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motifs V–IX at the C-terminal region (Table 1), indicating that this segment is specially important for Gasdermin A3 functions in the skin and hair follicles. Another common feature of these lines is a pronounced hair loss between the first and the third weeks of age. However, considerable differences have been reported regarding the severity of the phenotype, whether the anagen or the catagen stages of the first hair cycle are affected by the mutation, and whether the length of the hair shafts are affected or not. These differences might be due to dissimilarities in the genetic background of the various *Gsdma3* mutant lines, and also to the fact that they have been studied in a variety of laboratories employing different protocols.

While the exact function of Gasdermin A3 in skin and hair follicle physiology remains to be determined, mouse lines carrying mutations in the *Gsdma3* gene have been already useful for studying the mechanisms underlying hair follicle destruction in cicatricial alopecia [3]. Also, since there are some parallels with psoriasis (including an acanthotic and hyperkeratotic epidermis and the presence of numerous cells of the immune system in the dermis), a *Gsdma3* mutant line was employed as a model for evaluating therapies for this disease [4]. Thus, we believe that the newly described mouse line *Gsdma3*^{1359N} will be useful as an additional member of the allelic series for unraveling the functions of Gasdermin A3 in the skin and its appendages and to study a range of processes associated with different dermatological diseases.

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Letter to the Editor

Present situation of leprosy in Japan, 2006–2010: Analysis of drug resistance in new registered and relapsed cases by molecular biological methods

Keywords:
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Drug resistance;
New registered cases;
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Leprosy is a chronic infectious disease caused by an obligate intracellular pathogen *Mycobacterium leprae*. The present strategy for leprosy control is based on the multidrug therapy (MDT), recommended by the World Health Organization (WHO), which has successfully reduced the number of leprosy cases in the world.

Newly reported cases in Japan have markedly decreased during the last two decades. There have been fewer than 10 cases per year

in the recent three years. Amongst these newly registered cases, the proportion of imported cases. Relapse cases in Japan are limited to only a few in the recent years.

Although MDT is an effective treatment for leprosy, drug-resistance are known to occur for each agents. Rapid detection and control of such drug-resistant strains is essential in control of leprosy. However, the drug-resistance situation of *M. leprae* has not yet been well informed in Japan.

Table 1A

Number of newly registered patients in Japan. The number of newly reported leprosy in Japan between 2006 and 2010 shows decline whilst the proportion of imported cases increased. The percentage of non-Japanese patients in 2006, 2007, 2008, 2009 and 2010 were 85.7% (6/7), 91.7% (11/12), 57.1% (4/7), 100% (2/2) and 100% (4/4), respectively.

Year	Japanese	Non-Japanese	Ratio of Non-Japanese (%)
2006	1	6	85.7
2007	1	11	91.7
2008	3	4	57.1
2009	0	2	100
2010	0	4	100
Total	5	27	84.4

Table 1B

PCR result in newly registered patients. First, *hsp-70* PCR method was applied to detect *M. leprae* on 27 samples obtained from newly registered patients, excluding five cases registered in 2006. The positive rate was 85% (23/27). Then, mutation analyses on the DRDRs of *folP1*, *rpoB* and *gyrA* genes were applied to samples positive for *hsp-70* with PCR. Cases of mutations detected on *folP1*, *rpoB* and *gyrA* were 8.7% (2/23), 0% (0/23) and 4.3% (1/23), respectively.

Country	PCR		Mutation			
	Positive	Negative	No mutation	<i>fol P</i> (dapsone)	<i>rpo B</i> (RFP)	<i>gyr A</i> (quinolones)
Philippines	6	0	5	0	0	1
Brazil	6	1	5	1	0	0
Indonesia	3	1	3	0	0	0
Vietnam	1	0	1	0	0	0
Korea	1	0	0	1	0	0
Nepal	1	0	1	0	0	0
Thailand	1	0	1	0	0	0
Myanmar	0	1	0	0	0	0
Japan	4	1	4	0	0	0
Total	23	4	20	2	0	1
%	100	–	87	8.7	0	4.3

We investigated the present situation of leprosy in the aspect of drug-resistance mutation in new and relapse cases of leprosy by molecular biological methods. In this study, drug-resistant mutation was investigated amongst the patients presenting positive PCR tests in the years from 2006 to 2010. A total 49 patients (27 new and 22 relapse cases) met the criteria and included in this study.

For the detection of DNA of *M. leprae*, we performed PCR amplification of the *hsp-70* gene of *M. leprae* [1], and further tested the *hsp-70* PCR positive sample for drug-resistance determining regions (DRDRs) [2]. Mutations were measured on the *folP1* gene for dapsone [3], the *rpoB* gene for rifampicin (RFP), and the *gyrA* gene for quinolones [4,5]. Nested PCR conditions for drug resistance were different from that of RLEP-nested PCR [6,7].

The number of newly reported leprosy in Japan between 2006 and 2010 shows decline whilst the proportion of imported cases increased (Table 1A). Mutation analyses on the DRDRs of *folP1*, *rpoB* and *gyrA* genes were applied to samples positive for *hsp-70* with PCR (Table 1B). All of the drug resistant samples originated from imported cases (Table 1C).

All (22) of the relapse cases were Japanese nationals, and mutation analyses on the DRDRs of *folP1*, *rpoB*, and *gyrA* genes were performed (Table 2A). All of the drug resistant cases we confirmed were lepromatous leprosy, multibacillary (MB) leprosy case (Table 2B).

The mutation rate in relapse cases in Japanese was higher than that of newly detected cases. This phenomenon is most likely to be the result of prolonged administration of dapsone alone until the 1990s in Japan. The result indicated a strong correlation between mutation rate and relapse. Two possible reasons were conceived regarding the high positive rate of dapsone resistance in patients with relapse: reinfection by the primary drug resistant strain and reactivation of dapsone-resistant strains capable of persisting after chemotherapy, as discussed below. Although it is still unclear whether recurrences are caused by reinfection of *M. leprae* or by reactivation of persistent *M. leprae*, close correlation between drug resistance and relapse have been recognized likewise in several studies [8,9].

The sum of the mutation rates with relapsed case for *folP1*, both *fol P1* and *gyr A*, and *folP1* and *rpoB*, thus dapsone-resistant cases were 23% (Table 2A). This rate falls approximately in the mid portion of the ranges from other reports. Regarding other areas in Southeast Asia, mutation rates for *folP1* amongst the relapse cases were 26% (5/19) in the Philippines (Cebu), 8.3% (2/24) in Myanmar (Yangon), 10% (1/10) in Indonesia (North Maluku and North Sulawesi) [10], and 57% (8/14) in Vietnam (the central and highland regions) [7].

Table 1C

Drug resistant cases in newly registered patients. All of the drug resistant samples originated from imported cases. Case 1: a 32 year-old male from Brazil having borderline lepromatous leprosy presented *folP1* mutation. Case 2: a 69 year-old female from Korea having borderline lepromatous leprosy demonstrated *folP1* mutation. Case 3: a 24 year-old male from Philippines with lepromatous leprosy showed *gyrA* mutation. All of these cases drug resistant mutations were cases of multibacillary (MB) leprosy.

Case	Country	Age	Gender	Classification	Mutation
1	Brazil	32	M	BL ^a	<i>folP1</i> (dapsone)
2	Korea	69	F	BL	<i>folP1</i> (dapsone)
3	Philippines	24	M	LL ^b	<i>gyrA</i> (quinolones)

^a BL, borderline lepromatous leprosy.

^b LL, lepromatous leprosy.

Table 2A

PCR results of relapsed leprosy patients. The mutations detected on *fol P1*, *rpoB*, *fol P1/gyr A*, and *fol P1/rpo B* were 9.1% (2/22), 9.1% (2/22), 9.1% (2/22), 4.5% (1/22), respectively. These data are summed up that the percentage of dapsone-resistant cases was 23% (5/22), 14% (3/22) for RFP, and 9.1% (2/22) for quinolone.

