Enzyme-Linked Immunosorbent Assay for Detection of Antibody to Varicella-Zoster Virus

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Primary varicella-zoster virus (VZV) infection is a serious illness in immunocompromised individuals, and a rapid, sensitive, and reliable assay to identify high-risk VZV-susceptible patients would be clinically useful. An enzyme-linked immunosorbent assay (ELISA) for antibody to VZV was compared with the fluorescent antibody-to-membrane antigen (FAMA) assay and found to be similar in both sensitivity and specificity. The antibody titers determined by both assays were also similar. The absence of antibody detected by ELISA correlated with susceptibility to VZV infection. Because it is simple to perform and has equivalent sensitivity to FAMA, ELISA should be useful for VZV antibody testing in diagnostic and research laboratories.

Primary infection with varicella-zoster virus (VZV) is a serious and often fatal illness in immunocompromised individuals (5, 6, 13). Nosocomial outbreaks involving both hospital staff and patients often result in the exposure of a large number of high-risk individuals whose immune status to VZV is unknown (11, 12). Since the presence of serum antibody to VZV correlates with immunity to infection (2, 15), a rapid and reliable serological test for antibody to VZV would be very useful. By identifying people susceptible to VZV infection, such an assay would permit efficient institution of passive antibody therapy for immunocompromised patients and allow appropriate segregation procedures during nosocomial outbreaks to prevent secondary spread of infection (1).

Although several serological assays for VZV antibody have been developed, inherent difficulties in each of these methods have limited their usefulness for the routine diagnostic evaluation of immunity to VZV. For example, both classical virus neutralization and complement fixation assays have proven relatively insensitive for detecting individuals previously infected with VZV (3, 4, 20). The immune adherence hemagglutination assay (IAHA), although more sensitive than complement fixation, may yield a significant number of false-negative results (7). Moreover, cross-reacting antibodies to other herpesviruses have been noted with IAHA, virus neutralization, and complement fixation assays (18, 22). Fluorescent antibody-to-membrane antigen (FAMA), complement-enhanced neutralization, and radioimmunoassay (RIA) are considered the most specific and sensitive methods for detecting antibody to VZV (8, 9, 16, 21).

However, all of these assays are technically difficult, and RIA has the added disadvantages of requiring expensive equipment and presenting a radiation hazard.

The enzyme-linked immunosorbent assay (ELISA) is a rapid, sensitive, and reliable method of assaying antibody to a variety of antigens. Recently, an ELISA for antibody to VZV has also been reported, and results have been comparable to complement-enhanced neutralization, complement fixation, and IAHA for detection of immunoglobulin G (IgG) (7). The results of this study demonstrate that ELISA is also comparable in sensitivity to FAMA for detection of antibody to VZV. The absence of antibody detectable by ELISA appears to correlate with susceptibility to VZV.

MATERIALS AND METHODS

Tissue culture. Fibroblasts (FS-350Q), derived from human newborn foreskin, were utilized in passage 13 to 25. Cultures were grown in Eagle basal medium (BME) supplemented with 10% heat-inactivated (56°C for 30 min) fetal bovine serum, 50 U of penicillin per ml, and 50 µg of streptomycin per ml and maintained with BME containing 5% fetal bovine serum plus penicillin and streptomycin. Media for cells seeded into slide chambers for the performance of FAMA also contained 1 µg of amphotericin B per ml.

Virus. The strain of VZV (1294) used for these studies was isolated from the vesicle fluid of a normal child with uncomplicated varicella. A seed pool of this strain, passage 7 in FS-350Q, was maintained in liquid nitrogen in BME containing 20% fetal bovine serum and 8% dimethyl sulfoxide. Infected whole cells were utilized for the subculture and cryopreservation of virus (14, 17). For subculture, infected cells were inoculated at a ratio of approximately one infected to three uninfected cells.

Sera. A total of 144 sera were obtained from normal and immunocompromised individuals participating in studies of varicella (13, 19). In this group, 21 cases of clinical varicella developed. Five of these were further verified by seroconversion, using both assay systems. As a positive serological control, a 14-day convalescent plasma (ZIP) from an otherwise healthy 18-year-old woman with zoster was evaluated in each assay procedure. This serum is negative for antibody for herpes simplex virus types 1 and 2 and cytomegalovirus (13). All sera were heat inactivated at 56°C for 30 min before testing. Initial screening of sera was at a 1:4 or 1:5 dilution for FAMA and ELISA, respectively. Subsequent serial twofold dilutions were performed on sera initially diluted 1:5.

