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Natural or vaccine-induced antibody as a predictor of immunity in the face of natural challenge with influenza viruses

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SUMMARY

A study of influenza in residential schools provided the opportunity to assess the significance of antibody as a predictor of immunity. Five hundred and fifty-six pupils from 8 schools were included in the investigations, and the outcome for these children in 27 naturally occurring outbreaks of influenza was analysed. The outbreaks comprised 5 caused by strains of influenza A H3N2, 10 caused by strains of influenza A H1N1, and 12 caused by strains of influenza B. On 8 occasions a second outbreak of the same serotype occurred in a school.

There was a general correlation between the presence of antibody to the outbreak strain and protection from infection. For each of the three influenza virus serotypes the infection rate in those with no detectable antibody was approximately 80%. Those with past experience of the virus but no antibody to the outbreak strain experienced lower infection rates (62% overall) but the infection rates were lowest in those with intermediate and high level antibody to the challenge strain (18% overall).

Vaccine was used by three of the schools. The effect of antibody derived from recent experience, either natural or vaccine-induced, on subsequent challenge with a drifted strain i.e. one showing antigenic drift away from the previous strain, was compared. Intermediate or high level antibody to the challenge strain in those who had experienced a recent natural infection was associated with a low infection rate (9%). A similar level of antibody produced in response to vaccination was associated with a significantly higher infection rate (23%: $P < 0{\cdot}025$). Among the vaccinees who had produced such antibody the infection rate was highest (32%) in those who had responded to vaccine in the presence of antibody to the vaccine strain.

The evidence from this study indicates that whilst antibody surveys of populations may provide some information about susceptibility to challenge with new strains of influenza viruses, the circumstances of the induction of the antibody affect its value as a predictor of immunity.

INTRODUCTION

Infection or vaccination with influenza viruses induces the production of antibodies which react both with the strain providing the antigenic stimulus and often with drifted strains of the same serotype (Smith & Davies, 1976; Oxford *et al.* 1979; Grilli & Davies, 1981; Grilli, Davies & Smith, 1986).

When recently isolated strains show a significant antigenic drift two questions arise: is it likely that sizeable outbreaks of influenza will occur?; should the new strains be recommended for inclusion in the vaccine? To attempt to answer these questions antibody to the new virus in a sample of the population may be measured (Pereira & Chakraverty, 1977, 1982; Chakraverty *et al.* 1986) or the heterotypic response to the new strain following vaccination with a previous strain may be assessed (World Health Organization, 1988). The interpretation of the results depends on an estimate of the titre of antibody likely to correlate with immunity. From past experience it is reasonable to assume that those with no detectable antibody to the new strain will be more susceptible than those whose sera give high titres. Results between these extremes are common and the way the test is set up will affect the apparent titre. Attempts to correlate titre with immunity have depended on either artificial challenge experiments with attenuated strains (Hobson *et al.* 1972; Al-Khayatt, Jennings & Potter, 1984) or observing populations during outbreaks of influenza where both infection and attack rates can be assessed (Wesselius-de Casparis, Masurel & Kerrebijn, 1972; Delem & Jovanovic, 1978).

In population surveys the stimulus which induces antibody to a new and drifted strain is unknown. A study of influenza in residential schools provided an opportunity to observe the effect of antibody in the face of natural challenge during outbreaks. It was also possible to compare the predictive value of antibody resulting from recent or more remote infection with that stimulated by vaccination.

MATERIALS AND METHODS

Study design

Five hundred and fifty-six pupils from the 1980 or 1981 intake to eight schools were included in this study. The age range on entry was 10–13 years. Parental consent was obtained and a blood sample collected from every child. Further samples were collected annually throughout their school careers. The size of the cohort recruited from the intake in each school ranged from 22 to 124. Sickness and vaccination histories for each pupil were recorded. Each school followed its own vaccination policy. Of the 8 schools in the study, 3 used vaccine and 5 did not. Two of the schools used a disrupted virus preparation (Influvac: Duphar) which contained antigens from A/Bangkok/1/79 (H3N2), A/Brazil/11/78 (H1N1) and B/Singapore/222/79 strains. The remaining school used an alternative whole virus vaccine (MFV-Ject: Merieux) which contained the same viruses. Thirty-nine per cent of all the pupils in the study received at least one dose of vaccine.

