Analysis of the Leprosy Agents *Mycobacterium leprae* and *Mycobacterium lepromatosis* in Four Countries

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Key Words: Leprosy; Molecular diagnosis; Geographic medicine; *Mycobacterium lepromatosis; Mycobacterium leprae*

Am J Clin Pathol October 2014;142:524-532

DOI: 10.1309/AJCP1GLCBE5CDZRM

ABSTRACT

Objectives: *To differentiate the leprosy agents* Mycobacterium leprae *and* Mycobacterium lepromatosis *and correlate them with geographic distribution and clinicopathologic features.*

Methods: *Species-specific polymerase chain reactions were used to detect each bacillus in archived skin biopsy specimens from patients with leprosy from Brazil (n = 52), Malaysia (n = 31), Myanmar (n = 9), and Uganda (n = 4). Findings were correlated with clinical and pathologic data.*

Results: *Etiologic species was detected in 46 of the 52 Brazilian patients, including 36 patients with* M leprae*, seven with* M lepromatosis*, and three with both bacilli. The seven patients with sole* M lepromatosis *all had tuberculoid leprosy, whereas only nine of the 36 patients infected with* M leprae *exhibited this type, and the rest were lepromatous (*P *< .001). All patients with dual infections had lepromatous leprosy. Of the nine patients from Myanmar, six were test positive: four with* M leprae *and two with* M lepromatosis*. Of the Malaysian and Ugandan patients, only* M leprae *was detected in 27 of the 31 Malaysians and two of the four Ugandans.*

Conclusions: *The leprosy agents vary in geographic distribution. Finding* M lepromatosis *in Brazil and Myanmar suggests wide existence of this newly discovered species. The leprosy manifestations likely vary with the etiologic agents.*

Leprosy, also known as Hansen disease, is one of the oldest human infections that can be traced along global human dispersals during the past 100,000 years[1-3] and possibly to the hominid era millions of years ago.[4] *Mycobacterium leprae* has been known to be the leprosy agent since initial discovery in 1873.[5] In 2008, a new etiologic agent—namely, *Mycobacterium lepromatosis*—was recognized in two patients of Mexican origin who died of diffuse lepromatous leprosy (DLL).[6] Further analyses of 20 genes and pseudogenes revealed a 9.1% genetic difference between the two leprosy bacilli to substantiate a species-level divergence that occurred approximately 10 million years ago.[7] Thus, *M lepromatosis* is also ancient. The 9.1% sequence difference contrasts starkly with the clonal worldwide *M leprae* strains that vary by 0.005%, as revealed by extensive genome sequencing and multilocus typing.[1,2,8]

DLL is a unique, severe form of leprosy initially recognized by Lucio and Alvarado[9] in 1852 and further described by Latapi and Chevez-Zamora[10] in 1948. It is also called diffuse leprosy of Lucio and Latapi,[11,12] leprosy with Lucio's phenomenon,[13,14] or merely Lucio's leprosy. This form of leprosy shows a diffuse cutaneous infiltrate, with no nodule or plaque formation and frequent skin ulceration in the late stage. DLL is predominantly seen in patients from western and central Mexico and the Caribbean countries,[10,15,16] but rare cases have been reported elsewhere, including Asia (India, Iran, Malaysia, and Singapore),[17-21] the Pacific (Hawaii),[22] Europe (France),[23] North America (United States),[24] South America (Brazil),[25,26] and northern Africa (Tunisia).[27] All these reports were based on the clinical and pathologic features prior to recognition of *M lepromatosis*.

The discovery of *M lepromatosis* has led to an analysis of 120 Mexican leprosy cases to determine the etiologic agents using archived biopsy tissue.[28] The study confirmed and differentiated the mycobacteria in 87 cases. Of these, *M lepromatosis* alone caused 55 cases, *M leprae* alone caused 18 cases, and both species together caused 14 cases. *M lepromatosis* caused not only all 13 DLL cases specifically but also more cases of lepromatous leprosy (LL) and other clinical forms of leprosy. This study suggests that *M lepromatosis* is likely the dominant cause of leprosy in Mexico, and it coexists with *M leprae* in endemic areas. Both bacilli may cause dual infections in a patient.

Two recent case studies have independently corroborated this new cause of leprosy. Vera-Cabrera et al[29] reported a case of DLL in a Mexican woman due to *M lepromatosis;* they confirmed the new species and excluded the presence of *M leprae* from biopsied skin tissue by analysis of four genes. Jessamine and colleagues[30] reported a case of LL caused by *M lepromatosis* in a native Canadian man. The patient had manifested polyneuropathy for 2 years before the onset of a skin rash that led to biopsies and the diagnosis.

In addition to Mexico and Canada, *M lepromatosis* has been identified across the Pacific in Singapore, and the two patients who were ethnically Chinese died of dual infection with both leprosy agents.[21,31] In another study,[32] we showed that *M lepromatosis* also caused severe leprosy reactions, another common clinical feature of the disease. Therefore, *M lepromatosis* is the long-elusive second cause of leprosy.

