Buruli Ulcer and Mycolactone-Producing Mycobacteria

Kazue Nakanaga1*, Rie Roselyne Yotsu2, Yoshihiko Hoshino1, Koichi Suzuki1, Masahiko Makino1, and Norihisa Ishii1

1Leprosy Research Center, National Institute of Infectious Diseases, Tokyo 189-0002; and 2Department of Dermatology, National Center for Global Health and Medicine, Tokyo 162-8655, Japan

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CONTENTS:
1. Introduction
2. Epidemiology and symptoms of Buruli ulcer (BU)
   2–1. History
   2–2. Symptoms
   2–3. BU in Japan
3. Mycolactone and mycolactone-producing mycobacteria
   3–1. Isolation of mycolactone
   3–2. Discovery of mycolactone synthetase bearing plasmid pMUM001
   3–3. Role of mycolactone
   3–4. Other mycolactone-producing mycobacteria
4. Conclusion

SUMMARY: Buruli ulcer (BU) is an emerging human disease caused by Mycobacterium ulcerans, which mainly affects the extremities. It is most endemic in sub-Saharan Africa; however, it has been reported worldwide, including in some non-tropical areas. “M. ulcerans subsp. shinshuense” is proposed as a subspecies of M. ulcerans, which have been reported from Japan and China. A total of 35 BU cases have been reported as of November 2012. Although M. ulcerans is categorized as nontuberculous mycobacteria, it has some unique characteristics that could only be observed in this bacterium. It possesses a giant virulent plasmid, composed of 174-kbp nucleotides, coding polyketide synthase to produce macrolide toxin called mycolactone. The discovery of such a linkage of plasmid and its pathogenesis has not been reported in other human disease-causing mycobacteria.

1. Introduction

Buruli ulcer (BU) is a necrotizing skin disease caused by Mycobacterium ulcerans, belonging to nontuberculous mycobacteria (NTM) family. It has a unique characteristic of producing a macrolide toxin called “mycolactone” (1–5), which has not been identified in any other species of mycobacteria including Mycobacterium tuberculosis and Mycobacterium leprae. BU has been reported in at least 33 countries, and M. ulcerans disease is probably the third most common mycobacterial disease after tuberculosis and leprosy in some endemic areas among immunocompetent individuals (6). BU is categorized as a neglected tropical disease by the World Health Organization (WHO). The most endemic areas include sub-Saharan Africa, and a control strategy has been implemented to minimize morbidity and disability associated with BU especially in these areas (Fig. 1). The mode of transmission remains unclear; however, it is speculated that an aquatic environmental source is the origin of infection. Similarities in characteristics are found in other mycobacterial strains, which mainly infect frogs, fish, or turtles (7–9), and therefore, they must be differentiated when searching for M. ulcerans in environmental sources. In this review, the history of researches on M. ulcerans and other mycolactone-producing mycobacteria are described, focusing on mycolactone and their virulent plasmids.

2. Epidemiology and symptoms of BU

2–1. History
Historically, BU first gained notice from two foci. One focus was in Central Africa where the first related description was made by Sir Albert Cook. He described several cases of chronic large ulceration in Uganda in 1897. Later on, the disease was named after Buruli County in Uganda, where the first large epidemic was investigated in the 1960s (10,11). At present, sub-Saharan Africa, especially Cote d’Ivoire, Ghana, and Benin are the highest endemic countries to be controlled. The other focus is in southeastern Australia. In the first report, there was a series of unusual painless ulcers in a patient from Bairnsdale in 1935 (12). Thirteen years after the first report, MacCallum et al. made the first description of the mycobacterial infection in 1948 (13). The name M. ulcerans first appeared in a report by Fenner in 1952 (14). Currently, Bellarine Peninsula in Victoria is known to be the highest endemic area in Australia (15).
2-2. Symptoms
BU often starts as a painless papule or nodule, looking like an insect bite. However, it gradually leads to the destruction of skin and ulceration. Interestingly, despite their severity, the lesions are often painless. The ulcer usually presents with a yellow-whitish necrotic base with undermined borders and edematous surroundings. Major affected sites are the extremities and the face. If patients seek treatment during early stages, antibiotics (rifampicin and streptomycin as recommended by the WHO) can prove to be successful. Delayed treatment may cause irreversible deformity, long-term functional disabilities such as restriction of joint movement, extensive skin lesions, and sometimes life-threatening secondary infections (1–5).