The school medical officers were responsible for the clinical diagnosis of influenza. Throat swabs and, where appropriate, blood samples were collected from pupils with symptoms of influenza. The throat swabs were examined for the presence of viruses by local laboratories who also carried out primary serological tests for respiratory pathogens.

Influenza serology

Sera were examined for antibodies to influenza viruses by radial haemolysis. The technique used was a modification of that described by Oxford *et al.* (1979)

Table 1. *Persistence of antibody following natural infection or vaccination*

Type of stimulus	Influenza virus	Number with antibody, year 1	Number with antibody, year 3	Annual reduction in zone diam. (mm) based on mean values	Correlation coefficient for linear regression
Natural infection	A H3N2	49	48	0·24	−0·99
	A H1N1	64	63	0·25	−1·00
	B	54	52	0·25	−0·98
Vaccination	A H3N2	52	45	0·18	−0·99
	A H1N1	64	63	0·21	−1·00
	B	48	48	0·21	−1·00

and has been described in detail previously (Grilli & Davies, 1981; Grilli & Smith, 1983). An 8% suspension of sheep erythrocytes was used in the preparation of plates for A H3N2 and B viruses, and a 3% suspension of sheep erythrocytes for A H1N1 viruses. Strains representative of the major variants of the influenza virus serotypes A H1N1, A H3N2 and B which had been in circulation during the children's lifetimes were used. For A H1N1 viruses these were A/USSR/92/77, A/England/333/80 and A/Chile/1/83; for A H3N2 viruses, A/Hong Kong/1/68, A/Port Chalmers/1/73, A/Texas/1/77, A/Philippines/2/82 and A/Mississippi/1/85; for B viruses, B/England/21/68, B/Hong Kong/5/72, B/Singapore/222/79 and B/USSR/100/83. The strains were obtained from the National Institute for Biological Standards and Control, London and the Public Health Laboratory Service Virus Reference Laboratory, Colindale, London.

All sera from individual pupils were examined together at the end of the study. In our hands a 1·0 mm or greater increase in zone diameter between two consecutive sera has been shown to be statistically significant (Grilli & Davies, 1981) and was taken as evidence of infection or response to vaccine. These serological responses were based on antibodies detected in the annual sera. The antibody status of individuals exposed to influenza outbreaks was determined from specimens of blood collected in the preceeding autumn. Most of the outbreaks occurred in the spring term but there were two which occurred in December.

RESULTS

Persistence of antibody

The antibody status before outbreaks was assessed in sera collected up to 6 months before the outbreak. Serological evidence of infection or response to vaccination was obtained from sera collected up to 10 months later. It was therefore important to determine if these estimates truly reflected the status of the individual.

Antibody reacting with the outbreak strain detected in these sera had been induced by an earlier strain. Therefore the persistence of antibody to a strain representing the next antigenic drift to that providing the stimulus was assessed over 3 years for influenza viruses A H3N2, A H1N1 and B following natural infection or response to vaccine (Table 1). These data are confined to pupils for