These findings and worldwide reports of DLL indicate possible global existence of *M lepromatosis*. In this study, we examined such a possibility among patients with leprosy from Brazil, Myanmar, Malaysia, and Uganda. A convenient collection of archived skin biopsy samples was tested for both leprosy bacilli, aiming at their signatory gene sequences.

Materials and Methods

Patients, Tissue Sources, and DNA Extraction

This study used a convenient collection of archived skin biopsy tissue specimens from Brazil, Malaysia, Myanmar, and Uganda. There were a total of 96 specimens, with 52 from Brazil (Curitiba and surrounding areas in southern Brazil), 31 from Malaysia (mainly Johor Bahru), nine from Myanmar, and four from Uganda. Each specimen came from one patient with a clinicopathologic diagnosis of leprosy. Various leprosy types were included without selection.

These formalin-fixed, paraffin-embedded (FFPE) tissues were small, typically about 4 × 4 mm on cut surface. The tissue blocks, archived for 1 to 30 years (median 3 years), were retrieved from the original hospitals. They were wrapped individually for containment and sent to Houston for tests. To prevent cross-contamination, blocks from different countries were sectioned in separate batches at a research-only histology laboratory, and the cutting blade and forceps were changed or treated with DNase for each block. Depending on the size of tissue, two to five pieces of a 7-µm section were used for DNA extraction, and for those that tested negative, tissue samples were cut and tested once or twice more to minimize tissue sampling bias.

DNA extraction was done using a tissue kit (QIAamp DNA Mini Kit; Qiagen, Valencia, CA). Tissue sections were deparaffinized by xylene treatment, and the proteins then were digested with proteinase K, spiked with nonspecific carrier DNA, and loaded onto the silica-based mini-column. After washing, DNA was eluted in a buffer for testing. The exceedingly low quantity of DNA from the minute tissue sample allowed only one to three polymerase chain reactions (PCRs) in each case.

PCR and Differentiation of Species

The 16S ribosomal RNA (rRNA) gene, known for all described bacteria (~10,000 species), was selected as the PCR target in view of its conserved and variable regions.[6,33,34] The designs of PCR primers and assays were described previously.[28,31,32] In brief, two rounds of heminested PCRs were used to maximize detection sensitivity: the first-round PCR used primers AFBFO (5′gcgtgcttaacacatgcaagtc) and MLER4 (5′ccacaagacatgcgccttgaag) that were common to all mycobacteria (~150 species); the resulting amplicon, 171 base pairs (bp) in size and usually faint or subdetectable, were diluted (100-fold) and further amplified by two separate second-round PCRs using MLER4 and LPMF2 (5′gtctcttaatacttaaacctattaa) for *M lepromatosis* (142 bp) and MLER4 and LERF2 (5′ctaaaaaatctttttttagagatac) for *M leprae* (135 bp). The thermocycles were as follows: activation of enzyme at 95°C for 2 minutes; 35 cycles of denaturation (95°C for 20 seconds), primer annealing (58°C for 20 seconds for the first-round PCR or 48°C for 20 seconds for the second-round PCR), and extension (72°C for 40 seconds); and final extension for 5 minutes. A regular Taq polymerase was used. The target amplicons were examined by an agarose gel electrophoresis.

The heminested PCRs, with two 35 doubling cycles, enabled detection of as low as one to three copies of the target, an exquisite sensitivity shown previously in an *M leprae* PCR study.[35] The small amplicons suited fragmented DNA extracted from FFPE tissue. The design also ensured high specificity. As defined or noted previously,[6,34,36] the inner forward primers LPMF2 and LERF2 were based on the signatory 16S gene sequences for *M lepromatosis* and *M leprae*, respectively. These sequences were mainly AT nucleotides, and lack of these sequences in all other mycobacteria precluded cross-reaction. Either primer, when

DOI: 10.1309/AJCP1GLCBE5CDZRM

paired with the reverse primer MLER4 that was GC rich and typical for mycobacteria, would not pick up human sequences. As also shown in another earlier *M leprae* PCR study,[34] a primer similar to LERF2 detected *M leprae* only in experimental DNA prepared from cultures as well as tissue DNA from patients with leprosy.

The robust performance of the PCR assay has been noted in our recent studies.[31,32] The method also used each extracted DNA once (in the first-round PCR) for both organisms. To prevent carryover or cross-contamination, we kept benches for PCR setup and amplicon examination separate along with separate sets of equipment and reagents. Good molecular testing practice as required by regulation was also followed.

Efforts were made to sequence representative amplicons for verification of PCR specificity and detection of strain variation among different countries. The DNA sequencing was performed in a separate laboratory using the Sanger method on ABI sequencers (Applied Biosystems, Foster City, CA). A portion of the *M lepromatosis* 16S rRNA gene from a Brazilian sample was deposited in the GenBank as GQ900374.