2-3. BU in Japan
The first reported case of BU in Japan was a 19-year-old woman in 1980 (16). The causative agent was isolat-
Table 1. Characteristics of cases reported in Japan as of November 2012 (28,29)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Known isolate</td>
<td><strong>Mycobacterium ulcerans</strong> subsp. <em>shinshuense</em>**</td>
</tr>
<tr>
<td>International traveling</td>
<td>None</td>
</tr>
<tr>
<td>Mode of transmission</td>
<td>Unknown, not clear with aquatic environment(1)</td>
</tr>
<tr>
<td>Regional bias</td>
<td>Honshu island (northern limit, Akita Prefecture; southern limit, Kagoshima Prefecture; no case in Shikoku island)</td>
</tr>
<tr>
<td>Seasonal bias</td>
<td>Autumn and winter (unclear incubation period)</td>
</tr>
<tr>
<td>Age</td>
<td>2–84 years</td>
</tr>
<tr>
<td>male cases:female cases</td>
<td>12:23</td>
</tr>
<tr>
<td>Pain sensation</td>
<td>More outstanding in Japanese cases</td>
</tr>
<tr>
<td>Sensitivity against antibiotics</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Affected regions</td>
<td>Usually extremities (legs, arms, or face)</td>
</tr>
<tr>
<td>Size of ulcer</td>
<td>Mainly &lt;5 cm (category I)</td>
</tr>
</tbody>
</table>

(1): Three cases in one family member often contact with tiny stream on their property.

ed and classified as ‘‘*M. ulcerans* subsp. *shinshuense***’’ because it was closely related to *M. ulcerans* (17). The second reported case was a 37-year-old woman in 2003 (18). After the second report, there has been a steady increase, summing up to a total of 35 cases as of November 2012 (16–26) (Fig. 2). Among all cases, 23 (65.7%) subjects were female and 12 (34.3%) were male. The average age of those affected was 39.7 years (range, 2–84 years) for females and 43.3 years (range, 5–81 years) for males. Eleven cases were found in the Chugoku region, 9 in the Chubu region, 7 in the Tohoku region, 6 in the Kinki region, 1 in the Kanto region, and 1 in the Kyushu region (Fig. 3). Although there was no geographical focus in the distribution, 3 out of 4 cases in Fukushima Prefecture (Tohoku region) were family members. The highest number of cases (9 cases) was found in Okayama Prefecture (Chugoku region). There has also been one report from China of which *M. ulcerans* subsp. *shinshuense* was isolated from an ulcer (27). Hence, *M. ulcerans* subsp. *shinshuense* is now speculated to be an endemic species of *M. ulcerans* in east Asia (28,29). This subspecies has been isolated or at least confirmed through direct nucleotide sequencing in the identified cases. The characteristics of BU in Japan are shown in Table 1. Though there seem to be some differences in clinical presentation such as more painful cases in Japanese cases (Fig. 4), histopathological findings do not differ from those of other parts of the world. Unlike other mycobacterial infections, the histopathology of BU presents with poor formation of granulation tissue and little inflammatory cell infiltration surrounding the ulcerative lesion. Although there is extensive necrosis, the circumjacent epidermis is usually unaffected, and the lesions are usually deeper in the subcutaneous areas. Acid-fast bacilli are often evident on the lower dermis.

3. Mycolactone and mycolactone-producing mycobacteria

3–1. Isolation of mycolactone

From the very beginning, pathologists were certain that some toxic substances from *M. ulcerans* relate to the unique characteristic of the skin lesions in BU because histopathological changes were observed distant from where massive acid-fast bacilli were detected (30–32). In 1999, mycolactone was isolated as the cause of cytopathicity and cell-cycle arrest in cultured L929 murine fibroblasts (33). Guinea pigs produced lesions similar to those of BU in humans following purified mycolactone injection (34). Mycolactone is not a protein but a smaller molecule, which is composed of a 12-membered ring (macrolide) to which two polyketide-derived side chains are attached. It structurally resembles immuno-suppressants such as rapamycin, FK506, and cyclosporin A. The characteristic mixture of mycolactone congeners varies in clinical isolates from geographically different areas (35). The structure that
has the highest activity in mammalian cell lines is mycolactone A/B, which exists as a 3:2 equilibrating mixture; the major and minor components are Z-Δ4′,5′- and E-Δ4′,5′-isomers, respectively, on the unsaturated fatty acid side chain (36) (Fig. 5).