Mutation	Cases	%
No mutation	15	68.2
Dapsone (<i>folP1</i>)	2	9.1
RFP (<i>rpoB</i>)	2	9.1
Dapsone and quinolones (<i>folP1</i> and <i>gyrA</i>)	2	9.1
Dapsone and RFP (<i>folP1</i> and <i>rpoB</i>)	1	4.5
Total	22	100

Table 2B

Drug resistant cases in relapsed leprosy patients. Cases detected with *folP1* mutation included a 73 year-old male with history of dapsone use and 69 year-old female with history of dapsone and RFP use. Cases detected with *rpoB* mutation were a 77 year-old male with history of dapsone use and a 72 year-old male with history of dapsone and RFP use. Cases that showed both *folP1* and *gyrA* mutations were a 71 year-old male with a history of dapsone and RFP use and a 77 year-old female with history of dapsone use. The case that presented both *folP1* and *rpoB* mutations was a 72 year-old male with history of dapsone and RFP use.

Case	Age	Gender	Classification	Mutation	Past drug history		
					Dapsone	RFP	Quinolones
1	73	M	LL	<i>folP1</i>	+	–	–
2	69	F	LL	<i>folP1</i>	+	+	–
3	77	M	LL	<i>rpoB</i>	+	–	+
4	72	M	LL	<i>rpoB</i>	+	+	–
5	71	M	LL	<i>folP1</i> and <i>gyrA</i>	+	+	–
6	77	F	LL	<i>folP1</i> and <i>gyrA</i>	+	–	–
7	72	M	LL	<i>folP1</i> and <i>rpoB</i>	+	+	–

The mutation rates of the relapsed case for *rpoB* (including both mutation *folP1* and *rpoB*) were 13.6% (3/22) in this study (Table 2A). Mutation frequencies of the *rpoB* gene are also low in other reports. Regarding other areas in Southeast Asia, no cases of RFP-resistance have been detected in the Philippines, 1.9% (1/54) in Myanmar, and 3.3% (4/121) in Indonesia [10]. However, in Japan, the RFP-resistant rate is very high. The long-term use of drugs outside the standard MDT regimen in Japanese leprosy cases might have been instrumental in promoting this RFP-resistance.

There were two patterns we have speculated to be the cause of multi-drug resistance. First, spontaneous and under-dosage of RFP prescribed to patients receiving long-term dapsone therapy. Presenting dapsone-resistant *M. leprae* infection was noted. Second, spontaneous and under-dosage of either dapsone, RFP, or quinolone prescribed as monotherapy together with wrong combination of MDT.

Our study indicated high rates of drug resistance, especially, dapsone or RFP in patients amongst the relapse cases, when compared to the newly detected cases in Japan. Moreover, we have to do laboratory tests, include drug-resistant mutation test, and apply multi-drug resistance cases to administration of minocycline (MINO) or clarithromycin (CAM) instead of resistant drugs in Japan. Therefore, we suggest the importance of confirming the drug-resistant status of each leprosy patients through laboratory tests, such as drug-resistant mutation test. When encountering multi-drug resistant cases, the administration of MINO or CAM is most ideal.

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Letter to the Editor

Tyrosinase-related protein1 in mouse melanocytes at early embryonic stage

The key enzymes of the melanogenic pathway, encoded by the *tyrosinase* gene family, are tyrosinase, tyrosinase-related protein 1 (Tyrp1), and dopachrome tautomerase (Dct). Tyrosinase is the critical and rate-limiting melanogenic enzyme and is common to the pheomelanogenic and eumelanogenic pathways. The two other melanogenic enzymes, Tyrp1 and Dct, are active in the eumelanogenic pathway. The functions of Dct have been determined, but the biological role of Tyrp1 remains unclear. Microphthalmia-associated transcription factor (Mitf) is also considered to be a key transcription factor that regulates the expression of most melanogenic proteins.

Skin melanocytes are derived from neural crest (NC) cells that migrate into the dermis and epidermis during embryogenesis [1]. The primary culture method for melanocytes derived from the neural tube, *in vitro* primary culture of NC cells, has been reported in mice [2]. We previously performed the *in vitro* primary culture method derived from neural tube in mice embryos to investigate

melanoblasts in the early stage [3–5]. In the present study, we investigate the role of Tyrp1 independent of Mitf during the mouse embryonic stage using an *in vitro* primary culture method of wild-type and *Mitf^{mi-ew}* mutant mice.

C57BL/6 mice obtained from Japan SLC Co. Ltd. (Hamamatsu, Japan) were used at 9.5 days post-coitum and were mated in our laboratory. *Mitf* mutant embryos homozygous for the *Mitf^{mi-ew}* (eye-less white) allele that encodes a non-functional protein (background strain Naw) were used [6]. Homozygous mutant embryos were obtained by mating homozygous parents. Timed pregnancies were obtained by checking mating plugs, and the morning a plug was detected was defined as embryonic day 0.5. All mice were kept in a temperature- and humidity-controlled environment with a 12-h light–dark cycle in the Institute for Animal Research of St. Marianna University. This study was approved by the Animal Care and Use Committee of St. Marianna University, School of Medicine.

NC cell cultures were established as described by Ito and Takeuchi [2]. Trunk regions posterior to forelimb buds were dissected from embryonic day 9.5 embryos using tungsten

REVIEW ARTICLE

Buruli ulcer and current situation in Japan: A new emerging cutaneous *Mycobacterium* infectionRie R. YOTSU,¹ Kazue NAKANAGA,² Yoshihiko HOSHINO,² Koichi SUZUKI,² Norihisa ISHII²¹Department of Dermatology, National Center for Global Health and Medicine, and ²Leprosy Research Center, National Institute of Infectious Diseases, Tokyo, Japan**ABSTRACT**

Buruli ulcer (BU) is a new emerging disease and the third most common chronic mycobacterial infection in humans, caused by *Mycobacterium ulcerans*. Approximately 5000 cases are reported annually from at least 33 countries around the globe, but more from the tropical nations. A total of 32 cases have been reported from Japan sporadically since 1980. None of the cases were related to international travel. Of the total reported, *M. ulcerans* ssp. *shinshuense*, a subspecies speculated to be domestic to Japan or in Asia, has been isolated from 23 cases. The mode of transmission and its incubation period remain unclear, despite several proposed hypotheses, including several vectors and cutaneous wound as port of entry for the pathogen. *M. ulcerans* invades the skin, subcutaneous tissue, fascia and eventually forms extensive ulceration. Smear, culture, histopathology and polymerase chain reaction are established diagnostic tools to identify *M. ulcerans*. Multiple antimicrobial therapy is a commonly used therapeutic method, but patients often need extensive debridement and, at times, skin grafting, especially when diagnosis is delayed. Thus, expanding a system for improved awareness and diagnosis in Japan and Asia is important, together with elucidating the candidate vector and the mode of transmission. Here, to establish a base for future progress in better understanding of this infectious disease, we reviewed the characteristics of the disease together with an update of reported cases in Japan.

Key words: Buruli ulcer, *Mycobacterium ulcerans*, *Mycobacterium ulcerans* ssp. *shinshuense*, mycolactone, non-tuberculous mycobacteria.

INTRODUCTION

Buruli ulcer (BU) is a necrotizing skin and soft tissue infection caused by *Mycobacterium ulcerans*, categorized as a non-tuberculous mycobacteria (NTM). It is the third most common mycobacterial infection after tuberculosis and Hansen's disease (leprosy), and cases have been reported from at least 33 countries with the incidence rate highest in sub-Saharan Africa.¹ Despite its image as a disease confined to tropical areas, in recent decades, reports have also been made from sub-tropical and non-tropical nations including Australia, China and Japan.¹ In Japan, a total of 32 cases have been reported sporadically since 1980. Interestingly, it is now evident that pathogens isolated from Japanese and Chinese cases slightly differ from those of other countries.

The World Health Organization (WHO)² includes BU as a neglected tropical disease (NTD) primarily due to its disabling and stigmatizing complications, and is working toward better diagnosis, treatment and prevention. Moreover, research is promoted, for there are various issues still remaining to be uncovered including its vector, mode of transmission and pathogenesis.² The objectives of

this article are to: (i) review the current state of knowledge of Buruli ulcer; (ii) summarize the 32 cases reported in Japan; and (iii) propose future perspectives how these cases and diagnostic network in Japan may contribute to the better understanding and control of BU worldwide.

