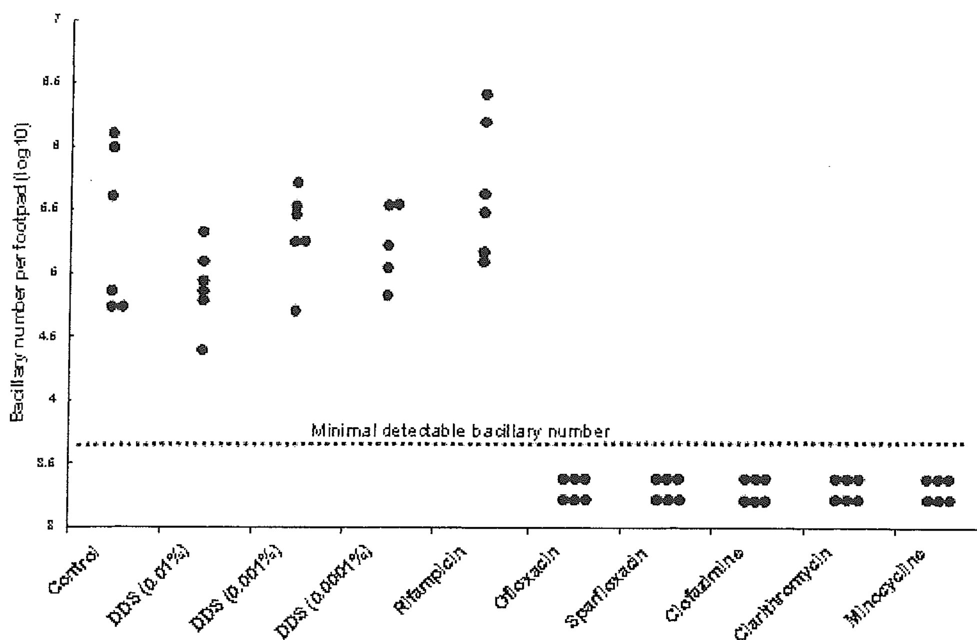


FIG. 1. Bacillary growth in footpads of mice administered anti-leprosy drugs.

0.01% rifampin, 0.01%, 0.001% and 0.0001% dapsone showed almost the same level of growth as observed in the control mice. No bacillary growth was noticed in footpads of mice treated with ofloxacin, sparfloxacin, clofazimine, clarithromycin, and minocycline (Fig. 1). According to the results of this mouse footpad assay, Kusastsu-6 was concluded to be resistant to rifampin and dapsone at high concentration, but susceptible to the other drugs mentioned above.

**Genetic analysis.** The expected PCR products of the *folP*, *rpoB* and *gyrA* gene was successfully obtained from Kusatsu-6. The sequencing results displayed a missense mutation in the *rpoB* gene, affecting the codon at position 516 (numbering system applied for *E.coli*), GAT → TAT, leading to an amino acid substitution, Asp → Tyr, simplified as Asp-516-Tyr (Fig. 2). Similarly, a missense mutation at codon 55 (CCC→CTC, Pro-55-Leu) in the *folP* gene was revealed. No mutation was found at codon 89 or 91 in the *gyrA* gene.

## DISCUSSION

Single point mutations within an 81-bp region in the *rpoB* gene involving 5 codons, Gly-513, Asp-516, His-526, Ser-531 and Leu-533 have been proved to lead to rifampin resistance in *Mycobacterium tuberculosis* ([16, 20]). The deduced amino acid sequence of this region presented 100% identity to that in *M. leprae* ([22]). According to the highly conserved nature of this region in the *rpoB* gene, six distinct mutations affecting 4 codons (Gly-513, His-526, Ser-531 and Leu-533) within this region carrying resistance to rifampin have been already clarified in *M. leprae* ([1, 5, 11, 13, 22]). Nevertheless, until now there has been direct evidence to explain whether the mutation at codon Asp-516 in the *rpoB* in *M. leprae* is linked to rifampin resistance, even if in *Mycobacterium tuberculosis* mutation at this codon Asp-516 confers rifampin resistance. To our knowledge, the present study is the first report to elucidate that this mutation at codon 516 is responsible for rifampin resistance in *M. leprae*.

As we know, the standard mouse footpad assay, which has been employed for drug resistance testing in leprosy for 40 yrs, requires not only long periods of at least 12 months to get results, but also requires considerable facilities, expertise and rigorous restrictions on the conditions of the *M. leprae* examined ([7, 8]). Therefore, it is necessary and urgent to establish rapid and routinely applicable approaches for the detection of drug resistance in leprosy. Recently, the characterization of the mutations at codons Gly-513, His-526, Ser-531 and Leu-533

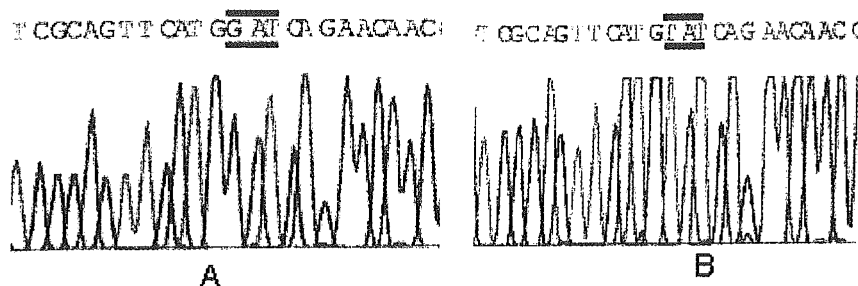


FIG. 2. rpoB sequencing result. (A) codon 516 GAT for amino acid Asp in wild type *M. leprae*. (B) codon 516 TAT for the substituted amino acid Tyr in Kusatsu-6.

have been used to set up simple and feasible alternative protocols. PCR-single strand conformation polymorphism (PCR-SSCP) ([6]), PCR-heteroduplex formation assay (PCR-HDF) ([22]), solid-phase hybridization to oligonucleotide capture probes ([7]) and Touch-Down PCR ([10]) have yielded satisfactory preliminary evaluations in leprosy. On the other hand, a comprehensive understanding of mutations in the *rpoB* gene correlated with rifampin resistance enhances the reliability and integrity of promising methods for drug susceptibility testing. This novel profile of the mutation at codon Asp-516 contributes to data on mutation patterns of rifampin resistant *M. leprae*, and is certainly worthwhile in the determination of drug susceptibility testing in leprosy. The multiplication of Kusatsu-6 in mouse footpads treated by rifampin indicated the full concordance with the missense mutation of Asp-516-Tyr in the *rpoB* gene.

In addition, the molecular mechanisms of dapsone- and quinolone-resistant *M. leprae* have been adequately described so far. Dapsone-resistant relevant mutations are limited at condons 53 and 55 in the *folP* gene ([9, 21]) whereas mutations reflecting resistance to quinolone affect codon 89 and 91 in the *gyrA* gene ([11]). Our results clearly demonstrated that the dapsone-resistant *M. leprae* harbored a missense mutation at codon 55 (CCC→CTC, Pro-55-Leu) in the *folP* gene. Mutations in the *folP* gene are commonly detected among Japanese relapsed leprosy cases because MDT was not applied to them until 2000, as we discussed previously ([13]). In spite of this, the lack of bacillary growth in the mice footpads administered ofloxacin and sparfoxacin was identical to the result of the *gyrA* sequencing. All the results presented complete agreement between *in vivo* susceptibility and genetic tests.

In conclusion, the verification of the mutation at condon Asp-516 in the *rpoB* gene is involved in rifampin resistance in *M. leprae*. This finding offers valuable information for molecular drug susceptibility testing in leprosy and hopefully will help to provide a useful tool for the further successful control of leprosy all over the world.

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# Polymorphism in the *rpoT* gene in *Mycobacterium leprae* isolates obtained from Latin American countries and its possible correlation with the spread of leprosy

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**Abstract**

The genotypes of *Mycobacterium leprae* isolates originating from Mexico, Peru and Paraguay were analysed for the polymorphism of short tandem repeats in the *rpoT* gene. The genotype with four copies of the six-base tandem repeats in the *rpoT* gene was prominently predominant in Mexico, but the genotype of all isolates from Peru and Paraguay contained three copies of the six-base tandem repeats. These obvious different distributions might reflect the spread of leprosy by the different strains of *M. leprae* harboured by the various human races that moved to the American continent, as has been demonstrated in other infectious diseases.
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*Keywords:* Leprosy; *Mycobacterium leprae*; *rpoT* gene; Genotyping; Latin American countries

## 1. Introduction

Genotyping of causative agents of infectious diseases is essential for epidemiological analysis of transmission. Until recently, it was believed that there is no genomic diversity among *Mycobacterium leprae* isolates, a useful feature for the analysis of leprosy transmission. However, in 2000, two independent studies reported some genomic polymorphisms suitable for genotyping of *M. leprae* [1,2]. Since then other polymorphic genomic regions which might be applicable for genotyping have been also revealed [3]. One of them is the different copy number of six-base tandem repeats in the *rpoT* gene of *M. leprae* [1]. Specifically, *M. leprae* isolates can be divided into two sub-genotypes based on the polymorphism in the *rpoT* gene, which contains either four or three copies of a six-base tandem repeat. Our previous study showed the prominent distribution of the genotype with four copies of the six-base tandem repeats in the *rpoT* gene in the East Asia, Korea and the main island of Japan [1]. For some infectious diseases, the distribution of microorganisms with specific genotype in specific geographical areas has been noticed to correspond with the worldwide movement of human races

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[4–8]. Studies of the genomic similarity of microorganisms, other than *M. leprae*, suggest that disease strains were distributed in Korea and the main island of Japan by one of the Mongoloid groups that migrated into these areas [6,9–11]. Our data with *M. leprae* [1] substantiated this observation.

Intrigued with this, it was of interest to determine the genotypes of *M. leprae rpoT* gene that exist in the American continent. Because of the complicated history of invasion, conquest and migration, the American continent consists of multiple races. The genotypes of *M. leprae rpoT* gene distributed in Latin American countries are investigated in this study.

