10	10
A2/Eng/12/64	40	**1960**	960	480	40
A2/Eng/10/67	80	1960	**2560**	320	80
A2/Tokyo/1/67	15	160	320	**1960**	40
A2/HK/1/68	30	20	60	15	**640**

* Reciprocal HI titre.

Table 2. *Incidence of haemagglutination inhibition antibody to influenza virus A2/Hong Kong/1/68 in human sera collections*

Date of serum	No. tested	No. positive	Positive (%)	Mean titre
June–Aug. 1966	97	0	0	—
Sept.–Oct. 1968	270	26	9·6	1/26
May–July 1969	454	160	35·2	1/175

Incidence in human sera of HI antibody to influenza virus A2/Hong Kong/1/68

Sera collected during 1966, the immediate pre-Hong Kong influenza virus period of 1968, and also after the Hong Kong influenza in 1969, were each titrated for HI antibody to influenza virus A2/Hong Kong/1/68. The results are shown in Table 2. No HI antibody at a titre of 1/6 was found in 97 serum specimens collected in 1966. These sera were all obtained from persons under 60 years of age at the time of collection, 68 samples being from adults aged 20 years or more. Among 270 sera from 1968, 26 (9·6%) contained HI antibody to influenza virus A2/Hong Kong/1/68 at a titre of 1/6 or greater. Actual antibody titres were relatively low, the arithmetic mean titre was 1/26, and only one specimen contained antibody at a titre of greater than 1/96. The distribution by age of HI antibody positive sera is shown in Fig. 1. In contrast, of 454 postepidemic serum specimens collected in 1969, 160 (35·2%) contained HI antibody to influenza virus A2/Hong Kong/1/68 at a titre of 1/6 or greater, and the arithmetic mean titre of the HI antibody positive

https://doi.org/10.1017/S0022172400042406 Published online by Cambridge University Press

sera was 1/175. Of the serum specimens with HI antibody, 70 (44 %) showed titres of 1/96 or greater.

Fig. 1. shows the incidence of HI antibody to influenza virus A 2/Hong Kong/1/68 at 1/6 or greater in pre- and postepidemic serum specimens, grouped by age. No HI antibody to influenza virus A 2/Hong Kong/1/68 was detected in sera collected in 1968 from persons aged 9 years or less. Variable percentages of sera with HI antibody to Hong Kong influenza virus (3–19 %) were found in the age groups 10–79 years. In the postepidemic sera collected in 1969, the incidence of HI antibody increased with age from 30 % in children of 9 or less to a maximum of 40 % for persons aged 20–29 years. It declined in each older decade to 21 % for persons aged 60–69 years and 24 % in those of 70–79 years.

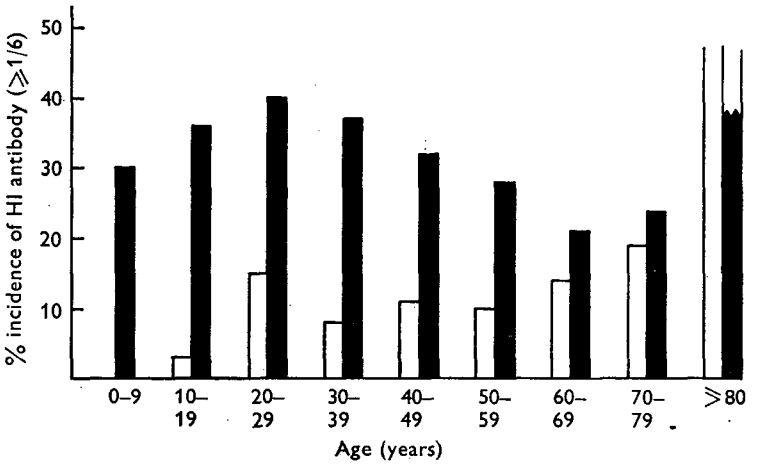

Fig. 1. Percentage incidence of haemagglutination-inhibition (HI) antibody to influenza virus A 2/Hong Kong/1/68 in human sera. □, Pre-epidemic (1968) sera; ■, postepidemic (1969) sera.

Only three pre-epidemic (1968) sera were available from persons aged 80 years or more; these contained HI antibody at titres of 1/18, 1/24 and 1/36. In view of the small number of samples, ten further sera collected in 1964 from persons aged more than 80 years were tested; eight contained HI antibody. Fourteen postepidemic sera (1969) were available from persons aged 80 years or more, and 12 contained detectable HI antibody to influenza virus A 2/Hong Kong/1/68. The arithmetic mean titre of these HI positive sera was 1/28; this finding was in striking contrast to the results obtained for younger persons (Table 2).

