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HUMORAL AND CELLULAR IMMUNE RESPONSE TO VARICELLA-ZOSTER VIRUS IN CHILDREN INOCULATED WITH LIVE ATTENUATED VARICELLA VACCINE

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SUMMARY Twenty-seven hospitalized children without a history of varicella were vaccinated with 500 plaque forming units of live attenuated varicella vaccine (Oka strain) and followed for four to five weeks at weekly intervals for the development of virus-specific cell-mediated immunity (CMI) and neutralizing (NT) antibody activity. These children had suffered from heterogeneous underlying diseases, such as acute leukemia, lymphoma, the nephrotic syndrome, and other chronic illnesses and were in various immunological states. Development of specific CMI, detected by lymphocyte transformation (LTF), was observed in 23 of the 27 children. The appearance of LTF activity in immunologically handicapped patients was delayed and/or rather suppressed compared with that of immunologically normal patients. An antibody response was detected in 26 of the 27 children. All of the vaccinated patients were protected effectively against a subsequent outbreak of varicella in the ward.

INTRODUCTION

It is well known that varicella may be serious or even fatal in high risk children with deficient immunity resulting from certain malignancies or from immunosuppressive therapy. The increased risk of varicella infection in these subjects is a serious problem for medical staff. A live attenuated varicella vaccine was developed (Takahashi et al., 1975) and used for preventing the spread of varicella in hospital (Takahashi et al., 1974) and for use in children with leukemia and other malignancies (Izawa et al., 1977). A remarkable protective effect of this vaccine was observed in these studies.

On the other hand, it has long been suspected that cell-mediated immunity (CMI) is important in recovery from infections caused by varicella-zoster (V-Z) virus, and increasing efforts are being focused on studies of specific

CMI against V-Z virus (Russel et al., 1972; Jordan et al., 1974; Ruckdeschel et al., 1976; Kamiya et al., 1977). Recently, we reported the sequential development of a cellular immune response to V-Z virus in natural infection and discussed its possible role in the recovery of patients from varicella (Kumagai et al., 1980). The present study was designed to investigate the development of both cellular and humoral immune responses to V-Z virus after inoculation of live attenuated varicella vaccine in order to characterize the temporal nature of the responses in patients in different immunological states.

MATERIALS AND METHODS

1. *Vaccine*

The live attenuated varicella vaccine used was the Oka strain of varicella virus (Takahashi et al., 1975) which had been passaged in human embryo lung (HEL) cells 11 times and then passaged in guinea pig embryo cells 6 to 7 times. The virus concentration was about $10^{2.0}$ $TCID_{50}$/0.1 ml and a dose of 0.5 ml/person was injected subcutaneously.

2. *Patients*

Twenty-seven children who had no history of varicella were vaccinated. They were a heterogeneous population with various kinds of diseases. They were divided into three groups according to their immunological status. Group 1 included 9 cases of immunologically handicapped children undergoing immunosuppressive therapy for underlying diseases, and children who were in remission for certain malignancies after intensive immunosuppressive therapy. Group 2 consisted of 11 patients who were not receiving immunosuppressive treatment but had diseases that may be related to immunological derangement. The remaining 8 subjects were probably immunologically normal patients with miscellaneous diseases (Group 3). Clinical data on these subjects are presented in table 1. Serial specimens of heparinized and clotted blood were obtained from these subjects and processed for assay of lymphocyte transformation (LTF) and antibody specific to V-Z virus.

3. *Preparation of V-Z virus antigen*

The details of our method for preparing V-Z virus antigen have been reported previously (Kumagai et al., 1980). Briefly, cultures of HEL cells, which were maintained in Eagle minimum essential medium (MEM) supplemented with 2% fetal calf serum were infected with the Kawaguchi strain of varicella virus (Takahashi et al., 1975). When a CPE of more than 80% of the entire monolayer had developed, the cultures were scraped into 1/20th of their original volume of glycine-buffered saline (pH 9.5). The suspension was sonicated and centrifugated, and the supernatant was dialyzed against MEM and stored at −80°C until use. The antigenic titer of this preparation by the complement fixation test was 1:32 with 4 units of V-Z virus immune human serum. For in vitro LTF assay, appropriated dilutions of virus antigens were prepared in RPMI 1640 medium (Grand Island Biological Co., N.Y., U.S.A.).

