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### ANTIBODY RESPONSES TO EARLY ANTIGENS OF VARICELLA-ZOSTER VIRUS (VZV) DURING VARICELLA AND ZOSTER

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The antibody responses to membrane and early antigens and thymidine kinase of varicella-zoster virus (VZV) were studied in sera during both varicella and zoster by a test with fluorescent antibody to membrane antigen (FAMA), staining the biochemically transformed cells by the immunofluorescent technique and neutralization of virus-specific thymidine kinase activity, respectively. Similar increases in FAMA antibody titers were demonstrated in sera from patients with varicella and zoster. IgM was detected in both groups, but appeared earlier during varicella than during zoster. Furthermore, the antibody titers to early antigens and virus-specific thymidine kinase were higher in patients with zoster than in those with varicella. These data suggest that different types of antibody responses occur during varicella and zoster.

Varicella is a common infectious disease in childhood, and the virus remains latent in nerve cells after primary infection. This latent virus is reactivated later in life and causes zoster. Varicella-zoster virus (VZV) induces virus-specific glycoproteins on the membrane, early antigens and some virus-

specific enzymes (Takahashi, 1985; Gelb, 1985). Antibodies to various antigens are reported to be induced in vivo during infection (Brunell et al., 1975; Gerna et al., 1979; Kallander et al., 1982). The antibody to early antigens was also demonstrated in the serum of patients with primary infection of

Epstein-Barr virus (EBV) and cytomegalovirus (CMV) (Henle et al., 1970; Thé et al., 1974). In this work, we measured the VZV-specific antibodies to early antigens in sera during varicella and zoster.

Human embryonic fibroblast (HEF) cells were grown in medium 199 with 10% calf serum plus  $\text{NaHCO}_3$  (0.075%), penicillin (100 units/ml) and streptomycin (100  $\mu\text{g}/\text{ml}$ ). The Kawaguchi strain of VZV was used. Sera from 28 patients with varicella and 16 patients with zoster were examined. The patients with varicella ranged from 6 months to 35 years of age, and those with zoster from 14 to 75 years of age. The diagnoses of varicella and zoster were based on clinical findings. Paired sera were taken from 17 of 28 patients with varicella and all those with zoster. A modification of the test for fluorescent antibody to membrane antigen (FAMA) was used. Briefly, HEF monolayers in 96-well microplates were overlaid with VZV-infected cells at 1 infected cell per 5 non-infected cells and the plates were incubated overnight at 37 C in a  $\text{CO}_2$  incubator. Then the cells were washed twice with serum-free medium and incubated with diluted sera for 1 hr in a  $\text{CO}_2$ -incubator at 37 C. They were then washed twice with serum-free medium and incubated with fluorescein conjugated anti-human immunoglobulin (Cappel Inc. USA) for 30 min at 37 C. Finally, they were washed twice with phosphate buffered saline (PBS) and examined under a fluorescence microscope.

Antibody to early antigen was detected with mouse L(O)C13 cells, a cell line that is biochemically transformed by VZV infection, and expresses early antigens, as reported previously (Yamanishi et al., 1981; Lopetegui et al., 1983). Cells were grown on 18 mm glass cover slides in selection medium containing  $6 \times 10^{-7}$  M aminopterin,  $5 \times 10^{-5}$  M guanosine,  $5 \times 10^{-5}$  M adenosine and  $1.6 \times 10^{-5}$  M thymidine supplemented with 10% calf serum. Then they were washed with PBS, air-dried, and fixed in a mixture of acetone and methanol

for 15 min at  $-20$  C and stored at  $-80$  C for staining. The anti-complement immunofluorescence technique was employed for staining, as described previously (Yamanishi et al., 1981). The fixed cover slips were overlaid with about 0.1 ml of a mixture of equal proportions of human complement (fresh anti VZV antibody-negative serum) diluted 1:10 and serum from a varicella or zoster patient, and incubated at 37 C for 30 min in a humidified chamber. The cover slips were then washed thoroughly with PBS and overlaid with fluorescein isothiocyanate conjugated goat anti-human complement  $\text{C}_3$  serum diluted 1:20 (Cappel Inc. USA). After a second incubation at 37 C for 30 min, the cover slips were washed with PBS, and examined in a fluorescence microscope.

