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COMPARATIVE ASSAY OF VARICELLA SKIN TEST ANTIGENS BY THE ELISA AND RPHA TESTS

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Summary. — The antigenic potency of the varicella skin test antigens was assayed by reversed passive haemagglutination (RPHA) test using sheep red blood cells coated with zoster convalescent serum and by enzyme linked immunosorbent assay (ELISA) using anti-VZV sera or monoclonal antibody to gpI followed by an anti-IgG β-galactosidase conjugate. Three kinds of varicella skin test antigens were compared: a soluble varicella skin test antigen, a modified soluble varicella skin test antigen, and a crude varicella skin test antigen. The RPHA test was suitable for the soluble and for modified soluble varicella skin test antigens but it was not suitable for the crude varicella skin test antigen. ELISA was applicable for all the three antigen types. It was possible to assess quantitatively the content of viral antigens in the same type of the skin test antigen, but not by comparing the different skin test antigen types. ELISA was more efficient in the quantitative assay of the amount of viral antigens than the RPHA test.

Key words: *varicella-zoster virus*; *skin test antigen*; *RPHA*; *ELISA*

Introduction

The skin test antigen for testing varicella-zoster virus (VZV) was developed and first characterized by Kamiya *et al.* (1977);its clinical utility in predicting the immunity to VZV has been further documented (Baba *et al.*, 1978; Hata, 1980; Asano *et al.*, 1981; Shiraki *et al.*, 1984; Tanaka *et al.*, 1984; Florman *et al.*, 1985; LaRussa *et al.*, 1985). Two types of skin test antigens have been introduced. The crude varicella skin test antigen can be prepared from the sonicated VZV-infected cells (Kamiya *et al.*, 1978; Baba *et al.*, 1978; Hata, 1980; Asano *et al.*, 1981; Shiraki *et al.*, 1984; Florman *et al.*, 1985; LaRussa *et al.*, 1985), while the soluble varicella skin test antigen was prepared from the supernatant of VZV infected culture which was found to contain mainly two major viral glycoproteins excreted from VZV-infected cells (Asano *et al.*, 1981; Shiraki *et al.*, 1982; Shiraki and Takahashi,

1982; Shiraki *et al.*, 1984; Tanaka *et al.*, 1984; Baba *et al.*, 1987). The antigenic potency of crude skin test antigen was assessed by complement fixation (CF) test and that of the soluble skin test antigen was established by RPHA test (Shiraki *et al.*, 1984). Although the latter is sensitive, the procedure is complicated and the value obtained is not distinct enough for comparing the antigenic potency of the skin test antigens because the system assays the antigens in two-fold serial dilutions. ELISA is easy and more convenient to perform than RPHA test. Monoclonal antibodies to viral glycoproteins have been prepared and also anti-VZV polyclonal sera are available for the serological assay. This study was designed to measure the antigen content in the skin test antigens by ELISA.

Materials and Methods

Skin test antigens. The crude varicella skin test antigen was prepared from sonicated extracts of VZV (Oka strain)-infected MRC-5 cells (Takahashi *et al.*, 1975; Kamiya *et al.*, 1978; Asano *et al.*, 1981; Shiraki *et al.*, 1984). Briefly, MRC-5 cells were inoculated with virus-infected cells at a ratio of 1 to 5 uninfected cells. After incubation for 48 hr at 37 °C, VZV-infected cells in a Roux bottle were washed three times with phosphate-buffered saline (PBS) and suspended in 5 ml of PBS. The cell suspension was sonicated and centrifuged at 1,500 xg for 10 min followed by heating at 56 °C for 30 min. The soluble varicella skin test antigen was prepared as follows (Asano *et al.*, 1981; Shiraki *et al.*, 1984): by 24 hr post-inoculation the VZV-infected MRC-5 cells were washed three times with PBS and then refed with enriched minimal essential medium without foetal bovine serum and phenol red for 24 hr. Culture fluids were collected and centrifuged at 1,500 xg for 10 min and then at 100,000 xg for 2 hr. The supernatants were heated at 56 °C for 30 min. The modified soluble varicella skin test antigen was prepared from the culture fluids in the same way as the soluble varicella skin test antigen but after three times freezing and thawing. The control antigens were prepared from noninfected cells. The protein concentration of each preparation was determined (Bradford, 1976). The antigenic potency of the skin test antigen was examined by intradermal injection into the forearm.