Relationship of influenza virus A 2/Hong Kong/1/68 HI antibody to HI antibody against other A 2 influenza viruses

A possible explanation for the small number of serum specimens collected in 1968 with HI antibody to influenza virus A 2/Hong Kong/1/68, as well as the relatively low titre of this antibody, was that inhibition might be due to cross-reacting antibody to heterologous A 2 influenza viruses. To investigate this possibility, sera with and without HI antibody to A 2/Hong Kong/1/68 influenza virus

were tested for HI antibody to other A 2 influenza viruses. The results are shown in Table 3. Among sera with HI antibody to A 2/Hong Kong/1/68, the arithmetic mean titre of HI antibody to A 2/Singapore/1/57; A 2/England/12/64; A 2/England/10/67 and A 2/Tokyo/1/67 was 1/71; 1/24; 1/22 and 1/23, respectively. The corresponding titres against these same viruses in sera without detectable HI antibody to A 2/Hong Kong/1/68 were 1/19; 1/16; 1/10 and 1/22, respectively. Thus, sera containing HI antibody to A 2/Hong Kong/1/68 had higher mean titres of HI antibody to three of the four A 2 influenza viruses tested than sera without demonstrable HI antibody to A 2/Hong Kong/1/68.

Table 3. *Haemagglutination-inhibition (HI) antibody to A 2 influenza viruses in sera with and without HI antibody to influenza virus A 2/Hong Kong*/1/68

	HI antibody to A 2 influenza virus strain			
HI antibody to A 2/Hong Kong/1/68	Virus strain	No. tested	No. positive sera (titres ⩾ 1/6)	Mean HI titre*
Positive (⩾ 1/6)	A 2/Sing/1/57	20	20	71
	A 2/Eng/12/64	17	13	24
	A 2/Eng/10/67	18	16	22
	A 2/Tokyo/1/67	13	13	23
Negative (< 1/6)	A 2/Sing/1/57	40	38	19
	A 2/Eng/12/64	40	31	16
	A 2/Eng/10/67	37	20	10
	A 2/Tokyo/1/67	40	38	22

* Reciprocal of arithmetic mean titre.

A further explanation for the presence of low titres of HI activity against influenza virus A 2/Hong Kong/1/68 in the 1968 sera was non-specific interference in the HI test caused by the presence in the sera of antineuraminidase antibody. A reaction between neuraminidase-inhibiting antibody, acquired during earlier influenza infection by virus with an identical neuraminidase, might cause steric interference of haemagglutination (Webster & Pereira, 1968); this would be interpreted falsely as indicating the presence of specific HI antibody to A 2/Hong Kong/1/68 virus. Therefore, sera collected in 1968 which inhibited A 2/Hong Kong/1/68 virus were tested for their ability to inhibit haemagglutination by the hybrid influenza virus FPV/A 2HK, which has the same neuraminidase as the former virus. No haemagglutination inhibition of this virus was found in the sera at a titre of 1/6 or greater. This result indicated that the presence of inhibitory action against influenza virus A 2/Hong Kong/1/68 in pre-epidemic sera was not due to steric inhibition by antineuraminidase antibody.

HI antibody to A 2 influenza viruses in sera from children

Haemagglutination-inhibition tests using five A 2 influenza viruses were carried out on 79 pre-epidemic sera (1968) and 75 postepidemic sera (1969) from children 6 months to 11 years. Fig. 2 shows that no detectable HI antibody to influenza A 2/Hong Kong/1/68 was found in pre-epidemic sera from children aged 6 months

https://doi.org/10.1017/S0022172400042406 Published online by Cambridge University Press

to 3 years whereas among 35 postepidemic sera, 11 (32%) contained HI antibody at titres of 1/12 or greater. The acquisition of HI antibody to influenza virus A2/Hong Kong/1/68 was not accompanied by an increase in the incidence or titres of HI antibody to other A2 influenza viruses (Fig. 2). No detectable HI antibody to influenza virus A2/Hong Kong/1/68 was found in pre-epidemic sera (1968)

