Geosmithia argillacea: An Emerging Cause of Invasive Mycosis in Human Chronic Granulomatous Disease

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Background. Chronic granulomatous disease (CGD) is an inherited disorder of the nicotinamide adenine dinucleotide phosphate oxidase that leads to defective production of microbicidal superoxide and other oxidative radicals, resulting in increased susceptibility to invasive infections, especially those due to fungi.

Methods. Geosmithia argillacea was identified from cultured isolates by genomic sequencing of the internal transcribed spacer region. Isolates previously identified as Paecilomyces variotii, a filamentous fungus closely resembling G. argillacea, were also examined.

Results. We identified G. argillacea as the cause of invasive mycosis in 7 CGD patients. In 5 cases, the fungus had been previously identified morphologically as P. variotii. All patients had pulmonary lesions; 1 had disseminated lesions following inhalational pneumonia. Infections involved the chest wall and contiguous ribs in 2 patients and disseminated to the brain in 1 patient. Four patients with pneumonia underwent surgical intervention. All patients responded poorly to medical treatment, and 3 died.

Conclusions. We report the first cases of invasive mycosis caused by G. argillacea in CGD patients. G. argillacea infections in CGD are often refractory and severe with a high fatality rate. Surgical intervention has been effective in some cases. G. argillacea is a previously underappreciated and frequently misidentified pathogen in CGD that should be excluded when P. variotii is identified morphologically.

Chronic granulomatous disease (CGD) is a rare inherited disorder involving defective nicotinamide adenine dinucleotide phosphate oxidase function, which leads to defective production of antimicrobial superoxide anion and related oxygen intermediates critical for host defense [1]. Clinically, CGD patients develop recurrent life-threatening infections and extensive tissue granuloma formation, autoimmune diseases, and inflammatory bowel disease, which may require immunosuppressive or immunomodulatory therapy. The pathogens that commonly cause infection in CGD include the bacteria Staphylococcus aureus, Serratia marcescens, Burkholderia cepacia complex, and Nocardia species and the fungi Aspergillus species, especially Aspergillus fumigatus and Aspergillus nidulans [2]. Other less common fungi, such as Paecilomyces species and Trichosporon inkin, are encountered more frequently in patients with CGD than among the general population, highlighting the fact that patients with CGD have a unique susceptibility pattern [1].

New pathogens are continually being identified as a result of improvements in microbiologic culture and identification techniques. The use of genomic sequencing and the increasing number of sequences available in databases have permitted identification
of emerging pathogens (eg, *Granulibacter bethesdensis*) [3] in CGD patients, as well as the reassignment of misidentified pathogens (eg, *Neosartorya udagawae*) [4, 5] to their correct taxa.

Here we describe, to our knowledge, the first report of invasive mycoses caused by another emerging pathogen, *Geosmithia argillacea*, in 7 CGD patients. This disease was aggressive, involving invasion across tissue planes or metastatic dissemination in 3 patients. As shown by genomic sequencing of stored fungal isolates, isolates from 4 of these patients had been misidentified originally as *Paecilomyces variotii*, which resembles *G. argillacea* morphologically. Correct identification has important therapeutic and prognostic implications.

**Materials and Methods**

**Isolates**

We reviewed microbiology data of isolates identified as *G. argillacea* or *P. variotii* from all CGD patients (Institutional Board Approved protocol 05-I-0123, 93-I-0119) obtained since 2000. Twenty-three isolates that were obtained from sterile biopsy sites or were repeat isolates were subjected to molecular sequencing to confirm their species identification. Isolates from cases 1 and 4 were grown and identified at another institution, and the isolate from case 7 was referred to the National Institutes of Health for identification. Selected isolates for each patient were kept in 2-mL sterile water vials at room temperature or lyophilized. Morphology was assessed macroscopically and microscopically from Sabouraud plates incubated at 28°C and/or Sabouraud and potato dextrose agar (PDA) slide cultures using lactophenol aniline blue preparations. Isolates from cases 2, 4, 6 and 7 have been deposited at the University of Alberta Microfungus Collection and Herbarium (as UAMH 10595, UAMH 9833, UAMH 10758, and UAMH 10905, respectively.