The assay of virus specific thymidine kinase activity in the cell extract and neutralization of enzyme activity were carried out as described previously (Ogino et al., 1977). Briefly, VZV-infected cells ( $1 \times 10^7$ ) were suspended in 1.0 ml of 0.15 M KCl in 0.05 M Tris buffer (pH 8.0) containing 3 mM 2-mercaptoethanol. The suspension was sonicated and centrifuged at 20,000 rpm, and the supernatant was used as the enzyme extract. A sample of 100  $\mu\text{l}$  of the enzyme extract was incubated with 50  $\mu\text{l}$  of inactivated serum of varicella or zoster for 2 h at 4 C. The enzyme assay mixture contained 0.2  $\mu\text{Ci}$  of  $^{14}\text{C}$ -TdR (56.6 mCi/mmol, Amersham), 5 mM ATP, 5 mM  $\text{MgCl}_2$ , cell extract, serum and 0.05 M Tris buffer (pH 8.0) to give a volume of 0.25 ml. The reaction was conducted at 38 C for 15 min and terminated by immersing the mixture in a boiling water bath for 2 min. The amount of phosphorylated  $^{14}\text{C}$ -TdR was determined by the DEAE-cellulose disc method. Under this condition, enzyme activity was linearly related to both the amount of enzyme extract and the time of incubation.

Sera from 28 varicella-patients and 16 zoster-patients were examined for antibody. The antibody responses to membrane antigen, early antigens and thymidine kinase during

TABLE 1. *Antibodies of varicella patients*

No.		Days after onset	FAMA		Nuclear Ag L(O)C13	Ab against TK
			total	IgM		
1	T.S.	3	256	8	<4	12.6 (a)
		9	64	8	<4	30.4
		23	64	4	<4	28.8
2	F.T.	17	64	<4	<4	12.9
3	M.T.	2	<4	<4	<4	1.2
		36	64	<4	<4	58.2
4	T.N.	29	512	<4	<4	n.t.
5	Y.O.	69	64	<4	<4	n.t.
6	A.A.	8	128	<4	<4	n.t.
		10	128	n.t.	<4	n.t.
		11	64	<4	<4	n.t.
7	M.A.	14	8	<4	<4	n.t.
		16	32	8	<4	n.t.
		18	128	n.t.	<4	n.t.
8	M.S.	2	<4	<4	<4	3.0
		9	256	8	<4	n.t.
		30	512	<4	<4	27.2
9	T.S.	2	<4	<4	<4	0
		9	256	16	<4	0
10	A.S.	4	4	<4	<4	0
		11	256	8	<4	0
		30	64	8	<4	n.t.
11	T.I.	3	4	<4	<4	4.1
		19	128	4	<4	37.3
12	H.I.	1	16	<4	<4	21.9
		8	512	4	<4	31.9
13	K.H.	2	4	8	<4	n.t.
		9	256	16	<4	n.t.
14	M.K.	4	<4	<4	<4	1.8
		11	512	16	8	6.7
		21	128	<4	8	25.7
15	Y.A.	7	32	<4	<4	n.t.
16	K.M.	1	<4	<4	<4	n.t.
		8	64	<4	<4	n.t.
17	K.K.	2	<4	<4	<4	n.t.
		8	64	<4	<4	n.t.
18	T.T.	30	128	4	8	6.1
19	M.K.	8	256	<4	<4	n.t.
20	T.T.	25	1,024	<4	<4	n.t.
21	T.K.	210	16	<4	<4	n.t.
22	Y.N.	45	64	<4	<4	n.t.
23	I.I.	20	32	<4	<4	n.t.
24	S.I.	19	128	<4	<4	n.t.
25	K.O.	2	<4	4	<4	12.0
		9	128	16	<4	8.0
26	S.K.	2	<4	4	<4	6.7
		9	256	32		11.8
27	Y.N.	7	256	32	<4	10.4
		17	256	4	<4	62.8
28	S.N.	2	128	4	4	35.3
		9	256	16	16	53.5

n.t.: not tested, (a): inhibition rate.

