Immunoglobulins M and G to Varicella-Zoster Virus Measured by Solid-Phase Radioimmunoassay: Antibody Responses to Varicella and Herpes Zoster Infections

A. M. ARVIN* AND C. M. KOROPCHAK

Department of Pediatrics, Division of Infectious Diseases, Stanford University School of Medicine, Stanford, California 94305

Both immunoglobulin M (IgM) and IgG antibodies to varicella-zoster virus (VZV) were detectable in a solid-phase radioimmunoassay with 125I-labeled goat antisera to human immunoglobulins. Primary infection with VZV was associated with early production of IgM and IgG antibodies and rapid development of lymphocyte transformation to VZV antigen. Among eight subjects with varicella tested 1 to 4 days after onset, seven patients had IgG and six patients had IgM antibodies; all patients had both IgG and IgM antibodies within 7 days. An IgM response was documented by radioimmunoassay in 18 of 26 patients with herpes zoster. VZV antibodies could be assayed by radioimmunoassay in unfractionated serum with commercial goat antisera to human immunoglobulins and commercial VZV antigen. VZV-specific IgG binding was present in all sera from 42 subjects with a VZV antibody titer of $\geq 1:8$ as determined by indirect immunofluorescence and cellular immunity to VZV as determined by lymphocyte transformation and who had had varicella at least 20 years before testing. The geometric mean titer was 1:6309, and titers were $\geq 1:16,384$ in 20 subjects. Antibody was present as determined by radioimmunoassay in 14 samples negative by complement fixation and in five samples negative by complement fixation and immune adherence hemagglutination. No specific binding was observed in 21 sera from subjects who were not immune to VZV as determined by indirect immunofluorescence or lymphocyte transformation despite the presence of herpes simplex or cytomegalovirus antibody indicated by complement fixation in 15 sera. High titers of VZV IgM antibody were detected in unfractionated sera despite the presence of high titers of VZV IgG antibody. The VZV radioimmunoassay provided a sensitive and practical method for measuring VZV IgG and IgM antibodies.

Primary infection with varicella-zoster virus (VZV) is common in early childhood. Varicella is usually mild in healthy children but life-threatening dissemination of the virus can occur in children with congenital immunodeficiency disorders or malignant diseases (7). A reliable method for determining susceptibility to varicella is important because the severity of the infection can be reduced by the prophylactic administration of zoster immune globulin to immunocompromised children (13). The indirect immunofluorescence methods for VZV antibody are sensitive, but these techniques require laboratory facilities for handling varicella virus, and their reliability can be affected by the subjective interpretation involved (26, 27). The standard serological methods, such as the complement fixation technique, have not been useful in detecting VZV antibody for prolonged periods after the primary infection (25). None of these methods has been easily adaptable to detect VZV immunoglobulin M (IgM) antibody in unfractionated serum.

The reactivation of latent VZV results in herpes zoster, which is a frequent occurrence among patients with malignancy and organ transplant recipients (18, 19). In addition to its clinical application, a very sensitive assay for VZV IgG and IgM antibodies might detect deficiencies in antibody production that contribute to the pathogenesis of severe primary varicella and to the reactivation of latent VZV virus in immunocompromised patients.

The solid-phase radioimmunoassay (RIA) has been a sensitive method for the detection of IgG antibody to herpesviruses, including VZV, herpes simplex, and cytomegalovirus (9, 10, 16), and has also been used to measure IgM antibody to cytomegalovirus (16). In this study, a simplified solid-phase RIA technique with commercially available reagents was employed to measure both IgG and IgM antibodies to VZV in
unfractionated serum samples. The RIA method was compared with other assays for VZV antibody, including complement fixation, indirect immunofluorescence assay (IFA), and immune adherence hemagglutination assay (IAHA). The VZV IgM and IgG responses of normal subjects were evaluated with the RIA technique during primary varicella and acute herpes zoster.

