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A study of acute respiratory disease in the community of Port Chalmers. II. Influenza A/Port Chalmers/1/73: intrafamilial spread and the effect of antibodies to the surface antigens

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SUMMARY

During the first year of a study of respiratory disease in the semi-isolated community of Port Chalmers, New Zealand, an epidemic of clinical influenza occurred from which the variant influenza A/Port Chalmers/1/73 (H3N2) was isolated. Within a selected group of 26 families, 59 (46 %) members had clinical or laboratory evidence of infection. During intrafamilial spread the infection frequency was highest for school-aged children (77 %), followed by female adults (67 %), infants (64 %) and male adults (41 %). The index infection in each family was a school-age child on 10 occasions, suggesting the role of this age group in the transmission of influenza A in this community. The secondary attack rate (SAR) of 58·3 % was higher than expected.

In sera taken before the 1973 epidemic, 59 % of family members had detectable HI antibody and 25 % NI antibody to A/England/42/72 while 38 % had detectable HI antibody and 8 % NI antibody to A/Port Chalmers/1/73. The relation between pre-existing antibody and infection frequency is discussed.

INTRODUCTION

Population immunity is important in the epidemiology of influenza A virus. The familiar sequence of an antigenically distinct subtype appearing and resulting in a world wide pandemic of disease, followed in subsequent years by epidemics of decreasing severity can be thought of as reflecting varying levels of population immunity (Kilbourne, Butler & Rossen, 1973). It is well established that antibody to the haemagglutinin antigens correlate with immunity to infection (Hirst, 1942; Kilbourne, Butler & Rossen, 1973), infection being prevented by virus neutralization. Antibody to the neuraminidase antigens is also important (Webster & Laver, 1971) correlating with resistance to clinical symptoms (Murphy, Kasel & Chanock, 1972) probably by limiting the extent of virus replication, through restricting the release of virus from the infected cell (Schulman, 1970).

An epidemic of clinical influenza occurred in Port Chalmers, New Zealand, during September and October 1973 from which influenza A viruses were isolated.

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Table 1. *Distribution by age of family members*

Age group (years)	No. of members, 1 July 1973
0–4	19
5–9	33
10–14	15
15–20	6
Mothers	26
Fathers	23
Grandparents	5
Total	127

Table 2. *Laboratory evidence of infection obtained in the 59 cases of influenza*

Method	No.	%
Positive by HI	32	54
Positive by CF	23	39
Positive by virus isolation	22	37
Positive by one or more methods	41	69

The surface antigens of the isolate A/Port Chalmers/1/73 (H3N2) differed antigenically from previous H3N2 strains, the antigenic 'drift' having occurred particularly in the neuraminidase antigen (World Health Organization, 1974). This variant was subsequently accepted as a prototype strain.

In this paper we report the activity of the variant as observed in a selected group of families during the initial and largest epidemic, and its subsequent presence in 1974 and 1975. An analysis was also undertaken to examine the effect of A/England/41/72 and A/Port Chalmers/1/73 haemagglutination inhibiting (HI) antibody and neuraminidase inhibiting (NI) antibody on the incidence of infection with the A/Port Chalmers/1/73 variant.

MATERIALS AND METHODS

Study population

A study of the epidemiology of acute respiratory disease in the semi-isolated community of Port Chalmers, New Zealand, was initiated in April 1973 and continued for 32 months. The intensive surveillance of a selected group of 26 families involved the weekly reporting of illness, the collection of specimens for pathogen isolation and the collection of sera at 6-month intervals. The family size ranged from 3 to 7, the mode being 5, and the distribution by age of family members on 1 July 1973 is shown in Table 1. During the study 552 sera were obtained (adults, 301; 5–20 years, 233; 0–4 years, 18). Eighty-six pairs of sera, taken in May and November 1973, were available and similar numbers were also available for succeeding 6-monthly periods. The methods of surveillance, collection

of specimens and pathogen isolation methods have been reported (Jennings, MacDiarmid & Miles, 1978).

Complement-fixation (CF) tests

Sera were heat-inactivated (56 °C for 30 min) and tested for the presence of complement-fixing antibody from an initial 1/8 dilution using the method of Bradstreet & Taylor (1962) adapted to the microtitre technique. Influenza A antigen and antiserum were supplied by Microbiological Associates Inc.