The above experiments, aimed at species assignment, were performed from 2009 through 2011. In 2013, all test-negative specimens were tested further by addition of a third second-round PCR using MLER4 and common inner primer MLEFO (5′gcaagtcgaacggaaaggtct), as designed earlier.[31,32] This PCR used the same first-round PCR mix saved initially, and the amplicon (156 bp) was sequenced to determine species. The purposes of this second-round common PCR were to detect a potential miss from species-specific PCRs earlier and to search for other mycobacterial agents.

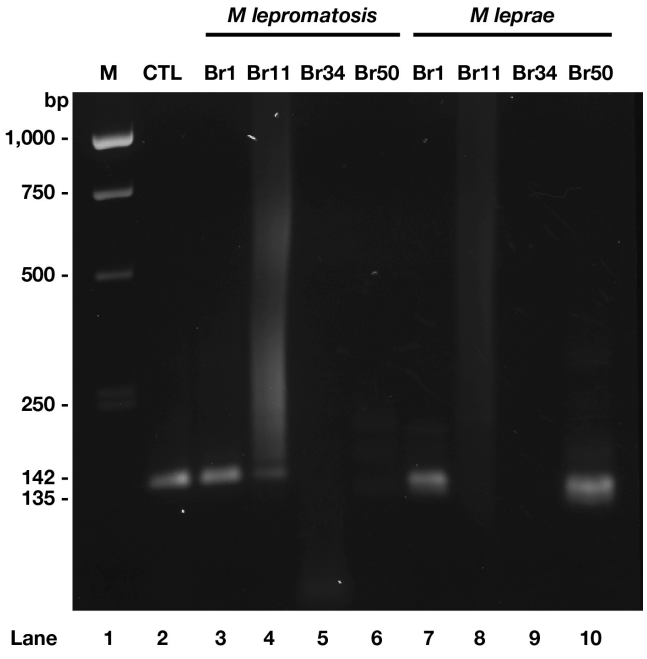

Image 1 Detection of *Mycobacterium lepromatosis* in Brazilian patients with leprosy: lane M for DNA size marker; lane CTL for *M lepromatosis* 142–base pair (bp) amplicon control; specimen Br1 with both *M lepromatosis* and *Mycobacterium leprae* (lanes 3 and 7); specimen Br11 with only *M lepromatosis* (lanes 4 and 8); specimen Br50 with only *M leprae* (135 bp) (lanes 6 and 10); and specimen Br34 negative for both bacilli (lanes 5 and 9).

Correlative Data and Analysis

The clinical and pathologic data included each patient's age and sex, the diagnoses, the description of the lesions, the biopsy date and body site, the load of acid-fast bacilli in the tissue smear or specially stained slides, the duration of tissue archiving (for laboratory quality assurance), and geographic location. These data were filled in by the local clinician and/or pathologist after the PCR analyses in the Houston laboratory had been completed. The histopathology slides were reviewed by at least one pathologist (B.W., X.Y.H., and/or F.M.A.) for diagnostic accuracy. When applicable, the Fisher exact test was used for statistical analysis.

Results

Brazilian Patients

The 52 Brazilian biopsy specimens were obtained from 2004 through 2010. The PCR analyses detected *M leprae* in 36 samples, *M lepromatosis* in seven samples, and both organisms in three; six specimens were negative for both organisms. Thus, among the 46 patients with species-confirmed infections, *M lepromatosis* caused or contributed to 10 (21.7%) infections. **Image 1** illustrates detection of each bacillus, both, or neither in four representative cases.

The clinical and pathologic features of the 52 patients upon etiologic differentiation are shown in **Table 1**. Patients included 31 men and 21 women with a mean age of 50 years. Those with single *M lepromatosis* included six women and one man, which was significantly different from those with *M leprae* infection (12 women and 24 men; $P = .015$). The clinicopathologic diagnoses were also significantly different: all seven patients with *M lepromatosis* manifested tuberculoid leprosy (TL), whereas only nine of the 36 patients with *M leprae* had TL and the rest had LL ($P < .001$). Microscopically, patients in the *M lepromatosis* group had rare to none acid-fast bacilli in the biopsy tissue compared with the heavy burden noted in 24 of 36 patients with *M leprae* ($P = .002$). Among patients with a clinical description of skin lesions, none in the *M lepromatosis* group had nodules (zero of five), whereas 15 of the 33 patients in the *M leprae* group had nodules, the difference being nearly statistically significant ($P = .136$). No trends in the mean

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Table 1
Clinical and Pathologic Features of the Brazilian Patients With Leprosy Sorted by the Etiologic *Mycobacterium*

