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A LIVE VARICELLA VACCINE IN A PEDIATRIC COMMUNITY

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A total of 663 children with various underlying diseases were immunized with a live varicella vaccine at the vaccine clinic of Osaka University Hospital during a period of seven years from October, 1975. Clinical reactions after vaccination occurred in 32.4% (24/74) of the children with malignancies and in 0.3% (2/591) of those in other groups. Vaccine-induced immunity was detected for more than 6 years, by FAMA (fluorescent antibody to membrane antigen) and IAHA (immune adherence hemagglutination) tests, and a skin-test for varicella-zoster virus (VZV). During an observation period of more than 7 years, clinical varicella developed in 12 children, 8 of whom were in the group with malignancies. Zoster occurred in only 4 (9.1%) of 44 vaccinees with acute leukemia, this incidence being significantly less ($p<0.05$) than that (21.6%, 8/37) in un-vaccinated leukemic children.

INTRODUCTION

Varicella is a benign, highly contagious childhood disease (Baba et al., 1982; Baba et al., 1984a). Occasionally, it can be fatal, especially when it develops in children who have a severe underlying disease or who have been treated with immunosuppressive drugs (Gershon et al., 1972; Feldman et al., 1975; Close and Houston, 1981). Pediatric wards contain ever increasing numbers of susceptible children. The ubiquity of varicella-zoster virus (VZV) makes it inevitable that occasionally an infected patient is admitted and the infection is not detected at the time of admission.

A live varicella vaccine has been successfully applied to normal children and to high risk subjects, such as those with leukemia (Ha et al., 1980; Arbeter et al., 1982; Brunell et al., 1982; Weibel et al., 1984). There are few reports, however, on intensive efforts to expand the

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potential advantages of using the vaccine for controlling VZV infection in hospitals. Increased vaccination of children in institutions should decrease the number of outbreaks of VZV infection and the incidence of clinical disease in these institutions. We set up a clinic for vaccination of children with underlying diseases in October, 1975. The results obtained up to September, 1982 are presented in this paper.

MATERIALS AND METHODS

1. *Population studied*

Children with various underlying diseases, including neuromuscular, congenital heart, blood and allergic diseases, malformation, digestive or prenatal disturbances and neoplasms have been treated in the outpatient clinic of the Department of Pediatrics, Osaka University Hospital. Children with no history of clinical varicella who were under well controlled conditions were immunized with live varicella vaccine. Before immunization, the immune status of individuals was checked by the following tests: skin-tests with PHA, PPD and varicella, assay of antibody activities to VZV and counts on the number of white blood cells.

2. *Vaccine*

Varicella vaccine of the Oka strain (Takahashi et al., 1975) with a history of 11 passages in human embryonic lung fibroblasts (HEL), 12 passages in guinea pig embryonic fibroblasts (GPE) and 3 to 9 passages in human diploid cells (WI-38 or MRC-5 cells) was used for vaccination. The viral dose used was 300 to 1,000 PFU/dose, but mainly 500 PFU/dose.

3. *Determination of VZV antibody*

The antibody activity to VZV in all specimens of serum was determined by tests of fluorescent antibody to membrane antigens (FAMA) and immune adherence hemagglutination (IAHA) antibody.

The modified procedure used for the FAMA test has been described previously (Baba et al., 1984b). Briefly, dilutions of 0.005 ml of test sera were transferred to Terasaki tissue culture plates and incubated with an equal volume of VZV (Kawaguchi strain) infected Vero cells for 60 min at 37 C. The cells were washed by dipping, and then 0.005 ml of a dilution of fluorescein conjugated goat antiserum to heavy-chain human IgG was added to each well and the cells were incubated at 37 C for 60 min. The cells in the plates were washed again and examined by fluorescence microscopy.