**MATERIALS AND METHODS**

**Subjects.** Sera were obtained from healthy subjects whose immunity to VZV was established by IFA and by the VZV-specific lymphocyte transformation assay (see below). The VZV-immune adults had had primary varicella 20 years or more before testing. Five of the subjects who were not immune by these criteria subsequently developed varicella. Sera were also obtained from otherwise healthy subjects with acute varicella or herpes zoster. The diagnosis was confirmed by VZV-specific direct fluorescence of epithelial cells from the skin lesions (24). Sera were tested for antibodies to herpes simplex and cytomegalovirus by the complement fixation technique (17).

**Antigen preparation.** One roller bottle (490 cm²) of confluent human foreskin fibroblast cells was inoculated with a VZV strain (passage 44) originally isolated from a patient with herpes zoster and kindly provided by Lucy Rasmussen. When 75% of the cells showed cytopathic effect, they were removed and used to infect five roller bottles containing confluent fibroblast monolayers. After 72 h, when 75% cytopathic effect was observed, the infected cells were removed with glass beads, washed once with phosphate-buffered saline (PBS), pH 7.2, suspended to a concentration of 5 x 10⁶ cells per ml and sonicated. After centrifugation at 400 x g to remove larger cell debris, the antigen was dispensed in working volumes and stored at -70°C. Control antigen was prepared from uninfected cells by the same procedure.

The commercial antigen was prepared for use as a VZV complement fixation antigen by Flow Laboratories, Inc., Rockville, Md.

**Varicella-zoster antibody testing by complement fixation, IAHA, and IFA and VZV lymphocyte transformation.** The VZV complement fixation assay was performed by a standard method using two units of antigen and two units of complement (17). For the IAHA assay (12, 19), serial dilutions of sera (0.025 ml) were incubated with 0.025 ml of VZV antigen in the wells of a microtiter “V” plate (Flow Laboratories) for 1 h at 37°C. The usual optimal dilution of antigen was 1:8 to 1:16. Complement was added at a dilution of 1:80 to 1:100. After incubation at 37°C for 40 min, diithiothreitol in Veronal-buffered saline and ethylenediaminetetraacetic acid (0.025 ml per well) and a 1.5% suspension of human “O” cells (0.025 ml per well) which had been screened for sensitivity in this assay were added. The plates were agitated for 1 min and left at room temperature for 1 h. All sera were also tested with control antigen. The antibody titer was read as the reciprocal of the maximum serial dilution resulting in 3+ hemagglutination.

Our modification of the IFA method has been described (1). Monolayers of human foreskin fibroblast cells grown in chamber slides (Lab-Tek Division, Miles Laboratories, Inc., Naperville, Ill.) were infected with VZV. When several foci of infected cells were visible in each well, but before the cytopathic effect was extensive, the slides were fixed in cold acetone for 5 to 10 min. The fixed slides were stored at -70°C. Beginning at 1:8, serial dilutions of the serum samples to be tested and known negative and positive sera were coded and added in triplicate to the slide wells. After incubation for 1 h at 37°C, the slides were washed three times with PBS, and fluorescein-conjugated guinea pig antihuman IgG (Microbiological Research Co., Bountiful, Utah) diluted 1:20 was added. After 1 h of incubation at 37°C, the slides were washed three times with PBS and examined under a fluorescence microscope. The titer was read as the highest dilution of serum which showed fluorescence of infected cells in at least two of the three wells.

VZV cell-mediated immunity was assessed by in vitro lymphocyte transformation to VZV antigen as described previously (1). Ficoll-Hypaque-separated peripheral blood mononuclear cells in 30% heat-inactivated human serum were incubated with VZV antigen for 5 days. Lymphocyte transformation was detected by comparing tritiated thymidine incorporation in antigen-stimulated cultures (³H-antigen) with control antigen cultures (³H-control). The results were expressed as a stimulation index (³H-antigen/³H-control). A stimulation index of at least threefold to VZV antigen was seen in normal seropositive subjects (1).

