Buruli Ulcer: Advances in Understanding *Mycobacterium ulcerans* Infection

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KEYWORDS

• Buruli ulcer • *Mycobacterium ulcerans* • Emerging disease • Skin disease • Mycolactone

Buruli ulcer (BU), the third most common mycobacterial infection in humans next to tuberculosis and leprosy, is an emerging infection caused by *Mycobacterium ulcerans*. BU is characterized by indolent, typically painless necrotizing skin lesions (**Figs. 1** and **2**A). Approximately 10% of patients develop bone involvement subjacent to skin lesions or metastatic osteomyelitis from lymphohematogenous spread of *M ulcerans* (see **Fig. 2**B). Pathogenesis is mediated by mycolactone, a diffusible, necrotizing, immunosuppressive, polyketide-derived macrolide toxin secreted by *M ulcerans*.[1] In 1962, the disease was named after Buruli County, Uganda, now called Nakasongola District, where the epidemic was documented first. Other names include Bairnsdale, Kakerifu, Kasongo, or Searls' ulcer.

EPIDEMIOLOGY

In 1998, the World Health Organization (WHO) recognized BU as a reemerging infectious disease in West and Central Africa, with a significant public health impact.[2] The reported incidence rates of BU are highest in West Africa, especially Benin, Ghana, and Côte d'Ivoire. However, BU is reported in about 30 countries (**Fig. 3**), and growing evidence suggests that BU is more widespread than earlier thought.[3] BU prevails in rural tropical wetlands, especially areas with stagnant water, including ponds and swamps. However, BU is also acquired without wetland exposure.

The rapid reemergence of BU, beginning in the early 1980s, particularly in areas where people are engaged in manual agriculture in wetlands, may be attributable to the man-made alterations to the environment, such as deforestation and other topographic alterations, which increase the amount of wetlands. Changes in global temperature and precipitation patterns further promote the reemergence of BU.

The WHO reports indicate that more than 5000 people are diagnosed with BU annually, but many cases are undiagnosed because of the geopolitical and socioeconomic factors in endemic countries. Children (5–15 years old) have the highest incidence of BU, with most lesions on the lower extremities. BU is a growing

Disclosure: The authors have nothing to disclose.

Disclaimer: The views expressed in this article are those of the author (D.S.W.) and do not reflect the official policy of the Department of the Army, Department of Defense, or the US government.

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Dermatol Clin 29 (2011) 1–8

doi:10.1016/j.det.2010.09.006

0733-8635/11/$ – see front matter. Published by Elsevier Inc.

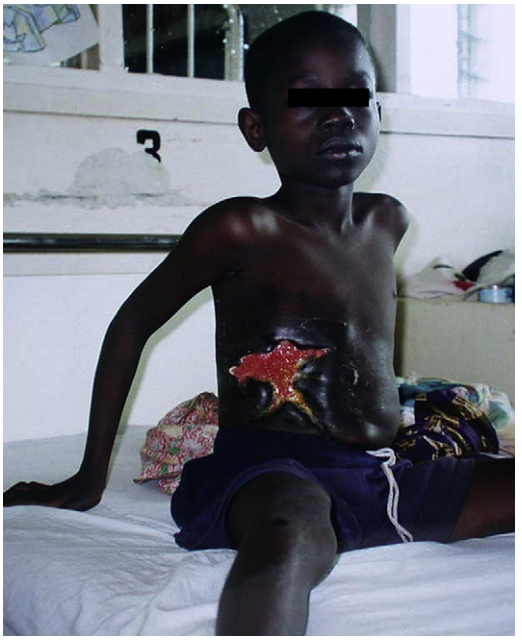

Fig. 1. Plaque of BU on the right flank of a Ghanaian boy. The lesion has characteristic rolled borders and is remarkably stellate, a feature of some plaques.

