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EFFECT OF HLA ON THE CELLULAR IMMUNE RESPONSE TO VARICELLA-ZOSTER VIRUS

YASUO CHIBA[1], JUNICHI MIURA and TOORU NAKAO

Department of Pediatrics, Sapporo Medical College, Chuo-ku S. 1, W. 16 Sapporo 060, Japan

AKEMI WAKISAKA and KOJI KUNIKANE

Department of Pathology, Hokkaido University School of Medicine, Kita-ku N. 15, W. 7 Sapporo 060, Japan

SUMMARY The effect of HLA on varicella-zoster virus (VZV)-specific lymphocyte transformation (LTF) was studied in 100 normal immune adults and 64 children who were immunized with live attenuated varicella vaccine. In the normal adults, a statistically significant association was observed between low responsiveness and the presence of A2 ($p<0.025$), and also between high responsiveness and the presence of Aw24 ($p<0.05$). A similar but clearer association, i.e. low responsiveness with A2 ($p<0.005$) and high responsiveness with Aw24 ($p<0.025$), was observed in the vaccinated children. In these children, Aw31 was also found to be related to low responsiveness ($p<0.05$). These results suggest that the VZV-specific cellualr immune response is in some way influenced by HLA.

INTRODUCTION

Induction of a varicella-zoster virus (VZV)-specific humoral and cellular immune response following chicken pox is important in recovery from the illness and in prevention of reinfection. The association of deficient immune function, particularly that of T-cells, with high susceptibility to severe VZV infection has been reported (Merigan and Stevens, 1971; Bloom and Rager-Zisman, 1975).

Recently, the lymphocyte transformation (LTF) response to some bacterial antigens was found to be controlled by genes that were closely linked to HLA (Sasazuki et al., 1978, 1980). Studies on the responses to vaccinia (De Vries et al., 1977) and influenza virus (Conningham-Rundles et al., 1979) have also suggested the presence of such regulatory mechanisms. However, little information is available on the effect of HLA on the VZV-specific LTF response. In the present study, we evaluated this in normal immune adults and in children who were immunized with live attenuated varicella vaccine.

1 Address reprint request to Yasuo CHIBA, Department of Pediatrics, Sapporo Medical College, Chuo-ku S. 1, W. 16. Sapporo 060, Japan

