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ANTIBODY RESPONSES TO INACTIVATED INFLUENZA VACCINES AND PARTIAL PROTECTIVE EFFECT OF ANTI-NEURAMINIDASE ANTIBODY AGAINST EPIDEMICS OF A(H3N2) AND A(H1N1) INFLUENZA

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SUMMARY A total of 195 students in a nursing school and a school of medical technology were immunized with inactivated influenza vaccine in divalent form as various combinations of types A and B in November and December, 1977. The seroconversion rates about one month after vaccination with type A and B influenza viruses were 20.0–94.1% and 29.4–77.8%, respectively. Soon after vaccination, there were successive epidemics of A(H3N2) and A(H1N1) influenza, starting in December, 1977. The incidences of A(H3N2) and A(H1N1) influenza were evaluated by the hemagglutination-inhibition (HI) antibody responses to H3 and H1 antigen in vaccinees given vaccine without H3 and H1, respectively. Students vaccinated with vaccine containing N1 but not H1 showed a much lower incidence of influenza than those given vaccine containing N2 but not H1 during the epidemic of A(H1N1) influenza: However, there was no significant difference in the incidences of influenza in groups given vaccine containing N2 but not H3 or N1 but not H3 in the epidemic of A(H3N2) influenza. Anti-N1 neuraminidase-inhibition (NI) antibody seemed to be evoked by vaccine containing N1 but not H1, when tested 1 month after vaccination, because the positive ratio of NI antibody was higher in the group given this vaccine than in the control group given vaccine containing neither N1 nor H1 and also than that in pre-vaccination sera.

INTRODUCTION

There are many reports on the effect of inactivated influenza vaccine in monovalent form (Fulk et al., 1970; Hennessy and Davenport, 1974; Parkman et al., 1977; Welty et al., 1977a, b), or in polyvalent form (Hilleman, 1969; Ruben and Jackson, 1972; Závadová et

al., 1972a, b), and on its prophylactic efficacy against natural infection or artificial challenge with various viruses (Knight et al., 1971; Wesselius-de Casparis, Masurel and Kerrebijn, 1972; Kilbourne, Butler and Rossen, 1973; Virelizier, 1975; Delem and Jovanovic, 1978). However, only HI antibody in the serum or nasal secretion has been examined, and there is no evidence that NI antibody plays any role in protection from infection, although there are reports that this antibody is evoked by vaccination or natural infection (Kilbourne, Christenson and Sande, 1968; Schild, 1969; Schulman, 1969; Fedson et al., 1971; Slepushkin et al., 1971; Hennessy, Minuse and Davenport, 1972; Murphy, Kasel and Chanock, 1972).

In Japan, starting at the end of 1977, there were successive epidemics of two types of influenza, A(H3N2) followed by A(H1N1), in one season. As described in this paper, just before the onset of these epidemics, inactivated influenza vaccines of divalent forms as various combinations of types A and B were administered to groups of volunteers, and the incidences of influenza in each group were compared according to the antigenicities of H and N in the vaccines. Evidence was obtained that anti-N1 NI antibody evoked by the vaccine was effective in protecting vaccinees against an epidemic of A(H1N1) influenza. This paper also discusses intra- and intertypic antigenic variations of N1 and N2.

MATERIALS AND METHODS

1. *Vaccines*

Inactivated influenza vaccines in divalent form were provided by the Committee for Study of Influenza Vaccine in Japan (President: Dr. Hideo Fukumi, NIH of Japan, Tokyo). The antigenic designation of the recombinant influenza A viruses were as follows: A/KIX-18(H0N2) from A/PR/8/34(H0)-A/Kumamoto/22/76(N2) and A/KIX-17 (H3N1) from A/Kumamoto/22/76(H3)-A/PR/8/34 (N1). The code numbers and compositions of the vaccines used are shown in Table 1.

