BIKEN JOURNAL Vol. 27, 23–29, 1984

SHORT COMMUNICATION

A SIMPLIFIED IMMUNOFLUORESCENCE TECHNIQUE FOR ANTIBODY TO VARICELLA-ZOSTER MEMBRANE ANTIGEN (FAMA)

KOICHI BABA, MORIATSU YOSHIDA, AKIO TAWA and HYAKUJI YABUUCHI

Department of Pediatrics, School of Medicine, Osaka University, Fukushima-ku, Fukushima, Osaka 553, Japan

KAZUHIRO MAEDA and MICHIAKI TAKAHASHI

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

(Received February 4, 1984)

Indirect fluorescent antibody to varicella-zoster membrane antigens (FAMA) was measured by a new technique. The procedure gives rapid, sensitive and accurate results and is suitable for use in diagnosis or screening of susceptibility to varicella-zoster virus (VZV) infection. The test procedure was simplified by using Terasaki tissue culture plates for the reaction and for direct observation by fluorescence microscopy. Preparations of VZV-infected Vero cells stored in liquid nitrogen could be used as antigen in this FAMA-test.

Rapid determination of susceptibility to varicella-zoster virus (VZV) is essential when subjects at high risk of severe varicella are included in a population in which an index case is diagnosed as having varicella or herpes-zoster. An outbreak of virus in such a community produces a crisis, with the prospect of increased morbidity and mortality (Weller, 1983). Prompt use of a live varicella vaccine in such a community is effective for reducing the size of the outbreak (Baba et al. 1978; Asano et al. 1982).

Routine use of some sensitive test for antibody such as the FAMA (fluorescent antibody to membrane antigen) test has long been required for screening for susceptibility. This test, is not however, generally available as a routine test, like the complement fixation (CF) test, because it requires many troublesome procedures, such as preparation of VZV-infected cells for each test. In this work, VZV-infected cells stored in liquid nitrogen could be used as antigen in the FAMA-test. We used Terasaki tissue culture plates (60 flat bottom wells, Falcon, 1950 Williams Dr., Oxnard, Ca., 93030) as a site for reaction of antibody with membrane antigens of cells infected with VZV. Moreover, the cells could

be stained on the plate, and examined directly in a Nikon inverted microscope model TMD, fitted with an Epi-fluorescence attachment and a B excitation module which contains a 470–490 nm band pass excitation filter, a 520 nm long pass barrier filter and a 510 nm dichroic mirror.

Monolayers of human embryonic lung fibroblasts (HEL) and Vero cells were propagated in medium (a mixture of equal volumes of Eagles' MEM and 199 medium containing 400 units of penicillin/ml and 200 mg of streptomycin/ml, and 10% heat-inactivated fetal calf serum). Medium with 2% fetal calf serum was used for cell maintenance. Fibroblasts infected with the Kawaguchi strain of VZV were placed on fresh monolayers of Vero or HEL cells. The cultures were maintained for about 48 h until a CPE (cytopathic effect) was observed in about 80% of the monolayer. Then the monolayers were beaded off into Hanks' balanced salt solution (HBSS), pH 7.4, and centrifuged at 1,000 *g* for 5 min. The supernatant was discarded and the pelleted cells were resuspended at a density of over $\geq 2 \times 10^6$ cells/ml in HBSS supplemented with 10% fetal calf serum plus 7% DMSO (dimethyl sulfoxide). Aliquots of 1.0 ml of cell suspensions were stored in liquid nitrogen until use. Noninfected cells, prepared in a similar manner, were used as controls. Aliquots of 0.005 ml of test sera diluted in microtiter plates (U plates, Cooke Engineering, Alexandria, VA) were transferred to Terasaki tissue culture plates and incubated with an equal volume of VZV-infected cell suspension in a humidified atmosphere. The plastic plates were dipped into 180 ml of HBSS to remove the supernatant of the cells adhering to the bottom of the plastic. The washed cells were incubated at 37 C for 30 min with 0.005 ml per well of a dilution of fluorescein-conjugated goat antisera to heavy-chain human IgG, IgA and IgM (Meloy Chemical Co., Springfield, VA). The plates were dipped into HBSS to remove the remaining conjugates from the cells, and then the cells were examined in a fluorescence microscope for fluorescent antibody to VZV. In each test run, coded preparations including dilutions of known positive and known negative sera were examined as additional controls.