VZV antibody determination. Antibody to VZV membrane antigen was assayed by a previously reported (13) modification of FAMA (21). Trypsinized monolayers of FS-350Q mixed with VZV-infected cells were seeded into tissue culture chamber slides. After 24 h, unfixed cells were exposed to the serum samples diluted in phosphate-buffered saline (PBS) and then incubated for 30 min at 37°C with fluorescein-conjugated goat antiserum to human immunoglobulins (Cappel Laboratories, Cochranville, Pa., lot 10460). The fluorescein conjugate was absorbed with mouse liver and FS-350Q and was utilized at a previously determined optimal dilution of 1:20 to 1:40 in PBS. Slides were mounted in glycerol buffer (pH 8.5) and inspected for membrane fluorescence. All samples were encoded for evaluation.

For ELISA, VZV antigen was prepared from 32-ounce (ca. 960-ml) bottles of FS-350Q at 72 h after infection (14). Control antigen was simultaneously prepared from uninfected cells. The virus-infected and control monolayers were each washed in Hanks basic salt solution (HBSS) and then scraped into 20.0 ml of HBSS. Cells were centrifuged at $250 \times g$ for 20 min at 4°C and resuspended in 2.5 ml of HBSS. With a Branson sonifier (model W140) fitted with a microtip, the cell suspension maintained on ice was sonically disrupted three times for 30 s at a power setting of 4. The sonicate was clarified at $250 \times g$ for 20 min at 4°C, and the supernatant fluid was stored at -20°C for use as antigen. The optimal dilution of antigen to be used in further serological testing was determined by block titration, using a ZIP as well as a normal serum. Control antigen was utilized in each assay at the same concentration used for VZV antigen.

ELISA was performed by a method similar to that of Forghani et al. (7). Briefly, polystyrene microtiter ELISA plates (Dynatech Laboratories, Windham, Maine) were labeled with 200 μ l of VZV and control antigens diluted in 0.06 M bicarbonate buffer, pH 9.6, for 24 h at 4°C. Plates labeled in this fashion could be stored for up to 3 months at 4°C (data not shown). After storage, the plates were washed with bicarbonate buffer (300 μ l per well), incubated for 30 min at 37°C, and then washed three times with PBS containing 0.05% Tween 20 (PBST). Test sera were diluted with PBST and incubated (200 μ l per well) at 37°C for 45 min. Wells were washed three times with PBST and then incubated with the previously determined optimal dilution (1:300) of horseradish peroxidase-conjugated antiserum to human IgG heavy and light chains (Cappel Laboratories) for 30 min at 37°C. After an additional four washes with PBST, enzymatic activity was

assayed with freshly prepared *o*-phenylenediamine (1 mg/dl) and 0.003% hydrogen peroxide in distilled water. After 30 min of incubation in the dark at room temperature, the reaction was terminated by the addition of 25 μ l of 8 N H₂SO₄ to each as well. Optical density was measured at 490 nm after a 1:3.5 dilution in PBST.

Statistical methods. Both ELISA and FAMA assays were performed by using encoded samples. Comparison of assay methods was by the least mean square method, and the correlation of titers was by linear regression.

RESULTS

ELISA reaction for sera lacking antibody to VZV. To establish reliable criteria for the ELISA antibody assay in identifying sera containing antibody to VZV, the variations in colorimetric reactions of sera lacking VZV antibody detectable by FAMA were examined. A total of 101 sera with VZV antibody titers of <1:4 by FAMA were evaluated by ELISA reaction, using both the VZV and control antigens. The absorbance readings of these serum samples diluted 1:5 were determined. The absorbance (mean $\pm$ standard deviation) for sera reacted with VZV antigen was 0.158 ± 0.085 and for control antigen was 0.124 ± 0.073 . An absorbance value of ≤ 0.30 was selected as the optical density to identify sera lacking antibody to VZV antigen. Similarly, sera with an absorbance of >0.266 against control antigen were invalid due to nonspecific reaction. With these criteria, only 3 of 101 sera would have been incorrectly categorized as containing antibody for VZV, and 9 of 101 sera were invalidated because of nonspecific reactions to control antigen.

Comparison of ELISA versus FAMA. A total of 144 sera were evaluated for antibody to VZV by both FAMA and ELISA. Table 1 summarizes the results of these studies. A total of 99 sera were negative by both assays, and 38 sera contained antibody by both assays. Four sera were negative by ELISA but positive by FAMA, and three individuals were positive by ELISA but negative by FAMA. Assuming that FAMA accu-

TABLE 1. Detection of antibody to VZV in 144 sera by ELISA and FAMA

ELISA	No. of sera with antibody by FAMA	
	Positive ^a (42)	Negative (102)
Reactive ^b	38 ^c	3
Nonreactive	4	99 ^d

^a Titer $\geq 1:4$. Total number of sera is shown within parentheses.

