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Infection with influenza A H1N1

1. Production and persistence of antibody

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SUMMARY

Three outbreaks of influenza caused by influenza A H1N1 occurred in a boys' boarding school in 1978, 1979 and 1983. The serological response to infection with variants of the H1N1 virus was studied by radial haemolysis and haemagglutination inhibition after primary infection and reinfection. The persistence of this antibody was also studied. Infection in 1978 resulted in the production of persistent antibody to both the haemagglutinin and neuraminidase of the homotypic strain. Antibody which cross-reacted with later variants of the virus was less frequently produced, the peak response was delayed and such antibody persisted less well. Infection in 1979 resulted in a similar response to that observed in 1978 after primary infection. Reinfection resulted in a broad response in all cases. In 1983 all infected boys produced antibody which reacted with the homotypic strain but only approximately one-third of primary infections produced antibody which reacted with the A/USSR/92/77 strain. The neuraminidase of the A/USSR strain failed to detect one third of the primary infections. Reinfection again resulted in a broad response.

INTRODUCTION

Strains of the influenza A H1N1 subtype first reappeared in May 1977 in Northern China after an absence of 20 years. During the next few months isolations of this subtype were reported in Hong Kong (Shortridge & Osmund, 1978) and the USSR (Zhdanov *et al.* 1978) and it spread rapidly round the world. In the United Kingdom the H1N1 virus was first isolated in January 1978 (Communicable Disease Surveillance Centre, 1978). Outbreaks in residential schools were reported in the Spring of 1978 among children who had not been born when the subtype was in circulation previously. Since 1978 H1N1 viruses have continued to circulate worldwide and there have been reports of isolations each winter (Chakraverty, Cunningham & Pereira, 1982). In the Winter of 1980–1 the strains isolated showed a drift away from the USSR-like strains towards A/England/333/80. There was evidence of reinfection in school children.

Since 1970 Christ's Hospital, Horsham, a boarding school for about 800 boys, has been participating in a long-term study of influenza. Since 1978 there have been three outbreaks caused by strains of A H1N1 in the school and this paper describes the production and persistence of antibody following infection in these outbreaks.

MATERIALS AND METHODS

Study design

A blood sample was collected with parental consent on entry at age 11 years from every boy who joined the school. Repeat samples were taken annually from certain boys to monitor asymptomatic infection. Boys who experienced symptoms of influenza during school terms were investigated by the school Medical Officer (Dr T. W. Hoskins) who collected throat swabs and acute sera when the boys first reported sick; convalescent sera were usually collected from all boys who had had symptoms at a convenient time during convalescence. In addition other sera were available which had been collected in relation to other laboratory investigations requested by the Medical Officer and which were not related to influenza.

Laboratory investigations

Throat swabs were collected into virus transport medium without antibiotics. Details of the laboratory investigations performed on these swabs have been reported elsewhere (Grilli & Smith, 1983).

Haemagglutination inhibition (HI) using strains of influenza A H1N1 was performed by the method described previously (Grilli & Smith, 1983). A titre of 20 was taken to indicate antibody to the haemagglutinin and a four-fold or greater rise in antibody titre as evidence of infection.

Radial haemolysis (RH) was performed in Hyland immunoplates (Travenol Laboratories, Thetford) with 2 mm diameter wells and 3 μl unit volumes of serum as described previously (Grilli & Smith, 1983). Sera were tested against three strains of the virus representative of drifts which had occurred over the study period. In addition antibody directed against the neuraminidase was investigated using a recombinant strain which contained an irrelevant haemagglutinin (H7) and the neuraminidase (N1) from the A/USSR/92/77 strain. An increase in zone diameter of at least 1 mm between paired sera tested on the same plate was taken to indicate a significant rise in antibody. The strains of influenza virus A H1N1 used in the serological tests were as follows: A/USSR/92/77 (A/USSR); A/England/333/80 (A/Eng/80); A/England/419/83 (A/Eng/83); and the recombinant A/equine/Prague/1/56 (H7) × A/USSR/92/77 (N1) (A H7N1).

