

Case report

Multiple recurrent abscesses in a patient with undiagnosed IL-12 deficiency and infection by *Burkholderia gladioli*

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ABSTRACT

We report the occurrence of two severe illnesses experienced by one patient over a 19 year period of time. Both illnesses were characterized by severe inflammation and tissue destruction. Signs and symptoms of the first illness were characteristic of lymphogranuloma venereum (LGV). The second illness mimicked scrofula. During the second illness the patient was discovered to have a rare immunodeficiency due to auto-antibodies to Interleukin (IL)-12 and infection by *Burkholderia gladioli*, a plant pathogen usually harmless in humans.

We were able to retrieve biopsies from the first illness to establish that *B. gladioli* was already present during the original presentation. That first illness lasted 5 year s, but she survived without the correct pathogen ever being identified, and without a diagnosis of immunodeficiency. After a remission of 10 year s, she experienced her second illness.

The responses to treatment before and after the correct diagnoses were established provide us with an excellent opportunity to consider and discuss how disease expression reflects complex relationships between host defenses and microbial characteristics.

Introduction

In this paper we discuss a patient who was found to be infected with *B. gladioli*, a plant pathogen generally harmless in humans. Our patient was also found to have auto-antibodies to Interleukin (IL)-12, a key immune-regulatory cytokine that bridges innate immunity with adaptive responses.

This patient has been previously reported [1], but our intention in this paper is to report on follow-up and outcomes following different types of interventions. This paper also summarizes clinical details of this patient's illness 15 years earlier from which we were able to retrieve biopsies and demonstrate the presence of *B. gladioli* in the original biopsies. By implication the organism may have sequestered in the patient's tissues for 10 year s of remission and both illnesses may have been associated with the same immunodeficiency.

Patient background

The patient was a 32 year old Cambodian female G2P1 who presented to prenatal clinic at 14 weeks gestational age with rectal bleeding for four weeks. She gave no history of abdominal pain or

previous GI complaints. Her previous pregnancy had been uneventful. She had worked in rice paddies in Cambodia before immigrating to the United States at age 13. She denied any history of childhood sexual abuse.

First illness

During pregnancy, the patient was treated symptomatically for inflammatory bowel disease on the basis of sigmoidoscopic visualization of multiple round masses that appeared to be hemorrhagic granulomas. Histology of these lesions showed granulation tissue with acute and chronic inflammation, but no specific findings of Crohn's disease. No acid-fast bacilli (AFB) or other pathogens were isolated, including chlamydia. She delivered at term *via* low vacuum extraction after a prolonged second stage of labor, and a fourth degree laceration through the rectum was repaired in layers.

Postpartum she developed severe right lower quadrant pain radiating to the right upper quadrant, as well as bloody diarrhea. Colonoscopy showed lymphoid aggregates in the terminal ileum without evidence of Crohns's disease. The rectum showed extensive ulceration with inflamed granulation tissue from 30 cm down to the

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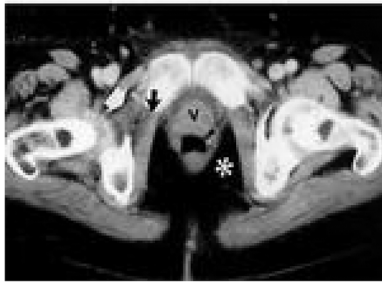


Fig. 1. Normal perianal anatomy. [Caption] CT scan through the female ischioanal space shows the vagina (v), rectum (arrowhead), internal obturator muscle (black arrow), external obturator muscle (large white arrow), and ischioanal fossa (*).

dentate line. Biopsies were non-diagnostic, but Computerized tomography (CT) was suggestive of lymphogranuloma venereum (LGV) because it showed bilateral inguinal adenopathy as well as diffuse circumferential nodular thickening of the rectum with marked perirectal soft tissue and inflammatory changes, extending into the ischioanal and presacral spaces, down to the level of the gluteus muscle, the obturator internus muscle, the piriformis muscle, the sciatic notch, and into the perineal region. Fig. 1 shows these CT anatomical landmarks in a normal female [2].

