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Human Cytomegalovirus (HCMV) Encephalitis in an Immunocompetent Young Person and Diagnostic Reliability of HCMV DNA PCR Using Cerebrospinal Fluid of Nonimmunosuppressed Patients

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Human cytomegalovirus (HCMV) encephalitis in adult nonimmunosuppressed patients has rarely been reported. We have diagnosed HCMV encephalitis in an anti-HCMV immunoglobulin G-negative, nonimmunosuppressed young woman by HCMV DNA PCR and virus isolation from cerebrospinal fluid (CSF). At the same time, HCMV antigen and HCMV DNA could be demonstrated in peripheral blood leukocytes, and the virus was isolated in fibroblast cultures. After 22 days of acute illness, the virus disappeared from the CSF. Remarkably, the patient did not generate detectable anti-HCMV antibodies within 5 months after the beginning of illness. To investigate the significance of HCMV DNA detection in CSF, samples of CSF, blood cells, and serum from 35 nonimmunosuppressed patients with various neurological disorders (but no herpes simplex virus central nervous system [CNS] disease) were tested for HCMV DNA, antigen, and antibodies. Eleven of these patients were found to be positive for virus DNA and/or antigen in peripheral blood leukocytes. Additionally, HCMV DNA was detected in the CSF of two patients with noninflammatory CNS diseases. A causative role of HCMV in the CNS diseases of these two patients was not evident. In summary, HCMV DNA amplification from CSF samples is a very suitable method to verify HCMV-associated encephalitis, but it should be taken into consideration that there are few cases of positive PCR with DNA from CSF without any known clinical correlative.

Human cytomegalovirus (HCMV)—like herpes simplex virus types 1 and 2 and varicella-zoster virus—can infect the parenchyma in the central nervous system (CNS) and peripheral nervous system. HCMV-caused meningoencephalitis is a common complication in newborns congenitally infected with the virus and in adults with a cellular immunodeficiency, e.g., transplant recipients and AIDS patients (15, 22).

In nonimmunocompromised patients, however, HCMV encephalitis has rarely been reported (4, 19–21). In these HCMV encephalitis cases, the clinical presentation was not uniform and specific changes in cerebrospinal fluid (CSF) did not always occur. No specific antibody pattern in CSF or serum could be associated with HCMV infection of the CNS. More recently, highly sensitive PCR conducted with CSF or brain biopsy specimens has been demonstrated to be suitable for rapid diagnosis of HCMV-associated CNS infections (5, 6, 11, 13, 27, 30, 34). In two studies, CSF from control groups of healthy, immunocompetent persons was investigated and found to be HCMV DNA negative (5, 30).

Our study concerns a case of HCMV encephalitis in a young female who was diagnosed by amplification of HCMV DNA and virus isolation from CSF as well as from peripheral blood cells. Remarkably, the patient eliminated the viral infection without generating any detectable HCMV-specific antibody response. In a parallel prospective study, we screened CSF and blood leukocytes from a number of patients with various nonviral CNS disorders for HCMV DNA and antigen as well as HCMV-specific antibodies.

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MATERIALS AND METHODS

Patients. CSF and blood specimens from a 23-year-old female student with clinical signs of acute encephalitis were investigated for HCMV DNA and antigen. In addition, CSF and blood samples of 35 neurological patients without any evidence of immunodeficiency were screened for HCMV DNA and pp65 antigen. Twelve of the patients were males, and 23 were females. They ranged in age from 24 to 77 years (median, 49 years; mean, 47 years). Four patients had infectious diseases of the CNS (none had herpes simplex or varicella-zoster virus infection), 8 suffered from suspected autoimmunological CNS inflammation, 3 had cerebrovascular disorders, 3 had idiopathic facial-nerve palsy, 3 had degenerative diseases of the CNS, 10 had disorders of the peripheral nervous system or myopathy, and 4 had tension headache or psychogenic pain syndromes.