## 2. Materials and methods

### 2.1. Source of M. leprae isolates and preparation of genomic DNA

A total of 72 *M. leprae* isolates were used in the present study. Twenty-seven samples from Mexico, 25 samples from Peru and 20 samples from Paraguay were examined. In Mexico, 2 samples from Sinaloa State, 15 samples from Jalisco State and 10 samples from Guanajuato State were collected. Samples from Peru and Paraguay were collected randomly from patients throughout the countries. Almost all of the patients were Mestizo. Twenty-four samples originated from Philippines were also included in the analysis (kindly supplied by Dr. A. Abraham, Dr. Jose N. Rodriguez Memorial Hospital, Philippines). Samples were collected from multibacillary cases [12], since paucibacillary specimens gave poor PCR products in the preliminary study, as reported by others [13]. Furthermore, leprosy disease types are defined by host immune response rather than causative strains. Slit-skin smear specimens were collected from the skin lesion of patient in the same manner as the routine slit-skin smear test for Bacterial Index examination. The sample on the disposable surgical blade was soaked in 70% ethanol and kept at a room temperature until use. The bacilli were removed from the blade and collected as a pellet by centrifugation at 10,000*g* for 20 min and then washed with phosphate-buffered saline. Template was prepared by treatment with lysis buffer as mentioned elsewhere [13].

### 2.2. rpoT genotyping by PCR and electrophoresis

PCR was carried out using a G mixture of FailSafe PCR System (EPICENTRE, Madison, WI, USA) in a 50 μl volume of reaction mixture. Primers A (5′-ATGCCGAACCGGACCTCGACGTTGA-3′) and B (5′-TCGTCTTCGAGGTCGTCGAGA-3′) (GenBank Accession No. AB019194) were used for amplification to span the 91 or 97-bp fragment containing the target region with three copies of the six-base tandem repeats or four copies of the six-base tandem repeats in the *rpoT* gene [1]. For comparing the differences of the repeats in the *rpoT* gene, 91- or 97-bp products were separated by electrophoresis in a 4% Meta Phore™ agarose gel (FMC Bioproducts, Rockland, ME, USA) using TBE (Tris/Borate/EDTA, pH 8.0) buffer at 50 V.

### 2.3. Sequencing

The numbers of six-base repeats were confirmed by direct sequencing. DNA samples for sequencing were recovered by MinElute Gel Extraction Kit (QIAGEN, GmbH, Germany) after electrophoresis of PCR products in 1.2% Seakem GTG agarose gel (Cambrex Bio Science Rockland Inc., Rockland, ME, USA). Samples were sequenced as described previously [14].

### 2.4. Genotyping of TTC repeats

Templates prepared for the *rpoT* genotyping were also analysed TTC repeats [2]. The target region was amplified and sequenced as described previously [14].

### 2.5. Confirmation of M. leprae

To identify of *M. leprae*, the *groEL* gene was amplified by PCR from the template samples using the method of Plikaytis et al. [15].

### 2.6. Ethical approval

Informed consent was obtained from all subjects, and the study was approved by the institutional ethics committee of National Institute of Infectious Diseases, Japan. Bacillary samples of slit-skin smears were collected when informed consent was obtained.

## 3. Results

### 3.1. Geographic distribution of M. leprae with different rpoT genotypes

PCR products of different sizes, 91- or 97-bp, were obtained according to the number of six-base tandem repeats in the *rpoT* gene (Fig. 1). Sequencing revealed that there were three copies of the six-base tandem repeats in the 91-bp PCR products and four copies in the 97-bp PCR products, respectively. No other *rpoT* genotype was detected. Of 27 samples from Mexico, only two samples from Jalisco state harboured three copies of the six-base tandem repeats. Both samples from Sinaloa state, 13 samples from Jalisco state and 10 samples from Guanajuato state revealed a genotype with four copies of the six-base tandem repeats. In contrast, all samples

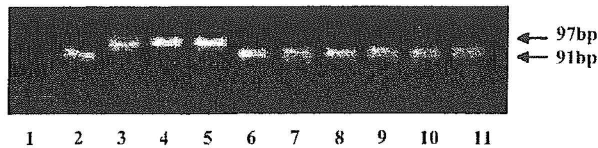


Fig. 1. The *rpoT* gene polymorphism in *M. leprae* obtained from Mexico, Peru and Paraguay. PCR products were resolved on a 4% Meta Phore agarose gel. Samples were: lane 1, the DNA size marker of 100 bp; lane 2, *M. leprae* with 3-copy of 6-bp tandem repeats; lane 3, *M. leprae* with four copies of the 6-bp tandem repeats; lane 4, isolate from Sinaloa state in Mexico; lane 5, isolate from Guanajuato state in Mexico; lane 6, isolate from Jalisco state in Mexico; lane 7, isolate from Peru; lane 8, isolate from Peru; lane 9, isolate from Peru; lane 10, isolate from Paraguay; lane 11, isolate from Paraguay.

Table 2
Frequency (%) of TTC genotypes in each country

| No. of repeats | Country | | |
|---|---|---|---|
| | Mexico | Peru | Paraguay |
| 9 | 5 | | 10 |
| 10 | 57 | 35 | 36 |
| 11 | 24 | 22 | 24 |
| 12 | 3 | 35 | 20 |
| 13 | 3 | 8 | 5 |
| 14 | 3 | | 5 |
| 18 | 5 | | |
| Total | 100% (27 cases) | 100% (25 cases) | 100% (20 cases) |

from Peru, Paraguay and the Phillipines showed three copies of the six-base tandem repeats (Table 1).

### 3.2. Frequency of each TTC genotype

The copy number of TTC repeats varied from 9 to 18 copies (Table 2). No biased distribution of some specific TTC repeat genotypes was recognized in these three countries. The 10-copy TTC genotype was the most frequent in all three countries.

### 3.3. Verification of bacterial materials

All samples examined showed the 364-bp PCR products of the *groEL* gene and indicated that samples contained *M. leprae*.

## 4. Discussion

The first stage of our study showed a prominent biased distribution of *M. leprae* with four copies of the six-base tandem repeats in the *rpoT* gene in the main island of Japan and Korea. The predominance of three copies of the same repeats in Okinawa, the island off the southern end of Japan, was associated with human migration [1]. It has been a very influential hypothesis that modern Japanese are derived from a base of Jomon ancestry compounded with later Yayoi immigrants who had migrated to Japan through the Korean Peninsula [16]. Additional data to support the hypothesis that *M. leprae* was spread by one of the Mongoloid lineage which migrated to Japan through the Korean Peninsula include the similarity of genetic marker of Korean and Japanese [17]; low seroprevalent frequency of HTLV-1 carriers in Koreans and Japanese [18]; and the resemblance between isolates from Korea and Japan on genotypes of JC virus, *Mycobacterium tuberculosis*, *Helicobacter pylori* and Hepatitis B virus [6,7,9–11].

In this study, the geographical distribution of *M. leprae* discriminated by *rpoT* gene polymorphism was compared in some Latin American countries. The most noticeable finding of this study was that even although all of the isolates from Peru and Paraguay harboured three copies of the six-base tandem repeats of *M. leprae rpoT* gene, in Mexico, was a predominance of bacilli with four copies of the repeats.

Genotyping of microorganisms distributed throughout the American continent and Asia has proved the concordant spread of some infectious diseases with the migration of human races [4–8]. It must be of interest to pursue whether there were any correlations between the genotypes of *M. leprae rpoT* gene distribution in the American continent and the spread of leprosy with the intercontinental movement of human races in the past because there have been reports that the movement of leprosy patients initiated the transmission of *M. leprae* [19]. Notably, the genotype of four copies of the six-base tandem repeats of the *M. leprae rpoT* gene was typically dominant in Mexico whilst in all isolates from Peru and Paraguay there were only three copies. According to a random analysis of *M. leprae rpoT* gene on an isolate from armadillo in the USA, three isolates from Brazil and an isolate from Haiti, it was supposed that *M. leprae* with three copies of the six-base tandem repeats existed on the American

Table 1
Number of each *rpoT* genotype isolated in the three Latin American countries

| *rpoT* Genotype | Mexico | | | Peru | Paraguay |
|---|---|---|---|---|---|
| | Sinaloa state | Guanajuato state | Jalisco state | | |
| 4-copy | 2 | 10 | 13 | | |
| 3-copy | | | 2 | 25 | 20 |

continent [1]. For this reason, the predominance of four copies of the six-base tandem repeats in Mexico was unexpected. These limited data might indicate that leprosy was introduced into Mexico and other countries in different ways, for example, from different human races such as from Africa by slaves, from Asia by different groups of Mongoloid people and from Europe countries by Caucasians. Such ideas have been explored in the context of other infectious diseases [4,5,8,20]. However, more samples from more other related countries would help to develop a more comprehensive correlation between the genotype of *M. leprae* and the history of introduction of leprosy to Latin American countries. This would with no doubt contribute to the study of anthropology and archaeology.

It is generally said that leprosy was introduced to Mexico from the Philippines during the Spanish colonial era. Until now, the preliminary study of all 24 isolates from the Philippines has shown three copies of the six-base tandem repeats, which does not support this view. More isolates of *M. leprae* from Mexico and other countries around Mexico for genotyping are required to resolve this point.

Polymorphism of TTC repeats exhibited a wide range of variation; however, the variation of samples from Peru was relatively limited, and there was no biased distribution of some specific genotypes in any countries. The bacilli with 10 copies of TTC repeats were detected most frequently and this was same with our previous results obtained in Indonesia [14].

Genotypes of *M. leprae* that are particularly distributed in some specific areas, other than three or four copies of six base tandem repeats in the *rpoT* gene, might facilitate the epidemiological and anthropological analysis of the origin and spread of leprosy. Unfortunately, even though microsatellite and minisatellite nucleotide sequences with polymorphism have been used for genotyping [3], none of them showed such biased geographical distribution as *rpoT* genotyping in our preliminary study (data ware not shown). Other short tandem repeats with polymorphism that are valuable for genotyping are under investigation for epidemiological analysis of leprosy transmission and the worldwide spread in the past.

