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EVALUATION OF THE EFFICACY OF SPLIT-PRODUCT TRIVALENT A (H1N1), A (H3N2) AND B INFLUENZA VACCINE

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ABSTRACT

The efficacy of split-product trivalent A(H 1 N 1), A(H 3 N 2) and B influenza vaccine in primary school childlen aged seven to 12 years was studied during 1980-1984. This vaccine was adequately immunogenic with infrequent reactions. Hemagglutination inhibition (HAI) antibody responses to A (H 3 N 2) virus were the most excellent among the components. The incidence of confirmed infection of the vaccinees were 6.5 % to 34.8% lower than those of the non-vaccinees in either years. As the result, the efficacy of vaccine was recognized ($x^2=76.34$, $p< 0.001$). However, this was not recognized in an entrant school's epidemic in 1984 caused by intense antigenic drift strain. The protective level of HAI antibody titer was 256, but increased to 521 in 1984 epidemic reflecting the high rate of isolates with intense antigenic drift.

INTRODUCTION

The antigenic structure of influenza A virus undergoes frequet alterations. In some years, the alterations relatively small (antigenic drift), while in others, they are drastic (antigenic shift) (Stuart-Harris and Schild, 1976 a). Furthermore, in addition to influenza A virus, the antigens of influenza B virus have been shown to undergo such antigenic drift (Schild et al., 1973). Because of this inconstancy in virus antigenic structures, influenza epidemics have annually experienced, resulting in a notable cause of mortality, morbidity and lost of productivity.

Since the Asian flu outbreak in 1957 in Japan, high population density has resulted in a rapid virus circulation among inhabitants, especially among school children, with the highest attack rates and primary disseminations of the virus in the home and community. In light of these epidemiological findings, the administration of whole virion vaccine to school children was initiated in 1962 as collective preventive service. The philosophic and conseptual basis for the vaccination policy in Japan originally differed from that in the United States in that vaccination was considered personal preventive service and udertaken primarily to protect the individuals at highest risk. Thereafter, the whole virion vaccine was changed to a current split-product trivalent vaccine in 1972, and annual vaccination has become compulsory for all school children aged three to 18 years since 1977 (Dowdle et al., 1980). Currently, 85% to 95% of approximately 21 million school children are receiving annual vaccinations according to the detailed program offered by the school physician and the school nurse. But in recent years, despite the considerable times and efforts expended in planning and enforcing the vaccination, misgivings have arisen concerning the efficacy of vaccine because of continuing influenza epidemics and the difficulty of distingushing, without laboratory tests, the infleunza from others caused by many different infectious agents.

Under these circumstances, the present study was originally undertaken in the fall of 1980 to evaluate the efficacy of the vaccine in 161 primary school children in Niigata Prefecture, Japan, and continued to 1984, including a total of 1995 children. During the study period, epidemics caused by antigenically different influenza viruses such as A (H 1 N 1) epidemics in 1981 and 1984, B epidemic in 1982 and A (H 3 N 2) epidemic in 1983, were experienced, and at the same time antigenic drift strains were frequently isolated. This gave us the opportunity to investigate retrospectively whether vaccination could induce protection from natural infection caused by antigenically different influenza viruses and strains with various degrees of antigenic difference from the vaccine strain.

We report here on the field studies of the vaccinees and a comparison group during these four influenza epidemics.

Study Population and Methods

Study population. A total of 1995 primary school children aged seven to 12 years in Niigata Prefecture, Japan, took part in this study from 1980 to 1984. All were in good health and none had a history of allergy. The participants were divided into two groups besed on their consent to vaccination (1464 vaccinees and 531 non-vaccinees). Informed consent was obtained from both participants and their parents or guardians through the school physician or the nurse, Because all but a few of the vaccinees received two doses of vaccine in a vaccination season, the vaccinees who received only one dose of vaccine were omitted, so a group of vacciees was the group receiving two doses of vaccine in this study, but the history of vaccinations before and during this study differed case by case. The number of participants by group, school and year were as follows: 116 children (85

vaccinees and 31 non-vaccinees) in the S schools in 1980-81; 625 children (467 vaccinees and 158 non-vaccinees) in the K, M, and S schools in 1981-82; 564 children (435 vaccinees and 129 non-vaccinees) in the K, M, and O schools in 1982-83 and 690 children (477 vaccinees and 213 non-vaccinees) in the H, K, and M schools in 1983-84.

Identical questionnaires were sent to all participants through the school nurse and fllled out to obtain information concerning adverse reactions to the vaccine after each vaccine administaion, and later concerning respiratory illnesses during winter.

Vaccine. The commercial split-product trivalent A(H 1 N 1), A(H 3 N 2) and B influenza vaccine (Denka Institute of Biological Science, Tokyo, Japan) was used throughout this study. The virus strains and these composition expressed as chick cell -agglutinating intact equivalent units (CCA)/ml in a vaccine were annually determined at the National Institute for Health of Japan as shown in Table 2.

Vaccine was administrated 0.5 ml at a time with two subcutaneous inoculations four weeks apart between October and November.

