Immunization of institutionalized asthmatic children and patients with psychomotor retardation using live attenuated cold-adapted reassortment influenza A H1N1, H3N2 and B vaccines

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Live attenuated cold-adapted reassortant (CR) influenza virus vaccines were evaluated in institutionalized asthmatic children and severe psychomotor-retarded (SPR) patients. Almost all the vaccinees were seropositive to the vaccine strains before immunization. Trivalent CR vaccine (containing A H1N1 (CR-125), A H3N2 (CR-149) and B (CRB-117)), bivalent CR vaccine (CR-125 and CR-149) and monovalent CRB-117 were inoculated to 19 asthmatic children and 36 and 16 SPR patients, respectively. Overall 49, 22, and 11% of vaccinees were infected by A H1N1, A H3N2 or B vaccine viruses, respectively, as indicated by significant haemagglutination-inhibition (HI) antibody titre rises 4 weeks after inoculation. No severe adverse reactions associated with CR vaccination were observed in the handicapped patients. A nosocomial outbreak of influenza A H1N1 occurred in the ward with asthmatic children, but none of the 19 CR-trivalent vaccinees became infected. However, five of 20 non-vaccinees in the same ward, and ten of 30 vaccinees in another ward that received inactivated split vaccine became infected. The CR vaccines demonstrated significant protective effects against natural exposure to the A H1N1 virus, and were well tolerated and safe when given to patients with bronchial asthma and severe psychomotor retardation.

Keywords: Influenza virus; cold-adapted reassortant; trivalent live vaccine; high-risk patient; nosocomial infection

At present, it is recommended in Japan that the influenza split virus vaccine be given annually to school children. However, the effect of current vaccination programmes on morbidity is insignificant, and that on mortality marginal. Also, the antibody responses to inactivated vaccine vary, depending on the immunological status of the vaccinee[1]. Accordingly, parenteral inactivated vaccines have been estimated to have an efficacy of only 60–80%[2].

In recent years, considerable effort has been focused on the development of a live attenuated influenza virus vaccine, as an alternative approach to prevent influenza. Vaccination by live virus involves exposure of susceptible individuals to infection with the attenuated virus, which stimulates secretory IgA and circulating IgG antibody responses, as well as other immune responses. One of the candidates, a temperature-sensitive (ts) reassortant vaccine, was evaluated but was associated with genetic instability in seronegative children[3,4]. A series of cold-adapted (ca) reassortants between the ca master strains, A/Ann Arbor/6/60(H2N2) and B/Ann Arbor/1/66, and recent wild-type isolates are now being intensively evaluated. The results indicate a strong ability to control influenza with the correct antigenicity, safety and efficacy. Several studies with live attenuated ca reassortant (CR) influenza A and B vaccines have shown these to be safe and antigenic in infants and young children[5–9]. The present study evaluates the live attenuated tri-, bi-, and monovalent combinations of CR vaccines in institutionalized asthmatic children and severe psychomotor-retarded (SPR) patients. This report details the relative absence of adverse reactions, significant serological responses and protective efficacy

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of the vaccines against a nosocomial outbreak of influenza A H1N1 observed in the winter of 1988–1989.

MATERIALS AND METHODS

Vaccine

The CR vaccine strains employed in this study were CR-125(H1N1), CR-149(H3N2) and CRB-117, which are genetic reassortants between ca master strains A/Ann Arbor/6/60(H2N2) and B/Ann Arbor/1/66, and their respective wild-type strains A/Kawasaki/9/86 (H1N1), A/Los Angeles/2/87(H3N2), and B/Ann Arbor/1/86. Each vaccine virus contains the haemagglutinin and neuraminidase genes of the wild-type strain and the remaining six internal genes from the parent ca donor strain. The vaccine preparations were provided by Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan; the titration of infectivity, safety testing in ferrets, and genotyping of vaccine preparations were performed at the University of Michigan. Experimental vaccines were prepared in specific pathogen-free (SPF) fertile chicken eggs inoculated by the allantoic route. The infected SPF allantoic fluid was concentrated and purified by centrifugation and ultrafiltration. Two CR vaccines, CR-125 and CR-149, were mixed and lyophilized as a bivalent form, while CRB-117 was lyophilized as a monovalent form. On administration of the trivalent CR vaccine containing both type A and B viruses (CR-AB), equal amounts of reconstituted influenza A bivalent (CR-A) and monovalent B(CR-B) vaccines were mixed. Reconstituted CR vaccines adjusted to contain 10^7 plaque-forming units (p.f.u.) per 0.3 ml of infectivity in Madin–Darby canine kidney (MDCK) cells for each virus were inoculated intranasally. Inactivated split vaccine, prepared by the Chemo-Sero-Therapeutic Research Institute to contain A/Yamagata/120/86(H1N1), A/Fukuoka/C29/85(H3N2), A/Sichuan/2/87(H3N2) and B/Nagasaki/1/87, was administered subcutaneously (0.5 ml) twice with a 2-week interval.