EPIDEMIOLOGY AND TRANSMISSION

The first report of BU dates back to 1897 when Sir Albert Cook described cases of chronic ulceration in Uganda. It took approximately half a century for it to be recognized as a mycobacterial skin infection; MacCallum (Australia) made the first definitive description of *M. ulcerans* in 1948.³ The disease was named after Buruli County, Uganda, where the first large epidemic was investigated in 1961.⁴

Since the early 1980s, this infection has been rapidly re-emerging along with rapid environmental such as deforestation, eutrophication, dam construction, irrigation, farming, mining and habitat fragmentation.⁵ Presently, the disease is reported from various

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parts of the world, at a rate of over 5000 new cases per year.^{1,2} However, diversity in the incidence rate according to regions and lack of awareness prevents accurate sketching of the demographic of BU.

Most cases of BU are found in people living in or around aquatic environments (e.g. wetland, river, reservoir). Therefore, though its vector and mode of transmission are still unidentified, it is hypothesized that *M. ulcerans* is acquired through environmental contact. There are a number of reports that have detected *M. ulcerans* DNA from environmental samples including water filtrates, soil, fish, turtles, frogs, snails and various insects.⁶⁻¹⁴ A recent published report by Lavender *et al.*¹⁵ provided some insights into the potential for mosquitoes to be involved in the transmission of the disease by testing mosquitoes for *M. ulcerans* DNA in an endemic area of southeastern Australia. The study revealed the infection rate per 1000 mosquitoes to be 1.86 (1.48-2.32) with the highest rate obtained from the location with the highest prevalence of the disease.¹⁵ However, these studies tested only for DNA, and this does not provide definite proof for it to be the reservoirs or vectors of *M. ulcerans*. Recently, three new cases of BU were found simultaneously from a family in Japan: a mother and her son and daughter. Close investigation of these kinds of cases may lead to further understanding of the epidemiology of the disease.

EPIDEMIOLOGY AND CURRENT SITUATION OF BU IN JAPAN

The first case of BU in Japan was reported by Mikoshiha *et al.*¹⁶ in 1982. It was a case of a 19-year-old woman who presented a chronic and necrotic ulcer on her left elbow. The case was considered to be an endemic infection, because she lacked history of international travel. Tsukamura *et al.*¹⁷ reported that the mycobacterium obtained from this ulcer showed a close resemblance to *M. ulcerans*, but with some differences. Later, with further research, he advocated this novel subspecies as "*M. ulcerans* ssp. *shinshuense*" in 1989.¹⁸

After a 21-year window period, the second case of BU was reported in 2003. Since then, there has been a steady increase in reported cases, summing up to a total of 32 as of October 2011 (Fig. 1). Amongst these cases, *M. ulcerans* ssp. *shinshuense*, a sub-

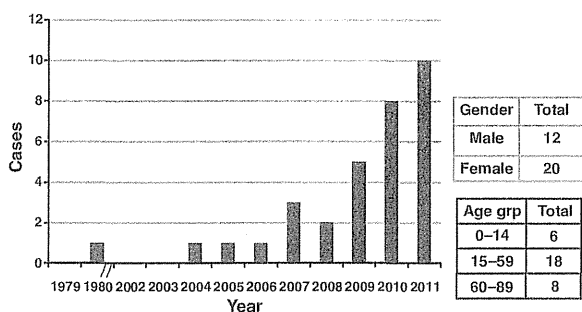


Figure 1. Buruli ulcer cases in Japan by year diagnosed.

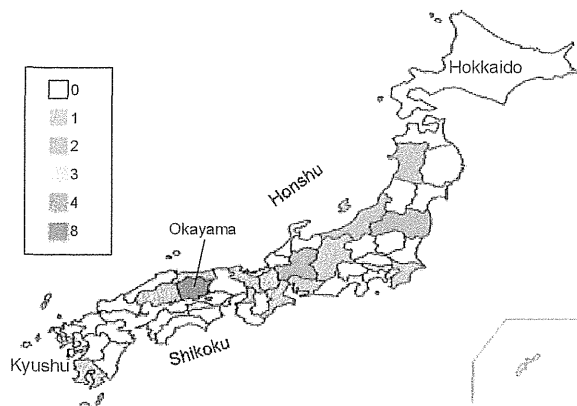


Figure 2. Distribution of Buruli ulcer cases in Japan: a total of 32 cases as of October 2011.

species speculated to be domestic to Japan or in Asia, has been isolated from 23 cases. Of the total, 12 cases (37.5%) were male and 20 cases (62.5%) were female. A tendency was towards middle-aged adults in our cases (Fig. 1). Our age distribution differs from that of other countries. Quek *et al.*¹⁹ reported that in southeastern Australia, there were more cases in patients over 60 years of age, while Debacker *et al.*²⁰ reported that the age distribution in Benin reached its peaks in the 10-14-year age group and amongst those older than 59 years.

All but one case were reported from the main and largest island of Japan, Honshu (Fig. 2). More cases were found from the central western regions of Japan, especially from Okayama Prefecture where eight cases have been identified so far. This prefecture is facing the inland sea, Seto, and the climate is somewhat similar to the Mediterranean Sea, dry and moderate throughout the year. The adjacent prefecture of Hiroshima, also reports one case.

It is interesting to note that 25 cases (86.2%) were diagnosed during autumn and winter (Fig. 3). Interpretation of these statistics needs to be carefully assessed, for the incubation period of this infection is not known; however, it may be a clue to the seasonal inclination.

BACTERIOLOGY

Mycobacterium ulcerans is an NTM that may be cultured *in vitro* showing optimal but very slow growth at 28-34°C on the Löwenstein-Jensen (or Ogawa) medium for mycobacterial culture. This predilection for lower culture temperature explains the skin being its main foci of infection and its limited systemic dissemination. The colonies of *M. ulcerans* are usually yellowish, rough and have well-demarcated edges. The yellowish color may also be observed in the dark.²¹ *M. ulcerans* produces a necrotizing immunosuppressive polyketide toxin, called mycolactone, that is responsible for its pathogenicity.²² There are six structural variants to mycolactone: A, B, C, D, E and F. Most cases of BU are positive for mycolactone A/B (Fig. 4), while few cases present C or D.

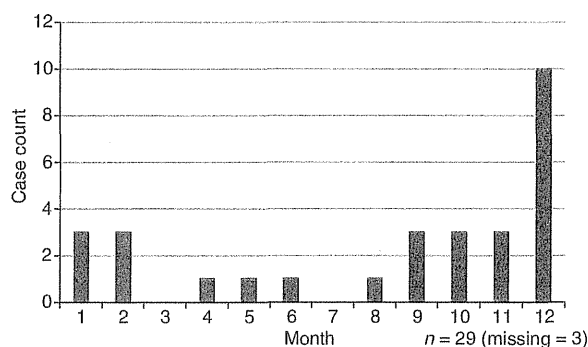


Figure 3. Month diagnosed with Buruli ulcer.

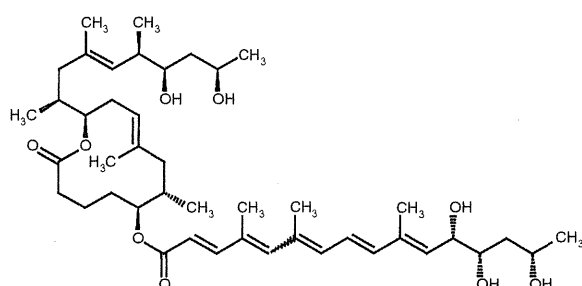


Figure 4. Mycolactone A/B.

PATHOGENESIS AND IMMUNOLOGY

Pathogenesis of *M. ulcerans* is closely related to the production of mycolactone. Mycolactone is a toxic lipid that is cytotoxic to fibroblasts, lipid cells, macrophages, and keratinocytes; inducing both apoptotic and necrotic changes in these cells. It is also known to suppress the local immune system.²² These two major functions explain the extensive progression of the ulcer with relatively low inflammatory response, both clinically and histopathologically. It is also speculated that mycolactone damages the peripheral nerves, resulting in the ulcers being painless.²³

CLINICAL MANIFESTATIONS

The common sites of the skin lesions are exposed parts of the body, particularly the extremities and the face. BU often starts as erythema or papule, which may resemble an insect bite (Fig. 5a). The lesion gradually develops into a painless nodule measuring a few centimeters in diameter (Fig. 5b). In a few days to several weeks, the papule or nodule perforates and forms an ulcer (Fig. 5c). The ulcer is often characterized by white or yellow necrotic tissue on the base, undermined borders and edematous surroundings. The lesion is not limited to a single focus, but when the ulcers are adjacent to each other, they may merge and form a massive ulcer. In rare cases, the ulcer invades the muscular layer.

