Molecular Detection of *Cellulosimicrobium cellulans* as the Etiological Agent of a Chronic Tongue Ulcer in a Human Immunodeficiency Virus-Positive Patient

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Ulcerations appeared on the tongue of a 48-year-old human immuno-deficiency virus-positive man. Histological findings of the biopsy specimen and the fact that the patient had resided in Louisiana led us to suspect “American histoplasmosis.” A new ulcer appeared while the patient was being treated with itraconazole, and the gene for 16S rRNA of *Cellulosimicrobium cellulans* was amplified. The lesions healed during treatment with oral penicillin and azithromycin.

**CASE REPORT**

The patient was a 48-year-old man, positive for human immunodeficiency virus since 1988. From 1994 on, he received antiretroviral treatment, which mostly consisted of two nucleoside inhibitors of the viral reverse transcriptase and one protease inhibitor. In November 2001, the antiretroviral treatment was stopped because of the development of a prominent cervicodorsal lipohypertrophy (buffalo neck). His CD4 count was above 250 cells/µl most of the time, despite high viral loads (>10^5 copies of RNA/ml) during time without antiretroviral treatment, and he had never suffered opportunistic infections. He had no history of aphthous ulcers or viral infections of the oral mucosa.

In January 2003, he presented with three deep ulcerations on the left side at the base of his tongue. The ulcerations were surrounded by a small inflammatory wall. Inflamed and sensitive regional lymph nodes were noted. At that time, his CD4 count was 365 cells/µl. A biopsy sample of the ulceration was taken, and histological examination revealed Malpighian coating and hyperplasia with some small areas of epithelial dysplasia. The chorion contained multiple inflammatory lymphoplasmocytic infiltrates with microabscesses and some histiocytic epithelioid granulomas without necrotic foci. No malignancies and no microorganisms were observed. Computer tomography of the oral cavity noted a prominence on the left side of the tongue and small normal lymph nodes along both sides of the neck. The patient’s tonsils, salivary glands, and other structures were normal.

On the basis of both the clinical and histological findings and the fact that the patient had resided in Louisiana for a short time 20 years ago, the diagnosis of “American histoplasmosis” was suggested. In spite of repeatedly negative serological tests for *Histoplasma capsulatum*, treatment with itraconazole (400 mg/day) was started in January 2003. By the end of June, a new ulcer of the left side of the tongue appeared. At this time (23 June 2003), the serum level of itraconazole had been determined at 1.67 mg/liter. New serological tests for histoplasmosis, leishmaniasis, and syphilis were performed but found to be negative. In the absence of new etiological data, the daily dose of itraconazole was increased to 1,000 mg, and a new biopsy specimen of the patient’s tongue was taken and sent to our microbiology laboratory to search for bacterial and fungal DNA.

The search for fungal DNA was undertaken by PCR using universal primers (ITS1 [5'-TCCGTAGTTGAACTCGGG-3'] and ITS2 [5'-TCCTCCGCTTATGATG-3']) targeting the 3' terminus of the 18S rRNA gene and the 5' terminus of the 23S rRNA gene, including the internally transcribed spacers ITS1 and ITS2 (21). The result of this PCR was negative.

The search for bacterial DNA by PCR was done using universal primers designed on the basis of conserved sequences of the *rrn* gene coding for 16S rRNA (13BS [5'-GCCCAGGAAA CGTATTAC-3'] and 91E [5'-TCAAAKGAATTGACGG GGC-3']) (15). The 16S rRNA gene sequence obtained was compared with those available in the GenBank, EMBL, and DDBJ databases with the gapped BLASTN 2.0.5 program obtained from the National Center for Biotechnology Information server (http://www.ncbi.nlm.nih.gov/BLAST/). This PCR data was used to provide evidence that *Cellulosimicrobium cellulans* was the etiological agent of the chronic tongue ulcer.

The patient was treated with oral penicillin and azithromycin for 2 weeks, and the ulcers healed.

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yielded a positive result with the amplification of a fragment of the expected size of 479 bp, which was subsequently sequenced. The nucleotide sequence of the amplified fragment was identified without ambiguity as the 16S rRNA gene of \textit{Cellulosimicrobium cellulans} (100\% identity of 452 nucleotides with the 16S rRNA gene of \textit{C. cellulans} strain AS 4 1333 [AY114178.1]).

As this result was unexpected, the patient was asked to consent to another biopsy. The amplification of bacterial DNA from this second specimen yielded exactly the same result, \textit{C. cellulans}, whereas cultures on sheep blood and chocolate agar (Bio-Rad France), incubated under normal and low-oxygen conditions, revealed very poor oropharyngeal flora consisting of \textit{Aerococcus viridans} and nonhemolytic streptococci. Following the diagnosis of infection due to \textit{C. cellulans}, an actinomycetal species, treatment with itraconazole was stopped and the patient was given penicillin V (2 × 10\(^6\) U/day) and azithromycin (500 mg/day for 3 days every fortnight) starting at the beginning of September 2003. With this treatment, the healing process was initiated, and 3 weeks later, the functional status of the patient’s tongue had become normal and the lesions had disappeared. This treatment was continued for 5 months. At the beginning of February 2004, the treatment was stopped, and since that time, no further ulceration of the patient’s tongue has been observed.

