Corneal Graft Rejection Complicated by Paracoccus yeei Infection in a Patient Who Had Undergone a Penetrating Keratoplasty

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A 68-year-old man who had undergone two penetrating keratoplasties of his left eye was admitted with early corneal graft failure. Culture of the anterior chamber fluid yielded Paracoccus yeei, a nonfermentative Gram-negative bacillus which thus far had only been implicated in ocular disease by means of PCR and 16S rRNA gene sequencing directly on patient material.

CASE REPORT

A 68-year-old male patient was admitted to the ophthalmology department of our hospital with suspected early corneal graft failure following a second penetrating keratoplasty (PKP). His medical history mentioned congenital rubella syndrome and congenital glaucoma of both eyes, which eventually led to blindness of the right eye. At age 52, the blind right eye had been enucleated due to severe ocular pain. At age 59, a cataract extraction plus intraocular lens implantation of the left eye had been performed, followed by a YAG laser capsulotomy for posterior capsular opacification after two years. At age 66, a trabeculectomy procedure followed by topical application of mitomycin-c to reduce fibrous proliferation had been performed to reduce increased intraocular pressure in the left eye. One year prior to the current admission, the patient had developed severe corneal decompensation of the left eye, for which he underwent a nonimmunotyped PKP. The latter operation was complicated by a corneal ulceration with Streptococcus pneumoniae, and a second nonimmunotyped PKP was performed the same year.

A year after the first PKP was performed, the patient presented himself at the outpatient clinic of our hospital with increasing pain and decreased visual acuity of his left eye. On ocular examination, the re-PKP showed signs of early corneal graft failure, with stromal edema in the upper third of the PKP. The lower part of the cornea had an irregular surface but was relatively translucent. Inflammatory cells were present in the anterior eye chamber. At that time, the patient was using rimexolone, dorzolamide-timolol, and fusidic acid in the left eye. Other medication included deslortadine inhalations once daily because of pollen-related allergic complaints. No signs of systemic illness were present. To reduce the process of graft rejection, oradexone was administered subconjunctivally. Also, 1% prednisolone eyedrops were given in 8 daily doses (dd), together with ofloxacin eyedrops.

Two days later, the patient was admitted to the ophthalmology department of our hospital with a necrotizing corneal ulcer. To test for the presence of microorganisms, an anterior eye chamber puncture was performed, revealing a nonfermentative Gram-negative bacillus and a coagulase-negative staphylococcus (the latter probably representing contamination of the sample). Thus, vancomycin and ceftazidime were injected intravitreally. Additionally, vancomycin and ceftazidime eyedrops were started in 6 dd because the corneal infiltration was spreading very fast in the cornea. Because the corneal lesion kept extending, intravenous vancomycin (2 dd of 1 gram) and ceftazidime (3 dd of 2 g) were added to the antibiotic regimen.

Despite intensive antibiotic therapy, the condition of the eye deteriorated, with extensive infiltration of the complete donor cornea, including the peripheral recipient cornea, rendering it impossible to perform a third PKP. In addition, ultrasonography of the eye showed a severe choroidal solution, also limiting the visual prognosis at the retinal level. Because of the very poor visual prognosis, the patient chose to have his only eye enucleated.

Microbiological examinations. After overnight incubation, culture of the aqueous humor on Columbia sheep blood agar yielded mucoid gray-white colonies. These were also recovered from GC-lect agar (BD) but not from anaerobically incubated medium. Gram staining showed Gram-negative cocccobacilli growing in pairs, with a vacuolated appearance. Furthermore, two colonies of coagulase-negative staphylococci grew on the Columbia sheep blood agar, one in the 2nd and one in the 3rd streak of the inoculating wire, but none on the GC agar or the anaerobically incubated agar plate; these were therefore regarded as contamination (although it was decided to include coverage for coagulase-negative staphylococci in the antibiotic regimen). The cultured Gram-negative bacillus was positive for oxidase and catalase. Biotyping with an API NH kit revealed the numerical code 3430, corresponding to Haemophilus influenzae (99.7%), and the API NE kit yielded the code 1243044, corresponding to Ochrobactrum anthrophi (99.6%); both determinations were unlikely considering the macroscopic morphology of the isolate. Its main biochemical characteristics are displayed in Table 1.

