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Specificity of Skin Test with Varicella-Zoster Virus Antigen in Varicella-Zoster and Herpes Simplex Virus Infections

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Specificity of the skin test with varicella-zoster virus (VZV) antigen was examined in guinea pigs infected with herpes simplex virus (HSV) type 1 or VZV and in children with a history of HSV infection who developed varicella. Infected guinea pigs responded positively only to homologous virus. No cross-reaction between HSV and VZV was detected in the skin test, as well as in the neutralization test in infected guinea pigs, suggesting that the VZV skin test is specific for immunity to VZV infection. Twelve children were infected with HSV during an HSV epidemic and subsequently developed varicella in institutional settings. During the 2.5-month period between the HSV and VZV infections, the immune status of the children to VZV was negative both in the skin test and in the antibody test, although antibody to HSV was detected by an immune adherence hemagglutination test. After VZV infection, all responded positively both in the skin test and in the antibody test (immune adherence hemagglutination test) to VZV. These results suggest that the VZV skin test is specific for immunity to VZV infection, not cross-reactive to HSV infection in humans. This specificity will be of value in screening susceptibility or immunity to VZV, irrespective of prior HSV infection.

Skin test antigen to varicella-zoster virus (VZV) was developed and first characterized by our group in 1977 (9). Since then, its utility as a predictor of immunity to VZV has been further documented (1, 3, 6, 8, 9, 11, 16–18, 20). It has been successfully used for the follow-up of vaccinees (including adults) of a live varicella vaccine or natural varicella. Its reactivity can be assessed in guinea pigs infected with live varicella vaccine (12, 17, 23). Although the usefulness of the skin test antigen for screening susceptibility to VZV infection has been documented, little information is available regarding whether the VZV skin test is cross-reactive with herpes simplex virus (HSV) infection, particularly in humans. Recently it has been reported that a VZV glycoprotein (gpII) is cross-reactive with HSV glycoprotein (gB) (4, 5, 10, 15). This study was designed to clarify the specificity of skin test antigen in relation to HSV infection in experimentally infected guinea pigs and in naturally infected children in institutional settings.

MATERIALS AND METHODS

Skin test antigens. The VZV soluble skin test antigens were prepared from supernatants of VZV (Oka strain)-infected MRC-5 cells (1). Briefly, MRC-5 monolayers were inoculated with virus-infected cells at a ratio of 1 to 5 uninfected cells. After incubation for 24 h at 37°C, the cells were washed three times with phosphate-buffered saline and fed with enriched minimal essential medium without phenol red for 24 h. Culture fluids were collected and centrifuged at 1,500 × *g* for 10 min and then at 100,000 × *g* for 2 h. The supernatants were heated at 56°C for 30 min and used as a soluble skin test antigen. This antigen contained about 10 μg of protein per ml, and its antigenic value was 1:8 in a reversed passive hemagglutination test (17). The control skin test antigen was prepared from uninfected cultures in the same manner. The second antigen was the VZV crude antigen prepared from sonic extracts of VZV (Oka strain)-infected cells (1, 12). The VZV-infected guinea pig cells were suspended in phosphate-buffered saline, sonicated, and centrifuged at 1,500 × *g* for 10 min. The supernatant was heated at 56°C for 30 min and used as a VZV crude skin test antigen. The HSV and control crude skin test antigens were prepared from HSV type 1 (HSV-1; Seibert strain)-infected and uninfected guinea pig cells, respectively, in the same manner. The antigenic value of each viral crude antigen was 1:8 in a complement fixation test.

Animal study. Inbred guinea pigs (Yodo strain) weighing about 200 g were inoculated with live varicella vaccine virus (19) (Oka strain, 15,000 PFU/ml per animal) or HSV-1 (Seibert strain, 10^7 PFU/ml per animal) subcutaneously. The skin test antigens used were the control and VZV soluble skin test antigens, prepared from supernatants of MRC-5 cell cultures, and the control, VZV, and HSV crude skin test antigens prepared from guinea pig cells. Skin test antigen (0.1 ml) was simultaneously inoculated intradermally into the backs of the animals 3 weeks after immunization with virus. Inoculation sites were examined for erythematous changes 8, 24, and 48 h later. The neutralization test titer was assayed as described previously (2, 15).