Antisera to VZV. 1. Zoster convalescent serum (1 : 512 as determined by the complement fixation test) (Shiraki *et al.*, 1982), 2. anti-VZV guinea pig serum (1 : > 400 as determined by the neutralization test) (Shiraki and Hyman, 1987), and 3. monoclonal antibody to gpI (Okuno *et al.*, 1983; Davison *et al.*, 1986). The monoclonal antibody (Cl 12) reacted with viral glycoprotein (gpI) and with glycoproteins of molecular weight of 55 kD and 45 kD. The soluble varicella antigen contained the 45 kD glycoprotein derived from the 55 kD glycoprotein; it is one of two major glycoproteins excreted from infected cells (Shiraki *et al.*, 1982; Shiraki and Takahashi, 1982; Okuno *et al.*, 1983).

Serological assay of the skin test antigens. The RPHA test was performed as described previously (Shiraki *et al.*, 1984). Briefly, formalin-fixed sheep red blood cells (F-RBC) were treated with tannic acid and then with zoster convalescent serum. Antigens were preincubated with 10% F-SRBC for 1 hr to form a final 1% suspension. Antigens were subjected to two fold serial dilution and incubated with antibody coated F-RBC. Antigenic titres of the skin test antigens were determined by their HA pattern.

Antigenic potency was assessed by two methods (direct and sandwich) of antigen coating on the ELISA plate. A 100 μl aliquot of the antigens was distributed to the wells of the ELISA plate (Greiner) and incubated at 4 °C overnight (direct method). The wells were washed and the surface was blocked with 1% bovine serum albumin (BSA) in PBS. Zoster convalescent serum or ascites of monoclonal antibody was diluted 1 : 50 with 1% BSA in PBS and then used as anti-VZV serum. After washing the wells, 100 μl of anti-VZV serum was applied and incubated for 1 hr at 37 °C. After washing the wells, anti-human IgG β-galactosidase conjugate (Amersham) or anti-mouse IgG β-galactosidase conjugate (Amersham) and nitrophenyl-β-D-galactopyranoside were used according to the manufacturer's instruction.

In the sandwich method, anti-VZV serum was coated first on the plate. Zoster serum or guinea pig serum was diluted 1 : 50 with PBS and 100 μl of diluted serum was distributed to

each well. After overnight incubation at 4 °C, the wells were washed, 100 μl overnight incubation at 4 °C, the wells were washed, 100 μl of antigen was added and incubated for 1 hr at 37 °C. After washing the wells, further reactions were performed in the same way as by direct method.

Results

Table 1 shows the protein concentration, the RPHA titre, and the antigenic potency assessed *in vivo* by the skin reaction. Each antigen possessed enough potency to induce the skin reaction. The antigenic potency of skin test antigens was well correlated with the RPHA titre except for the crude varicella antigen. The number of antigen molecules or particles determined the titre in the RPHA test. Although the crude varicella skin test antigen contained a higher protein concentration and a comparable skin test reactivity, the viral antigens were present as large aggregated forms. In RPHA test this may have resulted in a lower antigenic titre than with other antigens.

Table 2 shows the comparative antigenic potency of the skin test antigens assessed by the ELISA test. Antigens were coated on the ELISA plates and reacted with zoster convalescent serum and monoclonal antibody (Cl 12) (direct method). The soluble varicella antigen was well assessed by zoster serum and by monoclonal antibody. In repeated experiments the modified soluble skin test antigen was not efficiently detected by the zoster serum. The reason is not clear, but this might occur possibly due to less efficient antigen-fixation on the plate or due to poor antigenic presentation in this type of preparation to the antibody in zoster serum. The crude varicella antigen exhibited higher antigenic content in the sandwich method than in the direct method. This may be due to better antigen fixation on the ELISA plates coated with anti-VZV serum. Both the soluble and modified soluble varicella antigens were low in antigenic value by the sandwich method. Viral antigens in these preparations may be present as single molecules or

Table 1. Antigenic potency of skin test antigens

Antigens	Protein concentration (μg/ml)	RPHA titre	Skin reaction at 24 hours (mm)
Soluble control	9.5	1 : <1	ND
Soluble varicella	8.5	1 : $2^{3.7}$	15 × 15
Modified control	48.2	1 : <1	ND
Modified varicella	52.8	1 : 2^{4}	16 × 16
Crude control	85.4	1 : <1	ND
Crude varicella	92.8	1 : 2	15 × 18

ND = not done

The RPHA titres of the skin test antigens were the means of three assays.