Examination of CSF. CSF specimens were examined for cell count, cytology (Pappenheim stain), glucose, lactate, and protein concentration. Isoelectric focusing for the detection of oligoclonal immunoglobulin G (IgG) bands and determination of albumin and immunoglobulin quotient and possible intrathecal immunoglobulin synthesis were undertaken according to the methods of Reiber (26).

HCMV DNA isolation and PCR. DNA from 200 μl of CSF was isolated by proteinase K digestion (100 μg/ml for 1 to 2 h at 65°C) followed by phenol-chloroform extraction and ethanol precipitation. Alternatively, phenol-chloroform extraction was replaced with QIAamp blood kit columns (QIAGEN GmbH, Hilden, Germany). The DNA was then resuspended in 50 μl of Tris-EDTA buffer. For PCR, 10 μl of DNA solution, corresponding to 40 μl of CSF, was added per 50 μl of reaction mixture.

Mononuclear cells (peripheral blood lymphocytes [PBL]) were isolated from 5 ml of citrated blood by standard density gradient centrifugation and washed in phosphate-buffered saline, as described elsewhere (24). DNA from PBL was prepared by using the QIAGEN QIAamp blood kit; for PCR, 10 μl of DNA solution per 50 μl of reaction mixture was used.

The primers used for PCR were complementary to the coding region for the pp65 lower matrix protein generating a 132-bp fragment (12). The reaction mixture contained a 200 μM concentration of each deoxynucleoside triphosphate, 1× PCR buffer II, 2 mM $MgCl_2$, and 2.5 U of *Taq* polymerase (all from the Perkin-Elmer Corp., Norwalk, Conn.). Routinely, 40 cycles of amplification

were carried out in the following order: denaturation at 94°C for 30 s, primer annealing at 55°C for 30 s, and primer extension at 72°C for 1 min. The lower detection threshold was found to be at least 50 to 100 HCMV DNA copies per reaction. The amplification products were analyzed on a 2% agarose gel. The specificity of the PCR products was verified by Southern hybridization with the internal oligonucleotide probe CS3, terminally labeled with [γ-^{32}P]ATP (ICN Pharmaceuticals, Irvine, Calif.) (12).

Virus culture. For virus isolation, CSF or PBL were cocultivated with human embryonal lung fibroblasts (Fi301, 12th passage) and incubated at 37°C in Dulbecco's modified Eagle's medium containing 7.5% fetal calf serum. Medium was changed every 4 days.

Antigen detection. HCMV pp65 antigen in peripheral blood mononuclear cells was detected by centrifugation culture and indirect immunofluorescence with the monoclonal antibodies CMV-C10 and CMV-C11 (Clonab-CMV; Biotest, Dreieich, Germany), as described earlier (24), by immunocytology with monoclonal antibodies corresponding to immediate-early antigens (E13; Pasel & Lorei, Frankfurt, Germany) and early antigens (C10 and C11, Clonab-CMV [Biotest], and the CCH2-DDG9 mixture [Dakopatts, Copenhagen, Denmark]) and the alkaline phosphatase–anti-alkaline phosphatase (APAAP) technique (DAKO, Hamburg, Germany) for staining (10) or by immunocytological testing with the CMV-vue kit (INCSTAR, Stillwater, Minn.).

HCMV serology. Serum and CSF IgM and IgG anti-HCMV antibody concentrations were determined by enzyme-linked immunosorbent assay techniques (ETI-CYTOK-G, ETI-CYTOK-G Plus, and ETI-CYTOK-M [Sorin Biomedica, Saluggia, Italy] and CMV-IgM ELA assay [Medac, Hamburg, Germany]).

Flow cytometry. For quantification of circulating activated memory T cells, the expression of CD11a, CD57, and HLA-DR (fluorescein isothiocyanate labeled) on $CD8^+$ (phycoerythrin labeled) T-cell receptor α/β^+ (tricolor labeled) molecules was measured by flow cytometry (all from Becton Dickinson, Heidelberg, Germany).

