

BRIEF REPORT: UVEITIS CAUSED BY *TROPHYRYMA WHIPPELII* (WHIPPLE'S BACILLUS)

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WHIPPLE'S disease is a multisystem bacterial disease usually characterized by malabsorption, diarrhea, and polyarthritis. Ocular manifestations of Whipple's disease include blurred vision or visual loss with one or more of the following findings: vitritis, uveitis, retinitis, retinal hemorrhage, choroiditis, papilledema, optic atrophy, and keratitis.¹ In general, these ocular manifestations occur in patients who also have gastrointestinal or central nervous system involvement.

We report a case of chronic, bilateral uveitis with unusual ophthalmologic features. The patient had no clinical evidence of gastrointestinal disease, and light microscopy of repeated duodenal-biopsy specimens was negative. A preliminary diagnosis of Whipple's disease was made on the basis of vitreous aspiration and light microscopy. Electron microscopy revealed bacilli that resembled those of Whipple's disease. The diagnosis was confirmed by polymerase-chain-reaction (PCR) assay, which detected 16S ribosomal RNA gene (rDNA) sequences corresponding to the Whipple's disease bacillus (*Tropheryma whipplei*) in the vitreous² and a nearly identical gene sequence in the duodenal mucosa.

CASE REPORT

In November 1989, a 59-year-old white woman was referred because of bilateral uveitis. She had had seronegative arthritis involving the wrists, knees, and ankles for 10 to 20 years, although she had been without symptoms for approximately 6 months, with normal plain radiographs in June 1989.

On presentation, the patient's visual acuity was 20/25 in the right eye and 20/50 in the left eye. There were 1 to 2+ vitreous cells in the right eye, a few small dot hemorrhages in the macula, and slight engorgement of the optic-nerve head. The left eye had 3+ anterior vitreous cells, an engorged disk, and cystoid macular edema. A fluorescein angiogram showed leakage in both nerve heads. She had an erythrocyte sedimentation rate of 39 mm per hour, normal total hemolytic complement and angiotensin-converting enzyme levels, and negative results on antinuclear-antibody, rheumatoid-factor, rapid plasma reagin, and fluorescent treponemal antibody absorption tests.

Magnetic resonance imaging of the head revealed cortical atrophy.

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There was a negative reaction to purified-protein derivative (without controls), a normal chest radiograph, and negative serologic tests for Lyme disease. The uveitis responded poorly to corticosteroid injections. In February 1990 a diagnostic vitrectomy of the left eye revealed reactive mononuclear cells but no atypical cells. Cultures for bacteria, fungi, and acid-fast bacilli were negative.

Over the ensuing year, the patient had persistent inflammation in both eyes, with cystoid macular edema. Cytologic tests, cultures, and staining after a diagnostic pars plana vitrectomy of the right eye were unrevealing.

The patient lost 7 kg in one year and had 7.4 g of fat in a 24-hour stool collection. In November 1990 and May 1992, endoscopic biopsies of the duodenum and terminal ileum revealed normal histologic features. Examination of the cerebrospinal fluid was unremarkable. Despite local corticosteroid therapy and the systemic administration of cyclosporine and cyclophosphamide, visual acuity deteriorated in both eyes over the next three years, and bilateral epiretinal membranes with substantial macular distortion, cataracts, and white fluffy corneal endothelial precipitates developed. One of several subsequent ophthalmologic surgical procedures revealed a thick exudative material over the right pars plana inferiorly and fine neovascularization of the optic nerve head.

In March 1993 the patient was found to have iris nodules in the right eye (Fig. 1). Flocculent, inflammatory corneal debris persisted (Fig. 2), and there were new retinal nodular precipitates. Repeated vitreous examinations revealed lymphocytes, neutrophils, and foamy macrophages and slender rod-shaped structures suggestive of bacteria that were positive on periodic acid-Schiff (PAS) staining, but no malignant cells. A portion of vitreous obtained in March 1993 was studied by electron microscopy and PCR (see the Methods section).