**Molecular Identification**

Mycelial DNA was extracted with the UltraClean Microbial DNA Isolation kit (MoBio Laboratories) according to the manufacturer’s instructions for molds and quantified with a spectrophotometer (NanoDrop). The internal transcribed spacer (ITS) region was amplified using published primers ITS1 and ITS4 [6] using Ready to Go Beads (GE Healthcare) in a PTC-200 Thermal Cycler (MJ Research) with the following cycling parameters: 1 cycle of 95°C for 5 min, then 34 cycles of 60 seconds at 95°C, 60 seconds at 55°C, and 60 seconds at 72°C, followed by 10 min at 72°C.

Polymerase chain reaction (PCR) amplicons were purified with the Microcon YM-100 centrifugation filter device (Millipore) and sequenced on a 3100 sequencer (Applied Biosystems) using the same PCR primers. Results were analyzed using Lasergene software, version 7.0 (DNASTAR). Sequences were compared with GenBank sequences by means of nucleotide-nucleotide Basic Local Alignment Search Tool (BLASTn) and aligned to best-matched sequences by means of CLUSTAL W using MegAlign (DNASTAR). Phylogenetic trees were constructed using the Phylip 3.60 package [7]. Distance matrices based on the Kimura 2-parameter model were produced with the DNADIST program, and a neighbor-joining tree constructed with the NEIGHBOR program. Bootstrapping was performed using SEQBOOT (100 iterations).

An isolate from case 4 could not be regrown from the stored culture stock for sequence-based identification. Direct amplification and sequencing of the ITS region was performed on DNA isolated directly from the culture stock, producing a match for *G. argillacea*. Because this sequence was shorter than those of the other 6 isolates, it was not included in the phylogenetic analysis.

**Antifungal Susceptibility Testing**

Antifungal drug susceptibility testing was performed by means of broth microdilution at the Fungus Testing Laboratory, University of Texas Health Science Center, using the
RESULTS

Morphological Characteristics
Colonies on Sabouraud agar plates grew relatively slowly and appeared as cream to pale brown–colored powdery molds, with colonies reaching a diameter of 3 cm in 1 week at 28°C (Figure 1A). All isolates were thermophilic, displaying good growth at 42°C. Microscopic examination of the cultures revealed hyaline, rough septate conidiophores bearing monoverticillate, biverticillate, or terverticillate penicilli. Phialides were cylindrical with a tapering tip producing smooth-walled cylindrical or box-shaped conidia measuring 3.0–4.5 by 1.5–2.7 μm, which later appeared ovoid shaped at maturity (Figure 1B).

Molecular Analysis
The ITS sequences showed best match to G. argillacea AF033389 (type strain) and to 2 canine isolates, EU862337 and EU862335, with similarity to the type strain ranging from 97.9% to 98.5%. The ITS sequence of the isolate from case 7 differed in having an insertion of 31 base pairs that lowered the similarity to AF033389 to 93% as calculated with use of the Blast program. Besides this insertion, the sequence showed high similarity to the reference strains of G. argillacea. In the phylogenetic analysis, all clinical isolates grouped with reference strains of G. argillacea with high bootstrap support (Figure 2). Four clinical isolates (cases 1, 2, 5, and 6) had identical ITS sequences, as was evident from their clustering in the tree. In general, intraspecies similarities among the clinical isolates was in agreement with published values for clinical and reference isolates of the species [9–11].

Patients
Clinical histories are provided for 7 patients and summarized in Table 1.