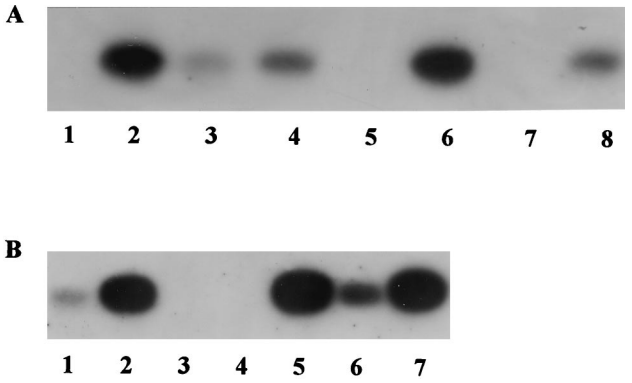

FIG. 1. Results of Southern hybridization of PCR products from CSF collected from the study patient on different days after hospitalization. DNA fragments were separated on a 2% agarose gel, transferred to a nylon membrane, and hybridized with the radiolabeled internal oligonucleotide CS3 according to the method of Gozlan et al. (12). (A) Lanes 1 and 7, buffer control; lane 2, 0.1 pg of AD169 control DNA; lane 3, 0.01 pg of AD169 control DNA; lane 4, positive control (CSF from an HCMV-infected child); lane 5, negative control (human placental DNA); lanes 6 and 8, DNA from 40 μl of CSF collected on day 1 after hospitalization. (B) Lane 1, 0.01 pg of AD169 control DNA; lane 2, 0.1 pg of AD169 control DNA; lane 3, negative control (human placental DNA); lane 4, buffer control; lane 5, DNA from CSF collected on day 8 after hospitalization; lane 6, DNA from CSF collected on day 17 after hospitalization; lane 7, DNA from PBL collected on day 17 after hospitalization.

RESULTS

HCMV encephalitis in a nonimmunosuppressed young woman. A formerly healthy, human immunodeficiency virus (HIV)-negative 23-year-old female student had suffered from upper respiratory infection for several days when she developed drowsiness, headache, and nausea. She then had at least six generalized epileptic seizures within several hours and was admitted to the hospital of the Charité Medical School, Berlin, Germany.

On the day of admission, she was awake and agitated but not oriented. She did not show meningism or any focal neurological signs. Her body temperature was 38.5°C. Cranial computed tomography, cranial magnetic resonance imaging (MRI), and cerebral angiography were normal at admission. On day 8, MRI showed temporomesial edema on both sides. On days 33 and 100, MRI showed slight temporal atrophy and hyperintense temporomesial lesions—probably gliosis—on both sides.

CSF examination showed a normal leukocyte count and normal protein and glucose concentrations on day 1, but it showed mild temporarily mononuclear pleocytosis on day 3 (17 megaparticles [Mpt]/liter) and on day 8 (38 Mpt/liter). Oligoclonal bands never appeared. Electroencephalography initially showed generalized slowing and increased irritability but did not reveal focal signs.

Although anticonvulsant drugs were administered immediately, the patient continued to have primarily generalized seizures and, after some days, also focal temporal-lobe seizures with secondary generalization, which merged into status epilepticus. From day 7 to day 39 after admission, the patient was ventilated on a respirator. On day 11, she began to be treated with barbiturate for coma for several weeks. The patient improved continuously, but 7 months after the commencement of the illness she still suffered from amnestic and concentration deficits and from drug-resistant, complex focal epilepsy.