4. *In vitro lymphocyte transformation*

A technique for whole blood microculture (Pauly and Han, 1976; Chiba et al., 1978; Scott et al., 1978; Patel et al., 1979), slightly modified for a specific lymphoproliferative response to virus antigen, was used. Heparinized venous blood (20 units of sodium heparin/ml of blood) was diluted 1:15 in RPMI 1640 medium supplemented with 60 μg of kanamycin sulfate/ml and 20 m*M* HEPES buffer. A 200-μl volume of the diluted blood with or without 20 μl of V-Z virus antigen was cultured in quadruplicate in an incubator under 5% CO_2 in air at 37°C for seven days. The cultures were labelled with a solution of 0.2 μCi of [^{3}H] thymidine/20 μl (specific activity 5 Ci/mmol) (the Radiochemical Centre, Amersham, England) for 24 h, washed with 3% acetic acid, and harvested in a multiple automated sample harvester (Labo Mash, Labo Science, Tokyo, Japan). The cultured cells were transferred to strips of glass fiber filter (Skatronas, Lierbyen, Norway) and processed for scintillation counting by the method described previously (Pauly and Han, 1976). Radioactivity was measured in a Packard LS 6024 liquid scientillation spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill, U.S.A.). Results are expressed as the ratio of [^{3}H]-thymidine incorporated into antigen-stimulated cultures to that incorporated into control cultures (stimulation index). A stimulation index of $\geqq$3.0 was considered as a significant response.

The optimal conditions for eliciting a maximal LTF response specific for V-Z virus were determined

TABLE 1. *Clinical data on patients investigated*

Subject	Age (year)	Sex	Underlying disease	Immunosuppressive therapy (per day)	Rash
Group 1					
M. Y.	7	F	ALL[a]	6 MP[b] 50 mg	
N. A.	7	M	ALL	6 MP 40 mg	
T. I.	4	M	Nephrotic synd.	Pred[c] 40 mg	
M. T.	1	F	ALL	Pred 5 mg	
T. S.	4	M	ALL	Pred 25 mg	(+)
N. S.	8	M	Hemosiderosis	Pred 25 mg	
Y. I.	3	M	ALL	6 MP 30 mg	
T. O.	5	M	Nephrotic synd.	Pred 50 mg	(+)
K. T.	5	F	Malig. lymphoma	VCR[d] 1.2 mg, Pred 30 mg	
Group 2					
K. T.	11	F	Chronic nephritis	—	
E. N.	7	M	Asthma	—	
H. T.	10	M	Nephrotic synd.	—	
S. K.	4	M	MCLS[e]	—	
K. M.	14	F	Nephrotic synd.	—	
J. M.	12	M	Asthma	—	
I. M.	12	M	Chronic nephritis	—	
S. O.	5	M	Nephrotic synd.	—	
H. M.	8	M	Rheumatic fever	—	
H. Y.	7	M	Nephrotic synd.	—	
M. K.	9	F	Chronic nephritis	—	
Group 3					
N. K.	4	F	Dwarfism	—	
Y. O.	3	F	Epilepsy	—	
M. S.	1	M	C.H.D.[f]	—	
C. K.	1	F	C.H.D.	—	
Y. S.	14	F	Fracture	—	
N. A.	8	F	Epilepsy	—	
Y. M.	8	F	Cerebral palsy	—	

[a] Acute lymphocytic leukemia.
[b] 6-Mercaptopurine.
[c] Prednisolone.
[d] Vincristine.
[e] Muco-cutaneous lymphnode syndrome.
[f] Congenital heart disease.

by both a dose-response study on the antigen and in vitro kinetics as described previously (Kumagai et al., 1980). On the basis of the data, lymphocytes were cultured for seven days with 1:16 and 1:64 dilutions of virus antigen, and the value for cpm in the culture without antigen was regarded as the control for lymphoproliferative activity. After inoculation of varicella vaccine, the LTF activity in response to V-Z virus antigen was followed for four to five weeks at weekly intervals.

5. *Determination of antibody to V-Z virus*

Neutralizing antibody was measured by the plaque reduction neutralization test using an indirect immunoperoxidase technique, essentially the same as that described by Gerna and Chambers (1976). Virus antigen was prepared by ultrasonic disruption of HEL cells infected with the Kawaguchi strain of varicella virus. Samples of virus solution diluted to 30 to 50 plaqueforming units (PFU)/0.1 ml in SPGA medium (Calnek et al., 1970) were mixed with equal volumes of serially dilutions of the test serum. After incubation at 37°C for 30 min, 0.2 ml portions of the serum-virus mixtures were inoculated in triplicate onto HEL monolayers grown in tissue culture multi-well plates (Linbro Scientific, Inc., Hamden, Conn., U.S.A.) and the plates were incubated at 37°C for 30 min. Then fresh medium was added to the cultures and they were incubated at 37°C for 72 h. The cells were fixed with absolute alcohol for 10 min and covered with 0.2 ml of a working dilution of the V-Z antibody positive serum. After incubation for 30 min at 37°C, the cells were washed three times with phosphate buffered saline (PBS). Then they were covered with 0.2 ml of the optimal dilution of anti-human IgG conjugate peroxidase (goat; MBL, Nagoya, Japan) and incubated for an additional 30 min. Enzymatic activity was detected after three washings with PBS using diaminobenzidine reagent with 0.01% H_2O_2 and black foci were counted. Checkerboard titration revealed that the optimal dilutions of the reference sera and conjugate were 1: 512 and 1: 200, respectively. Titers of NT antibody were expressed as the highest dilution of serum producing a $\geqq 50\%$ reduction in the foci count, compared with those of the virus controls.