A/USSR and A H7N1 were obtained from Dr G. C. Schild, National Institute for Biological Standards and Control, Hampstead, UK and A/Eng/80 was obtained from Dr M. S. Pereira, Central Public Health Laboratory, Colindale, UK. A/Eng/83 was a strain isolated during an influenza outbreak caused by influenza A H1N1 at Christ's Hospital in 1983.

Stock viruses were grown in the allantoic cavity of 10-day embryonated hens' eggs and the harvests were prepared by the method described previously (Grilli & Smith, 1983).

RESULTS

The outbreaks

Over the study period there were three outbreaks of influenza caused by influenza A H1N1 viruses. The first in 1978 occurred between 2 February and 1 March in a totally susceptible population. A total of 420 boys reported symptoms

of influenza. Ninety-eight per cent of the 251 cases investigated had laboratory evidence of infection. There was a high rate of infection throughout the school and it was estimated that 90 % of the 800 boys in school were infected; 50 % had clinical influenza and 40 % were either asymptomatic or had only minor respiratory symptoms.

The second outbreak occurred in 1979 between the end of February and the beginning of March. It was confined largely to those boys who had joined the school in October 1978, after the previous outbreak. There were 35 confirmed cases, 29 of which occurred in the new boys who had no evidence of previous infection. There was some evidence of asymptomatic infection and a small number of reinfections in boys with antibody before the outbreak. This outbreak and the one in 1978 have been described previously (Davies *et al.* 1982).

The third outbreak occurred in 1983 between 31 January and 27 February spanning a mid-term break from 18 to 20 February. Of the 757 boys in school during the outbreak 198 (26 %) had clinical influenza caused by influenza A H1N1. There were a further 10 boys with symptoms caused by influenza A H3N2 and 20 in whom infection with both influenza A H1N1 and A H3N2 was demonstrated. In addition to the symptomatic infections there were at least 80 other boys without clinical influenza who were also infected.

Response to infection

1978 Outbreak

Convalescent blood was collected in mid-March, 3–4 weeks after the boys were ill. In later April, about 9 weeks after the outbreak, blood was collected from some cases not bled in March and from a number of boys who had not experienced symptoms of influenza. In the summer term sera were available from a few cases of influenza B and parainfluenza virus infection. In October, 8 months after the outbreak, the 1976 and 1978 entry cohorts were bled. In late January 1979, there was an outbreak of influenza B and cases were investigated serologically. Thus sera collected at various times after the infection in the spring of 1978 were available and were examined by RH. Sera from 248 boys, known to have been infected, were compared using the four antigens. The proportion with antibody to each of these is shown in Fig. 1.

It is clear that some of the earliest sera collected 1 month after infection failed to react in the RH test. Two months after infection antibody to the homotypic haemagglutinin and to the neuraminidase could be detected in most of those infected and about a third now had detectable cross-reacting antibody. The maximum response to heterotypic strains was not seen until 5 months after infection and this was well maintained in sera collected at 8 and 11 months. About a quarter of the boys made only a homotypic response.

Of the 248 boys investigated by RH, 55 did not have symptoms. The extent of the response and the time at which antibody became detectable was not influenced by whether or not the infection resulted in symptoms of influenza.

The development of the response could be demonstrated in boys who were bled shortly after the outbreak and again in the summer term or in January 1979 before the influenza B outbreak (Table 1). A high proportion of those bled in March had not completed their antibody response as detected by RH.