HCMV-specific DNA in the CSF was detected by PCR on days 1, 8, and 17 after hospitalization (Fig. 1A, lanes 6 and 8; Fig. 1B, lanes 5 and 6). At the time of hospitalization, the PBL of the patient were found to be positive for HCMV antigen by immunocytology (the APAAP technique), by which HCMV immediate-early antigen as well as early antigen could be detected directly in fixed PBL. After immunocytological staining, 35 HCMV-antigen-positive cells were counted per 10^5 PBL. Infectious virus could be isolated on Fi301 cells from CSF collected at day 17 and from PBL obtained at day 34 after hospitalization. The virus was successfully passaged in fibroblast cultures. In Fi301 cells, the "CSF virus" and the "PBL virus" formed detectable cytopathic effects on days 20 and 14 postinfection, respectively; the sizes of cytopathic effects generated in the cell layer by the CSF virus were found to be smaller than those generated by the PBL virus. Nevertheless, in a comparison of the PCR-amplified "a" sequences of their genomes by restriction enzyme digestion (23), the viruses were found to be indistinguishable (data not shown).

Although the patient had not been treated with either ganciclovir or foscarnet, by day 22 after hospitalization the virus had been eliminated from the CSF but not from peripheral blood. HCMV DNA was verified in the patient's PBL for at least 4 months after the acute illness (data not shown). Anti-HCMV serum or CSF antibodies (IgM and IgG) were not detected on the day of hospitalization, during the acute phase of the illness, or 5 months after recovery. Furthermore, the patient did not show the typical signs of T-cell response in peripheral blood (cf. references 9 and 16) during follow-up, such as expansion of activated memory-type $CD8^+$ T cells ($CD8^+$ $CD11a^{bright}$ $CD57^{+/-}$ $HLA\text{-}DR^+$).

HCMV in nonimmunosuppressed patients with various neurological disorders. Thirty-five neurological patients were included in a parallel study whose purpose was to search for anti-HCMV IgG, HCMV antigenemia, and the presence of HCMV DNA in CSF and/or blood (Table 1). PBL from 32 patients were tested by PCR; in 11 cases (34%), HCMV DNA could be amplified. In addition, PBL from 4 (15%) of 26 patients tested for this parameter were also found to be HCMV antigen positive. Surprisingly, for 2 of the 35 patients we also found HCMV DNA in the CSF. One was a female patient with essential myoclonia, and the other was a woman with tension headache. Whereas both patients were also pos-

TABLE 1. HCMV monitoring of patients with neurological disorders other than HCMV encephalitis

Diagnosis	Anti-HCMV IgG in serum[a]	No. of patients	No. of patients positive/no. of patients examined		
			CSF HCMV PCR	Antigenemia[b]	PBL HCMV PCR
Meningitis or encephalitis	+	3	0/3	1/2	3/3
	−	1	0/1	0/1	0/1
Autoimmunologic CNS diseases	+	5	0/5	0/3	1/4
	−	3	0/3	1/3	1/3
Cerebrovasular disorders	+	2	0/2	0/1	0/1
	−	1	0/1	ND	0/1
Idiopathic facial-nerve palsy	+	1	0/1	0/1	0/1
	−	2	0/2	0/1	0/2
Sporadic essential myoclonia	−	1	1/1	1/1	1/1
Dengerative CNS diseases	+	1	0/1	0/1	0/1
	−	1	0/1	0/1	0/1
Peripheral nervous system or muscle disorders	+	3	0/3	0/3	1/3
	−	7	0/7	1/6	2/7
Tension headache or psychogenic	+	2	1/2	0/1	2/2
	−	2	0/2	0/1	0/1
Total[c]		35	2/35	4/26	11/32

[a] No anti-HCMV IgM antibodies were found.
[b] Detection of HCMV pp65 antigen in PBL by centrifugation culture or by immunocytological testing with the CMV-vue kit (INCSTAR). ND, not determined.
[c] The percentages of patients found to be positive by CSF PCR, for antigenemia, and by PBL PCR were 6, 15, and 34%, respectively.

itive for HCMV DNA in PBL, HCMV antigen could be detected only in the PBL of the patient with essential myoclonia. Interestingly, no antibodies were detected in the latter patient, while the patient with tension headache had IgG antibodies but was negative for HCMV antigen in the blood. For both patients, no HCMV-specific IgM antibodies could be obtained. CSF examination of these two patients did not show any signs of inflammation or disturbance of the blood-CSF barrier (Table 2). The cell counts in the CSF of the remaining 33 patients ranged between 0 and 213 Mpt/liter, with a mean value of 12.6 Mpt/liter.

