SUSCEPTIBILITY TO VARICELLA-ZOSTER VIRUS IN THAI CHILDREN AND YOUNG ADULTS

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INTRODUCTION

Varicella is usually a mild disease common in children, but can be serious and sometimes fatal especially in the immunocompromised patients (Finkel, 1961) and also cause complications in adults (Triebeausser *et al.*, 1967). Therefore, passive or active immunization in the prevention of varicella is necessary for susceptible individuals at high risk. Passive immunization with zoster immune globulin (ZIG) was shown to modify or prevent the infection of varicella-zoster virus (VZV) in contact cases who received ZIG within 72 hours after exposure to varicella (Gershon *et al.*, 1974). Recently, varicella vaccine has been available for active immunization (Takahashi *et al.*, 1981).

VZV induces both humoral (Zaia *et al.*, 1977) and cellular (Jordan *et al.*, 1974; Gershon *et al.*, 1976b) immune responses. In humoral study, the immune adherence hemagglutination (IAHA) test has been used successfully to detect VZ antibody (Gershon *et al.*, 1976a; Kositanont *et al.*, 1984). The *in vivo* VZ skin test has been used for the measurement of cell-mediated immunity (CMI) response to VZV (Kamiya *et al.*, 1977; Baba *et al.*, 1978).

The purpose of this study is to determine the susceptibility to VZV in children and young adults who are considered to be at high risk and the correlation between IAHA antibody and VZ skin test results.

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MATERIALS AND METHODS

During 1982-1983, a total of 224 subjects were studied at Siriraj Hospital. The study population was divided into 2 groups; the first group comprised of 53 immunocompromised pediatric patients, and 9 normal children, aged 3 to 13 years (mean $7.3 \pm$SD 2.8). These children suffered from acute leukemia, aplastic anemia, hookworm anemia, Hodgkin's disease, myeloproliferative disease, nephrotic syndrome, systemic lupus erythematosus (SLE) and β-Thalassemia. The second group consisted of 162 second-year medical students of Faculty of Medicine Siriraj Hospital, aged 17 to 27 years (mean $19.6 \pm$SD 1.2). The history of clinical varicella and herpes zoster was recorded from each subject.

The details of the procedure for IAHA test were as described elsewhere (Gershon *et al.*, 1976a; Kositanont *et al.*, 1984). Briefly, the test was performed by incubating inactivated serum with either VZV or control antigen, adding appropriate amount of complement and reincubating. Finally, dithiothreitol (Sigma Chemicals) and human RBC were added. The results were read after setting at room temperature for 2-3 hours. The hemagglutination pattern was considered to be positive, provided that nonspecific hemagglutination was not observed in the corresponding control antigen wells. The sera which

showed nonspecific agglutination were excluded. Antibody titers were expressed as the reciprocals of the highest serum dilution with positive result. The criteria for susceptible level determination was the IAHA antibody titer of < 2.

The method of VZ skin test was as described previously (Kamiya *et al.*, 1977; Baba *et al.*, 1978). A volume of 0.1 ml of VZV skin test antigen was injected intradermally in the forearm. The reaction was read at 24-48 hours by measuring the diameters of erythematous and/or induration areas. Both erythema and induration were measured in millimetres (mm). The criterion of at least 5 mm of erythema or 2.5 mm of induration was considered to be a positive result.

The VZV antigen for IAHA test was prepared from processed VZV-infected human fibroblast cell culture and uninfected one as control antigen (Gershon *et al.*, 1976a). A VZV skin test antigen was prepared by harvesting the VZV-infected human diploid cells, washing the cells with PBS, and sonicating the cells followed by centrifugation at 1,500 g for 20 minutes (Kamiya *et al.*, 1977). The reagents were kindly provided by Dr. M. Takahashi from Research Institute for Microbial Diseases, Osaka University, Japan.

Analysis of data was by the student "t" test, X^2 test and linear regression analysis.

RESULTS

The VZ antibody titers of 62 children and 162 young adults are shown in Table 1. Forty-two of 46 (91.3%) children who had negative history of varicella showed titers of < 2 as compared to 27 out of 46 (58.7%) young adults. In subjects with positive history, the IAHA titers of 12 out of 13 (92.3%) of children and 73 out of 95 (76.8%) of young

Table 1

VZ IAHA antibody titers according to history of varicella in children and young adults.

IAHA Antibody titers	History of varicella							
	Negative		Positive		Uncertain		Total	
	C	A	C	A	C	A	C	A
< 2	42 (91.3%)*	27 (58.7%)	1 (7.7%)	22 (23.2%)	3 (100%)	8 (38.1%)	46 (74.2%)	57 (35.2%)
2	0	1	0	10	0	1	0	12
4	0	3	0	14	0	3	0	20
8	1	5	0	11	0	2	1	18
16	0	4	0	20	0	4	0	28
32	0	3	1	14	0	3	1	20
64	1	2	2	2	0	0	3	4
128	0	1	1	2	0	0	1	3
256	0	0	4	0	0	0	4	0
512	2	0	1	0	0	0	3	0
1024	0	0	1	0	0	0	1	0
2048	0	0	2	0	0	0	2	0
Total	46	46	13	95	3	21	62	162

C = Children, A = Adults, *Percentage.

