Lack of Correlation between Virus Detection and Serologic Tests for Diagnosis of Active Cytomegalovirus Infection in Patients with AIDS

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The aim of this study was to evaluate the usefulness of serologic analysis for the diagnosis of active cytomegalovirus infection in patients with AIDS. Active cytomegalovirus infection was diagnosed by virus isolation from urine and saliva and detection of antigenemia. Serologic analysis was done by several conventional and innovative procedures. The results indicate no correlation between any of the most popular serologic procedures and virus detection by culture in urine or saliva or antigenemia.

Human cytomegalovirus (CMV) is a major cause of severe disease in patients with impaired immune defenses (for a review, see reference 3). In recent years, the number of patients with AIDS has increased markedly and CMV is one of the most important causes of morbidity and mortality in this population (1, 14).

Rapid and specific diagnosis of active CMV infection is critical with respect to the efficacy of antiviral therapy. Since clinical signs of infection are often absent in the early stage of infection, quick and reliable laboratory diagnosis is needed.

Diagnosis of active viral infection is best accomplished by either (i) direct detection of the virus by isolation in tissue culture or (ii) demonstration of the viral genome or viral antigens with DNA probes or immunological reagents, respectively.

Significant progress has been made in direct virus detection. In fact, the time required to detect CMV in culture has been shortened by the shell vial procedure (2), while DNA probes (9, 12, 20) or highly specific monoclonal antibodies (18, 21) are available for rapid detection of the viral genome or viral antigens in pathological materials.

Serologic analysis has also made significant progress and in different clinical settings has proved able to differentiate active or recent from past viral infections and primary from secondary viral infections (5–8, 11, 13, 16, 18, 23, 25).

Our aim was to evaluate the usefulness of serologic analysis in comparison with direct virus detection to identify an active phase of CMV replication in patients with AIDS, as recently carried out for a different group of CMV-infected patients (10).

Our study population consisted of two groups of human immunodeficiency virus-positive patients (Table 1). For patients in the first group, CMV was isolated from urine and/or saliva in two consecutive tests over a period of 1 month (two or three samples were obtained); in eight cases, antigenemia was also observed. For patients in the second group, the virus was not isolated nor was antigenemia detected over a period of 1 month (two or three samples were obtained). The sera used in this study were taken in conjunction with the second or third sample of CMV-negative or -positive pathological material.

Most serum samples and pathological materials were sent to our diagnostic laboratory for routine monitoring of CMV infection in human immunodeficiency virus-seropositive patients from the Section of Infectious Disease of the Division of Internal Medicine, Faenza, Italy.

Human embryo fibroblasts were grown in Eagle’s minimal essential medium with 10% fetal calf serum. The Towne strain was used in all of the experiments and was grown as previously described (7).

For virus isolation, the shell vial procedure was used (2). The inoculated cells were fixed 2 days after inoculation and stained in an indirect immunofluorescence (IIF) assay by using a monoclonal antibody that reacts with CMV-induced immediate-early antigen (E13 from Biosoft, Paris, France).

CMV antigen staining was performed as originally described by Schirm et al. (21) and modified by Revello et al. (18).

Enzyme-linked immunosorbent assays (ELISA) for detection of both immunoglobulin G (IgG) and IgM (4) were performed with two commercial antibody capture kits from Technogenetics (Hamburg, Germany). Tests were performed and results were interpreted as suggested by the manufacturer. Three calibration sera were used to divide the sera into four groups: negative sera were those that gave an ELISA optical density of <0.198, those with a low titer were in the range of 0.198 to 0.359, those with a medium titer were between 0.360 and 1.239, and all optical densities of >1.240 were considered high titters. For IgM detection, positive and negative sera were used as controls. Positive sera were those with an ELISA optical density of >0.210.

All IgM-positive sera were tested by latex agglutination (Rheuma-Wellco test; Wellcome, Dartford, England) to ascertain the presence of rheumatoid factor. No rheumatoid factor-positive sera were included in the present study.

Virus-induced early antigen and late antigen (LA) were prepared as described previously (5). IIF assays were done as described by Schmitz and Haas (22) for detection of total immunoglobulin reactivity to early antigen (5, 25) and for separate detection of IgG, IgM, and IgA against virus-induced LA (5, 24, 25).

Anticomplement immunofluorescence testing was performed as originally described by Revello et al. (17) for detection of IgM to the membrane of uninfected human embryo fibroblasts.

For immunoblotting (IB), protein extracts from purified
TABLE 1. Characteristics of the patients studied

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No. of patients in:</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Group 1</td>
</tr>
<tr>
<td>Total no. of patients</td>
<td>50</td>
</tr>
<tr>
<td>Females</td>
<td>12</td>
</tr>
<tr>
<td>Males</td>
<td>38</td>
</tr>
<tr>
<td>Mean age, range (yr)</td>
<td>34 (23-42)</td>
</tr>
<tr>
<td>Drug addicts</td>
<td>48</td>
</tr>
<tr>
<td>Homosexuals</td>
<td>2</td>
</tr>
<tr>
<td>With AIDS</td>
<td>39</td>
</tr>
<tr>
<td>With LAS-ARC&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pneumonia</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Persistent fever</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Retinitis</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Colitis</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Unknown</td>
<td>10</td>
<td>8</td>
</tr>
</tbody>
</table>

<sup>a</sup> LAS-ARC, lymphadenopathy syndrome–AIDS-related complex.

Viral particles were run in a 9% acrylamide gel and electrophoretically separated polypeptides were then transferred to nitrocellulose paper as previously described (7). The immune reaction with sera was done as previously described in detail (7) for both IgG and IgM (4, 15, 16). IB was also done for detection of IgM to recombinant proteins rp150 and rp52 (17).

Recombinant proteins rp150 and rp52 were produced as previously described (19), and protein extracts were run, transferred onto nitrocellulose, and tested with human sera as described for protein extracts from purified viral particles. Rp150 corresponds to fusion protein D1, which contains the last 25 amino acids at the carboxy terminus of phosphoprotein gp150 (molecular mass, 150 kDa) and is the major structural protein, while rp52 corresponds to fusion protein G2, which corresponds to amino acids 1071 to 1301 of the nonstructural DNA-binding protein (molecular mass, 52 kDa).

The results obtained (summarized in Table 2) show that the differences in the data obtained from the two groups of sera with all serological tests were statistically insignificant, with the sole exception of those obtained with IgA to LA (X<sup>2</sup> = 7.5). However, IgA to LA cannot be considered a serological marker of active viral replication in patients with AIDS, as only 10 of 50 patients excreting CMV had virus-specific IgA.

When each of the two groups was subdivided into patients with AIDS and those with lymphadenopathy syndrome–AIDS-related complex, the results did not differ nor did they change when we divided group 1 into two subgroups with respect to the presence or absence of antigenemia (data not shown).

The results obtained show that serologic analysis performed with both conventional and newer methods cannot distinguish between patients with AIDS who excrete CMV and those who do not.

Our results were not unexpected, because it is well known that patients with AIDS encounter several consecutive active CMV infections (1, 14), and therefore it is likely that the intervals between active infections are too short to allow CMV-specific immune reactivity to decrease or change during remission periods and become different from periods of active viral replication. However, we cannot exclude the possibility that our results are due to the deregulation of immunoglobulin production which is frequently observed during AIDS.

Our results strongly suggest that diagnosis of active CMV
infection in patients with AIDS should not be based on the currently available serologic tests.

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REFERENCES