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Antibody responses after repeated influenza A virus immunizations among schoolchildren in Japan

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SUMMARY

Antibody responses to influenza virus immunizations were examined among junior high school students. The students received two doses of a commercial split-product vaccine containing influenza A H1N1 during a 2-year period following the first appearance of H1N1 virus in the winter of 1977–78. In haemagglutination-inhibition (HI) tests, the students who had been infected with H1N1 virus in 1977–78 showed a better response and wider cross-reactivity to the drift strain than the students who had not experienced earlier H1N1 influenza infection. Neuraminidase-inhibition (NAI) antibody titres after immunization depended upon a history of natural infection with H1N1 virus, since students not previously infected showed no significant NAI antibody rise after immunization.

INTRODUCTION

At present in Japan, it is recommended that influenza vaccine is given annually to schoolchildren from kindergarten to senior high school age. This is on the basis of the theoretical possibility that mass immunization of children, in whom influenza epidemics generally begin, might diminish the intensity of epidemics in the general population (Francis, 1967). However, the lack of detailed data on the effect of annual immunizations on the reduction of influenza-associated morbidity among schoolchildren makes it difficult to evaluate the present policy (Dowdle *et al.* 1980).

During the 1977–78 pandemic of H1N1 influenza virus originating in North China, widespread outbreaks occurred in Japan and nearly 70–80% of the schoolchildren became infected. An A/Texas/1/77-like H3N2 virus also caused a simultaneous pandemic, resulting in dual infection with H1N1 and H3N2 viruses in some individuals (Yamane *et al.* 1978; Kendal *et al.* 1979). A second, but relatively small, outbreak of H1N1 influenza infection occurred in the winter of 1978–79, and a third in 1979–80. The present report describes the serological response to the three viral antigens, nucleoprotein (NP), haemagglutinin (HA) and

neuraminidase (NA), in these vaccinees when they were immunized according to the government recommendations. Our effort was focused on the effect of the presence or absence of immunological memory after natural infection on the antibody response to successive immunizations. Anti-NA antibody levels were also determined since anti-NA antibody may play a contributory role in preventing natural infections (Murphy, Kasel & Chanock, 1972; Monto & Kendal, 1973; Yamane *et al.* 1979*b*).

MATERIALS AND METHODS

Vaccine

The vaccine employed was an ether split-product vaccine commercially prepared by Saikin Kagaku Institute Company Ltd., Sendai. The first, 1978, vaccine contained 300 chick cell agglutinating (CCA) units per ml of A/USSR/92/77(H1N1), 200 CCA units per ml of A/Yamanashi/2/77(H3N2) and 200 CCA units per ml of B/Kanagawa/3/76. The second, 1979, vaccine, contained 200 CCA units per ml of A/USSR/92/77(H1N1), 300 CCA units per ml of A/Fukushima/103/78(H1N1) which showed an antigenic drift from the USSR strain in the HI test, and 200 CCA units per ml of B/Kanagawa/3/76. Thus, the second vaccine did not contain H3N2 antigen. Doses (0·5 ml) of the vaccine were given subcutaneously twice each winter with a 3-week interval.

Volunteers

Sixty-two vaccinees were selected at random from the students of Sendai Dai-ichi Junior High School. All the participants received two doses of vaccine, one in November and the other in December of 1978 and 1979. In total, six paired serum specimens were collected from each individual; at the beginning of November (before immunization) in 1978 and 1979, in the middle of January (3 weeks after the second dose of vaccine) in 1979 and 1980, and in the middle of March (when natural epidemics usually finish) in 1979 and 1980. These six paired sera collected from each individual were simultaneously tested for the presence of antibodies. The vaccinees were divided into two groups according to the results of the HI test against A/USSR/92/77(H1N1) strain using the sera collected in November, 1978, i.e. before the first immunization. One group consisted of those who were infected in the winter of 1977–78, and the other of those who were not. The former was seropositive (HI antibody titre $\geqslant 16$) against USSR strain, and the latter, seronegative (HI titre < 16).

Serological tests

(1) Anti-HA antibody titres were determined by a microtitre technique (Yamane *et al.* 1979*a*). The viruses used were A/USSR/92/77(H1N1), A/Fukushima/103/78(H1N1), A/Yamanashi/2/77(H3N2), A/New Jersey/8/76 (Hsw1N1) and B/Kanagawa/3/76.