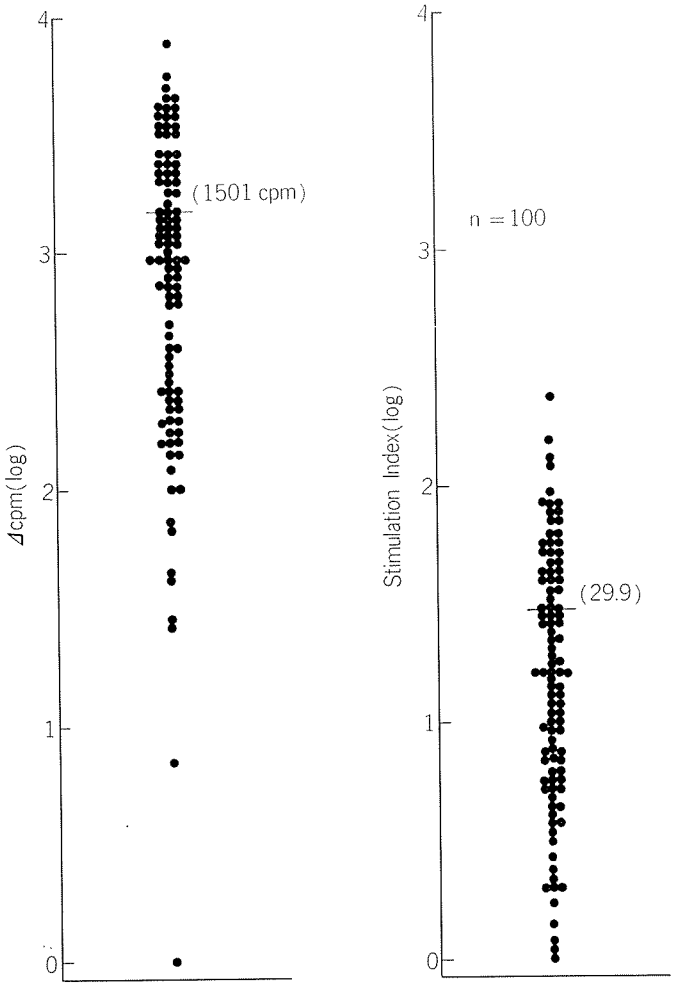

FIGURE 1. VZV-specific LTF response in 100 normal immune adults shown as Δcpm and the stimulation index.

METERIALS AND METHODS

1. *Subjects*

Two groups of subjects were studied. Group 1 consisted of 100 normal adults who were immune to VZV judging from their clinical history or the presence of VZV-specific immunofluorescent antibody to membrane antigen (Zaia and Oxman, 1977). Group 2 consisted of 64 initially seronegative normal children who were subsequently immunized with live attenuated varicella vaccine (Oka strain, 750 PFU/dose). The average ages of the subjects in groups 1 and 2 were 25.7 and 3.1 years, respectively. One or serial specimens of blood were obtained from these subjects and processed for assay of LTF and determinations of HLA.

2. *Preparation of virus antigen*

Human embryonic fibroblasts infected with VZV (Kawaguchi strain) were sonicated for 30 sec in glycine buffer solution (pH 9.5) and cell debris was removed by low speed centrifugation. The resulting supernatant was subjected to ultracentrifuga-

TABLE 1. *Relationship between VZV-specific LTF responses and HLA analyzed on the basis of the median (left) or mean (right) stimulation indices of normal immune adults*

Stimulation index (SI)		SI<16.2	SI≧16.2	SI<29.9	SI≧29.9
Number of cases (%)		50 (50.0)	50 (50.0)	66 (66.0)	34 (34.0)
HLA-A	2	22 (66.7)[a]	11 (33.3)	25 (75.8)	8 (24.2)
	11	10 (62.5)	6 (37.5)	13 (81.3)	3 (18.7)
	w24	24 (40.7)	35 (59.3)[b]	37 (62.7)	22 (37.3)
	26	11 (45.8)	13 (54.2)	16 (66.7)	8 (33.3)
	w31	12 (52.2)	11 (47.8)	17 (73.9)	6 (26.1)
	w33	10 (58.8)	7 (41.2)	14 (82.4)	3 (17.6)
HLA-B	7	4 (57.1)	3 (42.9)	6 (85.7)	1 (14.3)
	w22	1	3	2	2
	w35	8 (44.4)	10 (55.6)	10 (55.6)	8 (44.4)
	w38	1	0	1	0
	w39	5 (83.3)	1 (16.7)	5 (83.3)	1 (16.7)
	40	19 (48.7)	20 (51.3)	27 (69.2)	12 (30.8)
	w44	9 (75.0)	3 (25.0)	10 (83.3)	2 (16.7)
	w46	3	1	3	1
	w51	8 (44·4)	10 (55.6)	10 (55.6)	8 (44.4)
	w52	7 (35.0)	13 (65.0)	11 (55.0)	9 (45.0)
	w54	4 (44.4)	5 (55.6)	6 (66.7)	3 (33.3)
	w58	1	1	2	0
	w59	0	4	1	3
	w62	14 (66.7)	7 (33.3)	17 (81.0)	4 (19.0)
	SN-2	1	2	3	0
	others	5	2	2	5

[a] $\chi^2=5.47$, $p<0.025$
[b] $\chi^2=5.00$, $p<0.05$

tion at 100000×G for 3 h on a 20% (wt/vol) sucrose cushion, and the pellet was suspended at 1/20 of the original volume in RPMI 1640 medium. The complement fixation (CF) antigen titers of these preparations were 1: 16–1: 32 units.