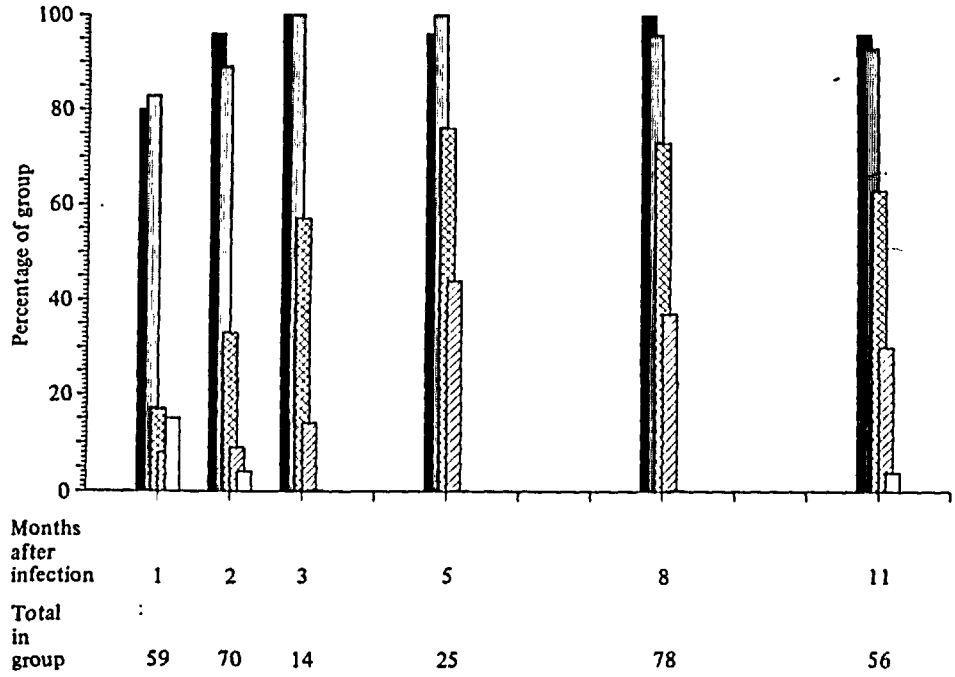

Fig. 1. The production of antibody following infection in 1978. The bars indicate the proportion of the group with antibody to the neuraminidase of A/USSR/92/77, ■; the haemagglutinin of A/USSR/92/77, ⊡; A/England/333/80, ⊠; and A/England/419/83, ⊘; and the proportion with no detectable antibody, □.

Table 1. *Development of antibody response in boys bled more than once*

Date of first serum (1978)	Total in group	Number (%) with 'late response'*	Nature of 'late response'		
			Homotypic only	Homotypic and cross-reacting	Cross-reacting only
March	30	24 (80)	7	12	5
April	24	14 (58)	2	9	3
May	12	3 (25)	—	2	1
June/July	4	0	—	—	—
October	16	0	—	—	—

* 'late response': development of antibody or increase in titre between the first convalescent serum and a later specimen.

The response to infection as measured by HI and RH was compared in 35 boys who were bled in March or April, and again in January 1979 (Table 2). HI was more efficient than RH at detecting the early response, especially antibody reacting with heterotypic strains. A higher percentage of the sera obtained in January 1979 reacted with A/Eng/80 and A/Eng/83 in both tests than of those obtained in March/April 1978. Two boys had no detectable antibody to the haemagglutinin in their early sera by either test, although they responded later. One boy had lost his response to the haemagglutinin in both tests by January 1979.

The relationship between the amount of antibody produced to the homotypic strain and the proportion with cross-reacting antibody was investigated. The

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Table 2. *Comparison of HI and RH for the detection of antibody in 35 boys following primary infection in 1978*

	Percentage with antibody to						Percentage without detectable antibody	
	A/USSR		A/Eng/80		A/Eng/83			
Date of serum	HI	RH	HI	RH	HI	RH	HI	RH
March/April 1978	94	86	37	17	29	3	6	14
Jan. 1979	94	97	66	63	57	31	6	3

Table 3. *Relationship between cross-reacting antibody and homotypic response as measured by radial haemolysis (RH)*

Antibody to A/USSR. Zone diameter (mm)	Proportion (%) with cross-reacting antibody in sera collected in				
	March 1978	April 1978	May–July 1978	October 1978	January 1979
< 5·5	0/18 (—)	3/24 (13)	4/10 (40)	4/11 (36)	5/12 (42)
5·5–6·4	4/24 (17)	12/23 (52)	10/16 (63)	14/18 (78)	11/15 (73)
⩾ 6·5	6/9 (67)	8/13 (62)	13/13 (100)	7/7 (100)	11/14 (79)

proportion of sera with cross-reacting antibody increased with increasing zone size to the homotypic strain (Table 3). However, it is clear that sera collected several months after infection and which gave small or moderate zone sizes, were more broadly reactive than sera with a similar amount of homotypic antibody but which were collected early during convalescence.

