TLR2 neutralizing antibody was able to suppress the PGN-mediated induction of HSL mRNA and protein levels (Fig. 4C vs. A and D vs. B, respectively), suggesting that the action of PGN is mediated through TLR2. Although activation of TLR4 by LPS enhanced HSL expression, *M. leprae* infection abolished LPS-induced HSL mRNA and protein levels (Fig. 4G vs. E and H vs. F, respectively). These results suggest that lipolysis is activated by TLR activation. However, infection of live *M. leprae* inhibits the TLR-mediated increase of HSL expression despite the fact that PGN is actually a component of the *M. leprae* cell wall. Therefore, *M. leprae* could potentially activate a hitherto unrecognized TLR-independent pathway that results in inhibition of TLR-mediated HSL activation in order to prevent the degradation of lipids in infected phagosomes.

3.4. Appearance of HSL mRNA in slit-skin smears correlates with clinical course of leprosy

All of the *in vitro* studies described herein indicated that *M. leprae* infection decreases HSL expression, which may correlate with the maintenance of a lipid-rich environment within the phagosome. To examine HSL expression in the skin lesions of leprosy patients, HSL mRNA levels were evaluated in slit-skin smear specimens by RT-PCR analysis. HSL mRNA was not detected in the five lepromatous leprosy (LL) patients nor in four out of seven borderline lepromatous leprosy (BL) patients, but was clearly detected in two of these patients (Fig. 5A, cases 8 and 12). Interestingly, these two cases, whose HSL mRNA levels were clearly detectable, exhibited a 'type 1 lepra reaction (or upgrading reaction)' after treatment (at one year for case 8 and three months for case 12), which is thought to be a cell-mediated, delayed-type of hypersensitivity immune response.

We also analyzed slit-skin smear samples from four patients who received MDT treatment, which consisted of diaphenylsulfone, clofazimine and rifampicin, as per WHO protocol. While HSL mRNA was not detected before treatment, HSL expression was induced (or recovered) after MDT treatment in all four cases (Fig. 5B). These results indicate that HSL expression is significantly suppressed following *M. leprae* infection in LL and BL patients; however, it reappeared in untreated patients who might have a potentially active immune response to *M. leprae* and in those whose bactericidal activity is enhanced by effective treatment.

4. Discussion

In this report, we first demonstrated that *M. leprae* suppresses the expression of HSL mRNA and protein in infected macrophages. Only live *M. leprae* could sustain suppressed levels of HSL, although phagocytosis itself only transiently decreased HSL levels. This situation is quite similar to the induction of lipid droplet-associated proteins, ADRP and perilipin, in macrophages infected with *M. leprae* as previously reported [9]. In that study, only live *M. leprae* infection could induce and maintain high expression levels of ADRP and perilipin. The present study provides another mechanism by

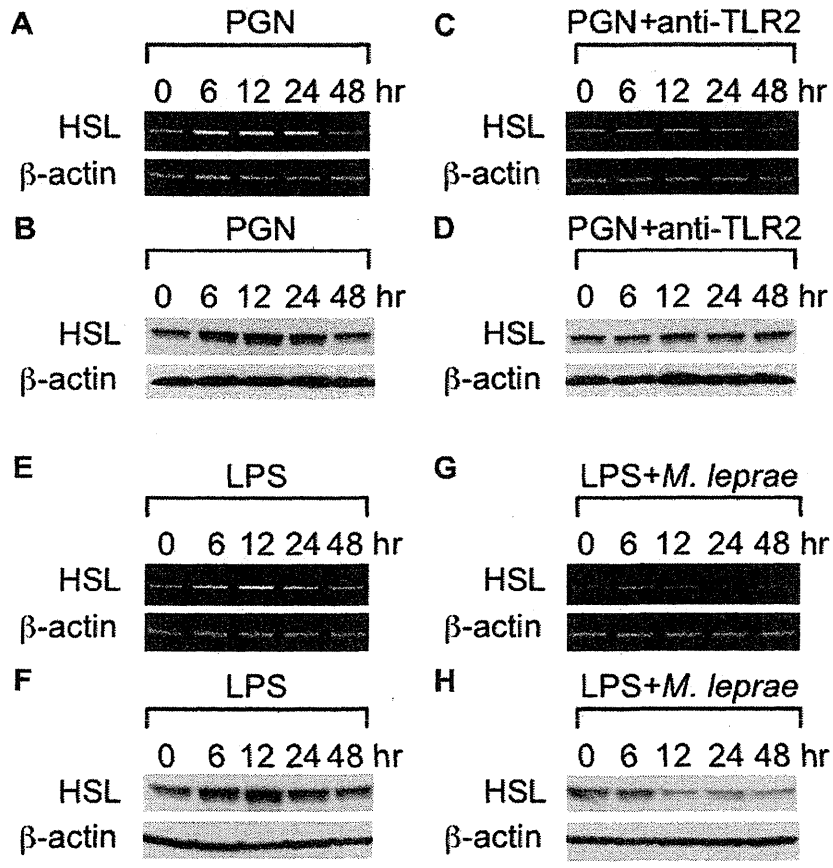

Fig. 4. TLR-mediated increase of HSL expression and its suppression by *M. leprae* infection. THP-1 cells were cultured in a six-well plate and treated with PGN (A and B), PGN plus TLR2 neutralizing antibody (C and D) or LPS (E and F) or LPS plus *M. leprae* (G and H). After incubating for the indicated time, mRNA expression or protein levels of HSL were evaluated by RT-PCR analysis (A, C, E and G) and Western blot analysis (B, D, F and H).