**RIA for varicella-zoster IgG and IgM antibodies.** The RIA for VZV IgG antibody was a modification of the method described by Friedman et al. (10). Commercial VZV antigen (Flow Laboratories) was diluted to 1:5 with PBS, pH 7.2, and antigens prepared in this laboratory were diluted to 1:8 to 1:10 for use in the assay. Three lots of the commercial antigen were used with equivalent results. VZV antigen (0.025 ml) or uninfected control antigen (0.025 ml) was added to each well of a polystyrene “V” plate (Dynatech Co., Alexandria, Va.), and the plate was allowed to dry overnight at room temperature. After one washing with PBS, the wells were filled with PBS containing 20% fetal calf serum and incubated for 1 h at 37°C. The wells were then rinsed twice with PBS and allowed to dry. Each serum sample to be tested was added to two wells containing antigen and to two control wells. The sera were diluted in PBS containing 10% fetal calf serum and 0.05% Tween 20 (J. T. Baker Chemical Co., Phillipsburg, N.J.) using a microtiter plate dilutor (Cooke Co., Alexandria, Va.). The plates were incubated at 37°C for 1 h and washed three times with PBS containing 0.05% Tween 20 before ¹²⁵I-labeled antihuman globulin was added.

The specific binding of VZV IgG antibody was assessed using goat antihuman IgG, specific for the Fc fragment (Cappel Laboratories, Downingtown, Pa.) that had been labeled with ¹²⁵I by a modified chloramine T method (20). ¹²⁵I-labeled monospecific goat antihuman IgM antibody (Tago Co., Burlingame, Calif.) purified by affinity chromatography was used to detect IgM antibody bound to the VZV antigen-coated wells. After labeling, 67 to 76% of the counts in
these preparations were precipitable with 10% trichloroacetic acid. The specific activity of the labeled preparations ranged from 8 to 10 μCi of $^{125}$I per microgram of protein.

For use in the RIA, the iodinated globulin was diluted to contain $2 \times 10^8$ cpm/ml. After preparation of the plates as described, $^{125}$I-labeled antihuman IgG or IgM (0.025 μl per well) was added to each well, and the plates were incubated for 1 h at 37°C. The plates were then rinsed five times with cold PBS containing 0.05% Tween 20 and allowed to dry. The wells were separated by passing a “hot wire” below the surface of the plate, transferred to counting vials, and counted for 1 min in a gamma scintillation counter (Beckman Instruments, Inc., Fullerton, Calif.). Controls used in each assay included known positive and negative sera and diluent alone. Specific antibody binding was considered to be present at each serum dilution for which the ratio of the mean counts per minute (cpm) of the two wells containing serum and VZV antigen to the mean cpm of the two wells containing serum and control antigen was $\geq 2.5$. The maximum cpm seen after the incubation of positive sera with antigen ranged from 6,000 to 15,000 cpm per well; the maximum cpm after the incubation of antibody-positive serum with control antigen ranged from 200 to 1,000 cpm per well. The incubation of negative serum or diluent with VZV antigen or with control antigen also produced counts in this range.

**Separation of IgG and IgM serum fractions.**

The methods used for the removal or separation of IgG and IgM antibody from the test sera included the adsorption of IgG by using staphylococcal protein A (3), the separation of IgG and IgM by using a gel filtration technique, and incubation with 2-mercaptoethanol (2). The gel purification procedure, kindly performed by Gregory Filice, used Bio-Gel A-5M, which is a 6% agarose gel (Bio-Rad Laboratories, Richmond, Calif.) to separate 7S and 19S globulins.

Rheumatoid factor. Sera that were positive in the VZV IgM assay were tested for rheumatoid factor with a commercial latex agglutination method (Calbiochem, La Jolla, Calif.) (21). VZV IgG and IgM antibody titers were assayed in sera from six adults with high titers of rheumatoid factor, ranging from 1:1,280 to 1:5,120, who had had varicella at least 20 years before testing.

