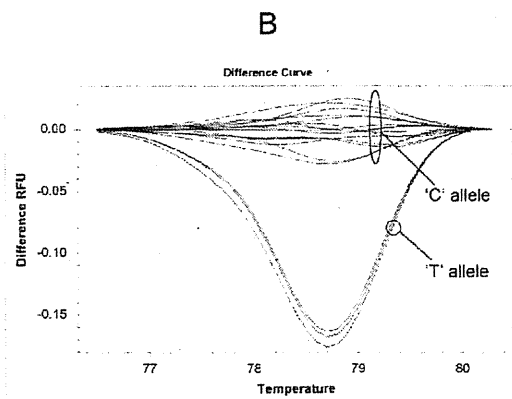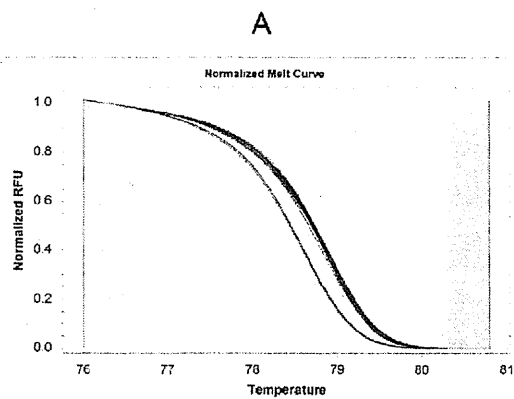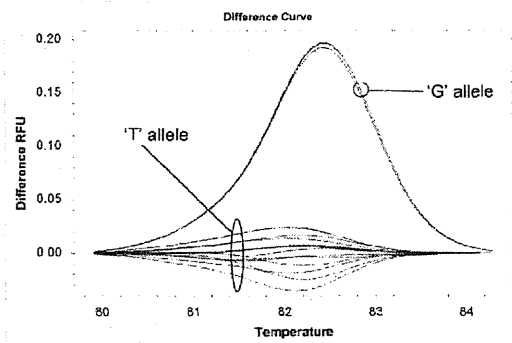A

B
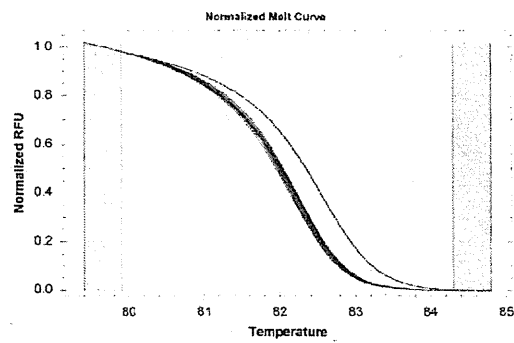
Locus 1
14,676

Locus 2
1,642,875

Locus 3
2,935,685
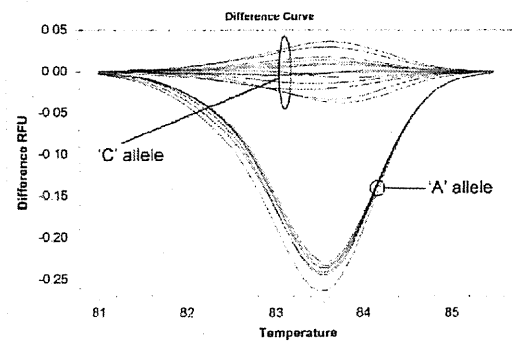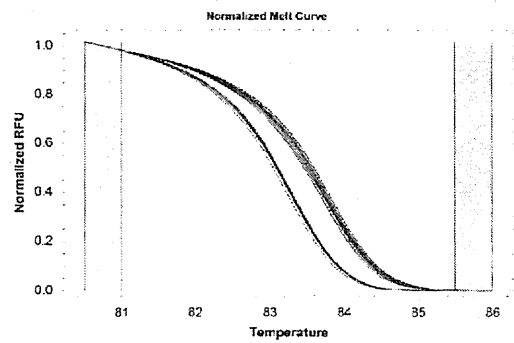
Table 1: Primer sequences for SNP typing by RT-PCR-HRM analysis