RESULTS

1. *Protective effect of vaccination against spread of varicella in the ward*

All the children inoculated with live attenuated varicella vaccine were protected from natural infection during subsequent outbreaks of varicella in the ward. Protection for more than 2 years was confirmed in 8 subjects, who came in close contact with other subjects with varicella. No exacerbation or relapse of basic illnesses, such as leukemia, lymphoma, or the nephrotic syndrome, which might be related to vaccination, was observed in this series of patients.

2. *Lymphoproliferative response and NT antibody*

Group 1: Immunologically handicapped patients.

Individual LTF activities of patients in group 1, expressed as the stimulation index and the NT antibody titer, are shown in Fig. 1. A significant antibody response was observed in all cases in this group. The geometric mean titer of NT antibody at four weeks (at three weeks in case K.T.) was $2^{4.7}$.

In 6 of 9 subjects, significant LTF activity was detected, although a marked variability in the response was observed. Thus, the LTF response in cases M.Y. and N.A. appeared as early as one week after vaccination, whereas, it was apparently delayed in cases T.I. and M.T.. In three cases (T.S., N.S., Y.I.), no

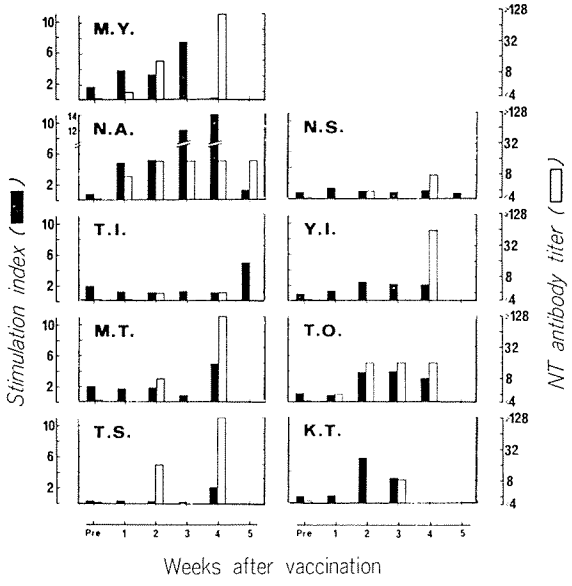

FIGURE 1. Lymphoproliferative activity expressed as the stimulation index, and titers of NT antibody to varicella-zoster virus in nine patients under immunosuppressive treatment.

LTF activity was detectable, in spite of elevation of antibody activity. The maximal stimulation index in patients of group 1 ranged from 1.4 to 14.0 and the geometric mean was 5.8.

In cases T.O. and T.S., mild eruptions appeared at 15 days and 18 days, respectively, after inoculation of vaccine. Herpes zoster developed in case T.S. 1 year after vaccination when he had a relapse of acute lymphocytic leukemia a few weeks before his death.

Group 2:

Data on the 11 subjects in this group are presented in Fig. 2. Although a similar variability in the development of LTF activity was observed in this group, a significant response was observed in all cases and three cases (K.T., E.N., H.T.) showed a positive LTF response in the first week after vacciantion. The maximal stimulation index in this group ranged from 3.6 to 73.8 and the geometric means was 23.2. In all cases, a significant rise in antibody titer was observed and the geometric mean at four weeks was $2^{4.4}$. No consistent relationship was observed between the degree of LTF activity and the antibody titer.

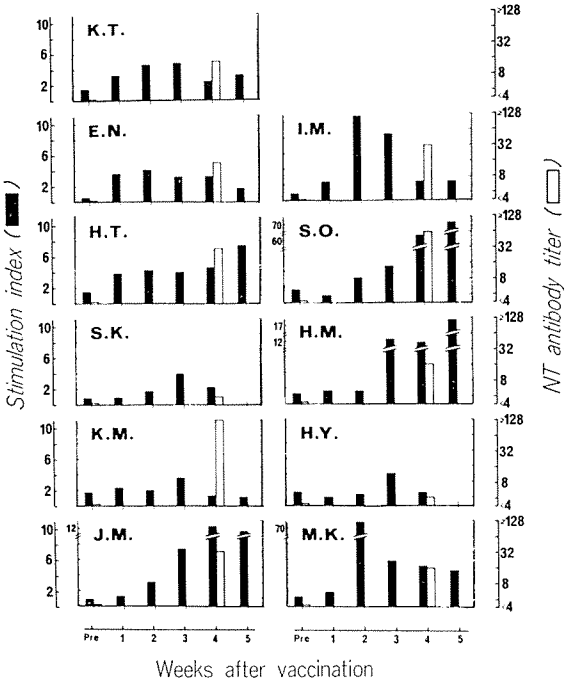

FIGURE 2. Lymphoproliferative activity expressed as the stimulation index, and titers of NT antibody to varicella-zoster virus in 11 patients with diseases possibly related to immunological derangement.

