Detection of Specific Immunoglobulin M Antibodies to Cytomegalovirus by Using Monoclonal Antibody to Immunoglobulin M in an Indirect Immunofluorescence Assay

MARIALUISA ZERBINI,* MONICA MUSIANI, GIOVANNA GENTILOMI, AND MICHELE LA PLACA

Institute of Microbiology, University of Bologna, 40138 Bologna, Italy

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The detection of immunoglobulin M (IgM) antibodies to cytomegalovirus-induced late antigens by an indirect immunofluorescence assay was improved by the use of monoclonal antibodies to human IgM. Nonspecific background fluorescence was absent, facilitating the reading of the slides and the detection of a specific fluorescence reaction in sera with low levels of specific IgM. Moreover, the indirect immunofluorescence assay with monoclonal antibodies to IgM proved more sensitive than the indirect immunofluorescence assay with polyclonal antibodies to IgM. The absorption of human sera on staphylococcal protein A avoided false reactions due to the presence of rheumatoid factor and high levels of specific IgG in test sera.

Human cytomegalovirus (CMV) can cause serious illness, including congenital malformations, mononucleosis syndrome after blood transfusion, and serious, sometimes fatal, complications in organ allograft recipients. Moreover, in some conditions, such as immunosuppression, malignancy, and pregnancy, reactivated infections are particularly frequent. The wide range of CMV infections emphasizes the need for rapid and reliable diagnostic techniques to detect active CMV infections. The demonstration of specific immunoglobulin M (IgM) class antibody in human sera is conventionally accepted for rapid laboratory diagnosis of most viral infections. Indirect tests, including immunofluorescence (IF) assays, radioimmunoassays (4, 9), and enzyme-linked immunosorbent assays (6), have been used to detect CMV-specific IgM. IF assays are routinely used for the detection of IgM to CMV, but the detection of specific IgM antibodies raises several problems, especially due to the presence in human sera of high levels of specific IgG (1) and occasionally of rheumatoid factor (RF) to human IgG (3, 10). The absorption of human sera with staphylococcal protein A (SPA) (12) or the use of separation columns (11) significantly improves the IF assay, but the low titers of IgM class antibody and the nonspecific background fluorescence can still limit the sensitivity and specificity of the IF assay. With the advent of monoclonal antibodies with specificity for individual epitopes on polypeptides, it has become possible to better examine the humoral immune response. In our study, we describe the results obtained with an indirect IF assay for the detection of IgM to CMV-induced late antigens (LA) with monoclonal antibody to human IgM.

Sera. We tested 49 sera from renal allograft recipients, as well as from pregnant women with serological signs of active or recent CMV infection, for the presence of IgM against CMV-induced LA. Of these sera, 41 were negative for RF (Rheuma-Wellecotest; Wellcome Research Laboratories), and 8 proved positive. Active or recent CMV infection was diagnosed serologically by the presence of a titer of anti-CMV-induced LA IgG ≥1/320, a titer of anti-CMV-induced early antigen IgG ≥1/20, and a titer of anti- immediate-early antigen IgG ≥1/20 (8). Each serum sample was divided into two portions. One serum aliquot remained untreated; the other one was absorbed on a suspension of SPA.

Treatment of sera with SPA. A 100-μg amount of SPA (Institute Virion Ltd, Diagnostic Laboratories) was reconstituted with 1 ml of distilled water plus 1 ml of 0.01 M phosphate-buffered saline (PBS; pH 7.4). A 500-μl amount of the SPA suspension was centrifuged at 2,000 × g for 10 min, and the pellet was suspended in 100 μl of undiluted serum. After an incubation of 40 min at 4°C with continuous agitation, the mixture was centrifuged for 30 min at 2,000 × g, and the supernatant fluid was used.

Human sera treated with SPA are hereafter referred to as absorbed test sera. With this preparation, a serum sample negative for IgM and positive for IgG against LA with a titer of 1/320 resulted in a titer <1/10 for IgG after absorption.

Antigen preparation. Human embryo fibroblasts were grown in Eagle minimum essential medium supplemented with 10% fetal calf serum and antibiotics and used between passage 15 and passage 20. The Towne strain of human CMV was used in all of the experiments. The virus was prepared and harvested as previously described (7). Human embryo fibroblast cultures, grown on cover slips, were infected with 2 PFU of CMV per cell.