SNP Target: Location [a,b]	Primer name	Primer Location [b]	Primer sequence (5'-3')
Locus1: 14,676	HRM14F	14601-14621	TGAACAGTCTCGTAACCGTG
	HRMM14R [c]	14721-14701	CAATGCATGCTAGCCTTAATG
Locus2: 1,642,875	HRM16F	1642813-1642836	CTCGTCACAAATCCGAGTTTGAAT
	HRM16R	1642925-1642902	GTAGTAGTCTTCCAAGTTGTGGTG
Locus3: 2,935,685	HRM29F	2935599-2935616	TGGTGTCGGTCTCCATCC
	HRM29R [d]	2935716-2935699	ACCGGTGAGCGCACTAAG

[a,c,d] per Monot *et al* (28)

[b] per *M. leprae* TN genome sequence (http://genolist.pasteur.fr/Leproma/)

Table 2: Comparison of RT-PCR Cycle threshold C(t) values and estimates of starting quantity

Sample type	Strains	DRDRs					
		$folP1^a$		$rpoB^b$		$gyrA^c$	
		C(t)	SQ(pg)^d	C(t)	SQ(pg)	C(t)	SQ(pg)
ADML^e	NHDP63(0.1pg)	22.02	1000.00	23.61	1000.00	22.87	1000.00
	NHDP63 (1pg)	25.36	100.00	27.41	100.00	26.41	100.00
	NHDP63 (10pg)	28.83	10.00	31.37	10.00	30.28	10.00
	NHDP63 (100pg)	32.29	1.00	35.25	1.00	33.95	1.00
	NHDP63 (1ng)	35.11	0.10	39.1	0.10	37.3	0.10
MFP^f	Airaku-2	26.17	59.97	28.66	51.13	27.22	64.48
	Airaku-3	26.63	43.98	28.99	41.29	28.12	36.43
	Amami	25.54	92.43	28.25	66.69	26.81	83.70
	Hoshizuka-4	29.20	7.60	31.58	7.84	30.55	7.81
	Kusatsu-3	28.61	11.36	31.26	9.60	29.96	11.42
	Kusatsu-6	26.64	43.60	29.13	37.81	28.07	37.61
	Ryukyu-6	27.44	25.24	29.56	28.59	28.53	28.11
	Zensho-2	26.49	48.24	28.85	45.26	27.56	51.82
	Zensho-4	26.84	38.04	29.44	30.91	28.12	36.44
	Zensho-5	27.40	25.95	29.97	22.06	29.05	20.28
	Zensho-9	26.72	41.14	29.39	31.85	28.25	33.60
	Zensho-15	28.03	16.92	30.69	13.80	29.35	16.75
	Gushiken	25.64	86.33	27.75	91.88	26.47	103.63
	Hoshizuka-5	27.74	20.50	29.92	22.67	28.72	24.99
	Indonesia-1	26.89	37.00	29.47	30.27	27.96	40.34
	Korea-3-2	27.48	24.63	29.44	30.96	28.51	28.56
	Thai-53	27.29	27.89	29.84	23.92	28.22	34.26
	Thai-311	25.80	77.56	28.18	69.74	26.77	85.79

^{a,b,c} The % efficiency, correlation of coefficient of determination R^2 and slope are 95.4%, 0.997 and 3.373 for $folP1$; 83%, 0.998 and 3.811 for $rpoB$ and 91.3%, 0.997 and 3.549 for $gyrA$

^{d} Starting quantity (SQ); all DNA templates were tested in triplicate for each target and quantitated according to the NHDP63 DNA standard curve.

^{e} ADML: Armadillo derived *M. leprae*.

^{f} MFP: Mouse foot-pad derived *M. leprae*.