Case 1. A 10-year-old boy with X-linked CGD diagnosed at 2 months of age had a medical history of pneumonia, lymphadenitis, and osteomyelitis of the left arm. At age 8, he received a diagnosis of right middle lobe pneumonia (Acremonium sp), which partially responded to caspofungin. Recurrence of symptoms during antifungal treatment led to a repeat fine-needle biopsy; culture of the specimen grew G. argillacea on the basis of the morphology, which was confirmed by genomic sequencing (University of Texas, San Antonio). Susceptibility
testing revealed resistance to voriconazole (mean inhibitory concentration [MIC], >16 μg/mL). The MICs for caspofungin, posaconazole, and amphotericin B were 0.5, 2, and 4 μg/mL, respectively, and antifungal therapy was switched to posaconazole and caspofungin. Voriconazole was subsequently added. Despite intense oral and intravenous antifungal therapy with therapeutic levels of both azoles for 8 months, the infection progressed to involve an adjacent rib and the chest wall (Figure 3Ai). The chest wall abscess was drained; calcofluor white stain revealed true septate hyphae (Figure 3B), and the cultures grew G. argillacea. Regular twice-weekly white cell transfusions were added. After almost 2 years of intense medical therapy, failure to control the infection led to a surgical resection of his right middle lobe. At 2 months postoperative follow-up, there was no evidence of disease progression in his lung and the rib osteomyelitis seems less intense.

**Case 2.** A 25-year-old white man had X-linked CGD that was diagnosed in infancy. He had a history of recurrent infections and abscesses treated medically and surgically. At age 25, after 3 years without infection, he developed a case of left lower lobe pneumonia; culture of a specimen grew *Phoma* species. Treatment with voriconazole and caspofungin resulted in improvement of the lung lesion. A year later, during continued caspofungin and voriconazole therapy, a left upper lobe infiltrate appeared that rapidly extended into the pleural cavity and adjacent chest wall (Figure 3Aii). A sample from surgical drainage of the purulent material grew a mold identified as *P. variotii*. Culture of samples from repeated lung biopsies, as well as biopsy of the mass and adjacent lymph node, again grew *P. variotii*. ITS sequencing revealed this to be *G. argillacea*. Failure to control the infection despite the prolonged intensive medical therapy led to an attempt at ex vivo retroviral-mediated

<table>
<thead>
<tr>
<th>Case</th>
<th>Diagnosis</th>
<th>Age/sex</th>
<th>Source</th>
<th>Sample date</th>
<th>Surgery</th>
<th>Antifungal therapy</th>
<th>MIC, μg/mL</th>
<th>Isolate Genbank access no.</th>
<th>Patient status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CGD, gp91phox</td>
<td>10/male</td>
<td>Lung, chest wall abscess</td>
<td>2009, 2010</td>
<td>Chest wall drainage, lung resection</td>
<td>Posaconazole, voriconazole, caspofungin</td>
<td>Amphotericin B, 4; itraconazole, NT; voriconazole, &gt;16; posaconazole, 2; caspofungin, 0.5; micafungin, &lt;0.015</td>
<td>HQ246724</td>
<td>Alive</td>
</tr>
<tr>
<td>2</td>
<td>CGD, gp91phox</td>
<td>25/male</td>
<td>Lymph node, BAL, tracheal aspirate, chest wall abscess</td>
<td>2005, 2007</td>
<td>Chest wall drainage, gene therapy</td>
<td>Voriconazole, caspofungin</td>
<td>Amphotericin B, 2; itraconazole, NT; voriconazole, &gt;8; posaconazole, 0.5; Caspofungin, &lt;0.03; micafungin, NT</td>
<td>HQ246725</td>
<td>Deceased</td>
</tr>
<tr>
<td>3</td>
<td>CGD, P22phox</td>
<td>18/male</td>
<td>Sputum (twice)</td>
<td>2010</td>
<td>None</td>
<td>Posaconazole, micafungin</td>
<td>Amphotericin B, 4; itraconazole, NT; voriconazole, &gt;16; posaconazole, 4; caspofungin, 0.5; micafungin, &lt;0.015</td>
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<td>Alive</td>
</tr>
<tr>
<td>4</td>
<td>CGD, P47phox</td>
<td>40/female</td>
<td>BAL, endotracheal aspirate, brain abscess, lung biopsy</td>
<td>2000, 2005</td>
<td>Craniotomies, lung resection</td>
<td>Itraconazole, amphotericin B, posaconazole</td>
<td>Amphotericin B, 2; itraconazole, 1; voriconazole, NT; posaconazole, NT; caspofungin, NT; micafungin, NT</td>
<td>...</td>
<td>Alive</td>
</tr>
<tr>
<td>5</td>
<td>CGD, gp91phox</td>
<td>24/male</td>
<td>Lung biopsies</td>
<td>2001, 2003</td>
<td>Lung resection</td>
<td>Itraconazole, posaconazole</td>
<td>Amphotericin B, 8; itraconazole, 4; voriconazole, &gt;8; posaconazole, 4; caspofungin, NT; micafungin, NT</td>
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<tr>
<td>6</td>
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<td>18/male</td>
<td>Lung biopsy</td>
<td>2006</td>
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<td>Posaconazole</td>
<td>NT</td>
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**NOTE.** BAL, bronchoalveolar lavage; CGD, chronic granulomatous disease; MIC, mean inhibitory concentration; NT, not tested.
gene therapy with a myeloreductive conditioning. The patient died almost 6 months after his gene therapy despite ongoing intensive antifungal therapy and granulocyte transfusions.