Features	No. of Patients by *Mycobacterium* Detected				
	Mycobacterium lepromatosis	*Mycobacterium leprae*	Both	Neither	Total
No. of patients (M:F)	7 (1:6)	36 (24:12)	3 (2:1)	6 (4:2)	52 (31:21)
Age, mean (range), y	54.3 (19-91)	51.6 (19-82)	44.0 (40-48)	38.0 (10-65)	49.9 (10-91)
Type of leprosy diagnosis					
Lepromatous leprosy		27	3	2	32
Tuberculoid leprosy	7	9		4	20
AFB load on microscopy					
Multibacillary		24	3	2	29
Paucibacillary (rare)	3	5			8
None identified	4	7		4	15
Skin lesions					
Nodules (± others)		15	3	2	20
Macules, plaques, others	5	18		4	27
Lack of data	2	3			5
Body site of the biopsy					
Face	1	2		2	5
Trunk, chest, abdomen	2	7	1	2	12
Arm, forearm, hand		15	2	2	19
Leg, thigh, ankle, foot	2	10			12
Lack of data	2	2			4
Geographic location					
Curitiba, State of Parana	4	31	2	5	42
Other areas of Parana	2	4	1	1	8
State of Santa Catarina	1	1			2

AFB, acid-fast bacilli.

age, skin biopsy sites, or geographic locations of the patients were noted between the two groups.

The three patients with dual infections accounted for 6.5% of all 46 species-confirmed cases. These patients all had LL with heavy bacillary burden and skin nodules that were features of *M leprae* infection. When these patients were included in the *M leprae* group for comparison with the *M lepromatosis* group for skin lesions, the difference in the presence of nodules was statistically significant (18 of 36 vs zero of five, $P = .056$). As shown in Image 1, the PCR target amplicons of case Br1, a dual infection, were strong to allow sequencing verification of the specificity of both amplicons. In addition, with use of common primers (MLEFO and MLERE) as designed and used earlier,[31,32] a longer 410-bp fragment of the 16S rRNA gene from this case was sequenced (GenBank accession GQ900374), which showed 100% matches with the *M lepromatosis* sequences of a Mexican strain (EU203590) and a Singaporean strain (GQ900372). This result thus suggests conservation of this gene and likely dominance of *M lepromatosis* in this dual infection. Histopathology of Br1 showed dense histiocytic infiltration and heavy bacillary burden on Fite stain **Image 2**. The amplicons of the second case with dual infections were also verified by sequencing analysis. The amplicons of the third cases were too weak to be sequenced directly, but their sizes (142 bp for *M lepromatosis* and 135 bp for *M leprae*) were distinct on gel electrophoresis.

For all seven TL cases with sole *M lepromatosis* infection, weaker PCR amplicons with some background smear compromised sequencing; case Br11 was an example (Image 1). Presumably, these technical issues came from the FFPE effect and the extremely low DNA quantity due to the minute size of the biopsy specimen and the low bacillary burden in tissue. But there should be no doubt about the specificity of the amplicon. Histopathology of one such case (Br14) showed a florid granulomatous reaction, perineural invasion, and rare acid-fast bacilli **Image 3**. In contrast, most specimens with *M leprae* showed the target amplicon strongly, as illustrated in Image 1, lane 10; a representative amplicon from another case was sequenced (153 bp) to show a full match with *M leprae*.

Myanmarese Patients

The nine biopsy specimens from Myanmar, obtained in 2007 and 2008, came from eight patients with LL and one with TL. The eight LL samples were all smear positive (bacteriologic index 2+ to 5+), whereas the TL sample was smear negative. The PCR tests confirmed the etiologic bacilli in six samples, all from patients with LL, including two samples with *M lepromatosis* and four samples with *M leprae*. **Image 4** illustrates detection of *M lepromatosis* in the two samples as well as one sample with *M leprae* and one with neither. The *M lepromatosis* amplicon was also weak but clear.

The patients with *M lepromatosis* were a man (Mr4) and a woman (Mr6) aged 22 and 55 years, respectively,