TABLE 2. *Antibodies of zoster patients*

No.		Days after onset	FAMA		Nuclear Ag L(O)C13	Ab against Tk
			total	IgM		
1	M.N.	5	256	<4	<4	0 (a)
		9	512	<4	16	58.6
2	M.H.	5	32	<4	4	15.1
		10	32	<4	<4	0.6
3	N.O.	4	128	<4	<4	11.4
		10	128	<4	4	9.1
		21	128	4	4	9.1
4	N.S.	7	128	<4	<4	n.t.
		14	512	16	32	n.t.
		70	256	4	4	n.t.
		100	128	<4	<4	n.t.
5	F.K.	7	256	8	8	39.5
		10	512	16	32	n.t.
		21	256	8	32	86.5
		45	256	4	32	60.1
		90	256	<4	8	29.6
6	S.K.	10	256	<4	<4	76.3
		14	256	<4	64	88.1
		50	128	<4	32	66.0
7	S.K.	3	16	<4	<4	12.2
		10	256	4	128	90.8
		21	128	<4	64	93.4
		120	128	<4	<4	21.0
8	S.K.	4	16	<4	<4	4.4
		7	1,024	4	32	66.3
		60	512	<4	<4	58.1
9	H.I.	4	8	4	<4	0.2
		14	512	4	64	59.6
		21	256	8	64	59.3
		90	256	8	64	13.3
10	M.N.	2	16	<4	4	7.8
		4	256	<4	16	49.7
		16	512	8	128	22.8
		53	256	<4	64	27.4
11	T.K.	3	64	<4	4	10.1
		12	512	<4	64	62.6
		28	512	<4	16	59.0
		50	512	4	32	n.t.
		8	512	4	128	n.t.
12	S.I.	14	512	16	64	n.t.
		6	32	8	<4	13.4
13	N.Y.	14	512	16	<4	14.5
		40	512	<4	<4	20.7
		4	16	<4	<4	8.2
14	A.N.	7	32	<4	<4	13.4
		15	128	<4	<4	81.6
		4	128	8	<4	n.t.
15	Y.N.	10	128	4	64	n.t.
		21	256	16	32	n.t.
		5	64	n.t.	n.t.	n.t.
16	M.O.	7	128	n.t.	<4	n.t.
		26	64	n.t.	16	n.t.

n.t.: not tested, (a): inhibition rate.

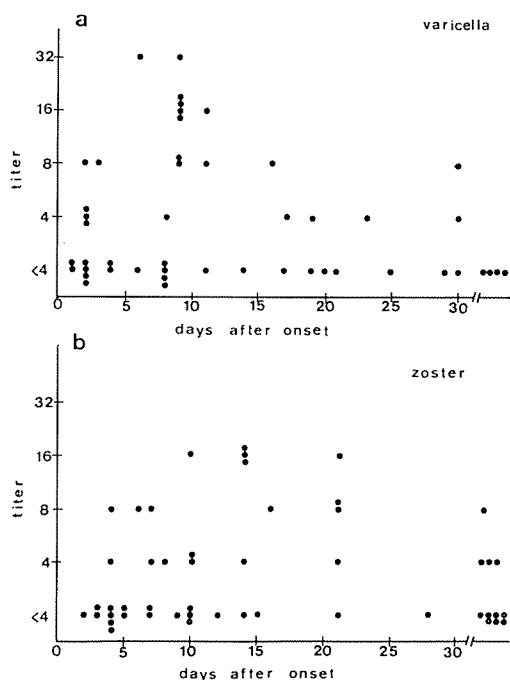


FIGURE 1. IgM antibody response to VZV in 28 varicella patients (a) and 16 zoster patients (b). IgM antibody to VZV was assayed as described in the text.

varicella and zoster are listed in Tables 1 and 2. Antibodies to membrane antigens were induced in the sera of both patients with varicella and those with zoster, and the maximum titer of each serum was between 1:64 and 1:1024 for varicella and between 1:32 and 1:1024 for zoster. Antibodies against membrane antigens appeared in the sera earlier during zoster than varicella infection. Low titers of antibodies (1:4 or less) were found in patients with varicella at the beginning of infection. On the other hand, relatively high titers of antibodies were detected in patients with zoster. IgM antibody, detected by the FAMA test, was induced during both varicella and zoster, and it reached a maximum titer earlier in varicella than in zoster (Fig. 1).

Antibody tests for early antigens in patients

with varicella and zoster were carried out with L(O)C13 cells. Three of 28 patients (10.7%) with varicella had detectable antibody to L(O)C13 antigens, their titers being 1:4 to 1:16, but the other 25 patients had no detectable antibody (less than 1:4), even though they had high titers of antibodies to membrane antigen detected by the FAMA test. In contrast, 14 of 16 zoster-patients (87.5%) had antibody to L(O)C13 antigens and their antibody titers were higher than those of patients of varicella. No correlation between titers to membrane antigen and early antigens was observed in patients with either varicella or zoster. Some of the sera were tested for ability to neutralize TK. Antibodies to VZV specific TK were detected in the sera of 3 of 12 varicella patients (25.0%), and in 8 of 12 patients with zoster (66.7%). Antibody to TK was detected in the second week of disease and became undetectable sooner than antibody to membrane antigen. The inhibition rate seemed to be greater in sera from patients with zoster than in those of patients with varicella.