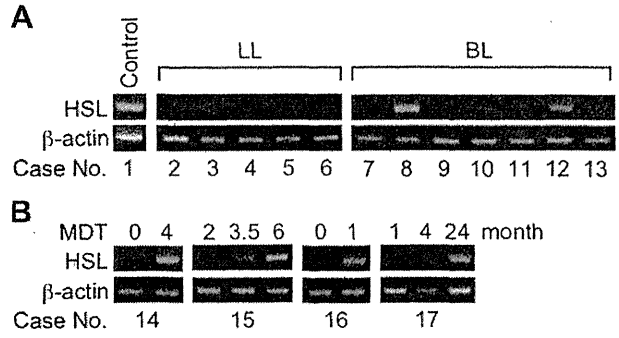

Fig. 5. Detection of HSL mRNA in slit-skin smear samples from leprosy patients. RNA was isolated from slit-skin smear specimens taken from five LL patients and seven BL patients for RT-PCR analysis (A). Cases 8 and 12 developed type 1 lepra reactions after MDT treatment. RNA isolated from four BL patients before and after MDT for the indicated period of time, was also used for RT-PCR analysis (B). The control sample was obtained from patients with skin granulomas where *M. leprae* was not found.

which *M. leprae* maintains host cell lipids, namely by suppressing their degradation.

Activation of the TLR signaling pathway by PGN increased HSL expression, indicating that activation of the innate immune response may induce lipid degradation that makes it difficult for *M. leprae* to survive within infected phagosomes. *M. leprae* infection not only suppressed HSL expression, but also inhibited the ability of PGN to increase HSL expression. We previously showed that PGN suppresses expression of ADRP and perilipin, and also significantly reduces expression of CORO1A, also known as tryptophan aspartate-containing coat protein (TACO), which contributes to the inhibition of lysosomal fusion and accounts for the survival of bacilli [9,16,17]. *M. leprae* infection invalidates all of these effects of PGN and thus maintains levels of CORO1A, ADRP and perilipin, and reduces HSL expression, thus ensuring a favorable phagosome environment for itself. These results showing that *M. leprae* and PGN, a cell wall component of *M. leprae*, have quite different effects are somewhat contradictory. It is plausible to speculate that some *M. leprae* components can activate a pathway that counteracts TLR signaling.

Suppression of HSL, in addition to the induction and phagosomal translocation of ADRP/perilipin and CORO1A, would reduce degradation of stored lipids, thereby maintaining the lipid-rich environment in the parasitized phagosome where *M. leprae* lives. *M. leprae* possesses only a small number of functional genes, which likely makes it difficult for the bacilli to survive without relying on host cell metabolism [14–16,22]. Therefore, *M. leprae* may regulate the expression of host genes that accumulate and maintain cellular lipids in order to utilize them as an essential nutrient for survival. Recent studies suggest that *Mycobacterium tuberculosis (M. tuberculosis)* persists within lipid-rich foamy phagosomes, while its translocation into the cytosol may relate to caseation and virulence [23–27]. Therefore, intracellular lifestyle and lipid requirements might differ substantially between *M. tuberculosis* and *M. leprae*, partly reflecting the massive gene decay in *M. leprae* [22]. Whether the small lipid droplets seen in *M. leprae*-infected cells (Fig. 1B) fuse with phagosomes containing *M. leprae* is still to be determined.

There was once debate over whether the lipids originate from *M. leprae* cell wall components or from the host [28,29]. It was suggested that lipids and fatty acids were important carbon sources for *M. leprae* in infected macrophages where the oxygen tension gradient is low [30]. It is now known that mycobacteria induce the accumulation of 1-palmitoyl-2-(5,6-epoxyisoprostane E2)-sn-glycero-3-phosphorylcholine (PEIPC), a host-derived oxidized phospholipid, and is similar to the formation of foamy cells found in atherosclerotic lesions [31,32]. Of interest, *M. tuberculosis* has a large number of proteins involved in lipid metabolism, including at least one HSL family protein. However, *M. leprae* seems to have a small number of such genes, and no HSL-like genes were identified (http://genolist.pasteur.fr/Leproma/).

