Cytomegalovirus (CMV) Retinitis Activity Is Accurately Reflected by the Presence and Level of CMV DNA in Aqueous Humor and Vitreous

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To evaluate the potential of ocular and systemic specimens to provide markers of active cytomegalovirus (CMV) retinitis, we examined the relationship between virologic and clinical aspects of CMV infections in AIDS patients with CMV retinitis. CMV polymerase chain reaction (PCR) analysis of 74 aqueous humor and vitreous specimens indicated that ocular specimens can provide accurate markers to differentiate active and inactive CMV retinitis (aqueous or vitreous PCR, \( P < .001 \)). Moreover, these markers were superior to extravascular measures, including plasma PCR (\( P = .08 \)) and blood and urine CMV cultures (\( P = .05 \)). A direct correlation was identified between the quantity of CMV DNA in aqueous humor or vitreous specimens and the corresponding surface area of active CMV retinitis (\( r^2 = .69 \) and \( .44 \), respectively). Thus, qualitative and quantitative PCR-based analyses of aqueous humor can provide valuable markers of CMV retinitis activity. Such assays could provide rapid and reliable tools to assist in management of patients with CMV retinitis in whom the view of the retina is obscured.

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Informed consent was obtained from all patients. Human experimentation guidelines of the U.S. Department of Health and Human Services and the University of California, San Diego, were followed.
Financial support: National Eye Institute (EY037366 to W. R. F.); Reprints or correspondence: Dr. Stephen A. Spector, University of California, San Diego, AIDS Ocular Research Unit. Ocular complications are common in individuals with AIDS and most frequently are the result of opportunistic viral infections. Accountable for \( \sim 95\% \) of infectious retinopathies in patients with AIDS, cytomegalovirus (CMV) is the most common AIDS-related ocular pathogen [1]. Prior to use of highly active antiretroviral therapy (HAART), up to 40% of human immunodeficiency virus (HIV)–positive individuals with CD4° cell counts of \(<50\text{ cells}/\mu\text{L}\) experienced CMV-related retinitis [2]. Although the current prevalence of CMV retinitis is lower, emerging resistance to HAART, with decline of CD4° lymphocytes below \(50\text{ cells}/\mu\text{L}\), has renewed concerns about the long-term management of CMV retinitis.

In the majority of cases, CMV retinitis can be readily diagnosed during routine, noninvasive ophthalmologic examination of the retina. However, other rarer pathogens, such as *Treponema pallidum*, varicella zoster virus, and *Toxoplasma gondii*, may cause clinical signs similar to those seen in CMV retinitis (reviewed in [1, 3]). Currently, differential diagnosis relies heavily on response to the initial treatment. A rapid and reliable assay for differentiation of active and inactive CMV retinitis could greatly assist diagnosis and reduce the delay in initiating appropriate therapy.

The objectives of this study were to determine whether CMV retinitis activity can be accurately monitored by analysis of either aqueous humor or vitreous specimens and whether the presence of CMV DNA in ocular fluids provides a marker for ocular CMV disease superior to that provided by systemic measures. If it can be determined that aqueous specimens are as sensitive and specific as vitreous specimens for identification of active CMV retinitis, then aqueous paracentesis, a safer procedure than vitreous collection and one that can be performed in an ophthalmologist’s office, could be applied readily to patients with retinitis of uncertain etiology.

Materials and Methods

Study population. The study population consisted of 27 AIDS patients with CMV retinitis who were followed at the University of California, San Diego, AIDS Ocular Research Unit. Ocular specimens included archived specimens from 4 patients and specimens from 23 consecutive patients undergoing surgery (between September 1996 and January 1998) for whom clinical evaluation and fundus photographs made possible conclusive designation of active versus inactive CMV retinitis. Surgeries were performed for implantation of a ganciclovir intraocular device (\( n = 27 \)), retinal detachment (\( n = 11 \)), cataract extraction (\( n = 3 \)), diagnostic biopsy (\( n = 1 \)), or epiretinal membrane removal (\( n = 1 \)). The population included two women and 25 men, with a median age of 40 years (range, 26–62 years). One patient had newly diagnosed CMV retinitis; the remaining 26 patients had received or were receiving systemic and/or local treatment for CMV retinitis (median systemic...
Treatment time, 13.5 months; range, 0.25–29 months). Current therapies at surgery included systemic therapy (n = 14), local therapy (implantation of a ganciclovir intraocular device or intravitreal injection of cidofovir within the preceding 5 weeks [n = 16]), or local therapy in combination with oral ganciclovir (n = 6). In 9 instances, patients were not receiving anti-CMV therapy at the time of surgery. Two patients in this study were reported as patients 1 and 2 in a previous study examining antiviral resistance [4]. The surface area of active retinitis was determined by examination of fundus photographs and reported as disc areas.