Fluorescent cells obtained by the specific reaction of antibody with membrane antigens of cells infected with VZV were seen as cells with bright fluorescent rings around their surface adhering to the plates. No fluorescence was observed, when infected cells were treated with conjugate but not antibody or when uninfected cells were examined. No autofluorescence of uninfected or infected cells was observed. Findings were similar with HEL cells and with Vero cells. A positive or negative reaction for specific antibody could be judged by examining hundreds of cells in one field of the fluorescence microscope (300×), as seen in Fig. 1.

The neutralization (NT) test was performed as described previously (Baba et al. 1978). Briefly, the Kawaguchi strain of varicella virus containing approximately 200 PFU/0.1 ml was mixed with equal volumes of serial dilutions of the test serum. After incubation at 37 C for 30 min, 0.2 ml portions of the mixtures were placed on HEL monolayers in plastic trays (Linbro, Hamden, Conn.) of 35 mm diameter and incubated at 37 C for 90 min to allow absorption of the virus. Then fresh medium was added, and the cultures were incubated at 37 C for five to six days until viral lesions were large enough to be counted. Neutralizing antibody titers were expressed as the highest dilution of the serum producing 50% or more reduction in the focal count observed with virus control.

The immune adherence hemagglutionation (IAHA) test was carried out by a modification of the technique of Gershon et al. (1976). Briefly, serial dilutions of heat-inactivated serum were made in Linbro microtiter U plates (0.025 ml/well). Dilutions were made in duplicate for VZV antigen and the antigen control (Flow Laboratories, Inc. U.S.A.) with 0.1% gelatin in veronal buffer, pH 7.4. Vol-

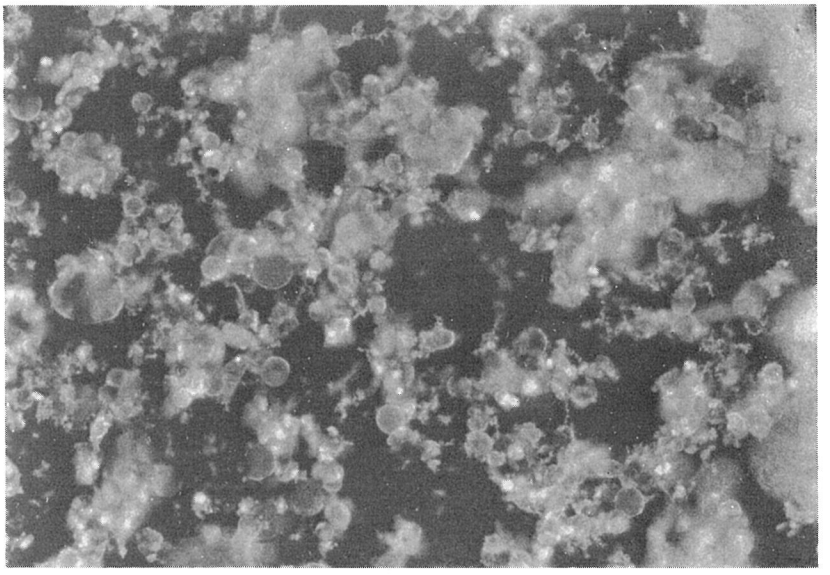

FIGURE 1. Fluorescent positive Vero cells for antibody against varicella-zoster membrane antigen (×300).