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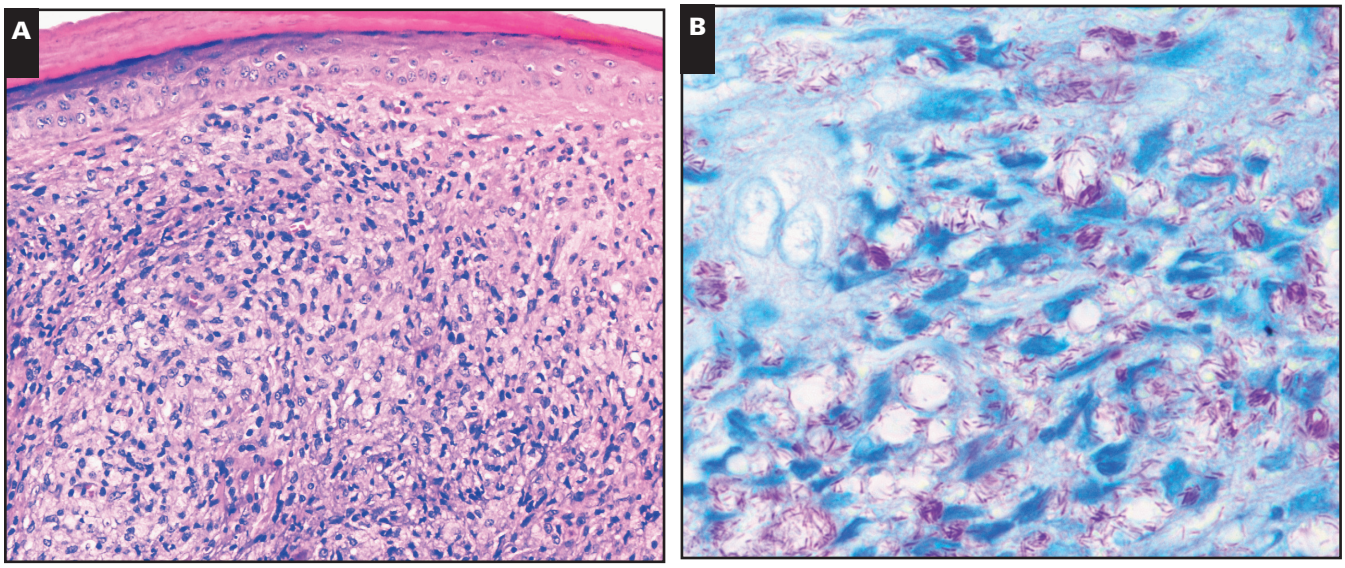
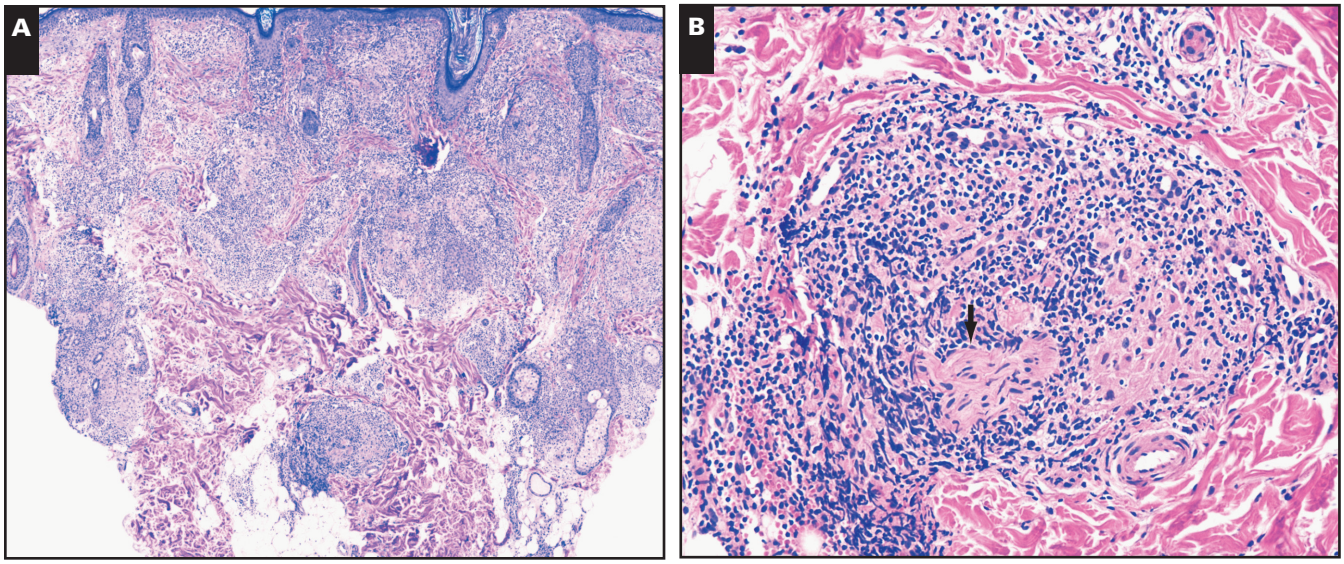


Image 2 Histopathology of dual infection by *Mycobacterium lepromatosis* and *Mycobacterium leprae* in a Brazilian patient (Br1). **A**, Dense histiocytic infiltration (H&E, ×200). **B**, Heavy bacillary burden (Fite, ×1,000).