The lipid degradation process in adipocytes involves both HSL and perilipin [33]. Both are polyphosphorylated by protein kinase A (PKA), and phosphorylation of perilipin is required for the translocation of HSL from the cytosol to the surface of the lipid droplet, which is a critical step in the lipolytic reaction [34]. Furthermore, there is growing evidence that both perilipin and comparative gene identification-58 (CGI-58) protein act as scaffold proteins on lipid droplets in adipocytes [35]. We demonstrated that live *M. leprae* not only suppresses HSL protein expression, but that it also phosphorylates two serine residues, Ser^{563} and Ser^{565}, which are essential for its action. HSL expression is modulated by energy level changes in a variety of situations, such as obesity [36], type 2 diabetes mellitus [37] and in cultured adipocytes [38,39]. However, there have been no reports that pathogenic microorganisms have the ability to modulate HSL expression. Since *M. tuberculosis* and *Mycobacterium avium* utilize host lipids [40,41], our finding may highlight an important mechanism by which these bacteria interact and modify host gene expression. Phosphorylation of HSL occurs at multiple sites, including Ser^{563}, which is believed to be mutually exclusive with phosphorylation of HSL at the non-PKA site Ser^{565} [19,20]. Therefore, the observed decrease of HSL phosphorylation might be due to down-regulation of PKA or induction of a non-functional kinase following infection with *M. leprae*.

HSL expression was not detected in slit-skin smear samples from any of the LL patients and most of the BL patients examined in this study. Two BL patients who showed detectable levels of HSL mRNA developed a type 1 lepra (or reversal) reaction, which is thought to be a Th1-type cellular immune response and is characterized by an acute inflammatory reaction that causes worsening of skin lesions, neuritis and other systemic complications that occur in patients who are immunologically unstable [42]. Therefore, the increase in HSL expression detected in untreated BL patients is potentially activation of an immune reaction. The lepra reaction is one of the major problems faced by clinicians during treatment, and there is currently no method of predicting this critical side effect of treatment. Our observation suggests that detecting HSL mRNA in slit-skin smear samples from untreated patients could potentially be a reliable, convenient and minimally invasive procedure by which to predict possible occurrence of the type 1 lepra reaction. In addition, MDT treatment results in a rapid induction of HSL expression. Consequently, HSL expression levels after MDT treatment might also be a marker for treatment efficacy without the need for complicated tests to evaluate drug resistance of the bacilli. Although early diagnosis and appropriate treatment provide a complete cure, delays in diagnosis and treatment result in severe deformities and disabilities. Therefore, evaluation of HSL levels from slit-skin smear samples may be a simple and accurate method for clinical examination. In both cases, however, analysis of more clinical samples is needed to validate the clinical usefulness of this metric.

In conclusion, we have shown that *M. leprae* infection suppresses host HSL expression, which helps to retain the lipid-rich environment necessary for the survival of the pathogen within the phagosome. In addition, the measurement of HSL expression from slit-skin smears may be a useful diagnostic tool for patient prognosis.

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REVIEW ARTICLE

Buruli ulcer and current situation in Japan: A new emerging cutaneous *Mycobacterium* infection

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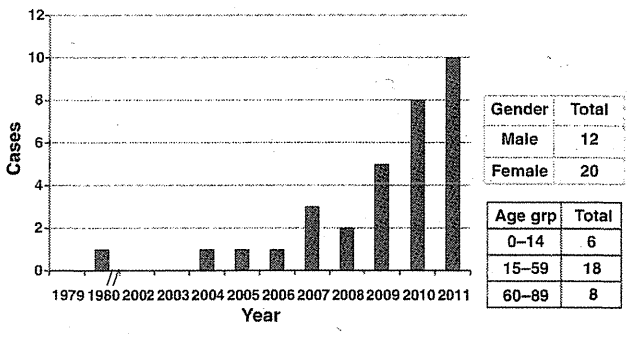
parts of the world, at a rate of over 5000 new cases per year.[1,2] However, diversity in the incidence rate according to regions and lack of awareness prevents accurate sketching of the demographic of BU.

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 Mikoshiba *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 subspecies 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.



Gender	Total
Male	12
Female	20

Age grp	Total
0–14	6
15–59	18
60–89	8

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

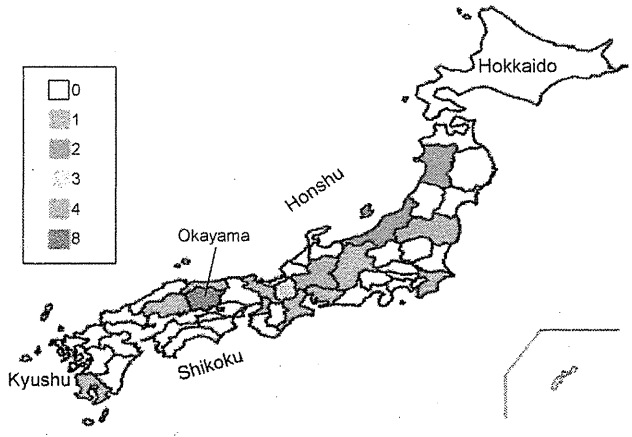


Figure 2. Distribution of Buruli ulcer cases in Japan: a total of 32 cases as of October 2011.

