

# Serologic and Polymerase Chain Reaction Analysis of Intraocular Fluids in the Diagnosis of Infectious Uveitis

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- **PURPOSE:** Infectious uveitis entities are usually rapidly progressive blinding diseases that can be prevented by prompt administration of specific antimicrobial therapy. With the aim of improving early diagnosis in patients with infectious uveitis, intraocular fluid samples from patients with sight-threatening posterior uveitis were investigated to determine the causative agent.
- **METHODS:** Thirty-eight patients with acquired immunodeficiency syndrome (AIDS) and retinitis, eight immunosuppressed patients with retinitis, 16 immunocompetent patients with acute retinal necrosis, and 22 immunocompetent patients with toxoplasmic retinochoroiditis were analyzed

Accepted for publication June 9, 1995; revised manuscript received Oct. 24, 1995.

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by polymerase chain reaction for the presence of herpesviruses and *Toxoplasma gondii* DNA and for local antibody production against these microorganisms.

- **RESULTS:** In patients with AIDS and retinitis, polymerase chain reaction was positive for cytomegalovirus DNA in 21 (91%) of the 23 ocular fluid samples obtained during active cytomegalovirus retinitis, whereas local antibody production analysis was negative in all cases. In acute retinal necrosis, varicella-zoster virus or herpes simplex virus could be established as the inciting agent in 81% of the cases, using the combination of both techniques. Polymerase chain reaction was positive in all samples obtained within two weeks after the onset of disease. *Toxoplasma gondii* DNA was detected in 4 of 13 samples (31%) from immunocompetent patients with active toxoplasmic retinochoroiditis; in each case, local antibody production was also detected. In contrast, no local antibody production was observed in two of three samples from transplant recipients that were positive for *T. gondii* DNA. All the control samples tested were negative for the above-mentioned tests.

- **CONCLUSIONS:** In patients with AIDS, polymerase chain reaction analysis is preferable above local antibody production in detecting the inciting agent of retinitis. In other cases, the combination of both techniques can make a valuable contribution to the diagnosis.

**H**ERPESVIRUSES AND *Toxoplasma gondii* are common inciting agents of ocular inflammation in immunosuppressed and immunocompetent individuals.<sup>1-3</sup> Cytomegalovirus is the major cause of retinitis in patients with acquired immunodeficiency syndrome (AIDS), and it is rarely seen in immunocompetent individuals.<sup>1</sup> Intraocular inflammation caused by other herpesviruses<sup>1,4</sup> and even infections with multiple herpesviruses have also been reported in patients with AIDS.<sup>5</sup> In immunocompetent patients, *T. gondii* is the most common infectious cause of posterior uveitis.<sup>3</sup> Varicella-zoster virus and herpes simplex virus are the major causes of acute retinal necrosis, a severe inflammatory eye disease associated with a poor visual prognosis.<sup>6</sup>

Because retinitis can progress rapidly,<sup>4,6</sup> early diagnosis and start with specific antimicrobial therapy is recommended.<sup>7</sup> Until now, the diagnosis of infectious uveitis entities generally has been based on clinical characteristics combined with results of serologic laboratory examination. However, fundus examination can be complicated because of vitreous haze or opacities, and various syndromes share similar clinical features. Detection of local antibody production is beneficial in several infectious uveitis entities,<sup>8-10</sup> but this indirect method is often negative early in the course of the disease.<sup>8,9</sup> Polymerase chain reaction is a specific method directly detecting DNA of microorganisms, and its advantages include increased sensitivity and more rapidly available results than with viral culture.<sup>11,12</sup> Polymerase chain reaction has already been used to detect various types of infectious uveitis.<sup>13-19</sup> To improve early diagnosis, we analyzed intraocular fluid samples from immunosuppressed and immunocompetent patients who had posterior uveitis with a presumed infectious cause for both local antibody production against herpesviruses and *T. gondii*. We also tested for DNA of these microorganisms by polymerase chain reaction. The results show that polymerase chain reaction analysis is preferred to serologic techniques in patients with AIDS and retinitis, whereas in patients with other conditions, the combination of both techniques increases the detection of inciting agents in uveitis.

## PATIENTS AND METHODS

A DIAGNOSIS OF UVEITIS WAS BASED ON CLINICAL CHARACTERISTICS according to the criteria of the Internation-

al Uveitis Study Group and the Research Committee of the American Uveitis Society (1994).<sup>20,21</sup> Samples from thirty-eight patients with AIDS and retinitis, eight immunosuppressed patients without AIDS who had retinitis, 16 patients with acute retinal necrosis, and 22 patients with toxoplasmic retinochoroiditis were included.

Blood and aqueous humor or vitreous fluid samples were collected simultaneously. Aqueous humor samples were obtained by performing a paracentesis for diagnostic purposes, and vitreous fluid samples were collected during a therapeutic or diagnostic pars plana vitrectomy. In total, 58 aqueous humor samples and 26 vitreous fluid samples were collected and, depending on the amount of fluid, tested for the presence of different herpesviruses and *T. gondii*. In five patients with acute retinal necrosis, a second ocular fluid sample was obtained at a later stage during the disease.

Patients without intraocular inflammation included control subjects with 13 aqueous humor samples obtained during cataract or glaucoma surgery and vitreous fluid samples collected during a therapeutic vitrectomy in seven cases of proliferative vitreoretinopathy and six cases of diabetic retinopathy. The control patients were informed of these procedures and their consent was obtained. Twelve control vitreous samples were obtained from tissue donors. No information was available concerning seropositivity for herpesviruses or *T. gondii*.