**RESULTS**

Both laboratory and commercial VZV antigen preparations were sufficiently adherent to the wells of the polystyrene test plate to provide an adequate antigen-coated surface for the detection of specific antibody binding. The optimal concentration of VZV antigen was determined by evaluating the specific binding detectable in seven sera with VZV antibody titers of 1:8 by IFA and no demonstrable VZV antibody by the complement fixation or IAHA assays. Using a 1:5 dilution of the commercial antigen and 1:8 to 1:10 dilutions of our laboratory antigen, antibody was detectable by RIA at serum dilutions of 1:256 or higher in sera with low positive IFA titers. Parallel testing of these sera showed equivalent VZV antibody titers with commercial antigen diluted 1:5 and our laboratory antigen diluted 1:8 in PBS.

With the commercial antigen, specific IgG binding was detected in all 42 sera from subjects remote from infection who had VZV antibody as determined by IFA (Fig. 1). No specific binding was observed in 21 sera from subjects who were not immune to varicella by IFA or in vitro lymphocyte transformation (Fig. 1). When $^{125}$I-labeled goat antihuman IgM was used to detect bound IgM, all 8 patients with acute varicella and 18 of 26 patients with acute herpes zoster had VZV IgM antibody (Fig. 1 and 2). None of the 15 subjects remote from infection who had VZV IgG antibody had detectable VZV IgM antibody (Fig. 1). No specific binding occurred when sera from nine subjects who were not immune to varicella were tested. Binding curves for $^{125}$I-labeled antihuman IgG and IgM in unfractionated serum from a subject with acute varicella are shown in Fig. 3.

Twelve serum samples from seropositive subjects were tested to determine the reproducibility of the assay. Of the 12 sera, 10 had IgG titers within a twofold dilution, and two samples showed a greater than fourfold variation in titer. As is the case with other methods, it was necessary to test paired sera in the same assay to obtain the most accurate comparison of VZV IgG and IgM antibody titers early and later in the course of acute infection (Fig. 2).

Some assays for the detection of viral antibody by solid-phase RIA have been based on a comparison of the $^{125}$I-labeled antihuman IgG bound after incubating the test serum with viral antigen and the binding measured when a known negative serum was incubated with the antigen (15). Specific VZV IgG binding could be assessed with reference to a standard negative serum without significantly altering the antibody titer in 20 seropositive subjects. However, the elimination of the incubation of each serum with control antigen produced false-positive results among subjects who had negative VZV IFA titers and no lymphocyte transformation to VZV antigen. Of 10 nonimmune subjects, 2 showed binding ratios greater than 2.5 at serum dilutions of 1:16 when binding was assessed in relation to a standard negative serum. The binding ratios were consistently less than 2.5 when the calculation was based on binding of the subject’s serum to control antigen.

The sensitivity of the VZV RIA for measuring IgG antibody was evaluated in 14 subjects who had no antibody to varicella as determined by complement fixation (Fig. 4). The VZV RIA
titers were lower in this group (geometric mean titer [GMT], 1:380) than in the unselected population of seropositive subjects (GMT, 1:6,309), but the GMT by VZV RIA was fourfold higher than the GMT measured by IFA. The RIA and IFA were equivalent in detecting seropositive subjects in this group and in the unselected population, but antibody was detectable by RIA in more dilute serum samples from the same individual. The IAHA failed to detect antibody in five sera that were positive by RIA and the GMT by RIA was 12-fold higher than the GMT by IAHA (Fig. 4).

Cross-reactivity with herpes simplex virus and cytomegalovirus antibodies did not appear to affect the VZV antibody results by RIA. Despite the presence of antibody to herpes simplex virus in six subjects and antibody to cytomegalovirus in nine subjects, VZV RIA titers were negative in those individuals who were VZV-susceptible as determined by IFA and lymphocyte transformation. When the assay was used to measure VZV IgG antibody in immune subjects with no recent VZV infection, the VZV GMTs were equivalent between groups that had antibody to herpes simplex virus or cytomegalovirus and those that did not. The VZV GMT in 17 herpes simplex virus-seropositive subjects was 1:2,454 compared with 1:5,623 in 18 herpes simplex virus-seronegative subjects; the VZV GMTs in 15 cytomegalovirus-seropositive and 17 cytomegalovirus-seronegative subjects were 1:3,238 and 1:5,254, respectively.

