JOURNAL OF CLINICAL MICROBIOLOGY, May 1984, p. 606–609
0095-1137/84/050606-04$02.00/0

Vol. 19, No. 5

Antibody Class Capture Assays for Varicella-Zoster Virus

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Received 22 December 1983/Accepted 7 February 1984

Pooled monoclonal antibodies to varicella-zoster virus (VZV) were used as "detector" antibodies in a four-phase enzyme immunofluorescence assay for determination of immunoglobulin M (IgM), IgA, and IgG antibodies to VZV. Polyclonal antisera specific for heavy chains of human IgM, IgA, and IgG were employed as "capture" antibodies on the solid phase. The antibody class capture assay (ACCA) for VZV IgM antibody detected high titers of virus-specific IgM in all patients with varicella and in 5 of 10 zoster patients. VZV IgM antibody was not detected in patients with primary herpes simplex virus infections or in other individuals without active VZV infection, with one exception, a patient with encephalitis who had other serological findings compatible with a reactivated VZV infection. VZV-specific IgA and IgG antibody titers demonstrable by ACCA were compared with those measured by solid-phase indirect enzyme immunofluorescence assay (EIFA). VZV IgA antibody titers detected in patients with varicella and zoster were variable and could not be considered to be reliable markers of active VZV infection. IgA antibody titers detected by ACCA tended to be higher than those demonstrated by solid-phase indirect EIFA in varicella and zoster patients. VZV IgG antibody titers detected by ACCA in patients with varicella, and to a lesser extent in zoster patients, were as high as or higher than those demonstrated by solid-phase indirect EIFA. However, ACCA was totally insensitive in detecting VZV IgG antibody in individuals with past infections with VZV and would not be a suitable approach for determination of immunity status to VZV.

In recent years methods for assay of virus-specific immunoglobulin M (IgM) antibody have been advanced which are based on the "capture" of IgM from the test serum by mu chain-specific antibody on a solid phase; virus-specific IgM antibody is detected by subsequent addition of viral antigen followed by enzyme- or radiolabeled viral IgG antibody (5, 10) or by labeled viral antigen (16, 17). This approach effectively separates IgM from IgG antibodies and some inhibitors and has overcome some of the problems with low sensitivity and specificity that have been encountered in viral IgM antibody assays employing viral antigen bound to the solid phase (3). In the present report we describe a four-phase "capture" assay for IgM antibody to varicella-zoster virus (VZV) which is based on the use of a pool of monoclonal antibodies to VZV (9) as detector antibodies. In addition, we compared the sensitivity of antibody class capture assays (ACCA) with that of solid-phase indirect enzyme immunofluorescence assays (EIFA) for detection of VZV antibody of the IgA and IgG classes.

MATERIALS AND METHODS

Immune reagents. The production and serological reactivities of VZV monoclonal antibodies have been described previously (9). The pool of monoclonal antibodies used as detector antibodies in the capture assays consisted of five antibodies (mouse ascitic fluids) with specificities for different glycoproteins of VZV (B. Forghani, K. W. Dupuis, and N. J. Schmidt, submitted for publication). Unlabeled heavy chain-specific goat antisera to human IgM, IgA, and IgG used for ACCA were from Cappel Laboratories, Cochranville, Pa. Alkaline phosphatase-conjugated heavy chain-specific goat antisera to human IgA and IgG used in the solid-phase indirect EIFA were from Miles Laboratories, Elkhart, Ind. Goat anti-mouse immunoglobulin was from Antibodies, Inc., Davis, Calif., and was labeled with alkaline phosphatase as previously described (7, 8). The optimal working dilution of each immune reagent for use in ACCA or in solid-phase indirect EIFA was determined by preliminary block titration.

Antigens. VZV antigens were prepared from infected human fetal diploid lung cell cultures harvested when they showed a 4-plus viral cytopathic effect. The cells were dislodged into the fluids by shaking with glass beads and then sedimented by centrifugation at 700 × *g* for 30 min. The supernatant fluid was removed and saved, the cells were resuspended in 1/10 of the original culture volume of the fluid and frozen and thawed three times, and the cell lysate was clarified by centrifugation at 700 × *g* for 30 min. The remaining infected culture fluid was then added back to the clarified lysate. Control antigen was prepared in parallel from uninfected cell cultures. Antigen prepared in this manner was used for ACCA and for most of the solid-phase indirect EIFA tests for IgA and IgG antibodies. Some EIFA tests were performed with antigens produced from lysates of infected cells as described previously (7); comparable results were obtained with the two types of antigen preparations. Optimal working dilutions of antigen were determined by block titrations against human sera known to be positive or negative for VZV antibodies. The working dilution of antigen used in ACCA was 1:1 or 1:10, and the working dilutions of antigens in solid-phase indirect antibody assays were 1:200 or 1:1,000, depending on the type of antigen preparation.