DISCUSSION

CMV encephalitis occurs very rarely in immunocompetent adults and does not seem to be associated with a defined clinical syndrome (4, 17, 19–21, 30). Therefore, diagnosis depends on intrathecal evidence of HCMV infection. The low sensitivity of virus culture techniques made this diagnosis very difficult.

The CSF in our patient did not show signs of inflammation, except a very mild pleocytosis on days 3 and 8 which could also have been due to the patient's being in status epilepticus (2). Diagnosis of CMV encephalitis was based on repeated amplification of virus-specific DNA fragments from CSF. Furthermore, virus-specific antigen and DNA could be detected in PBL by the APAAP technique, centrifugation culture, and PCR. The virus could be isolated from both peripheral blood and CSF on human embryonal lung fibroblasts. Absence of any detectable HCMV-specific antibodies may indicate a primary HCMV infection. However, there is some evidence that a small number of latently HCMV-infected people do not exhibit measurable antibody response (references 3, 28, 29, and 32 and our own unpublished data). Surprisingly, after 3 weeks of hospitalization the virus was eliminated from the CSF of our patient without specific antiviral therapy, and we did not detect CMV-specific antibodies until 5 months after the onset of the illness. This corresponds with the two of four PCR-positive patients reported by Studahl et al. (30) who were also negative for CMV-specific antibodies and with a patient reported by Pantoni et al. (19) who had only a low antibody titer in serum.

Patients with active systemic viral infection, including active HCMV infection, commonly show expansion of activated memory $CD8^+$ T cells. Initially, HLA-DR^+ $CD11a^{bright}$ $CD8^+$ T cells dominate; later on, the phenotype switches to HLA-$DR^{+/-}$ $CD11a^{bright}$ $CD57^+$ $CD8^+$. Up to 80% of activated $CD8^+$ T cells express this phenotype (9, 16). Interestingly, this typical T-cell response did not develop in our patient at any time. This means that the HCMV infection might have been eliminated only by nonspecific mechanisms, such as natural killer cells or interferon, or by a locally restricted cellular immune system.

One explanation for the clinical course of our patient may be that the virus was already present in latent form before the acute episode. HCMV reactivation might be induced by tumor necrosis factor alpha (cf. references 8 and 25) in the framework of the respiratory infection preceding the CNS symptoms. The selective defect in the specific immune response to HCMV might have allowed the spreading of the reactivated virus in the patient. Since the patient did not show any signs of immunodeficiency, the selective defect might have been caused by a tolerance phenomenon as a result of intrauterine CMV contact or to a genetically determined gap in the T-cell-receptor repertoire.

To verify the diagnostic reliability of HCMV DNA PCR for examination of CSF, we studied CSF and PBL from an independent control group of 35 nonimmunocompromised neurologic patients. None of the patients exhibited any signs of systemic or CNS inflammation. Eleven of 32 patients showed signs of an active HCMV infection outside the CNS, as determined by the detection of HCMV antigen and/or DNA in PBL. This rate was significantly higher (31 versus 7%) than that observed in healthy blood donors (8). In addition, HCMV DNA could be amplified from the CSF of two patients—one with essential myoclonia and one with tension headache. Because these patients were not admitted, we only had one CSF sample for our investigation. Contamination of CSF by HCMV DNA-positive peripheral blood cells could be ruled out, since the number of leukocytes in the CSF was below 5 Mpt/liter.