Table 3: RT-PCR-HRM assay for *M. leprae* DRDR mutation detection (DRDR assays)

Sample type	Template	*folP1* Reported DRDR genotype[a]	HRM Cluster[b]	HRM Cluster verification by sequencing[c]	*rpoB* Reported DRDR genotype	HRM Cluster	HRM Cluster verification by sequencing	*gyrA* Reported DRDR genotype	HRM Cluster	HRM Cluster verification by sequencing
ADML[d]	NHDP63	No mutation	WT		No mutation	WT		No mutation	WT	No mutation
MFP[g]	Airaku-2[e]	P(CCC)55L(CTC)	WT	No mutation	S(TCG)456L(TTG)	WT	No mutation	No mutation	WT	No mutation
	Airaku-3	T(ACC)53I(ATC)	V		No mutation	WT		No mutation	WT	
	Amami	P(CCC)55L(CTC)	V		No mutation	WT		No mutation	WT	
	Hoshizuka-4	P(CCC)55S(CTC)	V		S(TCG)456L(TTG)	V		A(GCA)91V(GTA)	V	
	Kusatsu-3	T(ACC)53I(ATC)	V		No mutation	WT		No mutation	WT	
	Kusatsu-6	P(CCC)55L(CTC)	V		D(GAT)441Y(TAT)	V		No mutation	WT	
	Ryukyu-6	No mutation	WT		No mutation	WT		A(GCA)91V(GTA)	V	
	Zensho-2	P(CCC)55L(CTC)	V		No mutation	WT		No mutation	WT	
	Zensho-4[f]	T(ACC)53I(ATC)	V		S(TCG)456L(TTG)	V		A(GCA)91V(GTA)	V	A(GCA)91V(GTA) No mutation
	Zensho-5[h]	P(CCC)55L(CTC)	V	Pro(CCC)55Leu(CTC) Thr(ACC)53Ile(ATC) No mutation	S(TCG)456L(TTG)	V	S(TCG)456L(TTG)	No mutation	WT	No mutation A(GCA)91V(GTA)
	Zensho-9	No mutation	V	Pro(CCC)55Leu(CTC)	H(CAC)451Y(TAC)	V	H(CAC)451Y(TAC)	No mutation	WT	
	Zensho-15	Unknown	V	Pro(CCC)55Leu(CTC)	Unknown	V	S(TCG)456L(TTG)	Unknown	V	A(GCA)91V(GTA)
	Gushiken	No mutation	WT		No mutation	WT		No mutation	WT	
	Hoshizuka-5	No mutation	WT		No mutation	WT		No mutation	WT	
	Indonesia-1	No mutation	WT	No mutation	No mutation	WT	WT	No mutation	WT	No mutation
	Korea-3-2	No mutation	WT		No mutation	WT		No mutation	WT	
	Thai-53	No mutation	WT		No mutation	WT		No mutation	WT	
	Thai-311	No mutation	WT		No mutation	WT		No mutation	WT	

[a] per Matsuoka, M. (22) , sequenced verified

[b] HRM Cluster is designated as WT for wild type or V for variant target sequence. NHDP63 with same sequences as in TN strain was considered as WT.

[c] Representative samples of each of the clusters were verified by PCR product sequencing and the genotypes detected are indicated.

[d] ADML: Armadillo derived *M. leprae*.

[e] The HRM clustering results were not concordant with expected genotypes for both *rpoB* and *folP1* genes for Airaku-2 (22). VNTR strain typing was performed for this strain which confirmed that it was indeed not Airaku 2 (44). However, the designation Airaku-2 was retained during the course of the study and in all Tables in this report.

[f] HRM assay for *gyrA* DRDR separated this strain from wild type and other mutants; DNA sequencing results showed C and T mixed allele (See **Figure 2**, *gyrA*, orange curves and **Figure 3**).

[g] MFP: Mouse foot-pad derived *M. leprae*

[h] HRM assay for *folP1* and *gyrA* DRDRs separated this strain from wild type and other mutants which share the same genotype, DNA sequencing show minor mixed alleles at codon 53 and 55 in *folP1* and codon 91 in *gyrA* (See **Figure 2**, blue curve in *folP1*-panel B and orange curve in *gyrA*-panel B and **Figure 3**).