3. *In vitro lymphocyte transformation (LTF)*

The method of whole blood microculture (Chiba et al., 1978) was employed. Briefly, 200 μl of heparinized blood diluted 1: 10 in RPMI 1640 medium was cultured with 20 μl of VZV antigen 2CF units) in quadruplicate in microculture plates. After incubation for 6 days in an incubator under 5% CO_2 in air at 37°C, the cultures were labelled with 0.2 μCi of ^{3}H-thymidine and harvested 24 h later. LTF activity was expressed either as the actual ^{3}H-thymidine incorporation into antigen-stimulated cultures (Δcpm) or as the ratio of cpm of the virus stimulated culture to that of the control culture without antigen (stimulation index; SI).

4. *Determinations of HLA*

HLA-A, -B and -DR locus typing of the subjects were performed by the standard technique of the National Institutes of Health. For statistical analyses, the Chi square test was employed.

RESULTS

1. *Normal immune adults*

The VZV-specific LTF responses expres-

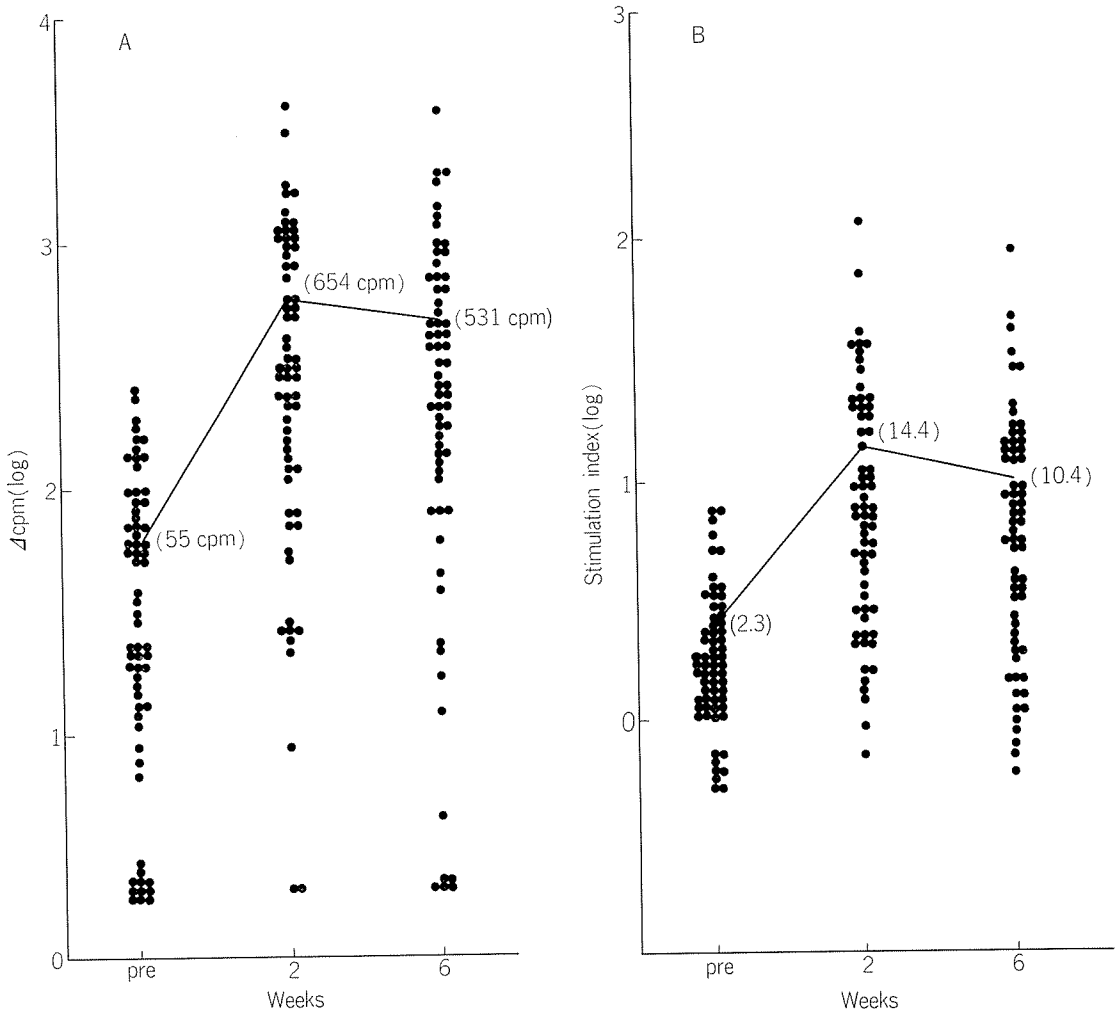

FIGURE 2. Development of VZV-specific LTF activity in 64 children immunized with varicella vaccine. LTF responses are expressed as Δcpm and as stimulation indeces.

sed as Δcpm and as SI in the immune adults are shown in Fig. 1. The Δcpm values ranged from 0 to 7575 cpm and the SI values from 0.2 to 240.9. There was no indication of the existence of distinct subgroups showing high or low responsiveness. Therefore, the median and the mean SI of the population were employed for statistical evaluation of the effect of HLA. As shown in Table 1, a significant association was observed between low responsiveness and the presence of A2 ($p<0.025$) and between high responsiveness and that of Aw24 ($p<0.05$), when the results were analyzed on the basis of the median, but not the mean, SI. The effect of HLA-DR antigens on the response was also evaluated in 83 subjects in this group in the same way, but no significant association was observed (data not shown).