At that time, the patient reported difficulty in concentrating, memory loss, and occasional loose stools. A neurologic examination was otherwise negative, and the serum albumin level was normal. After vitreous cytopathologic features consistent with Whipple's disease had been confirmed, another endoscopic duodenal biopsy was performed in May 1993. The histologic features and the results of PAS staining were again normal. A tissue sample was also studied by PCR (see the Methods section). The patient was given a 14-day course of intravenous penicillin G (24 million units per day) and intramuscular streptomycin (1 g per day). She gained 5 kg, and the ocular inflammation improved dramatically. Her visual acuity improved to 20/100 in the right eye and 20/40 in the left eye. Because of intolerance to trimethoprim-sulfamethoxazole, she was treated with oral penicillin V potassium (500 mg every six hours) and probenecid and continued to do well.

METHODS

Electron Microscopy

One hundred microliters of vitreous fluid was centrifuged at 10,000×g in a 0.5-ml microcentrifuge tube. The supernatant fluid was removed, and the pellet was fixed first in 1.5 percent glutaraldehyde and then in 2 percent osmium tetroxide, washed, dehydrated, and embedded in epoxy resin (LX-112, Ladd Research Industries, Burlington, Vt.). Thin sections were stained again in 2 percent uranyl acetate and 2 percent lead citrate and viewed in an electron microscope (H-600, Hitachi, Tokyo, Japan).

PCR Analysis

Samples of ocular and duodenal tissue were processed and analyzed by PCR as previously described,^{2,3} with the following modifications. Two hundred microliters of vitreous from a control patient with macular pucker and from the case patient were incubated overnight at 55°C with an equal volume of proteinase K digestion buffer. These samples were then subjected to sonic-wave disruption for five minutes in sealed tubes (Sonifier Cell Disruptor, Heat Systems Ultrasonics, Plainview, N.Y.). Finally, these vitreous samples were twice quick-frozen and thawed at 95°C for two minutes. Frozen and paraffin-embedded duodenal tissues from the case patient and from a patient with nontropical sprue were processed as described previously.² Because the combination of *T. whipplei* 16S rDNA primers W3FE and W2RB failed to generate a visible product with these samples, a new antisense *T. whipplei* 16S rDNA primer, W4RB, was designed from a

variable region closer than W2RB to W3FE. W4RB (5'CGGGA-TCCTGTGAGTCCCCGCCATTACGC3', corresponding approximately to *Escherichia coli* 16S ribosomal RNA [rRNA] positions 1155 to 1133) was used in conjunction with W3FE to amplify a 98-base-pair (bp) internal portion of the *T. whippelii* 16S rRNA gene (total amplicon length, 154 bp). With the hot-start technique, 40 PCR cycles were performed with an annealing temperature of 63°C, 20 nM of each primer, and 2 mM of magnesium ion.

The specificity and sensitivity of primers W3FE and W4RB were tested by analyzing K562 human cell DNA, digested formalin-fixed lymph-node tissue infected with *Bartonella* (formerly *Rochalimaea*) *henselae*, *Rhodococcus equi* cells, and *Dermatophilus congolensis* cells. All the samples were negative with these primers. Duodenal tissues from three patients with Whipple's disease (previously shown to be positive with primers W3FE and W2RB)² were strongly positive with W3FE and W4RB; *Arthrobacter globiformis* cells were weakly positive (data not shown). In serial dilutions of cloned *T. whippelii* 16S rDNA mixed with digested uninfected tissue, PCR reactions with primers W3FE and W4RB could detect approximately 100 16S rRNA gene copies (data not shown).

RESULTS

Vitreous fluid obtained in November 1991 and March 1993 was found on light microscopy to contain foamy macrophages with delicate structures and clumped material that were positive on PAS staining, findings consistent with a diagnosis of Whipple's disease. In addition, bacillary structures highly suggestive of *T. whippelii* were detected in the 1993 sample by electron microscopy. Some of the bacilli appeared to be undergoing cell division, suggesting that they were viable at the time of vitrectomy (Fig. 3).