Table 4: Sensitivity and specificity of HRM detection of DRDR mutations in clinical biopsy DNA samples

Target	Classification	Number of samples	Number of samples[a]				
			<0.1 pg	0.1-1 pg	1-10 pg	10-100 pg	100-1000 pg
folP1	True wild type	112	1	16	37	50	8
	True mutant	5	2	2	1	0	0
	False wild type	0	0	0	0	0	0
	False mutant	4	2	1	1	0	0
	Total	121	5	19	39	50	8
	Sensitivity[b]	100%	100%	100	100	na[d]	na
	Specificity[c]	96.50%	33%	94.10%	97.40%	100%	100%
rpoB	True wild type	115	2	12	34	59	8
	True mutant	0	0	0	0	0	0
	False wild type	0	0	0	0	0	0
	False mutant	6	3	3	0	0	0
	Total	121	5	15	34	59	8
	Sensitivity	na	na	na	na	na	na
	Specificity	95.04%	40%	80%	100%	100%	100%
gyrA	True wild type	115	2	16	36	55	6
	True mutant	0	0	0	0	0	0
	False wild type	0	0	0	0	0	0
	False mutant	6	4	2	0	0	0
	Total	121	5	18	36	55	6
	Sensitivity	na	na	na	na	na	na
	Specificity	95%	20%	88.90%	100%	100%	100%

[a] Number of samples grouped according to the starting concentration SQ (pg)

[b] Sensitivity is defined as number of true mutants/(number of true mutants + number of false wild types)

[c] Specificity is defined as number of true wild type/(number of true wild types + number of false mutants)

[d] 'na': not applicable as no true mutants were present in the samples set.

Table 5: Comparison of RT-PCR Cycle threshold C(t) values and estimates of starting quantity

Sample type	Strains	SNP Locus1[a]		Locus2[b]		Locus3[c]	
		C(t)	SQ(pg)[d]	C(t)	SQ(pg)	C(t)	SQ(pg)
ADML[e]	NHDP63(1ng)	21.95	1000.00	21.97	1000.00	21.01	1000.00
	NHDP63 (100pg)	25.40	100.00	25.34	100.00	24.24	100.00
	NHDP63 (10pg)	29.09	10.00	28.73	10.00	27.76	10.00
	NHDP63 (1pg)	32.70	1.00	32.24	1.00	31.14	1.00
	NHDP63 (0.1ng)	36.50	0.10	35.75	0.10	34.43	0.10
	Thai-53	27.14	26.40	27.42	20.71	26.09	29.48
	3039	27.16	25.97	27.15	24.60	26.16	28.16
	BR4923	27.25	25.36	27.18	24.36	26.24	26.79
MFP[f]	Airaku-2	26.35	58.92	26.30	53.48	25.25	53.64
	Airaku-3	27.08	37.60	27.14	30.74	25.90	34.52
	Amami	26.13	67.50	25.85	72.29	25.01	63.49
	Hoshizuka-4	29.95	6.47	29.52	6.19	28.73	5.00
	Kusatsu-3	29.17	10.40	29.32	7.11	28.00	8.22
	Kusatsu-6	27.30	32.85	27.16	30.02	26.08	30.61
	Ryukyu-6	28.17	19.35	27.90	18.30	26.58	21.74
	Zensho-2	27.28	33.35	27.73	20.67	25.62	41.90
	Zensho-4	27.70	25.73	27.26	28.14	26.41	24.31
	Zensho-5	27.82	23.87	27.65	21.76	27.02	16.04
	Zensho-9	27.38	31.21	27.12	30.92	26.35	25.33
	Zensho-15	28.68	14.11	28.45	12.79	27.64	10.52
	Gushiken	25.90	77.88	25.96	67.15	24.39	97.04
	Hoshizuka-5	28.04	20.87	28.03	16.79	26.59	21.60
	Indonesia-1	27.86	23.70	27.40	25.64	25.93	33.77
	Korea-3-2	27.93	22.40	28.05	16.58	26.46	23.56
	Thai-53	27.42	30.50	27.32	26.91	26.19	28.33
	Thai-311	25.95	75.25	25.88	70.67	24.84	71.41