Serological methods and virus isolation procedures. Three sets of sera from all participants, collected before vaccination (in October), four weeks after the second dose (in December), and four weeks after the epidemics (between March and April), were tested for hemagglutination inhibition (HAI) antibodytiters against the vaccine strains, unless otherwise noted. Before HAI titer was measured, one part of serum and three parts of the receptor-destroying enzyme (RDE; *Vibrio cholerae*) were mixed, incubated at 37℃ overnight, and then heated at 56℃ for 30 min. Thereafter, natural agglutinin was removed by adding 0.05 ml of packed red cells and lefting the mixture stand 20 min at room temperature. Serum became diluted at ratio of 1:4 after this treatment. Serial dilutions of 0.2 ml of the treated serum were incubated with equal volumes of the influenza virus containing 4 HA units. After the wells had stood for 1 hr at room temperature, 0.4 ml of 0.5% solution of chicken red cells was added to each well and the HAI titer was read. The HAI titer was expressed as the highest reciprocal dilution that could completely inhibit hemagglutination. It should be noted that a 64 HAI titer in this study is equivalent to a titer of 16 in the United States or European countries because of the manner of expression for HAI titer in Japan. The lower limit of detection was HAI titer of 32. A fourfold or greater rise in HAI titer was taken as proof of infection. In this study, an HAI titer giving $\leqq$10% incidence of infection was considered as the protective level for the reasons described in the results and discussion sections.

At the same time, to analyze the predominantly causative virus type or subtype, and the degree of antigenic difference between the vaccine and field strains in each epidemic, virus isolations were attempted using throat swabs collected from affected individuals including some of the participants in the four schools, and by inoculation them into the allantoic cavities of embryonated eggs. When an isolate was inhibited below 1: 256 dilution of the antibody originally containing 1024 HAI titer to the homologous vaccine strain, this isolate was defined as an intense antigenic drift strain in this study.

Statistical analysis. The x^2 with one degree of freedom and with or without Yates' s correction was applied to the difference between proportions using the observed values. The correlation between proportions was analyzed by the correlation coefficient with Fisher's transformation of 'r' to 'z'. In calculation of geometric mean titer (GMT), titer of <32 were considered as 0.

RESULTS

Vaccine reactions. Data concerning vaccine reactions were almost the same throughout the four vaccination seasons, so that the latest data obtained in 1983 are shown in Table 1 as representative. Data were returned on the quetionnaires from 96.6% and 96. 9% of the vaccinees after the first and second dose, respectively. The percentage of the vaccinees who had not complained of any local and systemic reactions was 62.7 (53.6 and 71.9 after the first the and second dose, respectively). As local reactions, redness, tenderness and local pain lasting at most three days were reported from 19.2%, 12.4% and 12.5% of the vaccinees, respectively. On the other hand, as systemic reactions, fever (37℃-38℃) lasting one to three days, malaise, headache, abdominal pain, diarrhea and vomiting were reported from 1.7%, 5.1%, 6.5%, 3.4%, 1.1% and 0.3% of the vaccinees, respectively, and less frequently than local ones. All the reactions including local and systemic ones, except abdominal pain, were less frequent after the second dose than the first dose.

Furthermore, to compare systemic symptoms at the intervaccination period with those after vaccinations, another questionnaire was sent to 690 children including both vaccinees and non-vaccinees in May, 1984 and data returned on the questionnaires from 662 of the children are shown in Table 1. 86.6% of reporting children were without any symptoms, but 1.8%, 2.7%, 6.3%, 6.8%, 1.4% and 0.5% of children reported to have complained of fever, malaise, headache, abdominal pain, diarrhea and vomiting, respectively. When the differences of incidences of such symptoms between after vaccinations and the intervaccination period were applied to x^2 test, a significant difference was recognized only in malaise ($x^2=5.52$, $p<0.025$), but not in fever, headache, diarrhea and vomiting ($\alpha=0.05$). In contrast, abdominal pain was rather more frequent at the intervaccination period ($x^2=9.99$, $p < 0.005$).

HAI antibody responses. Table 2 shows the patterns of HAI anti-body responses to each influenza virus antigen expressed as (1) the rate of participants with a fourfold or greater rise HAI antibody titer following two dose of vaccine (the rate of significant rise), (2) the ratio of GMTs between pre-and post-vaccination. The rates of significant rise were dispersed in a considerably wide range between 5.3% and 51.5%, but A (H3N2) induced the most excellent responses among the vaccine components in all the years. The GMTs at pre-and post-vaccination ranged from 59.3 to 172.4 and 173.6 to 347, 3, respectively. As a result, the maximum ratio was 4.47 and the minimum was 1.52. The mean ratios through the four vaccination seasons were 3.11 in A (H3N2), 2.35 in A (H1N1)

Table 1. Incidence of reactions following the first and second dose and comparison of incidence of systemic reactions with those at intervaccination period.