Subsequently, the isolate was identified by 16S rRNA gene sequencing (4). Bacterial DNA was isolated using Prepmann Ultra sample preparation reagent (ABI). DNA amplification...
and sequencing were performed on a 9800 fast thermal cycler (ABI). For the sequencing reaction, PCR mastermix from a Microseq 500 16S rRNA gene bacterial identification PCR KIT (ABI) was added to approximately 25 ng of bacterial genomic DNA in a total reaction mixture volume of 30 \mu l. The PCR assay included an initial step of 95°C for 10 s, followed by 30 cycles of 95°C for 0 s (the temperature was set to drop directly when the denaturation temperature was reached), 64°C for 15 s, and 72°C for 60 s. The amplification product was purified with a QIAquick PCR purification kit (Qiagen) and resuspended in 150 \mu l of MilliQ water (pH 8.0). Forward and reverse sequencing were performed with the forward and reverse sequencing mix in the Microseq 500 16S bacterial identification sequencing kit (Applied Biosystems) on 7 \mu l of purified DNA in a total volume of 20 \mu l. The sequencing assay consisted of a 1-min hold at 95°C, followed by 25 cycles of 96°C for 10 s, 50°C for 5 s, and 60°C for 60 s. The sequencing products were purified in Sephadex G50 spin columns and analyzed with a 3100 genetic analyzer (ABI). The 442-bp sequence obtained was analyzed using the MicroSeqID software, version 2.0, and the MicroSeq 500 database (ABI), but this did not yield a reliable identification. However, a BLAST search in GenBank identified the microorganism as Paracoccus yeei, with 100% similarity to the type strain G1212. The identity of the strain was later confirmed by commercial 16S rRNA gene sequencing at Scanbec GmbH (Halle/Saale, Germany). A 1,255-bp sequence with 100% similarity to P. yeei strain G1212 was obtained.

Susceptibility testing by Etest (BioMérieux) yielded MICs of 4 \mu g/ml for ceftazidime, 0.25 \mu g/ml for tobramycin, 16 \mu g/ml for piperacillin, 0.5 \mu g/ml for ciprofloxacin, and >32 \mu g/ml for vancomycin.

Ultimately, in our case, a diagnosis of corneal infection due to Paracoccus yeei was made.

Paracocci are Gram-negative cocci, diplococci or coccobacilli, with a high GC content and an obligately respiratory mode of growth (1). The genus Paracoccus is classified within the family Rhodobacteraceae and currently contains 29 recognized species. In 2003, based on biochemical tests, DNA-DNA hybridization, cellular fatty acid profile, and 16S RNA sequencing, Daneshvar et al. proposed Paracoccus yeeii (the name was later changed to P. yeei) as a separate species for 13 isolates in the CDC eugonic oxidizer group 2, all of which had been isolated from human samples (2). The morphology of the species in Gram stain was described as Gram-negative cocoid or diplococoid to coccobacillary forms, often appearing vacuolated or peripherally stained. The natural habitat of P. yeei is unclear. To date, the 16S rRNA gene sequences of four environmental P. yeei isolates have been submitted to GenBank (www.ncbi.nlm.nih.gov/GenBank/). These strains were isolated from a marine sediment, a spacecraft-associated clean room, a sweet pepper, and soil.

Since the species was proposed, only one case of human infection with P. yeei has been described: a 67-year-old male patient with a history of heart failure who developed bacteremia and bullous lesions on the leg with this microorganism (5). However, two previous reports suggested that P. yeei may cause eye disease. First, in the original article proposing the novel species, one of the thirteen strains was isolated from an eye, although no further clinical information was provided (2). Second, Drancourt et al. published a report in 2008 identifying a high proportion of fastidious microorganisms in 1,520 patients with uveitis of unknown etiology (i.e., culture negative) by means of PCR and 16S rRNA gene sequencing directly on ocular fluid. Among the microorganisms identified were five never before associated with ocular infections, one of these being P. yeei. Since no other microorganism was detected in the sample from this patient, the uveitis was attributed to P. yeei (3).

In conclusion, this is the first clinically documented case of culture-positive P. yeei (mixed) corneal infection, which occurred as an early complication in a patient with a penetrating keratoplasty. The natural habitat of the microorganism is largely unknown, making it difficult to speculate on how our patient acquired the strain. The wider availability of molecular techniques for species identification may yield an increasing number of reported infections with P. yeei.

**Nucleotide sequence accession number.** The 1,255-bp 16S rRNA gene sequence that confirmed the identity of the isolate was deposited in GenBank under accession number GU083584.
REFERENCES


