Use of the Cytomegalovirus (CMV) Antigenemia Assay for the Rapid Diagnosis of Primary CMV Infection in Hospitalized Adults

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We assessed the value of the cytomegalovirus (CMV) antigenemia assay for diagnosing primary CMV infection in adults. The CMV antigenemia assay was performed for 40 patients admitted to our unit over a 2-year period with unexplained fever and suspected primary CMV infection. Nine of the 10 patients with primary CMV infection had positive CMV antigenemia assays, and the results were available within 5 hours. All 10 patients had a mononucleosis-like syndrome. All but one of the 30 other patients had negative CMV antigenemia assays. A false-positive result was obtained for a patient with systemic lupus erythematosus. Overall, the CMV antigenemia assay was 90% sensitive and 96% specific for the diagnosis of primary CMV infection. Therefore, the CMV antigenemia assay appears to be a simple, rapid, inexpensive test for the diagnosis of primary CMV infection in hospitalized adults.

Primary cytomegalovirus (CMV) infection is usually asymptomatic in immunocompetent persons but may manifest as CMV mononucleosis in ~10% of adults and is characterized by fever, liver function test abnormalities, lymphocytosis with atypical lymphocytes, and a usually mild and self-limiting course [1]. However, severe primary CMV infection in immunocompetent individuals can result in multiorgan dysfunction and is associated with a high mortality rate [2, 3].

The diagnosis of CMV mononucleosis can be difficult [1]. Patients with primary CMV infection are often hospitalized with a wide variety of clinical diagnoses such as prolonged fever of unknown origin, and these patients may undergo unnecessary and potentially dangerous diagnostic procedures.

The CMV pp65 antigenemia assay was recently proposed as a reliable and sensitive method for the diagnosis of CMV disease in immunocompromised patients [4]. The purpose of our study was to assess the value of the CMV antigenemia assay for diagnosing CMV mononucleosis in hospitalized adults, most of whom were immunocompetent.

Patients and Methods

Patients

During the study period (1 February 1995 to 31 January 1997), 40 patients with unexplained fever were admitted to our unit, and the CMV antigenemia assay was performed on admission because primary CMV infection was suspected. Six patients with HIV infection who enrolled in this study were known to be negative for IgG antibodies to CMV on admission. Primary CMV infection was diagnosed by using the following criteria: (1) the presence of a mononucleosis-like syndrome characterized by fever, increased liver enzyme levels (serum alanine aminotransferase level, $\geq$40 U/L) and lymphocytosis with atypical lymphocytes on blood smears; (2) the presence of specific IgM antibodies to CMV or a fourfold change in titers of IgG antibodies to CMV; and (3) lack of serological evidence of an ongoing primary Epstein-Barr virus (EBV) infection, as indicated by the absence of antibodies to EBV or by the presence of IgG antibodies to EBV capsid antigen (EB-VCA) and to Epstein-Barr nuclear antigen (EBNA).

CMV Antigenemia Assay

The CMV antigenemia assay was performed with a commercially available kit (CINAkit; Argene Biosoft, Varilhes, France) according to the manufacturer's instructions. Polymorphonuclear leukocytes (PMNLs) were separated from 5 mL of heparinized blood by dextran sedimentation. After lysis of contaminated erythrocytes, a PMNL suspension containing 2×10^5 cells was cytocentrifuged, formaldehyde fixed, and processed in an indirect immunofluorescence stain with a combination of monoclonal antibodies (AC3 + AYM-1) to the CMV protein pp65 (as the primary antibody) and a fluorescein-conjugated F (ab′)2 goat antibody to mouse IgG and IgM (as the secondary antibody). CMV pp65–positive cells were counted under a fluorescence microscope by using a ×400 magnification. Positively stained leukocytes were easy to detect on the basis of an intense green nuclear fluorescence. The results were expressed as the number of positive cells per 2×10^5 leukocytes examined.

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Assays for CMV Antibodies

IgG antibodies to CMV were detected with an EIA (Enzygnost Anti-CMV/IgG; Behring, Marburg, Germany), according to the manufacturer's instructions. Antibody titers were determined by calculation with the alpha-method. A significant increase in IgG antibodies to CMV was defined as a fourfold or more increase in titer in a pair of samples tested in the same run. IgM antibodies to CMV were detected with use of an EIA (Enzygnost Anti-CMV/IgM; Behring), according to the manufacturer's instructions.

Assays for Antibodies to EBV

Tests for IgG antibodies to EB-VCA and EBNA and for IgM antibodies to EB-VCA were performed by using ELISAs (Clin-ELISA VCA IgM, VCA IgG, and EBNA IgG; Bio-Rad, Ivry sur Seine, France), according to the manufacturer's instructions.

Results

Patients with Primary CMV Infection

During the study period, a diagnosis of CMV mononucleosis was made for 10 patients (table 1). There were seven men and three women, and the mean age was 38 years (range, 28–68 years). Seven of the 10 patients had no evidence of immunosuppression. Two patients were infected with HIV, and one was receiving immunosuppressive therapy with azathioprine for Crohn's disease. Fever was a constant clinical finding and varied in duration from 3 days to 30 days (median duration, 17 days). All patients had atypical lymphocytosis and elevated serum transaminase levels. The course was self-limiting for nine patients. One HIV-infected patient (patient 7) had a severe primary CMV infection and recovered only after treatment with ganciclovir. Examination of a liver biopsy specimen from this patient with use of specific immunoperoxidase staining for CMV showed multiple typical CMV inclusions.