**Case 3.** An 18-year-old man with \( P_{22} \)phox-deficient autosomal recessive CGD had lung infections in childhood and, from age 14, inflammatory bowel disease complicated by rectal stricture resulting in subacute obstruction not controlled by oral prednisone therapy and repeated manual anal dilations. Adalimumab, a tumor necrosis factor-\( \alpha \) inhibitor, was added to control his inflammatory bowel disease. He developed diffuse mulch pneumonitis after exposure to aerosolized mold in a horticultural class [12]. Bronchoscopic biopsy and induced sputum samples grew several organisms, including *Actinomadura* species, *Aspergillus fumigatus*, and *G. argillacea*. The *G. argillacea* was susceptible in vitro to posaconazole. After 3 months of intense antifungal (posaconazole and micafungin) and antibacterial therapy and moderate-dose prednisone (<0.5mg/kg) therapy, the disseminated pulmonary nodules on computed tomography (CT) remained stable.

**Case 4.** A 40-year-old white woman with \( P_{47} \)phox-deficient autosomal recessive CGD was relatively healthy until age 30, when she received a diagnosis of left lower lobe pneumonia and, a few months later, an *A. fumigatus* abscess in the left psoas. Shortly thereafter, she developed brain lesions, and her antifungal therapy was changed from itraconazole to amphotericin B, with the addition of voriconazole when she developed severe pneumonia. A bronchoscopy-obtained isolate was identified morphologically as *G. argillacea* by L. Sigler, MD, at the University of Alberta Microfungus Collection and Herbarium (UAMH), Devonian Botanical Garden, Edmonton, Alberta,
Canada [13]. Therapy was changed to liposomal amphotericin B and oral voriconazole. However, a new pulmonary infiltrate developed, and isolates from the repeat bronchoscopy and a biopsy specimen from the brain lesion were referred to UAMH for morphological confirmation [13]. Molecular sequencing performed during the present study confirmed that these isolates matched *G. argillacea*. Multiple craniotomies were required to remove all cerebral lesions (Figure 4). The intracranial infection has not recurred.

**Case 5.** A 25-year-old black man with X-linked CGD had left eye enucleation for candidiasis in childhood, subcorneal pustular dermatitis, and recurrent cases of pneumonia. Pulmonary infiltrates in the left lower lobe were biopsied in 2001 and resected in 2003 but recurred 3 years later in the same area. Bronchoscopy and biopsies of the lung yielded isolates for each pulmonary event that grew a mold that was then morphologically identified as *P. variotii* but confirmed here by means of sequencing as *G. argillacea*. Posaconazole prophylaxis since 2006 has controlled his lung disease.

**Case 6.** An 18-year-old white man with X-linked CGD received a diagnosis at 2 years of age of recurrent bronchitis and skin abscesses. He had multiple infections, including *Burkholderia multivorans* infection and fungal pneumonia, hepatic abscesses, and severe inflammatory bowel disease associated with rectovesical fistula. Culture of a biopsy of a lung lesion at age 16 grew a mold initially identified as *P. variotii* but shown here to be *G. argillacea*. The patient was treated with posaconazole, but he died of uncontrolled inflammatory bowel disease and its complications. Culture of specimens obtained premortem and at autopsy grew *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, but there was no growth of fungus despite septate hyphae observed with gomori-methenamine silver stain.