umes of 0.025 ml of VZV antigen or antigen control were added to dilutions of serum. Plates were placed on a microshaker for 10 sec and then incubated for 1 h at 37 C. Subsequently, 0.025 ml of guinea pig complement was added, usually at a dilution of 1: 100. The plates were agitated for 10 sec and then incubated for 40 min at 37 C. DTT-VB-EDTA (0.025 ml, consisting of 1.5 g of dithiothreitol in 500 ml of 0.04 *M* EDTA in veronal buffer without gelatin) was added to all wells and the plates were shaken for 10 sec. Then a 1.5% suspension of human 0-type RBC in GVB (0.025 ml) was added to each well, and the plates were mixed to allow development of hemagglutination. The serum specimens used in this work were selected from those of 95 subjects obtained before, during and after 4 epidemics of varicella over a 6-year serveillance period in an institution.

The reactivities of VZV-infected HEL or Vero cells to antibody in sera obtained in the acute to convalescent phases of varicella infection were compared for class-specific antibody titers to VZV. The individual and geometric mean titers obtained with either the HEL or Vero cell system were not significantly different despite differences in the phases when sera were collected (Table 1). Vero cells were used as indicator cells in following experiments because they were readily available and were easy to maintain.

Serial dilutions of 10 representative sera from the previous experiment were incubated with different concentrations of cells at different temperatures (Table 2). The individual and geometric mean titers indicated that the reaction was most sensitive when carried out with 8×10^3 cells per well at 37 C. Use of 32×10^3 per well or more appeared to result in some false negative reactions; the reason for this is unknown but a presumably similar phenomenon has been described previously (Williams et al., 1974).

Twenty replicate tests on the same serum (No. 7 in Table 1) were performed to compare the re-producibility of the FAMA test with those of the NT and IAHA test (Fig. 2). The coefficient of variation (CV), i.e., the ratio of the standard deviation (SD) to the geometric

TABLE 1. *Reactivities of HEL and Vero cells infected with varicella-zoster virus to antibodies*

Serum No.	Days after onset	Acute stage sera incubated with					
		HEL cells infected with VZV			Vero cells infected with VZV		
		Ig G	Ig A	Ig M	Ig G	Ig A	Ig M
1.	2	4	2	<2	4	2	<2
2.	5	8	4	4	8	4	4
3.	5	32	4	4	32	4	4
4.	6	64	8	<2	64	8	2
5.	7	32	4	4	64	8	4
6.	7	256	32	8	128	32	8
7.	7	256	16	4	256	16	4
8.	7	128	16	8	256	16	4
Mean±SD	5.8±1.6	42.3±4.3	7.5±2.5	4.0±1.6	48.5±4.3	8.0±2.3	3.7±1.5

Incubation was done for 60 min at 37 C.

TABLE 2. *Sensitivities of the FAMA (fluorescent antibody to membrane antigens) test with different numbers of VZV-infected Vero cells at different temperatures*

VZV-infected Vero cells/well	8×10^3	8×10^3	8×10^3	16×10^3	32×10^3	64×10^3
Incubated temperature	4 C	20 C	37 C	37 C	37 C	37 C
Serum No.						
2.	<2	4	8	8	<2	<2
3.	4	32	32	32	<2	<2
4.	16	32	64	64	<2	<2
8.	64	256	256	128	128	<2
9.	64	128	128	128	<2	<2
10.	32	128	256	128	128	<2
11.	128	128	512	512	512	<2
13.	64	64	256	256	256	<2
14.	32	128	128	256	128	<2
16.	32	64	64	64	<2	<2
GMT±SD	26.0±3.5	64.0±3.0	104.0±3.2	97.0±3.0	19.7±10.6	<2

Incubation was done for 60 min.
Serum numbers correspond to those of Table 1.

mean titer (GMT) multiplied by 100, was 1.1% in the FAMA test, 2.5% in the NT test and 0.3% in the IAHA test, indicating that the expected reproducibility of this FAMA test is moderate and that results are comparable even when obtained in different test runs.