2. *Subjects and schedule of vaccination*

A total of 195 students in a nursing school and a school of medical technology, aged 18 to 26 years, were divided into 5 groups according to the kind of vaccine they were given, as shown in Table 1. These subjects were vaccinated twice, with an interval of 2 or 3 weeks between vaccinations, by subcutaneous injection of 0.5 ml of vaccine. Blood specimens for serological tests were taken 3 times: before vaccina-

TABLE 1. *Vaccines used and the time schedule for vaccination and bleeding*

Vaccine			No. of vaccinees	1st bleeding (before vaccination) (I)
Code	Virus present	CCA[a]		
V-1-77	A/KIX-18 (H0N2)	400	34	Oct. 19, '77
	B/Kanagawa/3/76	300		
V-2-77	A/KIX-17 (H3N1)	400	49	Oct. 26, '77
	B/Kanagawa/3/76	300		
V-3-77	A/Kumamoto/22/76 (H3N2)	400	45	Oct. 22, '77
	B/Kanagawa/3/76	300		
V-5-77	A/PR/8/34 (H0N1)	400	31	Nov. 19, '77
	B/Lee/40	300		
V-7-77	A/Adachi/2/57 (H2N2)	400	36	Nov. 19, '77
	B/Lee/40	300		

[a] Chick cell agglutinating units/ml.
[b] Not done.

tion (I), and 1 month (II) and 6 months (III) after vaccination. Specimen III could not be obtained from 49 persons given V-2-77 vaccine because they had graduated from the school when the specimen was taken.

3. *HI test*

HI antigens were either provided by the Committee for Study of Influenza Vaccine in Japan or prepared in this Laboratory in the normal way by cultivating the virus in the allantoic cavity of developing chicken eggs. Serum specimens were treated with RDE (receptor-destroying enzyme, Takeda Pharmaceutical Co., Osaka, Japan) at 37 C overnight and then heated at 56 C for 1 hr to eliminate non-specific inhibitors. For the HI test, the V-type microtiter plate method was employed and PBS (phosphate buffered saline) was used to dilute HI antigen or serum samples, and to suspend chicken red blood cells in 0.5%. An antibody increase of more than 4-fold at 1 month (specimen II) or 6 months (specimen III) after vaccination was regarded as indicating seroconversion.

4. *NI test*

The NI test was carried out according to the method of WHO (Aymard-Henry et al., 1973) in a small test tube. The A/KIX-17(H3N1) strain and fetuin (GIBCO, U.S.A.) were used as NI antigen and substrate, respectively, in the reaction.

RESULTS

1. *HI antibody responses to the strains contained in the vaccine*

The seroconversion rates and geometric mean titers of HI antibody to the strains in each vaccine are summarized in Table 2. The seroconversion rates against type A and B varied from 20.0 to 94.1%, and 29.4 to 77.8%, respectively, probably because of the potency of the vaccine or the antibody titer before vaccination. The Lee strain of type B seemed to have a higher potency than the Kanagawa strain of type B.

The difference between the geometric mean HI antibody titers in specimen I and those in specimens II and III represents the antibody response of the vaccinees. It is unknown why the geometric mean antibody titers in specimen I of groups V-1-77 and V-7-77 were so high against vaccine strain, B/Kanagawa and A(H2N2), respectively. The seroconversion rates tended to be lower in these groups than in other groups, possibly due to the higher prevaccination antibody titers.

Date			
1st vaccination	2nd vaccination	2nd bleeding (1 month after vaccin.) (II)	3rd bleeding (6 months after vaccin.) (III)
Nov. 9, '77	Nov. 30, '77	Dec. 14, '77	Jun. 7, '78
Nov. 16, '77	Dec. 7, '77	Jan. 14, '78	ND[b]
Nov. 16, '77	Dec. 7, '77	Jan. 28, '78	Jun. 7, '78
Nov. 19, '77	Dec. 3, '77	Jan. 10, '78	Jul. 1, '78
Nov. 19, '77	Dec. 3, '77	Jan. 10, '78	Jun. 20, '78

TABLE 2. *HI antibody responses of vaccinees as measured with homologous antigen contained in the vaccine*

Code of vaccine	Virus strain present	Seroconversion 1 month after vaccination (II) vs		Mean HI antibody titer (2^n) vs					
				Type A in			Type B in		
		Type A (%)	Type B (%)	I	II	III	I	II	III
V-1-77	A/KIX-18 (H0N2) B/Kanagawa/3/76	32/34[a] (94.1)	10/34 (29.4)	3.2	8.4	8.0	6.9	7.6	7.3
V-2-77	A/KIX-17 (H3N1) B/Kanagawa/3/76	31/49 (63.3)	16/49 (32.7)	0.9	5.7	NE[b]	2.0	4.0	NE
V-3-77	A/Kumamoto/22/76 (H3N2) B/Kanagawa/3/76	9/45 (20.0)	17/45 (37.8)	3.8	5.8	4.9	2.7	5.2	4.0
V-5-77	A/PR/8/34 (H0N1) B/Lee/40	13/31 (41.9)	21/32 (67.7)	0.3	4.1	2.4	2.5	5.7	5.1
V-7-77	A/Adachi/2/57 (H2N2) B/Lee/40	12/36 (33.3)	28/36 (77.8)	6.2	7.4	7.1	0.7	5.5	5.2

[a] No. of subjects showing more than 4-fold increase in antibody/No. vaccinated.
[b] Not examined.