TABLE 2. Diagnostic parameters for two patients positive for HCMV DNA in CSF

Patient		Diagnosis	CSF leukocyte count (Mpt/liter)	Albumin ratio (CSF/serum), 10^3	Oligoclonal bands	Test result for HCMV		
Gender	Age (yr)					Antigenemia	DNAemia	IgG in serum
Female	36	Sporadic essential myoclonia	3	3.8	−	+	+	−
Female	60	Tension headache	2	3.9	−	−	+	+

Furthermore, it is highly unlikely that carryover contamination occurred in this study for the following reasons: (i) use of negative controls in each test run; (ii) physical separation of specimen preparation, amplification, and analysis steps in PCR; and (iii) correlation of antigenemia with PCR results.

In similar studies by other authors, HCMV DNA could not be amplified from the CSF of anti-HIV-negative control subjects (1, 5, 7, 31), though in a prospective study Studahl et al. (31) detected HCMV DNA in the CSF of 5 of 64 seropositive patients with suspected viral infection of the CNS. Interestingly, clinical data suggested HCMV-associated neurological disease in only 4 of these 5 patients. The remaining patient was a 17-year-old boy with cerebral atrophy. Unlike our 2 patients with HCMV DNA-positive CSF, this patient was reported to have no markers of an active HCMV infection outside the CNS. Gozlan et al. (14) found 4 patients positive by CSF PCR in a group of 62 anti-HIV-positive patients with a normal $CD4^+$-cell count and a firm diagnosis of non-HCMV disease. Power et al. (22) could demonstrate HCMV genomic material in brain biopsy specimens of 2 of 46 patients by in situ hybridization. One of these patients suffered from intractable epilepsy secondary to mesial temporal sclerosis, and the other had human T-cell lymphotropic virus type 1 myelopathy. Furthermore, HCMV DNA has frequently been found in brain biopsy specimens from patients with chronic Rasmussen's encephalitis (18, 22); however, a causative role of HCMV or Epstein-Barr virus in this chronic CNS disease has been called into question (33).

Nevertheless, the highly sensitive PCR-mediated detection of HCMV DNA in CSF remains a suitable method for ante mortem diagnosis of HCMV-related neurological diseases as shown in our and many other laboratories, despite the fact that in a small number of cases positive PCR could not be correlated with an HCMV-associated CNS disease. The clinical significance of these findings remains unclear and needs further prospective evaluation. A positive HCMV PCR result with the CSF of immunocompetent patients requires careful interpretation with regard to the individual clinical context.