Reactions	Percent with or without reactions at indicated period of observation			
	First dose	Second dose	Total	Inter-vaccination
Without any reactions	53.6	71.9	62.7	86.6
Local reactions				
Redness	22.1	16.2	19.2	
Tenderness	13.7	11.0	12.4	
Local pain	16.3	8.7	12.5	
Systemic reactions				
Fever (37℃–38℃)	1.7	1.7	1.7	1.8
Malaise	5.6	4.5	5.1	2.7
Headache	6.3	6.7	6.5	6.3
Abdomical pain	3.3	3.5	3.4	6.8
Diarrhea	1.3	0.9	1.1	1.4
Vomiting	0.4	0.2	0.3	0.5

NOTE. Data were returned on the questionnaires from 461 (96.6%) and 462 (96.9%) of 477 vaccinees on about on the seventh day after the first and second dose, respectively, in 1983 and from 662 (95.9%) of 690 children including the vaccinees and non-vaccinees in May of 1984 during the intervaccination period.

Table 2. HAI antibody responses following the second dose by year amd virus antigen.

Year	Vaccine strain*	CCA*	Vaccinees			Non-vaccinees	
			No. of subjects	No. (%) with ≧fourfold rise	Change in GMT* (Ratio)	No. of subjects	GTM*
1980	A/Kuma. /37/79	200	85	30 (35.3)	73.5–245.6 (3.34)	31	61.4
	A/Bang. /1/79	200		39 (45.9)	59.3–265.0 (4.47)		33.4
	B/Kana. /3/76	300		34 (40.0)	69.1–174.9 (2.53)		51.3
1981	A/Kuma. /37/79	300	467	65 (13.9)	146.0–263.2 (1.80)	158	130.7
	A/Bang. /1/79	250		92 (19.7)	138.1–278.2 (2.01)		83.9
	B/Sing. /222/79	150		88 (18.8)	81.6–173.6 (2.14)		59.3
1982	A/Kuma. /37/79	250	435	53 (12.2)	126.2–221.3 (1.75)	129	100.4
	A/Niig. /102/81	300		224 (51.5)	78.2–308.2 (3.94)		41.4
	B/Sing. /222/79	150		23 (5.3)	155.4–235.6 (1.52)		147.0
1983	A/Kuma. /37/79	200	477	115 (24.1)	123.6–310.8 (2.51)	213	100.4
	A/Ishi. /7/82	350		127 (26.6)	172.4–347.3 (2.01)		108.4
	B/Sing. /222/79	150		92 (19.3)	140.1–308.7 (2.20)		93.7

NOTE. A/Kuma. /37/79 virus is H1N1 subtype and other A viruses are H3N2.
*Kuma.=Kumamoto, Bang.=Bangkok, Kana.=Kanagawa, Sing.=Singapore, Niig.=Niigata, Ishi.=Ishikawa. CCA=cell agglutinating intact equivalent units/ml. GMT=geometric mean titer.

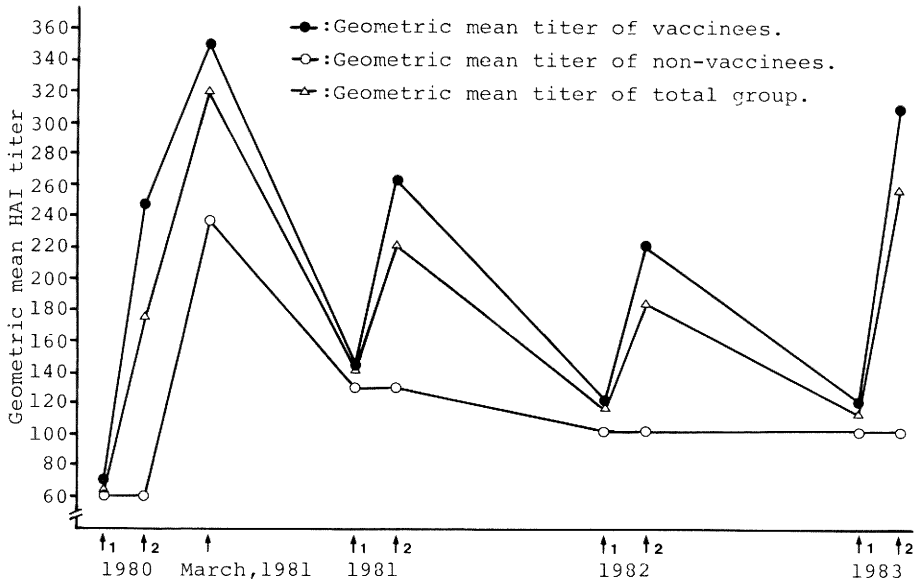

Fig. 1. Persistence of HAI antibodies to A/Kumamoto/37/79 (H1N1) virus following annual vaccinations and natural infection. Arrows numbered 1 and 2 (abscissa) indicate times when serum samples were collected before vaccination in October and after vaccination in December, respectively, in each year. March,1981 (arrow in abscissa) indicates the time when serum samples were collected after an A (H1N1) epidemic.

and 2.10 in B influenza virus antigen. In this category, A (H3N2) virus also induced the most excellent response. Furthermore, there was a tendency to show a poorer response to the same type or subtype virus antigen as the causative one during the pretracted epidemic; for example, the ratio of GMTs decreased from 3.34 in 1980 to 1.80 in 1981 after the A (H1N1) epidemic, from 2.14 in 1981 to 1.52 in 1982 after the B epidemic and from 3.94 in 1982 to 2.01 in 1983 after the A (H3N2) epidemic.