3–2. Discovery of mycolactone synthetase bearing plasmid pMUM001

Attempts to identify genetic bases of the virulent factor in M. ulcerans started by comparing closely related mycobacterial strain Mycobacterium marinum (37). This idea of subtraction was thought of because these two strains were genetically related species but M. This idea of subtraction was thought of because these two strains were genetically related species but M. marinum produced no mycolactone. A type I polyketide synthase (pkS) gene fragment was identified as an M. ulcerans specific gene based on genomic suppressive subtractive hybridization between M. ulcerans and M. marinum (38). In 2004, a giant plasmid, which bears mycolactone-producing enzymes, was identified using pkS probes identified through subtractive hybridization. These probes were hybridized using pulsed-field gel electrophoresed DNA or a BAC library of the M. ulcerans Ghana isolate Agy99. The plasmid pMUM001 is composed of 174,155 bp, as a 62.8% GC content, and its 81 protein-coding DNA sequences bears a cluster of genes for complete mycolactone synthesis (39,40). The discovery of pMUM001 had profound implications for mycobacterial research because mycobacterial plasmids have never been directly linked to virulence. Moreover, the M. tuberculosis complex, the most virulent mycobacterium for humans, is reported to have no plasmids.

3–3. Role of mycolactone

The role of mycolactone in M. ulcerans survival in nature is not clarified. On the other hand, during infection of humans, which causes BU, the function of mycolactone is thought to cause “painless lesions” and “poor acute inflammatory cellular infiltration.” Although its mechanisms have not yet been revealed, painless lesions were successfully demonstrated by M. ulcerans infection and/or purified mycolactone injection in mice. Nerve degeneration occurs through invasion of bacilli or mycolactone at the perineural and endoneurial level, inducing loss of pain sensation or hypesthesia (41,42). Mycolactone effectively suppresses the capacity of dendritic cells to secrete β-chemokines macrophage inflammatory protein (MIP)-1α, MIP-1β, regulated on activation, normal T cell expressed and secreted, as well as monocyte chemoattractant protein 1 and interferon γ-inducible protein 10, which may limit initiation of primary immune responses. Moreover, it inhibits the recruitment of inflammatory cells to the infection site (43).

3–4. Other mycolactone-producing mycobacteria

In 2001, there was an outbreak of a new granuloma-forming disease in a laboratory, which killed a number of experimental frogs (Xenopus tropicalis). Granulomas were identified systemically on the skin as well as in other areas; the causative agent was later identified as Mycobacterium liflandii (44). It formed orange-pigmented colonies after long cultivation, possessed giant plasmid pMUM002 similar to pMUM001, and had IS2404 and IS2606 like M. ulcerans. The product of pMUM002 has now been identified as mycolactone E (7). M. liflandii was initially thought to be the causative pathogen for BU; however, it is now known that it infects mainly frogs (45).

Some M. marinum strains isolated from fish are known to be Mycobacterium pseudoshottsii, which have IS2404, IS2606, and pMUM003 (similar to plasmid pMUM001), and produce mycolactone F (8). M. pseudoshottsii has been isolated in Japan, especially from various types of farm-raised fish such as yellow tail (Seriola quinqueradiata), greater amberjack (Seriola dumerili), sevenband grouper (Epinephelus septemfasciatus), striped jack (Pseudocaranx dentex), and yellowtail amberjack (Seriola lalandi) (46). M. pseudoshottsii were isolated from the kidney of the fish that were lethally affected. The genomes of some of these mycolactone-producing mycobacteria were analyzed, and interestingly, it is now evident that these species are very closely related in their evolution. What is more striking, differences in the genome sequencing of the plasmids for pMUM001, pMUM002, and pMUM003 correspond to differences in the structures of the unsaturated fatty acid side chains for each type of mycolactone, namely, A/B, E, and F (47,48).

4. Conclusion

Some researchers have reported that mycobacterial strains that have pMUM001-like plasmids and produce mycolactone should be considered a single species (49). This conclusion emerged from a number of genomic studies. Nonetheless, infection of these mycobacteria in fish or frogs, especially in places with high risk of infection such as fish farms, is causing a considerable anxiety in people. Therefore, there is a need to understand the possibility of infection from animals to humans, a need for invention of easy diagnostic tools, and a need to raise awareness among people about the mycobacteria.
with the right information.

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Conflict of interest None to declare.

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