Immunooassay with cytomegalovirus early antigens from gene products p52 and CM2 (UL44 and UL57) detects active infection in patients with chronic fatigue syndrome

S H Beqaj, A M Lerner, J T Fitzgerald

ABSTRACT

Aims: To investigate whether the use of recombinant early antigens for detection of antibodies to human cytomegalovirus (HCMV) gene products CM2 (UL44, UL57) and p52 (UL44) is specific in the diagnosis and differentiation of active HCMV infection in a subset of patients with chronic fatigue syndrome (CFS), a diagnosis which is often missed by the current ELISA assay that uses crude viral lysate antigen.

Methods: At a single clinic from 1999 to 2001, a total of 4774 serological tests were performed in 1135 patients with using two immunoassays, Copalis and ELISA. The Copalis immunoassay utilised HCMV early gene products of UL44 and UL57 recombinant antigens for detection of HCMV IgM antibody, and viral capsid antigen for detection of HCMV IgG antibody. The ELISA immunoassay utilised viral crude lysate as antigen for detection of both HCMV IgG and IgM.

Results: 517 patients (45.6%) were positive for HCMV IgG by both assays. Of these, 12 (2.2%) were positive for HCMV(V) IgM serum antibody by HCMV ELISA assay, and 61 (11.8%) were positive for IgM HCMV serum antibody by Copalis assay. The Copalis assay that uses HCMV early recombinant gene products CM2 (UL44, UL57) and p52 (UL44) in comparison with ELISA was 98% specific.

Conclusions: Immunoassays that use early antigen recombinant HCMV CM2 and p52 are five times more sensitive than HCMV ELISA assay using viral lysate, and are specific in the detection and differentiation of active HCMV infection in a subset of patients with CFS.
antibodies to HCMV by recombinant assay had abortive or incomplete HCMV infection that could not be detected by ELISA. Abortive herpesvirus EBV and HCMV infection may be essential to patients with CFS.

**METHODS**

From 11 June 1999 to 17 December 2001, 1135 patients with CFS were seen. Patients met CDC criteria for CFS. All CFS patients had complete medical history, physical examination and determination of their physical activity capability by the Energy Index Point Score (EI) which was validated by the Fatigue Severity Score. All CFS patients had EI point scores <5. Standard 12-lead ECG, 24-hour ECG monitoring and specific serological tests for Lyme disease, rheumatic fever and EBV were performed. If the ECG was abnormal, rest/stress myocardial perfusion and radionuclide ventriculography were performed.

**Immunoaassays**

Two immunoassays were used for detection of antibody to HCMV infection. ELISA immunoassay, that uses crude viral lysate as an antigen for detection of HCMV IgM and HCMV IgG antibodies (Diasorin, Stillwater, Minnesota, USA) and scattered light technology Copalis Multiplex assay, that uses recombinant early antigens p52 and CM2 (gene products of UL44 and UL57) for detection of IgM antibodies to HCMV were performed. VP antigen for detection of IgG antibodies to HCMV (Diasorin) was also used in the Copalis assay. Both assays were performed in our laboratory using commercial kits, and followed the manufacturer’s instructions.

**RESULTS**

**Clinical findings**

CFS patient demographics from this centre have been described previously. CFS patients with HCMV(V), HCMV(VP), CM2 sera, rheumatoid factor was negative, eliminating possibility of cross-reactivity with rheumatoid factor. Of 1135 CFS patients, 517 were positive for HCMV IgG antibodies by both ELISA immunoassay and recombinant Copalis immunoassay, confirming HCMV infection.

Of the 517 HCMV infected patients, 12 (2.2%) were positive for HCMV(V) IgM antibody by ELISA assay, and 61 (11.8%) were positive by Copalis multiplex assay, indicating active HCMV infection. Of the 61 CFS patients positive for IgM antibody by Copalis multiplex assays, 9 were p52 positive, 40 were CM2 positive, and 12 were positive for both combined p52, CM2 antigens (table 2). Interestingly, of the 12 CFS patients HCMV(V) IgM positive by ELISA assay, only three CFS patients had detectable Copalis HCMV, p52, CM2 multiplex assays. Therefore, 59 CFS patients’ active HCMV infection was detected only by HCMV p52 and/or CM2 assays.