Solid-phase indirect EIFA. Viral IgA and IgG antibody assays employing antigen in the solid phase were performed as described previously (7), using alkaline phosphatase-labeled antibodies to the heavy chains of human IgA or IgG and 4-methyl umbelliferyl phosphate as a fluorogenic substrate (8).

ACCA. Cups in microtiter plates (Immulon II round bottom; Dynatech Laboratories, Inc., Alexandria, Va.) were sensitized by the addition of goat antibodies specific for the heavy chains of human IgM, IgA, or IgG. Working dilutions were 1:1,500 for the IgM reagent, 1:1,000 for the IgA

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reagent, and 1:1,000 for the IgG reagent. The sera were diluted in 0.05 M bicarbonate buffer, pH 9.5, and added in a volume of 0.2 ml per cup. After overnight incubation at room temperature, the contents of the wells were aspirated, and the wells were washed twice with 0.01 M phosphate-buffered saline, pH 7.3 (PBS), containing 0.05% Tween 20, using a washer-aspirator (Dynatech). After the addition of 0.35 ml of 5% bovine albumin in PBS to the wells, plates were held for at least 4 h at room temperature and then washed once with PBS-Tween 20 buffer. The plates were used immediately or stored at −70°C.

Test sera, viral and control antigen, and the pooled VZV monoclonal antibodies were diluted in PBS with 1% bovine serum albumin. Two sets of serum dilutions, starting at 1:16, were prepared in the plates in volumes of 0.1 ml by using microdiluters. After incubation for 1 h at 37°C, the plates were washed three times with PBS-Tween 20 buffer. To one set of serum dilutions was added 0.1 ml of the optimal dilution of viral antigen, and to the other was added 0.1 ml of the same dilution of control antigen. After overnight incubation at room temperature, the plates were washed three times, and the optimal dilution (1:10,000) of the VZV monoclonal antibody pool was added in a volume of 0.1 ml. Plates were incubated for 2 h at 37°C and then washed three times with PBS-Tween 20 buffer. Alkaline phosphatase-labeled goat anti-mouse serum diluted 1:750 in PBS with 3% bovine serum albumin was added in a volume of 0.1 ml, and tests were incubated for 1 h at room temperature. The plates were then washed three times, and 0.1 ml of the fluorogenic substrate, 4-methyl umbelliferyl phosphate, was added at a concentration of 0.025 mg/ml in 10% diethanolamine buffer (pH 9.8) with 10^{-3} M $MgCl_2$. Tests were incubated at room temperature, and after 12 to 15 min the enzymatic action on the substrate was stopped by the addition of 0.05 ml of 1 M K_2HPO_4-KOH, pH 10.4. Readings were made visually by using a light box with UV illumination (8). Antibody endpoints were the highest serum dilutions showing clear-cut blue fluorescence with the viral antigen, and results were considered to be specific only when corresponding wells containing control antigen showed no fluorescence. Sera known to be positive and negative for the VZV class-specific antibody were included as controls in each run.

Absorption of test serum with aggregated human IgG. Based on previous results which indicated that rheumatoid factor could give false-positive or falsely high viral IgM antibody titers in the capture system (6, 18), sera were routinely absorbed with aggregated human IgG to remove rheumatoid factor before they were examined for viral class-specific antibodies. This procedure is described in detail elsewhere (6).

Sera examined. Sera examined in this study included those from 10 patients with a clinical diagnosis of primary VZV infection (varicella) and 10 with a diagnosis of reactivated VZV infection (zoster); the clinical diagnoses were confirmed by results of complement fixation tests. Also included were sera from six primary herpes simplex virus (HSV) infections; five of these patients had experienced previous infections with VZV, as evidenced by the presence of VZV IgG antibody in their acute-phase serum specimens. Additional sera were from 4 patients with diagnoses of encephalitis, 6 patients with clinical findings of rash, 3 patients with fevers of undetermined origin, 2 patients with lymphatic disease, 1 patient with gastroenteritis, 1 patient with influenza, 6 healthy adults with latent VZV and HSV infections, and 11 young children who were seronegative for VZV antibody by the anticomplement immunofluorescence test.