The radiological diagnosis of LGV was not followed because the patient was “lost-to-follow-up”. Over the next year the patient’s condition deteriorated with development of painful perirectal abscesses and draining fistulas to the buttocks. Repeat colonoscopy failed to demonstrate a fistulous opening, but three inflammatory masses in the rectum showed ulceration, granulation tissue, histiocytes and medium to large sized eosinophilic bodies. Gram stain, fungal stain, and AFB stains were negative. Tissue cultures and histopathology were negative for bacteria (aerobic and anaerobic), mycobacteria, and fungi. Serology was negative for fungi, amoebae, and schistosomiasis, but was borderline(1:64) for *C. trachomatis*. Tissue obtained from the terminal ileum showed normal histopathology and had negative tissue cultures.

The patient underwent several incision and drainages of her left buttocks abscesses, but she continued to deteriorate, developing fevers, anorexia, lethargy, and a leukocytosis of 27,000 with erythrocyte sedimentation rate (ESR) of 130 mm/hr. No organism could be identified. A right labial abscess resulted in an extensive surgical exploration, This



Fig. 3. Left neck draining lymphadenitis.

revealed several abscesses in the ischioanal fossae bilaterally communicating *via* fistulas to the buttocks, the vagina, and the labia majora. (Fig. 2)

All fistulous tracts and abscesses were debrided, irrigated, and packed with drains. Postoperatively she received irrigations and whirlpool treatments. Despite negative operative cultures and serology, the clinical presentation still appeared to be consistent with tertiary LGV, so doxycycline was prescribed orally for 6 months. Intravenous ampicillin was added because cultures grew *E. coli* and other enteric bacteria. She required a second extensive debridement procedure before the fistulas closed permanently and her ESR, initially 130, normalized.

Second illness

The patient was asymptomatic for ten years. She then presented with a large suppurative neck mass that appeared to be either a lymphoma or scrofulous lymphadenopathy. Histopathology showed an acute suppurative lymphadenitis with abscess formation. (Fig. 3) Many granulocytes were seen as well as histiocytes with intracellular Gram negative bacilli. The cultures grew *Burkholderia gladioli*. Treating clinicians assumed a mis-identification of the organism and presumed a diagnosis of melioidosis, caused by *Burkholderia pseudomallei*.

Despite treatment with targeted antimicrobials, the patient developed a contralateral neck mass two years later, which again grew *B.*