2. *Incidence of A(H3N2) influenza in groups given vaccine without H3*

The groups given V-2-77 or V-3-77 vaccine which contained H3-antigen were excluded in this analysis, because it was not possible to distinguish antibodies evoked by the vaccine from those evoked by natural infection. Increases in antibodies against H3-antigen in specimen II or III in the groups given vaccine without H3 were considered to indicate natural infection in the epidemic. Therefore, the incidences of influenza determined in this way in the groups given vaccine containing N1 and N2, respectively, were compared (Table 3) to determine the effect of anti-N2 NI antibodies on the incidence of A(H3N2) influenza. No significant difference was found between the groups given vaccine containing N2 and that containing N1, the values being 37.1% and 35.5%, respectively.

TABLE 3. *Effects of vaccines with N2 or N1 but without H3 against epidemic of A(H3N2) influenza*

Group	Vaccine code (Antigenicity of type A virus present)	Seronegative ratio[a] in		Incidence rate[b] observed in					
		I	(%)	II	(%)	III	(%)	Total	(%)
N2	V-1-77 (H0N2)	10/34	(29.4)	8/34	(23.5)	2/34	(5.9)	10/34	(29.4)
	V-7-77 (H2N2)	31/36	(86.1)	15/36	(41.7)	1/36	(2.8)	16/36	(44.4)
	Subtotal	41/70	(58.6)	23/70	(31.8)	3/70	(4.3)	26/70	(37.1)
N1	V-5-77 (H0N1)	25/31	(80.6)	8/31	(25.8)	3/31	(9.7)	11/31	(35.5)

[a] Subjects with HI antibody titers of less than 1 : 16 were regarded as seronegative.
[b] Evaluated as more than 4-fold increase of anti-H3 HI antibody titer after an epidemic of A(H3N2) influenza. A/Yamanashi/2/77(H3N2) was used as HI antigen.

3. *Incidences of A(H1N1) influenza in groups given vaccines without H1*

Similar comparisons were made of infection during the epidemic of A(H1N1) influenza. As shown in Table 4, group V-5-77 given vaccine containing N1 had a lower incidence rate (12.9%) than the other groups given vaccine containing N2, or not containing N1 (35.7%). Thus anti-N1 NI antibody induced by vaccine containing N1 had some protective effect against infection.

4. *Anti-N1 NI antibody response*

Blood specimens I or II of individuals in the groups given vaccine with N1 (V-2-77 or V-5-77) or without N1 (V-1-77) were tested for anti-N1 NI antibody (Table 5). The positive ratio of anti-N1 NI antibody was 19/30 (63.3%) for all the persons given vaccine containing N1, and this was significantly higher than that of the person given vaccine without N1 (6/15) or that in the prevaccination sera (total 12/45).

DISCUSSION

Two successive epidemics of different type A influenzas in the same season are rare, although epidemics of combinations of types A and B are fairly common. In this work inactivated influenza vaccines containing various antigens differing from those of these two type A epidemics were administered to students aged 18 to 26 years before the epidemics. The rates of infection of these students in the epidemics of A(H3N2) and A(H1N1) influenza could be compared because the immune status after vaccination should persist for at least 6 months, and because the epidemics seemed to occur between the times of blood samples, I and II, and II and III, respectively, judging from the data on the incidence rates shown in Tables 3 and 4.

The seroconversion rates of the homologous antibodies to the vaccinated strains were fairly high, but varied from 20.0 to 94.1% against type A and 29.4 to 77.8% against type B. This variation may be due in part to the type of vaccine. Among the type A vaccines, A(H0N2) was the most potent, and the B/Lee strain was more potent than the B/Kanagawa strain. The prevaccination antibody titers may also have affected the seroconversion rate.