**Specimen handling.** Ocular specimens were stored at −80°C within 4 h of collection. Prior to polymerase chain reaction (PCR) analysis, vitreous and aqueous specimens were heated at 94°C for 12 min and diluted 10-fold in PCR reaction buffer. Blood and urine specimens, collected on the day of surgery, were cultured on human foreskin fibroblast cells for CMV. Plasma was stored at −20°C prior to DNA extraction [5].

**PCR.** Qualitative and quantitative competitive PCR (QC-PCR) reactions were performed according to procedures described elsewhere [6, 7]. Reaction volumes of 100 μL contained 10 mM Tris-HCl at pH 8.3, 50 mM KCl, 2.5 mM MgCl₂, 200 μM dNTPs, 2 U of Taq polymerase (Boehringer Mannheim, Indianapolis), and 50 pmol of primers 459 and 627. QC-PCR reactions contained an additional 500 copies of competitor DNA. Qualitative PCR amplification consisted of 20 cycles of 95°C for 14 s, 61°C for 1 min, and 72°C for 1 min, followed by 20 cycles of 95°C for 15 s, 61°C for 1 min, and 72°C for 1 min. PCR products were detected by use of [³²P]-labeled probe 628, gel electrophoresis, and autoradiography. Control reactions containing 1280, 320, 80, 40, 20, or 10 copies of CMV EcoR-1 fragment D indicated that sensitivity was ≤40 copies.

Qualitative and quantitative PCR reactions contained either 0.1–1.0 μL of aqueous humor or vitreous or DNA extracted from 10 μL of plasma. All samples were run in duplicate and confirmed by independent PCR analysis of a fresh aliquot of diluted specimen. Negative vitreous and aqueous PCR results were confirmed by analysis of 5 μL of undiluted specimen.

**Results**

**PCR analysis of CMV DNA in aqueous humor and vitreous specimens.** To determine whether aqueous humor and vitreous specimens can provide accurate markers for HIV-related CMV retinitis activity, qualitative CMV PCR analyses of ocular specimens from 27 patients with CMV retinitis were compared in a double-masked analysis in which clinical assessment of retinitis activity was based on clinical examination and fundus photographs. A total of 74 ocular specimens were examined; specimens were available from both eyes of 1 patient with untreated, bilateral CMV retinitis and from 33 eyes of 26 additional patients with previously treated CMV retinitis. Sequential ocular specimens were available from 9 eyes of 6 patients who underwent multiple surgeries during the study period.

Of 21 vitreous specimens from eyes with clinically apparent active retinitis, 20 (95%) were CMV PCR positive. All 16 vitreous specimens from eyes with healed or quiescent retinitis were CMV PCR negative (P < .001, Fisher’s exact test; table 1). A similar analysis of 37 aqueous humor specimens was performed to determine whether fluid from the anterior chamber of the eye provided as sensitive a marker for CMV retinitis activity. Of 15 aqueous specimens from eyes with active retinitis, 14 (93%) were positive by CMV PCR. All 22 aqueous specimens from eyes with healed or quiescent retinitis were negative for CMV DNA (table 1). Identical PCR results were obtained from aqueous humor and vitreous for each of 14 patients from whom corresponding specimens were available.

For only 1 patient did the results of CMV PCR analysis not correspond to the clinically apparent status of CMV retinitis. Both vitreous and aqueous specimens from the same eye with active retinitis were CMV PCR negative. These results indicate that CMV PCR analysis of ocular specimens is both sensitive and specific for differentiation of active and inactive CMV retinitis.

**Analysis of systemic CMV.** To enable direct comparison of the sensitivity and specificity of ocular specimens with systemic specimens for identification of active CMV retinitis, we analyzed blood and urine specimens collected from the study population at the same time as the ocular specimens, for CMV DNA. Plasma CMV PCR results did not correlate with CMV retinitis activity (P < .08, Fisher’s exact test; table 1). However,
plasma PCR was a more sensitive marker for retinitis in patients who were not receiving systemic anti-CMV therapy than for retinitis in patients who were receiving such therapy (0.77 and 0.55, respectively; table 1). In six patients with active CMV retinitis, plasma PCR was negative. The median CD4⁺ cell count of these patients was 14 cells/mm³ (range, 4–31 cells/mm³), which indicates that these patients continued to be severely immunocompromised and that residual immune function was unlikely to be a factor in this discrepancy. At the time of specimen collection, 4 of these 6 patients were receiving systemic anti-CMV therapy, 1 had received an intravitreal cidofovir injection, and 1 was untreated.

There was no association between cultures of CMV from blood and active CMV retinitis (P = .18). A weak association was identified between CMV isolated from urine or from either blood or urine and CMV retinitis activity (P = .05 for both). However, the sensitivities of urine culture and combined culture analyses were weak (0.67 and 0.58, respectively). These findings, which permit a direct comparison of PCR-based ocular analyses and extraocular markers, indicate that ocular fluids from either the posterior or anterior segment of the eye can provide more effective markers for active CMV retinitis than systemic measures of CMV.