2. *Children immunized with varicella vaccine*

The sequential development of LTF ac-

TABLE 2. *Relationship between VZV-specific LTF responses and HLA analyzed on the basis of the median (left) or mean (right) stimulation indices of children immunized by varicella vaccine*

Stimulation index (SI)		SI<13.6	SI≧13.6	SI<19.5	SI≧19.5
Number of cases (%)		32 (50.0)	32 (50.0)	43 (67.2)	21 (32.8)
HLA-A	2	17 (73.9)[a]	6 (26.1)	21 (91.3)[d]	2 (8.7)
	11	5 (35.7)	9 (64.3)	7 (50.0)	7 (50.0)
	w24	17 (39.5)	26 (60.5)[b]	25 (58.1)	18 (41.9)[e]
	26	4 (36.4)	7 (63.6)	7 (63.6)	4 (36.4)
	w31	11 (78.6)[c]	3 (21.4)	12 (85.7)	2 (14.3)
	w33	4 (66.7)	2 (33.3)	5 (83.3)	1 (16.7)
HLA-B	7	2	1	2	1
	w35	3	2	4	1
	w39	3	0	3	0
	40	11 (45.8)	13 (54.2)	13 (54.2)	11 (45.8)
	w44	3 (33.3)	6 (66.7)	4 (44.4)	5 (55.6)
	w46	2	0	2	0
	w48	0	1	0	1
	w51	1	2	2	1
	w52	9 (56.3)	7 (43.7)	11 (68.8)	5 (31.2)
	w54	5 (45.5)	6 (54.5)	7 (63.6)	4 (36.4)
	w62	7 (70.0)	3 (30.0)	9 (90.0)	1 (10.0)

[a] $\chi^2=8.21$, $p<0.005$
[b] $\chi^2=5.74$, $p<0.025$
[c] $\chi^2=4.48$, $p<0.05$
[d] $\chi^2=7.84$, $p<0.01$
[e] $\chi^2=4.89$, $p<0.05$

tivity in 64 immunized children is shown in Fig. 2. In the different individuals, maximal responses were observed in either week 2 or 6 after vaccination, and so the higher of the two values for SI was employed for statistical evaluation. As shown in Table 2, a significant association was observed between low responsiveness and the presence of A2 ($p<0.005$) or Aw31 ($p<0.05$), and between high responsiveness and the presence of Aw24 ($p<0.025$) when the results were analyzed on the basis of median SI values. The association of A2 and Aw24 with low and high responsiveness, respectively, were somewhat clearer than those in normal immune adults and were even detected in analyses of mean SI values.

