BIKEN JOURNAL Vol. 25 89–95, 1982

SHORT COMMUNICATION

TRIAL OF SPLIT-PRODUCT TRIVALENT INFLUENZA VACCINE IN HIGH-RISK CHILDREN

AKIRA YAMADA, HITOSHI YAMAWAKI, NAOKI TSUDA, KOICHI BABA and HYAKUJI YABUUCHI

Department of Pediatrics, School of Medicine, Osaka University, Fukushima-ku, Fukushima, Osaka 553, Japan

AKIKO MAEDA

Laboratory of Virology, Osaka Prefectural Institute for Public Health, Nakamichi, Higashinari-ku, Osaka 537, Japan

KOICHI YAMANISHI

Department of Virology, Research Institute for Microbial Diseases, Osaka University, Yamadaoka, Suita, Osaka 565, Japan

(Received January 9, 1982)

Split-product trivalent influenza vaccine, containing H3N2, H1N1 and B, was given to 188 high-risk children and 25 healthy ones in the 4 year period from 1978 to 1981. A field trial involving 80 of these children was performed for evaluating the safety and immunogenicity of this vaccine. The antibody response to influenza vaccine in the patients was similar to that in normal children. The reaction to this vaccine of high-risk children was low, and no severe clinical reactions were observed after vaccination.

Infants with underlying diseases appear the most susceptible to the complication of influenza. So, immunization against influenza is most necessary in high-risk children and inactivated influenza virus vaccine has been recommended for children with special risk from infection with influenza virus because of an underlying chronic illness (Allison et al., 1977; Hall et al., 1977; Steinherz et al., 1980). Though split-product trivalent influenza vaccine has been administered safely to healthy children and adults since 1973 in Japan, high-risk children have often not been vaccinated because of their underlying conditions.

This study on whether patients with various diseases could safely receive split-product trivalent influenza vaccine, and vaccination trials was conducted under sufficient medical surveillance of hospitalized children. This paper describes the results of vaccination trials on high-risk children using split-product influenza vaccine.

Since 1976, 188 high-risk children have been inoculated with this vaccine, of which the strains were determined at the National Institute for Health of Japan before the winter

season. All patients received blood tests, a test of serum components by electrophoresis and a phytohemagglutinin (PHA) skin test before immunization. Eighty (55 patients and 25 healthy children) of the 126 children received two vaccinations between Oct. 1980 and March 1981, and were examined serologically. Sera were collected from the subjects just before each vaccination and one to two months after the second vaccination. The influenza split-product vaccine (Lot H228, manufactured by the Research Foundation for Microbial Diseases of Osaka University) used in this season contained 200 chick cell agglutinating (C.C.A.) intact equivalent units/ml of A/Bangkok/1/79 (H3N2), 200 C.C.A. intact equivalent units/ml of A/Kumamoto/37/79 (H1N1) and 300 C.C.A. intact equivalent units/ml of B/Kanagawa/3/76. Each subject received 0.5 ml of the trivalent vaccine subcutaneously at each vaccination. The serum antibody titer against influenza virus (A/Bangkok/1/79, A/Kumamoto/37/79, and B/Kanagawa/3/76) was determined by the HI (hemagglutination inhibition) microtiter method as described previously (Maeda et al., 1979). Nonspecific inhibitor was removed with the receptor destroying enzyme. A significant antibody response was defined as a fourfold rise

TABLE 1. *Diagnoses of immunized children*

Dignosis (No. of patients)	Vaccinated month and No. of patients			Total
	Oct. 1978 ≀ Mar. 1979	Oct. 1979 ≀ Mar. 1980	Oct. 1980 ≀ Mar. 1981	
Neuromuscular diseases				
Epilepsy (27), Febrile convulsion (10) Cerebral Palsy (8), Myasthenia gravis (2), Others (39)	6	31	41	86
Congenital heart diseases				
VSD[a] (16), ASD[b] (7), TGA[c] (10), TOF[d] (4), Others (4)	4	26	20	36
Neoplastic diseases				
Leukemia (4), Retinoblastoma (3), Neuroblastoma (2), Others (4)	0	6	9	13
Blood, Immunity or Allergy diseases				
Asthma (6), Eczema (6), Egg allergy (5), Kawasaki's disease (3), Agammaglobulinemia (2), Others (7)	4	17	13	29
Malformation and Inborn errors of metabolism				
AGS[e] (3), Down's syndrome (2), Hirschsprung's disease (2), Cleft palate (1), Glycogen storage diseases (1), Others (10)	1	16	15	19
Diseases of the kidney				
Nephrotic syndrome (4), Double kidney (1)	1	1	3	5
	16	97	101	188

