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Diagnosis of Human Cytomegalovirus-Induced Retinitis in Human Immunodeficiency Virus Type 1-Infected Subjects by Using the Polymerase Chain Reaction

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In 13 of 16 AIDS patients with retinitis, a herpesviruslike infection was diagnosed by clinical investigation. In 12 of the 13 patients, human cytomegalovirus (HCMV) DNA was detected in 5 μl of aqueous humor by using the polymerase chain reaction (PCR). In the aqueous humor of 12 control patients HCMV DNA could not be detected by PCR. PCR may be used to monitor specific antiviral long-term therapy in HCMV retinitis.

AIDS patients frequently suffer from bacterial or viral infections of the eye. Retinitis is in most cases caused by human cytomegalovirus (HCMV) infection, but other agents such as *Toxoplasma gondii* and herpes simplex virus have also been identified as causative agents (1, 9). Since HCMV infection of the eye causes lesions with the same appearance as those observed in herpes simplex virus retinitis, it is difficult to differentiate between these infections by mere clinical observation. However, an exact diagnosis is required for an appropriate therapy (3).

Unfortunately, in most AIDS patients the diagnosis of an acute HCMV infection cannot be made by routine virological procedures such as detection of specific immunoglobulin M antibodies (7). HCMV can be frequently isolated from the urine of patients without overt clinical symptoms, which is probably due to the latent status of HCMV in different human cells (10).

In postmortem eyes with HCMV retinitis, localized production of HCMV has been documented (6). We have therefore used the polymerase chain reaction (PCR) to detect HCMV in the aqueous humor of the eye to determine its diagnostic potential for eye infections.

On the basis of clinical investigation, 13 of the 16 human immunodeficiency virus type 1 (HIV-1)-infected retinitis patients had acute herpeslike retinitis, two had toxoplasmosis, and one patient had herpes zoster retinitis during a generalized case of herpes zoster. The 16 HIV-1-infected patients were classified as CDC IV, according to the Centers for Disease Control classification of AIDS stages. Samples of aqueous humor of the eye (50 to 80 μl), urine, saliva, and serum were obtained from all patients. In addition, from five patients vitreous body, together with aqueous humor, was collected following vitrectomy, which was carried out according to clinical requirements. All samples were taken before treatment with ganciclovir was initiated. After treatment the 13 patients showed a temporary clinical improvement. Aqueous humor specimens were obtained from 12 control patients without HIV-1 infection (10 cataract patients, 1 with acute retina necrosis, and 1 with opacity of the cornea).

HCMV strain Ad169 was propagated in human fibroblasts. Twofold dilutions were prepared to titrate the infectivity of the supernatant (8). Five days after virus inoculation a titer of 1.3×10^5 (2^{17}) 50% tissue culture infectious doses ($TCID_{50}$) per ml was obtained in the freshly harvested tissue culture supernatant.

The sequences of the five oligonucleotides (Table 1) were derived from the immediate-early (MIE) gene of HCMV. Two primers were identical to those used by Demmler et al. (2). Five microliters of undiluted aqueous humor was used for the amplification procedure. Urine and saliva, after low-speed centrifugation, were applied at a dilution of 1:4 in distilled water. Before amplification all materials were first heated at 94°C for 10 min to denature the DNA. The amplification mix (in a total of 25 μl) contained 75 pmol of each primer, 200 μM of deoxynucleoside triphosphate, 10 mM Tris (pH 8.3, supplemented with 50 mM KCl–2.5 mM $MgCl_2$–0.02% gelatin), and 2 U of *Thermus aquaticus* polymerase (*Taq*; Perkin-Elmer Cetus, Emeryville, Calif.) (11). The specific amplification conditions for the primer set we used were as follows: denaturation at 95°C for 30 s, annealing at 58°C for 30 s, elongation at 72°C for 90 s, for a total of 35 cycles. The amplified DNA fragment was detected by liquid hybridization with a ^{32}P-end-labeled oligonucleotide (11). In all amplification experiments, supernatants (5 μl each) from HCMV-infected and noninfected cells were used as positive and negative controls, respectively.