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 pathogenecity.[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.

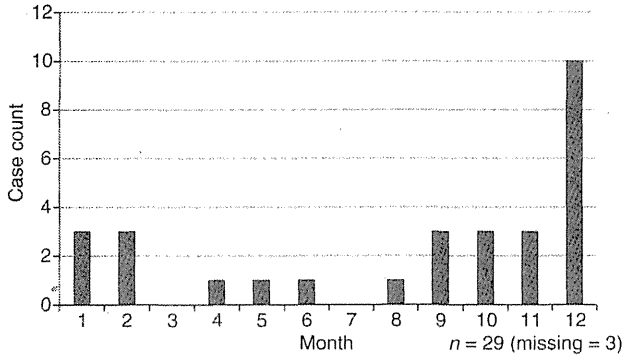


Figure 3. Month diagnosed with Buruli ulcer.

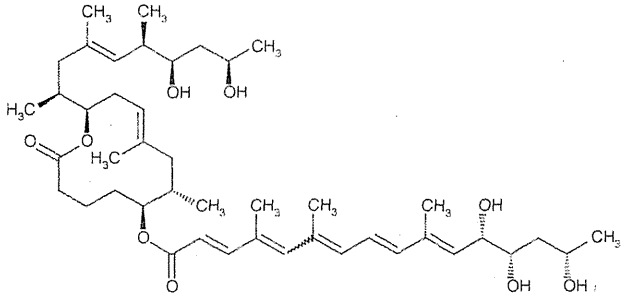


Figure 4. Mycolactone A/B.

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

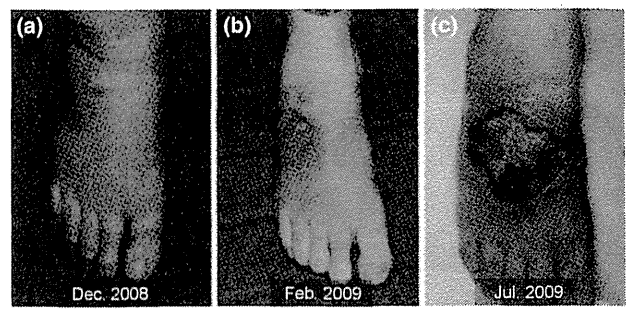


Figure 5. Clinical features. (a) Initial symptoms of Buruli ulcer. It often starts as erythema or papule. (b) The lesion gradually develops into painless nodule measuring few centimeters. The case in the photo is associated with redness and swelling. (c) In a few days to several weeks, the papule or 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 Tesshin 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 IS*2404* (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

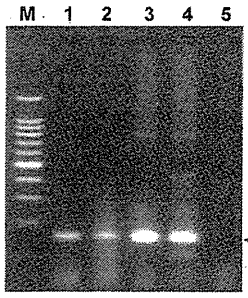


Figure 6. Detection of IS*2404* by polymerase chain reaction.

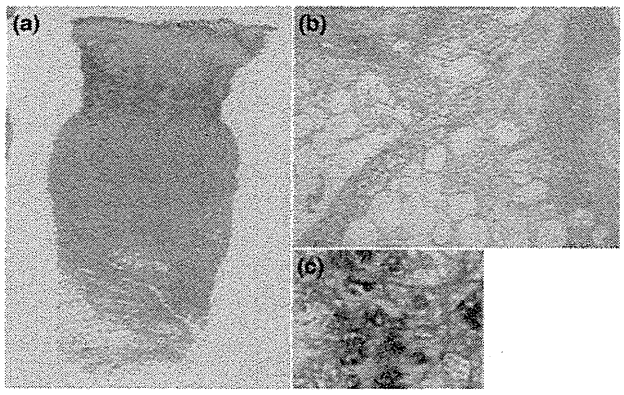


Figure 7. Histopathology. (a) Hematoxylin–eosin stain presents necrotic signs of the dermis, adipose tissue and occasional extension to the fascia. Granulomas or epithelioid cells are rare, as with caseous necrosis (original magnification ×40). (b) Infiltration of lymphocytes in the dermis and adipose tissue is relatively poor (original magnification ×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 ×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 IS*2404*
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 ulcer[27]

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

reactions". 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, postoperative 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 administration 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. *shinshuense* 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 (*M. u*), positive (*M. u* ssp. *s*)
Niacin accumulation	Negative
Toxin	Mycolactone
IS*2404* (PCR)	Positive
M. marinum in DDH†	Positive (misidentification)

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

Table 4. Characteristics of cases reported in Japan

Known isolate	*Mycobacterium ulcerans* ssp. *shinshuense*
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 likeliness 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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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 acanthothic 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 $Gsdma3^{I359N}$ 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:
Leprosy;
Drug resistance;
New registered cases;
Relapsed cases;
Dapsone;
Rifampicin

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. laprae* 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	*fol P (dapsone)*	*rpo B* (RFP)	*gyr A (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]	*folP1* (dapsone)
2	Korea	69	F	BL	*folP1* (dapsone)
3	Philippines	24	M	LL[b]	*gyrA* (quinolones)

[a] BL, borderline lepromatous leprosy.
[b] LL, lepromatous leprosy.