On the other hand, in the case of the non-vaccinees, the rises in HAI antibody titers were not observed before influenza epidemics, i.e., the GMTs ranged between 33.4 and 147.0, which one-half to one-eighth of those of the vaccinees at post-vaccination.

Persistence of HAI antibodies to A/Kumamoto/37/79/ (H1N1) virus antigen following annual vaccinations and a natural infection.

The fact that the single strain, A/Kumamoto/37/79, was used as an A (H1N1) component of the vaccine through this study provided an unique opportunity to assess the persistence of antibodies as a result of four annual vaccinations, and also as a result of an A (H1N1) epidemic in 1981 in the case of the non-vaccinees (Fig. 1). The initial GMT of 73.5 increased to 245.6 following vaccination in the fall of 1980, and further increased to 350.0 after the A (H1N1) epidemic in the winter of 1981. Thereafter, 1.8 to 2.5-fold rises by annual vaccinations were observed, such as from 146.0 to 263.2 in 1981, from 126.2

to 221.3 in 1982 and from 123.6 to 310.8 in 1983, but at the same time, these decreased to about one-half of the GMTs ten months after vaccination. On the other hand, the initial GMT of 61.4 of the non-vaccinees also increased to 238.9 following a natural infection in the winter of 1981, then decreased to 130.7 in the next ten months and finally in the fall of 1983, further decreased to 100.4 which was close to the initial GMT. After two doses of vaccine the GMTs of the vaccinees and entire groups were near to or more than the protective level of HAI antibody titer, but, with striking contrast, in the case of the non-vaccinees, this level of GMTs was not detectable except immediately after natural infection.

Efficacy of influenza vaccine. As shown in Table 3, four epidemics moderate in scale (about 33000 to 44000 reported patients and five to seven weeks duration) caused by different type or subtype influenza viruses, were experienced in Niigata Prefecture, Japan, during this study. At the same time, the strains with defined antigenic drift were isolated with the frequency of 8.3% to 68.8%. Under these circumstances, the incidences of confirmed infection were compared between two groups to evaluate the efficacy of vaccine. Although it has been reported that 40 HAI titer (equivalent to 128 or 256 HAI titer in this study) is a consistently protective level (50% protective level) (Meiklejohn et al., 1952; Hobson et al., 1973;Evans, 1973), 128 HAI titer did not always give 50% incidence of infection, but in contrast , more than 256 HAI titer gave almost $\leqq$10% incidence of infection. In light of these findings, an HAI titer giving $\leqq$10% incidence of infection was defined as the protective level of HAI antibody titer in this study. In this case, 10% was raised to as much as 11.6% incidence of infection at HAI titer in 1982 (in Table 4) from

Table 3. Number of isolates with defined antigenic drift* and characteristics of influenza epidemics in Niigata Prefecture, Japan, from 1981 to 1984.

				Characteristics of influenza epidemics		
Year	Virus type or subtype	No. of isolates	No. (%) of isolates with defined antigenic drift*	Predominantly caused by	Estimated duration (Weeks)	No. of reported patients
1981	A (H1N1)	60	5 (8.3)	A (H1N1)	7	43511
	B	3				
	A (H3N2)	0				
1982	B	61	18 (29.5)	B	7	33154
	A (H3N2)	1				
	A (H1N1)	0				
1983	A (H3N2)	106	37 (34.9)	A (H3N2)	6	34911
	A (H1N1)	0				
	B	0				
1984	A (H1N1)	16	11 (68.8)	A (H1N1)	5	38840
	A (H3N2)	0				
	B	0				

NOTE. *Defined as the antigenic drift strain that was inhibited by$\leqq$1: 256 dilution of the antibody containing 1024 HAI titer to the homologous vaccine strain.

Table 4. Comparisons of incidence of confirmed infection and prevalence of the protective level of HAI antibody titer between the vaccinees and non-vaccinees.

Year	Group	Incidence of infection								No. (%) with protective level	
		<32*	32*	64*	128*	256*	512*	≧1024*	Total (%)		
1981	Vaccinee	0/0	1/1	5/8	10/19	2/31	0/19	0/7	(21.2)	57/85	(67.1)
	Non-vaccinee	3/5	3/3	2/5	4/7	0/9	0/2	0/0	(38.7)	11/31	(35.5)
	Total (%)	(60.0)	(100)	(53.8)	(53.8)	(5.0)	(0.0)	(0.0)	(25.9)	68/116	(58.6)
1982	Vaccinee	5/5	9/10	45/76	50/133	22/167	4/60	0/16	(28.9)	243/467	(52.0)
	Non-vaccinee	13/18	13/22	20/43	10/46	0/23	0/4	0/2	(35.4)	29/158	(18.4)
	Total (%)	(78.3)	(68.8)	(54.6)	(33.5)	(11.6)	(6.3)	(0.0)	(30.6)	272/625	(43.5)
1983	Vaccinee	0/0	3/4	9/25	16/94	6/140	0/92	0/80	(7.8)	312/435	(71.7)
	Non-vaccinee	17/24	12/14	18/37	8/36	0/13	0/5	0/0	(42.6)	18/129	(14.0)
	Total (%)	(70.8)	(83.3)	(43.5)	(18.5)	(3.9)	(0.0)	(0.0)	(15.8)	330/564	(58.5)
1984	Vaccinee	0/0	5/6	22/26	51/72	66/173	16/153	1/47	(33.8)	200/477	(41.9)
	Non-vaccinee	6/7	26/34	30/47	34/56	13/52	1/14	0/3	(51.6)	17/123	(8.0)
	Total (%)	(85.7)	(77.5)	(71.2)	(66.4)	(35.1)	(10.2)	(2.0)	(39.3)	217/690	(31.4)

