Burkholderia pseudomallei Infection in a Puerto Rican Patient with Chronic Granulomatous Disease: Case Report and Review of Occurrences in the Americas

Susan E. Dorman, Vee J. Gill, John I. Gallin, and Steven M. Holland

Chronic granulomatous disease (CGD) is caused by one of several genetic lesions involving the phagocyte NADPH oxidase system; it is characterized by defective production of toxic oxygen metabolites by neutrophils and ineffective microbial killing. Patients with CGD characteristically have recurrent pyogenic infections, especially those due to organisms possessing catalase activity. Burkholderia species are important pathogens in patients with CGD. In such patients, Burkholderia cepacia can cause pneumonia, bacteremia, skin abscesses, cervical adenitis, and widely disseminated fatal infection [1–4]. The first reported cases of human disease caused by Burkholderia gladioli were in two North American patients with CGD. Both patients had B. gladioli pneumonia, which in one case was associated with B. cepacia bacteremia and sepsis syndrome [5]. A subsequent case of B. gladioli bacteremia in a European patient with CGD has been reported [6].

We report the occurrence of Burkholderia pseudomallei lymphadenitis and mediastinitis in a Puerto Rican patient with CGD. This is the first reported case of B. pseudomallei infection in a CGD patient from the Americas and is one of a small number of cases of melioidosis autochthonous to the Americas.

Case Report

An 11-year-old Puerto Rican boy with X-linked CGD was hospitalized because of 2 weeks' duration of productive cough, right neck pain, and fever that had begun in October while he was in Puerto Rico and had not decreased with oral amoxicillin/clavulanate therapy. He had a long history of recurrent bacterial and aspergillus pneumonias, pyogenic liver abscesses, osteomyelitis, sinusitis, and granulomatous gastritis. Two years before the current hospitalization, he had undergone right upper lobectomy for treatment of refractory pneumonia due to Nocardia otitidiscaviarum. Six months before admission, he had Phialophora melinii soft-tissue infection of the foot that was treated initially with amphotericin B and then with itraconazole, which he was taking at the time of admission. Other medications included trimethoprim-sulfamethoxazole and IFN-γ prophylaxis. He had been born to Puerto Rican parents in Naguabo, Puerto Rico, and continued to reside there on a farm. His only travel was to New York City and the Washington, D.C., area for medical care; no family members or close contacts had recently traveled.

Physical examination at the time of admission revealed a temperature of 38.5°C and a tender right supraclavicular mass. A chest CT revealed a right hilar mass, right hilar lymphadenopathy, right paraseptal lymphadenopathy, and a 2.5-cm right supraclavicular mass (Figure 1A). A direct biopsy specimen of the right supraclavicular mass and a transthoracic needle biopsy specimen of the hilar mass were obtained. Routine blood cultures were negative. Empirical therapy with ceftriaxone, trimethoprim-sulfamethoxazole, and amphotericin B and daily granulocyte transfusions were started. IFN-γ therapy was continued.

Direct smears were prepared from the right supraclavicular and hilar biopsy specimens. The biopsy specimens were submitted for routine, fungal, and mycobacterial cultures. Smears were stained with gram, calcofluor white, and auramine-rhodamine stains. Gram staining of the direct smears showed a few polymorphonuclear leukocytes but no organisms. Calcofluor white staining for fungi and auramine-rhodamine staining for mycobacteria were negative. After 2 days, cultures of both
the right supraclavicular and hilar biopsy specimens on the chocolate agar plates incubated at 35°C in 6% CO₂ yielded scant growth of a gram-negative rod.

A commercially available bacterial identification test (API NFT, bioMérieux Vitck, Hazelwood, MO) was used, as were conventional biochemical reactions for definitive identification. Identification and susceptibility tests were performed with use of the MicroScan Negative Breakpoint Combo (Type 8) panels (Dade International, Deerfield, IL). After 24 hours, results of the MicroScan system were nondefinitive, thus suggesting that the organism was a ‘rare biotype.’ Upon repeated testing, the MicroScan system made the identification of Chromobacterium violaceum (99.6% probability), while the API NFT system suggested B. pseudomallei as the presumptive identification.

The organism grew on blood and chocolate agars as creamy, white, entire, low convex colonies; after 4 days of incubation, colonies became dry and wrinkled. The organism was catalase-positive, weakly oxidase-positive, and nonfermentative. By conventional biochemical testing, it was capable of oxidizing glucose, mannitol, sucrose, lactose, and maltose. Triple sugar iron testing showed an acid slant with no change in the butt portion. The organism was motile and showed negative reactions when tested for growth on citrate; esculin and acetamide hydrolysis; production of indole, lysine, and ornithine decarboxylases; urease; acid from xylose; and utilization of o-nitrophenyl-B-D-galactopyranoside. The organism was positive for nitrate reduction to nitrite as well as for production of N₂ gas, arginine dihydrolase, gelatinase, and DNase. The organism had multiple (three to five) polar flagella and produced white pigment.

