BIKEN JOURNAL Vol. 27, 19–22, 1984

SHORT COMMUNICATION

DELAYED-TYPE HYPERSENSITIVITY AND IN VITRO LYMPHOCYTE RESPONSE IN GUINEA PIGS IMMUNIZED WITH A LIVE VARICELLA VACCINE

KIMIYASU SHIRAKI, KOICHI YAMANISHI and MICHIAKI TAKAHASHI

Department of Virology, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka 565, Japan

YOSHITANE DOHI

Department of Bacteriology, School of Medicine, Osaka University, Suita, Osaka 565, Japan

(Received February 7, 1984)

The relation of delayed type hypersensitivity (DTH) assessed by the Varicella-Zoster virus (VZV) skin test and lymphocyte transformation (LTF) with VZV antigen was investigated in guinea pigs immunized with live varicella vaccine virus, or heat-inactivated vaccine virus.

Guinea pigs immunized with live varicella vaccine virus showed positive DTH and LTF responses to viral antigen as well as a neutralizing (NT) antibody response, while those immunized with heat-inactivated vaccine virus showed only an NT antibody response of the same degree as that to live vaccine vrius. These results show the reliability of the skin test in assessing cell-mediated immunity (CMI) to VZV and the advantage of the live varicella vaccine over the inactivated one in immunizing guinea pigs.

It has been reported that the immune status of individuals with regard to clinical varicella can conveniently be determined by cell-mediated immunity assessed by the VZV skin test, and this test has been successfully applied to children who recieved a live varicella vaccine (Oka strain) for assessing their cellular immunity (Kamiya et al., 1977; Baba et al., 1978; Asano et al., 1981). It has also been found that guinea pigs are good experimental animals for use in studies on VZV infection (Yamanishi et al., 1980; Myers et al., 1980; Matsunaga et al., 1982). Antibody responses and skin reactive delayed-type hypersensitivity have been observed in guinea pigs inoculated with VZV adapted to guinea pig embryo cells. In this study, we investigated the correlation of DTH and LTF to VZV in guinea pigs immunized with a live varicella vaccine, and the difference in the immunologic responses to VZV with live and heat-inactivated varicella vaccine. Inbred guinea pigs (Yodo strain) were inoculated with live varicella vaccine virus (15,000 plaque forming units (PFU)/ml/

animal), or vaccine heat-inactivated at 56 C for 30 min. The antigens used in skin tests were a soluble antigen prepared from the culture fluid of VZV infected human diploid cells (Asano et al., 1981) and an antigen prepared from VZV-infected guinea pig embryo cells

TABLE 1. *Skin test reactivity to soluble skin test antigen and neutralizing antibody responses in immunized guinea pigs*

Immunization	Skin test reaction 24 h	Skin test reaction 48 h	Neutralizing antibody
A. Live vaccine			
No. 1	10×10 / —[a]	10×10 / —	1 : 16
No. 2	8×7 / —	8×6 / —	1 : 32
No. 3	15×15 / —	15×12 / —	1 : 16
No. 4	9×6 / —	7×7 / —	1 : 16
B. Heat-inactivated vaccine			
No. 1	— / —	— / —	1 : 32
No. 2	— / —	— / —	1 : 8
No. 3	— / —	— / —	1 : 16
No. 4	— / —	— / —	1 : 16
C. No immunization			
No. 1	— / —	— / —	<1 : 2
No. 2	— / —	— / —	<1 : 2
No. 3	— / —	— / —	<1 : 2
No. 4	— / —	— / —	<1 : 2

The skin test reaction is shown as (soluble varicella antigen) / (soluble control antigen)

Soluble skin test antigen prepared from human cells was used.

[a] The skin test reaction was <5 mm in diameter.

TABLE 2. *Lymphocyte transformation tests in immunized guinea pigs*

Immunization		Skin test reaction: human antigen[b]	Skin test reaction: G.P. antigen[c]	LTF (S.I.)[a]
A. Live vaccine				
No. 1	C.[d]	—[e]	—	1.44
	V.[d]	7×6	8×8	3.70
No. 2	C.	—	—	1.30
	V.	6×6	12×11	2.25
No. 3	C.	—	N.D.[f]	1.64
	V.	8×7		3.77
B. Heat-inactivated vaccine				
No. 1	C.	—	—	1.28
	V.	—	—	1.19
No. 2	C.	—	—	1.40
	V.	—	—	1.44
No. 3	C.	—	—	1.12
	V.	—	—	1.49
C. Heat inactivated vaccine with Freund's complete adjuvant				
No. 1	C.	—	—	1.12
	V.	6×6	10×8	2.00
No. 2	C.	6×7	—	0.90
	V.	6×6	12×12	3.49
No. 3	C.	—	—	1.09
	V.	7×8	12×11	4.43
D. No immunization				
No. 1	C.	—	—	1.06
	V.	—	—	1.17
No. 2	C.	—	—	1.56
	V.	—	—	1.63
No. 3	C.	—	—	1.21
	V.	—	—	1.14

[a] S.I. (stimulation index) is shown as the maximal value of $\left(\frac{^3\text{H-thymidine uptake in the presence of antigen}}{^3\text{H-thymidine uptake in the absence of antigen}}\right)$ at each dilution of antigen.