Sera from six immune subjects without recent VZV infection who had VZV IgG antibody titers ranging from 1:1,024 to 1:2.6 × 10³ and who also had high titers of rheumatoid factor were evaluated to determine whether rheumatoid factor produced a false-positive result in the VZV IgM assay. Rheumatoid factor, which is usually an IgM antibody against IgG, could attach to the VZV IgG bound to the VZV antigen. The ¹²⁵I-

Fig. 1. IgG and IgM antibody titers to VZV measured by RIA in nonimmune and immune subjects identified by VZV IFA and VZV lymphocyte transformation. The antibody titers are indicated along the vertical axis. The antibody titer is the highest dilution of the serum sample for which the specific binding ratio (defined as the mean cpm after incubation in two wells containing VZV antigen divided by the mean cpm after incubation in two wells with control antigen) is ≥2.5. The left panel shows the VZV IgG antibody titers in nonimmune subjects, immune subjects who had varicella more than 20 years before testing, and subjects with acute herpes zoster tested 2 to 14 and 21 to 35 days after the onset. The right panel shows the VZV IgM antibody titers in immune subjects who had varicella more than 20 years before testing and subjects with acute herpes zoster.
labeled antihuman IgM might then bind to the rheumatoid factor, resulting in a positive assay for IgM in serum that did not contain specific VZV IgM. However, five of the six sera tested were negative in the VZV IgM assay despite the presence of rheumatoid factor. One serum had an IgM titer of 1:16. This titer was lower than the VZV IgM titers of most patients with recent varicella or herpes zoster (Fig. 1 and 2). The IgM antibody detected in the patients with acute infection was considered VZV specific because none of the sera from these patients contained rheumatoid factor.

Both IgG and IgM antibodies to VZV were present very early in the course of primary varicella infection in normal subjects (Fig. 2). Among eight patients tested within 4 days after the onset of exanthem, seven had IgG antibody and six had IgM antibody. VZV IgG and IgM antibody titers continued to increase 7 to 16 days after the appearance of the rash in most patients. The production of VZV IgG and IgM antibody within a few days after the onset of clinical varicella was accompanied by a positive in vitro lymphocyte transformation response to VZV antigen in four patients. Antibody production preceded lymphocyte transformation in the other four patients. The only patient with no detectable VZV IgG, IgM, or lymphocyte transformation was tested just 12 h after appearance of the exanthem.

VZV IgM antibody was detectable by RIA in 18 of 26 patients with herpes zoster, including 13 patients tested 3 to 5 weeks after the onset of the infection (Fig. 1). All of the patients with herpes zoster had VZV-IgG antibody and positive lymphocyte transformation responses to VZV antigen.

The presence of high titers of VZV IgG antibody in patients with acute varicella and herpes zoster did not interfere with the detection of VZV IgM antibody in unfractionated serum.
Testing done after adsorption of serum with staphylococcal protein A and after gel filtration to remove IgG showed that the antibody detected with the VZV IgM procedure was present in the IgM fraction and that IgM titers were not increased by the removal of IgG (Fig. 3). VZV IgG antibody remained detectable after adsorption with staphylococcal protein A or gel filtration in some cases, reflecting the sensitivity of the RIA method. VZV IgG was measurable when the total IgG had been reduced to less than 0.9 to 1.8 mg/ml. IgM antibody was not detected after the treatment of VZV IgM-positive sera with 2-mercaptoethanol, but IgG antibody persisted (Fig. 3).