Ulcers caused by *M. ulcerans* are often documented to be painless, unless secondary bacterial infection exists at the site. In

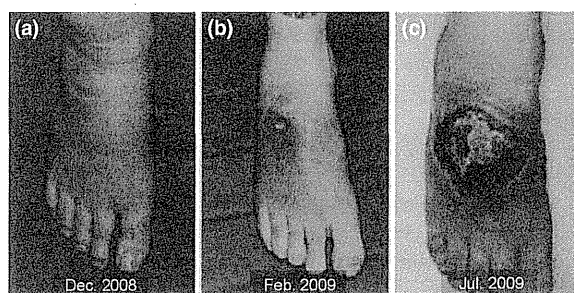


Figure 5. Clinical features. (a) Initial symptoms of Buruli ulcer. It often starts as erythema or papule. (b) The lesion gradually develops into painless nodule measuring few centimeters. The case in the photo is associated with redness and swelling. (c) In a few days to several weeks, the papule or nodule perforates and forms an ulcer. The ulcer is often characterized by white or yellow necrotic tissue on the base, undermined borders and edematous surroundings. Photos provided by Dr Teshin Watanabe of Tottori University, Japan.

contrast, approximately half of the cases confirmed with *M. ulcerans* ssp. *shinshuense* in Japan are reported with pain. Swelling of the regional lymph node and fever are usually absent, and the host's general condition is often well. BU rarely causes direct death, but when not treated early, the disease often results in permanent functional disability. A massive ulcer that lies across the joint, without successful skin grafting and intensive rehabilitation, may leave contracture of the joint.

LABORATORY TESTS

Direct smear or stamp test

Direct smear specimens obtained from the ulcer or stamped biopsy specimens are magnified with Ziehl–Neelsen (Z–N) stain.

Culture test

Fresh skin biopsy, purulent discharge fluid and swab obtained from the surface of the ulcer are the options for specimen. Both liquid media and Löwenstein–Jensen (or Ogawa) medium are used, and cultured at 25°C and 32°C. *M. ulcerans* forms yellowish rough colonies. Because we have experienced a successful isolation at 11 months of culture, we recommend that culturing is continued for at least 6 months.

Polymerase chain reaction (PCR) and other molecular biological studies

Polymerase chain reaction is the best method for early diagnosis. It is performed on a fresh biopsy or previously obtained paraffin block, and targets the high-copy insertion sequence IS2404 (Fig. 6).²⁴ A positive study will rule out *Mycobacterium marinum* or other non-*M. ulcerans* NTM. DNA–DNA hybridization is useful for culture-positive samples, but it cannot differentiate between *M. marinum* and *M. ulcerans*. Further, we perform 16S rRNA gene sequencing to separate *M. ulcerans* ssp. *shinshuense* from *M. ulcerans*.²⁴ Alternative methods include PCR targeting 174-kb plasmid pMUM001 and

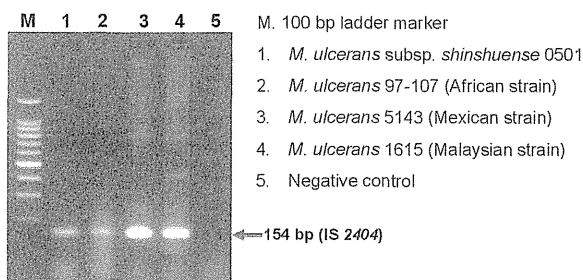


Figure 6. Detection of IS2404 by polymerase chain reaction.

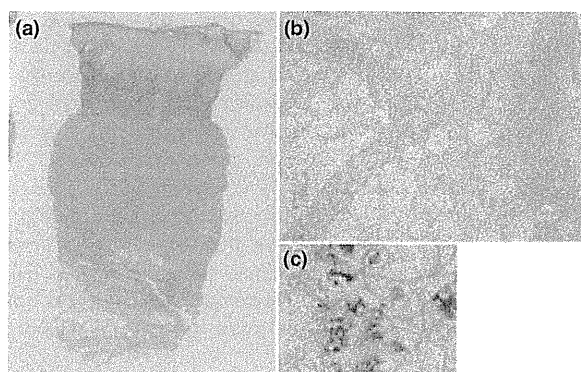


Figure 7. Histopathology. (a) Hematoxylin–eosin stain presents necrotic signs of the dermis, adipose tissue and occasional extension to the fascia. Granulomas or epithelioid cells are rare, as with caseous necrosis (original magnification $\times 40$). (b) Infiltration of lymphocytes in the dermis and adipose tissue is relatively poor (original magnification $\times 200$). (c) Ziehl–Neelsen stain often reveals the mycobacterium in the deep dermal layer to the adipose layer, which is often observed as clusters (original magnification $\times 400$). Paraffin block provided by Dr Yoichi Kato of Okazaki City Hospital, Japan.

drug sensitivity tests, but are not yet common. These sophisticated tests can only be performed at equipped reference institutes that are highly experienced in molecular techniques.

Histopathology

The specimen is to be obtained from a nodule or the rim of the ulcer. Hematoxylin–eosin stain presents necrotic signs of the dermis, adipose tissue and at times extending to the fascia (Fig. 7a,b). Infiltration of lymphocytes in the dermis and adipose tissue is relatively poor, suggesting an immunosuppressant effect of the mycobacteria or the mycolactone. Granulomas or epithelioid cells are rare, as with caseous necrosis. Z-N stain often reveals the mycobacterium in the deep dermal layer to the adipose layer, which often are observed as clusters (Fig. 7c).

DIAGNOSIS

The diagnosis of BU is definitive if *M. ulcerans* is isolated from the ulcer presenting in the exposed parts of the body. Performing all

Table 1. Criteria to diagnose Buruli ulcer

1. Skin eruption accompanying ulcer (regardless of presence of pain)
2. Tissue necrosis with poor inflammatory cell infiltration evident by histopathology
3. Polymerase chain reaction amplification of IS2404
4. Detection of acid-fast bacilli in a smear specimen
5. Histopathological confirmation of acid-fast bacilli

The case is defined Buruli ulcer if it fulfills criteria 1, 2 and 3. Criteria 4 and/or 5 are needed to confirm diagnosis.

tests – smear, histopathology, culture and PCR – is essential for accuracy (Table 1). However, it is known that culture alone may take a very long time, and its success rate is low. 16S rRNA gene sequencing is recommended only if there is a necessity to precisely identify *M. ulcerans*, because it is very time-consuming and expensive. In Japan, laboratory tests for *M. ulcerans* and *M. ulcerans* ssp. *shinshuense* are performed at the Leprosy Research Center (LRC), a division within the National Institute of Infectious Diseases (Tokyo, Japan).

Differential diagnosis for BU includes: cutaneous tuberculosis, leprosy, leishmaniasis, myiasis, diabetic ulcer, necrobiosis lipoidica, pyoderma gangrenosum, pressure sore, malignant skin tumor and trauma.

TREATMENT

There is yet no established treatment regimen. Antimicrobial therapy is the standard treatment, but only a limited number of antimicrobials show high efficacy for *M. ulcerans*, and usually require surgical intervention due to the presence of mycolactone.