[a] VSD ; Ventricular septal defects.
[b] ASD ; Atrial septal defects.
[c] TGA ; Transposition of great artery.
[d] TOF ; Tetralogy of Fallot.
[e] AGS ; Adrenogenital syndrome.

in antibody titer after vaccination. The HI titer was expressed as the reciprocal of the serum dilution. A titer of $<1:16$ was taken as 2^0 in calculation of geometric mean titers (GMTs).

Table 1 shows the diagnoses in high-risk children (86 had neuromuscular diseases, 36 congenital heart diseases, 13 neoplastic diseases, 29 blood, immunity or allergy diseases, 19 malformation and inborn errors of metabolism and 5 diseases of the kidney). In all, 188 patients were immunized with split-product trivalent vaccine between 1978 and 1981.

The immune responses to the three sets of antigens in the vaccine (A (H3N2), (H1N1) and B) were studied in 55 of 188 patients with various diseases, and 25 healthy children who received two immunizations with the vaccine. After the first vaccination, 74%, 64% and 46% of the children showed a fourfold or greater increase in HI titer to the three sets of antigens, and about 90% of the vaccinated children showed a fourfold or greater rise in antibody titer after the second immunization. The antibody responses in various groups were compared. Children in the blood, immunity or allergy group seemed to respond slightly less to antibody against B/Kanagawa/3/76, but there were no substantial differences in antibody responses of healthy children and patients (Table 2a, b, c). Individual antibody re-

TABLE 2-a. *Significant immune response[a] to A/Bangkok/1/79 (H3N2)*

Diseases	Antibody response after	
	first immunization	second immunization
Neuromuscular diseases	71% (12/17)	88% (7/8)
Congenital heart diseases	70% (7/10)	100% (3/3)
Neoplastic diseases	71% (5/7)	100% (4/4)
Blood, Immunity or Allergy diseases	67% (6/9)	67% (2/3)
Malformation and Inborn error of metabolism	100% (1/1)	100% (4/4)
Diseases of kidney	100% (1/1)	—
Subtotal	74% (37/50)	91% (20/22)
Healthy	76% (19/25)	90% (19/21)

[a] more than fourfold rise.

TABLE 2-b. *Significant immune response[a] to A/Kumamoto/37/79 (H1N1)*

Diseases	Antibody response after	
	first immunization	second immunization
Neuromuscular diseases	65% (11/17)	100% (8/8)
Congenital heart diseases	60% (6/10)	67% (2/3)
Neoplastic disease	57% (4/7)	100% (4/4)
Blood, Immunity or Allergy diseases	44% (4/9)	100% (3/3)
Malformation and Inborn error of metabolism	100% (6/6)	75% (3/4)
Diseases of kidney	100% (1/1)	—
Subtotal	64% (32/50)	91% (20/22)
Healthy	68% (17/25)	90% (19/21)

[a] more than fourfold rise.

TABLE 2-c. *Significant immune response[a] to B/Kanagawa/3/76*

Diseases	Antibody response after	
	first immunization	second immunization
Neuromuscular diseases	29% (5/17)	88% (7/8)
Congenital heart diseases	70% (7/10)	100% (3/3)
Neoplastic diseases	57% (4/7)	100% (4/4)
Blood, Immunity or Allergy diseases	22% (2/9)	67% (2/3)
Malformation and Inborn error of metabolism	67% (4/6)	75% (3/4)
Diseases of kidney	100% (1/1)	—
Subtotal	46% (23/50)	86% (19/22)
Healthy	52% (13/25)	81% (17/21)

[a] more than fourfold rise.

