Immunoassay with cytomegalovirus early antigens from gene products p52 and CM_2 (UL44 and UL57) detects active infection in patients with chronic fatigue syndrome

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ABSTRACT

Aims: To investigate whether the use of recombinant early antigens for detection of antibodies to human cytomegalovirus (HCMV) gene products CM_2 (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 patients 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 CM_2 (UL44, UL57) and p52 (UL44) in comparison with ELISA was 98% specific.

Conclusions: Immunoassays that use early antigen recombinant HCMV CM_2 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.

Chronic fatigue syndrome (CFS) is a major public health problem affecting young adults aged 35–65 in a female:male ratio of 4:1.[1] Although many groups have tried to relate CFS to different causes, the aetiology of CFS remains unknown.[2] We have studied patients with CFS intensively and demonstrated that CFS is associated with long lasting chronic viral infection, such as Epstein–Barr virus (EBV) and human cytomegalovirus (HCMV) infection.[3 4] We use Centers for Disease Control (CDC) CFS diagnostic criteria and have developed a diagnostic profile which includes several immunoassays based on viral gene products which determine CFS subset classification for individual patients.[5 6] Three CFS subsets are recognised based on viral infection: EBV subset; HCMV subset; and EBV-HCMV co-infection. Subset classification is necessary for selection of antiviral therapy and appropriate treatment.[7] Since latent herpesvirus infection with no active infection may be positive by DNA PCR, recognition of active infection may be facilitated by serological responses to early antigens.

Molecular genomic recombinant technology allows the development of immunoassays with antigens prepared from viral genes.[8–11] We have previously reported that use of recombinant antigens for detection of antibodies to EBV infection may assist in diagnosis and differentiation of EBV infection in patients with CFS.[3] Abortive EBV infection has been found in patients with CFS who have raised serum antibodies to early antigen (EA).[3] As there is no adequate immunoassay for diagnosis and differentiation of HCMV infection, we sought such HCMV early antigens because they might be specific in diagnosis and, therefore, facilitate ultimate treatment of patients with CFS.

There is no commercially available immunoassay that employs specific recombinant protein antigens for detection of HCMV serum antibody as a diagnostic tool for diagnosis and differentiation of HCMV infection, similar to that of the EBV immunoassay.[3] The only available serological assay for HCMV infection is an ELISA utilising crude human fibroblast tissue culture preparations of extracellular virus particles which are poorly characterised. Using a crude viral lysate as an antigen in immunoassay, detection of specific HCMV IgM antibodies is poor.[12 13] In our previous pilot studies, we reported that p52 and CM_2 recombinant early antigens, which are products of HCMV UL44 and UL57 genes, are specific for detection of IgM antibody to HCMV in patients with CFS.[4] The recombinant viral capsid antigen for HCMV(VP) also used in this assay is specific for detection of IgG antibodies to HCMV and is usually utilised for diagnosis of prior HCMV infection in all patients, including the CFS patient.[4] In this larger study, we tested 1135 patients with CFS for HCMV infection. Two immunoassays were used and the results were compared: Copalis immunoassay that uses recombinant early antigens and ELISA immunoassay that uses crude viral lysate antigen. We performed 4774 assays in 1135 patients with CFS from a single clinic during the period 1999–2001. A total of 517 patients (45.6%) were positive for antibodies to IgG by both assays. Of these, only 12 CFS patients (2.2%) were positive for IgM antibody by ELISA; 61 CFS patients (11.8%) were positive for IgM serum antibody to HCMV by recombinant CM_2 and p52 assays. We suspect that 61 CFS patients positive for IgM

Table 1 HCMV IgM recombinant rec-p52 (UL44) and rec-CM_2 (UL44, UL57) serum antibody titres in 1135 patients with chronic fatigue syndrome, 1999–2002 from an infectious diseases practice

	Serum HCMV antibody titres		
Cohort	**rec-p52**	**rec-CM_2**	**rec-p52,CM_2**
Mean (SEM) (4774 tests)	0.22 (0.62)	0.32 (0.72)	0.22 (0.62), 0.32 (0.72)
Number of patients with rec-p52, CM_2 serum antibody titres, 61 patients (5.4%) values >1.3*	9 (0.8%)	40 (3.5%)	12 (1.1%)

*Three of the 61 patients had positive serum HCMV IgM antibody titres to HCMV(V).
rec, recombinant antigen.

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.[5] All CFS patients had complete medical history, physical examination and determination of their physical activity capability by the Energy Index Point Score (EI)[14] which was validated by the Fatigue Severity Score.[15] 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.[16]

Immunoassays

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)[17 18] and scattered light technology Copalis Multiplex assay, that uses recombinant early antigens p52 and CM_2 (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.[18–20] 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.[16] CFS patients with HCMV(V), HCMV(VP), CM_2 and p52 raised serum antibody titres were clinically indistinguishable from all other CFS patients. Patients with raised serum titres to *Borrelia burgdorferi* or raised serum antibody titres to streptolysin 0 were also indistinguishable from the larger group of CFS patients. All CFS patients (96%, p<0.01) had abnormal oscillating T-waves indicative of CFS cardiomyopathy,[21 22] and 19% had abnormal cardiac wall motion.[16]

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 CM_2 (Diasorin) is indicative of IgM HCMV, and is specific for active infection.[23–29] A value >1.3 for both p52 and/or CM_2 was considered positive.

In 43 random positive HCMV IgM antibodies to p52, CM_2 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 CM_2 positive, and 12 were positive for both combined p52, CM_2 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, CM_2 multiplex assays. Therefore, 59 CFS patients' active HCMV infection was detected only by HCMV p52 and/or CM_2 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.[7 14] 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 CM_2 is rarely seen in immunocompromised

Table 2 HCMV rec-p52 (UL44) and rec-CM_2 (UL44, UL57) serum antibody titres in 61 patients with chronic fatigue syndrome

	Serum HCMV antibody titres				
Patient cohort	**c-IgG(v)***	**c-IgM(v)**	**c-(VP)**	**rec-IgM p52†**	**rec-IgM CM_2†**
rec-p52 (9 pts)	114 (10.6)‡	0 pt	13.9 (5.4)	2.2 (0.41)	
rec-CM_2 (40 pts)	104 (6.2)	3 pts	9.4 (1.7)		2.4 (0.29)
rec-p52,CM_2 (12 pts)	130 (16.4)	0 pt	13.9 (5.4)	2.4 (0.22)	3.1 (0.48)
Totals 61 pts		3 (7.3%) pts	61 pts (100%)	21 (34.4%) pts	52 (85.2%) pts

*c, conventional HCMV virion antigen, positive >20 .
†rec, recombinant HCMV antigen, positive >1.3.
‡Mean (SEM).
pt, patient.

patients (HIV or transplant patients) where HCMV viral titres indicating complete viral multiplication are high, and virus is easily detected in blood.[25–29] 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.[30 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.[33] 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 CM_2 for detection of IgM antibody (table 2). Both p52 and CM_2 antigens are non-structural products of HCMV genes UL44 and UL57, which are early HCMV genes.[10 20 24] The central portion of p52 is a major reactive protein of acute HCMV infection. The antigen CM_2 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 CM_2 positive and 12 were positive for both p52 and CM_2 antigens. This use of recombinant HCMV p52 and CM_2 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 CM_2 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 CM_2 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 CM_2 antibodies to p52 and CM_2 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.[33 34]

Conclusion

Raised IgM serum antibody titres to HCMV recombinant early antigens p52 and/or CM_2 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, CM_2 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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