**DISCUSSION**

The solid-phase RIA technique proved to be a simple and sensitive method for the measurement of VZV IgG and IgM antibodies. Using commercially available reagents, this method detected VZV antibody in very dilute serum samples in which no VZV antibody had been detected by IAHA or IFA. The RIA method was not subject to the false-negative results found with the IAHA technique in older persons without recent infection noted by Forghani et al. (8) and observed in the present study. The RIA was as reliable as the IFA in detecting antibody among subjects remote from infection and measured higher titers of antibody in these subjects. Among 21 VZV seronegative subjects who were susceptible by lymphocyte transformation, the IFA method gave one false-positive result compared with none by RIA. The false-positive IFA
titer was due to the difficulty of accurately judging differences in fluorescence between negative and low-positive sera. The endpoint in the RIA method was clear and eliminated the problem of subjective interpretation. Although the RIA was not compared with the VZV enzyme immunoassay described by Forghani et al. (8), the highest IgG titer observed with that assay was 1:2,048 in subjects remote from infection, whereas the mean titer for a similar population was 1:6,309 by RIA and 20 subjects had titers ≥1:16,384. Since sera from patients who were susceptible to varicella by lymphocyte transformation and IFA were consistently negative for VZV by RIA, and five of these sera were obtained from individuals who subsequently developed varicella, we believe that the method can be used clinically to identify VZV-susceptible patients.

An important advantage of the RIA technique was its application to the measurement of VZV IgM antibody. Although the IFA procedure has been used to measure VZV IgM antibody, competition between IgG and IgM for binding sites can cause difficulty with this method (6). The capacity to measure IgM antibody without fractionating the serum and the commercial availability of purified monospecific goat antihuman IgM antisera made the RIA a practical assay for VZV IgM antibody. With the monospecific antisera, specific binding ratios were as high for IgM as for IgG antibody without any alteration of the procedure. Although rheumatoid factor can interfere with the detection of specific VZV IgM antibody in a few instances, this problem should arise only with sera containing very high titers of rheumatoid factor. Even in these sera, false-positive IgM titers can be expected to be lower than titers observed during VZV infection. The phenomenon is not likely to be important in most clinical circumstances, since rheumatoid factor was not detected in patients with either varicella or herpes zoster. Preliminary studies show that the RIA technique can also be used to measure VZV-specific IgA antibody.

Sixty-nine percent of patients with herpes zoster had VZV IgM antibody as determined by RIA. These data substantiated the observations of Ross and McDaid (22) and Brunell et al. (4), who reported that VZV IgM antibody could be detected by IFA in patients with herpes zoster. Fifty percent of patients had IgM antibody as determined by RIA according to Ross and McDaid (22). Schmidt and Lennette found IgM antibody in only 1 of 22 patients tested by VZV neutralization with the IgM serum fraction (23). The VZV IgM data by RIA contrast with results obtained when the IAHA method was modified to measure VZV IgM. Using the IAHA method, Gerna et al. have suggested that a specific VZV IgM response occurs with varicella but not with herpes zoster (11). The VZV IgM titer we obtained in serum from subjects with herpes zoster were as high as those in patients with primary varicella in some cases. The presence of VZV IgM antibody does not differentiate primary varicella from generalized herpes zoster.

The studies of kinetics of the immune response to primary varicella showed that both IgM and IgG antibody and VZV-specific lymphocyte transformation develop very early in the course of clinical infection in otherwise healthy subjects. The simultaneous appearances of IgG and IgM antibodies within 4 days after the onset of varicella demonstrated by RIA contrast with the apparent delay in VZV IgM production assessed by the IFA method (5). Craddock-Watson et al. detected IgM antibody within 7 days in 31% of patients, when 100% of patients had high titers of IgM antibody by RIA within 7 days. In addition to comparing the antibody response to varicella and herpes zoster in normal subjects with the response in immunocompromised patients, the VZV RIA for IgG and IgM antibody will be useful to determine whether the antibody response to the varicella vaccine now being evaluated in high-risk patients is similar to that seen after natural infection (14). The sensitivity of the method will allow further investigation of the role of maternally acquired antibody in modifying varicella, the effect of reexposure of immune subjects to the virus, and other aspects of the natural history of VZV infection.

ACKNOWLEDGMENT

We gratefully acknowledge the assistance of Catherine Albin, Gregory Filice, Halsted Holman, and Janet Kahle.

LITERATURE CITED


other studied mans Cytomegalovirus specific live a bodies. J. immunoglobulin virus M.