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# Diversity of Potential Short Tandem Repeats in *Mycobacterium leprae* and Application for Molecular Typing

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**A recent advance in molecular typing for tracing the transmission of leprosy is the discovery of short tandem repeats (STRs) in *Mycobacterium leprae*. To substantiate polymorphic loci from STR as promising candidates for molecular typing tools in leprosy epidemiology, 44 STR loci including 33 microsatellites and 11 minisatellites were investigated among 27 laboratory strains by sequencing PCR products. Not all STRs were necessarily polymorphic. Thirty-two out of the 44 loci were polymorphic. Nine polymorphic loci were suitable for identifying genotypes according to the discriminatory capacity, stability, and reproducibility. All the strains were classified into independent genotypes by the selected nine loci. Three multicase households were subjected to molecular typing. *M. leprae* obtained from household cases showed identical copy numbers by TTC triplet alone, but the isolates from one family contact case were divided into different genotypes by adding eight other polymorphic loci. The combination of information from multiple loci allows increasing levels of discrimination and it is likely that the generation and documentation of data will result in the choice of a potential molecular typing tool for leprosy epidemiology.**

*Mycobacterium leprae* is an obligate intracellular parasite with tropism for macrophages and Schwann cells and the only species of mycobacteria to infect peripheral nerves (19). It causes leprosy, a chronic granulomatous infection of the skin and peripheral nerves with characteristic deformities and disability (1). It is generally accepted today that the worldwide implementation of standardized multidrug therapy for leprosy has decreased the number of registered leprosy cases from a peak of 10 to 15 million to a current total of less than 1 million. However, the annual confirmed new cases remain at 500,000 to 700,000. This continuing number suggests that effective multidrug therapy fails to disrupt the chain of leprosy transmission (26).

Even though leprosy is one of the oldest recorded diseases, the source for *M. leprae*, the portal of its exit/entry, and the mode of transmission are still under investigation. Some regard human beings as a host for the bacteria, while others are still considering more possibilities. It was proposed that the nasal mucosa are the exit/entry pathway of *M. leprae* (9, 17, 18). In light of the transmission mode, human-to-human direct contact was first generally accepted (6, 16), with time as airborne (23), as vector-borne (14, 25) and as vehicle-borne (3, 12) routes from evidence that has been obtained. All the progress in leprosy epidemiology was helpful in understanding the chain of transmission, yet at the same time was overwhelming.

In the past few years, studies focusing on leprosy transmission by molecular genotyping have shed new light on it (8, 10, 11, 22, 24, 27, 28). The most recent one was the report from Groathouse et al. (8). By in silico analysis, 44 promising polymorphic short tandem repeat (STR) sites, including both 33 microsatellite loci (repeat units of 1 to 5 bp) and 11 minisatellite (repeat units of >5 bp) were selected from the *M. leprae* TN genome sequence. Variable-number tandem repeats (VNTRs) at 9 of 11 STR loci from four clinical isolates of *M. leprae* were found. Clearly, it is urgent to accumulate extensive data for developing powerful typing tools to tracking the transmission of leprosy to finally reach a world free of leprosy.

In this study, we identified 32 polymorphic loci throughout the 44 STRs in a batch of 27 laboratory strains by sequencing and demonstrating the applicability and feasibility of nine potential loci acting as genetic markers to discriminate different *M. leprae* strains.

## MATERIALS AND METHODS

***M. leprae* strains.** A panel of 27 laboratory strains of *M. leprae* was subjected for genetic analysis. Strains were maintained by inoculation into nude mice footpads annually in our laboratory. All the strains used in this study were recovered from multibacillary cases. Excluding Thai-53, Thai-311 and Thai-237 were from Thailand, Indonesia-1 was from Indonesia, and Korea 3-2 was from Korea, and the others were from Japan.

All patients were from geographically distinct regions. Four strains, Thai-53, Kyoto-1, Zensho-4, and Korea3-2, and 17 samples, namely, the fourth generation of Thai-53 (Thai-53 4th), Thai-53 7th, Thai-53 11th, Kyoto-1 3rd, Kyoto-1 5th, Kyoto-1 7th, Kyoto-1 8th, Zensho-4 (biopsy specimen), Zensho-4 1st, Zensho-4 2nd, Zensho-4 3rd, Zensho-4 4th, Korea3-2 (biopsy specimen), Korea3-2 1st, Korea3-2 2nd, Korea3-2 3rd, and Korea3-2 4th (Table 6) were employed for the stability testing of loci. Partially purified bacterial materials were prepared from the inoculated footpads by deferential centrifuging and suspension in a phosphate buffered saline at concentration of $10^{5-6}$/ml.

**Primer selection.** Primer sets for the amplification regions of DNA containing the STR sites were referred to the study of Groathouse et al. (8). The sequences of primer pairs were listed (Table 1).

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TABLE 1. Primers used in this study[a]

| Primer | Sequence (5′ to 3′) | Repeat unit (bp) | Locus | Amplicon size (bp) | Accession no.[b] |
|---|---|---|---|---|---|
| RP1-1 | ATA ACC AAC CGT TTC GCT GC<br>GCA GAT TGC ACC CTG CAG TA | 1 | (T)8(A)6 | 108 | *ML0258-ML0259* |
| RP1-2 | ATG ATC CCG GTG TGA GCT CG<br>TCT CTG CAG CCC ACA CCC TG | 1 | (T)6(N)7(T)8 | 149 | ML0415/− |
| RP1-3 | AAG AAC GTA CAA TCA CC<br>AGC GAC CAC CCA CTA GCA TC | 1 | (A)9 | 145 | *ML1196-ML1197* |
| RP1-4 | TTG TGC TGG TCT CGT AGG CG<br>TGC CGA CCT CGG TCT GCT TC | 1 | (G)9 | 150 | ML0823/+ |
| RP1-5 | CGG ATA TAA TTA ACC TTC GG<br>GTT GCG GGT CTA CTT GGT TG | 1 | (C)9 | 131 | ML2238/− |
| RP1-6 | GGG CGA TGC CGC CTT CGA AC<br>CAG ACC CGG AAA CAG GAT AC | 1 | (G)10a | 128 | ML0266/+ |
| RP1-7 | AAT CCA AGC TGA TCG GCC AG<br>TGC CCT GCA ATG TGC CGA TG | 1 | (G)10b | 137 | ML0350/− |
| RP1-8 | GCG CAC TCT GTT AAT GAT GC<br>ACA CCG ACA ACC GGT TAC CG | 1 | (G)11 | 136 | ML1126/− |
| RP1-9 | TGG CCA TCG TGG TGC TGT TC<br>CAA CCT CGG CGG ACG CAT AC | 1 | (G)12 | 119 | **ML0946/+** |
| RP1-10 | TCG AGT TTT GGA CGG CAC CG<br>AGT GCT TGT GCG GTC CAC AC | 1 | (C)16(G)8 | 152 | *ML1647-ML1648* |
| RP1-11 | ACT TCG GCG ACT GCG GTA AC<br>GGT CAC TGG GCG TTG TGG TC | 1 | (C)20 | 159 | *ML0237-ML0238* |
| RP1-12 | GAA CTG GCC GGG TTA AAA GG<br>TGC GCC TTC ACT GTG AAA TG | 1 | (G)22 | 176 | ML0164/+ |
| RP2-1 | CAG GTC TTC GCG CCA ATT CT<br>CCC GCG ACT CCC TGG CTT GC | 2 | (CG)6 | 140 | **ML2472/−** |
| RP2-2 | GTG TTA CGC GGA ACC AGG CA<br>CCA TCT GTT GGT ACT ACT GA | 2 | (AC)8a | 124 | ML1285/− |
| RP2-3 | GAT GCG ACT ATC ACT CGC AC<br>GCT GGT TTC CTT CTA GTC CC | 2 | (AC)8b | 140 | *ML1824-ML1825* |
| RP2-4 | GCC TGG TGC CCG GAC AAT GC<br>ACT GAT CTC GCC GGC GCT GT | 2 | (AC)9 | 140 | *ML1227-ML1228* |
| RP2-5 | CAA CAG ATC GGT CGG AGA GG<br>CTG GGT AGC CAT CTG GCT GC | 2 | (CA)6 | 160 | *ML2106-ML2107* |
| RP2-6 | GAC CCG GTT GCT TCA TGT AT<br>GTA AGT TAC AAA CAA GCT GT | 2 | (TA)8 | 143 | *ML2676-ML2677* |
| RP2-7 | CGG AAA GTG TTG TGG TCG AC<br>CCA AAC ACT TAG TCC ACT AA | 2 | (TA)9 | 189 | *ML2375-ML2376* |
| RP2-8 | TAA TTT TCT TAA AGA TAG AG<br>TGT GAT CTT TGC ACT GTC GT | 2 | (TA)10 | 139 | *ML1450-ML1451* |
| RP2-9 | ATG AGC CGT AAG GAA TTA AC<br>ATG CTG CCA CTC ATT AGA GG | 2 | (TA)13 | 158 | *ML0235-ML0236* |
| RP2-10 | CTG AGT AGA CCA GTC GAC AG<br>ATC GCG GAG CAA CAA TTT CT | 2 | (AT)10 | 150 | *ML2476-ML2477* |
| RP2-11 | TTG GCG CTT CTG ATA TGG CT<br>GGC GAG CCC GTC TGG CTC GA | 2 | (AT15) | 164 | *ML0798-ML0799* |
| RP2-12 | TTA GCA GGA CGA TTG TAC AG<br>ACC CGG AAT TCC TCC AAG | 2 | (AT)17 | 160 | ML2183/− |
| RP2-13 | CCG ACT CGC CGA AGC GAA AC<br>CCG CGT TAG CAT AGG CAA T | 2 | (TA)18 | 119 | ML0830/− |
| RP3-1 | GTC ACG GCG CCG GGA ACG CA<br>CCG ATT CTG CTG GGC TGC TG | 3 | (ACC)5 | 150 | ML1645/− |
| RP3-2 | TCA CCA TCG ACG CTC CGG GT<br>TCG GCC TGG TTG TCT GCC TT | 3 | (GGT)5 | 161 | *ML2159-ML2160* |
| RP3-3 | CGT GAC AAT TCC TGG CAC AT<br>CGA GAA GTG TAC CAC CAT CC | 3 | (AGT)5a | 143 | ML1073/− |
| RP3-4 | GGC CAA ACG GTC AGG TTG TT<br>ACA TCG AAT CGC TGT TGT AC | 3 | (AGT)5b | 149 | ML1118/− |
| RP3-5 | GTA CGC CAA GGA CCG TGA CT<br>GGT TGT TGA TGT TGG TGG TG | 3 | (ACT)5 | 120 | ML2236/+ |
| RP3-6 | ACG CTG CGG TTT CGC AGC CT<br>AAT ATG CAT GCC GGT GGT | 3 | (GTA)9 | 148 | *ML2172-ML2173* |
| RP3-7 | GGA CCT AAA CCA TCC CGT TT<br>CTA CAG GGG GCA CTT AGC | 3 | (AGA)20 | 168 | *ML2344-ML2345* |
| RP5-1 | GAA GTT GAA TTC TTA TTG CC<br>GCG GAG GGC ATC ACA CAG AA | 5 | (CACCG)3 | 130 | *ML2158-ML2159* |