[b] Soluble skin test antigen prepared from human cells.

[c] Crude antigen prepared from guinea pig cells.

[d] C.: control antigen. V.: viral antigen.

[e] The skin test reaction was <5 mm in diameter.

[f] Not done.

(Matsunaga et al., 1982). Control antigens were prepared in the same manner from cells not infected with VZV. Their antigenic titers were 1: 8 in the reversed passive hemagglutination test (Shiraki et al., 1984) and in the complement fixing test respectively. Erythematous change of more than 5 mm in diameter 48 h after intradermal inoculation of the antigen was taken as a positive skin reaction. Infiltration of predominantly mononuclear cells into the site of a positive skin reaction was observed on histological examination (Shiraki et al., 1984).

The NT antibody titer was assayed as described previously (Asano and Takahashi, 1978).

The LTF test was performed by a modification of the whole blood microculture technique described by Pauly et al. (1976) and Kumagai et al. (1980). Heparinized whole blood obtained by heart puncture was diluted 1: 15 with RPMI 1640 and 1.5 ml of diluted blood was distributed into 24 wells microplates. Then 100 μl of diluted viral or control antigen prepared from syngeneic guinea pig cells or RPMI 1640 was added to each well and the plates were incubated at 37 C in a CO_2 incubator for 6 days. The cultures were labeled with ^{3}H-thymidine 2 μCi/well (97 Ci/mmol, Amersham) for the last 24 h.

The cultures were then mixed with 100 μl of 1% Nonidet P-40, filtered on glass filters and washed first with phosphate buffered saline, then with 5% trichloroacetic acid and finally with ethanol. Then the radioactivities on the glass filters were measured. The relatively long incubation period of 6 days was needed to evaluate the LTF response.

Guinea pigs were inoculated with live or heat-inactivated vaccine virus, and the skin test and serum assay were carried out 3 weeks later. As shown in Table 1, the cutaneous reaction was positive in guinea pigs inoculated with live virus, but not with heat-inactivated virus, whereas comparable NT antibody responses were observed in guinea pigs inoculated with live and inactivated vaccine virus.

Next, the skin test and LTF test were done in 3 groups of 3 guinea pigs each (Table 2): The first group was inoculated with live vaccine virus, and the second with heat-inactivated vaccine virus, while the third group was not inoculated. The skin test and LTF test were performed 2 weeks after inoculation.

Table 2 shows the results of the skin test and LTF test. Guinea pigs inoculated with live varicella vaccine virus gave positive reactions in the skin test and LTF test with viral antigen but not with control antigen, taking a stimulation index of more than 2 as a positive LTF response. Guinea pigs inoculated with heat-inactivated varicella virus or untreated animals did not give a positive skin test or LTF test with either viral or control antigen. Despite the similarity in the humoral antibody responses to live and heat-inact vated viruses, the CMI responses to the two were different; live varicella virus induced CMI but heat-inactivated varicella virus did not. Possibly when live varicella virus is inoculated, it replicates in guinea pigs, and so can be recognized by specific immunocompetent cells responsible for CMI to VZV.

REFERENCES

Asano, Y., Shiraki, K., Takahashi, M., Nagai, H., Ozaki, T., Yazaki, T. 1981. Soluble skin test antigen of varicella-zoster virus prepared from the fluid of infected cultures. J. Infect. Dis. 143: 684–692.

Asano, Y., Takahashi, M. 1978. Studies on neutralization of varicella-zoster virus and serological follow-up of cases of varicella and zoster. Biken J. 21: 15–23.

Baba, K., Yabuuchi, H., Okuni, H., Takahashi, M. 1978. Studies with live varicella vaccine and inactivated skin test antigen: Protective effect of the vaccine and clinical application of the skin test. Pediatrics 61: 550–555.

Kamiya, H., Ihara, T., Izawa, T., Yamada, A., Takahashi, M. 1977. Diagnostic skin test reaction with varicella virus antigen and clinical application of the test. J. Infect. Dis. 1977: 784–788.

Kumagai, T., Chiba, Y., Wataya, Y., Hanazono, H., Chiba, S., Nakao, T. 1980. Development and characteristics of the cellular immune response to infection with varicella-zoster virus. J. Infect. Dis. 141: 7–13.

Matsunaga, Y., Yamanishi, K., Takahashi, M. 1982. Experimental infection and immune response of guinea pigs with varicella-zoster virus. Infect. Immun. 37: 407–412.

Myers, M. G., Duer, H. L., Hausler, C. K. 1980. Experimental infection of guinea pigs with varicella-zoster virus. J. Infect. Dis. 142: 414–420.

Pauly, J. L., Han, T. 1976. Whole blood microculture assay of human lymphocyte function. J. Lab. Clin. Med. 88: 864–872.

Shiraki, K., Yamanishi, K., Takahashi, M. 1984. Biological and immunological characterization of varicella-zoster virus (VZV) skin test antigen. J. Infect. Dis. in press.

Yamanishi, K., Matsunaga, Y., Otsuka, T., Takahashi, M. 1980. Immune response of guinea pigs to varicella vaccine strain (Oka) and wild strains. Biken J. 23: 53–55.