(2) Anti-NA antibody titres were determined by a chemical NAI technique with a fetuin substrate using the following recombinants kindly donated by Dr A. P. Kendal, Center for Disease Control, Atlanta, GA, USA; A/equine/Prague/1/56(Heql)-A/USSR/90/77(N1) and A/equine/Prague/1/56(Heql)-A/Port

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Chalmers/1/73 (N2), to prevent the steric hindrance effect from HI antibodies (Center for Disease Control, 1975).

(3) Anti-NP antibody titres were determined by the reversed single radial immunodiffusion(r-SRD) technique, using an A/chicken/Germany 'N'/49 (Hav2Neq1) strain ('N' virus). Five microlitres of serum specimen were applied to the agarose gel containing 100 μg per ml virion protein of 'N' virus. After 18 h incubation at 37 °C, the diameter of the opalescent zone surrounding the well was measured. The antibody level to influenza A NP was expressed as the area of the zone and a 1·5 fold or greater change was regarded as significant. According to this criterion, an antibody rise was observed in natural infections, but not after immunization (Yamane *et al.* 1981).

Statistical analysis

The geometric mean titres (g.m.t.) of anti-HA and anti-NA antibodies were calculated and Student's *t* test was performed as previously described (Center for Disease Control, 1975).

RESULTS

Serodiagnosis of natural infection with influenza A virus during the period examined

Since all the volunteers received vaccinations for successive years, the serodiagnosis of natural infection was mainly on the basis of antibody response to NP antigen of influenza A virus (Yamane *et al.* 1981). When the sera collected in January and March of 1979 were examined, none showed a significant antibody rise to NP antigen, ruling out the occurrence of natural infection in these vaccinees (Fig. 1). The same result was also obtained in 1980. Thus an investigation of the changes of antibody levels after immunization, unimpeded by the effect of natural infection, could be performed.

The antibody response to H1 haemagglutinin after immunization

Figure 2 shows a 3 year trend of the geometric mean HI antibody titres to the A/USSR/92/77(H1N1) strain. In both the infected and non-infected groups, the geometric mean HI titres rose in parallel immediately after immunization. However, a difference was found in the ratio of significant HI antibody rises between the infected and the non-infected groups (Table 1). The group which had not experienced natural infection with H1N1 virus in the past showed a significantly lower HI titre than that which had been infected. The infected group had a g.m.t. of almost 400, whereas the g.m.t. of the non-infected group was 64. The results of the g.m.t. for the two groups were compared after two antigenic stimuli, i.e. the g.m.t. in January 1979 for the infected group and the g.m.t. in January 1980 for the non-infected group. The distribution of HI titres also indicated a difference between the two groups. The percentage of the vaccinees whose HI antibody titre was $\geqslant 64$ in the infected group was nearly 100 %, whereas in the non-infected group it was 60 % ($P < 0{\cdot}02$). Taking a titre of $\geqslant 128$, the frequency of the infected group was 95 % and in the non-infected group was only 40 % ($P < 0{\cdot}05$).

Another H1N1 antigen, A/Fukushima/103/78, was present in the second vaccine. In the first 1978 trial, only the infected group showed a marked response

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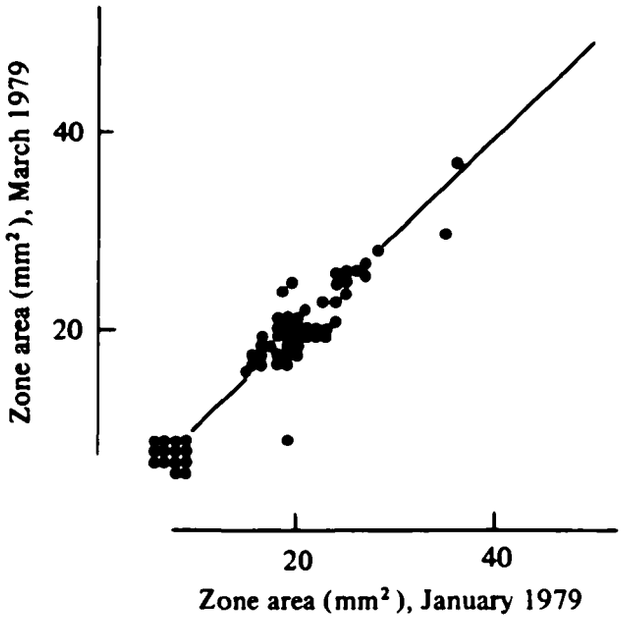

Fig. 1. Comparison of antibody levels to influenza A nucleoprotein by reversed single-radial-immunodiffusion between January 1979 and March 1979.