*Continued on facing page*

TABLE 1—*Continued*

| Primer | Sequence (5′ to 3′) | Repeat unit (bp) | Locus | Amplicon size (bp) | Accession no.[b] |
|---|---|---|---|---|---|
| RP6-1 | AAG CGT CGA TAC AAA GGC ACC GT<br>AGT AGC TTC GCCATC CTC GGT TT | 6 | 6-3a | 91 | **ML1022/−** |
| RP6-2 | GTT TGT CAA CAT TGG CAG GT<br>CTG GAA CCG CGT GGC CCA CT | 6 | 6-3b | 148 | **ML1918/−** |
| RP6-3 | CTA CTT GCG CGC CAC CGC CA<br>CCG TCG CCA GGT TTT GCA GA | 6 | 6-7 | 191 | **ML1505/+** |
| RP7-1 | CTG GGG CGC GCT CAA TCG CT<br>CGG GTT CGG GTG TAA CGA CA | 7 | 7-3 | 160 | **ML0213/+** |
| RP10-1 | GTA CGC CAC CAG GAC AAC TC<br>TGG CGG GCG TGC AGC CAA AC | 10 | 10-4 | 203 | ML0970/+ |
| RP12-1 | AGT AGC TTC CAT CCC CTC AT<br>GCG ACG AAA GCA TTT ACG GC | 12 | 12-5 | 180 | **ML1182** |
| RP15-1 | GCT GAG GTT AGG CGC CGA TC<br>TCG GCA GAG TCC TGG CCA TC | 15 | 15-3 | 195 | *ML2454-ML2455* |
| RP18-1 | GCT ATG GGC AGC CTG GGT AT<br>AGC CGG TTA CCA AGA TGG CA | 18 | 18-8 | 330 | **ML1334/+** |
| RP21-1 | TGT TGA AAT TTG GCG GCC AT<br>TGC AAG GAG TGC TCA GCT AT | 21 | 21-3 | 179 | ML0058/− |
| RP23-1 | CAG TCG CCC GGA TAC TGT TA<br>TAA ATC CGC TCC CAA ATC TT | 23 | 23-3 | 190 | *ML2469-ML2470* |
| RP27-1 | GTG CTG TGC CTG CCG TT<br>TCC CCA AAG CCG CCG AAT CC | 27 | 27-5 | 270 | **ML0568/+** |

[a] Primers were designed to amplify STRs reported by Groathouse et al. (8).
[b] Protein coding genes are in bold type. Intergenic regions are in italic type. Pseudogenes are underlined.

**Slit-skin smears from multicase household.** Eleven slit skin smears were collected as the same manner as that for Bacterial Index examination from leprosy patients in a total of three households. Among them, five smears from five patients in household I; four smears from two patients in household II, sample 6 from the left earlobe and sample 7 from the right earlobe within a single individual; sample 8 from the earlobe and sample 9 from the back in another individual; and two smears from two patients in household III (Table 7).

**Preparation of *M. leprae* DNA from strains and slit skin smears and sequencing analysis.** *M. leprae* templates from both strains and slit-skin smears were prepared by treatment with lysis buffer at 60°C overnight as described previously (13). PCR amplification of STR sites as well as sequencing analysis was performed under the same condition as described elsewhere using the listed primer pairs (10, 11, 12, 13). Briefly, target loci were amplified using a G mixture and a FailSafe PCR system (EPICENTRE, Madison, Wis.). DNA samples for sequencing were recovered with a MinElute gel extraction kit (QIAGEN GmbH, Hilden, Germany) after electrophoresis of PCR products. Samples were sequenced with a BigDye terminator cycle sequencing FS Ready Reaction kit (Perkin-Elmer Applied Biosystems, Norwalk, Conn.) and an ABI Prism 310 genetic analyzer (Perkin-Elmer). The nucleotide sequences obtained were analyzed using DNASIS software (Hitachi Software Engineering, Yokohama, Japan).

**Multiplication of *M. leprae* strains in nude mice footpad.** Inoculums of each strain were prepared from BALB/c-*nu*/*nu* nude mice which were inoculated with isolates of the third to fifth generations about 8 to 10 months before by Nakamura's method as described previously (15). Five-week-old male nude mice were injected with the inoculums containing $10^4$ bacilli/0.05 ml of Hanks' balanced salt solution. Bacillary number in the footpads of six nude mice at 10, 20, 30, 40, and 50 weeks growth were examined by Shepard's method (21).

**Ethical approval.** Informed consent was obtained from all subjects, and the study was approved by the institutional ethics committee of National Institute of Infectious Diseases, Tokyo, Japan. Bacillary samples of nasal mucus and slit skin smears were collected when informed consent was obtained. Animal experiments were conducted under the approval of the institutional animal experiment committee.

## RESULTS

**Allelic diversity of 33 microsatellite loci in *M. leprae* strains.** In the 27 *M. leprae* strains, 8 out of 33 selected microsatellite loci showed the same copy number of allele whereas the other 25 loci, (A)9, (G)9, (C)9, (G)10a, (G)10b, (G)11, (G)12, (C)16(G)8, (C)20, (G)22, (AC)8a, (AC)8b, (AC)9, (CA)6, (TA)8, (TA)9, (TA)10, (TA)13, (AT)10, (AT)15, (AT)17, (TA)18, (GGT)5, (GTA)9, and (AGA)20, presented at least two types of allele. All locus identifications are from Groathouse et al. (8) to maintain integrity. This result validated the comments from Groathouse group on the polymorphic (C)20, (AT)17, (TA)18, (GTA)9, and (AGA)20 and no diversity of (CG)6, albeit (AC)9 was also examined for considerable polymorphism here which was predicted as nonpolymorphic loci by them. However, the last repeat unit of some results was not easily defined for unknown reasons (Table 2).

**Allelic diversity of eleven minisatellite loci in *M. leprae* strains.** Throughout the 27 *M. leprae* strains, four out of 11 selected minisatellite loci showed no diversity while the other seven loci, 6-3a, 6-7, 12-5, 18-8, 21-3, 23-3, and 27-5, exhibited variable characteristics, which also verified Groathouse et al. (8) reports on the polymorphism of loci 6-7, 12-5, 18-8, 21-3, and 27-5 (Table 3).

**Variations of allelic diversity at STR loci in *M. leprae* strains.** Forty-four selected STR loci consisted of nine protein coding genes, 19 intergenic regions, and 16 pseudogenes. Polymorphism was revealed in six among nine (67%) protein coding genes, 16 out of 19 (84%) intergenic genes, and 10 of 16 (63%) pseudogenes. Almost all of the STR loci located in intergenic regions or in pseudogenes and were unlikely involved in biological functions. Overall, thirty-two out of 44 STR loci were polymorphic and 12 loci were invariable that might be of limited value as epidemiological markers. The variations were between 2 and 11 alleles (Table 4). Notably, at locus (CA)6, isolate Thai-237 differed from the other 26 strains by one repeat unit (Table 2). Similarly, at locus 23-3, isolate Hoshizuka-1 alone, and at locus 27-5, isolate Airaku-2 alone