[a,b,c] The % efficiency, correlation of coefficient of determination R^2 and slope are 90.3%, 0.994 and 3.579 for Locus 1; 91.7%, 0.998 and 3.538 for Locus 2 and 91.7%, 0.999 and 3.539 for Locus 3
[d] Starting quantity (SQ); All DNA templates were tested in triplicate for each target and quantitated according to the NHDP63 DNA standard curve.
[e] ADML: Armadillo derived *M. leprae.*
[f] MFP: Mouse foot-pad derived *M. leprae.*

Table 6: RT-PCR-HRM assay for *M. leprae* SNP typing. A: The expected RT-PCR-HRM cluster patterns for the three loci which generate four SNP types. **B**: SNP typing of MFP-LRC and armadillo derived reference strains based on the cluster pattern defined in **A**.

A

SNP type	Locus1	Locus2	Locus3
	Allele[a]/HRM Cluster[b]		
Type 1	C/1	G/2	A/2
Type 2	C/1	T/1	A/2
Type 3	C/1	T/1	C/1
Type 4	T/2	T/1	C/1

B

Sample type	Strain	HRM Cluster			SNP type
		Locus1	Locus2	Locus3	
MFP	Airaku-2	1	2	2	1
	Airaku-3	1[d]	2[d]	2	1[c]
	Amami	1	1	1	3
	Hoshizuka-4	1	1	1	3
	Kusatsu-3	1	1	1	3
	Kusatsu-6	1	1	1	3
	Ryukyu-6	1	1	1	3
	Zensho-2	1	1	1	3
	Zensho-4	1	1	1	3
	Zensho-5	1	1	1	3
	Zensho-9	1	1	1	3
	Zensho-15	1	1	1	3
	Gushiken	1	2	2	1
	Hoshizuka-5	1	1	1	3
	Indonesia-1	1[d]	2[d]	2	1[c]
	Korea-3-2	1	1	1	3
	Thai-53	1	2	2	1
	Thai-311	1[d]	2[d]	2	1[c]
ADML	Thai-53	1	2	2	1
	3039	1	1	2	2
	BR4923	2	1	1	4
	NHDP63	1	1	1	3

[a] The SNP alleles are indicated (28).
[b] NHDP63 allele is assigned to Cluster 1 and the alternative allele to Cluster 2.
[c] The SNP types are different from previous reports (22).
[d] Amplicons sequence verified

Table 7: Concordance of PCR-RFLP and RT-PCR-HRM methods for *M. leprae* SNP typing of clinical isolates.

Sample	Sample type	PCR-RFLP[a]		SNP type	HRM Cluster[b]		
		Locus 2/*CviKI*	Locus3/*BstUI*		Locus1	Locus2	Locus3
NP101	Clinical	-	-	1	1	2	2
NP103	Clinical	-	-	1	1	2	2
NP108	Clinical	-	-	1	1	2	2
NP109	Clinical	-	-	1	1	2	2
NP110	Clinical	-	-	1	1	2	2
NP111	Clinical	-	-	1	1	2	2
NP112	Clinical	-	-	1	1	2	2
NP114	Clinical	-	-	1	1	2	2
NP116	Clinical	-	-	1	1	2	2
NP117	Clinical	-	-	1	1	2	2
NP118	Clinical	-	-	1	1	2	2
NP119	Clinical	-	-	1	1	2	2
NP120	Clinical	-	-	1	1	2	2
NP123	Clinical	-	-	1	1	2	2
NP106	Clinical	-	-	1	V[c]/1	2	2
NP113	Clinical	-	-	1	V[c]/1	2	2
NP102	Clinical	+	-	2	1	1	2
NP104	Clinical	+	-	2	1	1	2
NP115	Clinical	+	-·	2	1	1	2
NP122	Clinical	+	-	2	1	1	2
NP124	Clinical	+	-	2	1	1	2
NP105	Clinical	+	-	2	1	1	2
NP121	Clinical	+	-	2	1	1	2
NP125	Clinical	nd[d]	-	2	1	V[c]/1	2
NP107	Clinical	nd[d]	-	1	1	2	2
NHDP63	ADML	+	+	3	1	1	1
Thai53	ADML	-	+	1	1	2	2
BR4923	ADML	+	+	4	2	1	1