These features were consistent with the identification of B. pseudomallei, and this identification was subsequently confirmed by the Centers for Disease Control and Prevention Special Bacteriology Reference Laboratory. A serum sample was tested for titers of antibodies to B. pseudomallei by EIA (performed by Specialty Labs, Santa Monica, CA); the sample was positive for both IgG (titer, 3.1; reference titer range, <2.0) and IgM (titer, 15.7; reference titer range, <2.0).

Antibiotic susceptibility testing by means of the Autoscan system (Dade International) showed that the organism was resistant to ampicillin, amikacin, aztreonam, cefazolin, cefoxitin, ciprofloxacin, gentamicin, ticarcillin, tobramycin, and trimethoprim-sulfamethoxazole and susceptible to ampicillin/subbac- tlam, ceftazidime, ceftriaxone, imipenem, piperacillin, and tetracycline. Susceptibility to chloramphenicol and doxycycline was determined by the Etest (AB BIODISK, Solna, Sweden).

On the basis of culture results and antibiotic susceptibility testing, therapy was changed to imipenem and doxycycline. Amphotericin B and trimethoprim-sulfamethoxazole therapy was stopped. After 1 week of the new therapy, the patient became afebrile. Granulocyte transfusions were discontinued after 2 weeks. A chest CT after 3 weeks of therapy showed significant decreases in the sizes of the right supraclavicular and hilar masses (Figure 1B). He received 6 weeks of intravenous imipenem and doxycycline therapy. Three weeks after his discharge from the hospital, while oral antibiotic therapy was still being prescribed, the patient developed fever and cough in Puerto Rico. He died en route to a hospital; permission for an autopsy could not be obtained.

Discussion

The genus Burkholderia includes five species known to cause human infection—B. cepacia, B. gladioli, Burkholderia pickettii (a rare cause of nosocomial urinary tract infection and bacteremia [7]), B. pseudomallei (the agent of melioidosis), and Burkholderia mallei (the agent of glanders, a disease of livestock and rarely humans [8]). Laboratory identification of B. pseudomallei can be difficult. This patient’s isolate was incorrectly identified as C. violaceum by the MicroScan system; however, absence of production of violet pigment was a strong
feature against the identification as *C. violaceum*, since all clinically significant isolates described have been pigmented. The isolate was correctly identified as *B. pseudomallei* by the API NFT system, and the identification was confirmed by conventional biochemical testing. Of the commercially available bacterial identification test kits, only the API NFT system has been adequately evaluated for accuracy in identifying *B. pseudomallei*, and it has been shown to be very accurate (>99%) in identifying known strains of this organism [9]. The antibiotic susceptibility pattern of the patient’s isolate was typical for *B. pseudomallei*, showing susceptibility to piperacillin, ampicillin/sulbactam, ceftazidime, imipenem, and tetracycline and resistance to aminoglycosides. Resistance to trimethoprim-sulfamethoxazole is increasing, especially in Thailand, where <20% of strains are susceptible [10].

*B. pseudomallei* and its role in human disease were first described by Whitmore and Krishnaswami in 1912 [11]. They described fulminant septicaemia with pneumonia and subcutaneous abscesses in a 10-year-old Burmese ‘‘morphia’’ addict. The classical spectrum of melioidosis (literally meaning “a resemblance to distemper of asses”) has since been demonstrated to include subclinical infection, acute local infection, indolent local infection, and fulminant septicaemia. The most common manifestations are pneumonia, lung abscess, soft-tissue infection, osteomyelitis, lymphadenitis, splenic abscess, liver abscess, and septicemia. Predisposing factors include diabetes mellitus, renal failure, alcohol abuse, and older age. Most human infections are probably acquired by inoculation of a preexisting skin lesion with contaminated soil or water or by inhalation of infectious dust particles. The incubation period of melioidosis can be as long as 26 years [12], and the incidence of relapse after apparent clinical resolution of primary infection has been reported to be 15% per year of follow-up [13].