Volunteers

Patients of the National Minami Fukuoka Chest Hospital were recruited to join the study, after informed consent was obtained from their parents. The hospital was divided into four wards: one ward (A, 50 beds) for asthmatic children, and three wards (B, C, and D, each with 40 beds) for the handicapped SPR children and young adults. A total of 142 volunteers – 39 in ward A, 36 in ward B, 37 in ward C and 30 in ward D – were enrolled between November 1988 and March 1989. Eight groups were formed and were randomized to receive one of the combinations of CR vaccines or inactivated split vaccine. In ward A, 19 were inoculated with the trivalent CR-AB vaccine, and the remaining 20 were enrolled as controls with no vaccination. In ward B, 17 were inoculated with bivalent CR-A vaccine, 16 with monovalent CR-B vaccine, and the remaining three were not vaccinated. In ward C, 19 were inoculated with bivalent CR-A, 16 with inactivated vaccine, and the remaining two were not vaccinated. In ward D, all 30 were immunized with inactivated vaccine (*Table 1*). Contraindications included in the study were known allergy to egg protein or chicken feathers.

Clinical observation

Observations were made for any associated adverse reactions, daily for one week. In ward A, fever, upper respiratory tract symptoms, and bronchial asthma attack were recorded by physicians. In wards B, C, and D, fever and objective signs were carefully examined. Body temperature was measured twice daily, in the morning and afternoon, until the end of March 1989, to monitor the outbreak of influenza.

Virus isolation

Pharyngeal swabs from vaccinees who developed body temperature >37.5°C within 1 week of inoculation were collected into Eagle's Minimum Essential Medium. Virus isolation was performed in MDCK cell cultures. Pharyngeal swabs were also collected until March 1989 from all the participants who developed influenza-like illness; virus isolation for these samples was done in MDCK cells and embryonated chicken eggs.

Table 1 Study population, and clinical and virological responses of patients with bronchial asthma and with severe psychomotor retardation to cold-adapted reassortant (CR) influenza virus vaccines

Ward[a]	Group	Type of vaccine	No. of vaccinees	Age (years) (mean ± s.d.)	No. of vaccinees with fever[d]	No. of vaccinees with virus shedding[e]
A	1	CR-AB[b]	19	11.1 ± 2.7	4[d]	2(A H1N1), 1(A H3N2)
	2	None	20	10.0 ± 2.3	3[d]	NT
B	3	CR-A[b]	17	23.1 ± 5.6	3	0
	4	CR-B[b]	16	21.4 ± 7.1	3	1(B)
	5	None	3	16.7 ± 3.3	0	NT
C	6	CR-A[b]	19	24.5 ± 5.1	8	2(A H1N1), 1(A H1N1 + A H3N2)
	7	Inactivated[c]	16	27.2 ± 6.3	1	NT
	8	None	2	24.0	0	NT
D	9	Inactivated[c]	30	13.3 ± 8.0	0	NT

NR, not tested

[a]Patients with bronchial asthma were in ward A, and those with severe psychomotor retardation were in wards, B, C and D

[b]CR-AB: trivalent, CR-125(A H1N1), CR-149(A H3N2) and CRB-117(B); CR-A: bivalent (CR-125 and CR-149); CR-B: monovalent (CRB-117)

[c]Inactivated split vaccine containing A/Yamagata/120/86(H1N1), A/Fukuoka/C29/85(H3N2), A/Sichuan/2/87(H3N2), and B/Nagasaki/1/87

[d]Fever (>37.5°C) within 7 days after inoculation. One of four vaccinees and all three unvaccinated children with fever in ward A were serologically confirmed to be infected with adenovirus type 11

[e]Pharyngeal swabs were collected from vaccinees who developed fever after inoculation. All isolates possessed both cold-adapted and temperature-sensitive phenotypes

Serological testing

Serum specimens were collected before vaccination, 4 weeks after vaccination, and at the end of March 1989 when the number of influenza infections abated. The standard haemagglutination-inhibition (HI) test was performed using the vaccine strains and the epidemic strain, A/Fukuoka/MF33M3/89 (H1N1), isolated from a hospital patient.