Group 3:

As shown in Fig. 3, a significant LTF response was detected in all cases, and in two (C.K. and Y.S.) a positive LTF response appeared as early as one week after vaccination. The maximal stimulation index in these

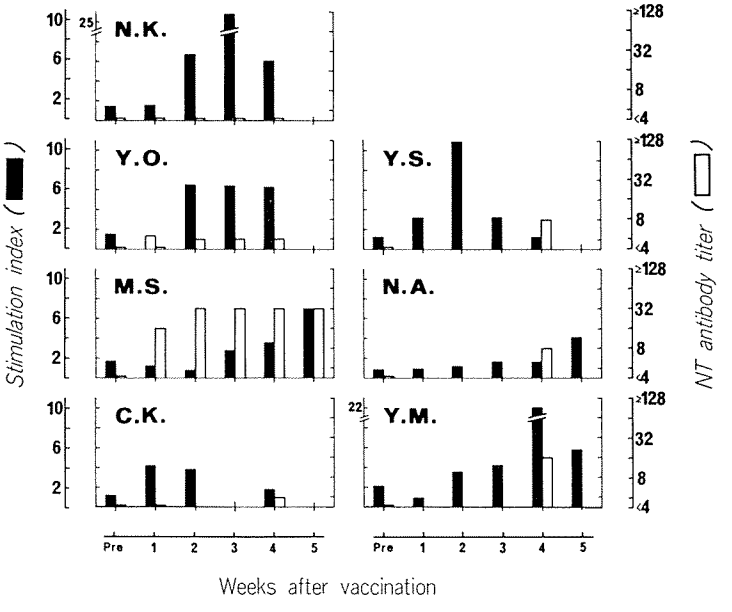

FIGURE 3. Lymphoproliferative activity expressed as the stimulation index, and titers of NT antibody to varicella-zoster virus in seven patients with normal immunological function.

subjects ranged from 4.1 to 25.9 and the geometric mean was 11.5. A significant rise in antibody titer was observed in 6 of 7 cases and the geometric mean at four weeks was 2^3. Varicella eruption did not develop in case N.K., who showed no antibody response, but she showed a lymphoproliferative response with a stimulation index of 25.9.

DISCUSSION

Recent clinical and epidemiological studies have demonstrated that immunization with live attenuated V-Z virus vaccine provides a high degree of protection against varicella. Clinical reactions are generally slight, and follow-up studies on neutralizing antibody activity in vaccinated subjects have suggested that this immunity is prolonged (Asano et al., 1975; Izawa et al., 1977).

In the present investigations, specific neutralizing antibody activity was found in 26 (96%) of 27 subjects after administration of 500 PFU of live attenuated vaccine. Slight clinical reaction (eruption) was observed in only 2 patients 15 to 18 days after vaccination. These two subjects had been treated with immunosuppressive drugs for underlying diseases. Therefore, these observations support the previous view that live attenuated V-Z vaccine affords sufficient immunity to susceptible children, including those who have suppressed immune function (Izawa et al., 1977).

Induction of specific CMI in these subjects was also examined in this study. The temporal nature and the degree of LTF activity following vaccination varied in different subjects, and the precise mechanisms involved in the responses are not known. However, in general, subjects who had been receiving immunosuppressive therapy (group 1) showed a delayed or suppressed LTF response after vaccination compared with that in subjects who were not under such treatment (groups 2 and 3). Therefore, the immunocompetent state of the host seems to be a major factor in determining the subsequent induction of specific cellular immunity.

We previously reported that the development of V-Z virus specific LTF activity was closely associated with recovery from this disease (Kumagai et al., 1980). Kamiya et al. (1977) recently reported consistent detection of delayed-type hypersensitivity to V-Z virus within a week after varicella vaccination. Asano et al. (1977) also observed that effective protection against varicella was achieved when the vaccine was administered within 72 h after exposure to the disease. These observations suggest that the protective efficacy of this vaccine is closely associated with subsequent induction of specific CMI. Therefore, delay in the development of the immunity in these immunosuppressed subjects may result in relatively ineffective protection when the vaccine is administered after exposure to the disease.

On the basis of these findings we recommend that patients who are highly susceptible to varicella should be immunized routinely when they are in remission, before an outbreak of varicella occurs.

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