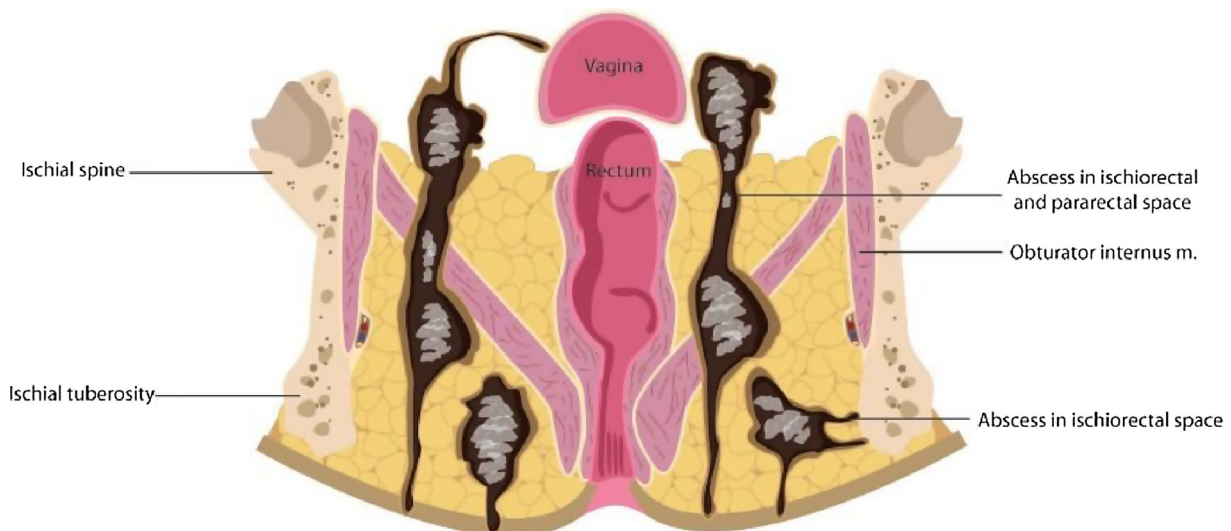


Fig. 2. Abscesses and Fistulas. [Caption] Diagram showing the location of bilateral abscesses in the ischioanal fossae with fistulas to the skin of the buttocks and through the levator into pararectal spaces and the vagina to the labia.

gladioli. This organism was confirmed by 16S ribosomal RNA analysis performed by the Centers for Disease Control and Prevention. Since *B. gladioli* was an unusual human pathogen, an extensive workup was conducted to rule out other co-pathogens and an immunological workup revealed an immunodeficiency due to auto-antibodies to IL-12, a cytokine that activates monocytes, macrophages, dendritic cells, neutrophils, natural killer cells, T-cells and B-cells.

The unusual coincidence of two rare clinical syndromes in one patient caused us to retrieve the paraffin blocks from her original rectal biopsy to be subjected to 16S rRNA analysis for *B. gladioli*. As predicted, the original rectal endoscopic biopsies obtained during her pregnancy before any antibacterial treatment, were also positive for *B. gladioli*.

Follow-up and outcomes

From 2010 to 2014 the patient received oral trimethoprim-sulfamethoxazole (TMP-SMX), levofloxacin, ertapenem, and thrice weekly injections of interferon-gamma (IFN-gamma). Nevertheless the suppurative lymphadenopathy of her anterior neck persisted.

She was enrolled in an NIH clinical trial using rituximab in an attempt to halt auto-antibody production. She had no clinical response or reduction in auto-antibody level following the first 3 doses of rituximab, but the suppurative lymph nodes on her neck healed after receiving the second cycle of 5 more doses. After the neck wounds closed, she was taken off IFN-gamma and ertapenem, but continued on TMP-SMX and levofloxacin. Since the neck wounds have recently recurred she has been restarted on another cycle of rituximab. If this is unsuccessful, alternatives such as adjunctive plasmapheresis or cyclophosphamide may be effective in curbing her auto-antibody production.

Patient perspective

The patient still has neck mobility problems and chronic pain, but she feels generally better and is looking forward continued improvement with another cycle of rituximab. She has headache and dizziness exacerbated by physical therapy. She uses a fentanyl patch and a heating pad for sleep.

Discussion

B. gladioli is a plant pathogen, indigenous to Southeast Asia and Northern Australia. It can affect onions, gladioli, iris, and sometime rice. *Burkholderia* organisms can be virulent and persistent because their virulence factors allow them to enter host cells, replicate intracellularly, and spread to nearby cells without alerting host immune system defenses. The subspecies *B. gladioli* is generally harmless in humans, but it has been reported to cause disease in some immunodeficient patients [3].

B. gladioli is less well studied than *B. pseudomallei* or *B. mallei*, because the latter are classified as potential bioterrorism agents. The cell walls of these more well-studied organisms contain modifications that reduce intracellular killing by neutrophils [4] and macrophages [5]. The lipopolysaccharide coat of *B. gladioli*, however, has been shown to be a potent stimulator of tumor necrosis factor (TNF)-alpha *in vitro* [6].

Virulence factors like flagellin, Type III Secretion Systems (T3SS) [7], adhesins [5], pili [5], and biofilm [8] have all been described for *B. pseudomallei* and *B. mallei*. Actin-based motility [7] and flagellae permit rapid transit through cells. Cell-to-cell membrane fusion with neighboring cells causes multinucleated giant cells (MNGC) spreading the organism silently. Some *Burkholderia* have been described to mutate [9] and sequester themselves inside the nuclei of host cells [10] permitting chronic asymptomatic carriage for years or even decades [11].