Statistical analysis

The geometric mean titres (GMTs) of HI antibodies before and after vaccination were statistically analysed by the paired t test. Student's t test was used to compare the GMTs between CR vaccine groups, inactivated vaccine groups, and non-vaccinated controls. For HI antibody titres of $<1:16$, 1:8 was used for calculation of GMT, and 1:2048 was used for titres of $\geq 1:2048$. The significant HI antibody responses among vaccinees against three vaccine strains were analysed by McNemar's test, and the infection rates of the groups were compared by Fisher's exact test.

RESULTS

Adverse reactions

An outbreak of pharyngoconjunctival fever caused by adenovirus type 11 was confirmed in ward A on 25 November 1988[10], 3 days after inoculation of trivalent CR-AB vaccine; examination of adverse reactions associated with CR-AB vaccination was therefore difficult. During the epidemic with adenovirus, four vaccinees and three non-vaccinees became febrile in ward A, of which one and three were later confirmed infected with the virus. However, development or worsening of a bronchial asthma attack was not apparent among vaccinees. Of 36 vaccinees with bivalent CR-A in wards B and C, 11 cases (31%) developed body temperature higher than 37.5°C on the third and fourth days after inoculation, with the highest body temperature of 38.8°C and the average, 38.1°C. Of 16 vaccinees with monovalent CR-B, 3 (19%) became febrile on the second to fifth days after inoculation (the highest body temperature was 38.1°C and the average 37.9°C). Febrile reactions among vaccinees improved within 1–2 days, and their general conditions were tolerable. In contrast, of 46 children immunized with inactivated split vaccine, only one developed elevated body temperature (37.8°C), on the first day after vaccination. Five non-vaccinees were not febrile during the examination period.

Virus isolation

Table 1 shows the degree of virus isolation from pharyngeal swabs of vaccinees who were febrile within 7 days after inoculation. In group 1, inoculated with trivalent CR-AB vaccine, three strains of influenza A virus (two strains of A H1N1 and one strain of A H3N2) were recovered from three cases. Four strains of influenza A virus were isolated from three cases in group 6, inoculated with bivalent CR-A vaccine: H1N1 and H3N2 from one case and two isolates of H1N1 from two others. One type B isolate was recovered from a febrile child in group 4, inoculated with monovalent CR-B vaccine. All isolates were confirmed to possess both ts and ca phenotypes.

Serological responses after vaccination

Table 2 summarizes the HI antibody titre response against each vaccine subtype. The GMTs of HI antibody against CR-125, CR-149 and CRB-117 were 1:300, 1:364 and 1:281 before vaccination, and 1:816, 1:516 and 1:320 after vaccination, respectively. Although no significant difference was observed between the GMTs of HI antibody against the three subtypes before vaccination, the GMT response to CR-125 following vaccination was greater than those to CR-149 and CRB-117. Seroconversions or significant antibody rises of fourfold or greater were also observed: 27 (49.1%) of 55 vaccinees with CR-125, 12 (21.8%) of 55 with CR-149, and four (11.4%) of 35 with CRB-117. To analyse the influence of pre-existing HI antibody on vaccine response, the vaccinees were classified into three groups according to their HI antibody titres before vaccination. All seronegative vaccinees to each subtype showed seroconversion. Significant HI antibody rises were also observed in subjects with HI titres of 1:16 to 1:64 before vaccination: 15 (88%) of 17 vaccinees for CR-125, five (36%) of 14 for CR-149, and three (27%) of 11 for

Table 2 Hemagglutination-inhibition (HI) antibody responses among vaccinees with cold-adapted CR influenza virus vaccines

Subtype of CR vaccine	No. of vaccinees	GMT[a] of HI antibody: Before vaccination[b]	GMT[a] of HI antibody: After vaccination[b]	HI titre before vaccination	No. responders[c]/ no. vaccinees	(%)
CR-125(A H1N1) (groups 1, 3, and 6)	55	299.8	815.7			
			$p < 0.001$	$<1:16$	1/1	
				1:16 ~ 1:64	15/17	27/55 (49.1)[d]
				$>1:64$	11/37	
CR-149(A H3N2) (groups 1, 3, and 6)	55	364.1	515.8			
			$p < 0.001$	$<1:16$	2/2	
				1:16 ~ 1:64	5/14	12/55 (21.8)[d]
				$>1:64$	5/39	
CRB-117(B) (groups 1 and 4)	35	281.4	320.0			
			$p < 0.01$	$<1:16$	1/1	
				1:16 ~ 1:64	3/11	4/35 (11.4)
				$>1:64$	0/23	