Haemagglutination-inhibition (HI) tests

Sera were treated for non-specific inhibitors with receptor-destroying enzyme (Wellcome Reagents Limited) and tested from an initial dilution of 1/10. Haemagglutination inhibition tests were carried out as described by Grist *et al.* (1966) adapted to the microtitre technique. Chick embryo allantoic fluid antigens were prepared in the laboratory with Influenza A/England/42/72 (MRC-7 strain) and A/Port Chalmers/1/73 (MRC-11 strain) obtained from Dr G. C. Schild, World Influenza Centre, London. Four haemagglutinating units of antigen and 0·5% fowl red blood cell suspensions were used.

Neuraminidase-inhibition (NI) tests

The neuraminidase assay of recombinant viruses and subsequent neuraminidase inhibition tests on serum samples were performed by the method of Aymard-Henry *et al.* (1973), as described by Palmer *et al.* (1975). Chick embryo allantoic fluid antigens were prepared in the laboratory with recombinant influenza viruses A/equi/Prague/1/56 (Heq 1) – A/England/42/72 (N2) (X38 strain) and A/equi/Prague/1/56 (Heq 1) – A/Port Chalmers/1/73 (N2) (X42 strain) obtained from Dr G. C. Schild. Antigens were stored in small volumes at −70 °C. Sera were heat-inactivated and used at a starting dilution of 1/10, the titre being calculated as the dilution that reduced neuraminidase activity by 50%.

RESULTS

Family members with antibody

The percentage of family members in each age group with detectable CF antibody at a titre 1/8 or greater to influenza A is shown in Fig. 1. The percentage of all members with antibody increased between May and November 1973. A decrease in the percentage with antibody occurred over 1975, detectable antibody being lost more rapidly by school-aged children (5–20 years) than adults.

Infection within families

Activity of the virus in 1973. Clinical influenza occurred in 17 of the 26 families. A total of 59 (46%) of the 127 members had influenza. Laboratory evidence of infection was obtained in 41 cases (Table 2). The illnesses were predominantly lower respiratory in 31 (52%) of the cases, upper respiratory in 24 (41%) and symptomless in 4 (7%). There was no evidence of any other virus involved.

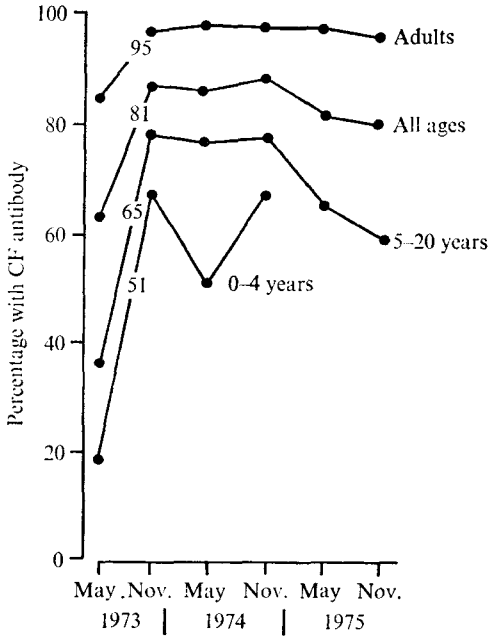

Fig. 1. Percentage of family members in each age group, in each year of the study with a CF antibody titre to Influenza A virus 1/8 or greater.

In Table 3 the age distribution of the family members with influenza in each year is summarized. In 1973 intrafamilial spread of influenza occurred in 15 families. Spread was determined by assuming that an illness occurring in a family member within 13 days of the onset of an illness in another was associated with the same pathogen (Cooney, Hall & Fox, 1972). Each member affected by illness was determined on the basis of both clinical diagnosis and laboratory evidence. The infection frequency was highest for the school-age group (5–20 years) The index case in each family was assumed to be the introducer of the disease and, in 10 families, this same age group (5–20 years) was probably responsible. Infants were the index cases on 3 occasions, female adults on 3 and male adults on 1 occasion.

Activity of the virus in 1974 *and* 1975. Clinical influenza occurred in two families in 1974 and in another two families in 1975 involving nine members (Table 3). The index case was a school-aged child in 3 families and an adult in 1 family.

During the study period, 68 (54 %) of the persons in the study had influenza. By calculation from the mean number of members in each age group for the entire study period, 38 % of infants, 90 % of school-aged children and 24 % of adults were infected. No cases, as far as could be ascertained, were reinfections with influenza A/Port Chalmers/1/73.