Table 2A
PCR results of relapsed leprosy patients. The mutations detected on *fol P1*, *ropB*, *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 (*folP1*)	2	9.1
RFP (*rpoB*)	2	9.1
Dapsone and quinolones (*folP1* and gyrA)	2	9.1
Dapsone and RFP (*folP1* and *rpoB*)	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 *gyaA* 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	*folP1*	+	–	–
2	69	F	LL	*folP1*	+	+	–
3	77	M	LL	*rpoB*	+	–	+
4	72	M	LL	*rpoB*	+	+	–
5	71	M	LL	*folP1* and *gyrA*	+	+	–
6	77	F	LL	*folP1* and *gyrA*	+	–	–
7	72	M	LL	*folP1* and *rpoB*	+	+	–

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 $Mitf^{mi\text{-}ew}$ 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 $Mitf^{mi\text{-}ew}$ (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

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ハンセン病の基礎医学分野における日韓協力について

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キーワード：基礎研究、日韓協力、ハンセン病

近年の日本におけるハンセン病新患の発見は、著しく減少し、2009年及び2010年の日本人からの新患発生は報告されていない。このような減少は韓国においても同様で、WHOへの報告に基づくと、同国の新患は2006年15例、2007年12例、2008年7例、2009年5例、2010年6例と極めて少数の報告にとどまっている。このようなハンセン病の現状を反映し、日本においてハンセン病に関する基礎研究を実施している研究機関、あるいは研究者は減少の一途をたどっており、研究体制に関しては厳しい状況にある。また日本以外の国々においても同様の問題が生じている。一方、世界に目を向けると、未だ多くの患者の発生がみられる国々が多数あり、そこには科学的根拠に基づいた手段により解決されるべき多くの課題が残されている。日本においてはハンセン病研究センターを中心として、研究が行われており、10余名の研究者がハンセン病に関する研究を継続している。あわせて、これまでに蓄積されてきた多くの知識と資源が維持されている。一方、韓国においてはカソリック医科大学のProfessor Gue-Tae Chaeあるはヨンセイ大学のProfessor Sang-Nae Choらが精力的に研究を継続している。このような現状において、より良いハンセン病対策構築のために日韓の研究者が協力して行うべき研究テーマは多々存在する。これまで両国間においては個人的な関係に基づいて研究資料の相互の提供等の共同研究が実施されてはきたものの、組織だった研究協力体制の構築には到っていない。シンポジュウムにおいては、日本において行われているハンセン病に関する基礎研究を紹介し、将来の共同研究の構築に向けて、その可能性と手段について考察した。

以下に日本において行われている基礎研究課題を列挙する。

1、薬剤耐性：これまでに報告された変異以外の薬剤耐性惹起変異の検索。研究に資するらい菌株の確立。PCR direct sequencing以外の方法による耐性菌の検出法の改良、開発。WHOによる薬剤耐性監視事業。耐性菌伝播状況調査に関する流行国との2国間協力。

2、ワクチン：らい菌抑制効果の改良。マウスfootpad法によるワクチン候補のスクリーニング。ワクチン効果発現の免疫学的解析。

3、診断方法：MMP-IIの抗体価測定における有用性の検証と流行地域への適用。Interferon-γアッセイの診断への応用。LAMP法の臨床サンプルへの適用。臨床検体の採取、保存方法の改良。

4、らい菌の病原性：神経障害発機序の解明のためのシュワン細胞の関与。低至適増殖温度のメカニズム。代謝機構の解析。細胞内増殖における脂質の利用。マクロファージ内増殖機構の解析。マクロファージ内におけるClofazimineの殺

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菌作用の解析。

5、遺伝子工学：蛍光蛋白質発現らい菌の開発。ワクチン効果増強のためのBCGの改変。マウスfootpad法に替わる*in vitro*薬剤感受性試験法の開発。

6、分子疫学：確立されたらい菌株を用いた全ゲノム解析。病原性にかかわる遺伝子あるいはgenotypingに有用である遺伝子領域の特定。感染様式並びに感染源の解明。墳墓中の資料からのらい菌遺伝子検出。

7、培養：人工培養条件の開発。培養細胞の利用。供試らい菌の活性。

以上、日本において実施されている研究について要約したが、ハンセン病対策上科学的に解決されなければならない、あるいは一層の研究が必要な課題は今もって多く残されている。例えば、宿主については、1、再発のメカニズム、再燃あるいは再感染の解明。2、再燃の予測。3、神経障害発生機序。4、感受性を規定する遺伝的背景。5、感染様式。6、LL型における免疫不全機構。7、LL型を発症するであろう個体に対し、真に有効なワクチン。8、抜本的感染防止策の構築。9、これまでに知られている動物以外の自然感染症例の有無。9、Lucio現象の解明。一方、らい菌については1、患者以外に存在し、感染源となる可能性。2、特異性と感受性を有する診断法に有用な抗原。3、病原性に関わる遺伝子の有無。4、薬剤耐性菌の伝播状況の把握。5、人工培養の開発。6、genotypingの改良。7、神経親和性、低温増殖性のメカニズム。等々が挙げられる。