By using the primers and the amplification conditions published by Demmler et al. (94°C, 120 s; 65°C, 90 s; 72°C, 60 s; 40 cycles) (2), viral DNA of HCMV was only detected in 3 of 16 eye specimens obtained from AIDS patients with posterior uveitis. To improve sensitivity, new primers were selected, which resulted in a shorter amplification product with a length of 110 bp. The previously published and newly selected primers (MIE6, MIE7) and the probe used for the detection of both amplification products are shown in Table 1. Both primer pairs failed to amplify cellular DNA. The sensitivity of the detection of HCMV by PCR was compared with virus isolation by using tissue culture supernatant containing 1.3×10^5 $TCID_{50}$/ml (1.3×10^2 $TCID_{50}$/μl). Tenfold dilutions were made and 5 μl of each dilution was amplified by using the published primers (MIE4, MIE5) at 40 and the newly selected primers (MIE6, MIE7) at 35 ampli-

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TABLE 1. Primers and probe used to detect amplification products

Primer or probe	Sequence (5′ to 3′)	Product length (bp)[b]	Nucleotide no.	$TCID_{50}$ detected by PCR for each primer pair
Primers				
MIE4[a]	AAGCGGCCTCTGATAACCAAGCC	435	1142–1166	6.5×10^{-2}
MIE5[a]	AGCACCATCCTCCTCTTCCTCTGG		1552–1576	
MIE6	AGTGTGGATGACCTACGGGCCATCG	110	1210–1267	6.5×10^{-3}
MIE7	GGTGACACCAGAGAATCAGAGGAGC		1352–1376	
PROBE MIE[a]	GAGGCTATTGTAGCCTACACTTTGG		1312–1336	

[a] Previously published (2).
[b] Length of the product obtained by the two primers.

fication cycles. Using primers MIE6 and MIE7, HCMV DNA was found at dilutions of up to 10^{-6} (corresponding to 6.5×10^{-3} $TCID_{50}$), which exceeded the amount of DNA detected by the published primers (8) by 10-fold (Table 1).

When primers MIE6 and MIE7 were used, in 12 of 16 AIDS patients with acute retinitis, HCMV DNA was found by amplification of aqueous humor. In three patients with HCMV retinitis, HCMV DNA was also detected in the urine (two patients) or in the saliva (one patient) while virus could not be isolated from any of the aqueous materials.

HCMV DNA was not detected in the aqueous humor specimens of 12 HCMV-seropositive patients without HIV-1 infection who had various ocular infections unrelated to HCMV. In three of the PCR-negative AIDS patients, other infectious agents (one patient with herpes zoster virus and two patients with *T. gondii* infection) which most likely had caused the underlying disease were identified. Remarkably, HCMV was isolated from the saliva of the patient with toxoplasmosis while HCMV was not found in the eye. This patient became HCMV DNA positive in the aqueous humor 4 months after the onset of toxoplasmosis.

Compared with earlier PCR protocols (2), the method described here detected a 10-fold lower amount of HCMV DNA. Besides, PCR was considerably more sensitive than virus isolation. In principle, longer sequences of about 400 bp can be amplified as efficiently as shorter ones containing only 100 bp. However, with shorter sequences, shorter annealing and melting temperatures and fewer cycles can be applied, which results in better preservation of the *Taq*. This may explain the improvements obtained with our primers.

Persistence of the HCMV has not been reported in the eye so far, an observation which is also supported by the absence of HCMV DNA in the aqueous humor of our control group. After completing this manuscript we became aware of a recent publication of Fox et al. (5), who were also able to detect HCMV DNA in the eyes of nine retinitis patients with AIDS by amplifying a 139-bp fragment of the pg 64 gene. In one negative control (vitreous body) a positive signal was obtained, while all 18 humoral fluids of control persons were PCR negative. We have now increased the number of our negative controls from 12 (4) to 15. All 15 humoral fluids were negative for HCMV DNA, although all patients had antibodies to HCMV. In principle, our data strongly support the findings of Fox et al. Obviously, PCR is considerably more effective in detecting HCMV in the eye than virus isolation, and it may provide a reliable method for the early diagnosis of HCMV retinitis in the immunocompromised host. The method may help to initiate an early therapy with an antiviral drug, thus improving the desolate conditions of HIV-1-infected subjects.

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