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REFERENCES

1. **Arribas, J. R., D. B. Clifford, C. J. Fichtenbaum, D. L. Comins, W. G. Powderly, and G. A. Storch.** 1995. Level of cytomegalovirus (CMV) DNA in cerebrospinal fluid of subjects with AIDS and CMV infection of the central nervous system. J. Infect. Dis. **172:**527–531.
2. **Barry, E., and A. Hauser.** 1994. Pleocytosis after status epilepticus. Arch. Neurol. **51:**190–193.
3. **Bevan, I. S., R. A. Daw, P. J. R. Day, F. A. Ala, and M. R. Walker.** 1991. Polymerase chain reaction for detection of human cytomegalovirus infection in a blood donor population. Br. J. Haematol. **78:**94–99.
4. **Chin, W., R. Magoffin, J. G. Frierson, and E. H. Lennette.** 1973. Cytomegalovirus infection. A case with meningoencephalitis. JAMA **225:**740–741.
5. **Cinque, P., L. Vago, M. Brytting, A. Castagna, A. Accordini, V. A. Sundqvist, N. Zanchetta, A. D. Monforte, B. Wahren, A. Lazzarin, and A. Linde.** 1992. Cytomegalovirus infection of the central nervous system in patients with AIDS: diagnosis by DNA amplification from cerebrospinal fluid. J. Infect. Dis. **166:**1408–1411.
6. **Cinque, P., L. Vago, H. Dahl, M. Brytting, M. R. Terreni, C. Fornara, S. Racca, A. Castagna, A. D. Monforte, B. Wahren, A. Lazzarin, and A. Linde.** 1996. Polymerase chain reaction on cerebrospinal fluid for diagnosis of virus-associated opportunistic diseases of the central nervous system in HIV-infected patients. AIDS **10:**951–958.
7. **Clifford, D. M., R. S. Buller, S. Mohammed, L. Robison, and G. A. Storch.** 1993. Use of polymerase chain reaction to demonstrate cytomegalovirus DNA in CSF of patients with human immunodeficiency virus infection. Neurology **43:**75–79.
8. **Döcke, W. D., S. Prösch, E. Fietze, V. Kimel, H. Zuckermann, C. Kluge, U. Syrbe, D. H. Krüger, R. von Baehr, and H. D. Volk.** 1994. Cytomegalovirus reactivation and tumor necrosis factor. Lancet **343:**268–269.
9. **Döcke, W. D., P. Reinke, G. Staffa, U. Settmacher, T. Höger, J. Groth, K. Lang, K. U. Lock, S. Prösch, V. Kimel, H. Warnke, R. von Baehr, and H. D. Volk.** 1994. An immune monitoring program for the management of immunosuppressive therapy in the early phase after transplantation. Transplantationsmedizin **6:**13–28.
10. **Fietze, E., S. Prösch, P. Reinke, J. Stein, W. D. Döcke, G. Staffa, S. Loening, S. Devaux, F. Emmrich, R. von Baehr, D. H. Krüger, and H. D. Volk.** 1994. Cytomegalovirus infection in transplant recipients: the role of tumor necrosis factor. Transplantation **58:**675–680.
11. **Flood, J., W. L. Drew, R. Miner, D. Jekic-McMullen, L. P. Shen, J. Kolberg, J. Garvey, S. Follandsbee, and M. Poscher.** 1997. Diagnosis of cytomegalovirus (CMV) polyradiculopathy and documentation of in vivo anti-CMV activity in cerebrospinal fluid by using branched DNA signal amplification and antigen assay. J. Infect. Dis. **176:**348–352.
12. **Gozlan, J., F. Caburet, C. Tancrede, and J. C. Petit.** 1992. A reverse polymerase chain reaction method for detection of human cytomegalovirus late transcripts in cells infected in vitro. J. Virol. Methods **40:**1–10.
13. **Gozlan, J., J. M. Salord, E. Roullet, M. Baudrimont, F. Caburet, O. Picard, M. C. Meyohas, C. Duvivier, C. Jacomet, and J. C. Petit.** 1992. Rapid detection of cytomegalovirus DNA in cerebrospinal fluid of AIDS patients with neurologic disorders. J. Infect. Dis. **166:**1416–1421.
14. **Gozlan, J., M. E. Amrani, M. Baudrimont, D. Costagliola, J. M. Salord, C. Duvivier, O. Picard, M. C. Meyohas, C. Jacomet, V. Schneider-Fauveau, J. C. Petit, and E. A. Roullet.** 1995. A prospective evaluation of clinical criteria and polymerase chain reaction assay of cerebrospinal fluid for the diagnosis of cytomegalovirus-related neurological disease during AIDS. AIDS **9:**253–260.
15. **Ho, M.** 1991. Cytomegalovirus: biology and infection, 2nd ed. Plenum Publishing Corp., New York, N.Y.