1979 Outbreak

The small outbreak in 1979 which occurred in late February/early March followed a large outbreak of influenza B in late January/early February. Samples of convalescent blood were collected on 28 March from those involved in both outbreaks. Most of those infected with A H1N1 had entered the school in October 1978 and had no evidence of previous infection, but there were a small number of reinfections in boys with antibody to H1N1 before the outbreak.

Response to primary infection. Infection in 1979 produced a pattern of response similar to that observed in 1978. Of 31 boys assessed in March, 30 had antibody to the neuraminidase and 29 to the haemagglutinin of A/USSR; of these, 17 had antibody reacting with heterotypic strains. Fifteen of the 31 boys were bled in 1980 (in relation to an outbreak of campylobacter infection), and 10 showed the development of, or increase in, antibody to heterotypic strains.

Response to reinfection. Twelve boys who were known to have antibody before the 1979 outbreak were shown to be reinfected. All produced antibody reacting with all four strains used.

1983 Outbreak

In 1983, many of the cases and the 1976 entry cohort were bled at the end of April, 2 months after the end of the outbreak. The 1980 entry cohort was bled in October 1983.

Table 4. *Response to primary infection in 1983*

Date of serum	Total number in group	Number (%) with antibody to				Number (%) with no detectable antibody
		A/Eng/83	A/Eng/80	A/USSR	A/H7N1	
April	33	22 (85)	21 (64)	12 (36)	22 (67)	5 (15)
October	22	22 (100)	21 (96)	15 (68)	22 (100)	—

Table 5. *Response to the haemagglutinin of A/Eng/83 following primary infection and reinfection measured by radial haemolysis*

Date of serum	Number in group	Number with antibody to A/Eng/83 RH zone dia. (mm)			
		< 6·0	6·0–6·9	7·0–7·9	⩾ 8·0
Primary infection					
April	28	11	14	2	1
October	22	10	6	5	1
Reinfection					
April	159	1	10	42	106
October	59	2	11	24	22

Response to primary infection. Boys who had no detectable antibody in their acute sera and no evidence of infection from earlier sera, and who developed antibody after the outbreak, were considered to have experienced primary infections. It is possible that a small proportion of them may have been infected before entering the school, but antibody was no longer detectable (see Persistence of antibody section, below). Their responses, detected by RH, are summarized in Table 4. Five of the 33 boys bled in April had failed to produce antibody detectable by RH. All sera with antibody detected by RH reacted with the homotypic strain but only about one-third of those infected produced antibody reacting with the most 'remote' heterotypic strain (A/USSR). The neuraminidase antigen failed to detect one-third of the infections. A higher proportion of the sera collected in October reacted with all the haemagglutinin antigens.

Response to reinfection. Boys with evidence of infection who had detectable antibody before the outbreak, whether or not this was still present in the acute serum, were considered to have experienced a reinfection. A total of 117 cases and 42 asymptomatic infections were detected among those bled in April, and a further 23 cases and 36 asymptomatic infections among those bled in October. After infection all had acquired antibody which reacted with all four antigens by RH. Twenty-one of these boys had no antibody in their acute sera.

Response to the homotypic haemagglutinin. There was a difference in the amount of homotypic antibody produced following primary infection or reinfection, but this was not related to whether or not infection was accompanied by symptoms of influenza. Table 5 illustrates this difference for those bled in April and October.