Table 3. *Distribution by age of members within families in which clinical influenza occurred*

Age group (years)	Family members with influenza: 1973 (17 families) No. Observed	1973 (17 families) No. (%) Infected	1974 (2 families) No. Observed	1974 (2 families) No. (%) Infected	1975 (2 families) No. Observed	1975 (2 families) No. (%) Infected
0–4	11	7 (64)				
5–20*	43	33 (77)	4	3 (75)	6	3 (50)
Female adults	18	12 (67)	2	1 (50)	2	1 (50)
Male adults	17	7 (41)	2	0	2	1 (50)
Total	89	59 (66)	8	4 (50)	10	5 (50)
SAR†		58·3		33·3		37·5

* Age group predominantly 5- to 14-year-olds.

† Secondary attack rate (SAR) calculated by assuming a single introduction of infection into each family:

$$\text{SAR} = \frac{\text{total no. infected} - \text{no. of families affected}}{\text{total no. observed} - \text{no. of families affected}} \times 100.$$

Distribution of HI antibody and NI antibody before the natural epidemic of A/Port Chalmers/1/73

Sera taken before the 1973 outbreak were tested for antibody to A/England/42/72. 54/91 family members (59%) had detectable HI antibody while 23/91 (25%) had detectable NI antibody. Only 25/91 (27%) had HI antibody titres greater than or equal to 1/40 to A/England (Table 4).

By comparison, 35/91 (38%) had pre-existing, presumably cross-reacting, HI antibody and 7/91 (8%) NI antibody to A/Port Chalmers, while only 12/91 (13%) had HI antibody titres 1/40 or greater to A/Port Chalmers. The geometric mean titres were 1/19·9 for A/England HI antibody and 1/10·3 for A/Port Chalmers HI antibody.

Relation of pre-existing A/England and A/Port Chalmers HI antibody and NI antibody to infection.

Family members with a fourfold or greater influenza A antibody rise by the complement fixation (CF) test or haemagglutination inhibition (HI) test, with or without virus isolations, were considered to have been infected. Twenty-seven per cent had significant CF antibody rises, 36% had significant HI antibody rises, while the combined results showed an overall infection rate of 33/91 (36%). A number of infected members would have been left out if only one of these criteria had been used.

The frequency of infection decreased with increasing serum HI antibody titres, shown in Table 4. Possession of detectable A/England HI antibody appeared to be associated with some protection against infection ($P < 0{\cdot}01$, $\chi^2 = 11{\cdot}34$ with 1 degree of freedom) and, similarly, with A/Port Chalmers HI antibody

Table 4. *Distribution of pre-existing HI antibody and NI antibody and their relation to infection with A/Port Chalmers/1/73 virus*

	A/England			A/Port Chalmers		
	No. of family members with titre	Infection frequency no. (%)		No. of family members with titre	Infection frequency no. (%)	
Serum HI antibody titre*						
< 10	37	21	(57)	56	26	(46)
10	12	4		13	4	
20	17	5	(22)	10	2	(20)
40	9	2		8	1	
⩾ 80	16	1		4	—	
Total	91	33		91	33	
Serum NI antibody titre*						
< 10	68	27	(40)	84	32	(38)
10	8	2		3	1	
32	7	3	(26)	3	—	(14)
100	7	1		1	—	
⩾ 320	1	—		—	—	
Total	91	33		91	33	

* Reciprocal titre.

Table 5. *Relation of pre-existing A/England and A/Port Chalmers HI antibody and NI antibody to infection with A/Port Chalmers/1/73 virus*

		A/England		A/Port Chalmers	
Serum HI Ab titre	Serum NI Ab titre	No. of family members with titre	Infection frequency no. of members (%)	No. of family members with titre	Infection frequency no. of members (%)
< 10	< 10	32	19 (59)	53	25 (47)
⩾ 10	< 10	36	8 (22)	31	7 (23)
< 10	⩾ 10	5	2 (40)	3	1 (33)
⩾ 10	⩾ 10	18	4 (22)	4	0
Total		91	33	91	33

($P = 0{\cdot}02$, $\chi^2 = 6{\cdot}53$ with 1 degree of freedom). However, there was little difference in the infection frequency among those either with or without HI antibody to A/Port Chalmers when compared with those members with or without HI antibody to A/England.

Similarly, the frequency of infection appeared to decrease with increasing serum NI antibody titres, however with both A/England and A/Port Chalmers NI antibody analysis, the numbers were too small to establish statistical significance.

The separate effects of A/England and A/Port Chalmers HI antibody and NI antibody

In an attempt to dissociate the effect of antibody to the haemagglutinin and neuraminidase antigens, family members were analysed for the presence or absence of antibody to each (Table 5). There was significant evidence of an association between A/England antibody titre and infection ($P < 0{\cdot}1$, $\chi^2 = 12{\cdot}25$ with 3 degrees of freedom). Both low A/England HI antibody and NI antibody levels were associated with a higher frequency of infection, while a high HI antibody level and a low NI antibody level was associated with a lower frequency of infection. Thus a significant independent protective effect of A/England HI antibody or NI antibody could not be detected. There was also a non-statistical suggestion of an association between A/Port Chalmers antibody level and resistance to infection.