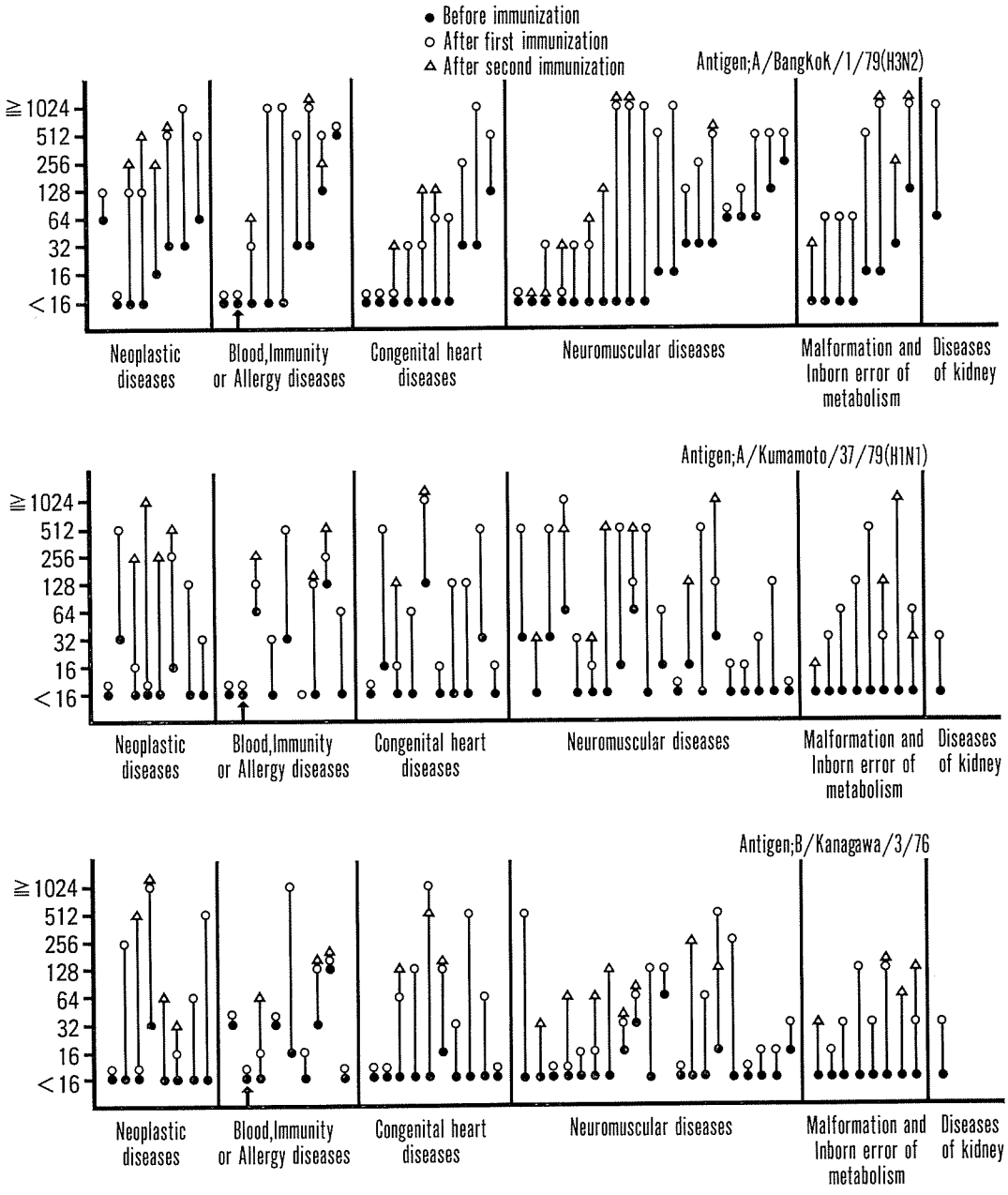

FIGURE 1. HI titers following immunization with split-product vaccine in children with various diseases. HI titers before immunization (●), after the first immunization (○) and after second immunization (△) are shown. The arrow indicates a child with agammaglobulinemia.

sponses after two doses of vaccine are shown in Fig. 1. Some children failed to response to one or two sets of antigens after the initial vaccination, but two doses of vaccine induced

