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

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ABSTRACT

Aims The purpose of this study is to demonstrate that the use of recombinant early antigens for detection of antibodies to 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) which is often missed by the current ELISA assay that uses crude viral lysate antigen.

Methods At a single clinic from 1999 – 2001 a total of 4,774 serologic tests were performed in 1135 CFS patients using two immunoassays; Copalis immunoassay and ELISA immunoassay. The Copalis immunoassay utilized 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 utilized viral crude lysate as antigen for detection of both HCMV IgG and IgM.

Results Of the total, 1135 CFS patients, 517 patients (45.6%) were positive for HCMV IgG by both HCMV IgG by both assays. Of these, twelve CFS patients (2.2%) were positive for HCMV(V) IgM serum antibody by HCMV ELISA assay, and 61 CFS patients (11.8%) were positive for IgM HCMV serum antibody by Copalis assays. 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 CFS patients.

INTRODUCTION

The chronic fatigue syndrome (CFS) is a major public health problem affecting young adults between ages 35 – 65 in a ratio of four females to one male.[1] Although many groups have tried to relate CFS to different causes, the etiology of CFS still remains unknown.[2] We have studied CFS patients intensively and demonstrated that CFS is associated with long lasting chronic viral infection such as Epstein-Barr virus (EBV) and cytomegalovirus HCMV infection.[3-4] We use CDC CFS diagnostic criteria and have developed a diagnostic profile which includes several immunoassays based upon viral gene products which determine CFS subset classification for individual patients.[5-6] Three CFS subsets are recognized based on viral infection: 1) EBV subset, 2) HCMV subset, and 3) 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 polymerase chain reactions, recognition of active infection may be facilitated by serologic 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 viral infection may assist in diagnosis and differentiation of EBV infection in CFS patients.[3] In this regard, abortive Epstein-Barr virus (EBV) infection has been found in CFS patients who have elevated 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 CFS patients.

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 EBV immunoassay.[3] The only available serologic assay for HCMV infection is an enzyme linked immunosorbent assay (ELISA) utilizing crude human fibroblast tissue culture preparations of extracellular virus particles which are poorly characterized. 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 CM₂ recombinant early antigens, which are products of HCMV UL44 and UL57 genes are specific for detection of IgM antibody to HCMV in CFS patients.[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 utilized for diagnosis of prior HCMV infection in all patients, including the CFS patient.[4] In this larger study, we tested 1135 CFS patients 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 4,774 assays in 1135 CFS patients from a single clinic during the period 1999-2001. Five hundred and seventeen patients (45.6%) were positive for antibodies to IgG by both assays. Of these, only twelve CFS patients (2.2%) were positive for IgM antibody by ELISA, and 61

CFS patients (11.8%) were positive for IgM serum antibody to HCMV by recombinant CM₂ and p52 assays. We suspect that 61 CFS patients positive for IgM antibodies to HCMV by recombinant assay had abortive or incomplete HCMV infection that could not be detected by ELISA assay. Abortive herpesvirus EBV and HCMV infection may be essential to CFS patients.

METHODS

From June 11, 1999 – December 17, 2001, 1135 CFS patients 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 Hr. ECG monitor and specific serologic tests for Lyme disease, rheumatic fever and EBV were done. If the ECG was abnormal rest/stress myocardial perfusion and radionuclide ventriculography were done.[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 to HCMV IgG antibodies (Diasorin, Stillwater, MN) [17-18] 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 done. VP antigen for detection of IgG antibodies to HCMV (Diasorin) was also used in Copalis assay.[18-20] Both assays were performed in our laboratory using commercial kits, and followed manufacturer's instructions.

RESULTS

Clinical Findings

CFS patient demographics from this center have been described.[16] CFS patients with HCMV(V), HCMV(VP), CM₂ and p52 elevated serum antibody titers were clinically indistinguishable from all other CFS patients. Patients with elevated serum titers to *Borrelia burgdorferi* or others with elevated serum antibody titers 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 CM₂ (Diasorin) is indicative of IgM HCMV, and is specific for active infection.[23-29] A value > 1.3 for both rec-p52 and/or rec-CM₂ was considered positive.

Table 1 HCMV IgM Recombinant rec-p52 (UL44) and rec-CM₂ (UL44, UL57) Serum Antibody Titers in 1135 Patients with Chronic Fatigue Syndrome, 1999-2002 From an Infectious Diseases Practice

Serum HCMV Antibody Titers			
Cohort	rec-p52	rec-CM ₂	rec-p52,CM ₂
mean \pm SEM (4774 tests)	0.22 \pm 0.62	0.32 \pm 0.72	0.22 \pm 0.62, 0.32 \pm 0.72
number of patients* with rec-p52, CM ₂ serum antibody titers, 61 pts (5.4%) values >1.3	9 (0.8%) pts	40 (3.5%) pts	12 (1.1%) pts

* Three of the 61 patients had positive serum HCMV IgM antibody titers to HCMV(V) rec= antigen: c=conventional virus lysate antigen.

In 43 random positive HCMV IgM antibody 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.

Table 2 HCMV rec-p52 (UL44) and rec-CM₂ (UL44, UL57) Serum Antibody Titers in 61 CFS Patients

Patient Cohort	HCMV				
	c-IgG(v)*	c-IgM(v)	c-(VP)	rec-IgM p52+	rec-IgM CM ₂ +
1) rec-p52 (9pts)	114 \pm 10.6**	0 pt	13.9 \pm 5.4	2.2 \pm 0.41	
2) rec-CM ₂ (40 pts)	104 \pm 6.2	3 pts	9.4 \pm 1.7		2.4 \pm 0.29
3) rec-p52,CM ₂ (12 pts)	130 \pm 16.4	0 pt	13.9 \pm 5.4	2.4 \pm 0.22	3.1 \pm 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 \pm SEM

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, nine patients were p52 positive, 40 patients CM₂ positive, and 12 patients 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 etiology of CFS still remains unknown. There is strong evidence that CFS is associated with chronic infections including HCMV, EBV, HH6, and other infections. We have previously demonstrated 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 the CFS disease. 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 demonstrate 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 (HIV or transplant patients) where HCMV viral titers 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, the EBV-IgM, which indicates active infection, disappears, but, on the other hand, the 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 the viral replication as it impairs DNA synthesis. Therefore, virus may express some genes and make some protein products, but not fully replicate.[33] Here, we demonstrate 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. As demonstrated here, 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 in these patients.

Of 517 HCMV(V) IgG positive CFS patients, 12 patients (2.2%) were positive for IgM antibody by ELISA assay, but 61 CFS patients (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 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 CM₂ is a chimeric protein product of fused UL44 and UL57 genes, which markedly increases sensitivity of the assay. This is demonstrated here: of 61 serum HCMV recombinant IgM positive CFS patients, 9 patients were p52 positive, “40 patients were CM₂ positive” and 12 patients were positive for both combined 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 prior study p52 and CM₂ HCMV IgM serum antibody titers 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 nonstructural 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

Elevated IgM serum antibody titers 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 etiology of CFS.

TAKE HOME MESSAGE

The p52, CM₂ recombinant IgM assay to early HCMV antigens is diagnostic of abortive HCMV infection and is specific and 5 x more sensitive than the current IgM ELISA HCMV conventional virus lysate assay. These results indicate active HCMV infection in 61 CFS patients, when only 12 of the CFS patients were shown to have active HCMV infection by the current ELISA assay with crude virus lysate antigen. Abortive herpesvirus infection is etiologic to CFS.

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COMPETING INTERESTS

Drs. Lerner, Beqaj and Fitzgerald hold US patents on diagnosis and treatment of CFS.

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