Polyradiculopathy Associated with Ganciclovir-Resistant Cytomegalovirus in an AIDS Patient: Phenotypic and Genotypic Characterization of Sequential Virus Isolates

Irene L. Smith, Mika Shinkai,* William R. Freeman, and Stephen A. Spector

To investigate the role of antiviral drug resistance in cytomegalovirus (CMV) disease progression, CMV cultured from an AIDS patient who developed progressive CMV disease despite continual anti-CMV treatment was characterized. In 7 CMV isolates, 1 ganciclovir-resistant strain predominated. Ganciclovir-resistant CMV, containing a UL97 mutation, was cultured from blood and urine before clinical indication of CMV central nervous system (CNS) disease, suggesting that the development of ganciclovir resistance preceded the dissemination of CMV to the CNS. Quantitative competitive polymerase chain reaction indicated that the isolation of ganciclovir-resistant CMV was concurrent with increased CMV DNA in plasma. The results suggest that antiviral resistance should be considered when selecting therapy for CMV CNS disease that develops in patients receiving treatment for CMV retinitis. In addition, plasma CMV DNA in AIDS patients receiving anti-CMV therapy may be a useful marker of disease progression and antiviral resistance.

Cytomegalovirus (CMV) diseases, including CMV-associated retinitis, colitis, and central nervous system (CNS) disease, are a significant cause of morbidity and mortality in persons with AIDS [1, 2]. Although CMV retinitis will usually respond to initial antiviral treatment with ganciclovir or foscarnet, retinal disease progression is common after prolonged treatment. In some cases, the progression is associated with development of resistance to antiviral drugs and concurrent disseminated CMV infection, including CNS disease (reviewed in [3]).

The use of an alternative antiviral for treatment of CMV disease that progresses during ganciclovir therapy has been advocated [4, 5]. However, because the relationship between drug resistance and dissemination of CMV disease is poorly understood, the rational development of alternative therapeutic strategies is difficult. In addition, because CMV strains may develop resistance to ganciclovir, foscarnet, and cidofovir (also known as HPMPC), an understanding of the pathogenesis of CMV dissemination in persons with AIDS is important.

As a step to understanding the relationship between the development of drug-resistant CMV and disease progression, we analyzed the clinical, virologic, and molecular aspects of CMV disease in an AIDS patient with disseminated CMV who died following 20 months of CMV treatment. Genotypic and phenotypic analyses indicated that the patient was infected with the same ganciclovir-resistant strain in his blood, urine, and cerebrospinal fluid (CSF). The presence of ganciclovir-resistant CMV was associated with an increase in the level of CMV DNA in plasma, and both the development of ganciclovir-resistant virus and an elevation in plasma DNA levels preceded the development of CMV-related CNS disease.

Material and Methods

Clinical summary (figure 1A–C). A 48-year-old white homosexual man with AIDS (37 CD4 lymphocytes/mm³) was given ganciclovir induction therapy (5 mg/kg twice daily) for CMV colitis. Shortly thereafter, CMV retinitis was identified in the patient. The retinitis was treated with and responded to the induction doses of ganciclovir. During a 5-month period, the patient underwent 3 episodes of ganciclovir induction treatment followed by maintenance therapy (5 mg/kg once daily) and was then switched to foscarnet induction therapy (90 mg/kg twice daily) followed by maintenance treatment of 120 mg/kg/day for reactivation of CMV retinitis. However, after 10 weeks, foscarnet therapy was discontinued because of drug intolerance, and ganciclovir therapy was reinstituted.

After 14 months of anti-CMV treatment, the patient had 4 CD4 lymphocytes/mm³, and retinitis progression in the right eye, and later the left eye, was not responding to ganciclovir or foscarnet treatment. Intracocular injections of cidofovir (20–40 μg) resulted in transient stabilization of retinitis; however, vision in the right eye was subsequently lost. After 17 months of anti-CMV therapy, the patient developed signs of polyradiculopathy, including pro-
gressive weakness of the lower extremities, urinary retention, and fecal incontinence. CMV was cultured from urine, blood, and CSF. The polyradiculopathy did not respond to induction doses of ganciclovir or foscarnet, and the patient died 3 months after the first symptoms of CNS disease appeared.

Cells and virus. CMV isolates cultured from peripheral blood, urine, and CSF specimens were established and maintained in human foreskin fibroblasts. Viral cultures were maintained in Dulbecco’s MEM (DMEM) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, 0.01% streptomycin, and 2.5 μg/mL amphotericin B (DMEM-10) and stored as cell-associated stocks in DMEM-20, 10% dimethyl sulfoxide under liquid nitrogen.

Polymerase chain reaction (PCR) analysis. The procedure for qualitative PCR analysis of plasma specimens has been described previously [6]. In quantitative competitive PCR (QC-PCR) reactions, plasma PCR conditions were modified by the addition of 1000 copies of a competitor DNA template. The competitor DNA sequence is identical to the fragment amplified in the qualitative plasma PCR except for the insertion of a 20-bp sequence. Fragments amplified from the internal competitor and from the CMV DNA present in the plasma were hybridized to a 32P-end-labeled probe and resolved by polyacrylamide gel electrophoresis. A standard curve was calculated using least squares analysis, and CMV genome copy number in plasma specimens was quantitated using the ratio of wild type to competitor PCR product.