Table 2

VZ skin test according to history of varicella in young adults.

History of varicella	VZ skin test		Total	X^2 test
	Neg.	Pos. (%)		
Negative	34	12 (26.1)	46	
Positive	38	57 (60)	95	$p < 0.001$ $X^2 = 14.23$
Uncertain	11	10 (47.6)	21	
Total	83	79 (48.8)	162	

Table 3

Relationship between VZ IAHA antibody and VZ skin test results in young adults.

VZ IAHA antibody	VZ skin test		Total	X^2 test
	Neg.	Pos.		
Negative	47	10	57	$p < 0.001$
Positive	36	69	105	$X^2 = 34.31$
Total	83	79	162	

adults were 32 to 2048 and 2 to 128 respectively. The titers of ≥ 2 were also detected in 13 of 21 (61.9%) young adults with uncertain history. The overall susceptible persons to VZV were 74.2% of (62) children and 35.2% of (162) young adults. The number of immune individuals increased with advancing age. The GM titer of 213.8 in children as compared to that of 11.0 in young adults showed significant difference ($p < 0.001$, t test).

Unfortunately, CMI to VZV was not studied in these children, but in young adults it was found that 26.1%, 60% and 47.6% showed positive VZ skin test in those with

Table 4

Age distribution of varicella occurrence in young adults who had positive history of varicella.

Age (years)	No. (%)
1-5	28 (29.5)
6-10	48 (50.5)
11-15	10 (10.5)
16-20	5 (5.3)
not known	4 (4.2)
Total	95 (100)

negative, positive and uncertain history of varicella respectively (Table 2). The overall positive VZ skin test was 48.8%. There was a relationship between the VZ skin test and history of varicella ($p < 0.001$, X^2 test).

Table 3 shows a correlation between VZ antibody and VZ skin test, especially in the negative group ($p < 0.001$, X^2 test).

According to history of varicella and herpes zoster in young adults, 80% of 95 had developed varicella at the age of 1 to 10 years (Table 4). VZ antibody titers against years after varicella attack in 91 young adults who could recall the time of previous infection were analyzed (Fig. 1). Antibody titers varied from < 2 to 128 and were not statistically different in the past years of 1 to 25 ($r = -0.06$). Four out of eight subjects with herpes zoster history had antibody titers of 16 and the other four cases had titers of < 2, 4, 8, and 64. Similar data of VZ skin test against years after varicella attack are shown in Fig. 2. The positive percentage were 62.5, 53.8, 60 and 63.6 at the various past-year intervals of 1-5, 6-10, 11-15 and 16-20. Of 8 subjects with positive history of herpes zoster, only 50% showed positive results.

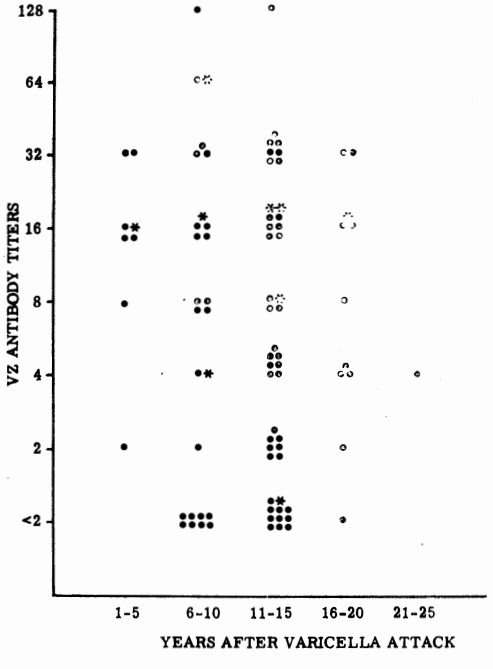

Fig. 1—Correlation between VZ antibody titers and years after varicella attack in young adults with positive history of varicella (●) or herpes zoster after varicella (*).

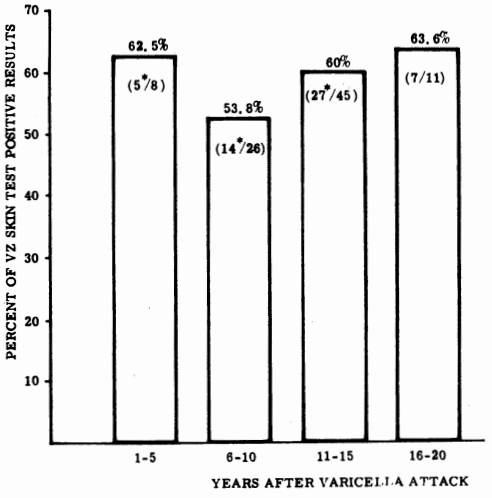

Fig. 2—Percent of positive VZ skin test against years after varicella attack in young adults with positive history of varicella. Subjects with positive history of herpes zoster after varicella (*).