Commonly selected oral antimicrobial agents are the combination of two or three from the following: rifampicin (RFP) 450 mg/day, clarithromycin (CAM) 800 mg/day and levofloxacin (LVFX) 500 mg/day. Streptomycin (SM) 15 mg/kg per day via i.m. route can be adopted. The WHO recommend RFP and SM dual therapy for 8 weeks: a regimen widely used in the endemic countries at present.²⁵ Recently, Nienhuis *et al.*²⁶ conducted a trial of 4 weeks of RFP + CAM after 4 weeks of RFP + SM, and found no significant difference with the WHO recommendation. The significance of this study lies in the result that it presented the possibility of minimizing: (i) the duration needed for daily access to health facilities; and (ii) number of doses of i.m. injections which is a burden for many patients, particularly children. It also lessens the risk of acquiring other infectious diseases such as HIV/AIDS and hepatitis B. In Japan, we recommend the RFP + CAM + LVFX triple therapy, which has shown good outcome and compliance in our cases. This regimen consists only of oral antimicrobials, thus making it possible to completely overcome the shortfalls of i.m. injections. Our sensitivity test also supports this regimen, in which the three antimicrobials showed higher sensitivity to the mycobacterium compared to other choices.²⁷

It is important to note that during antimicrobial therapy, new skin lesions may develop, a phenomenon known as “paradoxical

Table 2. Categories of lesions in Buruli ulcer²⁷

Category I	A single lesion <5 cm in diameter. Most category I lesions may completely heal with antibiotic treatment
Category II	A single lesion between 5 and 15 cm in diameter. Some category II lesions may completely heal with antibiotic treatment
Category III	A single lesion >15 cm in diameter, multiple lesions, lesion(s) at critical sites (eye, breast, genitalia) and osteomyelitis. In addition to antibiotics, most category III lesions require surgery (excision, skin grafting or amputation in severe cases). However, multiple small lesions and lesions located at critical sites may heal with antibiotics alone

reactions¹¹. It is most likely, but still remains to be formally researched, that the decrease in the production of mycolactone due to the therapy enables the hosts' immune system to recover and leads to this phenomenon.²⁸

The size of the ulcer is crucial in the determination of a therapeutic plan. The WHO categorizes clinical features into three stages in order to facilitate treatment selection and follow up (Table 2).²⁹ Category I is a single lesion of less than 5 cm in diameter; category II is a single lesion between 5 and 15 cm; and category III is a single lesion of more than 15 cm in diameter. Surgical intervention (debridement) including skin grafting is inevitable in cases not responding to antimicrobial therapy. The WHO does not endorse definitive indication of surgical treatment for ulcers in category I, but we suggest surgery of any ulcer larger than 1 cm in diameter, after completion of 4-week antimicrobial therapy to minimize bacterial colonization. We recommend that the excision is at least 2–5 cm away from the margin and deep enough to reach the fascia. If skin grafting is necessary, it should not be avoided. In either case, post-operative antimicrobial therapy should not be shorter than 4 weeks. If an ulcer of less than 1 cm in diameter does not respond to 2 weeks of antimicrobial therapy, we determine this as an indication of surgical intervention.

When the lesion extends above a joint and surgical intervention has been chosen, strict adherence to the rehabilitation schedule is imperative in order to prevent contracture and permanent functional disability.

PREVENTION/IMPLICATIONS FOR VACCINATION STRATEGIES

Despite the existent of contradictory reports, a few studies suggest the benefit of bacillus Calmette–Guérin (BCG) administration.^{30–34} It leads to prevention of BU within 6–12 months post-administration, or if vaccinated in childhood, it may prevent aggravation into osteomyelitis.^{32,33} BCG vaccine coverage in Japan between 2005 and 2007 was 96.6–98.7% (Control Program Support, The Research Institute Tuberculosis, Japan Anti-Tuberculosis Association). In our cases, history of BCG administration was not confirmed. However, none of the cases of BU in Japan extended into osteomyelitis. We cannot speculate if this is the result of the scheduled BCG adminis-

tration in childhood, characteristics of *M. ulcerans* ssp. *shinsuense*, different living conditions, onset and timing of treatment, or simply by chance.

IMPLICATIONS OF THE JAPANESE CASES AND FUTURE PERSPECTIVES

Recently, we are experiencing an increase of newly reported cases of BU in Japan. Though there may be an actual rise in the endemicity of the disease itself, we believe that the cases of BU reported from Japan were limited until the present for several other reasons: (i) low awareness of the disease amongst the clinicians in Japan; and (ii) NTM, including *M. ulcerans*, are not infections designated by government ordinance, and so the Japanese Ministry of Health, Welfare and Labor does not mandate clinicians and laboratories to report or keep track of the case statistics. We have been conducting activities and developing an information network, thus increasing awareness and improving the diagnostic process. This effort, together with the fact that diagnosis is often made by the same clinician and facilities, led us to this realization. It is evident that BU already existed in Japan in the 1980s.¹⁶ We speculate that there could have been cases treated with antimicrobials under the diagnosis of *M. marinum* or other bacterial infection. Moreover, considering the overlooked cases, there may be more cases waiting to be diagnosed and treated nationwide.

To the extent of our knowledge, the pathogen of BU in Japan and China is a different subspecies of *M. ulcerans*, distinctive from those from other countries. *M. ulcerans* ssp. *shinsuense* was isolated from the very first reported case in Japan.¹⁷ It is not yet clear if this subspecies clinically acts in a different manner, other than some of its laboratory findings (Table 3). So far, dermatological characteristics, including nodule and ulcer forming, non-healing ulcer and the common need of surgical intervention, seem to be similar to the disease caused by the authentic *M. ulcerans* reported elsewhere. Pain seems to be more outstanding in Japanese cases, but our cases are yet too small to draw out any conclusion (Table 4). Interestingly, van der Werf *et al.*³⁵ mentioned that less subcutaneous tissue involvement was seen in Australian cases at the initial stage when compared to those in Africa.

Table 3. Bacteriological characteristics of *Mycobacterium ulcerans*

Culture temperature	28–34°C
Growth rate	4 weeks (slow grower)
Characteristic of colonies	Yellow, rough
Pigmentation in dark	Positive (yellow)
Urease activity	Negative (<i>M. u.</i>), positive (<i>M. u</i> ssp. <i>s</i>)
Niacin accumulation	Negative
Toxin	Mycolactone
IS2404 (PCR)	Positive
<i>M. marinum</i> in DDH [†]	Positive (misidentification)

M. u., *Mycobacterium ulcerans*; *M. u* ssp. *s*, *Mycobacterium ulcerans* ssp. *shinsuense*. [†]DNA–DNA hybridization using DDH Mycobacteria (Kyokuto Pharmaceuticals, Tokyo, Japan). PCR, polymerase chain reaction.

Table 4. Characteristics of cases reported in Japan

Known isolate	<i>Mycobacterium ulcerans</i> ssp. <i>shinshuense</i>
International traveling	None
Mode of transmission	Unknown, not clear with aquatic environment
Regional bias	Honshu Island (awareness of dermatologists unknown)
Seasonal bias	Autumn and winter (unclear incubation period)
Age	8–81 years
Male : female	3:5
Pain sensation	More outstanding in Japanese cases
Sensitivity against antibiotics	Sensitive
Affected regions	Extremities
Size of ulcer	Mainly <5 cm (category I)

These endemic cases reported outside the African and South-American continents, including those reported from Australia and Japan, possibly indicate a high likelihood of the presence of BU in other subtropical regions. In 2000, Faber *et al.*³⁶ reported one case of BU with a history of travel to China, the isolate of which was identified as *M. ulcerans* ssp. *shinshuense*. This important case suggests the possibility of existence of *M. ulcerans* ssp. *shinshuense* in Asian countries other than Japan. Hence, rapid awareness of this disease among clinicians is needed worldwide despite the countries' present status, in order for us to better understand this disease and for the better treatment of patients with persisting ulcer.

CONCLUSIONS

Buruli ulcer is a new emerging mycobacterial infection seen in many countries, yet not much is known about the disease including its epidemiology and bacteriology. Our 32 cases are unique in that they were all infected within Japan. There is a large possibility that there could be overlooked cases in Japan, and moreover, this leads us to hypothesize the possibility of hidden cases in other countries which have never experienced BU before. Thus, raising awareness and promoting further research is demanded in this field worldwide.

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A Case of Mycobacterial Skin Disease Caused by *Mycobacterium peregrinum*, and a Review of Cutaneous Infection

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Key Words

Mycobacterium peregrinum · Rapidly growing mycobacterium · Skin · Therapy

Abstract

An 83-year-old Japanese man presented with a 2-month history of symptomatic nodules on the left hand. He was not in an immunocompromised condition and reported no causal events. A biopsy specimen demonstrated granulomatous tissue with mixed cell infiltration consisting of neutrophils, histiocytes, lymphocytes, and multinuclear giant cells. No bacillus was detected by PAS, acid-fast stain, immunofluorescent stain or polymerase chain reaction analysis. The isolate was found to be a rapidly growing mycobacterium after 4 weeks of incubation at 25°C on an Ogawa egg slant. *Mycobacterium peregrinum* was isolated by DNA-DNA hybridization analysis, 16S rRNA gene sequence, and by its production of 3-day arylsulfatase. The patient received 200 mg oral minocycline for 28 weeks. The lesion disappeared after 10 weeks of this treatment.