The sample of vitreous obtained in March 1993 was analyzed by PCR with oligonucleotide primers designed from variable regions of the *T. whippelii* 16S rRNA gene sequence. Because a previously described specific primer pair (W3FE and W2RB)² failed to generate a visible product from these samples, a new antisense primer (W4RB) was designed, with the expectation that a smaller target would increase the sensitivity of detection. This hypothesis was confirmed (W3FE and W2RB, 10,000 targets; W3FE and W4RB,

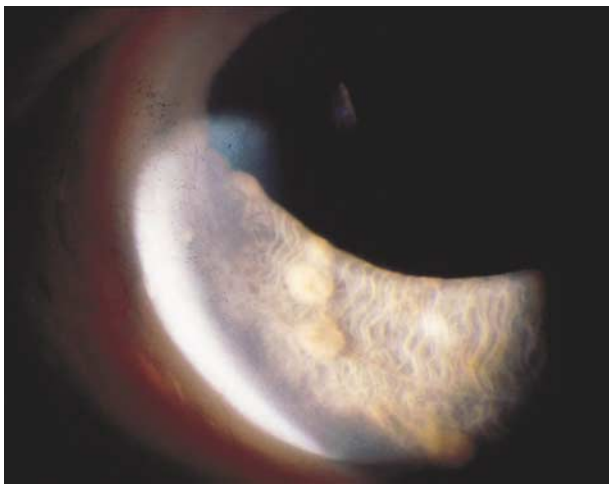


Figure 1. Slit-Lamp Photograph Taken in March 1993, Revealing Multiple Tan Iris Nodules in the Right Eye.



Figure 2. Slit-Lamp Photograph of the Right Eye, Revealing Flocculent, Inflammatory Debris That Adhered to the Posterior Corneal Surface and Eventually Obscured the Optical Axis.

100 targets) (data not shown). Primers W3FE and W4RB generated a visible product of the expected size (154 bp) from the patient's vitreous on several occasions (Fig. 4, lane 5), but not from control vitreous (Fig. 4, lane 3) or from other control samples. In addition, fresh-frozen duodenal mucosa from the case patient generated a visible PCR product of the expected size with W3FE and W4RB (Fig. 4, lane 6). The histologic features of this duodenal tissue, obtained in May 1993, before antibiotic treatment, were entirely normal (data not shown). In contrast, duodenal tissue from a patient with nontropical sprue was negative by PCR (Fig. 4, lane 4). The PCR products amplified from the patient's vitreous and duodenal tissue with W3FE and W4RB were cloned and sequenced. The DNA sequence of the vitreous product (98 internal nucleotide positions) was identical to the corresponding segment of the previously described *T. whippelii* 16S rDNA sequence (GenBank M87484). The sequence of the duodenal PCR product differed at one nucleotide position. This unpaired nucleotide position is invariant among at least 90 percent of the members of the bacterial gram-positive division, suggesting a *Taq* polymerase error in this cloned PCR product; however, we cannot rule out the presence of a variant strain or species.

DISCUSSION

Whipple's disease was first described in 1907 in a patient with migratory polyarthritis, cough, diarrhea with malabsorption, weight loss, and mesenteric lymphadenopathy.⁴ The disease most often involves the gastrointestinal tract and its lymphatic drainage, the heart, and the central nervous system. The diagnosis has been based on the finding of macrophages containing PAS-positive, diastase-resistant inclusions within the lamina propria of the small intestine. Similar PAS-positive mononuclear-cell infiltrates are found in other affected tissues, but the diagnosis of extraintestinal Whipple's disease has traditionally relied on visual demonstration of the unusual bacillus associated with the disease. This



Figure 3. Electron Micrograph Taken in March 1993, Revealing a Bacillary Structure in the Vitreous.

The organism appeared to be undergoing cell division ($\times 88,320$). (Figure provided through the courtesy of Darlene J. Whitney, Stanford University.)

organism has never been reproducibly cultivated or purified in vitro; however, it has been identified from its 16S rDNA sequence, amplified from tissues of patients with Whipple's disease.^{2,5} The organism is an actinomycete for which the name *T. whipplei* has been proposed.