RESULTS

ACCA. Figure 1 summarizes ACCA results on patients with VZV infections and in the other groups described above. The titers shown are the highest detected for each patient, and for all of the patients with VZV infections the highest antibody titers were seen in convalescent-phase sera collected between 12 and 30 days after the onset of illness.

All of the patients with primary VZV infection (varicella) produced high titers of virus-specific IgM antibody. On the other hand, only 5 of the 10 patients with reactivated VZV infection (zoster) showed IgM antibody responses, and titers tended to be lower than those seen in varicella. Only one individual in the group without clinical VZV infection showed IgM antibody. This was a 29-year-old male with a clinical diagnosis of encephalitis. VZV complement fixation tests showed an antibody titer rise from 1:16 to 1:64, and anticomplement immunofluorescence tests showed an antibody titer increase from 1:128 to 1:512. Furthermore, a VZV IgM antibody titer of 1:128 was demonstrated in both sera by indirect immunofluorescence staining. It seems possible that this patient had an activated VZV infection which could have produced encephalitis, or the VZV infection may have been activated by infection with another, unidentified agent which was the cause of the encephalitis. Serological results for other viruses commonly associated with encephalitis (HSV, mumps, and arboviruses) were negative.

VZV-specific IgA antibody responses demonstrated by ACCA were highly variable in the patients with varicella or zoster; several patients with either primary or reactivated infection showed low or negative IgA antibody responses. The encephalitis patient described above who showed a VZV IgM antibody response also showed an IgA titer to VZV of 1:64. A low VZV IgA titer of 1:16 was seen in a patient with encephalitis who had a serological diagnosis of measles virus infection.

The VZV IgG antibody titers detected by ACCA were generally high for the patients with active infection, with the exception of two zoster patients. However, this test system was markedly insensitive for detection of VZV IgG antibody in individuals with past infection (Fig. 2).

Comparison of ACCA and solid-phase indirect EIFA for VZV IgA and IgG antibodies. For those individuals who had class-specific VZV antibodies, Fig. 2 compares convalescent-phase IgG and IgA antibody titers obtained in the

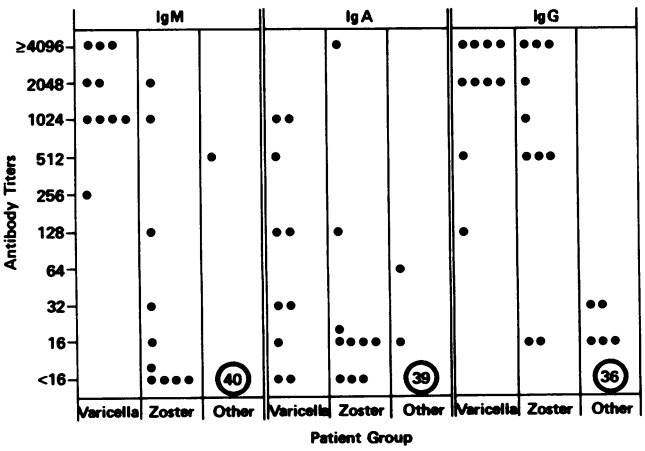

FIG. 1. VZV antibody levels detected by ACCA. See text for description of individuals in "other" category.

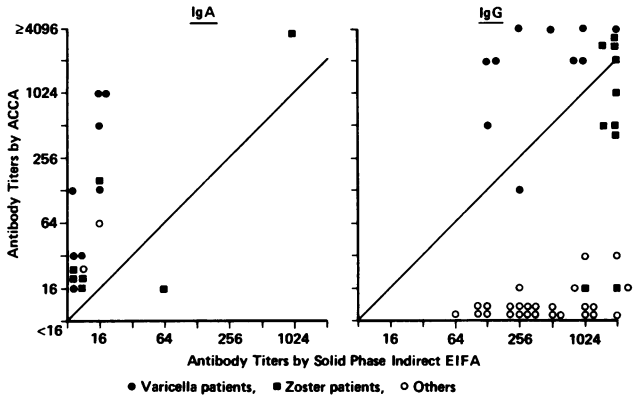

FIG. 2. Comparison of VZV IgA and IgG antibody titers obtained by ACCA and by solid-phase indirect EIFA.