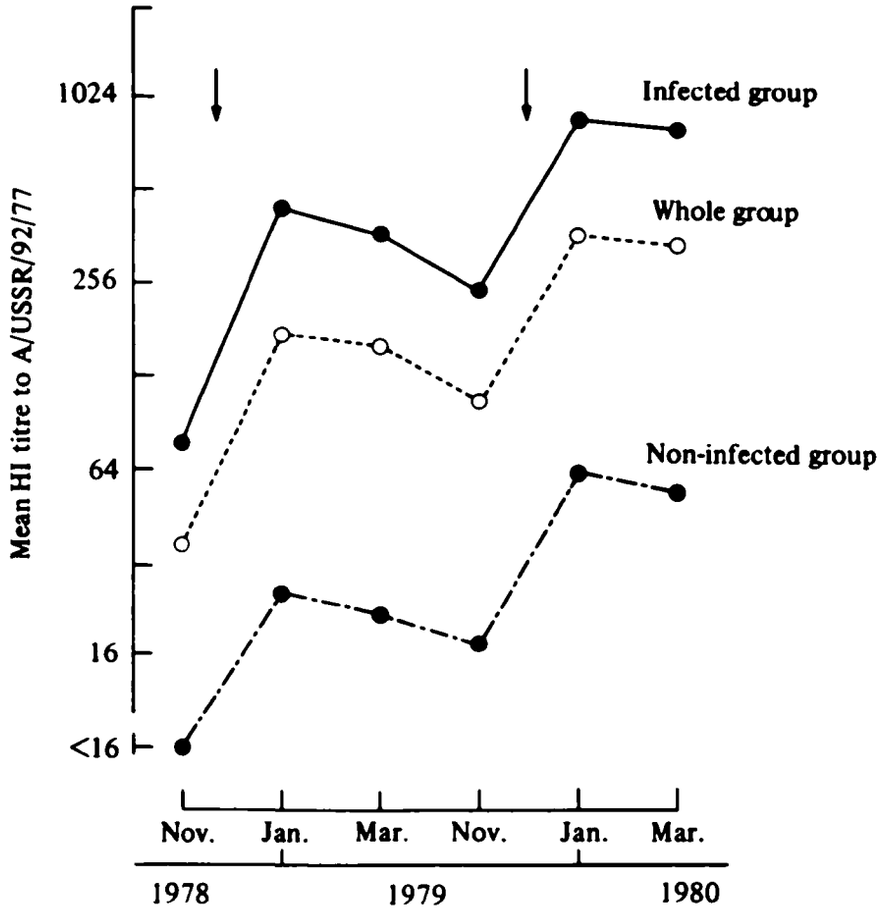

Fig. 2. The changes of the g.m.t. of HI antibody to A/USSR/92/77(H1N1) from November 1978 to March 1980, classified according to the presence or absence of natural infection history with H1N1 virus. Arrows indicate the dates of immunizations.

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Table 1. *Summary of the geometric mean titres of haemagglutination-inhibiting (HI) and neuraminidase-inhibiting (NAI) antibodies and the incidence of significant antibody rises after immunization in* 1978 *and* 1979

	Geometric mean titre in						% with significant antibody rises after	
	Nov., 1978	Jan., 1979	Mar., 1979	Nov., 1979	Jan., 1980	Mar., 1980	Immunization in 1978	Immunization in 1979
HI antibody titres to								
A/USSR/92/77 (H1N1)								
Whole group	36·6	184·9	151·4	110·4	371·5	354·8	69·4	61·3
Infected group	79·3	478·6	371·5	245·5	933·3	851·1	83·3	71·4
Non-infected group	8·0	26·2	22·9	20·9	64·6	57·5	45·0	50·0
A/Fukushima/103/78 (H1N1)								
Whole group	11·5	33·9	29·5	24·0	134·9	117·5	50·0	71·0
Infected group	14·5	64·6	55·0	38·0	199·5	177·8	71·4	71·4
Non-infected group	8·0	9·8	8·5	8·1	34·7	36·3	10·0	65·0
A/Yamanashi/2/77 (H3N2)								
Whole group	170·0	320·4	212·6	154·2	153·8	136·3	69·4	0·0
B/Kanagawa/3/76								
Whole group	47·9	64·6	58·9	53·7	70·8	66·1	4·8	3·2
NAI antibody titres to								
A/USSR/90/77 (N1)								
Whole group	4·0	6·8	6·1	5·2	7·0	7·1	21·0	4·8
Infected group	8·1	17·5	14·6	11·7	17·8	17·5	33·3	7·1
Non-infected group	1·1	1·6	1·2	1·1	1·2	1·3	0·0	0·0
A/Port Chalmers/1/73 (N2)								
Whole group	79·4	83·2	70·8	70·8	69·2	61·7	0·0	0·0

Seronegatives, < 16 in HI and < 2 in NAI tests, were calculated as 8 and 1, respectively.
Significant antibody rises were estimated as fourfold or greater rises in HI titres and as threefold or greater rises in NAI titres.