TABLE 2. Allelic diversity of microsatellite loci in *M. leprae* strains

| Strain | Copy no. of repeat element(s) at locus[a]: | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | (T)8(A)6 | (T)6(N)7(T)8 | (A)9 | (G)9 | (C)9 | (G)10a | (G)10b | (G)11 | (G)12 | (C)16(G)8 | (C)20 | (G)22 | (CG)6 | (AC)8a | (AC)8b | (AC)9 | (CA)6 |
| Zensho-2 | 8 & 6 | 6 & 7 & 8 | 8 | 9 | 10 | 10 | 10 | 13† | 9 | 16† & 7 | 16† | 17† | 6 | 7 | 8 | 7 | 6 |
| Airaku-2 | 8 & 6 | 6 & 7 & 8 | 8 | 9 | 10 | 9 | 9 | 13 | 14† | 12† & 8 | 9 | 14† | 6 | 9 | 6 | 8 | 6 |
| Airaku-3 | 8 & 6 | 6 & 7 & 8 | 8 | 9 | 10 | 9 | 11 | 11 | 10 | 16† & 8 | 13† | 18† | 6 | 10 | 6 | 8 | 6 |
| Ryukyu-2 | 8 & 6 | 6 & 7 & 8 | 8 | 9 | 12 | 7 | 9 | 14 | 11 | 10 & 8 | 8 | 16† | 6 | 12 | 7 | 8 | 6 |
| Zensho-9 | 8 & 6 | 6 & 7 & 8 | 8 | 9 | 9 | 9 | 11 | 13 | 10 | 16† & 8 | 8 | 19† | 6 | 9 | 7 | 8 | 6 |
| Gushiken | 8 & 6 | 6 & 7 & 8 | 9 | 9 | 10 | 12 | 11 | 10 | 9 | 14† & 8 | 9 | 16† | 6 | 11 | 7 | 7 | 6 |
| Thai-53 | 8 & 6 | 6 & 7 & 8 | 9 | 9 | 10 | 12 | 11 | 10 | 9 | 14† & 8 | 9 | 17† | 6 | 11 | 7 | 7 | 6 |
| Thai-311 | 8 & 6 | 6 & 7 & 8 | 8 | 10 | 10 | 9 | 11 | 10 | 9 | 16† & 8 | 13† | 16† | 6 | 9 | 6 | 8 | 6 |
| Amami-1 | 8 & 6 | 6 & 7 & 8 | 8 | 9 | 9 | 8 | 9 | 11 | 9 | 17† & 8 | 8 | 12† | 6 | 8 | 7 | 8 | 6 |
| Indonesia-1 | 8 & 6 | 6 & 7 & 8 | 9 | 9 | 13† | 11 | 9 | 14 | 9 | 18† & 8 | 12† | 9 | 6 | 11 | 7 | 9 | 6 |
| Thai-237 | 8 & 6 | 6 & 7 & 8 | 9 | 9 | 9 | 9 | 11 | 9 | 9 | 14† & 8 | 9 | 13† | 6 | 9 | 7 | 7 | 7 |
| Zensho-12 | 8 & 6 | 6 & 7 & 8 | 9 | 9 | 9 | 10 | 9 | 11 | 9 | 12 & 7 | 10 | 14† | 6 | 10 | 7 | 10 | 6 |
| Hoshizuka-1 | 8 & 6 | 6 & 7 & 8 | 8 | 9 | 12† | 9 | 10 | 13 | 9 | 10 & 7 | 10 | 14† | 6 | 8 | 8 | 7 | 6 |
| Hoshizuka-5 | 8 & 6 | 6 & 7 & 8 | 8 | 10 | 12† | 9 | 10 | 13 | 11 | 14† & 7 | 17† | 13† | 6 | 10 | 8 | 7 | 6 |
| Izumi | 8 & 6 | 6 & 7 & 8 | 8 | 9 | 10 | 13† | 11 | 11 | 9 | 17† & 7 | 9 | 17† | 6 | 11 | 8 | 7 | 6 |
| Kanazawa | 8 & 6 | 6 & 7 & 8 | 8 | 9 | 10 | 9 | 13† | 14† | 9 | 16† & 7 | 11 | 13† | 6 | 6 | 8 | 7 | 6 |
| Keifu-4 | 8 & 6 | 6 & 7 & 8 | 8 | 9 | 11 | 9 | 10 | 11 | 9 | 14† & 7 | 16† | 12† | 6 | 8 | 10 | 7 | 6 |
| Kitazato | 8 & 6 | 6 & 7 & 8 | 8 | 9 | 9 | 13† | 12 | 13† | 10 | 15† & 7 | 15† | 14† | 6 | 9 | 7 | 7 | 6 |
| Zensho-4 | 8 & 6 | 6 & 7 & 8 | 8 | 9 | 10 | 11 | 12 | 11 | 9 | 17† & 7 | 14† | 14† | 6 | 8 | 8 | 7 | 6 |
| Kusatsu-3 | 8 & 6 | 6 & 7 & 8 | 8 | 9 | 10 | 12 | 10 | 10 | 12† | 18† & 7 | 13† | 12† | 6 | 9 | 8 | 8 | 6 |
| Kyoto-2 | 8 & 6 | 6 & 7 & 8 | 8 | 9 | 11 | 11 | 9 | 13 | 10 | 19† & 7 | 10 | 15† | 6 | 6 | 8 | 7 | 6 |
| Oku-4 | 8 & 6 | 6 & 7 & 8 | 8 | 9 | 9 | 10 | 13† | 14 | 9 | 10 & 8 | 15† | 14† | 6 | 9 | 8 | 7 | 6 |
| Zensho-5 | 8 & 6 | 6 & 7 & 8 | 8 | 9 | 12 | 11 | 11 | 13 | 11 | 16† & 7 | 13† | 14† | 6 | 10 | 8 | 7 | 6 |
| Kusatsu-6 | 8 & 6 | 6 & 7 & 8 | 8 | 9 | 10 | 10 | 12 | 10 | 10 | 17† & 7 | 14† | 16† | 6 | 9 | 8 | 7 | 6 |
| Korea3-2 | 8 & 6 | 6 & 7 & 8 | 8 | 9 | 11 | 11 | 13† | 12 | 9 | 12† & 7 | 9 | 18† | 6 | 7 | 8 | 7 | 6 |
| Hoshizuka-4 | 8 & 6 | 6 & 7 & 8 | 8 | 9 | 10 | 10 | 11 | 13 | 9 | 19† & 7 | 10 | 10 | 6 | 8 | 8 | 7 | 6 |
| Kyoto-1 | 8 & 6 | 6 & 7 & 8 | 8 | 9 | 11 | 10 | 12 | 13 | 9 | 13† & 8 | 11 | 18† | 6 | 7 | 8 | 7 | 6 |

[a] †, difficult to read the sequence across the junction of stretch of repeat.

had one copy difference allele (Table 3). Additionally, at locus 12-5, a five-copy-repeat unit was obtained from *M. leprae* TN, but all of the strains in this study showed three- and four-copy-repeat units instead of the five-copy-repeat unit (Table 3).

**Comparison among the loci fall within protein coding genes with two variations.** Locus 6-3a is in the *rpoT* gene coding RNA polymerase sigma factor in *M. leprae* contained two variations, three- and four-copy alleles (2, 13). Twelve strains, Zensho-2 to Zensho-12, harbored the three-copy allele whereas 15 strains, Hoshizuka-1 to Kyoto-1, had a four-copy allele in the *rpoT* gene (Table 3). Geographic distribution of each genotype of *M. leprae rpoT* revealed the distinguished distribution in several countries in the world (13, 10). Intriguingly, we compared the copy numbers of the other two protein coding genes 12-5 and 18-8 having two alleles with the strains carrying three-copy and four-copy *rpoT* that were closely associated with geographic distributions and no correlation was exploited.

**Discriminatory capacity, stability, and reproducibility of nine potential polymorphic loci detected among serial passage strains by nude mice.** Nine polymorphic microsatellite loci, (AC)9, (AC)8b, (AC)8a, (TA)10, (AT)17, (AT)15 (GTA)9, (TA)18, and (AGA)20 (also named TTC) were selected for discriminatory capacity analysis. All 27 strains were divided into two groups, three-copy *rpoT* and four-copy *rpoT*, based on the *rpoT* polymorphism. By adding the conjunction of 9 loci, they were distinguished from each other (Table 5). Then stability and reproducibility testing was carried out among them through serial passage strains by nude mice owing to the nature of susceptibility of replication slippage of microsatellite (28). One generation designated approximately 1 year, these strains have been in passage for 4 to 11 years. Not only the identical profile of allele was shared among the different generations of the strains at each locus, but also the copy number of repeats was in agreement with that in the repeated experiment of the same strains, which ensured these polymorphic microsatellite loci were highly stable and reproducible (Tables 1 and 6). The stability of TTCwas done previously (11)]. It was the rationale for these nine polymorphic microsatellite loci to be a significant source of informative markers for the identification and discrimination of *M. leprae* strains.

**Application of polymorphic microsatellite loci for multicase households.** Based on the condition of leprosy patients living in same household and possessing the identical TTC pattern, eleven smears from three household were chosen (Table 7). Five bacterial materials from household I shared identical 13-copy of TTC repeat were also subjected to loci (AC)8a, (AC)8b, (AC)9, (TA)10, (AT)15, (AT)17, (TA)18, and (GTA)9 and presented the identical copy number of 12, 7, 10, 10, 13, 15, 14, and 9, respectively. In household II which had 12 copies of the TTC repeat, four smears at the above loci showed equal repeats of 10, 7, 9, 10, 13, 13, 11, and 11. There was an exact match by copy number at each locus between samples 6 and 7 as well as samples 8 and 9 within one individual. In household III, two smears harboring eight copies of the TTC repeat, the copy number of loci (AC)8a, (AC)8b, (AC)9, (TA)10, (AT)15 and (TA)18 was 10, 7, 9, 8, 16, and 15. respectively. Strikingly, at locus (AT)17, a difference of 9 and 10 copies and a mismatch of 11 and 12 copies at locus (GTA)9 were found in these two smears, respectively.