**Case 7.** A 25-year-old man with X-linked CGD was relatively healthy until he developed pneumonia. Biopsy of the pulmonary lesion yielded isolates identified as *P. variotii*, and he initially responded to posaconazole therapy. A year after cessation of treatment, he developed severe headaches. CT of his head revealed ring enhancing lesions that were thought likely fungal. A repeated culture of the persistent lung infiltrate yielded an isolate identified as *P. variotii*, which was subsequently re-identified as *G. argillacea*. Posaconazole therapy was re instituted, but he died 6 months later.

**DISCUSSION**

The genus *Geosmithia* contains numerous species formerly classified as *Penicillium*. *G. argillacea* was described in 1969 as a new thermotolerant *Penicillium* species [14]. In 1979, Pitt created the genus *Geosmithia* to distinguish isolates previously known as *Penicillium* species but that (1) formed conidia borne as cylinders from cylindrical, rough-walled phialides lacking narrow necks, as in *Penicillium* and *Paecilomyces*, and (2) produced conidia that were not typically green [15]. The teleomorph of *G. argillacea*, *Talaromyces eburneus*, was first described in 1994 by Yaguchi et al [16, 17], who isolated it from soil samples in Taiwan.

The inability of CGD patients to generate microbicidal reactive oxidants predisposes them to infections due to filamentous fungi, such as *Aspergillus*, that are major causes of morbidity and mortality [1, 18]. To our knowledge, this is the first report of invasive mycosis due to *G. argillacea* in humans, all of whom had CGD. Six of our 7 CGD patients from whom *G. argillacea* was isolated suffered severe, protracted infection, which extended across tissue planes from lungs to adjacent ribs in 2 patients (Figure 3A) and involved dissemination to the brain in 1 patient (Figure 4), despite antifungal therapy (Figure 3A) (Table 1). Surgical resection was required in 5 patients. This aggressive disease pattern and refractoriness to medical therapy from *G. argillacea* resembles the courses seen with several other rare invasive filamentous molds in CGD patients, including *A. nidulans*, *Aspergillus viridinutans*, *N. udagawae*, and *Chrysosporium zonatum* that tend to disseminate and invade adjacent bone [5, 18–21].

Retrospective identification of *G. argillacea* for isolates dating back to 2000 that were previously identified as *P. variotii* highlights the morphological similarities between these 2 organisms. Features helpful for presumptive identification of *G. argillacea* include restricted growth at lower temperature (<28°C); beige-buff–colored colonies; rough texture of stipes (conidiophores), metulae, and phialides; and characteristic cylindrical or box-shaped conidia (Figure 1). The distinctive roughness of the fruiting structure, along with the cylindrical shape of the conidia, which is more evident in early stages of conidial growth, provides a valuable initial clue for suspicion of *G. argillacea*. At later stages of maturity, the conidia adopt an ovoid shape, which resembles those of *P. variotii* (Figure 1). The species is differentiated from *Paecilomyces lilacinus*, which also displays rough stipes, by lack of a mauve color on PDA and from *Paecilomyces crustaeus* (teleomorph *Thermoascus crustaceus*), which also produces cylindrical conidia and is thermotolerant, by the lack of production of a teleomorph (ascomata) in culture. It is differentiated from *Merryhilda ingelheimensis* (teleomorph *Hamigera avellanea*) by its rough stipes and metulae, its cylindrical phialides and conidia, and the lack of any reverse reddish brown pigments or a teleomorph in culture. The *T. eburneus* teleomorph of *G. argillacea* is heterothallic and fails to form in culture without mating to appropriate additional strains.