NOTE. Incidence of infection is given as a number with≧fourfold rise in antibody titer after epidemic/a number with indicated antibody titer before epidemic. The protective level of HAI antibody titer is defined as a titer giving≦10% incience of infection in the total group (256 HAI titer in 1981–1983 and 512 HAI titer in 1984). * =HAI titer.

the nature of the two-fold dilutions. Data are summarized in Table 4.

Epidemic in 1981: As a total, 30 of 116 (25.9.%) children showed seroconversions against the vaccine strain, A/Kumamoto/37/79 (H1N1) virus. The percent incidence of infection of the vaccinees and non-vaccinees were 21.2and 38.7, respectively. The difference between the groups was not significant ($\alpha = 0.05$) . The percentages of the vaccinees and non-vaccinees with the protective level of HAI antibody titer (256) were 67.1 and 35.5, respectively, and the difference in these prevalences was significant ($x^2 = 9.34$, $p < 0.005$).

Epidemic in 1982: As a total, 191 of 625 (30.6%) children showed seroconversions against the vaccine strain, B/Singapore/222/79 virus. The percent incidences of infection of the vaccinees and non-vaccinees were 23.9 and 35.4, respectively. The difference between the two groups was not significant ($\alpha = 0.05$). The percentages of the vaccinees and non-vaccinees with the protective level of HAI antibody titer (256) were 52.0 and 18.4, respectively, and the difference in these prevalences was significant ($x^2 = 54.48$, $p < 0.001$).

Epidemic in 1983: As a total, 89 of 564 (15.8%) children showed seroconversions against the vaccine strain,A/Niigata/102/81 (H3N2) virus. The percent incidences of infection of the vaccinees and non-vaccinees were 7.8 and 42.6, respectively. The difference between the two groups was significant ($x^2 = 90.77$, $p < 0.001$). The percentages of the vaccinees and non-vaccinees with the protective level of HAI antibody titer (256) were 71.7 and 14.0, respectively, and the difference in these prevalences was significant ($x^2 = 103.97$, $p < 0.001$).

Epidemic in 1984: As a total, 271 of 690 (39.3%) children showed seroconversions

against the vaccine strain, A/Kumamoto/37/79 (H1N1) virus. The percent incidences of infection of the vaccinees and non-vaccinees were 33.8 and 51.6, respectively. The difference btween the two groups was significant ($x^2=19.76$, $p<0.001$). The percentage of the vaccinees and non-vaccinees with the protective level of HAI antibody titer (512) were 41.9 and 8.0, respectively, and the difference in these prevalences was significant ($x^2=78.71$, $p<0.001$). As a whole, the incidences of infection of the vaccinees were always lower than those of the non-vaccinees in either years, and also the difference between two groups was statistically significant ($x^2=76.34$, $p<0.001$). The protective level of HAI antibody titer giving $\leqq 10\%$ incidence of infection was 256, but 512 in the 1984 epidemic when isolates with intense antigenic drift were at the highest frequency, as much as 68.8%, compared with those of 8.3% to 43.9% in other years, as shown in Table 3. The prevalences of the vaccinees with the protective level of HAI antibody titer were significantly higher than those of the non-vaccinees in all of the years. The correlations between the incidences of infection and the prevalences of the protective level of HAI antibody titer were recognized in the vaccinees ($r=0.929$, $p<0.1$), and the total group ($r=0.905$, $p<0.1$), but to a certain degree, this was not recognized in the non-vaccinees ($r=0.641$).

Comparison of the efficacy of vaccine between the H and K schools in 1984 epidemic. During 1984 epidemic, 16 strains of A (H1N1) virus were isolated. Two of them were each independently isolated from a child in the H and a child in the K school (refferred to as the H and K strain, respectively). In the antigenic structures, the H strain was more closely related to the vaccine strain (A/Kumamoto/37/79) than the K strain because

Table 5. Comparison of efficacy of vaccine between the H and K schools during an A (H1N1) epidemic in 1984.