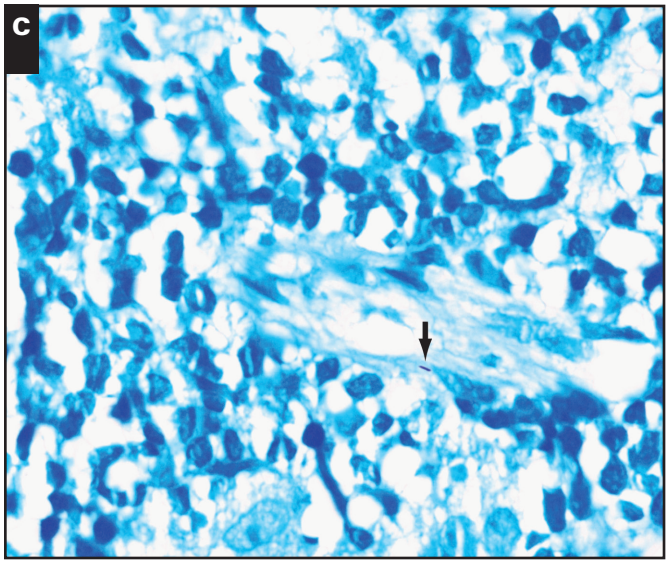


Image 3 Histopathology of *Mycobacterium lepromatosis* infection in a Brazilian patient (Br14). **A**, Panoramic view of the biopsy specimen with dense granulomatous infiltration (H&E, ×40). **B**, Perineural infiltration (arrow) (H&E ×200). **C**, Single acid-fast bacillus (arrow) (Fite, ×1,000).

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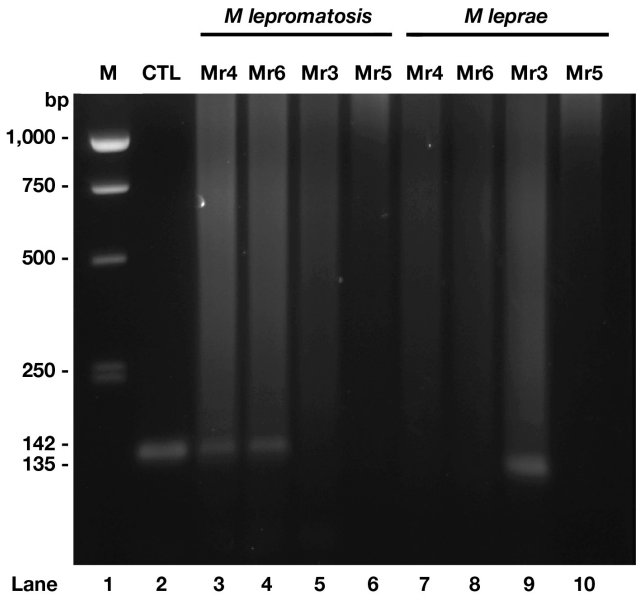

Image 4 Detection of *Mycobacterium lepromatosis* in Myanmar patients with leprosy: lane M for DNA size marker; lane CTL for *M. lepromatosis* 142–base pair (bp) amplicon control; specimens Mr4 and Mr6 positive for *M lepromatosis* (lanes 3 and 4) and negative for *Mycobacterium leprae* (lanes 7 and 8); specimen Mr3 positive for *M leprae* (135 bp) (lane 9) and negative for *M lepromatosis* (lane 5); and specimen Mr5 negative for both bacilli (lanes 6 and 10).

and the biopsy samples were from the ear and arm. It was unknown whether these patients had skin nodules. Histopathologic features of case Mr6 are shown in **Image 5**; they demonstrated dense histiocytic infiltration and heavy *M lepromatosis* burden on Fite stain, findings consistent with LL. The four patients with *M leprae* were three men aged 23, 27, and 27 years and one woman aged 45 years, and the biopsy samples were obtained from the arm (two patients) and chin and waist (one of each). The above results demonstrate the presence of *M lepromatosis* in a region of continental Asia where the prevalence of leprosy has been high.

Patients From Malaysia and Uganda

The 31 specimens from a Malaysian hospital, obtained from 2003 through 2011, came from patients of a few nearby countries: 26 patients from Malaysia provinces, three patients from Indonesia, and one each from Myanmar and Nepal. They included 21 male and 10 female patients with a mean age of 37 years (range, 5-83 years). The PCRs confirmed *M leprae* in 27 (87.1%), including 17 with LL and 10 with TL, and 15 showed a positive bacteriologic index of 1+ to 4+ on smear. No case with *M lepromatosis* was detected. The diagnoses of those unconfirmed cases included two cases of LL and two cases of TL, and three of the four had a negative bacteriologic index.

The four biopsy specimens from Uganda were obtained from 1979 through 1990, and all four patients carried the diagnosis of LL. *M leprae* was detected in two samples, but no *M lepromatosis* was detected. The patients with *M leprae* were a 45-year-old woman and a 50-year-old man. Review of the histopathology revealed florid cutaneous granulomata in the tissues of both *M leprae* cases and one case without etiologic confirmation.