Infection with *B. gladioli* has been described in patients with cystic fibrosis, chronic granulomatous disease, organ transplants, and other immunodeficiencies [3]. Likewise, our patient had auto-antibodies

against IL-12 rendering her immunodeficient. IL-12, also known as interferon-gamma inducing factor, stimulates CD 4 T cells and NK cells to secrete IFN-gamma, stimulating phagocytosis and cytolysis, especially for intracellular organisms. Our patient's serum, when studied *in-vitro*, showed no IFN-gamma activity [1].

This was thought to be of central importance for her disease, so IFN replacement was used initially in addition to targeted antimicrobial therapy. Surprisingly, this treatment did not produce the expected results. Her suppurative lymphadenopathy resolved only after she was treated with rituximab, along with antimicrobials. This treatment lowered her anti IL-12 antibody titer, suggesting a key role for IL-12 beyond its ability to induce IFN-gamma production. Since our patient is the only reported patient in the literature with auto-antibodies to IL-12, we must speculate on the basis of clinical presentation rather than any other kind of evidence.

Our patient's first illness resembled LGV, an illness now known to be caused by the L₁₋₃ serovars of *Chlamydia trachomatis*. Patients with LGV generally present with rectal pain and bleeding. Histology shows a granular or ulcerative proctitis that is frequently misdiagnosed as inflammatory bowel disease because of its similarity to Crohn's disease of the rectum [12].

The hallmark of LGV is extensive tissue destruction, probably mediated by chronic and excessive proinflammatory chemokines [13], originating from the inflammasome. This inflammation, if left untreated, can progress to perirectal and ischioanal abscesses, and rectovaginal or anal fistulas. The similarity of our patient's first illness to LGV suggests that perhaps IL-12 plays an important role in recognition and signaling related to the inflammatory cell wall characteristics of *B. gladioli*.

The second illness was a suppurative lymphadenitis in the neck with draining sinuses and significant necrosis. This presentation mimicked scrofula, a clinical entity generally caused by *M. tuberculosis*, but also associated with non-tuberculous mycobacteria, *Histoplasma capsulatum*, *Coccidioides immitis*, *Toxoplasma gondii*, and *Bartonella spp.* Scrofula was well-known in the pre-antimicrobial era, and associated with miraculous cures by the "Royal Touch" [14].

In the modern era, most patients with scrofula have tuberculosis, but also are frequently immunodeficient. Patients with auto-antibodies to IFN-gamma and other defects in the IL-12/IFN-gamma signaling pathway are especially susceptible to disseminated infection by mycobacterial diseases as well as other opportunistic and intracellular pathogens [15,16]. Patients with Mendelian susceptibility to mycobacterial diseases (MSMD), for example, have a genetic defect in the IL-12 pathway which renders them susceptible to tuberculous and non-tuberculous mycobacteria. Our patient was incapable of defending against *B. gladioli*, an organism that like *M. tuberculosis*, lives inside of macrophages, reproduces therein, and hides from host immunity. A major difference is that *M. tuberculosis* typically becomes walled off by host defenses in granulomas that contain MNGCs. Our patient, however, did not make any MNGCs, as seen in the many biopsies she had during her first illness.

Conclusions

We have presented a case of a patient with two severe illnesses that were both characterized by intense inflammatory destruction reminiscent of clinical syndromes from the pre-antibiotic era. Eventually a diagnosis of auto-antibodies to IL-12 was made, and the microbe responsible for her inflammation was shown to be *B. gladioli*. Generally harmless in humans with intact immunity, this microbe has an LPS coat that can be a potent stimulator of TNF-alpha *in vitro*. Generally the pro-inflammatory effect of excess TNF-alpha is modulated by anti-inflammatory and regulatory cytokines, but our patient had an IL-12 dysfunction that seemingly permitted rampant and relentless inflammation. Treatment with rituximab stopped the production of auto-antibodies to IL-12 and restored a semblance of normalcy to her

immune system. This case highlights the important regulatory role of IL-12 in preventing runaway inflammation.

Disclosures

Dr. Vigliani has no disclosures. Dr. Cunha has no disclosures.

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