^b Titer $\geq 1:5$.

^c Five patients who had household exposures to VZV did not develop varicella.

^d Twenty patients developed varicella after exposure to VZV.

FIG. 1. Comparative evaluation of sera for immunoglobulin activities to VZV virus by ELISA and FAMA. The linear regression line determined for antibody-positive sera ($r = 0.86$) indicates close agreement of these two systems.

rately reflected the presence of VZV antibody in these 144 sera, ELISA was 90% sensitive and 97% specific.

To further test the specificity of ELISA and FAMA, ZIP that was reactive at a dilution of 1:64 in both tests was absorbed three times with either VZV-infected or control FS-350Q cells at 37°C for 30 min. After absorption, the serum exposed to VZV-infected cells had become negative by both FAMA and ELISA, whereas the sera treated with control cells remained reactive in both assay systems.

To compare antibody titers determined by ELISA and FAMA, endpoint titrations of 19 sera reactive in both assay systems were performed to examine the antibody determinations of the two assay methods. As Fig. 1 illustrates, there was a close correlation of ELISA and FAMA antibody titers ($r = 0.86$).

Clinical correlation. Of the 98 patients determined to be seronegative by ELISA and FAMA, 20 subsequently developed clinical varicella. Paired acute and convalescent sera were available from five of these patients, and seroconversion was demonstrated by both assay systems. Of the 33 ELISA-positive, FAMA-positive sera, there were 4 household VZV exposures and no subsequent clinical varicella.

DISCUSSION

Although VZV serology has been performed by a variety of methods, virus neutralization with complement enhancement, FAMA, and RIA assays have to date yielded the most sensitive results. Although these assays are thought

to correlate well with immunity to VZV, they are time consuming, technically difficult, and require specialized virological support facilities. In addition, RIA requires the use of radioactive materials. A practical, sensitive, and reliable assay for antibody to VZV is needed for rapid assessment of immune status to VZV. Our findings and the studies of others suggest that ELISA fulfills these criteria. Forghani and co-workers previously reported that ELISA for IgG antibody to VZV correlated closely to complement-enhanced virus neutralization but yielded somewhat higher titers (7). In addition, ELISA appeared more sensitive than either complement fixation or IAHA and yielded fewer heterotypic reactions to other herpesviruses. Hachem et al. recently reported that ELISA compared favorably with FAMA for detection of IgM antibody to VZV, but the former yielded somewhat higher titers (10). Our study demonstrated that ELISA for immunoglobulin to VZV also compares favorably with FAMA in both specificity and sensitivity in identifying sera containing antibody to VZV. Moreover, ELISA yielded antibody titers similar to those derived by FAMA.

There were seven discrepancies between the two assays. Three sera were reactive by ELISA but not by FAMA, and four sera were FAMA reactive but negative by ELISA. It is not clear whether these differences represent differences in the antigens detected by the two assays or represent the limits of sensitivity of each assay system.

Previous studies have indicated that antibody detectable by FAMA correlates with immunity to VZV. The clinical information available in this study indicates that seronegativity by both assay methods correlates with susceptibility to VZV infection. Too few individuals were available to confirm whether antibody detectable by ELISA might be protective, but the correlation with FAMA suggests that this occurs.

The utility of any serological assay depends upon its sensitivity and specificity which, in turn, are reflections of the criteria established to identify reactive and nonreactive sera. The data presented in this study represent information derived as ELISA was implemented in our laboratory. At the inception of this assay, we noted that 9% of serum samples were eliminated due to nonspecific reactions to control antigen. This situation is analogous to the anti-complementary activity of sera seen in the complement fixation assay and does not affect test sensitivity or specificity. As ELISA has subsequently been performed in our laboratory employing a multi-channel automated washer, this nonspecificity has diminished. Thus, none of the last 70 sera evaluated showed nonspecific reactions to control antigen.

A number of unique advantages of ELISA make it a candidate for use in diagnostic laboratories. ELISA reagents can be easily prepared in the clinical laboratory or may be commercially available. Once prepared and standardized, these reagents can be stored for long periods of time. The antibody assay can be run in as little as 3 h, employing no elaborate equipment or unnecessary exposure to radiation. In addition, the test system can accommodate a large number of samples, and automated systems are available. All of these qualities lend themselves for use in both hospital and epidemiological settings.

ELISA for immunoglobulin to VZV is a sensitive and specific assay which compares favorably with complement-enhanced virus neutralization and FAMA. The ease of performance and preparation of reagents are additional advantages which should permit testing of antibody to VZV in diagnostic and research settings.

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