ACCA system with those obtained by indirect EIFA employing VZV antigen in the solid phase. Titers of IgA antibody demonstrated by ACCA were higher than those detected by solid-phase indirect EIFA, with a single exception. For the varicella patients, titers of IgG antibody demonstrated by ACCA tended to be higher than those demonstrated by solid-phase indirect EIFA. With two exceptions, IgG titers for zoster patients were comparable by ACCA and solid-phase indirect EIFA. On the other hand, IgG levels measured by ACCA were low or negative in the group of individuals without current VZV infections, whereas titers ranging from 1:64 to 1:2,048 were detected by the solid-phase indirect EIFA.

DISCUSSION

Mouse monoclonal antibodies to human IgM could not be employed as capture antibodies in the four-phase system developed for determination of VZV IgM, as was done for assay of IgM antibodies to certain other viruses (6). This was because unlabeled mouse monoclonal antibodies to VZV were used to demonstrate binding of viral antigen to VZV-specific IgM on the solid phase, and the labeled antibodies to mouse IgG which were used to detect these would have reacted directly with the capture antibodies in the absence of a virus-specific IgM reaction, giving false-positive results. However, results obtained using a polyclonal anti-human mu chain reagent of goat origin proved to be satisfactory from the standpoint of sensitivity and specificity. A pool of monoclonal antibodies to VZV directed against different viral glycoproteins was used, rather than individual monoclonal antibodies, to impart broader reactivity, and perhaps stronger binding affinity, to the test system.

Sensitivity of the VZV IgM antibody assay was evidenced by the fact that high titers of virus-specific IgM were demonstrated for all of the patients with varicella. Only one-half of the patients with zoster showed an IgM antibody response, and titer levels varied widely. The frequency with which IgM antibody responses to VZV have been demonstrable in patients with zoster has shown wide variation in studies employing different assay procedures (1, 4, 11, 12, 14, 15, 17). However, in recent studies with sensitive immunoassays which have taken into account the possible role of rheumatoid factor in producing false-positive results, IgM antibody responses have been demonstrable in 50 to 80% of zoster patients studied (1, 4, 11, 17). More comparative studies are needed on the sensitivity and specificity of different assay systems for detection of VZV IgM antibodies in zoster patients, and such studies should provide a better understanding of the humoral immune response to reactivated VZV infections. In the present studies the importance of using uninfected control antigens in viral ACCA systems was indicated by the fact that three individuals without current VZV infections reacted with the control antigen to the same level as to viral antigen, and in the absence of an uninfected antigen control, the test results would have been misinterpreted as being specific for VZV IgM antibody.

Specificity of the VZV IgM antibody assay was indicated by the fact that negative results were obtained with sera from initial HSV infections, from patients with clinical illnesses other than VZV infection, from adults with latent VZV infection, and from young children who were seronegative for VZV IgG antibodies. The only individual without a clinical diagnosis of varicella or zoster who showed positive results in the IgM assay had other serological findings which would suggest the possibility of a reactivated VZV infection. Gershon et al. (11) have demonstrated VZV IgM antibody in individuals without clinical evidence of infection and have suggested that "silent" reactivations of VZV may occur.

The ACCA for VZV IgA antibodies did not show promise for detection of active VZV infection, since IgA antibody responses in the varicella and zoster patients varied considerably in frequency and magnitude. Low titers and variable IgA antibody responses to VZV have also been noted in certain other studies (2, 4, 13).

The sensitivity of ACCA depends on a high proportion of the total immunoglobulin class being virus specific. This is the case with viral IgM antibody in acute viral infections. Although ACCA for VZV IgA antibodies gave variable titers for varicella and zoster patients, the titers obtained by this procedure were higher than those determined by solid-phase indirect EIFA, indicating that a fairly high proportion of the total serum IgA is virus specific in many VZV infections. VZV IgG titers determined by ACCA were comparable to those obtained by solid-phase indirect EIFA for varicella patients and to a lesser extent for zoster patients. On the other hand, ACCA was totally insensitive for determination of VZV IgG elicited by past infections, which apparently constituted only a small proportion of the present IgG class of the individuals. Thus, ACCA is not a suitable approach for determination of VZV immunity status.

ACKNOWLEDGMENT

These studies were supported by Public Health Service grant AI-15990 from the National Institute of Allergy and Infectious Diseases.

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