DISCUSSION

This study indicates immune status to VZV in Thai children and young adults. Measurement of immunity to VZV has been performed by study on the humoral or cell-mediated immunity. The antibody titers determined by the IAHA, neutralization (NT) and fluorescent antibody to VZV-induced membrane antigen (FAMA) tests were comparable (Yamada *et al.*, 1979). The IAHA is as sensitive as the FAMA test and both are more sensitive than CF test (Gershon *et al.*, 1976a; Yamada *et al.*, 1979; Kositanont *et al.*, 1984). Although some positive results by FAMA were not detected by IAHA test (Kalter *et al.*, 1977), the IAHA test has been acceptable to

be sensitive for assessing the immune status of individuals (Yamada *et al.*, 1979) and less sophisticated technique.

Susceptibility to VZV was found in 74.2% of children and 35.2% of young adults by IAHA antibody determination. The history of varicella in children given by their parents was quite accurate, based on our finding that 92.3% of these children with positive history were seropositive. Uncertain history and also VZ immunity detected in young adults with negative history of varicella revealed that the history could not indicate their immune status. Susceptibility testing is needed to determine whether high risk adults are immune by assessing antibody and/or CMI response. Although immune individuals increase with advancing age, some adults are still susceptible to VZV.

The IAHA titers of ≥ 8 were comparable to NT antibodies, however 16 of 84 sera, with titers of < 8 were positive NT antibodies (Forghani *et al.*, 1978). IAHA antibody titers of < 2 in most children who were susceptible to varicella infection have been reported (Yamada *et al.*, 1979). The presence of detectable IAHA antibody appears to protect individuals from clinical varicella. However, the absence of IAHA antibody in individuals, especially in old adults, may not mean that they are susceptible. VZ skin test has been used for assessing CMI response to VZV (Kamiya *et al.*, 1977). Therefore, the presence of either IAHA antibody or positive VZ skin test may indicate immunity to VZV. In the present study, 115 of 162 (71%) young adults had immunity to VZV regardless of humoral or CMI response. Thus, VZ antibody by IAHA test and CMI response by VZ skin test should be useful for assessing VZ immunity. In addition, IAHA antibody and VZ skin test results show close relationship.

The antibody level and persistence of positive VZ skin test are not actually related to the time of previous varicella infection. It means that past years of varicella attack are not be the only factor for resulting in antibody decline, but there are others due to their immune responses to VZV, herpes zoster occurrence and/or subclinical reinfection. High-titered antibody in herpes zoster cases have been reported (Miller *et al.*, 1970; Gershon *et al.*, 1974). In the study, individuals with history of herpes zoster showed rather high level of antibody titers. However, one subject had undetectable antibody, this could be explained that the herpes-zoster like lesion was due to herpes simplex virus as has been reported (Mok, 1971). The increment in VZ antibody was demonstrated in immune subject who exposed to VZV as a result of subclinical reinfection (Arvin *et al.* 1983). The pattern of CMI response was also reported by the same study, 71% of VZV immune subjects who had house-hold exposure to VZV showed an increase in lymphocyte transformation in response to VZV. Persistence of cellular and humoral immunity to VZV was maintained by re-exposure to the virus.

In the present study, 80% of subjects had developed varicella during 1 to 10 years of age. Preblud and D' Angelo (1979) also reported that 80% of the cases occur during the first decade of life. The 8.8% of herpes zoster cases found in this study were similar to those reported by Miller *et al.*, (1970). From this study, 74.2% and 29% of children and young adults respectively would be susceptible persons at high risk, thus varicella vaccination is recommended. The efficacy of varicella vaccine in these group has been investigated and the results would be published soon.

SUMMARY

During 1982-1983, susceptibility to varicella-zoster virus (VZV) in 224 Thai subjects

at high risk for varicella infection was studied. The immune adherence hemagglutination (IAHA) and VZ skin test were carried out to determine VZV immunity in immunocompromised children and young adults. The history of varicella and herpes zoster from each subject was recorded. The mean ± SD age in children and young adults were 7.3 ± 2.8 and 19.6 ± 1.2. Negative IAHA test was found in 74.2% of 62 children and 35.2% of 162 young adults. The increase in immune individuals was demonstrated with advancing age. Response to VZ skin test showed positive results in 79 of 162 (48.8%) young adults. The seronegativity was related to the negative VZ skin test ($p < 0.001$, X^2 test). Regardless of antibody detection or VZ skin test, 47 of 162 (29%) young adults were susceptible. According to the positive history of varicella and of herpes zoster obtained from 95 young adults, 80% had developed varicella during 1 to 10 years of age and 8.8% had positive history of herpes zoster. The findings suggest that the IAHA and VZ skin test should be used together for assessing VZ immunity. Varicella vaccination is highly recommended for susceptible persons who may develop severe illness.

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