Antibiotic therapy for *B. pseudomallei* infection should be based on the severity of clinical disease, host factors, and results of antimicrobial susceptibility testing. For severe disease, including septicaemia, combination therapy is recommended. Ceftriaxone plus trimethoprim-sulfamethoxazole has recently been advocated on the basis of good in vitro activity of these agents and favorable clinical response in small numbers of treated patients [14, 15]. However, in vitro, the carbapenems imipenem and meropenem have been shown to have more rapid bactericidal activity against *B. pseudomallei* than ceftriaxone [16] and longer postantibiotic effects than other β-lactam agents [17]. For severe disease, several weeks of combination intravenous therapy followed by prolonged oral therapy is recommended. Our patient responded well to 6 weeks of intravenous imipenem of the fetus in utero or during parturition, cannot be excluded. A 5-week course of chloramphenicol and kanamycin therapy
Table 1. Summary of data on reported cases of confirmed melioidosis acquired in the Americas.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Location</th>
<th>Age (y)/sex</th>
<th>Predisposing factor(s)</th>
<th>Infection(s)</th>
<th>Therapy</th>
<th>Outcome</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>[25] 1960</td>
<td>United States</td>
<td>20/M</td>
<td>None</td>
<td>Acute septic arthritis</td>
<td>Chloramphenicol, novobiocin, sulfisoxazole</td>
<td>Survived*</td>
<td>Travel history and location of residence at time of onset of symptoms not stated in initial report, but later report documented Panama as country of acquisition of infection. Identification confirmed at Walter Reed Army Institute of Research.</td>
</tr>
<tr>
<td>[26] 1964</td>
<td>Ecuador</td>
<td>30/M</td>
<td>None</td>
<td>Soft-tissue infection, lymphadenitis</td>
<td>None reported</td>
<td>Died†</td>
<td>Bacterial isolate studied in detail—reported results consistent with <em>Burkholderia pseudomallei</em> but not confirmed by reference laboratory.</td>
</tr>
<tr>
<td>[27] 1980</td>
<td>United States</td>
<td>21/M</td>
<td>Facial trauma</td>
<td>Orbital wound</td>
<td>None</td>
<td>Survived*</td>
<td>Not a pathogen. <em>B. pseudomallei</em> identification confirmed at Centers for Disease Control and Prevention.</td>
</tr>
<tr>
<td>[29] 1986</td>
<td>United States</td>
<td>72/M</td>
<td>None</td>
<td>Pneumonia, splenic abscess, bacteremia</td>
<td>Cefoxitin, gentamicin</td>
<td>Died‡</td>
<td>Lived in Mexico entire life before 4-week trip to California. Identification of <em>B. pseudomallei</em> confirmed at California State Department of Public Health Laboratories.</td>
</tr>
<tr>
<td>[31] 1997</td>
<td>Guadeloupe</td>
<td>4/M</td>
<td>None</td>
<td>Pneumonitis, peritonitis</td>
<td>Ceftazidime, trimethoprim-sulfamethoxazole</td>
<td>Survived*</td>
<td></td>
</tr>
<tr>
<td>[PR] 1998</td>
<td>United States</td>
<td>11/M</td>
<td>CGD</td>
<td>Lymphadenitis, mediastinitis</td>
<td>Imipenem, doxycycline, cefixime, granulocyte transfusions</td>
<td>Died‡</td>
<td>Identification of <em>B. pseudomallei</em> confirmed at Centers for Disease Control and Prevention.</td>
</tr>
</tbody>
</table>

NOTE. CGD = chronic granulomatous disease; PR = present report; SLE = systemic lupus erythematosus. Identity of isolates from cases reported by Garry and Koch [32], Beamer et al. [33, 34], and McDowell and Varney [35] were not confirmed; these cases have been omitted from the table.

* Survived *B. pseudomallei* infection.
† Died of *B. pseudomallei* infection.
‡ Cause of death unknown.

was then administered with general clinical improvement, but culture of a biopsied skin nodule ~5 weeks into this therapy yielded *B. pseudomallei*. His treatment was changed to an oral sulfa preparation, which he received as maintenance therapy, and subsequently his condition was reported to be well 15 months later.

*Burkholderia* species, including *B. cepacia* [1–4] and less frequently *B. gladioli* [5, 6], cause devastating infection in patients with CGD. These organisms are otherwise uncommon pathogens, except for *B. cepacia* in patients with cystic fibrosis. In 1975, Bottone et al. [1] reported the inability of peripheral blood leukocytes from patients with CGD to kill *B. cepacia* in vitro. *Burkholderia* species are catalase-positive and therefore are able to destroy bacterial hydrogen peroxide. Although this feature may account in part for the pathogenicity of these organisms in patients with CGD, *Pseudomonas aeruginosa*, which is also catalase-positive, has rarely been found to be a pathogen in patients with CGD. This finding suggests that the
presence or absence of catalase activity does not fully account for the pathogenicity of Burkholderia species in patients with CGD.

Speert et al. [3] subsequently showed that B. cepacia, but not P. aeruginosa, was resistant to neutrophil-mediated nonoxidative killing. The mechanism by which B. cepacia resists nonoxidative killing is not known. However, resistance to neutrophil-mediated nonoxidative killing may contribute to the pathogenicity of B. cepacia in patients with CGD who already have a defect in neutrophil-mediated oxidative killing of microbes.

These observations may extend to B. pseudomallei, which has been shown in vitro to be resistant to some methods of neutrophil-mediated nonoxidative killing, including the defense against human neutrophil peptide-1 [42]. B. pseudomallei has also been shown to be resistant to the cationic peptide protamine, thus suggesting resistance to other physiologically relevant compounds such as cecropins and magainins [42]. B. pseudomallei can survive and multiply in vitro within polymorphonuclear leukocytes and mononuclear leukocytes from healthy human donors [42, 43] and in rat alveolar macrophages [42]. Further studies to explore the interactions between Burkholderia species and phagocytes are under way in our laboratory. We conclude that B. pseudomallei, like B. cepacia and B. gladioli, should be considered a potential pathogen in patients with CGD.

Acknowledgment

The authors thank Ms. Denise Williams for her assistance in identifying Burkholderia pseudomallei at the National Institutes of Health.

References