Introduction

Mycobacterium (M.) peregrinum, an opportunistic, rapidly growing mycobacterium (RGM), belongs to the *M. fortuitum* group. Because patients with cutaneous infection due to *M. peregrinum* are rare, the clinical information on this mycobacterium is limited [1, 2].

Case Report

An 83-year-old Japanese man presented with a 2-month history of symptomatic nodules on the left hand. He was not in an immunocompromised condition and reported no causal events related to infection, including preceding trauma, fish breeding or circulating bath. On examination, an erythematous plaque with an ulcer and pustules 3 cm in size was seen on the dorsal aspect of the left hand (**fig. 1**). The superficial lymph node was not palpable. The results of laboratory examinations were within the normal range. Chest X-ray ruled out any pulmonary problems. A biopsy specimen from the hand demonstrated granulomatous tissue with mixed cell infiltration consisting of neutrophils, histiocytes, lymphocytes, and multinuclear giant cells (**fig. 2**). No bacillus was detected by PAS, acid-fast stain, immunofluorescent stain or polymerase chain reaction analysis. The isolate was found to be an RGM after 4 weeks of incubation at 25°C on an Ogawa egg slant. *M. peregrinum* was isolated by DNA-DNA hybridization analysis, 16S rRNA gene sequence, and by its production of 3-day arylsulfatase [3].

The patient received 200 mg oral minocycline (MINO) for 28 weeks. The lesion progressively shrank during the treatment and then disappeared after 10 weeks of treatment, leaving only a few scars. No recurrence was observed 1 year after his first visit.

Discussion

In recent years, the pathogenic potential of RGM has gained attention. Although they are not usually pathogenic in humans, they can cause disease in individuals with or without an immunocompromised condition and in people who have had traumatic accidents, such as tsunami survivors with late-onset skin or soft-tissue infections [4]. *M. abscessus* and *M. fortuitum* are the most commonly detected RGM in skin infections, and *M. peregrinum* is rarely identified. Thus, the clinical information, especially regarding treatment, has not been fully examined. **Table 1** lists the cases of *M. peregrinum* with skin lesions that have been reported [2, 4–8]. There were no patients with immunocompromised conditions. All patients were successfully treated with combinations of sparfloxacin (SPFX) and MINO; amikacin (AMK), imipenem-cilastatin (IMP/CS) and levofloxacin (LVFX); AMK, clarithromycin (CAM) and ciprofloxacin (CPFX); and CAM and LVFX, except our patient, who was treated with MINO monotherapy. For treatment of infections caused by *M. fortuitum* group mycobacteria including *M. peregrinum*, the Johns Hopkins Antibiotics Guide recommends oral monotherapy with sulfonamide, doxycycline (DOXY), or CAM for limited or localized wound infections and the combination of at least 2 agents including AMK plus either a β -lactam or quinolone for severe infections of the skin, soft tissue or bone, and for pulmonary disease [9]. Han et al. [1] reported that antimicrobial susceptibility varied among the species of RGM. The results in 105 strains showed that AMK was most active against *M. abscessus*, *M. chelonae* and the *M. fortuitum* group. CAM was the second most active drug for most RGM, except in the case of *M. fortuitum*. Less than half of the strains showed susceptibility to DOXY and MINO, and none of the 6 strains of *M. peregrinum* or *Clostridium septicum* showed sensitivity to these 2 antibiotics. Because most of these strains were isolated from respiratory sources or blood cultures in cancer patients, the data might not apply to cases of skin infection in patients without an immunocompromised condition. Although monotherapy with MINO was effective in our case, combination therapy should be performed routinely. Further case reports involving clinical evaluations of antibiotics are necessary to determine an appropriate therapy for rare mycobacterial infections.

Table 1. Patients with *M. peregrinum* skin lesions

No.	Year	Age/ sex	Country	Site of infection (causing event)	Location	Immuno- deficiency	Chronic disease	Antibiotics	Duration of treatment	Surgical procedure	Ref no.
1	1983	NR	USA	after mammoplasty	NR	–	NR	NR	NR	NR	5
2	1998	45/M	Japan	skin and soft tissue	left arm	–	–	SPFX, MINO	15 weeks	–	2
3	1998	NR	France	skin and soft tissue (subcutaneous insulin infusion)	NR	–	NR	NR	NR	NR	6
4	1999	>65/F	Japan	skin	NR	NR	NR	NR	NR	NR	10
5	2003	67/F	Japan	skin	back of left hand	NR	NR	NR	NR	NR	11
6	2006	NR	Spain	skin and soft tissue (mesotherapy)	NR	–	NR	CPFX	3–4 months	–	7
7	2009	58/F	Japan	skin and soft tissue (reconstruction of abdominal wall)	right chest wall	–	NR	AMK, IMP/CS, LVFX	5 weeks	removal of artificial sheet	8
8	2008	40/M	Sweden	skin	NR	–	NR	AMK, CAM, CPFX	3–6 months?	suture	4
9	2011	78/M	Japan	skin	back of right hand	–	chronic gastritis	CAM, LVFX	12 weeks	–	12
Present case	2011	83/M	Japan	skin	back of left hand	–	hypertension	MINO	28 weeks	–	

NR = Not recorded.

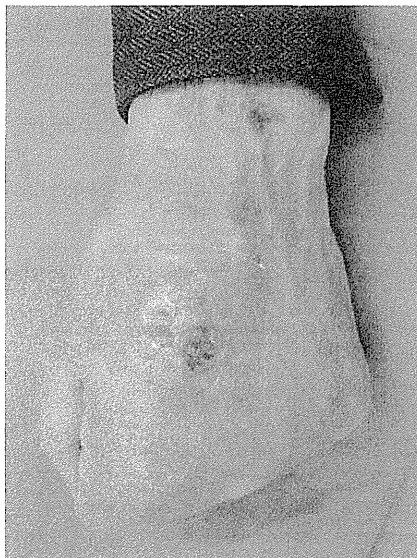


Fig. 1. Plaque with ulcer on the left hand.

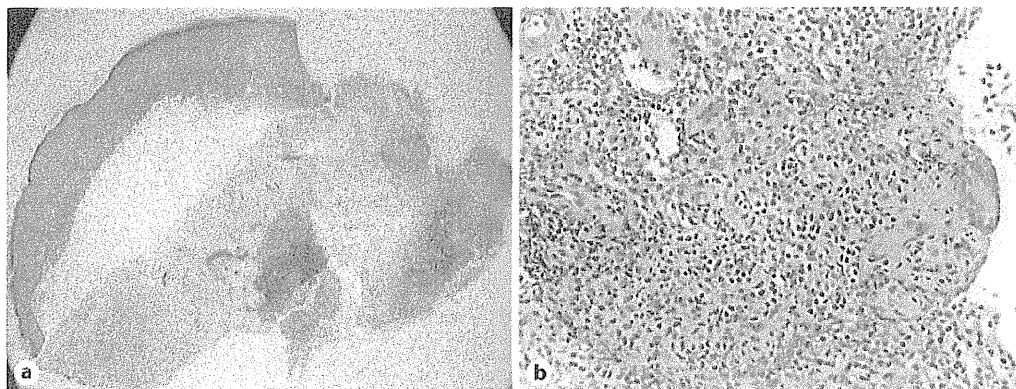
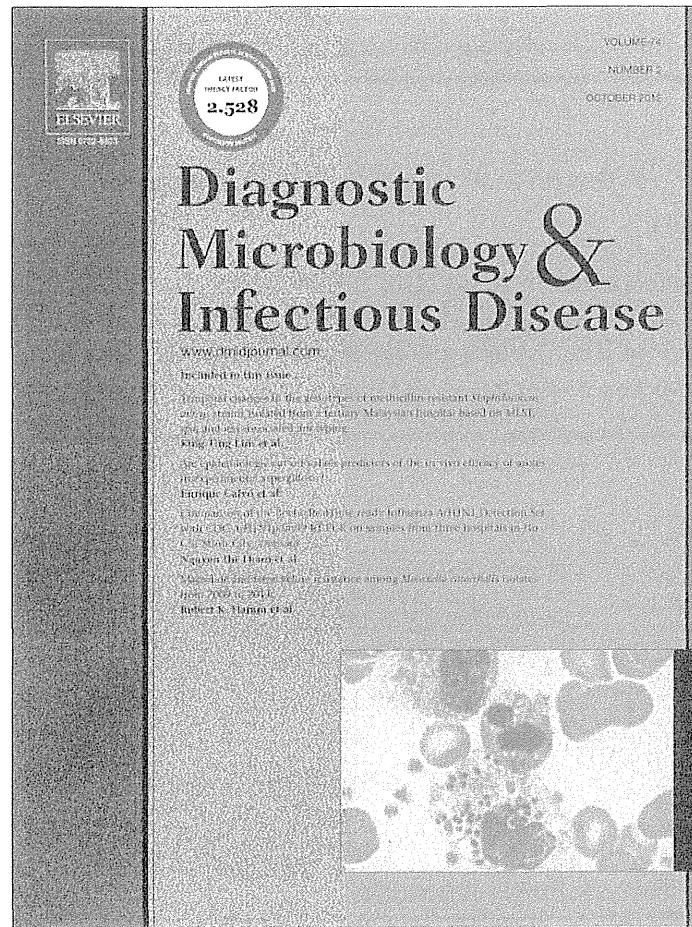