Blood samples were obtained from these patients 3–32 days after the onset of fever (median time, 13 days). IgM antibodies to CMV were detected during the acute phase of illness in nine of 10 patients. For the single patient in whom IgM antibodies to CMV were not detected, the titer of IgG antibodies to CMV increased from zero during the acute phase of illness to 4,800 U/L during convalescence. Five of 10 patients also had fourfold increases in titers of IgG antibodies to CMV. Only single serum samples were available for analysis from the five remaining patients. Serological data were consistent with a previous EBV infection (i.e., presence of VCA-specific IgG and antibodies to EBNA) for all but one patient. That patient did not have antibodies to EBV (VCA-specific IgG and IgM).

Results of CMV antigenemia assays were available within 5 hours after blood was obtained from these patients. The assays were positive for nine (90%) of 10 patients; the median number of positive cells was 9 per 200,000 cells examined (range, 1–650 positive cells). The levels of CMV antigen were low (<20 positive cells) in seven of nine patients. IgM antibodies to CMV were not detected in the patient with a negative CMV antigenemia assay.

Patients Without Primary CMV Infection

Of the 30 patients without CMV mononucleosis, four were infected with HIV (table 2). Final diagnoses included a wide

Table 1. Characteristics of patients with primary cytomegalovirus infection.

Patient no.	Age (y)/ sex	Previous immunosuppression	Time of blood sampling (d)*	Titer of IgG antibodies to CMV (U/L)	IgM antibodies to CMV	CMV antigenemia†	IgG antibodies to EB-VCA	EBNA
1	39/M	None	32	1,600	+	16	+	+
			60	6,300				
2	32/M	None	8	1,500	+	1	+	+
3	68/M	None	12	3,600	+	9	+	+
4	28/F	Azathioprine therapy	11	0	+	2	+	+
5	35/M	None	15	9,000	+	10	+	+
6	35/M	None	3	0	+	3	+	+
			120	8,100				
7	40/M	HIV infection	21	0	+	650	+	+
8	34/F	None	7	0	−	0	+	+
			21	4,800				
9	41/F	HIV infection	15	0	+	3	+	+
				2,100				
10	37/M	None	18	1,600	+	45	−	−

NOTE. CMV = cytomegalovirus; EB-VCA = Epstein-Barr viral capsid antigen; EBNA = Epstein-Barr nuclear antigen; + = positive; − = negative.
* After onset of clinical disease.
† Number of antigen-positive cells per 200,000 cells examined.

variety of diseases such as viral, bacterial, and parasitic infections; autoimmune disorders; and tumors. The origin of fever was never determined for two patients. Fifteen of the 30 patients had serological test results consistent with a previous CMV infection (i.e., detection of IgG antibodies to CMV and lack of IgM antibodies to CMV). Fourteen other patients lacked both IgG and IgM antibodies to CMV. The CMV antigenemia assays were negative for these 29 patients.

One patient (patient 2, table 2), however, had a high level of CMV antigen along with IgM and IgG antibodies to CMV, suggesting primary CMV infection. Nevertheless, because of an unusual clinical course, with the development of cutaneous vasculitis, a diagnosis of systemic lupus erythematosus (SLE) was suspected and confirmed by the presence of antinuclear and anti-DNA antibodies. When we reviewed the slides, we found that blood leukocytes stained with monoclonal antibodies to the pp65 CMV protein showed only weak, noncharacteristic staining. We observed similar staining of blood leukocytes when we used fluorescein-conjugated goat antibody to mouse alone. Furthermore, repeated CMV shell vial cultures and PCR assays for detection of CMV DNA in plasma remained negative for this patient.

Discussion

Rapid laboratory confirmation of primary CMV infection remains a serious obstacle in clinical practice [3]. Serological tests are usually used for the diagnosis of CMV mononucleosis, but they have a poor sensitivity and specificity [5]. To detect a fourfold increase in the titer of IgG antibodies to CMV, pre- and postinfection sera are needed. Furthermore, the test for IgG antibodies to CMV is positive for <50% of patients with CMV mononucleosis [1]. It is also a slow procedure that only allows a diagnosis after several weeks of illness. The test for IgM antibodies has a higher sensitivity, but false-positive results may be obtained for patients with rheumatoid factors or Epstein-Barr virus infection [1], and IgM antibodies may persist for up to 1 year after initial infection [1].

Blood leukocyte cultures are considered to be highly specific for the diagnosis of CMV infection in immunocompromised

Table 2. Characteristics of patients without primary cytomegalovirus infection.