Because it has been shown that aqueous humor and vitreous fluid contain polymerase chain reaction inhibitory factors,<sup>22</sup> DNA was isolated from ocular fluid samples by using silica particles, according to the method of Boom and associates.<sup>23</sup> Fifty  $\mu$ l of ocular fluid was used for DNA isolation, and afterward the template DNA was diluted in 50  $\mu$ l of distilled water. The amplifiability of the DNA was tested by adding 12.5 fg of plasmid-containing modified human  $\beta$ -globin DNA, and no polymerase chain reaction inhibitory activity was detected. Primers for cytomegalovirus, varicella-zoster virus, herpes simplex virus, and *T. gondii* were selected from published sequences (Table 1).<sup>11,12,24-26</sup> We performed a single polymerase chain reaction and chose the outer primers from publications describing a nested polymerase chain reaction. All primers were tested for the optimal annealing temperature and magnesium<sup>2+</sup> concentra-

TABLE 1

PRIMER SEQUENCES AND POLYMERASE CHAIN REACTION CONDITIONS FOR HERPESVIRUSES AND *Toxoplasma gondii*

POLYMERASE CHAIN REACTION	PRIMER SEQUENCES (5'→3')	POSITION	MAGNESIUM <sup>2+</sup> (mmol)	ANNEALING TEMPERATURE (°C)	REFERENCE
Cytomegalovirus	AGCTGCATGATGTGAGCAAG GAAGGCTGAGTTCTTGGTAA	1767-1786 1894-1913	3.0	56	Cathomas and associates <sup>12</sup>
Varicella zoster virus	TACGGGTCTTGCCGGAGCTGGTAT AATGCCGTGACCACCAAGTATAAT	51,066-51,090 51,314-51,338	2.0	60	Mahalingam and associates <sup>24</sup>
Herpes simplex virus 1	ATCACGGTAGCCCGGCCGTGTGACA CATACCGGAACGCACCACACAA	19-43 218-239	4.0	66	Aurelius and associates <sup>25</sup>
Herpes simplex virus 2	AACTCGGGRSCGTACTGYTT* CGGGAACGCGCCGGCCCAAC	1203 1600	5.0	70	Nahass and associates <sup>11</sup>
<i>Toxoplasma gondii</i>	GGCATTCTCGTTGAAGATT CCTTGCCGATAGGTCTAGG	Ribosomal DNA	2.0	58	Cazenave, Cheyrou, and Begueret <sup>26</sup>

\*R indicates A or G, S indicates G or C, Y indicates C or T.

tion (Table 1). Uracil DNA glycosylase was used to control carryover contamination.<sup>27</sup> The amplification mixture contained 0.4 pmol of 3' and 5' primer (Isogen Bioscience bv, Amsterdam, The Netherlands), 0.2 mmol of dUTP (Sphaero Q, Leiden, The Netherlands), dATP, dCTP, dGTP (Life Technologies, Breda, The Netherlands), 0.1 unit of *Taq* polymerase (Sphaero Q), 0.1 unit of uracil DNA glycosylase (Life Technologies), and 5 µl of isolated template DNA solution, diluted to a final volume of 50 µl with distilled water. The polymerase chain reaction was performed in a Biometra Trio-Thermoblock (Westburg, Leusden), as follows: the samples were incubated for ten minutes at 37 C and afterward at 95 C for five minutes, then 40 cycles were performed for one minute at 95 C denaturation, one minute at the optimal temperature for annealing, and one and a half minutes at 72 C for elongation. After the last cycle, the samples were incubated for seven minutes at 72 C for final elongation.

One fifth of the polymerase chain reaction product was subjected to electrophoresis on a 1.8% agarose gel, stained with ethidium bromide, and photographed. The gel was subjected to Southern blotting. The oligonucleotide probes (20 pmol) specific for the amplified fragments were labeled with 1.9 MBq of

adenosine 5'-[γ-<sup>32</sup>P] triphosphate (ATP) by the method of Maniatis and associates.<sup>28</sup> After hybridization, the nylon membranes were washed and exposed to x-ray film at -80 C for 24 hours.<sup>28</sup> For varicella-zoster virus and cytomegalovirus, the oligonucleotide probes were designed with the following sequences: varicella-zoster virus: CTC ACT ACC AGT CAT TTC TAT CCA TC; and cytomegalovirus: GGC CTT AGC CTG CAG TGC AC. For herpes simplex virus 1, the probe was used as previously described, with the following sequence: TAC GAG GAG GAG GGG TAT AAC AAA GTC TGT.<sup>25</sup> For *T. gondii*, no Southern blotting was performed because of the short length of the polymerase chain reaction product, and for herpes simplex virus 2, no appropriate probe was available. In these cases, we identified the polymerase chain reaction product by ethidium bromide staining after electrophoresis on agarose gel and comparing the length of the product of experimental samples with a positive control.

Using known amounts of plasmid containing viral DNA, 20 target molecules were detected for cytomegalovirus DNA and varicella-zoster virus DNA, and 35 target molecules for herpes simplex virus 1 DNA. For herpes simplex virus 2 and *T. gondii* DNA, the detection limit of the polymerase chain reaction was