Whipple's disease with ocular involvement is rare but well described. In general, most cases of uveitis are probably not caused by *T. whipplei*. In his review of 696 patients with Whipple's disease,¹ Dobbins found that 19 had evidence of ocular involvement. The most common ocular manifestations have included uveitis, vitritis, retinitis, and retrolbulbar neuritis. In our patient, the diagnosis of ocular Whipple's disease was based on the traditional findings of a mononuclear-cell infiltrate in the vitreous composed primarily of foamy macrophages, as well as delicate intracellular and extracellular rod-like bacillary structures detected on both silver and PAS staining and by electron microscopy. The diagnosis was confirmed by PCR-based detection of *T. whipplei* 16S rDNA in the vitreous. A PCR-based diagnostic approach to Whipple's disease has been applied to duodenal tissue, lymph node, pleural-fluid cells, and peripheral blood.^{2,6-8} We have extended this application to the diagnosis of ocular disease. PCR-based methods may be useful in chronic inflammatory ocular syndromes involving fastidious microbial pathogens. In this case of Whipple's disease, PCR detection of *T. whipplei* suggests that ocular involvement was due to local bacterial infection rather than an immune-mediated process.⁹

The unique ophthalmologic features of this case of ocular Whipple's disease were the development of white corneal endothelial precipitates, iris nodules, pars plana exudation, retinal nodular precipitates, and disk neovascularization. Early or atypical presentations

of extraintestinal Whipple's disease can mimic features of sarcoidosis and have led to confusion in a number of cases.¹⁰⁻¹³ The anterior-segment findings, especially iris nodules and greasy keratic precipitates, can be quite similar to those found in sarcoidosis. *T. whipplei* may share antigenic or structural features with other organisms that are capable of eliciting a sarcoidosis syndrome.^{14,15}

Our patient had only scant clinical evidence of extraocular Whipple's disease. Although her weight loss, anorexia, and brief intermittent episodes of loose stools were consistent with gastrointestinal involvement, three upper endoscopies with small-bowel biopsies performed over a four-year period failed to reveal gross or microscopical evidence of Whipple's disease. Other cases of extraintestinal Whipple's disease without clinical manifestations of gastrointestinal disease have been described, especially cases involving the central nervous system.¹⁶⁻²¹ In addition, the intestinal pathologic lesions of Whipple's disease may be patchy²² or only submucosal.²³ However, we know of no previous case of ocular disease in the absence of marked central nervous system and gastrointestinal manifestations.¹

The role of bacterial replication and pathologic features in the pathogenesis of gastrointestinal Whipple's disease has been unclear.¹⁰ We have documented the presence of *T. whipplei* DNA in the duodenal mucosa of a patient with extraintestinal Whipple's disease who had no clinical, endoscopic, or histologic evidence of intestinal involvement. This finding suggests either an intestinal portal of entry for *T. whipplei* or strong tissue tropism; it also suggests that the presence of this organism or its DNA in the intestinal mucosa is not necessarily associated with a pathologic process. Our finding may explain some cases of malabsorption in the setting of normal histologic features.²³

Whipple's disease was considered uniformly fatal until empirical trials indicated the therapeutic efficacy of

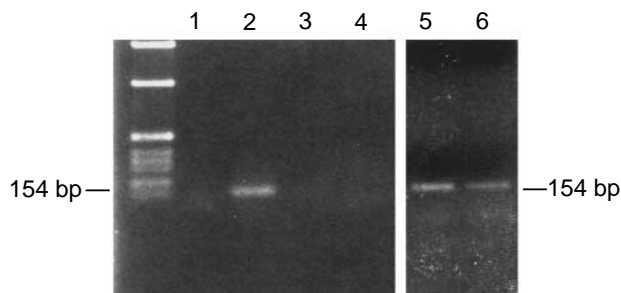


Figure 4. PCR Detection of *T. whipplei* with Primers W3FE and W4RB.

Lane 1 shows the results with PCR reagents only; lane 2, cloned *T. whipplei* 16S rDNA; lane 3, vitreous from a patient with macular pucker; lane 4, duodenal mucosa from a patient with non-tropical sprue; and lanes 5 and 6, vitreous and duodenal mucosa, respectively, from the study patient. A 1-kb DNA ladder (Life Technologies, Gaithersburg, Md.) serves as a size marker on the far left.

systemic antibiotics.²⁴⁻²⁸ Most current recommendations include the use of antibiotics that penetrate the central nervous system.¹ In patients with visual impairment, it would be prudent to use antimicrobial agents that penetrate the vitreous. Furthermore, our findings suggest that PCR may be the most sensitive indicator of persistent organisms in patients treated for this disease.

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