Fig. 2. Biopsy specimen demonstrated granulomatous tissue with mixed cell infiltration consisting of neutrophils, histiocytes, lymphocytes, and multinuclear giant cells.

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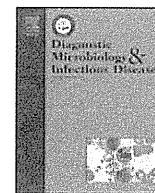


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Bacteremia due to *Mycobacterium massiliense* in a patient with chronic myelogenous leukemia: case report[☆]

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ABSTRACT

Bacteremia due to *Mycobacterium massiliense* is rare. We present here the case of a 58-year-old man with chronic myelogenous leukemia complicated by bacteremia caused by *M. massiliense*. The microorganisms were identified as *M. massiliense* by sequencing analysis, having initially been misdiagnosed as *M. abscessus* by DNA-DNA hybridization.

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1. Introduction

Rapidly growing mycobacteria (RGM) are usually considered to be saprophytes and are widely distributed in the environment, particularly in dust, watery soil, and water-distribution systems (Brown-Elliott and Wallace, 2005; Katoch, 2004). The *Mycobacterium abscessus*–*M. chelonae* group is responsible for one of the commonest RGM infections in humans. However, since the 1980s, *M. abscessus* and *M. chelonae* have generally been regarded as separate species (Brown-Elliott and Wallace, 2005). *M. abscessus* is commonly associated with wound infections and abscess formation, and causes chronic lung disease, often in immunocompromised patients (Han et al., 2007). *M. massiliense* is closely related to *M. abscessus* and was previously indistinguishable from the *M. chelonae*–*M. abscessus* group species by the DNA-DNA hybridization (DDH) method or by partial 16S rRNA gene sequencing (Simmon et al., 2007). We present here a case with chronic myelogenous leukemia that was complicated by bacteremia caused by *M. massiliense*. The microorganisms had initially been misdiagnosed as *M. abscessus* by DDH, but were identified as *M. massiliense* by sequencing analysis. We discuss the importance of sequencing analysis of the *hsp65* and *rpoB* genes and

of the 16S-23S rRNA internal transcribed spacer (ITS) region for diagnosis of *M. massiliense*.

2. Case report

A 58-year-old man was admitted to hospital with abdominal pain. He had no significant history. Clinical examination revealed hepatosplenomegaly. Laboratory findings revealed elevated white blood cells (130,000/ μ L). Chronic myelogenous leukemia in the accelerated phase with a minor bcr-abl fusion gene was diagnosed by bone marrow aspiration examination and fluorescence in situ hybridization analysis. He was treated with the molecular target drugs imatinib and dasatinib, but proved to be resistant. He was admitted to hospital several times for pneumonia caused by *Aspergillus* spp.

After 1 year and 9 months, he had a fever of 39.0 °C with the status of bone marrow suppression (white cell count, 800/ μ L) due to oral administration of busulfan. He was not harboring an indwelling venous catheter or any other vascular device at hospital admission. In the blood culture taken on the day of hospital admission (day 1) and in the smear of the sputum taken on day 9, mycobacteria were detected, but no contamination with other bacteria was observed. As shown in Fig. 1, he was treated with meropenem (MEPM) for 13 days from day 1 because screening for bacteria was not performed on hospital admission. Rifampicin (RFP), ethambutol (EB), and clarithromycin (CAM) were added from day 10 after admission because of the detection of mycobacteria. MEPM was stopped on day 14. Then, on day 16 after hospital admission, amikacin (AMK) was added because of the detection of what was thought to be

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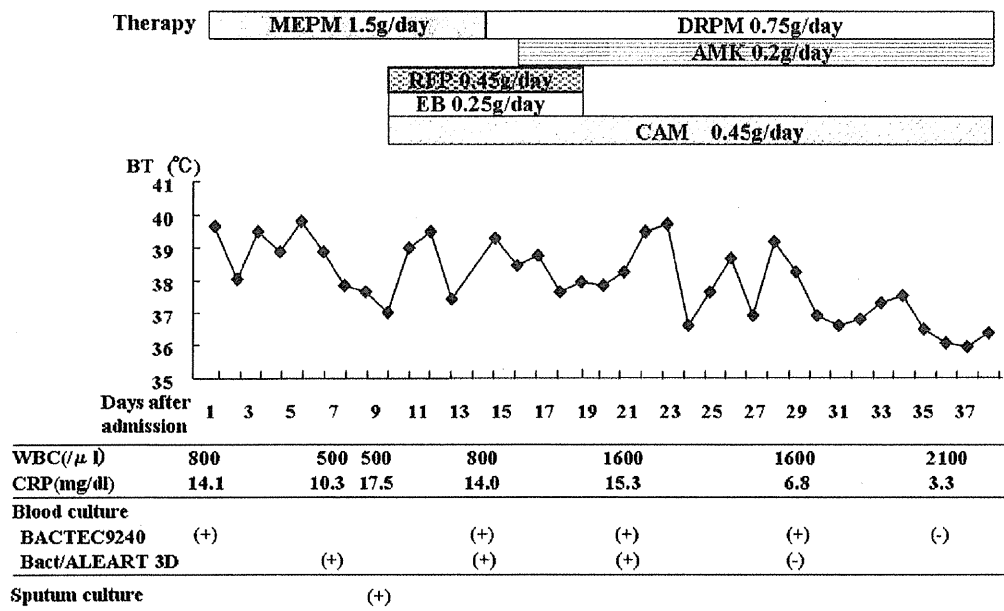


Fig. 1. Clinical course and dynamics of treatment with antibiotics, body temperature, laboratory data (white blood cells [WBC] and C-reactive protein [CRP]), microbiological results for blood culture and sputum, and detection of mycobacteria. MEPM = Meropenem; DRPM = doripenem; AMK = amikacin; RFP = rifampicin; EB = ethambutol; CAM = clarithromycin.

Mycobacterium abscessus (these microorganisms were finally identified as *M. massiliense*) (Fig. 1). His clinical status showed rapid improvement for 3 weeks. Mycobacteria were not detected in blood culture on day 36 after hospital admission.

After 2 years and 1 month from his first hospitalization, he was again admitted to hospital for blood transfusion. A computed tomography scan, however, suggested septic emboli due to fungal infection in the right lower lobe of the lung. Despite treatment with combined antibiotic and antifungal therapy, he died 5 months later due to progressive respiratory failure. During his last hospital admission, mycobacteria were detected in blood cultures on days 1 and 2, because of failure to take his medication before hospital admission, but not in blood culture on day 9.

At autopsy, multiple infections caused by *Aspergillus* spp. were found in the lungs, heart, kidneys, esophagus, brain, and colon, but granulomatous inflammation was not found. *M. massiliense* was not detected in the lung, liver, kidney, heart, spleen, or brain by polymerase chain reaction (data not shown).

3. Materials and methods

Blood cultures were collected on days 1, 7, 14, 21, 29, and 36 of the hospital admission 1 year and 9 months after his first hospitalization.