\section*{Discussion}

Lesions of the oral cavity in human immunodeficiency virus-positive patients are common, as immunosuppression predisposes the patient to oral bacterial and fungal opportunistic infections (2, 7). Oral manifestations may consist of either pseudomembranous, hyperplastic lesions or atrophic erythematous ulcerations. They are generally very painful and create dysphagia. The most common oral ulcerations are recurrent aphthae due to infection by herpes simplex virus type 1 or cytomegalovirus (7). \textit{Candida} spp. are the most frequently found fungi, but other fungi, such as \textit{Cryptococcus neoformans} and \textit{H. capsulatum}, may cause oral manifestations, including palatal and lingual ulcers and granulomas and nodular ulcerative lesions (7, 14, 17). Bacteria known to cause oral ulcerations include \textit{Treponema pallidum} and some actinobacteria, especially \textit{Mycobacterium tuberculosis} (20).

\textit{C. cellulans} is a gram-positive rod and belongs to the \textit{Actinomycetales} order. It is found in soil and decaying plant material. It is relatively avirulent and rarely associated with infection in humans (6). When it has been isolated from clinical samples, it has been associated with the presence of foreign bodies and it is generally found in immunocompromised patients.

The species has undergone several taxonomic changes since its first description in 1923 (1). It was first known as \textit{Brevibacterium fermentans}, and two case reports have been published describing meningitis and sepsis due to this bacterium in infants and children (3, 5). Some years later, \textit{B. fermentans} was renamed \textit{Oerskovia xanthineolytica}, and a few case reports were published on infections with \textit{Oerskovia} spp., such as endocarditis, pyonephrosis, endophthalmitis, pneumonia, meningitis, parenteral nutrition-related septicemia, catheter-related septicemia, and peritonitis (12). The genera \textit{Oerskovia} and \textit{Cellulomonas} were united into the unique genus \textit{Cellulomonas} in 1982, and \textit{O. xanthineolytica} became \textit{Cellulomonas cellulans} (19). Finally, in 2001, \textit{Cellulomonas cellulans} was reclassified as \textit{Cellulosimicrobium cellulans} (16).

\textit{C. cellulans} is known to grow well on standard synthetic media and to be susceptible to a variety of antibiotics, including penicillins, macrolides, and glycopeptides. The treatment given to our patient was based on this knowledge and not on the basis of an antibiotic susceptibility test, since the strain was not isolated. An explanation for this absence of growth could be that \textit{C. cellulans} was no longer viable on synthetic media due to the action of itraconazole which the patient had received for 5 months prior to the molecular diagnosis. Itraconazole is an antifungal agent belonging to the azole group; its cellular target is a cytochrome P450 (CYP) (11). CYP enzymes have been identified in several bacteria, such as \textit{Pseudomonas putida}, \textit{Methylcoccus capsulatus}, and \textit{Streptomyces} spp., whereas \textit{Escherichia coli} and streptococci do not possess CYP enzymes (10). The completion of several genome projects revealed that pathogenic bacteria, such as \textit{M. tuberculosis} and \textit{Rhodococcus} spp., also possess such enzymes, and it now seems that species of the \textit{Actinomycetales} order are particularly rich in CYP enzymes (4, 9, 13). Moreover, an ortholog of the specific P450 CYP enzyme (CYP51) has been identified in \textit{M. tuberculosis}, \textit{Mycobacterium smegmatis} (8, 18), and \textit{Rhodococcus} spp., and it has been postulated that a CYP51-like P450 enzyme was present in an ancestral actinomycete, from which it may have been inherited by the members of this order (10). Thus, \textit{C. cellulans} should possess a CYP51 enzyme.

As nothing was known of the activity of itraconazole against \textit{Actinomycetales} bacteria, we tested its in vitro activity against two \textit{C. cellulans} type culture collection strains (CIP 103404 [ATCC 12830] and CIP 101063). Itraconazole MICs were 128 mg/liter for the two strains of \textit{C. cellulans}. These itraconazole MICs, which resembled the MICs found for fluconazole against different mycobacteria, were notably higher than the itraconazole concentration found in the patient’s serum (1.67 mg/liter) after a 5-month treatment with 400 mg/day. Therefore, an in vivo inhibitory effect of itraconazole on \textit{C. cellulans} as the cause of the absence of growth of this bacterium on synthetic media is unlikely.

In conclusion, with the etiological diagnosis yielded by molecular biology methods, the infection was controlled, and the patient was cured.

\section*{REFERENCES}