TABLE 2—*Continued*

| Copy no. of repeat element(s) at locus[a]: | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (TA)9 | (TA)10 | (TA)13† | (AT)10 | (AT15) | (AT)17 | (TA)18 | (ACC)5 | (GGT)5 | (AGT)5a | (AGT)5b | (ACT)5 | (GTA)9 | (AGA)20 | (CACCG)3 |
| 8 | 14 | 23 | 9 | 14 | 15 | 15 | 5 | 4 | 5 | 5 | 5 | 14 | 9 | 3 |
| 12 | 7 | 19 | 7 | 15 | 14 | 17 | 5 | 4 | 5 | 5 | 5 | 10 | 13 | 3 |
| 10 | 7 | 21 | 7 | 18 | 13 | 17 | 5 | 4 | 5 | 5 | 5 | 11 | 13 | 3 |
| 6 | 10 | 22 | 7 | 19† | 13 | 13 | 5 | 4 | 5 | 5 | 5 | 8 | 14 | 3 |
| 7 | 13 | 15 | 7 | 21 | 16 | 12 | 5 | 4 | 5 | 5 | 5 | 11 | 14 | 3 |
| 8 | 11 | 23 | 8 | 13 | 13 | 20 | 5 | 5 | 5 | 5 | 5 | 11 | 14 | 3 |
| 9 | 11 | 23 | 8 | 13 | 13 | 21 | 5 | 5 | 5 | 5 | 5 | 11 | 14 | 3 |
| 12 | 7 | 19 | 7 | 14 | 11 | 19 | 5 | 4 | 5 | 5 | 5 | 9 | 25 | 3 |
| 6 | 10 | 24 | 8 | 17 | 10 | 15 | 5 | 4 | 5 | 5 | 5 | 9 | 15 | 3 |
| 8 | 10 | 19 | 9 | 14 | 13 | 10 | 5 | 5 | 5 | 5 | 5 | 9 | 15 | 3 |
| 9 | 11 | 18 | 9 | 11 | 15 | 10 | 5 | 5 | 5 | 5 | 5 | 9 | 16 | 3 |
| 10† | 12 | 22 | 9 | 16 | 18 | 16 | 5 | 6 | 5 | 5 | 5 | 9 | 11 | 3 |
| 9 | 15 | 15 | 8 | 21† | 14 | 13 | 5 | 4 | 5 | 5 | 5 | 15 | 9 | 9 |
| 14 | 13 | 19 | 8 | 15 | 14 | 12 | 5 | 4 | 5 | 5 | 5 | 15 | 9 | 3 |
| 8 | 12† | 16 | 8 | 13 | 13 | 16† | 5 | 4 | 5 | 5 | 5 | 11 | 9 | 3 |
| 9 | 14 | 19 | 8 | 17 | 13 | 11 | 5 | 4 | 5 | 5 | 5 | 12 | 10 | 3 |
| 7 | 11 | 19 | 9 | 21† | 15 | 19 | 5 | 4 | 5 | 5 | 5 | 13 | 10 | 3 |
| 14† | 13 | 18 | 8 | 12 | 12 | 14 | 5 | 4 | 5 | 5 | 5 | 16 | 10 | 3 |
| 8 | 10 | 17 | 9 | 20 | 13 | 15 | 5 | 4 | 5 | 5 | 5 | 13 | 10 | 3 |
| 10 | 9 | 24 | 8 | 13 | 12 | 17 | 5 | 4 | 5 | 5 | 5 | 16 | 11 | 3 |
| 14 | 16 | 18 | 9 | 18 | 15 | 14 | 5 | 4 | 5 | 5 | 5 | 16 | 11 | 3 |
| 10 | 13 | 19 | 9 | 14 | 15 | 12 | 5 | 4 | 5 | 5 | 5 | 13 | 11 | 3 |
| 8 | 12† | 18 | 8 | 14 | 14 | 14 | 5 | 4 | 5 | 5 | 5 | 18 | 11 | 3 |
| 12 | 16 | 19 | 8 | 18 | 12 | 17 | 5 | 4 | 5 | 5 | 5 | 16 | 16 | 3 |
| 11 | 15 | 20 | 8 | 17 | 14 | 15 | 5 | 4 | 5 | 5 | 5 | 14 | 13 | 3 |
| 10 | 12† | 20 | 8 | 20† | 14 | 17 | 5 | 4 | 5 | 5 | 5 | 13 | 12 | 3 |
| 12 | 12 | 23 | 9 | 15 | 12 | 16 | 5 | 4 | 5 | 5 | 5 | 15 | 9 | 3 |

TABLE 3. Allelic diversity of minisatellite loci in *M. leprae* strains

| Strain | No. of copies at locus: | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 6-3a | 6-3b | 6-7 | 7-3 | 10-4 | 12-5 | 15-3 | 18-8 | 21-3 | 23-3 | 27-5 |
| Zensho-2 | 3 | 3 | 7 | 3 | 4 | 3 | 3 | 7 | 2 | 2 | 5 |
| Airaku-2 | 3 | 3 | 6 | 3 | 4 | 4 | 3 | 7 | 2 | 2 | 3 |
| Airaku-3 | 3 | 3 | 6 | 3 | 4 | 4 | 3 | 8 | 1 | 2 | 5 |
| Ryukyu-2 | 3 | 3 | 6 | 3 | 4 | 4 | 3 | 8 | 2 | 2 | 5 |
| Zensho-9 | 3 | 3 | 6 | 3 | 4 | 4 | 3 | 8 | 2 | 2 | 5 |
| Gushiken | 3 | 3 | 6 | 3 | 4 | 4 | 3 | 7 | 3 | 2 | 5 |
| Thai-53 | 3 | 3 | 6 | 3 | 4 | 4 | 3 | 8 | 3 | 2 | 5 |
| Thai-311 | 3 | 3 | 6 | 3 | 4 | 4 | 3 | 8 | 2 | 2 | 5 |
| Amami-1 | 3 | 3 | 6 | 3 | 4 | 3 | 3 | 8 | 2 | 2 | 5 |
| Indonesia-1 | 3 | 3 | 6 | 3 | 4 | 4 | 3 | 8 | 3 | 2 | 5 |
| Thai-237 | 3 | 3 | 6 | 3 | 4 | 4 | 3 | 8 | 3 | 2 | 5 |
| Zensho-12 | 3 | 3 | 7 | 3 | 4 | 4 | 3 | 8 | 3 | 2 | 5 |
| Hoshizuka-1 | 4 | 3 | 6 | 3 | 4 | 3 | 3 | 7 | 2 | 3 | 5 |
| Hoshizuka-5 | 4 | 3 | 6 | 3 | 4 | 3 | 3 | 7 | 1 | 2 | 5 |
| Izumi | 4 | 3 | 6 | 3 | 4 | 3 | 3 | 7 | 1 | 2 | 5 |
| Kanazawa | 4 | 3 | 10 | 3 | 4 | 3 | 3 | 7 | 2 | 2 | 5 |
| Keifu-4 | 4 | 3 | 6 | 3 | 4 | 3 | 3 | 7 | 2 | 2 | 5 |
| Kitazato | 4 | 3 | 6 | 3 | 4 | 3 | 3 | 7 | 1 | 2 | 5 |
| Zensho-4 | 4 | 3 | 9 | 3 | 4 | 3 | 3 | 7 | 3 | 2 | 5 |
| Kusatsu-3 | 4 | 3 | 6 | 3 | 4 | 3 | 3 | 7 | 2 | 2 | 5 |
| Kyoto-2 | 4 | 3 | 8 | 3 | 4 | 3 | 3 | 7 | 3 | 2 | 5 |
| Oku-4 | 4 | 3 | 5 | 3 | 4 | 3 | 3 | 7 | 2 | 2 | 5 |
| Zensho-5 | 4 | 3 | 6 | 3 | 4 | 3 | 3 | 7 | 1 | 2 | 5 |
| Kusatsu-6 | 4 | 3 | 6 | 3 | 4 | 3 | 3 | 7 | 2 | 2 | 5 |
| Korea3-2 | 4 | 3 | 7 | 3 | 4 | 3 | 3 | 7 | 1 | 2 | 5 |
| Hoshizuka-4 | 4 | 3 | 9 | 3 | 4 | 3 | 3 | 7 | 2 | 2 | 5 |
| Kyoto-1 | 4 | 3 | 6 | 3 | 4 | 3 | 3 | 7 | 3 | 2 | 5 |

**Linkage between the growth rate of *M. leprae* in nude mice footpad and allelic diversity.** Shepard et al. (20) reported the hereditary fast-slow growth difference among *M. leprae* strains in conventional mouse footpads, which was also observed in nude mice footpads in our laboratory. However, in this study, the growth curve of *M. leprae* in nude mice footpads gave no difference between strains with three-copy *rpoT* (Zensho-2, Airaku-3, and Thai-53) and those with four-copy *rpoT* (Izumi and Zensho-4) except Kyoto-2, which revealed the copy number of *rpoT* was irrelevant to the fastidious growth. In addition, no correlation was found between the VNTR of the other protein coding genes and *M. leprae* growth.

## DISCUSSION

Poor understanding of leprosy transmission has hindered us from eradicating the disease even though *M. leprae* was identified as the pathogen as early as 1873 by Hansen (19). The unusual biological traits of being especially slow growing as well as the inability to culture *M. leprae* in vitro have partially accounted for the setbacks of detailed studies on leprosy. However, the determination of the complete *M. leprae* TN genome sequence (4) is a breakthrough for leprosy research, which has served as a public domain used by the Groathouse study group for the screening of potential VNTR for molecular typing (8). Tandem repeats are usually classified among satellites (spanning megabases of DNA, associated with heterochromatin), minisatellites (repeat units in the range from 6 to 100 bp, spanning hundreds of base pairs) and microsatellites (repeat units in the range from 1 to 5 bp, spanning a few tens of nucleotides) (5, 7). Both microsatellite and minisatellite loci

TABLE 4. Variation of allelic diversity at STR loci in *M. leprae* strains[a]

| No. | Locus | No. of repeats and range | No. | Locus | No. of repeats and range | No. | Locus | No. of repeats and range |
|---|---|---|---|---|---|---|---|---|
| 1 | (T)8(A)6 | No variation | 16 | (AC)**9*** | 7, 8, 9, 10 | 31 | (GTA)**9*** | 8, 9, 10, 11, 12, 13, 14, 15, 16, 18 |
| 2 | (T)6(N)7(T)8 | No variation | 17 | (CA)6 | 6, 7 | 32 | (AGA)20* | 9, 10, 11, 12, 13, 14, 15, 16, 25 |
| 3 | (A)9 | 8, 9 | 18 | (TA)8 | 6, 7, 8, 9, 10 | 33 | (CACCG)3 | No variation |
| 4 | (G)9 | 9, 10 | 19 | (TA)9 | 6, 7, 8, 9, 10, 11, 12, 14 | **34** | **6-3a** | 3, 4 |
| 5 | (C)9 | 9, 10, 11, 12 | 20 | (TA)10* | 7, 9, 10, 11, 12, 13, 14, 15, 16 | **35** | **6-3b** | No variation |
| 6 | (G)10a | 7, 8, 9, 10, 12, 13 | 21 | (TA)13 | 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 | **36** | **6-7** | 5, 6, 7, 8, 9, 10 |
| 7 | (G)10b | 9, 10, 11, 12, 13, | 22 | (AT)10 | 7, 8, 9, 10, 12, 14 | **37** | **7-3** | No variation |
| 8 | (G)11 | 10, 11, 12, 13, 14 | 23 | (AT15)* | 13, 14, 15, 16, 17, 18, 19, 20, 21 | 38 | 10-4 | No variation |
| 9 | **(G)12** | 9, 10, 11, 12, 14 | 24 | (AT)17* | 10, 11, 12, 13, 14, 15, 16, 18 | **39** | **12-5** | 3, 4 |
| 10 | (C)16(G)8 | 7, 8 & 10, 12, 13, 14, 15, 16, 17, 18, 19 | 25 | (TA)18* | 11, 12, 13, 14, 15, 16, 17, 19 | 40 | 15-3 | No variation |
| 11 | (C)20 | 8, 9, 10, 11, 12, 13, 14, 15, 14, 15, 16 | 26 | (ACC)5 | No variation | **41** | **18-8** | 7, 8 |
| 12 | (G)22 | 9, 10, 12, 13, 14, 15, 16, 17, 18, 19 | 27 | (GGT)5 | 4, 5, 6 | 42 | 21-3 | 1, 2, 3 |
| **13** | **(CG)6** | No variation | 28 | (AGT)5a | No variation | 43 | 23-3 | 2, 3 |
| 14 | (AC)8a* | 6, 7, 8, 9, 10, 11, 12 | 29 | (AGT)5b | No variation | **44** | **27-5** | 3, 5 |
| 15 | (AC)8b* | 6, 7, 8, 10, | 30 | (ACT)5 | No variation | | | |