DISCUSSION

The origin of the variant, influenza A/Port Chalmers/1/73 (H3N2) is obscure. It was first isolated during an epidemic of clinical influenza in Perth, Western Australia (World Health Organization, 1973). Two weeks later it was introduced into Dunedin by University students returning from the August vacation. Infections with it rapidly reached epidemic proportions, spreading to Port Chalmers 7–10 days later during the third week of September (Jennings, MacDiarmid & Miles, 1978), and appearing in other parts of New Zealand at the same time.

CF antibodies are useful as an index of recent infection as they are the first antibodies to appear, and unless subsequently stimulated anew by reinfection, decline in titre. In 1973 the percentage of all family members with CF antibody at a titre of 1/8 or greater increased from 62% to 87%, reflecting a high incidence of infection by influenza A virus. Detectable CF antibody persisted in 98% of adults for at least 2 years and in 78% of the 5- to 20-year age group for at least 12 months. Both the lower percentage of family members with CF antibody in May, 1973 and the relative permanence of antibody after the 1973 epidemic suggest the near absence of influenza A from the Port Chalmers community in the previous 1-2 years.

In 1973 the total infection rates, 36·8%, 61·6%, 40% and 29·2% respectively, for 0–4, 5–20, adult female and adult male age groups were highest for the school-aged children. During intrafamilial spread this age group had the highest infection rates and were also the most frequent introducers of infection into the family unit (10 out of 17 occasions) suggesting the importance of schools in this community for the spread of influenza. It might be expected that the highest infection rates would occur among those with the least immunity, however in family studies this has not always been the case. During inter-pandemic epidemics in Washington (Philip *et al.* 1961), the infection rates were highest for children under 6 years although the introducers of infection into families were predominantly school-aged children, while in Seattle (Foy, Cooney & Allan, 1976) infection rates were highest for the 10–19 years age group. During the Asian pandemic in Cleveland

(Jordan, 1961) the highest infection rates were in school-aged children and they were also the more important introducers of infection into the families, while with influenza A/Hong Kong in Cirencester (Hope-Simpson, 1970) the infection rates were similar for all age groups with adults being the more important introducers. In Seattle (Hall, Cooney & Fox, 1973) the highest infection rates for influenza A/Hong Kong were in pre-school children under 6 years, while there was little difference between the rates for all other age groups. The infection rate for school-aged family members was relatively low and the authors did not consider this to be consistent with this group serving as major introducers. Their observations were consistent with the highest infection rates occurring in the age group with the least prior immunity and suggested an important role for pre-school children in the community spread of influenza. With a later age for starting school in the United States, it is possible that a much higher proportion of pre-school children in Seattle go to kindergarten than in Port Chalmers. Although the number of pre-school children tested serologically was small, the evidence supports the role of school-aged children as introducers of infection into the families studied, and as an important age group in the community dissemination of influenza A.

The size and rapid spread of the A/Port Chalmers epidemic in 1973 could be explained by the large proportion of susceptible individuals within the community. Only 27% of this group had HI antibody titres 1/40 or greater to A/England, and assuming that high titres of HI antibody are required for protection (Kilbourne *et al.* 1973), this population was even susceptible to infection with a homologous A/England strain. The SAR of 58·3% within the families in 1973 was higher than that reported during the Seattle Virus Watch; Asian outbreak 28·3% and Hong Kong outbreak 24·1% (Hall *et al.* 1973). In Seattle families (Foy *et al.* 1976) during the 1973 A/England epidemic the SAR was estimated as 27%. This latter epidemic caused higher infection rates among school-children than the A/Hong Kong epidemic of 1968–9 and the authors speculated that this may have been related to the protective effect of the neuraminidase antibody acquired in an epidemic of Asian influenza in 1968. Influenza A/Port Chalmers/1/73 has undergone antigenic 'drift' in both the haemagglutinin and neuraminidase antigens, there being about a 32-fold reduction in NI antibody titre with A/Port Chalmers from A/England (World Health Organization, 1974). Thus it is possible that the high SAR of influenza A/Port Chalmers within this group of families relates to the lack of influenza virus activity in this community in the preceding two years in that the lack of pre-existing neuraminidase antibody has failed to prevent the spread of influenza A virus.

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