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Intraocular Detection of *Bartonella henselae* in a Patient with HLA-B27 Uveitis

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Bartonella henselae **uveitis was diagnosed in a 40-year-old woman with underlying HLA-B27 uveitis on the basis of immunodetection and molecular detection of the organism, a Marseilles genogroup and a CAL-1 genotype strain, in the vitreous fluid. This case illustrates that *B. henselae* should be included in the differential diagnosis of uveitis and the usefulness of immunodetection for rapid and specific diagnosis.**

CASE REPORT

A 40-year-old woman had been diagnosed with HLA-B27 uveitis in 1989. Her family history included ankylosing spondylitis in two first cousins, one of whom (a female) had had typical HLA-B27 uveitis. The patient owned a cat during childhood and again had contacts with a domestic cat in 2001. She denied receiving any arthropod bite. At that time, the patient presented with acute recurrences of nongranulomatous uveitis, which was controlled with topical steroids. Cataract extraction and intraocular lens implantation were performed on the right eye in December 1998. Starting in March 1999, severe bilateral relapses occurred after treatment with 30 mg of prednisone per day, and the patient was referred to our ophthalmology department in February 2000. Visual acuities at the initial examination were hand motion (oculus dexter [O.D.]) and 20/200 (oculus sinister [O.S.]). Biomicroscopy showed mutton fat keratic precipitates in both eyes with 2+ flares and cells in the anterior chambers of both eyes (Fig. 1A); laser flare photometry confirmed a severe anterior uveitis, with 324 photons/ms in the O.D. and 214 photons/ms in the O.S. The ocular pressures were 28 mm Hg in the O.D. and 25 mm Hg in the O.S., despite maximal local and systemic medications. Many giant cells were seen at the surface of the right intraocular lens. Vitritis was present in the right eye, and fundus examination was impossible in the left eye because of a complete papillary secclusion. The erythrocyte sedimentation rate was 42 mm in the first hour, immunologic investigations were noncontributory, and the angiotensin-converting enzyme level was normal. Anterior chamber paracentesis analyses excluded herpesvirus infection. Because the patient had contact with tuberculosis patients and the purified protein derivative skin test result was positive, treatment with rifampin in combination with isoniazid, ethambutol, and pyrazinamide was initiated for 6 months; and methylprednisolone pulses were started thereafter. Immunosuppressive medications were discontinued, whereas systemic prednisone (1 mg/kg of body weight/day) was initiated. Corticosteroids at doses greater than 20 mg/day and antibiotics controlled the ocular inflammation. In February 2002, a diagnostic vitrectomy was performed after a bilateral relapse with posterior pole involvement, papillitis, and macular edema dramatically decreased the patient's visual acuity. Immunodetection incorporating an anti-*Bartonella henselae* polyclonal rabbit antibody was performed as reported previously (7). Briefly, we used a polyclonal rabbit anti-*B. henselae* antibody at a 1:2,000 dilution and the immunoperoxidase method with amino-ethylcarbazole as the chromogen (Histostain-Plus kit; Zymed, Montrouge, France), followed by counterstaining with Mayer's hematoxylin. The assay was completed within 3 h. Immunocytochemical analysis showed clusters of immunopositive microorganisms with an extracellular distribution in the vitreous fluid (Fig. 2A). Negative controls (normal rabbit serum and vitreous fluid collected at necropsy from three people who did not have cat scratch disease [CSD]) showed no immunoreactivity (Fig. 2B). PCR amplification of the *Bartonella* internal transcribed spacer (ITS) region and the *Bartonella pap31* gene of the isolate from the vitreous fluid were performed as described previously (12, 15). Amplification and sequencing of the ITS were performed by using primer pair 16SF-23S1 (12); a 269-bp fragment of the *groEL* gene was amplified by seminested PCR with three primers (primers HSPps1, HSPps2, and HSPps4) and was sequenced as described previously (15). The amplicons that were obtained had sequence identity with the *B. henselae* sequence and indicated that the organism was a Marseille genogroup and CAL-1 genotype isolate. Also, a portion of the *B. henselae rpoB* gene, which encodes the rifampin resistance mutation, was amplified and sequenced on the basis of previous work (10) by incorporating PCR primers 1464 F (5′-GAT AAA AGA 4CG TAT GTC CTC GGTT-3′) and 2268 R (5′-AGG ATC TAAATCTTCTGTCGCACGA-3′) and sequencing primers SEQ F (5′-CGTCATGCCACAGGA TTT-3′) and SEQ R (5′-CAGCTCCTGAATCGCGA-3′). *rpoB* sequence analysis disclosed no mutation associated with rifampin resistance. Negative controls (vitreous fluid collected at necropsy from three people who did not have CSD and sterile distilled water) remained negative. The patient's serum exhibited an immunoglobulin G titer of 1:50 against *B. henselae* Houston strain, as determined by microimmunofluorescence as-