Patient no.	Age (y)/ sex	Final diagnosis	CMV antigenemia	IgG antibodies to CMV	IgM antibodies to CMV
1	58/M	Diverticulitis	−	+	−
2	42/M	Prostatitis	−	−	−
3	58/F	Rickettsial infection	−	+	−
4	53/F	Salmonellosis	−	−	−
5	61/M	Q fever	−	−	−
6	25/F	Cat-scratch disease	−	−	−
7	59/F	Tuberculosis	−	+	−
8	33/M	Tuberculosis*	−	−	−
9	69/F	*Mycobacterium avium* infection	−	+	−
10	39/M	Infectious mononucleosis	−	−	−
11	23/M	Primary HIV infection	−	+	−
12	30/M	Primary HIV infection	−	+	−
13	43/M	Acute hepatitis B	−	+	−
14	26/M	Acute hepatitis B	−	+	−
15	51/M	Yellow fever	−	−	−
16	36/M	Dengue	−	+	−
17	23/M	Schistosomiasis	−	−	−
18	43/M	Malaria	−	+	−
19	30/M	*Pneumocystis carinii* pneumonia*	−	−	−
20	33/F	Systemic lupus erythematosus	+	+	+
21	48/M	Cerebral vasculitis	−	+	−
22	27/M	Sarcoidosis	−	−	−
23	34/M	Crohn's disease	−	+	−
24	48/M	Angioimmunoblastic lymphadenopathy	−	−	−
25	34/M	Lymphoma*	−	−	−
26	36/M	Drug fever*	−	−	−
27	24/M	Intravenous drug use	−	−	−
28	33/M	Factitious fever	−	+	−
29	39/M	Fever of unknown origin	−	+	−
30	36/M	Fever of unknown origin	−	+	−

NOTE. CMV = cytomegalovirus; + = positive; − = negative.
* Patient was also infected with HIV.

individuals. Of the 10 patients with CMV mononucleosis reviewed by Cohen and Corey [2], four had positive CMV blood cultures. However, to our knowledge, no propective study has been performed to assess the value of CMV viremia for the diagnosis of primary CMV infection.

Several studies have shown that the CMV antigenemia assay is superior to conventional methods for the diagnosis of CMV disease in transplant recipients and HIV-infected patients [6–15]. In these studies, the CMV antigenemia assay was more sensitive than the shell vial culture and serological assays [6–16]. CMV antigen detection has an overall sensitivity of 85%–100% for diagnosing CMV infection in these immunocompromised patients. Moreover, the assay is superior to conventional culture techniques and serological methods. CMV antigenemia usually precedes the clinical manifestations of CMV disease, whereas blood cultures become positive and antibody responses appear after the onset of CMV disease [8, 10, 17]. The CMV antigenemia assay has other advantages over culture techniques; it is relatively simple to perform and provides results with negligible delay [7]. Furthermore, quantitation of CMV antigenemia may also provide an estimate of the viral load, which might be helpful in evaluating the efficacy of antiviral therapy [14].

On the other hand, only limited data regarding the value of the CMV antigenemia assay for the diagnosis of primary CMV infection are available [4]. Our study showed that the CMV antigenemia assay had good sensitivity for diagnosing CMV mononucleosis, since it was positive for nine of 10 patients. However, three of the 10 patients with primary CMV infection in our study were immunocompromised and may have been more likely than immunocompetent persons to have a positive test. Although we did not perform cultures or PCR to confirm the diagnosis, the clinical and laboratory features of these 10 patients were highly suggestive of CMV mononucleosis.

In addition, the sensitivity of the CMV antigenemia test was similar to that of detecting IgM antibodies to CMV, since the only patient with a negative CMV antigenemia assay also lacked IgM antibodies to CMV. The results of the CMV antigenemia assay were available within 5 hours of blood sampling, which avoided the use of unnecessary and costly diagnostic procedures. However, low levels (<20 positive cells) of CMV antigen were found in seven of nine patients. This might be the only restriction to the use of antigenemia as a marker of CMV infection, since slides need to be examined carefully to detect this low level of CMV antigen. We emphasize that technical skills are required for an accurate reading of the slides. For immunocompetent patients, the timing of sample collection with regard to the onset of fever might also be critical because CMV antigen disappears as the immune response develops [8]. On the other hand, one of our patients still had a positive CMV antigenemia assay 32 days after the onset of clinical disease.

In our study, only one of the 30 blood samples from patients without primary CMV infection was falsely positive (96% specificity). This patient had SLE, but such a false-positive result has not been observed for other patients with SLE (C. Scieux, personal data).

Recent studies indicate that PCR might be the most sensitive and specific assay for the early diagnosis of CMV infection [8, 10, 11, 17, 18]. In one study, CMV DNA was detected in 33 of the 35 initial serum samples from immunocompetent patients with active CMV infection [18]. However, the CMV PCR assay is not yet standardized, and its cost is approximately fivefold higher than that of the CMV antigenemia assay. On the contrary, the CMV antigenemia assay is commercially available and does not require sophisticated laboratory equipment. We therefore think that CMV antigenemia appears to be the most appropriate biological marker for the rapid diagnosis of primary CMV infection in hospitalized adults with CMV mononucleosis-like syndromes. Additional studies are needed to determine whether our findings may be generalized to outpatients or children.

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