Overall PCR Performance

Of the 96 specimens, the PCRs detected etiologic agent(s) in 81 (84.4%). Three additional specimens, one each from Brazil, Malaysia, and Myanmar, were excluded from final correlative analyses because the clinical and pathologic diagnoses were inconsistent with leprosy and the PCRs were also negative. Among the 81 positive results, 79 were detected by initial species-specific PCRs performed from 2009 through 2011, and two were from recent repeat tests of the 20 negative specimens by addition of a second-round common primer PCR. The common amplicons of the two specimens, both from Brazil and of tuberculoid leprosy with rare or no AFB under microscopy, were sequenced, which matched *M leprae*. Thus, the species-specific PCRs missed these cases, at a miss rate of 2% of all specimens. This result could be explained by the lower melting temperature of the AT-rich specific primers LPMF2 and LERF2; a potential solution would be to extend each primer by adding one or two nucleotides.

Discussion

By using differential PCRs, we have analyzed the etiologic agents of leprosy among patients from Brazil, Malaysia, Myanmar, and Uganda. The results showed dominance of *M leprae* with variable detection of *M lepromatosis* in these countries: while *M lepromatosis* was not detected in all 27 species-confirmed cases from Malaysia or in the two cases from Uganda, the new agent caused or contributed to 10 of 46 cases from Brazil and two of six cases from Myanmar. These results, together with the likely dominance of *M lepromatosis* in Mexico[28] and the detection in Canada[30] and Singapore,[31] suggest wide cross-continental existence of *M lepromatosis*, the second leprosy agent known only for several years.

The design of heminested PCRs has largely resolved the technical challenge in working with an exceedingly low quantity of fragmented DNA from FFPE specimens—truly like picking a needle in a haystack. The PCRs were also highly specific in view of amplicon sequences and no reaction with human DNA. Furthermore, the parallel testing for both acid-fast bacilli that are identical on stain and microscopy ensured test objectivity and allowed us to equally assume the etiologic role and to estimate the relative prevalence of each bacillus

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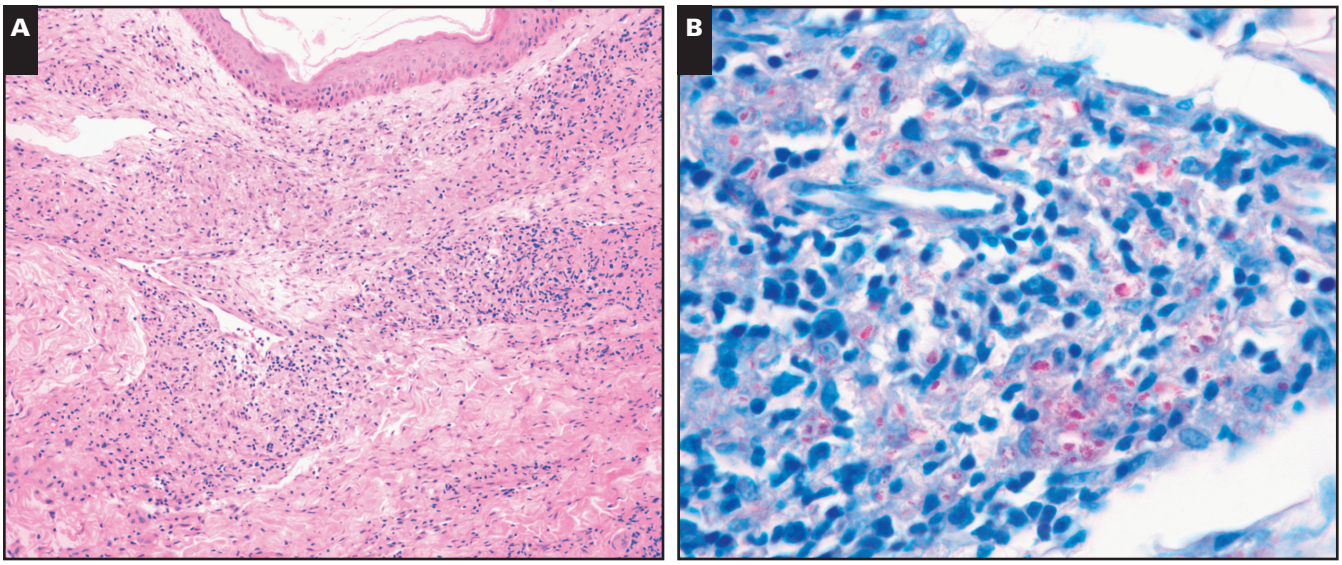

Image 5 Histopathology of *Mycobacterium lepromatosis* leprosy of Myanmar patient Mr6. **A**, Dense histiocytic infiltration of the skin (H&E; ×200). **B**, Heavy bacillary burden (Fite; ×1,000).

in different countries. For instance, finding dominance of *M leprae* in the present study contrasted the dominance of *M lepromatosis* found in our Mexico study. Therefore, this reliable PCR method should be useful for future studies.

As of the end of 2011, Brazil reported 33,955 new cases of leprosy, with an incidence rate of 1.7 per 10,000 population, the highest among all countries worldwide.[37] The most endemic regions were in the north, in the Amazon region and the northeastern part of Brazil, and these areas were a source of disease dissemination to other parts of the vast country.[38] Our detection of *M lepromatosis* in 10 (21.7%) of 46 species-confirmed cases suggests that this agent is likely not rare in southern Brazil. This finding thus raises the question: where did *M lepromatosis* in Brazil originate from?