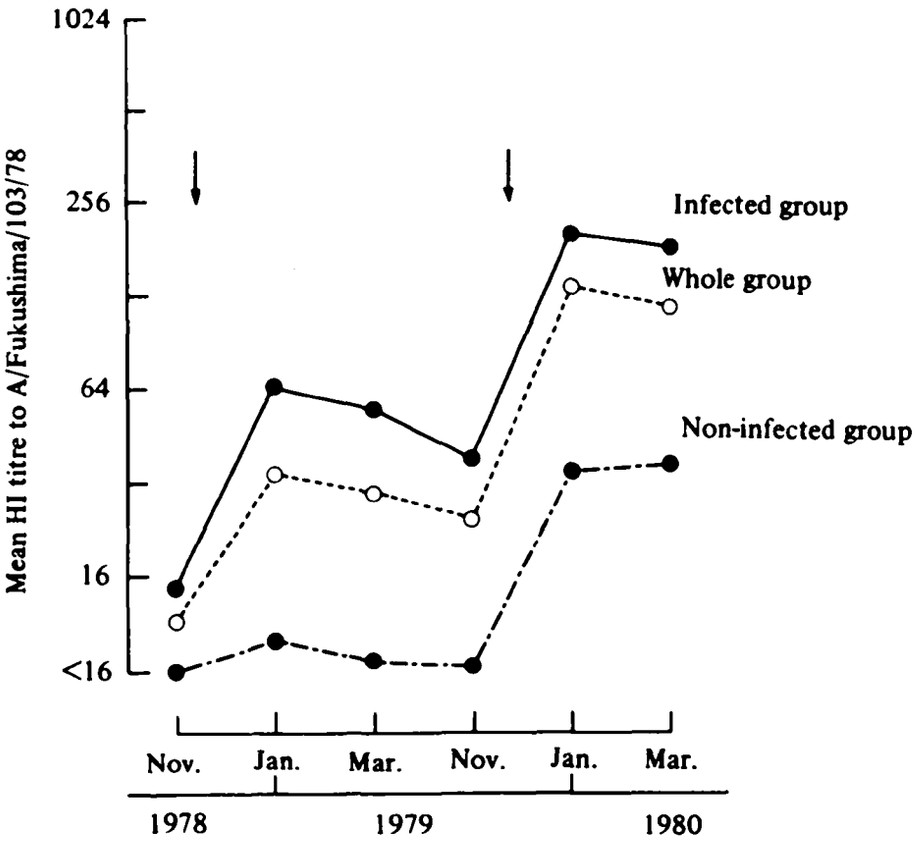

Fig. 3. The changes of the g.m.t. of HI antibody to A/Fukushima/103/78(H1N1) from November 1978 to March 1980. Arrows indicate the dates of immunizations.

Table 2. *The acquisition of HI activity to A/New Jersey/8/76 (Hsw1N1) virus after the two immunizations containing A/USSR/92/77 (H1N1) virus*

	Number of students (percentage) with HI titre of					
	< 16	16	32	64	128	256
Infected group						
1978-immunization						
Before immunization	42 (100)	0	0	0	0	0
After immunization	31 (74)	5 (12)	5 (12)	1 (12)	0	0
1979-immunization						
Before immunization	37 (88)	4 (10)	1 (2)	0	0	0
After immunization	30 (71)	4 (10)	4 (10)	1 (2)	3 (7)	0
Non-infected group						
1978-immunization						
Before immunization	20 (100)	0	0	0	0	0
After immunization	20 (100)	0	0	0	0	0
1979-immunization						
Before immunization	20 (100)	0	0	0	0	0
After immunization	20 (100)	0	0	0	0	0

to this heterologous A/Fukushima/103/78 strain as the result of cross-reactivity (Fig. 3). However, immunization of the non-infected group resulted in a negligible response to Fukushima strain. When they received the second dose of vaccine containing the Fukushima strain in 1979, both infected and non-infected groups showed a marked antibody rise to the Fukushima strain in parallel to response to the USSR strain. This presumably indicates a priming effect, in the non-infected group, of the USSR strain contained in the first year vaccine.