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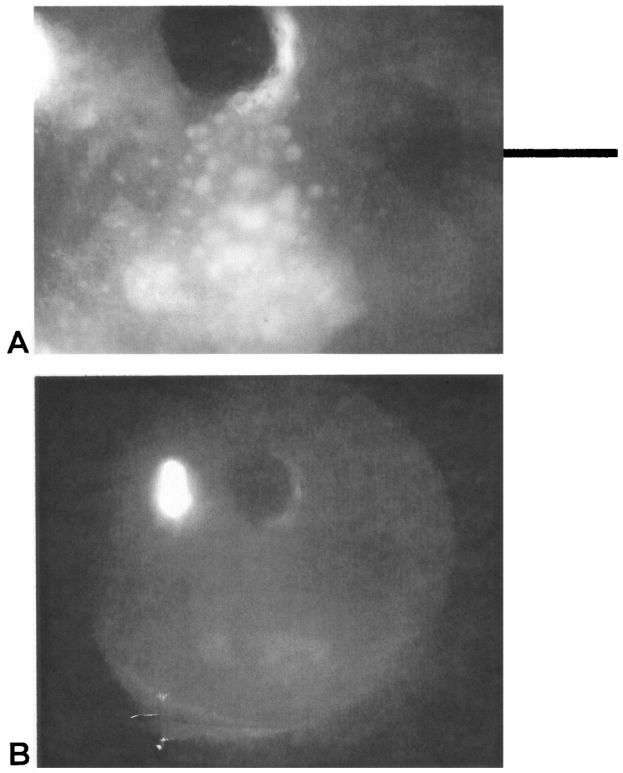

FIG. 1. Mutton fat keratic precipitates (bar) associated with a granulomatous uveitis before (A) and 6 months after (B) antibiotic treatment for *B. henselae* uveitis.

say (8). It reacted against two bands of 80 and 45 kDa when *B. henseale* Houston and Marseille strains were incorporated as antigens in a Western blot assay. When the diagnosis of *B. henselae* uveitis was made, antibiotic therapy was changed to rifampin at 900 mg/day in combination with sulfamethoxazole-trimethoprim (4,800/960 mg/day); and for the first time since 1998, the patient's ocular inflammation was controlled and corticosteroids were progressively tapered off, and the patient was monitored for 6 months. In October 2002, the antibiotic treatment was stopped; visual acuities were was 20/100 for the O.D. and 20/80 for the O.S.; laser flare values were 32 and 17 photons/ms for the right and left eyes, respectively; and the ocular pressure was normal at 17 mm Hg with local therapy. However, endothelial insufficiency with corneal edema in the right eye, a subcapsular cataract in the left eye that resulted in low visual acuity, and uveitis relapsed and prompted reintroduction of antibiotic treatment in November 2002, with an excellent response in March 2003 (Fig. 1B).