The serological responses of patients after the onset of varicella were examined by three methods as shown in Fig. 3. The antibody responses detected by the FAMA test and the

in sera of patients in actute to convalescent phases of varicella infection

Serum No.	Days after onset	Convalescent stage sera incubated with					
		HEL cells infected with VZV			Vero cells infected with VZV		
		Ig G	Ig A	Ig M	Ig G	Ig A	Ig M
9.	10	256	16	4	128	16	4
10.	14	512	16	8	256	64	8
11.	15	128	8	8	512	16	8
12.	21	256	16	4	512	16	8
13.	28	64	8	2	256	16	4
14.	28	128	8	4	128	8	4
15.	35	128	16	4	128	8	4
16.	40	64	8	2	64	8	2
Mean±SD	23.9±10.0	157.6±2.0	11.3±1.4	4.0±1.6	194.0±2.0	14.9±1.9	4.9±1.6

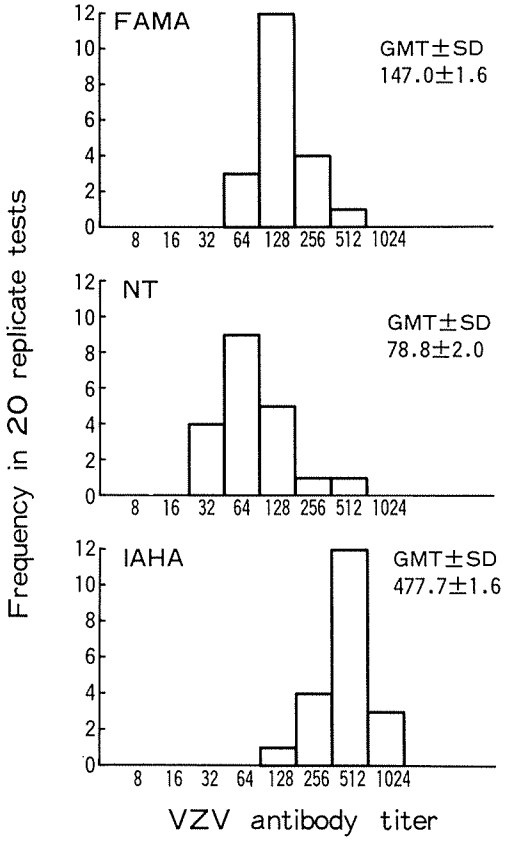

FIGURE 2. Distribution and frequency of antibody titers of the same serum obtained in 20 replicate tests by each of the indicated methods.

NT test were remarkably similar; the responses reached a peak within 2 to 3 weeks after the onset of a rash, and specific antibody persisted at a moderate level for over 6 months. In contrast, IAHA antibody showed a rapid rise within one week and rapid decline within 2 months after the onset of varicella infection. These findings suggest that FAMA antibody remains high during the convalescent phase and may persist at a comparable level to NT antibody for a long time.

The standardized technique for the FAMA test was as follows: aliquots of 0.005 ml of test sera diluted in microtiter plates were transferred to Terasaki tissue culture plates and incubated with an equal volume of VZV-infected Vero cells (8×10^3/well) for 60 min at 37 C in a humidified atmosphere. The cells were washed by dipping the plate in HBSS and then stained with 0.005 ml of a dilution of fluorescein-conjugated antiserum and examined for FAMA antibody to VZV.

This technique requires only a small quantity (0.005 ml) of serum for detection of each class-specific antibody to VZV. Vero cells are easier to grow and maintain than HEL cells. Storage of infected Vero cells in liquid nitrogen did not appear to decrease their ability to adhere to the flat bottom of Terasaki tissue culture plates and did not appear to in-