public health problem, with psychosocial and socioeconomic implications in endemic regions. Up to 60% of patients with BU suffer from disabling and stigmatizing sequelae, including scarring, contractures, and bone destruction.[4] Minimizing disability through treatment, both antimicrobial and surgical, and physiotherapy is, therefore, important in BU management. Imported BU is occasionally diagnosed in the United States, Canada, and Europe.[5]

BU is directly related to environmental factors and thus considered noncontagious.[6] The most possible mode of transmission is local, minor, often unnoticed skin trauma that permits inoculation of *M ulcerans*. The estimated incubation period is 2 to 3 months. Because *M ulcerans* DNA is detectable in some aquatic insects, the role of insects as vectors that infect humans by biting is under investigation.[7] In Australia, some investigators propose that BU is a zoonosis transmitted by mosquitoes from indigenous marsupials (eg, possums and koalas) to humans. *M ulcerans* DNA was found in mosquitoes during an outbreak of BU in humans in Australia, and the seasonal incidence of BU in humans correlates with that of notifiable arthropod-borne diseases in Victoria.[8] In Africa, terrestrial mammals are being investigated as reservoirs of *M ulcerans*.[9]

Risk factors for BU within endemic areas include failure to wear protective clothing, exposure to unprotected natural water sources, and inadequate care of minor skin wounds.[10,11] Human immunodeficiency virus seropositivity may increase the risk for BU or be associated with aggressive BU.[12]

BCG vaccination has some effect on BU. Several reports suggest that BCG vaccination provides some protection against BU, for 6 to 12 months after vaccination, and that neonatal BCG vaccination reduces the risk of BU osteomyelitis in those who acquire BU as children or adults.[13–15] However, a case-control study concluded that BCG vaccination is not protective against BU.[16] Prophylactic and therapeutic vaccines based on DNA engineering and virulence factors, including mycolactone, are under study (BuruliVac Project).[17] Intravenous immunoglobulin to neutralize mycolactone is not available.

MICROBIOLOGY OF *M ULCERANS*

Standard and real-time polymerase chain reaction (PCR) techniques have been used to identify *M ulcerans*, primarily by detecting 2 *M ulcerans* insertion sequences (IS*2404* and IS*2606*), in the environment in Australia and West Africa.[18] Improved *M ulcerans* DNA extraction procedures enhance environmental

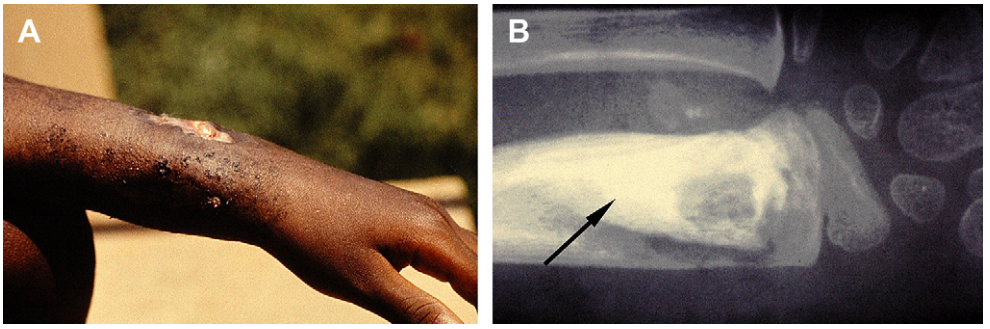

Fig. 2. (*A*) Plaque of BU on the forearm of a Congolese boy invaded the deep tissues and bone, causing contiguous osteomyelitis. (*B*) Radiograph shows contiguous reactive osteitis and necrosis of the cortex of the radius, with formation of a large sequestrum (*arrow*).