B. henselae, the bacterial agent of CSD, has been implicated as the cause of several ocular conditions, including Parinaud's oculoglandular syndrome, neuroretinitis, optic neuritis, the retinal white spot syndrome, focal retinal vasculitis, brand retinal arteriolar or venular occlusions, focal choroiditis, serous retinal detachment, peripapillary angiomatous lesions, anterior uveitis, vitritis, and pars planitis (9). For most patients with CSD uveitis, however, the diagnosis has been made on the basis of a single, elevated anti-*B. henselae* serum antibody titer (9, 11). Because of cross-reactivity between *B. henselae* and other microorganisms responsible for uveitis and since the seroprevalence of *B. henselae* in uveitis patients is similar to that in the general population, these diagnoses remain presumptive (1, 11). Also, the frequency with which infections with *Bartonella* species other than *B. henselae* produces uveitis is unknown; and a direct role of the latter species in the pathology of CSD uveitis has been questioned, as only one patient has been diagnosed with CSD uveitis on the basis of direct demonstration of *B. henselae* in ocular fluid by observation by electron microscopy and heminested PCR (4).

CSD uveitis was definitively diagnosed in our patient by the detection of *B. henselae* antigen and three *B. henselae*-specific sequences in the vitreous fluid. Laboratory contamination did not occur, as the vitreous was the only sample from the patient that was processed in the laboratory at that time. It was 1 of 100 vitreous fluid specimens that we examined as part of a collaborative project on the microbiology of chronic uveitis. Furthermore, specific antibodies were found by two different techniques in serum samples from several patients. *B. henselae*-associated posterior segment complications are usually reported as unilateral, although bilateral optic disk swelling and macular star formation have been reported in the course of CSD in some patients (13). In the patient described here, ocular manifestations were bilateral and the intraocular presence of *B. henselae* in one eye was firmly confirmed. Because of the underlying HLA-B27 uveitis, we cannot definitely confirm uni- or bilateral infection. Also, the coincidence of the *B. henselae* uveitis and HLA-B27 uveitis rendered the timing of infection and the role of prednisone therapy difficult to determine precisely; the course of *B. henselae* uveitis was chronic, with the diagnosis established probably 3 years after initial contamination. In the patient described here, immunohistochemical examination of the vitreous fluid was used for the first time for the rapid and specific diagnosis of *B. henselae* uveitis. We previously applied this technique for the rapid diagnosis of Whipple's disease uveitis, and immunohistochemistry now appears to be a first-line technique for the rapid and specific diagnosis of uveitis due to rare and fastidious cultured microorganisms for which specific polyclonal or monoclonal antibodies are available (3). The use of immunochemistry and PCR investigations may be considered for cases of undocumented, chronic uveitis prior to the initiation of immunosuppressive treatment. In the patient described here, diffuse immunostaining did not allow precise localization of *B. henselae* in a specific cell; rather, bacteria were seen extracellularly. We do not know if this resulted from cell lysis after intracellular growth or from extracellular growth. Also, sequence analysis of selected genomic targets allowed the genotyping of *B. henselae*. Indeed, two genogroups (Marseilles and Houston) and several genotypes are recognized for *B. henselae*, but the respective spectra of pathogenicities of the various genogroups and genotypes have not been established (2, 6). Direct diagnosis of CSD uveitis has been reported only once, with the CSD diagnosed by electron microscopic observation of bacilli further identified as *B. henselae* by heminested PCR of the vitreous fluid (4). That strain was not genotyped. Also, *B. henselae* retinitis was diagnosed in a human immunodeficiency virus-infected patient by microscopic observation of bacilli identified after sequencing of the 16S rRNA gene from a retinal biopsy specimen by