Blood culturing was performed using a BACTEC 9240 blood culture system (Becton Dickinson, Franklin Lakes, NJ, USA) and/or a Bact/ALEART 3D blood culture system (bioMérieux, Marcy l'Etoile, France). The diagnosis of mycobacteria was performed by DDH (DDH Mycobacteria; Kyokuto Seiyaku, Tokyo, Japan). Furthermore, these microorganisms were examined by sequencing analysis using the *hsp65* (416–816 bp; accession no. M15467) and *rpoB* (1068–1476 bp; accession no. L27989) genes and the 16S–23S rRNA ITS region. A drug-susceptibility test was performed using BrothMIC NTM (Kyokuto Seiyaku, Tokyo, Japan).

4. Results

In the day 1 blood culture, microorganisms were detected only as bacteria staining poorly by Gram stain in the Plus Aerobic/F bottles of the BACTEC 9240 blood culture system after about 5 days of

incubation, and these were positive for acid-fast bacilli, but no contamination with other bacteria was observed. They were misdiagnosed as *M. abscessus* by DDH using a colony taken after 3 days of incubation in isolated 5% sheep blood agar and 2% Ogawa egg slants, and were considered the sole pathogen. However, these microorganisms were finally identified as *M. massiliense* by sequencing analysis using the *hsp65* (416–816 bp) and *rpoB* (1068–1476 bp) genes and the 16S–23S rRNA ITS region. The smear of the sputum collected on day 9 was positive for acid-fast bacilli. A drug-susceptibility test revealed the following susceptibilities of the isolated *M. massiliense* to various drugs, expressed as the MIC: streptomycin, >128 μ g/mL; EB, 64 μ g/mL; kanamycin, 16 μ g/mL; isoniazid, >32 μ g/mL; RFP, >32 μ g/mL; levofloxacin, 16 μ g/mL; CAM, 0.25 μ g/mL; ethionamide, >16 μ g/mL; and AMK, 16 μ g/mL.

5. Discussion

We present here a case with the status of bone marrow suppression in which bacteremia due to *M. massiliense* was detected. In our case, the pathogen associated with the bacteremia could be detected early on as a mycobacterium (indeed, on day 6 of the hospital admission occurring after 1 year and 9 months from his first hospitalization) by careful evaluation of an aerobic culture. Although the mycobacteria were initially misdiagnosed as *M. abscessus* by the DDH method, they were found to be susceptible to CAM and AMK. Therefore, the patient showed rapid improvement. After this, the mycobacteria were finally identified as *M. massiliense* by sequencing analysis of the *hsp65* and *rpoB* genes and of the 16S–23S rRNA ITS region. On the basis of the present case, we can emphasize 2 important points: i) although in routine practice mycobacteria would not be expected to be grown in bacterial cultures, this should be required in immunocompromised patients; and ii) when *M. abscessus* is detected by the DDH method, sequencing of the *hsp65* and *rpoB* genes and of the 16S–23S rRNA ITS region should be performed for the differential diagnosis of such species as *M. massiliense* because of differences in susceptibility to various antibiotics (Nakanaga et al., 2010).

In a case with *M. abscessus*, the clinical outcome is poor because of its resistance to several antibiotics (Griffith et al., 1993). Moreover, a delay in diagnosis may allow the disease to worsen. In the present

case, mycobacteria were not detectable on day 36 of hospital admission because of the early detection of mycobacteria and their susceptibility to the antibiotics used. However, this rapid improvement may have been due to treatment not of *M. abscessus* infection, but of *M. massiliense*. Very recently, Koh et al (2011), who examined 81 patients with *M. massiliense* lung disease and 64 patients with *M. abscessus* lung disease, reported that the proportion of patients with sputum conversion and maintenance of negative sputum cultures was higher in patients with *M. massiliense* infection (88%) than in those with *M. abscessus* infection (25%). Moreover, they found inducible resistance to CAM (MIC, ≥ 32 $\mu\text{g}/\text{mL}$) in all the tested *M. abscessus* isolates ($n = 19$), but in none of the *M. massiliense* isolates ($n = 28$). This report supported our treatment of the present case.

Although the incidence of infection with the *M. abscessus*–*M. chelonae* group is high in the lungs, a few cases have been reported of disseminated infection. Zelazny et al. (2009), who examined 27 cases with *M. abscessus* and 11 cases with *M. massiliense*, reported that pulmonary infection was found in 23 cases (85.2%) of *M. abscessus* infection and in 7 cases (63.6%) of *M. massiliense* infection, while disseminated infection was found in 3 cases (11.1%) of the former infection and 2 cases (18.2%) of the latter. In the present case, the focus of the bacteremia was unclear. However, mycobacteria were detected in sputum on day 7 of hospital admission. In view of the above findings, one candidate for the focus may be the lung.

In conclusion, we report a case with bacteremia due to *M. massiliense*. An etiologic diagnosis of *M. massiliense* could not be

made immediately, and mycobacteria would not be expected to be grown in routine bacterial cultures, even rapidly growing species. In immunocompromised patients, however, careful examination, including for mycobacteria, is needed. Furthermore, accurate diagnosis of mycobacteria needs to be made because of differences in susceptibility to various antibiotics.

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REVIEW ARTICLE

Laboratory procedures for the detection and identification of cutaneous non-tuberculous mycobacterial infections

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ABSTRACT

There is evidence that the incidence of cutaneous non-tuberculous mycobacterial (NTM) infection is increasing worldwide. Novel culture methods and new analytical procedures have led to significant advancements in understanding the origin and progression of NTM infections. Differential identification of NTM isolates is important because culture characteristics and/or sensitivity to anti-mycobacterium drugs vary between different mycobacterial species. In this manuscript, we describe the latest diagnostic techniques for cutaneous NTM infection and show how these methodologies can be used for the diagnosis of Buruli ulcer in Japan.

Key words: 16S rRNA gene, Buruli ulcer, cutaneous infection, non-tuberculous mycobacterial infection, polymerase chain reaction, species identification.

INTRODUCTION

The basis for the increase in the number of cases of cutaneous non-tuberculous mycobacterial (NTM) infection is unknown. It has been attributed to an increase in the total number of these patients or to better detection and/or reporting methods.^{1–5} Insight into the origin and progression of NTM infections has been significantly advanced due to the employment of novel methods for the culture and analysis of NTM. However, the diagnosis of a cutaneous NTM infection in less experienced hospitals is occasionally delayed, or not made at all, because the causative agent can be difficult to isolate due to its variety of growth characteristics. To date, most cutaneous NTM infections have been caused by *Mycobacterium marinum*^{6,7} or by groups of rapidly growing mycobacterial (RGM) species, but sometimes a rare mycobacterium can cause the disease.^{8–10} Different strains of mycobacteria generally exhibit different characteristics in culture and/or sensitivity to anti-mycobacterial drugs. Therefore, accurate identification of the causative agent is important for the treatment of NTM infections. In this manuscript, we describe the latest molecular diagnostic techniques for cutaneous NTM infection and present a case study that used these methodologies for BU diagnosis.

NTM IN DERMATOLOGY

Causative agents of dermatological infections

Approximately 30 mycobacterial species have been identified as causative agents of cutaneous infection (Table 1). These

species of mycobacteria, with the exception of the *Mycobacterium tuberculosis* complex and *Mycobacterium leprae*, are classified as NTM. They are categorized into four groups based on their growth rate (slow growing mycobacterial [SGM] and RGM species) and photochromogenicity. The identification of mycobacterial species is now rapid and relatively simple. Some of the pathogens identified as causative agents are used as a designation of the disease (e.g. cutaneous *Mycobacterium massiliense* infection).

Clinical symptoms of cutaneous NTM infection

A number of cutaneous diseases, such as erythema, nodules, erosion and ulcers, have been attributed to NTM infection. The point of entry is thought to be via minute cutaneous wounds in which bacteria are attached. Although a visceral NTM infection may cause cutaneous infection in immunocompromised patients, it is not yet clear how many bacteria are required for pathogenicity. There is usually only one cutaneous lesion; however, multiple skin lesions have been observed when pathogenic microbes spread through the lymph fluid. Most infected skin lesions are on exposed areas such as the hands, feet or face, supporting the premise that small external wounds are the penetration pathway. For example, in cutaneous *M. marinum* infection, also known as fish tank granuloma, approximately 80% of the patients have eruption(s) in finger, hand and/or wrist joints. Patients do not experience the pain or itching usually associated with eruptions, but inflammation does sometimes lead to tenderness and/or spontaneous pain.^{11,12}

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