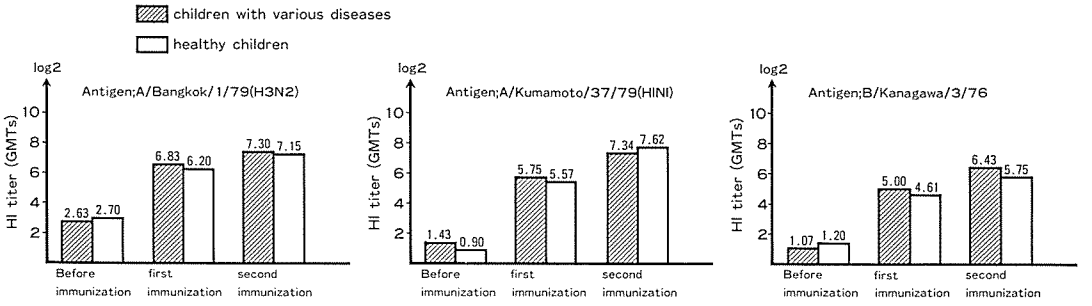

FIGURE 2. Comparison of HI antibody responses to trivalent influenza vaccine in children with various diseases and healthy children. Hatched columns represent titers of children with various diseases. Clear columns represent titers of healthy children.

antibody responses. Not much difference was detected in the antibody responses in different groups. The only exception to this trend was the antibody response of a case of X-linked agammaglobulinemia (Bruton's type) who failed to response to any antigens (Fig. 1). But even in this case no side reaction (fever or indulation) was observed.

The immunogenicities of the trivalent vaccine for healthy children and patients are shown in Fig. 2. Before vaccination, the GMTs of antibody to A/Bangkok/1/79 were $1:2^{2.63}$ for patients and $1:2^{2.70}$ for healthy children, and they increased to $1:2^{6.83}$ and $1:2^{6.20}$ after the first injection and to $1:2^{7.30}$ and $1:2^{7.15}$ after the second. Similar trends were observed in the responses to A/Kumamoto/37/79 and B/Kanagawa/3/76. However the antigenicity of B/Kanagawa/3/76 was lower than those of the other antigens.

Local and systemic reactions were monitored for 48 h after each vaccination. A febrile reaction was observed in 2 of 213 vaccinees. On child, who had a transposition of the great artery, developed a fever with a maximum temperature of 38.9 C within 8 h after vaccination and this fever continued for two days. Another child, who had an atrial septal defect, had a febrile reaction with a maximum temperature of 39.0 C 2 days after the booster injection and this reaction continued for two days. Two other children (one with Hirschsprung's disease and one healthy child) had local reaction at the site of injection and this reaction persisted for one to two weeks. Two unusual reactions that might be attributable to the vaccine were also noted. A child with a ventricular septal defect developed a fever 5 days after the initial and second immunizations. Another child of 20 months old with tetralogy of Fallot, developed temporary cyanosis 2 weeks after the second injection. However, 2 months later, she received a radical operation and her clinical course after operation was fair.

Influenza immunization of children with neoplastic diseases has been reported by Allison et al., (1977) and Steinherz et al. (1980). In our studies, 13 children with neoplastic diseases were immunized during a 4 year period. They were vaccinated when they had finished the chemotherapy course or operation at a time when they were not receiving chemotherapy. Sera from 8 children who were immunized in 1980 were collected. The immune responses of these children to split-product vaccine are shown in Table 3. Some cases did not response serologically to a single immunization, but all patients showed a significant response after the second vaccination, like those in other groups, and no side reactions were observed.

Studies have been made on the immunogenicities and clinical reactions of several kinds

TABLE 3. *Application of influenza split vaccine to children with neoplastic diseases*

	Age	Sex	Diagnosis	Therapy	Antibody titer			Side reaction
					before immmunization	first immunization	second immunization	
Case 1	5	M	ALL	CWS[a]	(<16, <16, <16)[b]	(128, <16, <16)	(256, 256, 512)	(−)
Case 2	4	F	Neuroblastoma	Finished	(16, <16, <16)	ND[c]	(256, 256, 64)	(−)
Case 3	3	M	Retinoblastoma	Finished	(64, <16, <16)	(512, 32, 512)		(−)
Case 4	5	F	Retinoblastoma	CWS	(<16, 32, <16)	(<16, 512, 256)		(−)
Case 5	4	F	Craniopharyngioma	Finished	(32, 16, <16)	(512, 256, 16)	(512, 512, 32)	(−)
Case 6	3	M	Teratocarcinoma	Finished	(<16, <16, 32)	(128, <16, >1024)	(512, >1024, >1024)	(−)
Case 7	6	M	Ganglioneuroma	Finished	(32, <16, 16)	(>1024, 128, 64)		(−)
Case 8	3	F	Neuroblastoma	Finished	(64, <16, <16)	(128, <16, <16)		(−)