Clinical study. Clinical application of the VZV skin test and collection of serum specimens were carried out for 12 children of 6 months to 2 years of age. The children had contracted chicken pox 2.5 months after acquiring acute herpetic gingivostomatitis, which had prevailed in an institution in Osaka, Japan. One patient had an HSV infection 4 days before acquiring chicken pox. Antibody titer was assayed by immune adherence hemagglutination (IAHA) to VZV and HSV (22). The skin test was performed by injecting 0.1 ml of a soluble skin test antigen of VZV intradermally into the forearm. The skin reaction was evaluated by measuring the erythematous change 30 h after injection and was taken as positive when the diameter of erythema or indura-

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TABLE 1. Cross skin tests with VZV and HSV-1 antigen in guinea pigs

Guinea pig[a]	Skin antigen[b]	Cutaneous reaction (mm) at:			Neutralization test antibody
		8 h	24 h	48 h	
Immunized with VZV vaccine					
1	HSV-1 (g)	–[c]	–	–	
	VZV (g)	15 × 15	15 × 15	10 × 10	
	Control (g)	–	–	–	HSV < x2
	VZV (h)	16 × 15	18 × 16	16 × 15	VZV x16
	Control (h)	–	–	–	
2	HSV-1 (g)	–	–	–	
	VZV (g)	15 × 15	14 × 13	10 × 10	
	Control (g)	–	–	–	HSV < x2
	VZV (h)	19 × 17	18 × 17	13 × 13	VZV x16
	Control (h)	–	–	–	
3	HSV-1 (g)	–	–	–	
	VZV (g)	16 × 16	11 × 11	10 × 9	
	Control (g)	–	–	–	HSV < x2
	VZV (h)	16 × 16	15 × 15	15 × 12	VZV x16
	Control (h)	–	–	–	
Immunized with HSV-1					
1	HSV-1 (g)	13 × 10	15 × 15	10 × 8	
	VZV (g)	–	–	–	
	Control (g)	–	–	–	HSV > x64
	VZV (h)	–	–	–	VZV < x2
	Control (h)	–	–	–	
2	HSV-1 (g)	10 × 10	15 × 10	7 × 5	
	VZV (g)	–	–	–	
	Control (g)	–	–	–	HSV > x64
	VZV (h)	–	–	–	VZV < x2
	Control (h)	–	–	–	
3	HSV-1 (g)	11 × 10	13 × 12	9 × 8	
	VZV (g)	–	–	–	
	Control (g)	–	–	–	HSV > x64
	VZV (h)	–	–	–	VZV < x2
	Control (h)	–	–	–	

[a] Animals were immunized with 15,000 PFU of VZV per animal or with 10^7 PFU of HSV-1 per animal. One guinea pig (no. 4) died 5 days after injection with HSV-1 and is not included here.
[b] The antigens were prepared in guinea pig embryo fibroblasts (g) or human diploid cells (h).
[c] –, Cutaneous reaction of 0 or <3 mm in diameter.

tion was 5 mm or larger and that at the control site was less than 5 mm.

RESULTS

Results of skin test in infected guinea pigs. A positive skin reaction was observed only with homologous antigen, and negative skin reactions were observed with heterologous or control antigen (Table 1). There was no difference in the degree of reaction to the antigens prepared either from guinea pig embryo cultures or from human cell cultures. A rise of neutralization test titer was confirmed only with homologous virus.

Skin test with VZV antigen in children infected with HSV and subsequently with VZV. The clinical diagnosis of HSV or VZV infection among the children was confirmed by significant antibody response after each infection (Fig. 1). Since one child had a clinical HSV infection just before contracting varicella, no significant rise in antibody to HSV was detected when the antibody appeared in the other 11 cases (Fig. 1A). Rises in HSV antibody titer were fourfold or more in 2 of 12 children when they had chicken pox after HSV infection. No cross-reactive IAHA antibody to VZV was detected in any of the 12 children during and after HSV infection (Fig. 1B). VZV skin test reactivity (erythema size in millimeters) in the children remained negative for 2 months after acute herpetic gingivostomatitis, and then it turned positive during and after exposure to epidemic varicella (Fig. 2). One child, negative at the test, was confirmed positive when tested again 3 months after onset.

DISCUSSION

The immunological relationship between VZV and HSV is one of the important subjects for serodiagnosis of both viruses. Immune response to either virus infection occasionally causes a rise in heterologous as well as homologous virus antibody titer (21). This was observed only in patients having prior infection with heterologous virus (13, 14). Antigenic cross-reactivity was reported by immunofluorescence assay (7). It was subsequently clarified that major antigenic crossing was present in viral glycoproteins, but that no cross-neutralization of virus was detected with hyperimmune sera (15). The cross-reactivity of gpII of VZV and gB of HSV in a precipitation test was recently confirmed (4, 5, 10). However, a soluble skin test antigen of VZV