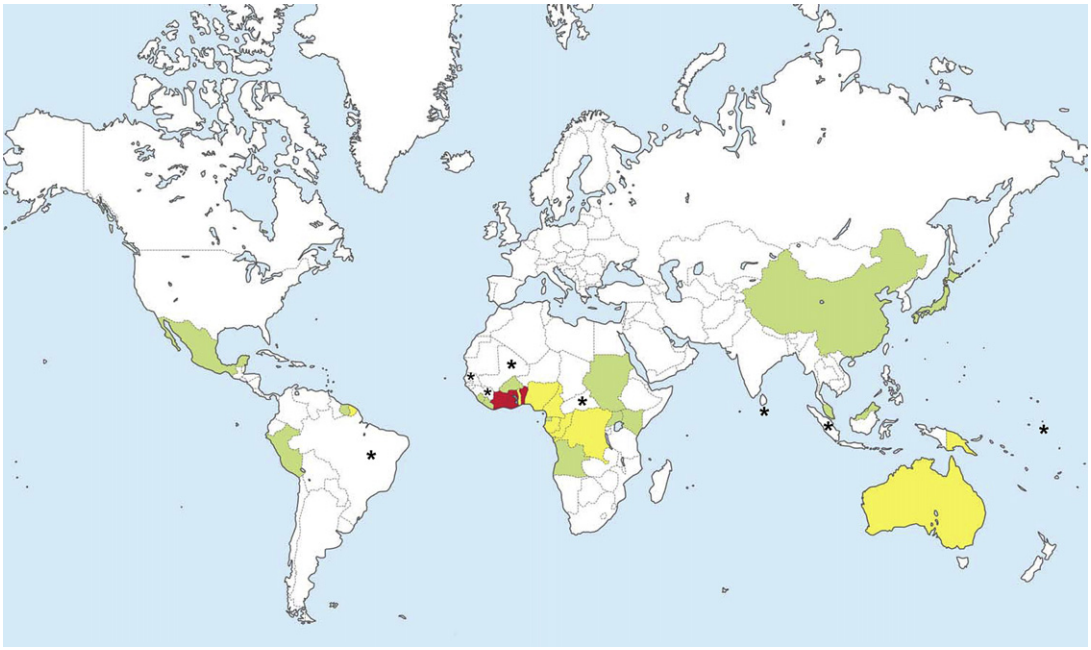

Fig. 3. Distribution of BU by country, as of 2010. Relative endemicity is denoted as high (*red*), moderate (*yellow*), and low (*green*); asterisks denote countries with suspected cases. Imported BU is occasionally diagnosed in the United States, Canada, and Europe.

detection, thereby advancing the understanding of reservoirs.[19] Portaels and colleagues[20] reported the first direct isolation of *M ulcerans* from nature in 2008 from a water strider, an aquatic insect that does not bite humans.

Unlike *M leprae* and *M tuberculosis* (the pathogens for leprosy and tuberculosis, respectively), *M ulcerans* produces a necrotizing, immunosuppressive, polyketide-derived macrolide toxin, called mycolactone.[1] Genes in a virulence plasmid of *M ulcerans*, controlled by SigA-like promoters,[21] encode for the synthesis of mycolactone. Identification of SigA-like promoters led to the development of *M ulcerans*–green fluorescent protein. This tagged protein linking fluorescence with toxin gene expression is a potential tool for studying BU pathogenesis and transmission.[21]

M ulcerans shares some environmental, molecular, and clinical features with *M marinum*, a water-associated organism that causes granulomatous skin lesions in humans, often called "swimming pool" or "fish tank" granuloma. Comparative genomics indicate that *M ulcerans* likely diverged from *M marinum*, acquiring a 174-kb virulence plasmid (pMUM001) with genes coding for mycolactone production and 10 proteins, all potential targets for vaccine development or serodiagnosis.[22] Accordingly, phenolic mycosides of *M ulcerans* and *M marinum* are identical, and sequences for the 16S ribosomal RNA (rRNA) gene are nearly identical.[6] As *M ulcerans* evolved toward becoming an intracellular organism, like *M marinum*, nonessential genes were lost, which may have increased the pathogenicity.[22]