**DISCUSSION**

The aetiology of CFS remains unknown. There is strong evidence that CFS is associated with chronic infections including HCMV, EBV, HHV6, and other infections. We have previously shown that patients with either HCMV or EBV, or co-infection, suffer from CFS, and that classification of infection is significantly important for diagnosis and treatment of CFS. However, classification of infection can be performed only by use of specific diagnostic tests. We have previously reported that use of recombinant antigens for detection of antibody to EBV in patients with CFS is specific for diagnosis and monitoring the antiviral treatment of disease. Likewise, here we show that use of recombinant antigens to early HCMV genes is specific for diagnosis of HCMV infection in patients with CFS. Serum antibody to p52 and CM2 is rarely seen in immunocompromised patients with chronic fatigue syndrome (CFS).

**Table 1** HCMV IgM recombinant rec-p52 (UL44) and rec-CM2 (UL44, UL57) serum antibody titres in 1135 patients with chronic fatigue syndrome, 1999–2002 from an infectious diseases practice

<table>
<thead>
<tr>
<th>Mean (SEM) of 4774 tests</th>
<th>0.22 (0.62)</th>
<th>0.32 (0.72)</th>
<th>0.22 (0.62)</th>
<th>0.32 (0.72)</th>
</tr>
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<tbody>
<tr>
<td>Number of patients with rec-p52, CM2 serum antibody titres</td>
<td>9 (0.8%)</td>
<td>40 (3.5%)</td>
<td>12 (1.1%)</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2** HCMV rec-p52 (UL44) and rec-CM2 (UL44, UL57) serum antibody titres in 61 patients with chronic fatigue syndrome

<table>
<thead>
<tr>
<th>Patient cohort</th>
<th>Ser HCMV antibody titre</th>
<th>rec-IgM p52</th>
<th>rec-IgM CM2</th>
</tr>
</thead>
<tbody>
<tr>
<td>rec-p52 (9 pts)</td>
<td>114 (10.6)</td>
<td>0 pt</td>
<td>2.2 (0.41)</td>
</tr>
<tr>
<td>rec-CM2 (40 pts)</td>
<td>104 (6.2)</td>
<td>3 pts</td>
<td>2.4 (0.29)</td>
</tr>
<tr>
<td>rec-p52, CM2 (12 pts)</td>
<td>130 (16.4)</td>
<td>0 pt</td>
<td>3.1 (0.48)</td>
</tr>
</tbody>
</table>

**Serology findings**

There were 4774 serum specimens from 1135 CFS patients tested for antibodies to HCMV IgG and HCMV IgM by both ELISA immunoassay and Copalis HCMV multiplex assays (table 1). A positive Copalis assay for HCMV p52 or CM2 (Diasorin) is indicative of IgM HCMV, and is specific for active infection. A value >1.5 for both p52 and/or CM2 was considered positive.

In 43 random positive HCMV IgM antibodies to p52, CM2 sera, rheumatoid factor was negative, eliminating possibility of cross-reactivity with rheumatoid factor. Of 1135 CFS patients, 517 were positive for HCMV IgG antibodies by both ELISA immunoassay and recombinant Copalis immunoassay, confirming HCMV infection.