In this communication, we demonstrated VZV IgM antibody by the FAMA test in varicella and zoster patients. Although IgG responses are characteristic of the second antigenic stimulation, an IgM response also occurred in zoster. IgM antibody seemed to appear later during zoster than during varicella infection (Fig. 1) and the titer was lower in zoster than in varicella. Schmidt and Arvin (1986) also reported detection of IgM in varicella and zoster by solid-phase radioimmunoassay.

VZV abortively infects mouse cells and expresses only immediate early and early antigens in the cells (Yamanishi et al., 1981; Lopetegui et al., 1985). We used biochemically transformed cells (L(o)c13) as a source of immediate early and early antigens in this experiment. We previously detected 7 VZV-specific polypeptides in this transformed cell by immunoprecipitation with anti-VZV monkey hyperimmune serum (Lopetegui et al.,

1983, 1985). Gerna et al. (1979) detected antibody to early protein in sera of patients with varicella and zoster by the immunoperoxidase antibody technique and found that the antibody appeared 3–5 days after the onset of infection, reaching a peak during the second week. They also reported that antibody to early antigen could be detected in the sera of patients with varicella and zoster. In our experiments, antibody to early antigens was detectable in a smaller proportion of the patients with varicella than of those with zoster, and it appeared earlier and reached a higher titer during zoster than during varicella. This discrepancy between our results and those of Gerna et al. may be due to use of different methods. Probably after the onset of varicella, antigens are memorized in memory cells and the reproduction of antibody to VZV is boosted in zoster. Since high titers of antibodies to late protein of VZV are produced during varicella infection, no significant difference in the antibody titers to late antigens was detected during varicella and zoster. On the contrary, only a small amount of antibodies to early antigens are produced during varicella infection and the onset of zoster may have a marked booster effect on their production. Weigle and Grose (1984) detected a polypeptide with a molecular weight of 32,000 in the sera of zoster patients. It is unknown whether this corresponds to the early antigens detected during zoster in our experiment.

VZV specific TK is also an early antigen (Ogino et al., 1977). We detected VZV-TK blocking antibodies mainly in the sera of zoster patients (Table 2, Fig. 2). Kallander et al. (1982) also reported that antibodies to VZV specific TK were detectable in sera from patients with zoster. These findings are consistent with the results on antibodies against early antigens described above.

Antibodies to early antigen and TK were detected in the serum of patients No. 28 with

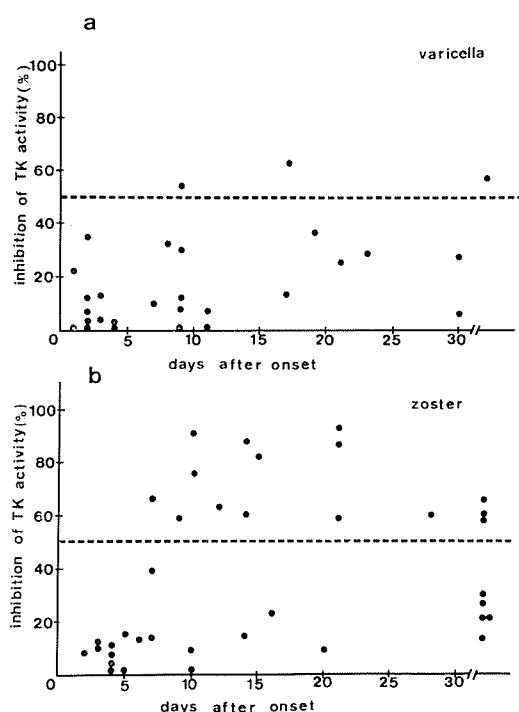


FIGURE 2. Inhibition of viral TK activity by sera from 28 varicella patients (a) and 16 zoster patients (b). TK activity was assayed as described in the text.

varicella on the second day after the onset of disease. This patient (4 years old) had a history of varicella 6 weeks after birth and she was then reinfected with varicella. The finding of antibodies to early antigen in this patient is analogous to the finding of antibodies to early antigens in most patients with zoster.

These results indicate that different types of antibody responses occurred during varicella and zoster. Varicella can be distinguished from zoster by an antibody test against early antigens, although usually this is not necessary, because the clinical manifestations of varicella and zoster are different.

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