Gene sequences of the 3′ end of the 16S rRNA of *M ulcerans* vary by geographic origin, dividing *M ulcerans* broadly into African, American, Asian, and Australian strains, with many substrains on each continent.[23] Each major strain generally differs in clinical presentation, mycolactone type and virulence, and host immune responses.[24] Mycolactone type coding by geographic origin includes A/B (Africa, the most pathogenic), C (Asia, Australia), and D (Asia).[1]

Molecular genetic techniques are slowly unraveling the evolution of *M ulcerans*. *M ulcerans* isolates from localized foci within endemic regions often show a high degree of genomic similarity (ie, clonal populations) with a lack of insertional-deletional genomic polymorphisms, underscoring a requirement for single-nucleotide polymorphism (SNP) analysis to differentiate substrains of *M ulcerans* within those areas.[25] Identifying SNPs and establishing SNP typing assays are increasingly defining the microepidemiology, genetic diversity, and evolution of *M ulcerans*.[26,27] For example, SNP analyses of *M ulcerans* in Ghana differentiate 54

M ulcerans strains into 13 SNP haplotypes, yet a geographically focal transmission.[27,28]

PATHOGENESIS AND IMMUNITY

Initial infection is primarily related to 2 properties of *M ulcerans*: optimal growth at temperatures (30°C–33°C) slightly below the core body temperature and production of mycolactone. The temperature requirement of *M ulcerans* favors the development of lesions in cooler tissues, especially the skin and subcutaneous tissue. Mycolactone destroys tissues by apoptosis and necrosis (**Fig. 4**) and suppresses host immune responses.[1]

Mycolactone profoundly suppresses elements of innate and adaptive cell-mediated immunity, thereby enhancing progression of BU. Mycolactone inhibits macrophages, monocytes, B cells, and T cells at least, in part, by inhibiting production of interleukin (IL)-1, IL-2, IL-6, IL-8, IL-10, tumor necrosis factor α, and interferon-γ (IFN-γ).[29,30] The immunosuppressive effects of mycolactone extend beyond skin lesions to circulating leukocytes and lymphoid organs.[31] Peripheral whole-blood samples from patients with active BU, when stimulated with mitogens, produce comparatively smaller amounts of helper T cell (T_H) 1, T_H2, and T_H17 cytokines.[32]

The clinical and histopathologic features of BU suggest an immunologic spectrum of host responses over time, which may be relevant for vaccine strategies. Early progressive ulcers generate abundant IL-10 with little inflammation (T_H2 response) and numerous, often extracellular, *M ulcerans* (**Fig. 5**) within areas of coagulation necrosis. Necrosis reflects mycolactone-induced death of tissue and inflammatory cells. In contrast,

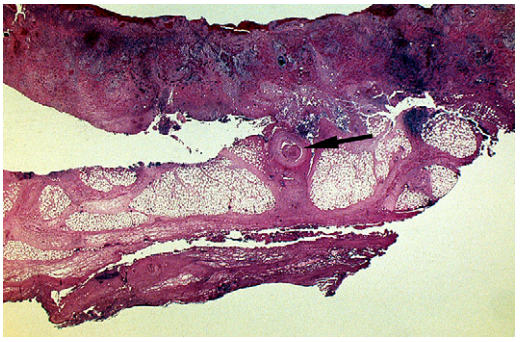

Fig. 4. Microscopic section of the undermined edge of a major BU. Note contiguous coagulation necrosis of the panniculus and fascia and vasculitis with thrombosis of a medium-sized vessel (*arrow*) (hematoxylin-eosin, original magnification ×40). A mild host inflammatory response is consistent with a toxin-mediated process.