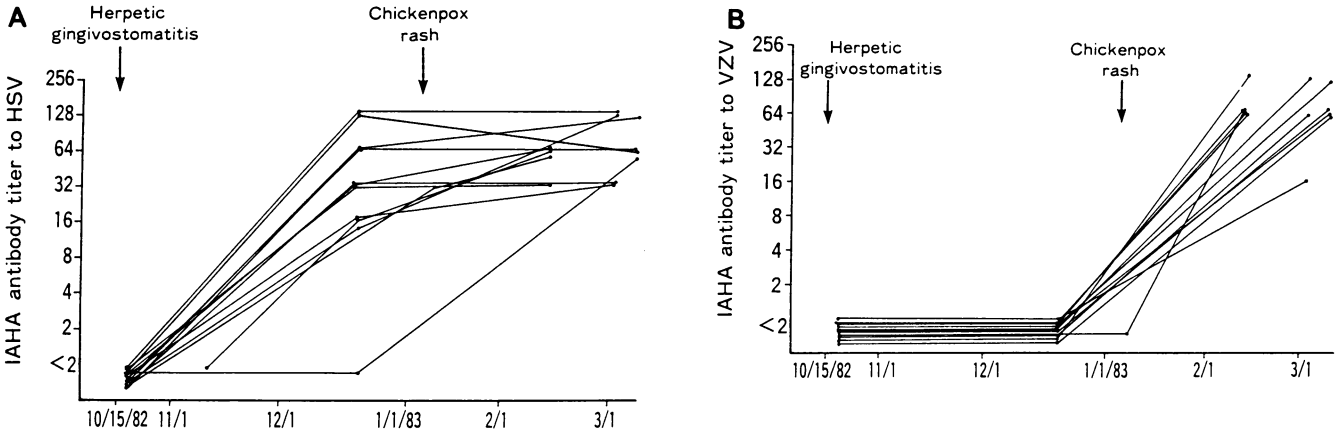

FIG. 1. IAHA antibody response to HSV-1 (A) and VZV (B) in 12 children who were exposed to HSV and then varicella infection. Arrows indicate the onset of outbreak of each epidemic of HSV or VZV. One child had HSV infection just before acquiring chicken pox. All children developed IAHA antibody to HSV and subsequently produced IAHA antibody to VZV after they had varicella infection.

contains two major glycoproteins with molecular weights of 115,000 and 45,000, and these were different species from the glycoprotein that was cross-reactive with HSV. Positive reactivity of VZV or HSV soluble skin test antigen was observed in guinea pigs immunized with homologous virus, but no cross-reactivity was observed with heterologous virus (17).

These observations were further confirmed in the present study in infected guinea pigs by using crude skin test antigens prepared from guinea pig embryo fibroblasts infected with either virus containing at least a viral cross-reactive glycoprotein. No cross-reaction was observed with cross-reactive crude antigens. This result suggested that the cross-reactive glycoproteins of both viruses could be detectable serologically but not detectable by blastogenic response to viral antigen in a short incubation period (24) or in the skin tests examined in this study. Cross-reactive determinant(s) of viral glycoprotein may play no or little role in neutralization or cellular immunity, although cross-reactivity could be detected by immunoprecipitation.

It is particularly important that in clinical tests, cutaneous reactivity to VZV antigen in the 12 children who were previously infected with HSV remained negative until they developed chicken pox, while positive reactions were observed after chicken pox. These results suggest that cross-reactions between the two viruses are too limited to affect the specific immune response to each, including clinical reactions, which should warrant the clinical use of the VZV skin test for screening susceptibility to VZV infection.

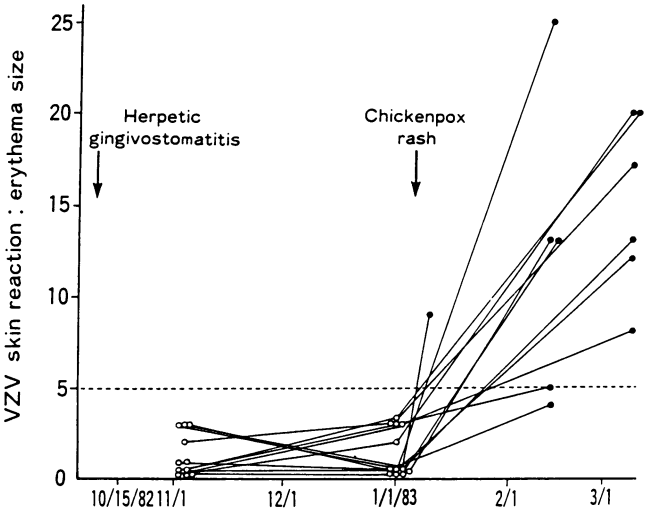

FIG. 2. Cellular immune response detected by varicella skin test in 12 children who were exposed to HSV and then varicella infection. Arrows indicate the onset of outbreak of each epidemic of HSV or VZV. Skin test reactivity to VZV (measured by erythema size in millimeters) in all children remained negative 2.5 months after HSV infection and turned positive during and after development of clinical varicella. Symbols: ○, before rash; ●, after rash.

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