Because our results indicate that all stored *P. variotii* isolates from CGD patients from sterile sites or recurrent isolations since
2000 are *G. argillacea*, this raises the possibility that some of the *Paecilomyces* infection in CGD patients in previously published reports may have been caused by *G. argillacea* [22–24]. Furthermore, similar misidentification may also occur with isolates from non-CGD patients [9]. We have molecular confirmation of *G. argillacea* from formally identified *P. variotii* isolates from 3 non-CGD patients (unpublished data), all of whom had underlying immune suppression. A large-scale, complete genomic sequencing of all *P. variotii* clinical isolates would be important for gaining an understanding of species-specific pathogenicity in different population groups, as well as their susceptibility profiles.

ITS sequencing to compare sequences with those of reference strains provided a definitive method for identification of the isolates as *G. argillacea* (high similarity, typically >97%; Figure 2) and unambiguous differentiation from *P. variotii* (low similarity, <77%; Figure 2) and from other morphologically similar organisms, such as *P. crustaceus*, *P. lilacinus*, and *M. ingelheimense* (not shown, low similarity, usually in the range 59%–84%). Phylogenetic analysis of the ITS sequences showed clustering of our clinical isolates with 3 reference strains of *G. argillacea*, which was supported by high bootstrap values in the phylogenetic tree. ITS sequencing was recently shown to have sufficient interspecific variation for identification of *G. argillacea* and differentiation from morphologically related species [9]. It is interesting to note the presence of an insertion sequence of 31 base pairs in the ITS sequence of the isolate from case 7. Such insertion sequences, which highlight the intraspecies ITS sequence heterogeneity, have been described in other molds [25, 26] but not in *G. argillacea*.

Apart from the previous presentation of case 4 [13], to our knowledge, there has been no detailed report on *G. argillacea* as a human pathogen. Thus far, its only association with malarial disease has been a single case of systemic mycosis in a German shepherd dog with extensive granuloma formation [11]. Very recently, Houbraken et al used genomic sequencing to identify 4 strains of *T. eburneus* (teleomorph of *G. argillacea*) among 34 isolates morphologically identified as *Paecilomyces* species [9]. These isolates were obtained from blood cultures during a pseudo-outbreak in a hospital, from a cystic fibrosis patient, and from the peritoneal dialysis fluid and blood of a patient; however, no clinical information associated with those isolates was provided [9]. Two recent publications report the isolation of *G. argillacea* among many other pathogens in sputum and bronchoalveolar lavage aspirates from patients with cystic fibrosis [10, 27]. Despite chronic colonization in these patients, there was no clinical correlation of pulmonary deterioration with *G. argillacea* isolation, nor did the fungus present a clinical problem even in patients undergoing lung transplantation, thus suggesting that *G. argillacea* is not a pathogen in cystic fibrosis.

The *G. argillacea* isolates from most of the patients in this report, as well as those described in the reports of patients with cystic fibrosis, exhibited broad resistance to most oral antifungals, such as itraconazole, voriconazole, and in some, posaconazole (Table 1). In vitro susceptibility was limited to echinocandins, such as micafungin or caspofungin. However, the results of in vitro susceptibility testing of molds may not correspond with clinical outcomes, as was the case in these patients, and breakpoints with proven relevance have yet to be identified or approved by CLSI or any other regulatory agency. Additional studies are needed to correlate clinical outcome with susceptibility data. However, surgery helped achieve local control or cure in several patients.

We conclude that obtaining diagnostic tissue samples from CGD patients to correctly identify pathogens is essential. *G. argillacea* is a previously underappreciated and frequently misdiagnosed pathogen in CGD. When *P. variotii* is identified phenotypically, molecular approaches should be used to exclude *G. argillacea*, because the aggressive pattern of *G. argillacea* infection to invade across tissue planes and disseminate, as well as its refractoriness to medical treatment, warrants intensive appropriate antifungal therapy and consideration of early surgery for resectable lesions.

**Acknowledgements**

**Financial support.** This work was supported by the National Institutes of Allergy and Infectious Diseases Division of Intramural Research and the Clinical Center of the National Institutes of Health. L. Sigler receives grant support from the Natural Sciences and Engineering Research Council of Canada (NSERC).

**Potential conflicts of interest.** All authors: no conflicts.

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