列記した課題について、日韓の研究者が共同で当たるにはどのような方策があるのかを考えると、現状はかなり厳しい状況にあるといわざるを得ない。しかしながら、日韓の間には既に、Korea-Japan International Symposium on Microbiology等の意見交換の場がある。我々ハンセン病研究に携わる者が、このような機会を利用してお互いの意見交換から始めることも将来の上記課題に対しての定期的意見交換あるいは共同研究体制の発展につながる方策であると考える。双方の若手研究者の受け入れ、お互いのハンセン病学会への参加も提案された。

シンポジュウムにおいては具体的な事業の開始について結論を得ることは無かったものの、相互協力について協議するワーキンググループを設置することが提案され、賛同を得た。

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Collaboration between Korea and Japan for basic research on leprosy

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New case detection in Japan has been markedly decreased and same trends have been also shown in Korea. Despite of unfavorable circumstances, research activities are still continuing and we have the accumulation of knowledge on leprosy both in Japan and Korea.

Following basic studies for leprosy on going in Japan were reviewed. 1. Analysis of drug resistance mechanism and its application for clinical samples. 2. Establishment of early diagnostic technique. 3. Clarification of mechanisms of neuropathy. 4. Analysis of *in vivo* growth mechanisms of *Mycobacterium leprae*. 5. Molecular epidemiology of leprosy. 6. Searching for new anti leprosy drugs. 7. Developing vaccine. 8. *In vitro* cultivation. Other subjects as follows was proposed as prospective studies. 1. Mechanisms of relapse. 2. Establishing diagnostic tool of reaction and preventive measures. 3. Clarification of immunological mechanisms of anergy in LL case.

The possibility of future collaboration between Korea and Japan to solve remaining problems in the clinical field was discussed and a course of action for collaboration was deliberated.

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2011年における世界のハンセン病の現況について

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世界のハンセン病の疫学は各国の保健担当の部署から世界保健機関（WHO）に報告される。報告されたデータはWHOによってまとめられ、速報的に週間疫学記録（weekly epidemiological record: WER）に掲載される。2011年初頭のデータが2011年9月にWERに掲載された（WER (No36) 86: 389-399, 2011）。現在、WHOにより行われている「ハンセン病による負荷のさらなる軽減のための強化された世界戦略（2011年－2015年）」は新規患者における第2級障害の患者の減少を指標として遂行されている。国内や国際機関の協力による継続的なハンセン病制圧計画の結果、ハンセン病の発生は世界的に減少している。今後は合併症や後遺症の管理を向上させ、そして地域の病気に対する意識を高めることによって人々が病気の初期に診断に訪れるようにし、ハンセン病による負荷を減少させることが必要である事などが述べられている。

2011年の世界ハンセン病状況

WHOにより行われている「ハンセン病による負荷のさらなる軽減のための強化された世界戦略（2011年－2015年）」[1]は現在、ハンセン病が流行する国々のハンセン病制圧計画により各国に適合され実行されている。この戦略の目的は第2級障害を伴う新患数を2010年末に比して2015年末までに35%以下にすることにある。

本戦略では世界的な目標を、第2級障害（外観的に障害の分かる症例）を有する新患の発生を減少させることに設定することにより、ハンセン病に対する診断の遅れを減少させ、MDTによる迅速な治療を行うことに拍車をかけることが期待される。これらの活動は新患の発生を減少させ、結果として一般社会へのハンセン病の伝染を減少させることが期待される。

この目標には国家によるハンセン病政策の維持と本病のさらなる減少に向けた進展のモニターが期待されている。それはさらにGOとNGO、ハンセン病に罹患した人々および回復した人々と彼らの住む社会とのパートナーシップを通じた長期間

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2）Reports from the European Region were not available.

の責務履行の維持を促進することが期待されている。

2011年、第1四半期の終わりまでに、130の国と区域がWHOへハンセン病の現況報告を行った。内訳は、アフリカ地域36、アメリカ地域27、南東アジア地域10、東地中海地域の22、西太平洋地域の35である[2]。登録された有病率や新患発生率、新患中の第2級障害者の比率などの指標の計算には、国連人口部門による2010年の人口データを用いた[3]。

Table 1は130の国と地域から報告された2010年の世界における新規ハンセン病患者数と有病率である。2010年に発見された新患数は228,474人であり、2010年初頭の登録患者数は192,246人であった。

Table 2は2004 − 2010年におけるWHO地域別新患数を表している。2004 − 2010年においては東地中海地域を除く全地域で患者数減少は毎年続いている。東地中海地域の南スーダンではハンセン病制圧計画の展開地域の拡大に伴い、より良いサービスが提供されるようになったことで以前より新患数が増加した。