The IAHA test was carried out by a modification of the technique of Gershon et al. (1976). Briefly, serial dilutions of heat-inactivated serum were made in duplicate in Limbro microtiter U-plates (0.025 ml/well) with 0.1% gelatin in veronal buffer (GVB) at pH 7.4. Volumes of 0.025 ml of VZV antigen or antigen control were added to dilutions of serum. Plates were placed on a microshaker for 10 sec and then incubated for 60 min at 37 C. Subsequently, 0.025 ml of guinea pig complement at a dilution of 1: 100 was added. The plates were agitated for 10 sec and then incubated for 40 min at 37 C. DTT-VB-EDTA (0.025 ml, consisting of 1.5 g of dithiothreitol in 500 ml of 0.04 *M* EDTA in veronal buffer without gelatin) was added to all wells and the plates were shaken for 10 sec. Then a 1.5% suspension of human 0-type RBC in GVB (0.025 ml) was added to each well, and the plates were mixed to allow development of hemagglutination.

4. *VZV skin-testing*

The culture fluid of VZV-infected human diploid cell (MRC-5) monolayers was centrifuged at 100,000 *g* for 2 h. The supernatant was incubated at 56 C for 30 min and used as the skin-test antigen. Control skin-test antigen was prepared in the same way from uninfected monolayers. The test and control antigens were injected intradermally into different sites in the forearm, and the size of erythema and induration was evaluated 30 h later. Based on previously published data (Kamiya et al., 1977; Baba et al., 1978), a cutaneous reaction was interpreted as positive if the diameter of erythema or induration was 5 mm or more at the injection site of test antigen and that at the control site was less than 5 mm.

RESULTS

1. *Clinical and immunological responses after vaccination*

As shown in Table 1, a total of 663 children classified into 6 groups (Table 2) were vaccinated. Clinical reactions after vaccination were observed in 24 of 74 children with malignancies. These children showed low grade

TABLE 1. *Clinical and serological responses in vaccinated children with underlying diseases*

Diseases	Number of vaccinated children	Clinical reaction	Immunological response (IAHA test and/or VZV skin test)
Nervous system	230	0	173/184 (94.0%)
Heart	126	0	92/96 (95.8%)
Malignancies	74	24/74 (32.4%)	54/57 (94.7%)
Immunologic abnormalities	119	1/119 (0.8%)	89/95 (93.7%)
Congenital abnormalities, digestive disorders	57	0	46/49 (93.9%)
Kidney, metabolic diseases	57	1/57 (1.8%)	46/48 (95.8%)
Total	663	26/663 (3.9%)	500/529 (94.5%)

Oct., 1975 to Sept., 1982

TABLE 2. *Grouping of underlying diseases*

Diseases of Nervous or Neuromuscular Systems

Epilepsy, Febrile convulsion, Cerebral palsy, Hydrocephalus, Cerebellar ataxia, Facial nerve palsy, Encephalitis, Myasthenia gravis, Muscular dystrophy etc.

Congenital Heart Diseases

Ventricular septal defects, Atrial septal defects, Tetralogy of Fallot, Transposition of the great arteries, Single ventricle etc.

Disease of Malignancies

Acute leukemia, Malignant lymphoma, Neuroblastoma, Hepatoblastoma, Rhabdomyosarcoma, Wilms tumor.

Immunological Diseases

Asthma, Egg allergy, Atopic dermatitis, Kawasaki diseases (MCLS), Purpura, Congenital agammaglobulinemia, Juvenile rheumatoid arthritis etc.

Congenital or Prenatal Abnormalities and Digestive Disorder

Down's syndrome, Hirschsprung's diseases, Chronic diarrhea, Liver cirrhosis, Hepatitis, Banti's syndrome etc.

Diseases of the Kidneys, Endocrine System or Metabolism

Nephrotic syndrome, Glomerulonephritis, Congenital adrenal hyperplasia, Hypothyroidism, Addison diseases, Tay-Sachs diseases, Sandhoff's diseases, Glycogen storage diseases etc.

rash and fever, which were not clinically troublesome, 2 to 3 weeks after vaccination. Clinical reactions were rare in other groups. Immunological responses assessed by the IAHA test and/or the VZV skin test were observed in 94–96% of the vaccinees in each group. Results of follow-ups of immunity in the 40 vaccinees who were followed for more than 6 years are shown in Figs. 1 and 2. Antibody was occasionally undetectable by the IAHA test, but consistently detected by the FAMA test (Fig. 1). In the skin test with VZV antigen, all but one vaccinees gave positive results (Fig. 2). Thus both humoral and cellular immunity to VZV induced by vaccination lasted for a long time.