Strain used for		Incidence of infection by indicated HAI antibody titer							No. (%) with 256 HAI titer	
HAI test	Group	≦32	64	128	256	512	≧1024	Total(%)	at pre-epidemic	
—— H school ——										
A/Kuma*	Vaccinee	1/2	1/3	2/9	3/35	2/59	0/16	(7.3)	110/124	(88.7)
	Non-vaccinee	9/16	5/17	5/14	3/20	0/5	0/1	(30.1)	26/73	(35.6)
	Total (%)	(55.6)	(30.0)	(30.4)	(10.9)	(3.1)	(0.0)	(15.7)	136/197	(69.0)
——K school——										
A/Kuma*	Vaccinee	4/4	18/20	41/53	48/98	11/59	1/15	(49.4)	172/249	(69.1)
	Non-Vaccinee	1/1	3/3	3/6	2/5	0/0	0/1	(56.3)	6/16	(37.5)
	Total (%)	(100)	(91.3)	(74.6)	(48.5)	(18.6)	(6.3)	(49.8)	178/265	(67.2)
A/Dune.	Vaccinee	93/134	29/75	9/35	0/5	0/0	0/0	(52.6)	5/249	(2.0)
	Non-vaccinee	7/10	2/4	0/2	0/0	0/0	0/0	(56.3)	0/16	(0.0)
	Total (%)	(69.4)	(39.2)	(24.3)	(0.0)			(52.8)	5/265	(1.9)

NOTE. Incidence of infection is given as a number with ≧fourfold rise in antibody titer after epidemic/a number with indicated antibody titer. A/Dune. (=A /Dunedin/6/83) virus was antigenically identical to an isolate from the K school and showed defined antigenic drift as mentioned in Table 3. *=A/Kumamoto/37/79 (a vaccine strain).

the antibody containing 1024 HAI titer to the homologous vaccine strain showed 512 HAI titer to the H strain, but showed only 128 HAI titer to K strain. In addition, it was demonstrated by cross HAI test that the K strain was antigenically identical to A/Dunedin/6/83 virus. As shown in Table 5, in the case of the H school, the percent incidences of infection of the vaccinees and non-vaccinees were 7.3 and 30.1, respectively, and the differences of these incidences were significant ($x^2=18.14$, $p<0.001$). The protective level of HAI antibody titer was 256. On the other hand, in the case of the K school, the percent incidences of infection of the vaccinees and non-vaccinees were 49.4 and 56.3, respectively, when assayed to the vaccine strain and also 52.6 and 56.3, respectively, when assayed to A/Dunedin/6/83 virus. The sifnificant differences of the two proportions were not recognized in both cases ($\alpha=0.05$). Furthermore, the protective level of HAI antibody titer against the vaccine strain was markedly increased to 1024, although 256 HAI antibody titer was a consisitently protective level against the A/Dunedin/6/83 virus. The lack of the efficacy of vaccine in the K school reflected the lower prevalence of the protective level of HAI antibody titer, because the percentage of the vaccinees with 256 antibody titer to the vaccine strain was as high as 69.1, but only 2.0 to the A/Dunedin/6/83 virus.

Reported respiratory illnesses during influenza epidemics. All the participants in this study were given identical questionnaires in March of each year to obtain the information concerning respiratory illnesses during winter. In this study, the respiratory illness was defined as an acute febrile (≧37℃) respiratory illness lasting more than one day, with or

Table 6. Incidence of reported respiratory illnesses and inapparent influenza infection by year and group.

Year	Group	No. of subjects reporting	No. (%) with respiratory illnesses	other respiratory	confirmed influenza	No. (%) with inapparent influenza
1981	Vaccinee	76/85	65 (85.5)	49 (75.4)*	16 (24.6)*	0/11 (0.0)
	Non-vaccinee	28/31	25 (98.3)	15 (60.0)	10 (40.0)	0/3 (0.0)
	Total	104/116	90 (86.5)	64 (71.1)	26 (28.9)	0/14 (0.0)
1982	Vaccinee	443/467	294 (66.4)	175 (59.5)	119 (40.5)	11/149 (7.4)
	Non-vaccinee	147/158	113 (76.9)	65 (57.5)	48 (42.5)	3/34 (8.8)
	Total	590/625	407 (69.0)	240 (59.0)	167 (41.0)	14/183 (7.7)
1983	Vaccinee	422/435	248 (58.8)	219 (88.3)	29 (11.7)	4/174 (2.3)
	Non-vaccinee	100/129	68 (68.0)	39 (57.4)	29 (42.6)	9/32 (28.1)
	Total	522/564	316 (60.5)	258 (81.6)	58 (18.4)	13/206 (6.3)
1984	Vaccinee	471/477	348 (73.9)	198 (56.9)	150 (43.1)	9/123 (7.3)
	Non-vaccinee	208/213	153 (73.6)	63 (41.2)	90 (58.8)	17/55 (30.9)
	Total	679/690	501 (73.8)	261 (52.1)	240 (47.9)	26/178 (14.6)

NOTE. Inapparent influenza infection is defind as infection with seroconversion among subjects who did not report respiratory illnesses. *Percentage in parenthesis indicates proportion of subjects who reported respiratory illnesses.

without any of the following symptoms - nasal discharge, chills, cough, muscle ache, headache and unusual fatigue. The data are summarized in Table 6. The rates of returned questionnaires were 89.7% to 98.4%and almost the same in the two groups except in 1983. Although 60.5% to 86.5% of reporting children answered to have respiratory illnesses with almost equal frequency in the two groups, 18.4% to 47.9% of them were confirmed as influenza by singnificant rises in HAI titer, suggesting the difficulty in recognizing influenza without laboratory tests when it was caused by other causative agents. Among the reporting children without respiratory illness, a maximum of 14.6% were considered as inapparent influenza infections and the rates of inapparent infection were always higher in the non-vaccinees than in the vaccinees throughout all three influenza epidemics.