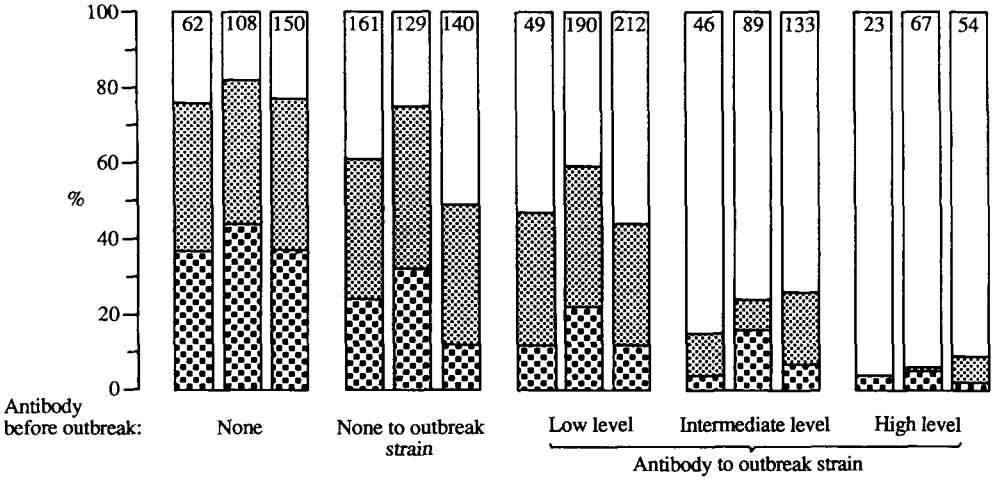

Fig. 1. Relationship between antibody status and infection and clinical attack rates in outbreaks. Each column shows the total number of pupils in the group together with the infection ( ) and clinical attack ( ) rates for that group. For each antibody category the first column shows these data for influenza virus A H3N2, the second for influenza virus A H1N1 and the third for influenza virus B. Antibody to the outbreak strain is sub-divided by amount: low level, defined by RH zone diameters $< 5{\cdot}5$ mm for A H3N2 and B, and $< 6{\cdot}5$ mm for A H1N1; intermediate level, defined by RH zone diameters 5·5–6·4 mm for A H3N2 and B, and 6·5–7·4 mm for A H1N1; high level, defined by RH zone diameters $\geqslant 6{\cdot}5$ mm for A H3N2 and B, and $\geqslant 7{\cdot}5$ mm for A H1N1.

whom there was no evidence of clinical or subclinical infection during the 3 years. Overall, less than 4% of those who produced antibody reacting with the drifted strain had no detectable antibody to this strain 3 years later. In those who retained detectable levels there was a direct relationship between the mean zone diameter and time. In all cases the fall in antibody over 3 years represented $< 1{\cdot}0$ mm change in zone diameter, equivalent to less than a twofold fall in titre.

Antibody status and fate

The relationship between antibody status, determined by examination of sera collected before an outbreak, and fate in the outbreak was assessed. The attack rates are based on clinical cases with laboratory evidence of infection and represent 91% of those classified as 'influenza' by the school medical officers.

Twenty-seven outbreaks were analysed. The overall infection rate in the cohorts in each outbreak ranged between 22 and 77% and in 17 outbreaks it exceeded 50%. Five outbreaks were caused by A H3N2; 341 children were assessed – 52% were infected and 21% had clinical influenza. Ten outbreaks were caused by A H1N1; 583 children were assessed – the overall infection rate was 55% and the attack rate 25%. Twelve outbreaks were caused by influenza B; 689 children were assessed – the overall infection rate was 46% and the attack rate 16%.

The pre-outbreak antibody status of an individual could be assigned to one of five categories (see Fig. 1).

(i) No detectable antibody to the serotype.

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(ii) Detectable antibody to earlier strains but not to the outbreak strain.
(iii) Antibody to the outbreak strain at three levels:
  (*a*) low level (approximately equivalent to a titre of less than 40 by haemagglutination inhibition (HAI));
  (*b*) intermediate level (equivalent to a titre of 40–80);
  (*c*) high level (equivalent to a titre of 160 or more).

Antibody to the outbreak strain in these pre-outbreak sera was derived from natural or vaccine experience of earlier strains than those causing the outbreaks.

The zone diameters representing antibody titres to the A H1N1 strains are larger than those for A H3N2 and B, reflecting the lower red cell concentration used (see Methods).

It will be seen that about 80% of children with no detectable antibody were infected and that about half of those infected were clinical cases. Those with antibody to earlier strains had somewhat lower infection rates and attack rates. Compared with this group, children with low level antibody to the outbreak strain were not significantly better protected, either in terms of infection rate or attack rate, with the exception of those exposed to A H1N1 outbreaks where the infection rate was lower ($\chi^2 = 8{\cdot}977$, $P < 0{\cdot}005$). Children with intermediate or high levels of antibody had the lowest infection and attack rates. It is of interest to note that, although increasing amount of relevant antibody was associated with lower infection rates the proportion of those infected who had clinical symptoms did not decrease with increasing antibody. Taking all types together 73(32%) of 228 with low level antibody, 25(40%) of 63 with intermediate level antibody and 5 out of 10 of those with high-level antibody who were infected became clinically ill.