[a] Protein coding genes are in bold type. Pseudogenes are underlined. Intragenic genes are in standard type. *, selected loci as the combined one for genotyping.

have been selected for this study to substantiate the polymorphic loci as promising candidates used as the molecular typing tools for leprosy epidemiology.

In order to generate comprehensive and reliable data, a battery of 27 laboratory strains was used to develop PCR systems to amplify 44 STR target loci and the PCR products were sequenced. Unexpectedly, the results disclosed that not all the STR loci were polymorphic. Some of the loci were with

TABLE 5. Discriminatory capacity of polymorphic microsatellite loci[a]

| Strain | No. of repeats | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 6-3a | (AC)9 | (AC)8b | (AC)8a | (TA)10 | (AT)17 | (AT)15 | (GTA)9 | (TA)18 | (AGA)20 or TTC |
| Gushiken | 3 | 7 | 7 | 11 | 11 | 13 | 13 | 11 | 20 | 14 |
| Thai-53 | 3 | 7 | 7 | 11 | 11 | 13 | 13 | 11 | 21 | 14 |
| Thai-237 | 3 | 7 | 7 | 9 | 11 | 15 | 11 | 9 | 10 | 16 |
| Zensho-2 | 3 | 7 | 8 | 7 | 14 | 15 | 14 | 14 | 15 | 9 |
| Thai-311 | 3 | 8 | 6 | 9 | 7 | 11 | 14 | 9 | 19 | 25 |
| Airaku-2 | 3 | 8 | 6 | 9 | 7 | 14 | 15 | 10 | 17 | 13 |
| Airaku-3 | 3 | 8 | 6 | 10 | 7 | 13 | 18 | 11 | 17 | 13 |
| Amami-1 | 3 | 8 | 7 | 8 | 10 | 10 | 17 | 9 | 15 | 15 |
| Zensho-9 | 3 | 8 | 7 | 9 | 13 | 16 | 21 | 11 | 12 | 14 |
| Ryukyu-2 | 3 | 8 | 7 | 12 | 10 | 13 | 19 | 8 | 13 | 14 |
| Indonesia-1 | 3 | 9 | 7 | 11 | 10 | 13 | 14 | 9 | 10 | 15 |
| Zensho-12 | 3 | 10 | 7 | 10 | 12 | 18 | 16 | 9 | 16 | 11 |
| Kitazato | 4 | 7 | 7 | 9 | 13 | 12 | 12 | 16 | 14 | 10 |
| Kanazawa | 4 | 7 | 8 | 6 | 14 | 13 | 17 | 12 | 11 | 10 |
| Kyoto-2 | 4 | 7 | 8 | 6 | 16 | 15 | 18 | 16 | 14 | 11 |
| Kyoto-1 | 4 | 7 | 8 | 7 | 12 | 12 | 15 | 15 | 16 | 9 |
| Korea3-2 | 4 | 7 | 8 | 7 | 15 | 14 | 17 | 14 | 15 | 13 |
| Zensho-4 | 4 | 7 | 8 | 8 | 10 | 13 | 20 | 13 | 15 | 10 |
| Hoshizuka-4 | 4 | 7 | 8 | 8 | 12 | 14 | 20 | 13 | 17 | 12 |
| Hoshizuka-1 | 4 | 7 | 8 | 8 | 15 | 14 | 21 | 15 | 13 | 9 |
| Oku-4 | 4 | 7 | 8 | 9 | 13 | 15 | 14 | 13 | 12 | 11 |
| Kusatsu-6 | 4 | 7 | 8 | 9 | 16 | 12 | 18 | 16 | 17 | 16 |
| Zensho-5 | 4 | 7 | 8 | 10 | 12 | 14 | 14 | 18 | 14 | 11 |
| Hoshizuka-5 | 4 | 7 | 8 | 10 | 13 | 14 | 15 | 15 | 12 | 9 |
| Izumi | 4 | 7 | 8 | 11 | 12 | 13 | 13 | 11 | 16 | 9 |
| Keifu-4 | 4 | 7 | 10 | 8 | 11 | 15 | 21 | 13 | 19 | 10 |
| Kusatsu-3 | 4 | 8 | 8 | 9 | 9 | 12 | 13 | 16 | 17 | 11 |

[a] Strains with identical *rpoT* genotypes were discriminated by the combination of STRs with polymorphism. All isolates were divided into independent genotypes.

TABLE 6. Stability of polymorphic microsatellite loci detected among strains subjected to serial passage in nude mice

| Generation | No. of copies at locus: | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | (AC)8a | (AC)8b | (AC)9 | (TA)10 | (AT)15 | (AT)17 | (TA)18 | (GTA)9 |
| Thai53 4th | 11 | 7 | 7 | 11 | 13 | 13 | 20 | 11 |
| Thai53 7th | 11 | 7 | 7 | 11 | 13 | 13 | 20 | 11 |
| Thai53 11th | 11 | 7 | 7 | 11 | 13 | 13 | 20 | 11 |
| Kyoto-1 3rd | 7 | 8 | 7 | 12 | 15 | 12 | 16 | 15 |
| Kyoto-1 5th | 7 | 8 | 7 | 12 | 15 | 12 | 16 | 15 |
| Kyoto-1 7th | 7 | 8 | 7 | 12 | 15 | 12 | 16 | 15 |
| Kyoto-1 8th | 7 | 8 | 7 | 12 | 15 | 12 | 16 | 15 |
| Zensho-4[a] | 8 | 8 | 7 | 10 | 20 | 13 | 15 | 13 |
| Zensho-4 1st | 8 | 8 | 7 | 10 | 20 | 13 | 15 | 13 |
| Zensho-4 2nd | 8 | 8 | 7 | 10 | 20 | 13 | 15 | 13 |
| Zensho-4 3rd | 8 | 8 | 7 | 10 | 20 | 13 | 15 | 13 |
| Zensho-4 4th | 8 | 8 | 7 | 10 | 20 | 13 | 15 | 13 |
| Korea3-2[a] | 7 | 8 | 7 | 15 | 17 | 14 | 15 | 14 |
| Korea3-2 1st | 7 | 8 | 7 | 15 | 17 | 14 | 15 | 14 |
| Korea3-2 2nd | 7 | 8 | 7 | 15 | 17 | 14 | 15 | 14 |
| Korea3-2 3rd | 7 | 8 | 7 | 15 | 17 | 14 | 15 | 14 |
| Korea3-2 4th | 7 | 8 | 7 | 15 | 17 | 14 | 15 | 14 |

[a] Biopsy specimen.

no polymorphism which may have little or no long-term epidemiological value. In bacteria, loci containing a tandem repeat from the microsatellite class have been called simple sequence contingency loci. Altered number of repeats allows reversible on and off states of expression for the corresponding gene. In such an extreme situation, the microsatellite is not suitable for strain identification, epidemiological, or phylogenetic studies (7). The slight allele shift found in a few loci, (CA)6, 23-3, 27-5, and 12-5, was on one hand supposed to be a result of replication slippage, which can lead to an increase or a decrease in the copy number of the repeat element during cell division and inclined to be attributed to the locus with no polymorphism, and on the other hand, there might be different subpopulations of *M. leprae*.

As far as we are concerned, the nine combined microsatellite loci with strong discriminatory capacity which have been also tested for its stability and reproducibility in this study were the most likely and reliable sites for genetic diversity for *M. leprae*. Our previous study showed the 10-copy TTC repeat was the predominant genotype in a leprosy-endemic village (11). Nonetheless, by TTC alone, no conclusion could be drawn that bacteria were from the same population. Meanwhile, the multicase household which was examined the identical genotype for TTC alone does not mean an identical *M. leprae* isolate at all. As a matter of fact, when eight polymorphic loci rather than TTC, (AC)8a, (AC)8b, (AC)9, (TA)10, (AT)15, (AT)17, (TA)18, and (GTA)9, were added to the genotype, *M. leprae* could be further subdivided into two populations. Moreover, all 27 strains could also be identified individually by these combined nine polymorphic loci. Hence, this group of locus is

TABLE 7. Application of potential polymorphic loci for household leprosy patients

| Patients[a] | Sample no. | No. of copies at polymorphic locus[b]: | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | TTC or (AGA)20 | (AC)8a | (AC)8b | (AC)9 | (TA)10 | (AT)15 | (AT)17 | (TA)18 | (GTA)9 |
| Son* | 1 | 13 | 12 | 7 | 10 | 10 | 13 | 15 | 14 | 9 |
| Father | 2 | 13 | 12 | 7 | 10 | 10 | 13 | 15 | 14 | 9 |
| Sister | 3 | 13 | 12 | 7 | 10 | 10 | 13 | 15 | 14 | 9 |
| Brother | 4 | 13 | 12 | 7 | 10 | 10 | 13 | 15 | 14 | 9 |
| Brother | 5 | 13 | 12 | 7 | 10 | 10 | 13 | 15 | 14 | 9 |
| Son* | 6 (left earlobe) | 12 | 10 | 7 | 9 | 10 | 13 | 13 | 11 | 11 |
| | 7 (right earlobe) | 12 | 10 | 7 | 9 | 10 | 13 | 13 | 11 | 11 |
| Brother | 8 (earlobe) | 12 | 10 | 7 | 9 | 10 | 13 | 13 | 11 | 11 |
| | 9 (back) | 12 | 10 | 7 | 9 | 10 | 13 | 13 | 11 | 11 |
| Son* | 10 | 8 | 10 | 7 | 9 | 8 | 16 | **9** | 15 | **11** |
| Brother | 11 | 8 | 10 | 7 | 9 | 8 | 16 | **10** | 15 | **12** |

[a] *, supposed index case.
[b] STRs with variation in the sane TTC repeat strains were indicated by boldface type.

anticipated to a wide application for clinical samples as a preliminary molecular typing system in leprosy epidemiology.