Comparison of primary responses in 1978, 1979 and 1983

The response to the haemagglutinin following primary infection with A H1N1 is compared for the three outbreaks in Table 6. In all three outbreaks sera collected early in convalescence were less broadly cross-reactive than those collected later.

Table 6. *Response to the haemagglutinin following primary infection in 'early' and 'late' sera*

Infection in	Date of serum	Total in group	% with antibody by RH to: Homotypic strain only	Cross-reacting strains: Total	Cross-reacting strains: 'Remote'*	None
1978	March	59	66	17	8	17
	April	70	56	33	9	11
	October	37	30	68	30	3
1979	March	31	39	55	26	6
	Late '79/80	21	14	76	71	5
1983	April	33	21	64	36	15
	October	22	4	96	68	—

* 'Remote': A/Eng/83 strain for 1978 and 1979; A/USSR strain for 1983.

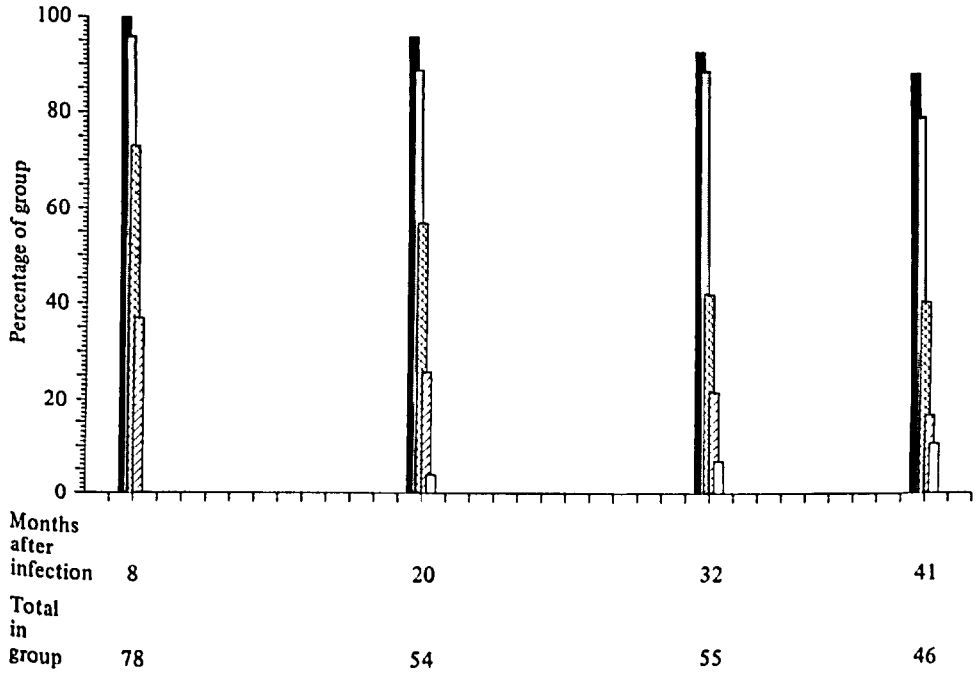

Fig. 2. The persistence of antibody following infection in 1978. The bars indicate the proportion of the group with antibody to the neuraminidase of A/USSR/92/77, ■; the haemagglutinin of A/USSR/92/77, ▦; A/England/333/80, ▩; and A/England/419/83, ▨; and the proportion with no detectable antibody, □.

Persistence of antibody following infection

Persistence of antibody could be studied in those who were bled at various times after infection in 1978 and from whom no evidence of reinfection was obtained in the interim. Fig. 2 shows the proportion with detectable antibody to the four antigens estimated at intervals up to 3 years after infection. Antibody to the haemagglutinin of A/USSR gradually declined so that by July 1981, 41 months

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after infection, it was no longer detectable in 20% of boys. Antibody to the neuraminidase persisted longer while antibody to heterotypic strains declined more rapidly. Three years after the maximum response antibody could not be detected in 11% of those who had been infected in 1978. Haemagglutination inhibition was less efficient than RH in detecting evidence of infection in the past; 34% of those assessed in July 1981 had no detectable antibody by HI.