16. **Kern, F., S. Ode-Hakim, H. Nugel, K. Vogt, H. D. Volk, and P. Reinke.** 1996. Peripheral T cell activation in long-term renal transplant patients: concordant upregulation of adhesion molecules and cytokine gene transcription. J. Am. Soc. Nephrol. **7:**2476–2482.
17. **Mahieu, L. M., M. Ieven, K. J. van Hoeck, P. M. Parizel, and K. J. van Acker.** 1997. Diagnosis of cytomegalovirus meningoencephalitis by polymerase chain reaction in an immunocompetent infant who recovered after treatment with ganciclovir. Clin. Infect. Dis. **24:**520–521.
18. **McLachlan, R. S., J. P. Girvin, W. T. Blume, and H. Reichman.** 1993. Rasmussen's chronic encephalitis in adults. Arch. Neurol. **50:**269–273.
19. **Pantoni, L., D. Inzitari, M. G. Colao, E. De Mayo, P. Marini, and F. Mazzota.** 1991. Cytomegalovirus encephalitis in a non-immunocompromised patient: CSF diagnosis by in situ hybridization cells. Acta Neurol. Scand. **84:**56–58.
20. **Perham, T. G. M., E. O. Caul, S. K. R. Clarke, and A. G. F. Gibson.** 1971. Cytomegalovirus meningo-encephalitis. Br. Med. J. **2:**50.
21. **Phillips, A. C., W. L. Fanning, W. G. Diter, and C. V. Phillips.** 1977. Cytomegalovirus encephalitis in immunologically normal adults. JAMA **238:**2299–2300.
22. **Power, C., S. D. Poland, W. T. Blume, J. P. Girvin, and G. P. Rice.** 1990. Cytomegalovirus and Rasmussen's encephalitis. Lancet **336:**1282–1284.
23. **Prösch, S., U. Bechstein, G. Witt, V. Kimel, W. Teterin, R. Cziesnek, and D. H. Krüger.** 1994. HCMV strain comparison by RFLP analysis of genomic virus DNA and of a PCR-amplified DNA fragment. Clin. Diagn. Virol. **2:**17–28.
24. **Prösch, S., V. Kimel, I. Dawydowa, and D. H. Krüger.** 1992. Monitoring of patients for cytomegalovirus after organ transplantation by centrifugation culture and PCR. J. Med. Virol. **38:**246–252.
25. **Prösch, S., H. D. Volk, P. Reinke, K. Pioch, W. D. Döcke, and D. H. Krüger.** 1998. Human cytomegalovirus infection in transplant recipients: role of TNFα for reactivation and replication of human cytomegalovirus. Monogr. Virol. **21:**29–41.
26. **Reiber, H.** 1980. The discrimination between different blood-CSF barrier dysfunctions and inflammatory reactions of the CNS by a recent evaluation graph for the protein profile of cerebrospinal fluid. J. Neurol. **224:**89–95.
27. **Rolfs, A.** 1993. Rapid detection of cytomegalovirus structures in cerebrospinal fluid and brain biopsies by polymerase chain reaction. Acta Neurol. Scand. **88:**236–238.
28. **Söderberg-Nauclér, C., K. N. Fish, and J. A. Nelson.** 1997. Reactivation of latent human cytomegalovirus by allogenic stimulation of blood cells from healthy donors. Cell **91:**119–126.
29. **Stanier, P., D. L. Taylor, A. D. Kitchen, N. Wales, Y. Tryhorn, and A. S. Tyms.** 1989. Persistence of cytomegalovirus in mononuclear cells in peripheral blood from blood donors. Br. Med. J. **299:**897–898.
30. **Studahl, M., A. Ricksten, T. Sandberg, and T. Bergström.** 1992. Cytomegalovirus encephalitis in four immunocompetent patients. Lancet **340:**1045–1046.

31. **Studahl, M., T. Bergström, K. Ekeland-Sjöberg, and A. Ricksten.** 1995. Detection of cytomegalovirus DNA in cerebrospinal fluid in immunocompetent patients as a sign of active infection. J. Med. Virol. **46:**274–280.
32. **Taylor-Wiedeman, J., J. G. P. Sissons, L. K. Borysiewicz, and J. H. Sinclair.** 1991. Monocytes are a major site of persistence of human cytomegalovirus in peripheral blood mononuclear cells. J. Gen. Virol. **72:**2059–2064.
33. **Vinters, H. V., R. Wang, and C. A. Wiley.** 1993. Herpesvirus in chronic encephalitis associated with intractable childhood epilepsy. Hum. Pathol. **24:**871–879.
34. **Wolf, D. G., and S. A. Spector.** 1992. Diagnosis of human cytomegalovirus central nervous system disease in AIDS patients by DNA amplification from cerebrospinal fluid. J. Infect. Dis. **166:**1412–1415.