The anti-N2 NI antibody produced by administration of vaccine containing N2 did not appear to have any significant effect in preventing infection with A(H3N2) influenza. This might be partly explained by drift of N2-antigenicity in the vaccine strain from that in the virus that caused the present epidemic,

TABLE 4. *Effects of vaccines containing N1 or N2 but not H1 against epidemic of A(H1N1) influenza*

Group	Vaccine code (Antigenicity of type A virus present)	Seronegative ratio[a] in		Incidence rate[b] observed in					
		I	(%)	II	(%)	III	(%)	Total	(%)
N1	V-5-77 (H0N1)	28/31	(90.3)	1/30	(3.2)	3/31	(9.7)	4/31	(12.9)
N2	V-1-77 (H0N2)	31/34	(91.2)	0/34	(0)	11/34	(32.4)	11/34	(32.4)
	V-3-77 (H3N2)	42/45	(93.3)	0/45	(0)	18/45	(40.0)	18/45	(40.0)
	V-7-77 (H2N2)	36/36	(100)	0/36	(0)	12/36	(33.3)	12/36	(33.3)
	Subtotal	109/115	(94.8)	0/115	(0)	41/115	(35.7)	41/115	(35.7)

[a] Subjects with HI antibody titers of less than 1 : 16 were regarded as seronegative.

[b] Evaluated as more than 4-fold increase of anti-H1 HI antibody titer after an epidemic of A(H1N1) influenza. A/USSR/92/77(H1N1) was used as HI antigen.

TABLE 5. *Anti-N1 NI antibody titers of individual vaccinees*[a]

Case No. in each vaccine group	Code of vaccine administered					
	N2 group		N1 group			
	V-1-77(H0N2) tested in		V-2-77(H3N1) tested in		V-5-77(H0N1) tested in	
	I	II	I	II	I	II
1	<2	2.0	<2	<2	<2	2.1
2	3.0	3.2	<2	3.0	<2	<2
3	<2	<2	<2	2.4	2.6	3.0
4	<2	<2	2.4	2.9	<2	<2
5	3.2	2.6	3.2	4.5	<2	5.4
6	2.4	3.4	<2	<2	2.2	3.5
7	2.6	2.8	<2	2.7	<2	4.0
8	<2	<2	2.1	5.8	<2	<2
9	<2	<2	<2	<2	<2	2.7
10	<2	<2	<2	<2	2.2	2.8
11	2.0	3.4	<2	<2	<2	3.2
12	<2	<2	<2	<2	2.3	3.3
13	<2	<2	<2	2.0	<2	<2
14	<2	<2	<2	2.2	<2	<2
15	<2	<2	<2	2.2	<2	2.2
Positive ratio	5/15	6/15	3/15	9/15	4/15	10/15

[a] A/KIX-17(H3N1) was used as NI antigen.

because antigenic variations in the N of A(H3N2)-type influenza virus (Schild et al., 1974; Werner, Thraenhart and Kuwert, 1978) and other influenza viruses (Schulman and Kilbourne, 1969) have been reported. Another possible explanation for the failure of anti-N2 NI antibody to prevent infection is that the A(H3N2) epidemic began so soon after vaccination that anti-N2 NI antibody may not yet have been formed. This possibility is supported by the fact that the rates of infection of the N2- and N1-vaccine groups were similar in the period between blood specimens I and II, but somewhat different in the period between specimens II and III (4.3% in the N2-group and 9.7% in the N1-group) (Table 3) when anti-N2 NI antibody had been formed. Precise comparison is difficult, however, because the epidemic had passed its peak by the latter period and only a few subjects were infected with A(H3N2).

On the other hand, the anti-N1 NI antibody produced by vaccine containing N1 appeared to be somewhat effective in preventing infection with A(H1N1) influenza (Table 4). This might be because the drift of N1-antigenicity was slight, as suggested by the report of Kendal et al. (1978) that the N1-antigenicity of the current epidemic strain resembled that of A/PR/8/34(H0N1), which we used in this study, as well as those of strains isolated in the epidemics of 1950–1951, although some drift within A(H1N1) influenza viruses has been reported (Paniker, 1968). It seems unlikely that the low rate of infection with A(H1N1) in the N1-vaccine group was caused by interference or cross-protection resulting from A(H3N2) infection in this group,

because the rate of infection with A(H3N2) in the group was not especially high, judging from the data in Table 3. Further analysis of N-antigenicities and further studies on the protective effects of purified N-antigen from various strains are necessary.

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