Drug sensitivity. Antiviral drugs were provided by the following suppliers: ganciclovir, Roche Bioscience (Palo Alto, CA); foscarnet, Astra Pharmaceutical Products (Westborough, MA); and cidofovir, Gilead Sciences (Foster City, CA). Drug IDso values were determined by plaque reduction assays, using a 0.3% agarose overlay to inhibit secondary plaque formation, as described previously [7]. Quadruplicate wells of human foreskin fibroblasts, in 24-well cluster dishes, were infected with ~100 CMV-infected cells and maintained at ganciclovir concentrations of 1, 5, 10, 25, and 50 μM or at foscarnet concentrations of 200, 400, 600, 800, and 1000 μM. Ganciclovir and foscarnet resistance are defined by IDso values ≥9 and ≥300 μM, respectively (unpublished data).

Heterogeneity in CMV isolates. PCR and restriction fragment length polymorphism (RFLP) were used to distinguish clinical strains of CMV [8]. RsaI digestion products of a 2-kb fragment amplified from infected-cell DNA were resolved in 2% agarose.

Sequence analysis. UL97 gene sequences were determined using the fmol DNA sequencing system (Promega, Madison, WI) with 32P-end-labeled primers. A sequencing template was PCR-amplified from infected-cell DNA using primers 5’-kin-43 (5’-GGTAGCTAGTGCAGCCTTAGG-3’) and 3’-kin-2376 (5’-AGACAGACGCAGCGTGCAA-3’). Sequencing primers were spaced ~300 nt apart.

Results

Detection and quantification of CMV DNA during disease progression. Of 7 sequential plasma specimens obtained over the course of the patient’s treatment for CMV disease, 5 were positive for CMV DNA by plasma PCR analysis. Of note, each of the 5 positive specimens was drawn in the later stages of
UL97 encodes a protein with phosphotransferase activity that is required for the activation of ganciclovir, and mutations in UL97 are associated with ganciclovir resistance of clinical CMV isolates [7, 10–12]. To determine if the ganciclovir resistance of blood-, urine-, and CSF-derived CMV isolates each arose from the same genetic alteration, the UL97 gene sequence was determined. A single amino acid substitution of valine for methionine at aa 460, resulting from an alteration at nt 1378 (adenine to guanine), was detected in the carboxy-terminal region of the phosphotransferase, a substitution shown previously to confer ganciclovir resistance [11].

Discussion

Lumbosacral polyradiculopathy associated with CMV infection of the CNS occurs in ~1% of persons with AIDS [1]. The clinical presentation and diagnosis of this syndrome have been described, and strategies for treatment have been proposed [1]. Anti-CMV therapy has been found to stabilize or improve the symptoms of polyradiculopathy in 30%–50% of patients [2, 3]. Treatment failure has been associated with the development of antiviral drug resistance (reviewed in [13]). The presence of ganciclovir-resistant CMV in the CSF of patients with polyradiculopathy has been reported and typically occurs in persons who have received several months of ganciclovir treatment for CMV retinitis [4, 5, 14].

Issues critical to the development of effective treatments for CMV infections that progress to the CNS include the identification of early markers of disease progression and an understanding of the relationship between the dissemination of infection and the development of drug resistance. In this report, we described a patient with AIDS who developed progressive CMV disease, including involvement of the CNS, despite extensive antiviral treatment.

CMV isolates cultured from multiple sites, including the CSF, were ganciclovir resistant and of the same strain, as determined by RFLP analysis. Further, CMV cultured from multiple sites were found by direct sequencing to contain the same methionine-to-valine mutation at aa 460 within the phosphotransferase gene (a mutation previously shown to confer ganciclovir resistance). Quantitative PCR of plasma CMV DNA demonstrated an increase in virus load with subsequent spread to the CNS, with the level of CMV DNA in the CNS being 30-fold the level in plasma. Although the patient had recurrent CMV retinitis, CMV DNA was not detected by culture or PCR analysis of plasma during the initial reactivations. The subsequent detection of CMV DNA in plasma coincided with the appearance of ganciclovir-resistant virus.

Multiple strains of CMV and strains with different drug sensitivities can simultaneously infect persons with AIDS [9, 15]; however, the patient in this report had 1 predominant CMV strain cultured from his blood, urine, and CSF. Ganciclovir-resistant virus was isolated from this patient prior to clinical manifestations of CMV dissemination to the CNS. Therefore,
the development of drug resistance in CMV appears to have preceded virus dissemination. Detection of the same genetic alteration, known to confer ganciclovir resistance in CMV cultured from the blood, urine, and CSF, supports this supposition. Moreover, the nature of disease progression and the lack of heterogeneity in CMV isolated from different sites argues against the notion that CMV-related neuropathies may have developed from reactivation of CMV within cells of the CNS.

Others have suggested that the selection of ganciclovir-resistant CMV could occur in the CSF because ganciclovir concentrations reach only 40%–50% of corresponding plasma levels [5]. Our findings indicate that CMV levels within CSF can be many times greater than levels in plasma. Although drug resistance in this patient developed prior to infection of the CNS, high virus load within the CNS may be an additional risk factor for selection of CMV strains that are resistant to antiviral therapy.

In this report, we provide evidence that multifocal CMV disease can be attributed to the dissemination of 1 drug-resistant CMV variant. Our findings suggest that CMV resistance to antiviral drugs should be considered when patients develop CNS disease while on treatment for CMV retinitis. In addition, quantification of CMV DNA in plasma and genotypic analysis of CMV DNA in persons with AIDS may provide useful markers of CMV disease progression and emergence of antiviral resistance.

Acknowledgments

We thank T. C. Meng, Linda Meixner, and Cheryl Jarman for thorough clinical evaluations and Rhett Jiles and Carrie McNeil for excellent technical assistance.

References