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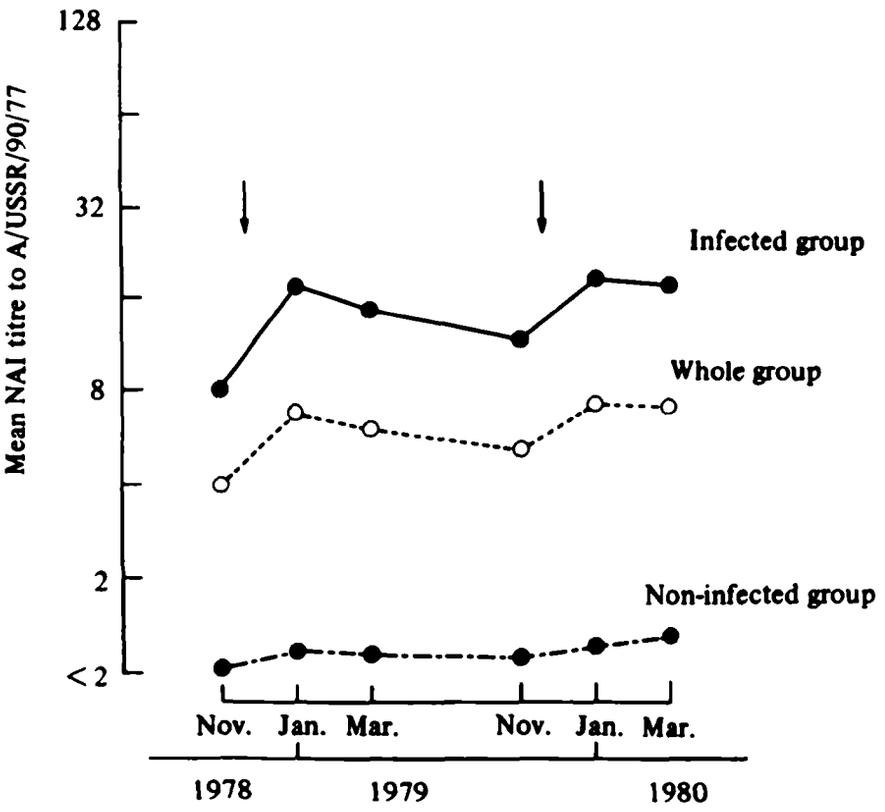

Fig. 4. The changes of the g.m.t. of NAI antibody to A/USSR/90/77(N1) from November 1978 to March 1980. Arrows indicate the dates of immunizations.

Cross-reactivity to Hsw1 antigen of the sera obtained after H1N1 influenza immunizations

A comparison was made between the HI antibody against A/New Jersey/8/76 (Hsw1N1) in the infected and non-infected groups. The vaccinees whose sera showed significant HI reactivity to Hsw1 antigen was limited to the infected group, and no member of the non-infected group showed cross-reactivity to Hsw1 antigen (Table 2).

The antibody response to N1 neuraminidase after immunizations

Figure 4 shows the g.m.t.s of NAI antibody during the 3 years of the study. Only the infected group possessed an antibody titre before immunization and this group also showed a good response to N1 antigen after the immunizations of 1978 and 1979. On the other hand, the non-infected group did not have significant antibody titres or rises in titre, even after the second immunization. Contrary to the HI antibody (Fig. 2), a booster effect of repeated immunizations on the NAI antibody response was not evident. The g.m.t.s of NAI antibody in the infected group after the first and second immunization were almost identical (16), whereas both g.m.t.s were < 2 in the non-infected group. The absence of a booster effect of vaccine on NAI titres was also demonstrated when the distribution of NAI titres in the infected group was compared before and after immunization in each of the 2 years (data not shown).

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The antibody response to Hong Kong influenza and influenza B viruses

The g.m.t.s of HI and NAI antibodies of the sera tested against H3N2 antigen were maintained at higher levels than those of H1N1 viruses (Table 1). This presumably reflects past antigenic stimulation either by natural infection or artificial immunization. However, the antibody levels gradually decreased after the first immunization, because of the omission of Hong Kong virus from the second vaccine. When the changes of HI and NAI titres were compared, an apparent difference was found between them. A sharp rise between pre- and post-immunization HI titres in 1978–79 was found, whereas no such rise could be demonstrated for the NAI titres. As with the results of H1N1 immunizations, it was clearly demonstrated that H3N2 immunization had no booster effect on NAI antibody titres.