Natural and vaccine-induced antibody

On eight occasions a second outbreak of the same serotype occurred in a school. The outcome in the second outbreak was analysed in children who had experienced a recent primary infection (those with no detectable antibody on entry), those who had a recent reinfection and those who had not been infected since 1980. The results were compared with those obtained from schools using vaccine, where children were divided into those making a primary response to vaccination, those making a secondary response (those who had antibody to the serotype before vaccination) and those who did not respond to vaccination and were not infected before an outbreak. Twenty-three vaccinated children who were shown to have been infected after vaccination and before an outbreak were excluded. The interval between two outbreaks of the same serotype varied between 2 and 6 years and the interval between vaccination and outbreak was between 2 and 5 years. Three of the second outbreaks were associated with A H1N1, four with influenza B and one with A H3N2. On three occasions the infection rate in the second outbreak was higher than in the first, on three occasions the infection rates in both outbreaks were similar and in two schools the infection rate in the first outbreak was higher than in the second. In every case challenge in second outbreaks and challenge following vaccination was with a strain which had undergone antigenic drift from the one associated with first outbreaks or was in the vaccine. Inspection of the data showed a similar pattern for all serotypes and the results presented in Table 2 are for all types together.

Table 2. *Effect of antibody to the outbreak strain on fate, related to previous experience for all types of influenza virus*

Antibody to outbreak strain		Experience: Recent: Primary stimulus	Recent: Secondary stimulus	Not recent*	Total
None	NV†	17(4)/30 [57]‡	19(5)/29 [66]	92(24)/125 [74]	128(33)/184 [70]
	V	2(2)/3	3(2)/10	18(8)/23 [78]	23(12)/36 [64]
Low level§	NV	24(8)/53 [45]	16(2)/60 [27]	38(18)/53 [72]	78(28)/166 [47]
	V	6(2)/18	20(7)/23 [87]	11(1)/14	37(10)/55 [67]
Intermediate level	NV	0/11	7(2)/38 [18]	2(1)/13	9(3)/62 [15]
	V	2(1)/22 [9]	26(12)/62 [42]	3(2)/10	31(15)/94 [33]
High level	NV	0/5	0/22 [< 5]	0/2	0/29 [< 4]
	V	0/12	5(3)/48 [10]	1/7	6(3)/67 [9]
Total	NV	41(12)/99 [41]	42(9)/149 [28]	132(43)/193 [68]	215(64)/441 [49]
	V	10(5)/55 [18]	54(24)/143 [38]	33(11)/54 [61]	97(40)/252 [39]

* That is: not infected in school/no response to vaccine.

† NV, not vaccinated; V, vaccinated

‡ $\frac{a(b)}{c}$ [d], where a = number of infections, b = number of cases, c = total in group, d = % infected, for groups in excess of 20 pupils.

§ For definitions of low, intermediate and high level antibody see legend for Fig. 1.

Those children who had had a recent primary stimulus by natural infection generally had little or no antibody to the drifted strain causing the second outbreak whereas, of those making a primary response to vaccine 34 out of 55 (62%) had intermediate or high titre antibody to the outbreak strain. There were no infections in the 16 children who had naturally derived antibody at this level and 2 infections in the 34 vaccinated children.

Children who had been recently reinfected and who had antibody to the outbreak strain had a low infection rate (23 of 120, 19%) and only four of these infections were symptomatic. On the other hand children who made a secondary response to vaccine by producing and retaining antibody to the outbreak strain, often at high titre, had a higher infection rate (51 of 133, 38%) and 22 of those infected had clinical influenza.

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Table 3. *Infection rates in vacccine responders related to pre-vaccination antibody*

Antibody to outbreak strain pre-outbreak	Antibody to vaccine strain before vaccination	
	Absent	Present
Low*	$\frac{11(3)}{25}$ [44]†	$\frac{15(6)}{16}$ [94]
Intermediate	$\frac{6(2)}{39}$ [15]	$\frac{22(11)}{45}$ [49]
High	$\frac{0}{21}$ [< 5]	$\frac{5(3)}{39}$ [13]
Total	$\frac{17(4)}{85}$ [20]	$\frac{42(20)}{100}$ [42]

* For definitions of low, intermediate and high level antibody see Legend for Fig. 1.
† See footnote for Table 2. (‡).