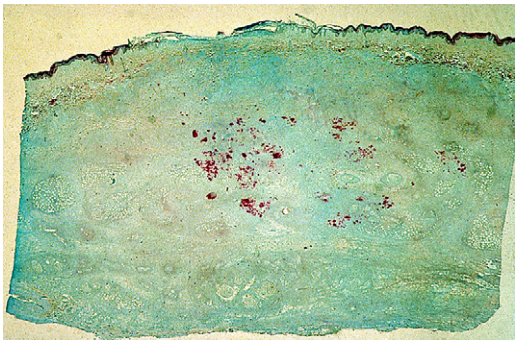

Fig. 5. Microscopic section of early nodule of BU showing clumps of extracellular acid-fast bacilli (AFB, *red*) in the center of widespread necrosis (Ziehl-Neelsen stain, original magnification ×40). Necrosis extends far beyond the AFB, supporting the notion that *M ulcerans* produces a diffusible necrotizing toxin.

mature or resolving BU lesions, especially under treatment with antibiotics, contain IFN-γ within granulomatous inflammation, organizing lymphoid aggregates, and typically intracellular *M ulcerans*, consistent with a T_H1, delayed-type hypersensitivity (DTH) response.[1,33,34] DTH in these patients, but not those with early BU or uninfected persons, is verified by skin test reactivity against burulin, a sonicate of *M ulcerans*.[35] Minor BU may self-heal early, suggesting elements of high host resistance.

CLINICAL FEATURES AND DIAGNOSIS

BU presents as a spectrum of localized or disseminated clinical forms, with variable natural history (**Fig. 6**). Early lesions are usually papular, nodular, or edematous, progressing to ulcers with rolled borders, spreading laterally. Most ulcers are painless unless secondarily infected. Fever and lymphadenopathy are rare. Experienced workers may

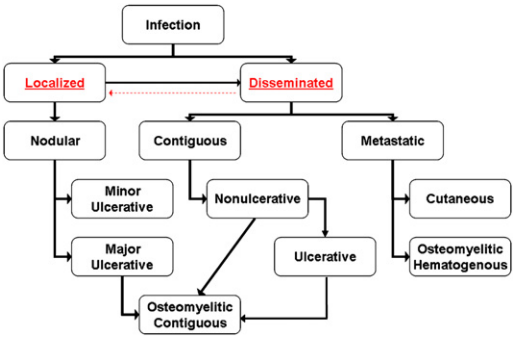

Fig. 6. Proposed classification and natural history of untreated clinical forms of active BU.

correctly diagnose some BU lesions on clinical features alone, but there is often discord between clinical impression and laboratory results because of incorrect clinical diagnosis, inadequate sampling, or laboratory errors. Important entities in the differential diagnosis of ulcerative and edematous BU include tropical phagedenic ulcer and necrotizing fasciitis, respectively.[36] Both these conditions, unlike BU, are painful. Many other conditions resemble BU, underscoring the importance of laboratory confirmation. Radiographic examination is indicated when bone involvement is suspected.

The 4 diagnostic laboratory tests for BU are (1) direct smear (with acid-fast stains auramine O or Ziehl-Neelsen), (2) culture, (3) histopathology, and (4) PCR. Estimated sensitivities for these techniques range from 60% or lesser to more than 90%. PCR, currently available only in research laboratories, is considered the most reliable method for all lesion subtypes,[37] followed by histopathology, culture, and direct smear. Lesion sampling techniques include swabbing, punch biopsy, and, as a less-invasive alternative to biopsy, fine-needle aspiration (FNA). PCR is highly sensitive when applied to swabbed material from ulcers and to biopsies and FNAs of nonulcerative lesions.[38] The targets of PCR, IS*2404* and IS*2606*, may be present in other pathogenic mycobacteria, such as *M marinum*; so clinical features or variable number of tandem repeat assays may discriminate *M ulcerans* from other species.[39]

Among the non-PCR diagnostic methods, histopathology is useful to confirm BU or generate a differential diagnosis when unconfirmed. Culture is recommended for tracking treatment response, often a concern in clinical trials.[38] Pharmacologic assays to detect mycolactone in the tissues infected with *M ulcerans* may become a diagnostic adjunct to culture.[40] Direct smears are useful at the community level. Rapid diagnostic tests for use in the field, to detect mycolactone or *M ulcerans*—specific proteins in lesional or other biologic fluids, are in early development.