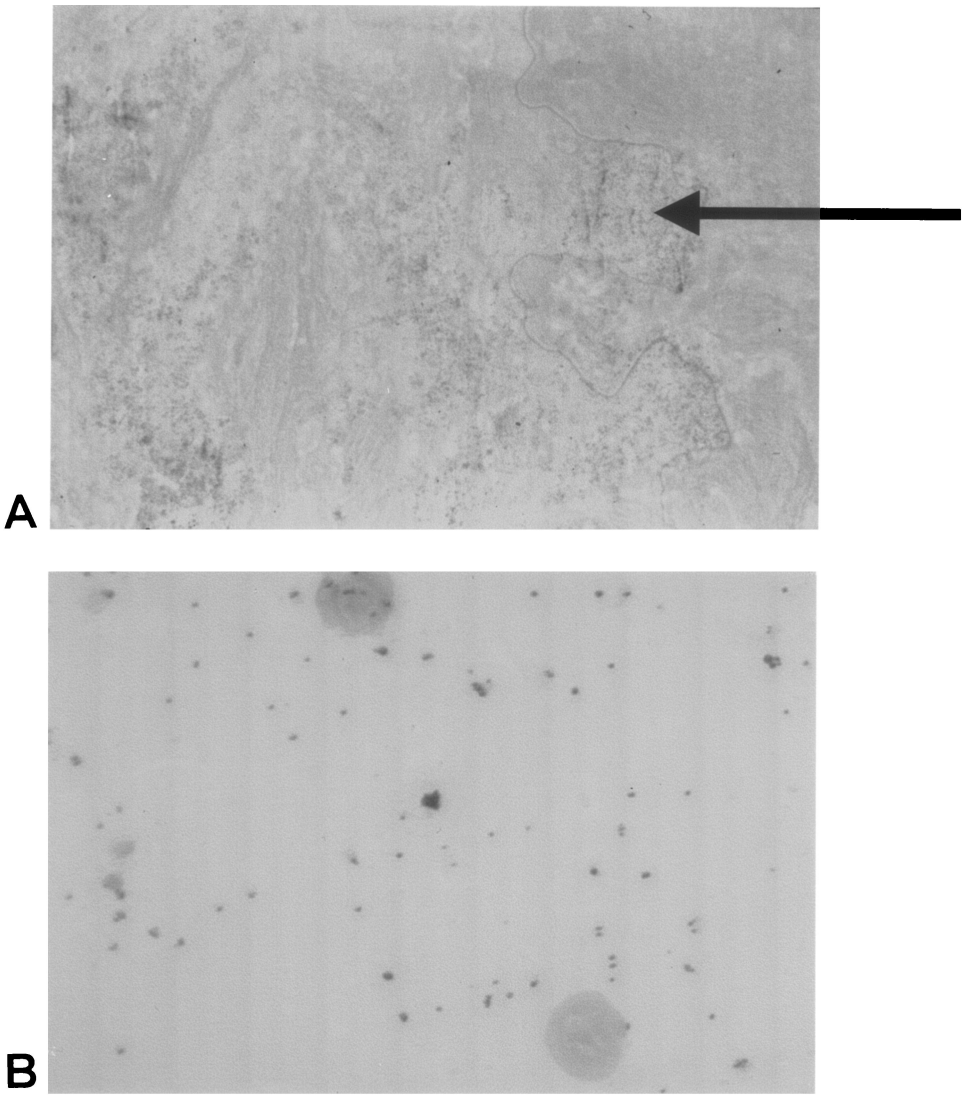

FIG. 2. (A) Detection of *B. henselae* as immunopositive material in the vitreous fluid of the patient (arrow) by immunocytochemical analysis (polyclonal rabbit anti-*B. henselae* antibody was used at a dilution of 1:2,000 with hematoxylin counterstain; magnification, ×250); (B) lack of detection of *B. henselae* in negative control vitreous fluid.

PCR (14). Otherwise, most cases have been diagnosed serologically (11). Nine HLA-B27 patients with CSD uveitis were reported previously, and the prevalence of this haplotype was found to be significantly higher in these patients than in patients with miscellaneous uveitis and the general population (5, 11). HLA-B27 patients may be more susceptible to *B. henselae* uveitis; or *B. henselae* infection may exacerbate previous HLA-B27-related uveitis, as reported for other gram-negative bacteria such as *Serratia*, *Salmonella*, and *Klebsiella*. It has been debated whether uveitis is due to the direct ocular involvement of *B. henselae* or a secondary immune reaction (11). Our case report and a previous one (4) clearly demonstrate that *B. henselae* reaches the eye and suggest that CSD uveitis results directly from *Bartonella* infection. These data support the recommendation that these patients be treated with antibiotics to achieve concentrations in the intraocular space effective against *B. henselae*.

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