[a]GMT, geometric mean titre
[b]GMTs before vaccination against three subtypes were not significantly different
GMT after vaccination against CR-125 was statistically higher than those against CR-149 and CRB-117 ($p < 0.01$ by Student's t test). The GMT against each subtype rose significantly after vaccination by the paired t test
[c]Serological responders were defined as those showing fourfold or greater rises in HI titres
[d]Responder rate for CR-125 was significantly higher than that for CR-149 ($p < 0.01$ by McNemar's test)

Table 3 Protective effect of cold-adapted reassortant (CR) influenza virus vaccines against the nosocomial outbreak of influenza A H1N1

Ward	Group	Type of vaccine	No. of vaccinees	HI titre before outbreak		No. of patients with febrile illness		No. of patients with infection[a] (%)	
A	1	CR-AB	19	<1:16	0	0	0	0	0 (0)
				1:16 ~ 1:64	2	0		0	
				>1:64	17	0		0	
	2	None	20	<1:16	3	0	6[b]	0	5 (25.0)[c]
				1:16 ~ 1:64	9	4		4	
				>1:64	8	2		1	
D	9	Inactivated	30	<1:16	0	0	10[b]	0	10 (33.3)[d]
				1:16 ~ 1:64	16	7		7	
				>1:64	14	3		3	

[a]Infection cases were defined as those showing fourfold or greater rises in HI antibody titres against A H1N1 virus (A/Fukuoka/MF33M3/89) isolated in the outbreak

[b]One of six febrile patients in ward A, and one of ten in ward D were not serologically confirmed as having influenza A H1N1 infection

[c]The infection rate of unvaccinated controls (group 2) was statistically higher than that of CR-AB vaccinees (group 1) by Fisher's exact test ($p < 0.05$)

[d]One of ten cases had a subclinical infection. The rate of children immunized with inactivated split vaccine (group 9) was statistically higher than that of CR-AB vaccinees (group 1) by Fisher's exact test ($p < 0.01$)

CRB-117. In contrast, significant HI antibody titre rises were infrequent in those with titres >1:64 before vaccination: 11 (30%) of 37 vaccinees for CR-125, five (13%) of 39 for CR-149, and none of 20 for CRB-117. No significant HI antibody titre rise to each vaccine strain was observed in the 25 non-vaccinees.

Among the 46 vaccinees with inactivated split vaccine, the GMTs of HI antibody to the vaccine strains, A/Yamagata/120/86(H1N1), A/Fukuoka/C29/85(H3N2), A/Sichuan/2/87(H3N2), and B/Nagasaki/1/87 were 1:197, 1:276, 1:73, and 1:145, respectively, before vaccination. After immunization, the GMTs rose to 1:661, 1:773, 1:551, and 1:467; significant HI antibody titre rises were observed in 27 cases (59%), 26 (57%), 31 (67%), and 25 (54%), respectively (data not shown).

Protective effect against outbreak of influenza A H1N1

A nosocomial outbreak of influenza-like illness was observed in wards A and D in the winter of 1988–1989, with influenza H1N1 strains isolated from the children. As shown in *Table 3*, all the infected children were clustered in only two groups, non-vaccinees and recipients of inactivated vaccine. During the period from 29 December, 1988 to 14 January 1989, five cases were serologically confirmed as influenza H1N1 in ward A; ten other cases were confirmed in ward D in the period 8–16 January. Fourteen of these 15 cases were febrile, with body temperatures of 38.2–41.0°C persisting for 2–10 days, and developed various upper respiratory tract symptoms. Of 19 vaccinees given trivalent CR-AB in ward A, none showed clinical illness or serological response to H1N1, but five (25%) of 20 non-vaccinees became infected. Of 30 patients given inactivated vaccine, ten (33%), including one with subclinical infection, were shown by serology to be infected with H1N1. No influenza activity was observed in wards B and C, and there was no significant HI antibody rise to the current H1N1 subtypes.

DISCUSSION

This report has documented that, first, no severe adverse reactions are observed after vaccination with CR vaccines (bivalent A, monovalent B, and trivalent A and B) to high-risk seropositive and seronegative institutionalized patients with bronchial asthma and SPR. Secondly, none of the CR vaccinees became infected during an outbreak of influenza A H1N1.