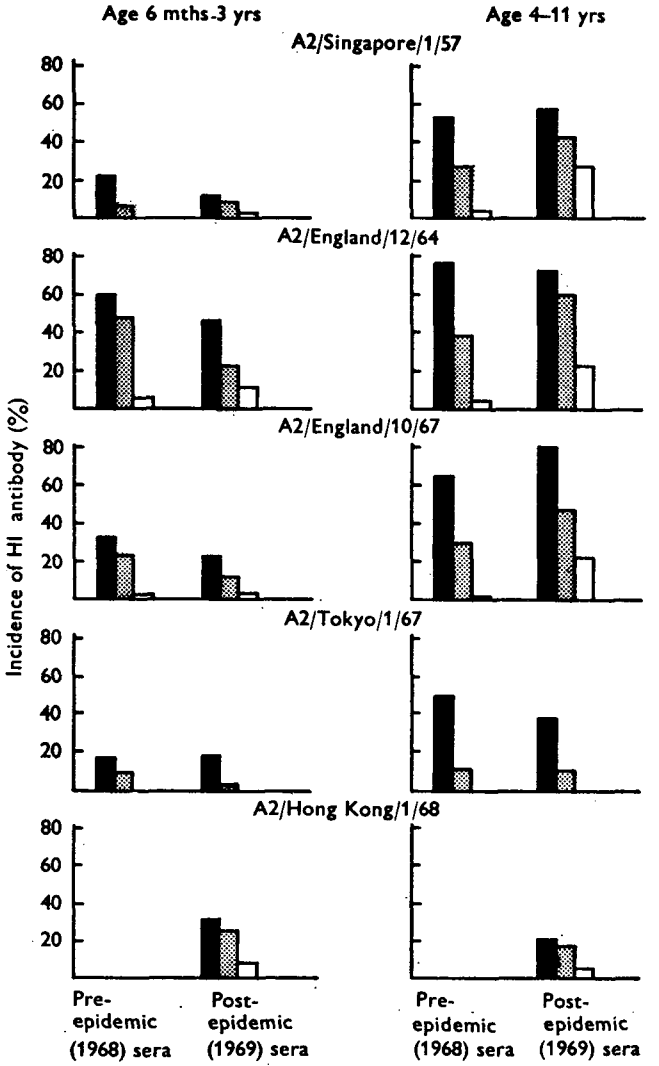

Fig. 2. Percentage incidence of haemagglutination-inhibiting (HI) antibody to A2 influenza viruses in pre- and postepidemic children's sera. HI antibody at titres of: ■, ⩾ 1/12; ▦, ⩾ 1/48; □, ⩾ 1/192.

from children aged 4–11 years, whereas 20% of postepidemic sera (1969) from similarly aged children contained antibody at titres of 1/12 or greater. During the epidemic period there was no significant increase in incidence of HI antibody to other A2 influenza viruses. However, the incidence of HI antibody at titres equal to or greater than 1/48 and 1/192 to influenza viruses A2/Singapore/1/57;

A 2/England/10/64 and A 2/England/12/67, increased during this period. HI antibody titres to influenza virus A 2/Tokyo/1/67 were unaffected by the influenza epidemic of 1968–9.

DISCUSSION

Tests made in our laboratory with five A 2 influenza virus strains and homologous ferret antiserum agreed with the findings of Coleman *et al.* (1968) that the haemagglutinin of influenza virus A 2/Hong Kong/1/68 was widely divergent from that of previously isolated A 2 influenza viruses. Only low levels of cross-reactivity with the other A 2 influenza viruses were found.

The results of haemagglutination tests with pre-epidemic human sera collected in 1968 and influenza virus A 2/Hong Kong/1/68 were different for persons aged less than 11 years, 12–79 years and 80 years or more. The low titres of HI antibody found in sera from persons aged 10–79 years could have three explanations. First, since the neuraminidase of influenza virus A 2/Hong Kong/1/68 is identical with that of earlier occurring influenza strains (Schulman & Kilbourne, 1969), steric inhibition of haemagglutination might have been caused by a reaction between the neuraminidase of influenza virus A 2/Hong Kong/1/68 and serum anti-neuraminidase (Webster & Pereira, 1968). No evidence to support this view was found.

Secondly, the HI activity to influenza virus A 2/Hong Kong/1/68 in pre-epidemic sera from persons aged 10–79 years represented cross-reacting HI antibody induced by previous infecting A 2 influenza viruses. Sera with demonstrable HI antibody to influenza virus A 2/Hong Kong/1/68 had higher mean HI titres to three or four A 2 influenza viruses tested than did specimens with no HI antibody to A 2/Hong Kong/1/68 virus. The largest difference was found for HI antibody to influenza virus A 2/Singapore/1/57, though ferret antiserum against influenza virus A 2/Singapore/1/57 showed little cross-reactivity with A 2/Hong Kong/1/57 virus. If the HI antibody to influenza virus A 2/Hong Kong/1/68 found in sera collected in 1968 was cross-reacting HI antibody, it was acquired since 1966, as sera collected in that year contained no detectable HI antibody against influenza virus A 2/Hong Kong/1/68.