It is thought that tandem repeats located within the regulatory region of a gene can constitute an on/off switch of gene expression at the transcriptional level (7). In spite of this, the intergenic or interpseudogenic location of the microsatellite loci makes it unlikely that a difference in repeat copy number would itself confer any biological advantage, but it is possible that it acts as a marker for some biologically distinct subpopulation of bacteria and that it is carried along during selective expansion of this subpopulation (28). On the basis of this, the repeat unit which was differed by one copy number at (AT)17 and (GTA)9 loci when multicase household were subjected to the nine combined polymorphic loci indicated the presence of two subpopulations of *M. leprae* which have different dominant genotypes and presumably have propagated independently of each other. With regard to the fully matching polymorphism profile in each sample from the same dwelling at each polymorphic microsatellite loci, it was possible that the patients were infected by the same strain of *M. leprae* that survived in some common infectious sources. Taking these into consideration, there might be certain publicly shared infectious sources other than multibacillary cases played a role in the transmission of leprosy.

The unique function of *rpoT* was precisely evaluated in tracking the possible worldwide spread of leprosy and its contribution to the study of anthropology and archaeology (10, 13). We analyzed the other two loci with characteristics common to locus 6-3a in *rpoT* and found no regularity and significance such as *rpoT* had.

Young et al. (28) compared microsatellite profiles for samples taken from different anatomic sites from the same individual and observed that while skin, blood, and nasal cavity samples consistently generated matching profiles, frequent mismatches were found when bacteria in skin and nerves were compared. Mostly, we obtained identical repeats from different skin lesions on the same individual such as samples 6 (left earlobe), 7 (right earlobe) and 8 (earlobe) and 9 (back). Rarely, different skin lesions from the same patient exhibited mismatch profiles at the TTC locus (data was not shown). This probably suggested different bacterial populations coexisted instead of resulting from replication slippage. Nevertheless, the underlying mechanisms are awaiting to be elucidated.

Molecular typing systems are undergoing rapid technical improvements. Advance in the understanding of the biological basis of microbial biodiversity at subspecies levels will improve the conceptual framework required for proper epidemiologic interpretation of disease transmission. Wider application of these systems will surely contribute to the epidemiology of leprosy transmission and, therefore, allow for more effective control and prevention strategies to reach a world free of leprosy.

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# ハンセン病基礎医学研究のトピックス

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ハンセン病はWHOの多剤併用療法の導入により、現在では「治る病気」として認識されるに至った。化学療法の普及と日本国内の新規患者の発症率から、ともすれば安易に考えられがちであるが、ハンセン病をサイエンスの観点から見た時、その重要性は極めて高い状態にある。ハンセン病及びらい菌を取り巻く基礎医学は、今尚未開発のまま取り残されている重要課題が山積みされている。ここでは、基礎医学上の最近のトピックスを紹介したい。

## はじめに

ハンセン病は有史以来、差別と偏見の対象となってきた慢性感染症である。抗酸菌属に分類されるらい菌の感染により発症し、皮膚および末梢神経を主病巣とする。1982年WHOは、多剤併用療法を推奨・開始した。本治療法は、薬剤耐性らい菌を抑制することを目的として開始されたが、当初期待した以上に奏効し、ハンセン病の制圧も視野に入るまでに至った。世界のハンセン病登録者数は順調に減少しているものの、新規ハンセン病患者の抑制は期待された以上には減少していない。真の意味でのハンセン病の制圧には、信頼に足るワクチンの開発が強く望まれる。また、薬剤耐性らい菌は日に日に増加しており、今後世界的レベルでの対処が必要となると想定される。一方、基礎医学分野では、Cole等によりらい菌の全遺伝子配列が決定され、生化学・免疫学等の分野の進展とともに、ハンセン病に関する基礎医学も急速に発展し、有史以来のらい菌の謎も少しずつ解き明かされていくものと期待される。

本稿では、ハンセン病およびらい菌に関する最新の知見を紹介することを目的とする。ハンセン病制圧への一助となれば幸いである。

## I．自然免疫

表皮や粘膜上皮などの被覆上皮は病原体の侵入を防ぐ物理的および生物学的障壁として働くが、病原体がそれらを超えて生体内に侵入した際に最初に発動するのが自然免疫系による生体防御反応である。すなわち、マクロファージなどの食細胞は、感染初期の段階において異物を認識し、貪

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食・殺菌するとともに炎症性サイトカインやインターフェロンなどを産生する。そのような異物の認識に関わる手段として、細胞表面のスカベンジャー受容体やマンノース受容体を初めとするいくつかの機構が知られていたが、近年、Toll様受容体（TLR）がクローニングされ、その自然免疫系における重要性が認識されてきた[1)]。

TLRはⅠ型の膜貫通型受容体であり、現在までに11種が知られている。その発現は、マクロファージなどの免疫担当細胞に限らず、上皮細胞を初めとする生体内の多くの細胞に見い出されることが報告され、病原体が持つ様々な分子パターン（pathogen-associated molecular patterns：PAMPs）を認識するパターン認識受容体（pattern recognition receptors：PRRs）として機能することが明らかにされてきた。

TLRが認識するPAMPsは、大別するとリポ多糖（LPS）やペプチドグリカン（PGN）などの細菌由来成分（TLR1, 2, 5, 6, 11などが認識）と、二本鎖RNA（dsRNA）や非メチル化CpGモチーフを持つDNAなどの核酸成分（TLR3, 7, 8, 9などが認識）とに分けることができる。しかしながら、TLRは機能やリガンドの情報からではなく、ホモロジー検索によって先に遺伝子群がクローニングされたものであり、それらが認識しうるリガンドの全容は未だ明らかではない。また、一つのTLRが認識できるリガンドは必ずしも一種類に限定するものではなく、様々な分子パターンと幅広い親和性を持つと考えた方がより合目的でもある。

刺激前の状態においては、一部のTLRは細胞膜に発現し、その他は細胞内に存在するが、最終的には異物を含んだエンドゾーム膜などに局在変化するものと考えられる。TLRの細胞内ドメインは、インターロイキン1受容体（IL-1R）とホモロジーが高くToll-IL-1R（TIR）ドメインと呼ばれる。TLRが異物を認識すると、このTIRドメインに結合するアダプター分子群が活性化し、シグナルが伝達される。抗酸菌が持つリポアラビノマンナン（LAM）やペプチドグリカン（PGN）は、マクロファージ等が細胞膜に発現しているTLR2がTLR1およびTLR6などと共同することによって認識される。その結果として、TIRドメインに結合するMyD88（myeloid differentiation factor 88）を介したシグナル伝達経路を活性化しNF$\kappa$Bの誘導を経てIL-1やTNF-$\alpha$などの炎症性サイトカイン遺伝子の転写を誘導することが示されている[2)]。

## Ⅱ．マクロファージとサイトカイン

### 1．ハンセン病におけるマクロファージの関与

らい菌は細胞内寄生菌としてマクロファージやシュワン細胞内で増殖する。マクロファージは下等動物から高等動物に至るまで広く存在し、体内に侵入した細菌などを貪食して排除する機能を有する。また、サイトカインなどの免疫調節分子を産生する他、抗原提示能を有し、さらには抗腫瘍作用も持ち合わせるなど多機能細胞である。

ハンセン病はきわめて特異な臨床スペクトラムを呈する疾患である。サイトカイン産生の観点からみると、TT型ではTh1型サイトカインであるIL-2とIFN-$\gamma$が主に発現しており、LL型ではTh2型サイトカインであるIL-4、IL-5、IL-10が強く発現している。マクロファージが主体となり産生するサイトカインはTT型ではIL-1$\beta$、TNF$\alpha$、GM-CSF、IL-6、LL型ではIL-10である。

### 2．マクロファージが産生するサイトカイン

マクロファージはグラム陰性菌のLPSなど細菌菌体成分等の刺激によりIL-1やTNF$\alpha$など炎症性サイトカイン、さらに、IL-6、IL-8、IFN$\alpha$/$\beta$等も産生する。抗酸菌にはLAMのような糖脂質を含む細胞壁菌体成分が存在し、LPSと同様にマクロファージからのサイトカイン産生を誘導する。らい菌を貪食したマクロファージからはTNF$\alpha$やIL-10が産生されるが、LAMで前処理するとマクロファージの活性化が抑制される。マクロファージはマンノースなどの糖に対する受容体やフィブロネクチン等を介してらい菌を認識し貪食する。オプソニン化されたらい菌を補体受容体やFc受容体を通じて貪食する機構もある。その過程でTNF$\alpha$やIL-1を産生する。しかし、*M. bovis* BCGなど他の抗酸菌と比較するとTNF$\alpha$の産生量は低く、その原因はPGL-Ⅰなどの脂質成分によるサイトカイン産生抑制作用によると考えられる。