Table 4. *Relationship between immune status of population, size of outbreak and infection rate in susceptibles*

Group	Total pupils at risk	Infection rate (%) mean (and range)	Pupils with no antibody to outbreak strain		Pupils with no antibody to serotype	
			% of total	Infection rate (%)	% of total	Infection rate (%)
1	507	32 (22–47)	31	45	12	56
2	564	53 (49–57)	43	70	21	77
3	542	66 (58–77)	66	78	26	89

Children whose antibody to the outbreak strain was induced by infection before entry had a high infection rate; 40 of 68 (59%) in the unvaccinated and 15 of 31 (48%) in those who did not respond to vaccine.

The 185 children who responded to vaccine and had antibody to the outbreak strain before an outbreak have been analysed according to their pre-vaccination status in relation to the vaccine strain (Table 3). The highest infection and attack rates are seen in those who had pre-existing antibody to the vaccine strain.

The effect of the immune status of the population on outbreak size

The 27 outbreaks were stratified according to infection rates, into 3 groups of 9 (Table 4). The highest overall infection rates and the highest infection rates among susceptibles were associated with the groups which had the highest proportion of children without relevant antibody (Group 3). However, even in the smallest outbreaks (Group 1) the infection rate among those with no evidence of past experience of the serotype was 56%.

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DISCUSSION

The levels of pre-outbreak antibody in this study were assessed in sera collected some months before the outbreaks. Other studies on vaccinated groups of adults have shown that antibody persists well during the year following vaccination (Cate *et al.* 1983; Jennings *et al.* 1985). Previous investigations on school children have shown that whilst antibody may decline abruptly from a peak titre achieved shortly after natural infection or vaccination, it is lost much more slowly thereafter (Smith & Davies, 1976; Grilli & Davies, 1981). The small loss of antibody observed in this study over 3 years suggests that the antibody detected in pre-outbreak sera would not have declined significantly before the challenge. Surveys used to assess the likely susceptibility of a population to a drifted strain, or to evaluate the likely effectiveness of a vaccine, are in any case necessarily made on sera collected some time before an unpredictable event.

The validity of comparing antibody status before an outbreak with subsequent fate depends on the demonstration that the population has been effectively challenged. When, during an outbreak, the infection rate was over 50% there seems no reason to doubt this. The evidence from the current study shows that, even in the smallest outbreaks, nearly 60% of those with no detectable antibody to the serotype were infected. Thus, in this type of population, when an outbreak occurs susceptible members of the population will be effectively challenged, although the size of the outbreak will be influenced by the proportion who are susceptible.

The study has confirmed that there is a relationship between past experience of a serotype and protection from infection. In particular, the presence of antibody to the outbreak strain was associated with lower infection and attack rates compared to those observed in pupils without such antibody. However, the nature of this past experience was an important factor. A 'significant' titre of antibody to the outbreak strain (intermediate or high level in this series) generally arose from recent reinfection in the unvaccinated and was associated with a low infection rate (10%). The effectiveness of antibody of similar titre derived from vaccination was dependent on the pre-vaccination experience of the individual. Those who lacked antibody to the vaccine strain before vaccination and who produced a good response had a low infection rate on challenge with a heterotypic strain (10%): protection comparable to that observed among the unvaccinated with similar titre antibody. However, in those who made a similar response but who had had antibody to the vaccine strain before vaccination, the infection rate was 32%. Overall the infection rate in vaccinees with intermediate or high level antibody to the outbreak strain was 23%.

The predictive value of antibody surveys on populations which include vaccinated individuals depends to some extent on knowing whether the population under surveillance has acquired antibody by natural infection or vaccination and if vaccination, what the baseline experience of the population was prior to that vaccination.

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Sussex; Downside School, Bath; Marlborough College, Wiltshire; Queen Margaret's School, Yorkshire; Rugby School, Warwickshire; Stonyhurst College, Lancashire; Wellington College, Berkshire), the staff of laboratories concerned with the primary investigations, and the staff of the Guildford Public Health Laboratory.

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