Of the 517 HCMV infected patients, 12 (2.2%) were positive for HCMV(V) IgM antibody by ELISA assay, and 61 (11.8%) were positive by Copalis multiplex assay, indicating active HCMV infection. Of the 61 CFS patients positive for IgM antibody by Copalis multiplex assays, 9 were p52 positive, 40 were CM2 positive, and 12 were positive for both combined p52, CM2 antigens (table 2). Interestingly, of the 12 CFS patients HCMV(V) IgM positive by ELISA assay, only three CFS patients had detectable Copalis HCMV, p52, CM2 multiplex assays. Therefore, 59 CFS patients’ active HCMV infection was detected only by HCMV p52 and/or CM2 assays.
patients (HIV or transplant patients) where HCMV viral titres indicating complete viral multiplication are high, and virus is easily detected in blood.20–29 In contrast, HCMV infection in immunocompetent patients is usually well controlled. HCMV maintains infection in immunocompetent patients by its latency, awaiting an opportunity to reactivate infection.20–31 However, in CFS patients, who are otherwise immunocompetent, complete virus, or abortive multiplication may be present. In the CFS patient, herpesvirus multiplication occurs in part without full virus assembly.

We have previously proposed this model in CFS patients with EBV infection.32 After treatment with valacyclovir, EBV-IgM, which indicates active infection, disappears, but EBV-EA remains in the patient’s serum for a longer period or may never disappear, indicating some continued EA formation.33 Antiviral therapy is effective only during viral replication as it impairs DNA synthesis. Therefore, the virus may express some genes and make some protein products, but not fully replicate.34 Here, we show that use of recombinant antigens in detection of antibody to HCMV gene products is a significant improvement in detection and differentiation of HCMV infection in CFS patients. There was excellent correlation between ELISA and Copalis assays for HCMV IgG serum antibodies, indicating HCMV infection in these patients. However, significant differences were seen in detection of HCMV IgM antibodies.

Of 517 HCMV(V) IgG positive CFS patients, 12 (2.2%) were positive for IgM antibody by ELISA assay, but 61 (11.8%) were positive by recombinant assay, indicating significant improvement in detection and differentiation of HCMV infection in CFS patients. The sensitivity of the recombinant assay is increased by use of the chimeric antigens, p52 and CM2, for detection of IgM antibody (table 2). Both p52 and CM2 antigens are non-structural proteins of HCMV genes UL44 and UL57, which are early HCMV genes.10–24 The central portion of p52 is a major reactive protein of acute HCMV infection. The antigen CM2 is a chimeric protein product of fused UL44 and UL57 genes, which markedly increases sensitivity of the assay. This is shown here: of 61 serum HCMV recombinant IgM positive CFS patients, 9 were p52 positive, 40 were CM2 positive and 12 were positive for both p52 and CM2 antigens. This use of recombinant HCMV p52 and CM2 antigens to detect IgM HCMV serum antibody is thus the best method to detect active HCMV infection in immunocompetent individuals.

In addition, these results confirm our previous findings that p52 and CM2 serum antibodies are specific in diagnosis of HCMV abortive infection in CFS patients similar to those infected with EBV.4 In that study, p52 and CM2 HCMV IgM serum antibody titres were present in this HCMV subset of CFS patients, but not in control non-CFS patients. In turn, the presence of p52 and CM2 antibodies to p52 and CM2 non-structural antigens may account for difficulties in detecting HCMV DNA in blood or cardiac biopsies in these CFS patients, consistent with the paradigm of incomplete or abortive viral multiplication. Abortive viral multiplication in immunocompetent CFS patients may be unique.35–34

Conclusion
Raised IgM serum antibody titres to HCMV recombinant early antigens p52 and/or CM2 indicate unique abortive HCMV infection in a subset of CFS undetectable by previous HCMV assays to crude structural antigens. Abortive herpesvirus infection may be a major aetiology of CFS.

Take-home messages

- The p52, CM2 recombinant IgM assay to early human cytomegalovirus (HCMV) antigens is diagnostic of abortive HCMV infection.
- It is specific and five times more sensitive than the current IgM ELISA HCMV conventional virus lysate assay.
- Results of this study indicate active HCMV infection in 61 patients with chronic fatigue syndrome (CFS); only 12 were shown to have active HCMV infection by the current ELISA assay with crude virus lysate antigen.
- Abortive herpesvirus infection is aetiological to CFS.

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Competing interests: All authors hold US patents on diagnosis and treatment of chronic fatigue syndrome.

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