DISCUSSION

It has been reported that split-product influenza vaccine was adequately immunogenic while maintaining a lower incidence of reactions compared with those of whole virion vaccine (Gross et al., 1977a, 1977b). The adverse reactions reported in this study were also infrequent, and, even if reported, were chiefly local ones, mild in nature and of short duration. The incidences of all reactions but abdominal pain were less frequent after the second dose than after the first dose. Such findings were similar to those reported by other (Gross et al., 1977b; Bernstein et al., 1983). For systemic symptoms, it is noteworthy that most of them were reported at the intervaccination period with almost equal frequency, suggesting, that careful considerations are required to judge whether the systemic reactions are induced by vaccination or not. Concerning antibody responses, it should be noted that various factors influenced the responses during this study. The most likely factors may have been considerably high HAI titer induced by natural infection, persisting until the next vaccination season, and resulting in a poorer response to the same type or subtype virus antigen as the causative one during the influenza epidemic (Stuart-Harris et al., 1976b; Foy et al., 1981a). Furthrmore, quantitatively unfixed vaccine antigens might be cited as potential factors (Gross et al., 1977b, 1980). However, in general, the responses to A (H3N2) virus were the most excellent among the vaccine components through the four vaccination seasons. These findings coincided with those reported by other (Bernstein et al., 1982; Gross et al., 1983). In addition, it was confirmed that split-product trivalent influenza vaccine had adequately induced antibody production because the GMTs of the vaccines were two to eight-fold higher than those of the non-vaccinees to any of the vaccine antigens following two doses of vaccine.

At each epidemic during this study, about 61% to 87% of the children reported respiratory illnesses, but only about 18% to 48% of them were influenza confirmed by serological diagnosis. At the same time, virus strains with various degrees of antigenic variations were isolated in Niigata Prefecture, Japan. Briefly, A (H1N1) virus was first introduced in 1978, thereafter about 70% of isolates in the 1981 epidemic were antigenic

variants with two to four-fold lower immunoreactivity to the antibody against the vaccine strain (A/Kumamoto/37/79), and about 69% of isolates in the 1984 were further antigenic variants with four to eightfold lower immuno-reactivity to the antibody. A (H3N2) virus has successively undergone antigenic variation since its first appearance in 1968, and of 106 isolates in the 1983 epidemic, only about 10% were antigenically related to the vaccine strain (A/Bangkok/1/79). The B virus has also successively undergone antigenic variations since first isolation of B/Hong Kong/72 strain, and about 30% of isolates in the 1982 epidemic were antigenic variants with four to eight-fold lower immunoreactivity to the antibody against the vaccine strain (B/Singapore/222/79). Under these circumstances, the efficacy of the vaccine was examined by the comparison of the incidences of confirmed infection between the two groups. These incidences were 7.8% to 33.8% and 35.4% to 51.6% in the vaccinees and non-vaccinees, respectively, resulting in 6.5% to 43.8% lower incidences in the vaccinees in either epidemics. As a whole, the efficacy of vaccine was recognized ($x^2=76.34$, $p<0.001$). Indeed, the efficacy of vaccine should be assessd using data based on the incidence of infection confirmed of either serological or clinical diagnosis (Stuart-Harris et al., 1976b), but we have no exact data to determine which method of assessment is more accurate. However, at least we can say that misgivings concerning the efficacy of vaccine shoud not be derived from the public eatimation in the absence of the incidence of confirmed infection. It is noteworthy that the rates of inapparent influenza infection of the non-vaccinees were always higher than those of the vaccinees in this study, suggesting the recommendation of serological diagnosis rather than clinical diagnosis to avoid the underestimation of the efficacy of vaccine. But, in contradiction to these findings, it has been shown that, when levels of HAI antibody have been increased as a result of vaccination, four-fold rises in antibody titer might be difficult to detect upon infection (Stuart-Harris et al., 1976b) resuling in the overestimation of the efficacy of vaccine. To overcome this discrepancy, we are studying the detection of type-specific CF antibody in addition to HAI antibody using single radial complement fixation (SRC-Fix) test (Niwayama et al., 1981), because pre-epidemic CF antibody levels are low and almost equal in both vaccinees and non-vaccinees so that dectection of rises of CF antibody may be a highly sensitive indication of recent past infection, as previously reported by others (Stuart-Harris et al., 1976b; Hall et al., 1973; Foy et al., 1981b) and confirmed by our unpublished studies.