On day 4 after infection, cells were fixed in acetone and stored at −20°C. Specific fluorescence reaction for CMV-induced LA scattered inside 70 to 80% of the cells. Negative control antigens were prepared from uninfected human embryo fibroblast cells processed as described for LA preparation.

IF assays. In the first IF assay, the antigen preparation was incubated with absorbed and serially diluted test serum at 37°C for 3 h. After three washes with 0.15 M PBS (pH 7.4), fluorescein-conjugated rabbit polyclonal antibody to human IgM μ chains (Dako), diluted 1/20, was added and incubated at 37°C for 45 min. After another three washes in PBS, cells were mounted in glycerol-PBS (1:1, vol/vol) and examined under a UV-light microscope.

The second IF assay for the detection of IgM to CMV LA was performed as follows. Absorbed test sera were incubated with the antigen preparation at 37°C for 3 h, and, after three washes in PBS, monoclonal antibody to human IgM (Miles Scientific, Div. Miles Laboratories, Inc.) was added at a dilution of 1/40 and incubated for 45 min at 37°C.

* Corresponding author.
FIG. 1. Distribution of titer values of IgM to CMV LA by IF in 41 absorbed test sera negative for RF when monoclonal antibody to IgM was used versus values obtained when polyclonal antibody to IgM was used.

were then washed with PBS and incubated with fluorescein-conjugated goat anti-mouse IgG at a dilution of 1/40. Cells were washed three times in PBS and mounted in glycerol-PBS.

The third IF assay was performed on human test sera which had not been absorbed with SPA. Cells were incubated with untreated serum for 3 h at 37°C; then, after three washes in PBS, the monoclonal antibody to human IgM was added, and this mixture was incubated at 37°C for 45 min. Cells were then washed, and fluorescein-conjugated goat anti-mouse IgG was added. Cells were then processed as described above.

The optimal working dilution of each immune reagent for IF assays was determined by preliminary block titration. Negative and positive reference sera for IgM to CMV-induced LA (1-401 [LA-IgG negative, LA-IgM negative], 2-21 [LA-IgG titer of 1/320; LA-IgM negative], and 3-182 [LA-IgG titer of 1/320, LA-IgM titer of 1/80]) were included in every test. All tests were performed in triplicate, and the titer was read as the highest dilution of serum which showed fluorescence of infected cells in all three samples. All tests were read in a "blind" fashion.

The detection of IgM against CMV-induced LA with an anti-IgM monoclonal antibody was compared with the detection of IgM with an anti-IgM polyclonal antibody in 41 absorbed sera which were negative for RF. The distribution of values of the sera tested by the two assays is shown in Fig. 1; a significant correlation at the 5% level was observed ($r = 0.34$). The test with the monoclonal antibody, however, was easier to evaluate because of the complete absence of nonspecific background fluorescence and proved more sensitive. In fact, out of 41 serum samples examined with monoclonal antibody, 11 with a onefold rise in titer, 4 with a twofold rise, 2 with a threefold rise, and 2 with a fourfold rise proved positive with respect to titers as determined with polyclonal antibody to IgM. In only one sample was a onefold decrease in titer noted in the assay with monoclonal antibody to IgM in comparison with the test with polyclonal antibody. Moreover, three sera which proved negative when tested with polyclonal antibody to IgM were positive when assayed with the monoclonal antibody. The increased sensitivity of the assay with monoclonal antibody was statistically significant, with a $t$ value of 4.07 ($P < 0.01$).

The detection of IgM with monoclonal antibody in 41 SPA-absorbed serum samples negative for RF was further compared with the detection of IgM with monoclonal antibody in the same sera not previously absorbed on SPA. The distribution of values is shown in Fig. 2. Out of 41 absorbed serum samples examined, 10 with a onefold rise in titer with respect to the same unabsorbed test samples, 12 with a twofold rise, 6 with a threefold rise, and 2 with a fourfold rise proved positive. Moreover, eight sera which proved negative when tested before absorption on SPA proved positive after absorption.