Immunization against influenza has been recommended in high-risk patients, especially those with chronic pulmonary diseases, including bronchial asthma[11–14]. Prevention against nosocomial influenza is also very important among institutionalized SPR patients. However, the efficacy of the presently available inactivated influenza split vaccine is marginal. CR vaccines are now being intensively evaluated as a more effective influenza vaccine, and their efficacy in healthy adults and children has been well reported[15,16]. The possible risk of adverse effects to patients with chronic pulmonary diseases following administration of the CR vaccine has been a concern, but Atmer *et al.*[17] reported that the CR vaccine did not have any untoward effects on pulmonary function after nasal administration to adults with bronchial asthma. In the present trial of trivalent CR vaccine to high-risk children, no bronchial asthma attacks associated with vaccination were observed; however, the unforeseen nosocomial outbreak of adenovirus type 11[10] did not permit a definitive evaluation of the side effects related to CR vaccination. Among SPR patients, body temperatures >37.5°C were observed in 31% of CR-A vaccinees and 19% of CR-B vaccinees, but the duration was limited to 1–2 days and their general conditions were tolerable. In Japan, it is very difficult to evaluate the CR vaccine in seronegative volunteers because most children are seropositive through the fiat of annual immunizations with inactivated vaccine. Although the number of seronegative children was small in our study, no severe side effects associated with the CR vaccines were observed among them. Therefore, the CR vaccine is well attenuated for SPR patients as well as asthmatic children.

The CR vaccines, both types A and B, have been proven to be genetically stable, and do not show any transmission in spite of virus shedding after vaccination[5,9,18,19]. In this study, influenza viruses (A H1N1 and H3N2, and B) were recovered following inoculation, but all the isolates possessed both ts and ca phenotypes. Also, no secondary transmission to non-vaccinees within the same wards was documented, and this was serologically confirmed.

Influenza outbreaks with different type A and B viruses have been frequently encountered recently, and multivalent inactivated vaccines have therefore been routinely recommended. For CR vaccine development, interference of viral replication when different subtypes are used in combination may become a problem; however, to date, immunological responses comparable to monovalent vaccine have been reported for bivalent type A vaccine (H1N1, H3N2)[18] and trivalent vaccine (H1N1, H3N2, and B) in children[20]. In this study, significant increases in HI antibody titres to each subtype were lower (CR-125 49.1%, CR-149 21-8%, and CRB-117 11.4%) than those reported so far. It is likely that these results are mainly due to pre-existing antibody levels among vaccinees, rather than interference between viruses. Indeed, all seronegative vaccinees showed seroconversion, and no significant differences in HI antibody response to each vaccine strain were confirmed when comparison was made between trivalent CR-AB (group 1), bivalent CR-A (groups 3 and 6), or monovalent CR-B (group 4) vaccinees. Thus, the development of multivalent CR vaccines may be practical, resulting in a vaccine that is potentially more effective against outbreaks of influenza.

Thus far, studies on antigenicity and efficacy of CR vaccines have been performed mainly on seronegative adults and children. However, seropositive subjects also frequently become infected with influenza, and the CR vaccine, in practice, wil be used blindly, independent of pre-existing antibody status. Therefore, antigenicity and efficacy in seropositive subjects are important determinants and need to be investigated. Clements *et al.*[5] reported good serological response to CR-A vaccine in seropositive adults. In children, reports on vaccination with monovalent CR-B vaccine[9] and trivalent CR-AB (H1N1, H3N2, and B) vaccine[20] showed moderate rises in antibody titres. In the present study, vaccinees who possessed relatively low HI antibody titres (1:16 to 1:64) to H1N1, H3N2 and B before vaccination responded better than those with higher HI antibody titres ($>1:64$). The CR vaccine response greatly depends on pre-existing antibody titres before vaccination; protective immunity is mainly achieved in susceptible persons.

The protective effects of CR-A H3N2 vaccine[15,19] and CR-A H1N1 vaccine[21] against natural influenza outbreaks have been documented. Our present report is the first indication of the protection offered by the trivalent CR vaccine against a nosocomial outbreak of influenza (H1N1) in institutionalized patients. The outbreak of influenza was observed in ward A for asthmatic children and ward D for SPR patients. In ward A, 25% of 20 non-vaccinees were serologically shown to have had influenza, whereas no infection was confirmed among the vaccinees with trivalent CR-AB. In ward D, all SPR patients were immunized with inactivated split vaccine, and were shown to have relatively high HI antibody titres, with GMT of 1:661 to H1N1, but 33% of 30 vaccinees became infected with H1N1. Antigenic differences between the epidemic H1N1 strain (A/Fukuoka/MF33M3/89), CR-125 (A/Kawasaki/9/86), and the inactivated vaccine strain (A/Yamagata/120/86) were negligible. It should be noted that the CR trivalent vaccine prevented influenza in ward A, but the inactivated vaccine did not in ward D. It might be difficult to make a final conclusion at this time as to the relative efficacy of the two vaccine types, because comparative studies were not performed in the same wards. However, the inactivated split vaccine clearly did not provide protective immunity in this study.

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