Although it has been reported that 40 HAI titer (equivalent to 128 or 256 HAI titer in this study) was a consisitently protective level (50% protective level) (Meiklejohn et al., 1952; Hobson et al., 1973;Evans et al., 1973), 128 HAI titer did not always give$\leqq$50% incidence of infection, but 256 HAI titer gave almost$\leqq$10% incidence of infection in this study. These results might be due to the antigenic variations between the virus strain used to measure HAI titers and that emerging in the current epidemic. On the basis of these findings, for the vaccination policy to function as collective prevention service in Japan, and also to further clarify antigenic drift as the potential factor in the relation

between the incidence of infection and HAI antibody titer, HAI titer giving≦10% incidence of infection was defined as the protective level in this study. Such a defined protective level of HAI antibody titer was 256 in the three epidemics, but increased to 512 in 1984 epidemic and typically to 1024 in the K school's epidemic. It is considered that the protective level of 512 HAI titer in the 1984 epidemic reflected that as much as 68.8% of isolates showed intenese antigenic drift. These results suggest that 256 HAI titer is a consistently protective level against influenza caused by the virus strains that were closely related to the vaccine strain in the antigenic structures, but an HAI titer of at least 512 is required to protect from influenza caused by the virus strains with intense antigenic drift. Furthermore, as shown in the K school's epidemic, in the majority of the vaccinees a vaccine containing A/Kumamoto/37/79 virus did not stimulate HAI antibody production up to the protective level, resulting in the lack of protection. These findings show that the efficacy of the vaccine depends on the degree of similarity between the vaccine strain and the circulating strain. Therefore, as pointed out by Couch et al. (1979), it is important to evaluate the relative effectiveness of cross-reacting antibodies in protection from influenza.

Although GMTs of the vaccinees showed a repetition of rises following vaccination and decreases with the lapes of time, GMTs after either vaccination were near to or more than the protective level. On the other hand, the high GMT of the non-vaccinees induced by natural infection considerably decreased in the next ten months and futher decreased to the almost initial low GMT with the lapse of three years. These finding show that duration of immunity induced by either vaccination or natural infection is usually regarded as lasting for less than a year, as reported by others (Kilbourne et al., 1974; Lerman et al., 1980; Clark et al., 1983), and also supports the recommendation for annual vaccination, although some reports have said that immunity lasts for more than a year (Foy et al., 1973), depending on the subjects-who had been primed by prior exposure to the homologous or related virus antigens (Mackenzie et al., 1975; Potter et al., 1973) - and annual re-vaccination with inactivated influenza A vaccine confered no long-term advantage (Hoskins et al.,1979).

In light of these findings, indeed, it is essential to select the vaccine strains that will match the "closeness-of-fit" relation (Nolan et al., 1981; Schulman, 1975; Davenport, 1979; Tyrrell et al., 1979) based on the accurate predictions derived from the current national surveillance system in Japan. Furthermore, to improve the efficacy of the influenza vaccine under the current vaccination program in Japan, we conclude that we should make more efforts to promote the receiving rates of vaccine among adults in addition to school children, and encourage them to receive two dose of vaccine as time requires, in order to maintain a sufficient level of HAI antibody titers for protection from influenza caused by intense antigenic drift, as collective prevention.

SUMMARY

A total of 1955 children (1464 vaccinees and 531 non-vaccinees) in four primary schools in Niigata Prefecture,Japan, were studied to evaluate the efficacy of split-product trivalent A (H1N1), A (H3N2) and B influenza vaccine during 1980-1984. The adverse reactions were infrequent, and if reported, they were chiefly local ones, mild in nature and of short duration. The incidences of systemic reactions were almost equal to those found at the intervaccination period. The antibody responses expressed as a fourfold or greater rise in hemagglutination inhibition (HAI) antibody titer and a ratio of geometric mean titer (GMT) between pre-and post-vaccination were the most excellent to A (H3N2) among the vaccine components. As a whole, the GMTs of the vaccinees following two doses of vaccine were two to eight-fold higher than those of the non-vaccinnes. In all four epidemics (H1N1 epidemics in 1981 and 1984, B epidemic in 1982 and H3N2 epidemic in 1983), 61% to 87% of children reported respiratory illnesses and 18% to 48% of them were influenza confirmed by sero-conversions. Among children without respiratory illness, a maximum of 14.6% were considered inapparent influenza infection and the rates of inapparent infection of the non-vaccinees were always higher than those of the vaccinees. Throughout these four epidemics, the incidences of confirmed infection of the vaccinees (7.8% to 33.8%) were 6.5% of 34.8% lower than those of the non-vaccinees (35. 4% to 51.6%). As a result, the efficacy of vaccine was recognized ($x^2=76.43$, $p<0.001$). However , this was not recognized in an entrant school's epidemic in 1984 caused by intense antigenic drift strain. The protective level of HAI antibody titer giving$\leqq$10% incidence of infection was 256, but increased to 512 in the 1984 epidemic, reflecting the high rate of isolates with intense antigenic drift. The GMTs exceeding or near the protective level following vaccinations decreased below this level with the lapse of ten months, the considerably high GMT induced by natural infection also decreased in the ten months, supporting the recommendation for annual vaccination. In light of these findings, it is essential to select the vaccine strain that will match the "closeness-of fit" relation based on the accurate predictions derived from a current national surveillance system in Japan. Furthermore, to improve the efficacy of the influenza vaccine under the current vaccination program, we conclude that we should make more efforts to promote the receiving rates of vaccine among adults in addition to school children, and encourage them to receive two doses as time requires, in order to maintain a sufficient level of HAI antibody titers for protection from influenza caused by intense antigenic drift strains, as collective prevention.

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