A similar assessment of the persistence of antibody 3 years after primary infection in 1979 was made on 26 boys. Four had been reinfected in the intervening period and 3 of the remaining 22 had residual antibody to the neuraminidase only. Sera collected in 1982 were also abailable from nine boys who were reinfected in 1979. All had antibody to A/USSR haemagglutinin and neuraminidase and to A/Eng/80. Four no longer had detectable antibody to A/Eng/83.

Of a total of 233 boys known to have experienced reinfection in 1983, 29 (12%) had no detectable antibody in their pre-outbreak serum and a further 41 (17%) had residual antibody to the neuraminidase only.

DISCUSSION

It appeared that primary infection with A H1N1 strains provided a relatively poor antigenic stimulus. Studies on the persistence of antibody after infection in 1978 showed that the antibody response took some months to develop fully and declined over the next 3 years, occasionally to undetectable levels.

The apparently slow development of antibody after infection in 1978 was more marked when assessed by RH than by HI (Table 2). Twenty per cent of sera collected less than 4 weeks from the onset of illness were negative by RH. These sera may have contained specific IgM which fails to react in this test (Strannegård, Grillner & Lindberg, 1975). To detect the response by HI the test was set up to achieve optimum sensitivity by ensuring the removal of non-specific inhibitors from the sera and using the lowest practicable antigen dose. Even so over half the convalescent sera had titres of only 20, the minimum accepted as evidence of infection.

The presence of cross-reactive antibody was related to the amount of antibody produced to the homotypic strain. However, for any given amount of homotypic antibody, sera collected late in convalescence were more broadly cross-reactive than sera collected early. This relationship was observed in all three outbreaks. The greater breadth of reactivity of the April 1983 sera compared with the April 1978 sera (Table 6) may, in part, be a reflection of the better response to the homotypic strain; only 18% had zones of less than 5·5 mm diameter in 1983 compared with 40% in 1978.

The response to reinfection was assessed in 1979 and 1983 and was characterized by the rapid production of high titre, broadly reactive antibody. If RH is used to diagnose infection, the strain used and the time of collection of the convalescent serum is not critical in reinfections; whereas with primary infections it is important to use the homotypic strain and to examine a late convalescent serum collected at least 6 weeks after onset if a significant proportion of infections are not to be missed.

It is likely that all those infected in 1978 responded to the neuraminidase of

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A/USSR. As with antibody to the haemagglutinin this response was not always detectable in sera collected early during convalescence. In 1979, 30 of 31 infected boys bled in March, had neuraminidase antibody. In 1983 a neuraminidase recombinant specific to the infecting strain was not available. One third of boys bled in April who had experienced a primary infection had no detectable antibody to the neuraminidase of A/USSR. These results suggest that there has been some antigenic drift in the neuraminidase antigen of A H1N1 strains since 1978.

Following infection in 1978 the proportion with antibody gradually declined. Cross-reacting antibody tended to disappear first and antibody to the neuraminidase persisted longest. After 3 years about 10% had lost all evidence of infection when tested by RH. Although HI was found to be more efficient than RH at detecting the antibody response in early convalescent sera, it may fail to detect small amounts of antibody remaining from past infection. A similar finding with influenza B has been described by Chakraverty (1980). Serological surveys using the HI test are likely to underestimate the experience of a population to A H1N1. Even using RH 30% of boys who were reinfected in 1983 would have been classified as primary infections had earlier sera not been available and the neuraminidase antigen not been used.

It has been demonstrated (Davies, Grilli & Smith, 1984) that infection with influenza A H1N1 in 1978 did not prevent reinfection with later strains of this subtype and this is confirmed by the very large number of reinfections observed in the 1983 outbreak. The relation of previous experience to fate in 1983 and the predictive value of antibody determinations is described in the accompanying paper (Davies, Grilli & Smith, 1986).

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