[a] Cell wall skelton of BCG.
[b] HI titer against A/Bangkok/1/79, A/Kumamoto/37/79 and B/Kanagawa/3/76, respectively.
[c] Not done.

of influenza vaccines (whole virus vaccine, split-product vaccine and purified hemagglutinin-neuraminidase vaccine) and on the appropriate dosage and schedule for high-risk children (Wright et al., 1976; Allison et al., 1977; Hall et al., 1977). Allison et al. (1977) first planned to use bivalent split-product A influenza vaccine or purified hemagglutinin-neuraminidase (HANA) bivalent A influenza vaccine, and monovalent influenza B vaccine for high-risk children. However, when they inoculated the bivalent A vaccine and monovalent B vaccine separately (2 doses of bivalent A vaccine and one dose of B vaccine), HANA influenza vaccine seemed to be less immunogenic than split-product vaccine. So later they gave only split-product vaccine at the second vaccination, and found that the children showed significant immune responses. Similar experiments were made by Hall et al. (1977) who used one dose of bivalent split-product influenza A vaccine (A/New Jersey/76 and A/Victoria/75 strains) in high-risk children. Split-product influenza virus vaccine was less effective than whole virus vaccine to elicit antibody in persons who had not previously encountered the antigens in the vaccine (Potter et al., 1977). However, split-product vaccine was less reactogenic than whole vaccine and two doses of vaccine could induce an adequate antibody level (Potter et al., 1980). Gross and Ennis (1977) also suggested in their paper that a two doses schedule of the split-product vaccine might be more acceptable for unprimed children, and they reported that the two doses of vaccine should be given approximately 1 month apart.

Patients with cardiac diseases developed fever as observed in this study. Hall et al. (1977) also found that a slightly greater percentage of children with cardiac diseases had fever than patients with pumonary and hematologic disorders after immunization with bivalent vaccine.

We conclude from this study that split-product trivalent influenza vaccine can be administered safely to patients with various diseases. All children with various diseases tested, except one with agammaglobulinemia, gave a similar antibody response to normal subjects. Studies in the next influenza epidemic will show whether our vaccination procedure for high-risk patients is effective.

REFERENCES

Allison, J. E., Glenzen, W. P., Taber, L. H., Paredes, A., Webster, R. G. 1977. Reactogenicity and immunogenicity of bivalent influenza A and monovalent influenza B virus vaccines in high-risk children. J. Infect. Dis. 136: Supplement 672–676.

Gross, P. A., Ennis, F. A. 1977. Influenza vaccine: split-product versus whole-virus types-how do they differ? N. Engl. J. Med. 296: 567–568.

Hall, C. B., Lee, H., Alexson, C., Meagher, M. P., Manning, H. A. 1977. Trial of bivalent influenza A vaccine in high-risk infants. J. Infect. Dis. 136: Supplement 648–651.

Maeda, A., Oishi, I., Minekawa, Y., Kunita, N., Funahashi, S., Yagura, H. 1979. Antibody responses to inactivated influenza vaccines and partial protective effect of anti-neuraminidase antibody against epidemics of A(H3N2) and A(H1N1) influenza. Biken J. 22: 91–98.

Potter, C. W., Clark, A., Jennings, R., Schild, G. C., Wood, J. M., McWilliam, P. K. 1980. Reactogenicity and immunogenicity of inactivated influenza A(H1N1) virus vaccine in unprimed children. Report to the medical research council committee on influenza and other respiratory virus vaccines. J. Biol. Stand. 8: 35–48.

Potter, C. W., Jennings, R., Clark, A. 1977. The antibody response and immunity to challenge infection induced by whole, inactivated and ether-Tween split influenza vaccines. Dev. Biol. Stand. 39: 323–328.

Steinherz, P. G., Brown, A. E., Gross, P. A., Braun, D., Ghavimi, F., Wollner, N., Rosen, G., Armstrong, D., Miller, D. R. 1980. Influenza immunization of children with neoplastic diseases. Cancer 45: 750–756.

Wright, P. F., Dolin, R., La Montagne, J. R. 1976. Summary of clinical trials of influenza vaccines-II. J. Infect. Dis. 134: 633–638.