[a] PCR-RFLP assay (31)

[b] NHDP63 allele is assigned to Cluster 1 and the alternative allele to Cluster 2.

[c] V: HRM automatically called these three strains NP106, NP113 and 125 into a different cluster (variant). When the melting curves were manually examined, NP113 and NP125 were in the same cluster as that of NHDP63, while NP106 appeared to belong to a different cluster. Locus1 amplicons of NP106 was sequenced, and the SNP allele, C was same as that of NHDP63 and TN strains.

[d] not determined due to low amount of amplicon

1

REVIEW ARTICLE

Current status of leprosy: Epidemiology, basic science and clinical perspectives

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ABSTRACT

Leprosy has affected humans for millennia and remains an important health problem worldwide, as evidenced by nearly 250 000 new cases detected every year. It is a chronic infectious disorder, caused by *Mycobacterium leprae*, that primarily affects the skin and peripheral nerves. Recent advances in basic science have improved our knowledge of the disease. Variation in the cellular immune response is the basis of a range of clinical manifestations. The introduction of multidrug therapy has significantly contributed to a decrease in the prevalence of the disease. However, leprosy control activities, including monitoring and prevention programs, must be maintained.

Key words: diagnosis, disability, leprosy, *Mycobacterium leprae*, social stigma.

INTRODUCTION

Leprosy, or Hansen's disease, is a chronic infectious disease caused by the acid-fast bacterium *Mycobacterium leprae*. Norwegian physician Gerhard Armauer Hansen identified the bacillus in the patients in 1873, making leprosy the first disease ascribed to a bacterial origin. Leprosy usually affects the dermis of the skin and peripheral nerves, but has a wide range of clinical manifestations. It can be progressive and cause permanent damage if left without treatment. Divided into paucibacillary (TB; tuberculoid pole) or multibacillary (MB; lepromatous pole), depending on the bacillary load, the disease manifests first in discoloration of the skin and then in rashes and nodules. The introduction of dapsone (diphenyl sulfone, DDS) in 1941 brought the first effective therapy, and multidrug therapy (MDT) was introduced by the World Health Organization (WHO) in 1981 to limit the development of drug resistance. Endemic leprosy has declined markedly and the disease is now rare in most industrialized countries. It is still a major public health problem in developing countries, where hundreds of thousands of new cases are diagnosed each year. In many of these countries, social stigmatization is an additional burden. Therefore, it is important that control activities continue if the disease burden and damaging impacts of leprosy are to be reduced. Dermatologists should be familiar with leprosy and other diseases needed for differential diagnosis.

EPIDEMIOLOGY

The WHO publishes an annual report on the worldwide incidence of leprosy, including the number of new cases, prevalence and disabilities.[1] The detection of new cases by the WHO has declined from 514 718 in 2003 to 244 796 in 2009, but the rate of decrease is getting smaller each year. Among 244 796 new cases in 2009, 16 countries that reported 1000 or more new cases accounted for 93% of the total. These countries and the number of cases detected in 2009 are: India (133 717 cases), Brazil (37 610 cases), Indonesia (17 260 cases), Bangladesh (5239 cases), the Democratic Republic of the Congo (5062 cases), Ethiopia (4417 cases), Nepal (4394 cases), Nigeria (4219 cases), Myanmar (3147 cases), the United Republic of Tanzania (2654 cases), Sudan (2100 cases), Sri Lanka (1875 cases), the Philippines (1795 cases), China (1597 cases), Madagascar (1572 cases) and Mozambique (1191 cases).