Development of mean LTF activity in relation to the possession of these antigens is shown in Fig. 3.

The relations between the responses and HLA-DR antigens were not evaluated in this group.

DISCUSSION

The mechanism by which VZV-specific immunity is maintained is poorly understood. However, a recent study showed that exposure of previously immune subjects to VZV can induce a secondary response of virus specific antibody and lymphocyte proliferation (Arvin et al., 1983). Physiological decrease of the VZV-specific LTF response with advancing

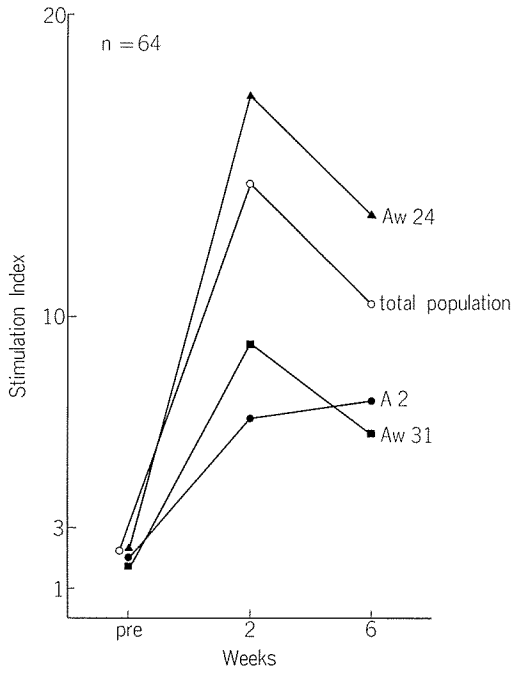

FIGURE 3. Development of VZV-specific LTF activity in 3 groups of children who had either HLA-Aw24, A2 or Aw31 and in the total population after immunization with varicella vaccine.

age is also thought to be a factor determining the levels of virus-specific immunity in humans (Berger et al., 1981).

The present study demonstrated the possible associations of the VZV-specific LTF response with HLA. Namely, in the group of immune adults, the presence of A2 antigen was associated with low responsiveness, while that of Aw24 was related to high responsiveness. These associations were more clearly demonstrated in vaccinated children. In the vaccinated children, Aw31 was also found to be associated with low responsiveness. In general, however, these associations were rather weak and no distinct subgroups showing high or low responsiveness were observed. The complex antigenic nature of VZV and differences in the histories of exposure to VZV in individual adults may have obscured strong association with a particular HLA specificity.

We could not evaluate the effects on the LTF response of antigens in the DR region. But by analogy with murine models, the HLA-D or DR region is suspected to have genes that are in close association with immune responsive or suppressive genes. For example, in the LTF response to tetanus toxoid in humans, a significant association was demonstrated between low responsiveness and HLA-DHO (Sasazuki et al., 1978). Moreover, in a study on the response to candida, high responsiveness was found to be associated with Dwl (Nose et al., 1980). Furthermore, Bergholtz and Thorsby (1978), and Berle and Thorsby (1980) demonstrated that the LTF responses to PPD and HSV in allogeneic combinations of lymphocytes and antigen presenting cells were maximal when these cells shared identical DR antigens. Thus, these observations suggest that genes in the HLA-D or -DR region, or other genes closely linked to them, may have roles in control of the response. The possible existence of such genes and the mechanisms of their effects on the VZV-specific LTF response require study.

A number of studies have demonstrated the importance of a virus-specific cellular immune response in protection against VZV infection (Weller, 1983). Herpes-zoster infection is generally associated with a suppressed LTF response, even in subjects without any particular immunologic defect (Russell et al., 1972). Recently, it has also been suggested that the second attack of varicella may actually occur in subjects who were previously immune to VZV (Gershon et al., 1984). Possibly selective decline of VZV immunity may be related to the occurrence of such reinfection. The present results suggest that HLA could be one factor influencing the level of the VZV-specific LTF response and affecting the protective ability of the host.

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