Both the 1978 and 1979 vaccines contained 200 CCA units per ml of influenza B/Kanagawa/3/76 strain. However, the HI antibody response to influenza B was poor (Table 2), as previously reported (Clark *et al.* 1970; Wright, Bryant & Karzon, 1980). A fourfold or greater antibody response to B/Kanagawa HA antigen was seen in only 3–5% of the students tested, and the geometric mean HI titres remained unchanged at approximately 50–70.

DISCUSSION

A programme of annual influenza immunization has been recommended and carried out in Japan since 1960 (Dowdle *et al.* 1980). This assumes that the high incidence of influenza-associated morbidity among schoolchildren plays a role in extending outbreaks of influenza in the general population. Thus routine immunization of schoolchildren against influenza may benefit the community through reducing influenza transmissions (Francis, 1967). However, problems still exist in the present immunization programme, such as antigenic mismatching due to recent antigenic drift or shift between the vaccine strain and the epidemic strain. This study was initiated to evaluate the effect of the current influenza immunization programme in Japan after the appearance of a new antigenic strain, the A/USSR/77 (H1N1) virus. The schoolchildren examined were classified into two groups according to the presence or absence of pre-existing antibodies acquired by natural infection with H1N1 virus. This allowed us to estimate the anamnestic antibody response during successive immunizations with A/USSR/92/77 virus.

Although post-vaccination HI antibody levels differed according to the presence or absence of a previous history of natural infection with H1N1 virus, the ratios of significant antibody rises were fairly high and the HI antibody titres would be expected to be protective (Feery, 1979). However, in terms of cross-reactivity of the HI antibody acquired after repeated immunizations to the various antigenic drift strains, a difference was found between persons who had been naturally infected and persons who had only been exposed to H1N1 virus in the form of vaccine. Recent studies reported that following immunization with either inactivated whole or split vaccines, most adults responded mainly by producing a cross-reactive or common antibody among Hong Kong viruses (Webster *et al.* 1976; Schild *et al.* 1977; Oxford *et al.* 1979), probably indicating original antigenic sin after vaccination with influenza viruses (Hennessy, Davenport & Francis, 1955;

Davenport & Hennessy, 1956, 1957). In the present studies, the poorer cross-reactivity to some drift strains, including Hsw1 antigen, among the people who had not been infected may be explained by the lack of immunological memory to a cross-reactive antigenic determinant which the natural infection may induce but the artificial immunization may not.

In the studies on Hsw1 immunization, it was demonstrated that individuals who received New Jersey Hsw1 immunization responded to the USSR H1 strain, especially those primed with the H1 antigen (Masurel & André, 1978; Sarateanu & Ehrengut, 1978; Foy *et al.* 1980). In the same way, those who had been infected with H1N1 virus and then were given the H1N1 vaccine showed significant HI reactivity to Hsw1 antigen. However, the possibility that this is due to steric hindrance by the NAI antibody of the HI antibody still exists. Although our observations indicate that the cross-reactivity of HI antibody is more likely, further study using recombinant antigens, i.e. Hsw1Neq1, is required to determine to what extent steric hindrance is involved.

The results described here demonstrate that a history of natural infection greatly influences the NAI antibody response to the successive immunizations. The role of NAI antibody in the natural challenge of the virus was not so great as that of the HI antibody. However, recent studies have indicated a significant relationship between NAI antibody level and protection or modification of illness (Murphy *et al.* 1972; Monto & Kendal, 1973; Yamane *et al.* 1979*b*). The present Japanese split vaccine is clearly not effective in inducing an NAI antibody response in persons not primed with natural infection. Moreover, no booster effect is evident in persons primed in the past. The limited NAI antibody response following immunization with split vaccine indicates the difficulty in achieving immunity to viral neuraminidase by immunization. This difficulty is compounded by the recent findings that the NA activity of some concentrates may be unstable even over a period of a few months, and that different batches of various concentrates prepared with similar strains may vary in NA activity (Kendal, Bozeman & Ennis, 1980). The results of this study indicate that NAI antibody titres in individuals depend mainly on previous natural infection. If this is the case, it may be necessary to use a live attenuated influenza vaccine or to use a sufficient dose of NA antigen in vaccines to ensure a good NAI antibody response.

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