A third explanation is that the introduction of influenza virus A 2/Hong Kong/1/68 into the population predated the serum collection. This explanation cannot be excluded. However, the absence of detectable HI antibody in children less than 11 years of age and the low mean titre of HI antibody in seropositive specimens did not support this view.

Most of the small number of specimens from individuals aged 80 years or more contained HI antibody to influenza virus A 2/Hong Kong 1/68, and this result is in agreement with previous findings (Masurel, 1969; Marine & Workman, 1969; Zakstelskaja, 1969). The presence of pre-epidemic HI antibody to A 2/Hong Kong/1/68 in this age group has been interpreted as evidence of influenza infection by a virus antigenically similar to or identical with this influenza virus in the years 1890–5 (Davenport, Minuse, Hennessy & Francis, 1969). The alternative view that it merely represents heterotypic antibody built up by successive A 2 virus infec-

tions has to be considered. However, the sharp change from a relatively low incidence of HI antibody in those aged less than 80 years to a high incidence for older persons, argues against this view. In addition, Masurel (1969) detected a high incidence of HI antibody to A2/Hong Kong/1/68 virus in sera from persons aged 70 years and more which had been collected before the first A 2 virus isolation in 1957.

No indication of the epidemic caused by influenza virus A 2/Hong Kong/1/68 in 1968–9 could have been obtained from observations of children's sera collected before the epidemic, and using A 2 viruses available at that time. Haemagglutination tests with postepidemic human sera collected in the summer of 1969 showed that only one-third of the population had experienced clinical or subclinical infection by Hong Kong influenza virus. The occurrence of an influenza epidemic in Sheffield in the following winter (1969–70) showed that this figure was indeed too low to give population immunity. Further surveillance of the immune status of the population is required to determine the level of immunity necessary to protect the community against successive influenza epidemics by a particular virus strain.

We wish to thank The Wellcome Trust for support for one of the authors (S.J.M.), and Professor Sir Charles Stuart-Harris and Professor M. G. McEntegart for their advice and criticism, and Mrs. K. Spinks for excellent technical assistance.

REFERENCES

Coleman, M., Dowdle, W. R., Pereira, H. G., Schild, G. C. & Chang, W. K. (1968). The Hong Kong/68 influenza A 2 variant. *Lancet* ii, 1384.

Davenport, F. M., Hennessy, A. V. & Francis, T. Jr. (1953). Epidemiologic and immunologic significance of age distribution of antibody to antigenic variants of influenza virus. *Journal of Experimental Medicine* **98**, 641.

Davenport, F. M., Minuse, E., Hennessy, A. V. & Francis, T. Jr. (1969). Interpretations of influenza antibody patterns of man. *Bulletin of the World Health Organization* **41**, 453.

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

Masurel, N. (1969). Relation between Hong Kong virus and former human A 2 isolates and the A 2/Equi2 virus in human sera collected before 1957. *Lancet* i, 907.

Pereira, M. S., Chakraverty, P., Pane, A. R. & Fletcher, W. B. (1969). The influence of antigenic variation on influenza A 2 epidemics. *Journal of Hygiene* **67**, 551.

Schild, G. C. & Stuart-Harris, C. H. (1965). Serological epidemiological studies with influenza A viruses. *Journal of Hygiene* **63**, 479.

Schild, G. C. & Stuart-Harris, C. H. (1967). Antibodies in children's sera for serologically different recently isolated strains of influenza viruses A 2 and B. *British Medical Journal* iv, 82.

Schulman, J. L. & Kilbourne, E. D. (1969). Independent variation in nature of haemagglutinin and neuraminidase antigens of influenza virus: distinctiveness of haemagglutinin antigen of Hong Kong/68 virus. *Proceedings of the National Academy of Sciences of the United States of America* **63**, 326.

Webster, R. G. & Pereira, H. G. (1968). A common surface antigen in influenza viruses from human and avian sources. *Journal of General Virology* **3**, 201.

World Health Organization (1953). Expert Committee on Influenza. *World Health Organization Technical Report Series* No. 64.

Zakstelskaja, L. Ja. (1969). Influenza in the U.S.S.R.: New antigenic variant A 2/Hong Kong/1/68 and its possible precursors. *American Journal of Epidemiology* **90**, 400.

Zhdanov, V. M. (1967). The 1967 outbreak of influenza B in the U.S.S.R. *Lancet* i, 263.