The effect of the presence of RF on the assay with monoclonal antibody to IgM was evaluated in the eight sera positive for RF which were tested for IgM by CMV LA with monoclonal antibody before and after absorption on SPA. The results shown in Table 1 demonstrate that unabsorbed sera could yield increased titers of IgM antibody and false-positive reactions. To control the effectiveness of the absorption on SPA of sera positive for RF, the positive control serum supplied with the Rheuma-Wellcotest kit was mixed at the dilution of 1/20 with four sera which were negative for RF but positive in the IgM test with monoclonal antibody. These mixed sera were then absorbed on SPA, and when tested in parallel with absorbed samples without added RF, they yielded the same results.

To assess the reproducibility of our assay with monoclonal antibody against IgM, 10 sera were selected. Eight sera had anti-CMV IgM titers ranging from 1/5 to 1/80, and two were negative. Five replicate samples of each of these sera were tested at different times to confirm reproducibility. In all of the 10 sera retested, differences in titer were less than or equal to onefold the expected antibody value. To test the
TABLE 1. Effect of RF on IF assay for the detection of anti-CMV LA IgM by use of monoclonal antibody to IgM

<table>
<thead>
<tr>
<th>RF-positive serum sample</th>
<th>Anti-CMV-induced LA IgM titer of:</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>SPA-absorbed serum</td>
<td>Unabsorbed serum</td>
</tr>
<tr>
<td>1</td>
<td>1/10</td>
<td>1/20</td>
</tr>
<tr>
<td>2</td>
<td>1/40</td>
<td>1/160</td>
</tr>
<tr>
<td>3</td>
<td>Negative</td>
<td>1/20</td>
</tr>
<tr>
<td>4</td>
<td>Negative</td>
<td>1/20</td>
</tr>
<tr>
<td>5</td>
<td>1/20</td>
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<td>1/10</td>
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<td>7</td>
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* Eight serum samples positive for RF were tested before and after absorption on SPA.

specificity of our assay, five sera which were positive with a titer of 1/20 for IgM to CMV LA were assayed for IgM to Epstein-Barr-induced virus capsid antigens and for herpes simplex type 1 virus-induced antigens and proved negative. Our study shows that the use of monoclonal antibody to IgM improves the IF technique for the determination of IgM to CMV-induced LA in test sera previously absorbed on SPA. The availability of monoclonal antibody to IgM can overcome some of the problems that have been encountered in the past with reagents of low specificity and sensitivity. In fact, with polyclonal antisera to IgM, a background fluorescence was noted in all the samples, and it was necessary to compare the results of each serum against viral antigens with those of serum against uninfected control antigens so that nonspecific reactivity of the test sera could be recognized and false-positive readings could be avoided. On the other hand, the absence of nonspecific background fluorescence observed when the monoclonal antibody to IgM on absorbed test sera was used allows a more rapid and certain evaluation of the results and allows detection of specific positive reactions at low dilutions of test sera. In fact, three sera which proved negative when tested with polyclonal antibody to IgM were positive when assayed with the monoclonal antibody. Moreover, specific positive reactions with both techniques had higher specific-IgM levels when tested with the monoclonal antibody to IgM. It is possible that the three-step technique which used the monoclonal antibody to IgM is able to amplify the specific reaction more than is the two-step technique which uses polyclonal antisera to IgM (2, 5).

The use of monoclonal antibody to IgM, however, cannot exclude the procedure of absorption of test sera on SPA. In fact, the results obtained when the monoclonal to IgM in sera negative for RF was used were lower titers of anti-CMV LA IgM and some false-negatives in unabsorbed versus absorbed sera. These observations suggest that, even in excess of antigen, anti-CMV LA IgG molecules compete with specific IgM and bind to antigenic sites more effectively than does specific IgM. The absorption of test sera on SPA is also helpful to avoid the interference of RF (12). In fact, the removal of IgG from test samples avoids false-positive reactions or false increased levels of IgM antibody due to the presence of RF. The sensitivity, specificity, and reliability of the IF assay for IgM to CMV LA in absorbed sera when a monoclonal antibody to IgM is used suggest that the use of monoclonal antibody to IgM may offer a useful tool not only in IF techniques but also in other assays to detect specific IgM.

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LITERATURE CITED