Table 3は2010年に1,000人以上の新規患者数が報告された17カ国について2004年からの新患数を示した。これらの17カ国は2010年の世界新患数の95%を占めた。

Table 4は新患数が年間100人以上の国を対象として、WHO地域ごとの新規患者について多菌型患者（multibacillary: MB）の割合、および女性、15才未満の小児、第2級障害者の割合のそれぞれ最高と最低の国々を示す。

新規患者中のMB患者の割合はアフリカ地域で

Table 1. Registered prevalence of leprosy and number of new cases detected in 130 countries or territories, by WHO region, 2010 and end of first quarter 2011

WHO region[a]	No. of cases registered (prevalence/ 10 000 population), first quarter of 2011	No. of new cases detected (new-case detection rate/100 000 population), 2010
African	27 111 (0.38)	25 345 (3.53)
Americas	33 953 (0.38)	37 740 (4.25)
South-East Asia	113 750 (0.64)	156 254 (8.77)
Eastern Mediterranean	9 046 (0.17)	4 080 (0.67)
Western Pacific	8 386 (0.05)	5 055 (0.28)
Total	**192 246 (0.34)**	**228 474 (3.93)**

[a] Reports from the European Region were not available.

Table 2. Trends in the detection of new cases of leprosy, by WHO region, 2004-2010

WHO region[a]	No. of new cases detected						
	2004	2005	2006	2007	2008	2009	2010
African	46 918	45 179	34 480	34 468	29 814	28 935	25 345
Americas	52 662	41 952	47 612	42 135	41 891	40 474	37 740
South-East Asia	298 603	201 635	174 118	171 576	167 505	166 115	156 254
Eastern Mediterranean	3 392	3 133	3 261	4 091	3 938	4 029	4 080
Western Pacific	6 216	7 137	6 190	5 863	5 859	5 243	5 055
Total	**407 791**	**299 036**	**265 661**	**258 133**	**249 007**	**244 796**	**228 474**

[a] Reports from the European Region were not available.

[3] *World population prospects: the 2006 revision*, Vol. 1. *Comprehensive tables*. New York, United Nations, 2007:578–586.

[4] This report includes cases from both Sudan and South Sudan because the data were submitted before the separation took place.

は、コンゴの61.72%からケニアの99.21%まで及ぶ。アメリカ地域では、この割合はブラジルの40.88%からキューバの83.06%まで、南東アジア地域ではバングラデシュの42.33%からインドネシアの80.96%まで、東地中海地域ではイエメンの61.95%からエジプトの88.38%まで、西太平洋地域では、キリバスの29.67%からフィリピンの93.92%までに及ぶ。

新規患者中で女性が占める割合は、アフリカ地域ではマリの20.11%からブルキナファソの

Table 3. Trends in the detection of leprosy in 17 countries reporting≥1000 new cases during 2010, and number of new cases detected annually since 2004

Country	No. of new cases detected						
	2004	2005	2006	2007	2008	2009	2010
Angola	2 109	1 877	1 078	1 269	1 184	937	1 076
Bangladesh	8 242	7 882	6 280	5 357	5 249	5 239	3 848
Brazil	49 384	38 410	44 436	39 125	38 914	37 610	34 894
China	1 499	1 658	1 506	1 526	1 614	1 597	1 324
Democratic Republic of the Congo	11 781	10 369	8 257	8 820	6 114	5 062	5 049
India	260 063	169 709	139 252	137 685	134 184	133 717	126 800
Ethiopia	4 787	4 698	4 092	4 187	4 170	4 417	4 430
Indonesia	16 549	19 695	17 682	17 723	17 441	17 260	17 012
Madagascar	3 710	2 709	1 536	1 644	1 763	1 572	1 520
Mozambique	4 266	5 371	3 637	2 510	1 313	1 191	1 207
Myanmar	3 748	3 571	3 721	3 637	3 365	3 147	2 936
Nepal	6 958	6 150	4 235	4 436[a]	4 708[a]	4 394[a]	3 118[a]
Nigeria	5 276	5 024	3 544	4 665	4 899	4 219	3 913
Philippines	2 254	3 130	2 517	2 514	2 373	1 795	2 041
Sri Lanka	1 995	1 924	1 993	2 024	1 979	1 875	2 027
Sudan	722	720	884	1 706[b]	1 901[b]	2 100[b]	2 394[b]
United Republic of Tanzania	5 190	4 237	3 450	3 105	3 276	2 654	2 349
Total (%)	**388 533 (95)**	**287 134 (96)**	**248 100 (93)**	**241 933 (94)**	**234 447 (94)**	**228 786 (93)**	**215 938 (95)**
Global Total	**407 791**	**299 036**	**265 661**	**258 133**	**249 007**	**244 796**	**228 474**

[a] New cases detected from mid-November 2009 to mid-November 2010.
[b] The number of cases for 2007–2010 includes data from southern Sudan.