2. *Incidences of clinical varicella and zoster in vaccinees*

The follow-up study showed that, 12 vaccinees, 8 with malignancies, contracted clinical varicella (Table 3). All the vaccinees who de-

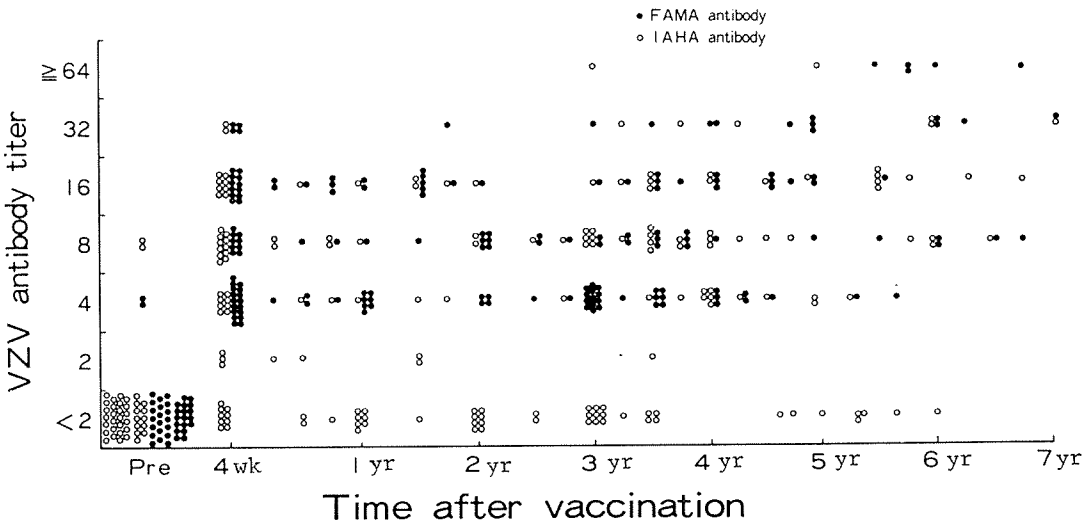

FIGURE 1. Serological follow-up of vaccinated children by the FAMA and IAHA tests (40 cases with underlying diseases).

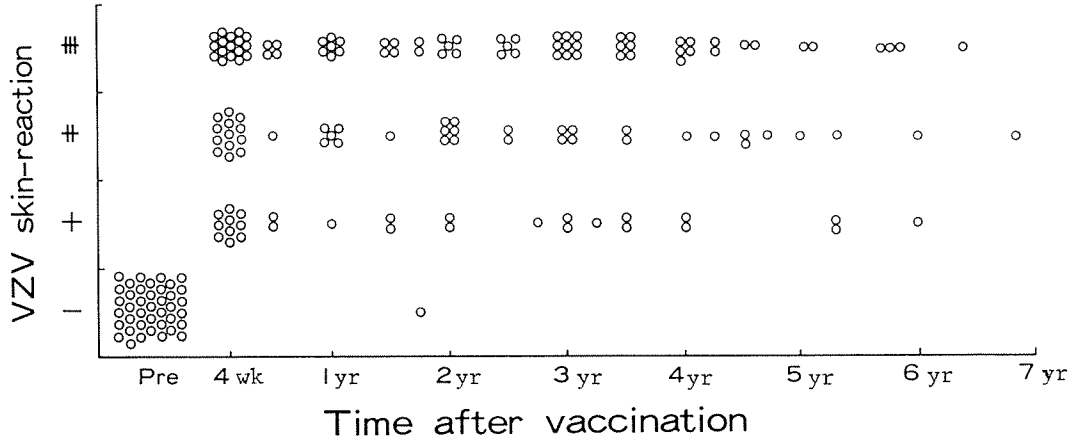

FIGURE 2. Immunological follow-up of vaccinated children by the skin test with varicella antigen (40 cases with underlying diseases).

veloped clinical varicella were immune-suppressed, but their clinical symptoms were all mild with a countable rash and/or low grade fever and with no adverse effect on treatment of their underlying diseases. Only 4 cases with acute leukemia contracted herpes-zoster.