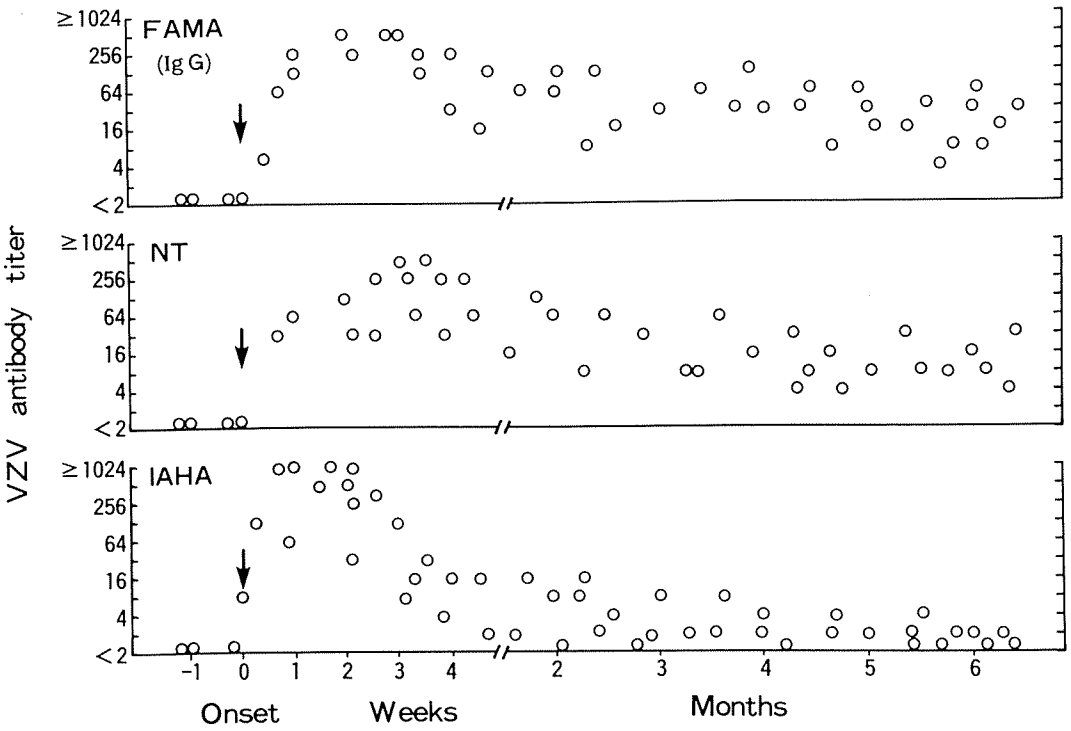

FIGURE 3. Antibody titers of individuals who developed varicella obtained by the three indicated methods.

crease their nonspecific fluorescent reactions. By dipping the whole plate into HBSS, the process of centrifugation for washing cells was avoided. Direct examination of the cells on plates with a fluorescence microscope does not involve troublesome techniques, such as sealing cells under a coverglass with nail polish. In addition, it is easier to judge whether the serum dilution is positive or negative for antibody by observation of cells adhering to the surface of the plastic plate than by observation of cells suspended on a slide glass, as done in another technique (Williams, 1974).

The method described is simple, rapid, accurate and easy enough for routine use in diagnosis of VZV infection or screening for susceptibility to VZV infection.

REFERENCES

Asano, Y., Hirose, S., Iwayama, S., Miyata, T., Yazaki, T., Takahashi, M. 1982. Protective effect of immediate inoculation of a live varicella vaccine in household contacts in relation to the viral dose and interval between exposure and vaccination. Biken J. 25: 43–45.

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

Gershon, A. A., Kalter, Z. C., Steinberg, S. 1976. Detection of antibody to varicella-zoster virus by immune adherence hemagglutination. Proc. Soc. Exp. Biol. Med. 151: 762–765.

Weller, T. H. 1983. Varicella and herpes-zoster:

Changing concepts of the natural history, control, and importance of not-so-benign virus. N. Engl. J. Med. 309: 1434–1440.
Williams, V., Gershon, A. A., Brunnel, P. A. 1974. Serologic response to varicella-zoster membrane antigens measured by indirect immunofluorescence. J. Infect. Dis. 130: 669–672.