Regardless of the test or sampling method, at least 2 sites per lesion suspicious for BU should be sampled; this process increases sensitivity over a single sample by up to 25%. When confronted with possible new geographic foci of BU, confirmation by PCR and at least 1 of the other 3 tests is advised.

TREATMENT

Historically, treatment of BU has been surgical excision of the affected tissues, correction of wound defects, and, if available, rehabilitative physiotherapy. Bone and joint lesions are given priority. By the 1970s, rifampin was known to heal most small BU lesions. However, until 2005, antibiotics remained a largely perioperative adjunctive therapy, aimed at reducing dissemination and recurrence or minimizing tissue excision.[41] The role of adjunctive antibiotic therapy for BU that is otherwise surgically excised remains unclear.[42] Other treatment methods, such as local heat, explored decades ago, may become practical as application systems are simplified.[43]

In 2004, with increasing BU incidence and limited surgical resources in Africa, supported by experimental and encouraging preliminary human data,[44] the WHO advocated a provisional antibiotic regimen for BU, comprising oral rifampin (10 mg/kg) plus intramuscular streptomycin (15 mg/kg), both given daily for 8 weeks under supervision.[45] Amikacin (15 mg/kg) can be substituted for streptomycin, administered intramuscularly or intravenously. Important contraindications and side effects for these drugs are described elsewhere.[45] As general guidelines, patients with lesions less than 5 cm in diameter (category I, small) receive antibiotics alone and those with lesions 5 to 15 cm in diameter (category II, moderate) receive 4 weeks of antibiotics and then undergo surgery, if necessary, followed by 4 more weeks of antibiotics. Patients with lesions more than 15 cm in diameter (category III, advanced) are treated with antibiotics for at least 1 week before surgery; the antibiotics are then continued for a total of 8 weeks. Follow-up of all patients is advised for an additional 10 months to assess for cure and complications.

Case series studies of rifampin plus streptomycin for small and moderate BU conducted in Benin and Ghana concluded that most lesions resolve after 8 weeks of treatment.[46,47] In 2010, Nienhuis and colleagues[48] reported the first randomized trial of rifampin plus streptomycin for early limited BU, defined as lesions of less than 6 months' duration comprising nodules or ulcers less than 10 cm in diameter. Rifampin plus streptomycin given daily for 8 weeks, or for 4 weeks, followed by rifampin plus clarithromycin (both oral) daily for 4 weeks, all without surgery, healed BU in more than 90% of patients by 1 year. These results, coupled with experimental data in mice and a case report describing resolution of advanced BU after 8 weeks of rifampin plus clarithromycin,[49] support studies of fully oral, less-toxic regimens, such as rifampin plus clarithromycin or rifapentine plus moxifloxacin.[50]

For advanced BU, rifampin plus streptomycin therapy is under investigation. In a study in the Democratic Republic of Congo, 61 patients with

PCR-positive ulcers (longest diameter>10 cm) were treated with daily rifampin plus streptomycin, treatment was extended to 12 weeks, and surgery was performed 4 weeks after antibiotics treatment was begun; 98% were classified as cured after 2 years.[51] Further studies in patients with large BU lesions, coordinated by the WHO, will aim to determine the best time for surgery within the course of antibiotics.

In some BU lesions, treatment with antibiotics may cause temporary immune-mediated inflammation with clinical worsening, proposed as a paradoxic sign of treatment success.[52] Awareness may prevent unnecessary treatment changes, reduce surgeries, and improve the accuracy of treatment trials.

ACKNOWLEDGMENTS

The authors thank Siripan Phatisawad for making the map.

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