The incidence of zoster in the vaccinees with leukemia and leukemic children who had experienced natural varicella were compared. The two groups had been receiving the same kind of chemotherapy. As shown in Table 4, herpes-zoster developed in 4 of 44 vaccinees and 8 of 37 children after natural varicella infection. The clinical symptoms in all vaccinees were mild, whereas 2 of the 8 children after natural varicella infection had moderate

TABLE 3. *Incidences of clinical varicella and zoster in vaccinated children with underlying diseases during a 7 year follow-up period*

Types of underlaying disease	Number of vaccinated children	Clinical varicella	Zoster
Nervous system	230	1	0
Heart	126	0	0
Malignancies	74	8 (mild)	4
(Acute lymphatic leukemia)	(44)	(4) (mild)	(4)
Immunologic abnormalities	119	0	0
Congenital abnormalities, digestive disorders	57	2	0
Kidney, metabolic diseases	57	1	0
Total	663	12	4

Oct., 1975 to Sept., 1982

TABLE 4. *Incidence of zoster in vaccinated acute leukemic children*

Group	Total	Zoster	Percent incidence zoster
Vaccinated group	44	4 (all mild)	9.1
Natural varicella group	37	8 (2 moderate 6 mild)	21.6

Oct., 1975 to Sept., 1982

clinical symptoms with severe neuralgia. These results suggest that in the vaccinees zoster was less frequent or less severe ($p<0.05$) than that in non-vaccinated children.

DISCUSSION

VZV infection in hospitalized children, and especially immunosuppressed children, can be serious or fatal. Special emphasis has been placed on techniques for rapid screening of susceptibility and preventive measures when an index case of the disease is recognized in the ward. Zoster immune globulin (ZIG) (Gershon et al., 1974) and some chemical drugs, such as acyclovier (Peterslund et al., 1981), are effective in modifying the clinical course, but only when they are used during the early stage of development of varicella; these drugs have no prophylactic effect. Prompt use of a live varicella vaccine is effective for reducing the size of the outbreak in an institution or hospital (Baba et al., 1978; Asano et al., 1982). But, subjects whose underlying diseases are in an active stage or who are receiving immunosuppressive therapies, such as large doses of steroid, anticancer chemical drugs, radiation or an operation, are usually not vaccinated until they become in a stable condition. To reduce serious further complications, such as pneumonia and encephalitis due to nosocomial VZV infection in these cases, it is essential to immunize their contacts in the community, so possibly reducing the number of outbreaks. Previous success using the vaccine on leukemic children during continued therapy (Ha et al., 1980) encouraged us to use the vaccine on children with other different ungerlying diseases. To realize this aim, we organized an infection control team and set up a vaccination clinic in our outpatient clinic. Patients under well-controlled conditions visiting the clinic were vaccinated with informed consent of their patients. After vaccination, clinical and seroepidemiological surveys of varicella infection in the population were made for more than 6 years. All but

two of the children showing clinical reactions after vaccination had neoplasms. It is noteworthy that the reactions were mild and in all cases recovery occurred without further complications. Acquisition of vaccine-induced immunity resulted in solid protection against subsequent varicella infection. Exceptions were seen in 12 of 663 vaccinees who were reinfected on secondary exposure to VZV. Of these 12 exceptions, 8 had malignancies. In the future, we plan to give booster vaccinations to children with malignant disorders to protect them more effectively from clinical varicella. The occurrence of zoster after vaccination was limited to leukemic children, and its incidence was less than in leukemic children after natural infection with varicella. It seems inevitable that zoster develops in a certain portion of subjects infected with either attenuated or wild VZV.

We conclude from the present results that live varicella vaccine (the Oka strain) is safe and very effective for protection of children with various underlying diseases against varicella infection.

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