Quantification of CMV DNA in ocular and extraocular specimens. To further characterize the association of CMV DNA with CMV retinitis activity, we determined levels of CMV DNA in 31 ocular specimens and 9 plasma specimens from patients with active retinitis by use of QC-PCR. In 26 paired vitreous and aqueous specimens that had been collected from patients with active CMV retinitis, levels of CMV DNA in vitreous were found to be significantly higher than levels of CMV DNA in corresponding aqueous humor (median CMV DNA in vitreous, 3.5 × 10³ genomes/µL; in aqueous humor, 3.2 × 10² genomes/µL; P < .005 [Wilcoxon signed rank test; figure 1A]). Eight corresponding vitreous and plasma pairs were quantified for CMV DNA (median CMV DNA in plasma, 7.3 genomes/µL). In all 8 cases, levels of CMV DNA in plasma were lower than those in corresponding vitreous. In 4 of 5 cases, plasma CMV DNA levels were lower than those of corresponding aqueous humor specimens.

Comparison of ocular CMV DNA levels and extent of retinitis activity. To determine whether the amount of CMV DNA present in ocular fluids correlates with the extent of CMV retinitis, we compared CMV DNA levels with an assessment of the surface area of active retinitis (figure 1B, 1C). For 18 vitreous specimens and 13 aqueous specimens, the corresponding area of active CMV retinitis is reported in disc areas, a measure equivalent to the area of the optic disc, ~2 mm². A linear correlation was identified between area of active retinitis and log₁₀ of the corresponding CMV DNA levels in vitreous (r² = .44, P = .003) and aqueous (r² = .69, P < .001) humors (figure 1B, 1C).

Discussion

PCR amplification of CMV DNA from both vitreous and aqueous specimens can provide highly sensitive and specific markers to differentiate active and inactive CMV retinitis. Among 74 ocular specimens examined, CMV PCR reflected CMV retinitis activity in all but 2. Both of these specimens were obtained from the same patient, whose vitreous and corresponding aqueous specimens were PCR negative in the presence of active retinitis. Active and progressive CMV retinitis in this patient had stabilized and appeared to be healing in response to intravitreal cidofovir treatment administered 43 days prior to specimen collection. However, clinical examination and fundus photographs indicated that the patient continued to have a persistent low-grade retinitis at the time of specimen collection. Thus, PCR-based analysis of ocular specimens may not reflect active CMV retinitis during transition stages of retinitis (i.e., during early reactivation or during healing). In these situations, “low grade” CMV retinitis may not release sufficient CMV DNA into ocular fluids for detection by PCR.

Determination of CMV DNA levels in ocular specimens enabled identification of a direct correlation between the clinically assessed area of active CMV retinitis and CMV DNA levels in vitreous and aqueous specimens. Clearly, PCR-based analysis of a single ocular specimen provides little information regarding the dynamics of CMV DNA levels relative to retinitis activity. However, this result supports the notion that the presence of CMV DNA in ocular fluids is greatly influenced by current retinitis activity and is not attributable to highly stable CMV DNA that persists for long time periods within aqueous humor or vitreous.

By analysis of corresponding ocular and extraocular specimens, we found that neither culture of CMV from blood or urine nor PCR amplification of CMV DNA from plasma is as accurate as PCR of CMV DNA from aqueous humor or vitreous for determination of CMV retinitis activity. This finding is consistent with reports by other groups [8–10] and with the observation that pretherapy, but not posttherapy, systemic CMV markers are sensitive for prediction of retinitis [7, 11, 12].

Our results indicate that, whereas ocular specimens accurately reflect the status of CMV retinitis, they do not necessarily reflect the status of systemic CMV infection. Similarly, during retinitis treatment, plasma CMV DNA PCR may not reflect the status of retinitis. This will certainly be the case when local therapy for CMV retinitis is used and has also been observed in patients treated with intravenous cidofovir [13] and unpublished data). The possibility that residual immune function was suppressing systemic CMV infection in patients with active retinitis and CMV PCR-negative plasma was ruled out by the low CD4⁺ cell counts in this patient group.

A concern in taking any clinical specimens for diagnosis or monitoring of disease is the safety of collection procedures. Although we found vitreous PCR and vitreous QC-PCR to
provide excellent markers, the surgical procedure required for collection entails some associated risk. On the other hand, PCR detection of CMV DNA in aqueous humor appears to be as indicative of retinitis activity as that in vitreous, and serious complications associated with aqueous paracentesis, when it is performed by an experienced ophthalmologist, are uncommon [14, 15].

In summary, our results indicate that CMV PCR of aqueous as well as vitreous is an excellent marker for active retinitis. Moreover, CMV DNA levels in ocular fluids are indicative of the extent of CMV retinitis. This assay should provide a valuable tool for the differential diagnosis of retinitis and provide a means of quantitative assessment of CMV retinitis activity.

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References