Table 4. Profile of newly detected cases reported by countries with ≥100 new cases, by countries with highest and lowest proportions, and WHO region, 2010

WHO region[a]	% cases of multibacillary leprosy among new cases	% of females among new cases of leprosy	% of children among new cases of leprosy	% of new leprosy cases with grade-2 disabilities
African	Democratic Republic of the Congo, 61.72; Kenya, 99.21	Mali, 20.11; Burkina Faso, 48.44	Niger, 1.34; Liberia, 17.43	Cameroon, 4.89; Madagascar, 21.64
Americas	Brazil, 40.88; Cuba, 83.06	Argentina, 24.86; Dominican Republic, 46.53	Argentina, 0.85; Dominican Republic, 16.67	Bolivia, 3.23; Paraguay, 13.01
South-East Asia	Bangladesh, 42.33; Indonesia, 80.96	Myanmar, 33.24; Sri Lanka, 44.35	Bangladesh, 5.46; Indonesia, 11.20	Nepal, 2.82; Thailand, 14.81
Eastern Mediterranean	Yemen, 61.95; Egypt, 88.38	Egypt, 35.74; Sudan, 42.94	Pakistan, 6.06; Yemen, 18.29	Yemen, 7.37; Sudan, 22.81
Western Pacific	Kiribati, 29.67; Philippines, 93.92	Marshall Islands, 13.64; Kiribati, 45.60	China, 2.95; Marshall Islands, 44.55	Marshall Islands, 0; China, 22.51

[a] Reports from the European Region were not available.

48.44%まで、アメリカ地域では、アルゼンチンの24.86%からドミニカ共和国の46.53%まで、南東アジア地域では、ミャンマーの33.24%からスリランカの44.35%まで、東地中海地域ではエジプトの35.74%からスーダンの42.94%まで、西太平洋地域ではマーシャル諸島の13.64%からキリバスの45.60%までである。

新規患者中の子供の割合は、アフリカ地域ではニジェールの1.34%からリベリアの17.43%まで、アメリカ地域ではアルゼンチンの0.85%からドミニカ共和国の16.67%まで、南東アジア地域では、バングラデシュの5.46%からインドネシアの11.20%まで、東地中海地域ではパキスタンの6.06%からイエメンの18.29%まで、西太平洋地域では中国の2.95%からマーシャル諸島の44.55%までである。

新規患者中の第2級障害者数の割合は、アフリカ地域ではカメルーンの4.89%からマダガスカルの21.64%まで、アメリカ地域ではボリビアの3.23%からパラグアイの13.01%まで、南東アジア地域ではネパールの2.82%からタイの14.81%まで、東地中海地域ではイエメンの7.37%からスーダンの22.81%まで、西太平洋地域ではマーシャル諸島の0%から中国の22.51%に及ぶ。

Table 5は2005から2010年における新規患者中の第2級障害者数と人口10万人あたりの割合の動向を表している。世界的には新規患者中における第2級障害者の割合は人口10万人あたり0.23であった。世界的には2010年における新患で第2級障害を伴う患者数は13，000人以上であった。2010年には人口10万人あたりの第2級障害罹患率は西太平洋地域の0.03からアフリカ地域の0.40まで開きがある。

Table 6は2004－2010年の間で全世界での再発症例数の推移を示したものである。2010年の再発症例数は2009年よりわずかに少なくなっているが、それは2010年にブラジルが再発症例数を報告していないからである。

Table 7は130の国と地域における2011年第1四半期末のハンセン病登録患者数、2010年に

Table 5. Number of cases of leprosy (rate/100 000 population) with grade-2 disabilities detected among new cases, by WHO region, 2005-2010

WHO region[a]	Year[b]					
	2005	2006	2007	2008	2009	2010
African	4 562 (0.62)	3 244 (0.46)	3 570 (0.51)	3 458 (0.51)	3 146 (0.41)	2 685 (0.40)
Americas	2 107 (0.25)	2 302 (0.27)	3 431 (0.42)	2 512 (0.29)	2 645 (0.30)	2 423 (0.27)
South-East Asia	6 209 (0.37)	5 791 (0.35)	6 332 (0.37)	6 891 (0.39)	7 286 (0.41)	6 912 (0.39)
Eastern Mediterranean	335 (0.07)	384 (0.08)	466 (0.10)	687 (0.14)	608 (0.11)	729 (0.12)
Western Pacific	673 (0.04)	671 (0.04)	604 (0.03)	592 (0.03)	635 (0.04)	526 (0.03)
Total	**13 886 (0.25)**	**12 392 (0.23)**	**14 403 (0.26)**	**14 140 (0.25)**	**14 320 (0.25)**	**13 275 (0.23)**

[a] Reports from the European Region were not available.
[b] Values are numbers (rate/100 000 population).

Table 6. Number of relapsed cases of leprosy worldwide, 2004-2010

Year	No. of countries reporting	No. of relapsed cases of leprosy
2004	40	2 439
2005	44	2 783
2006	41	2 270
2007	43	2 466
2008	49	2 985
2009	122	3 120
2010	117	2 113