The origin of the leprosy bacilli has been traced based on recently published data on *M leprae* genomes, *M lepromatosis* discovery, evolution of the leprosy bacilli, and human evolution.[4] In that study, we proposed that the leprosy bacilli (ie, both species and their last common ancestor) have been parasites of humans or early hominids for up to 20 million years, within which they underwent a long adaptive reductive evolution to reach the present lean genomes and strict parasitism. Both bacilli have spread globally along human dispersals.

In Mexico, the century-long record of DLL[9,10,12] and the likely dominance of *M lepromatosis* have led us to the hypothesis that the disease came with the first American settlers from Asia around 13,000 years ago.[28] Finding *M lepromatosis* in Myanmar in this study and in Singapore earlier[32] supports this Asian origin. Finding it in Brazil accords with further American spread from North to Central America, such as Costa Rica, where DLL has been endemic,[15] and to South America, such as the Amazon region of Brazil. The Canadian man infected with *M lepromatosis* had no significant history of exposure or travel to endemic areas,[30] which raises a likelihood of transmission of this agent in Canada, where aboriginal peoples also live. In the Brazilian Amazon, leprosy has been endemic for at least a century[38] and is still prevalent.[39] Thus, should future study find this bacillus and DLL in this region, particularly if also dominant, it would substantiate this hypothesis.

For yet unclear reasons, six of the seven Brazilian patients with single *M lepromatosis* infection were women, in comparison to more men with *M leprae* infection. This sex preference was not observed in the Mexico study, however.[28] The seven patients were all diagnosed with TL with rare or no acid-fast bacilli identified on microscopy, as opposed to mostly LL diagnoses with *M leprae*. This result differed from the Mexico study in which the 55 cases of single-agent *M lepromatosis* infection included 34 diagnoses of LL and 13 of DLL, for a total of 47 (85.5%) cases.[28] Thus, in view of the clinical reports of DLL in Brazil,[25,26] it has yet to be confirmed that *M lepromatosis* was the causative agent. Given the same agent and testing method, why were the clinical manifestations different in the two countries? One potential difference was ethnicity: most southern Brazilians are European descendants, whereas most Mexicans have the native and more recent Spanish heritages. Unfortunately, ethnicity data were not available in either study for analysis. Again, future studies from the Amazon region as well as other areas of ethnically diverse Brazil may offer insight into this aspect. Host immunity and immunogenetic background are well known to affect leprosy manifestations and susceptibility.[16,40]

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Lack of skin nodules is a recognized feature of DLL, based on extensive clinical experience with Mexican patients[9,10,14] and our recent studies that also established *M lepromatosis* as the specific cause of DLL.[6,28,31,32] In addition, the LL and TL cases caused by *M lepromatosis* that accounted for 42 of the 55 cases in the Mexico study also showed rare instances of nodular lesions.[28] In consistence, the Brazilian patients with sole *M lepromatosis* infection did not show skin nodules, although the small number of cases required confirmation. Among patients with *M leprae* infection, however, skin nodules occurred in about half of the Brazilians in this study and in one-third of the Mexicans.[28] The Canadian patient with LL caused by *M lepromatosis* had no skin nodules either, which likely contributed to the 2-year diagnostic delay in this nonendemic country.[30]

The findings on *M leprae* infection have thus far shown consistency—that is, this agent mainly caused LL in 27 of 36 patients from southern Brazil, in 17 of 27 from Malaysia, in four of four from Myanmar, in both patients from Uganda, and in 15 of 18 from Mexico,[28] for a total of 65 (74.7%) of 87 confirmed cases. It is well known that the clinical forms of leprosy vary widely across continents or countries. For instance, in India and Africa, 90% of leprosy cases are tuberculoid; in Mexico, 80% to 90% of cases are lepromatous (LL and DLL); and in Southeast Asia, the two forms are equally distributed.[16] In view of the chronicity of leprosy and variable lead time to diagnosis, some variations in clinical diagnoses and/or stages of infection are not unexpected. However, more cases from various countries need to be examined in the future to verify the *M leprae*–LL specificity and to determine whether *M lepromatosis* and/or dual infection is the main cause of variations.

In summary, the significance of *M lepromatosis* appears beyond Mexico and DLL. It is so far another cause of leprosy in the Americas and Asia. Leprosy, a clinical entity known to humanity for millennia, is caused by *M leprae*, *M lepromatosis*, or both.

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This work was supported in part by a University Cancer Foundation grant from The University of Texas M. D. Anderson Cancer Center (MDACC) and by the National Institutes of Health grant CA16672 for M. D. Anderson's core facilities for DNA analysis and research histology.

Acknowledgments: We thank the staff of our core facilities for assistance in histology and gene sequencing and Bryan Tutt for editorial assistance.

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