One of the authors with a history of varicella underwent skin tests. The skin test antigens (0.1 ml) were injected intradermally into the forearm. The cutaneous reaction was examined at 8, 24, and 48 hr after injection.

Table 2. Antigenic assay of the skin antigens by ELISA test

Antigens	Direct method		Sandwich method (monoclonal antibody)	
	Zoster serum	Monoclonal antibody (Cl 12)	Zoster serum coating	Guinea pig serum coating
Soluble control	29	105	8	38
Soluble varicella	248	207	88	96
Modified control	34	121	24	44
Modified varicella	95	181	121	97
Crude control	38	61	59	82
Crude varicella	220	635	1,071	961

The values of the ELISA assay were expressed as the absorbance × 1,000.
Monoclonal antibody (Cl 12) was used as the second serum to detect viral antigens in the sandwich method.

oligomeric forms, and not as large aggregated form of viral antigens, which resulted in a limited number of antigenic sites in a single viral antigen. This may cause competition or blocking of the reactive site by polyclonal antibody versus monoclonal antibody.

Table 3 shows the results of comparative antigenic assay of soluble varicella skin test antigens assessed by direct method using zoster serum. Antigen H was the soluble control skin test antigen. The titres of the antigens in the RPHA test ranged from 1 : 4 to 1 : 8, and all antigens had enough reactivity to induce skin reaction (more than 15 mm in diameter). The value of the ELISA reading of the antigens ranged from 130 to 411 corresponding to a 1 : 4 titre in the RPHA test, and 548 and 795 for a 1 : 8 titre in the RPHA test. The values between the RPHA and ELISA tests were correlated. The ELISA test determined the amount of viral antigens in the skin test antigens more precisely than the RPHA test.

Table 3. Comparative antigenic assay of the soluble varicella skin test antigens

Antigens	A	B	C	D	E	F	G	H
Protein concentration (μg/ml)	18.1	24	16.8	10.2	11.5	13.1	9.6	6.4
RPHA	1 : 4	1 : 4	1 : 4	1 : 8	1 : 4	1 : 4	1 : 8	1 : <1
ELISA	384	411	130	795	176	250	548	29

Antigens A, B, C, D, E, F, G were the soluble varicella skin test antigens. Antigen H was the soluble control skin test antigen. Antigenic value in the ELISA test was determined by direct method. The value of the ELISA test was expressed as the absorbance × 1,000.

Discussion

The varicella skin test antigens were examined for their antigen content by the RPHA and ELISA tests. The soluble varicella skin test antigen with the RPHA titre of 1 : 4 or higher could induce skin reaction (Shiraki *et al.*, 1984). Although RPHA test is useful in evaluating the antigen content, it is not suitable for quantitative comparison of the antigenic value of each preparation because of the rather rough titration as shown in Table 3. This study was designed to quantify then antigen amount by ELISA. It is difficult to compare the antigen content of skin test antigens in a single assay because of their diversity in the antigenic form, but it is possible to compare antigen amount of each preparation by the same assay method.

The soluble varicella skin test antigen contains two major soluble viral glycoproteins, and this antigen can be assessed by direct method by zoster serum or monoclonal antibody. The crude varicella skin test antigen can be assessed by both the direct and sandwich methods. The modified skin test antigen may contain two major glycoproteins and the other viral proteins degraded from viral particles and infected cells by freezing and thawing. This antigen seems to be less efficient for ELISA but can be assessed by combination of the direct and sandwich methods. ELISA is technically far easier and cheaper than the RPHA test. Antigenic assay by ELISA makes it possible to compare quantitatively the amount of viral antigen of the soluble varicella antigens as shown in Table 3. ELISA test can be used for standardization of the amount of skin test antigen content.

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