The proportion of new cases with multibacillary leprosy ranged from 32.70% in the Comoros in Africa to 95.04% in the Philippines. The proportion of females among newly detected cases ranged from 6.50% in Ethiopia to 59.11% in the Central African Republic. The proportion of children among new cases ranged from 0.60% in Argentina to 30.30% in Papua New Guinea. Grade 2 disabilities in new cases ranged from 1.45% in Liberia to 22.8% in China. As the number of new cases declines, the damaging

1

impact of the disease on the physical, social and economic well-being of individuals and families affected by leprosy are also expected to decline.

Very few new leprosy patients are registered in developed countries. When leprosy is detected, it is primarily found among immigrants from countries where the disease is still endemic. There is an association between the incidence of leprosy and socioeconomic factors such as gross national product (GNP), personal housing expenditures and the number of persons per household, suggesting that improvements in socioeconomic conditions greatly contribute to the reduction of leprosy.[2] The proportion of children under the age of 15 years among newly detected cases would be a good indicator of the situation in a country/region. Similarly, the proportion of cases with grade 2 and visible disabilities among newly detected cases would be a reflection of early detection and treatment.

BACTERIOLOGY AND GENOMICS

Mycobacterium leprae is an obligate intracellular parasite that cannot be cultivated *in vitro*. It grows very slowly with an approximate generation time of 12–14 days. The inability to cultivate *in vitro* and the lack of animal models have been major disadvantages for leprosy research. However, the availability of the *M. leprae* genome sequence has contributed to knowledge of the disease. The first genome sequence of *M. leprae*, completed in 2001,[3] revealed that only half of the small genome contains protein-coding genes, while the remainder consists of pseudogenes and non-coding regions (Fig. 1). The number of pseudogenes is much larger in the *M. leprae* genome than in other mycobacteria,[4] and the number and proportion are exceptionally large in comparison with other pathogenic and non-pathogenic bacteria and archaea.[5,6] Many of the *M. leprae* pseudogenes are the result of stop codon insertions thought to be caused by the dysfunction of sigma factors or the insertion of repetitive sequences derived from transposons.[7–9] Despite this genetic damage, a specialized intracellular environment free from evolutionary competition has allowed the organism to survive.[3,10,11] It has been speculated that *M. leprae* has lost over 1500 genes from its genome and that non-coding regions are functionally silent and useless.[12] However, analyses have demonstrated that some of the pseudogenes and non-coding regions are highly expressed at the RNA level, and that expression of these RNA in clinical samples
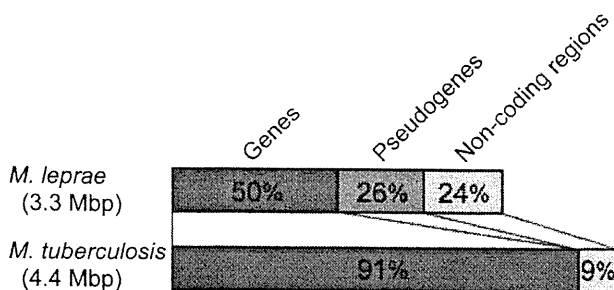
shows varying patterns among patients, suggesting as yet unknown functions.[13–16]

Single nucleotide polymorphisms (SNP) and short or variable number tandem repeats have been used for *M. leprae* genotyping. SNP analysis revealed four primitive subtypes of *M. leprae* and the number is increasing as the analysis progresses.[17–19] Some reports have also presented the possibility of dual infections or phenotypically distinct strains of *M. leprae*; however, these situations are still somewhat obscure.[20,21]

TRANSMISSION AND PATHOLOGY

It is evident that humans are the major reservoir of *M. leprae* infection, while naturally occurring infection has been reported in wild animals, including the nine-banded armadillo and several species of primates.[22–32] A recent study found that the same genotypic strain of *M. leprae* was detected at high incidence in wild armadillos and leprosy patients in the southern USA, suggesting that leprosy may be a zoonosis in regions in which armadillos serve as a reservoir.[33]

Although transmission of *M. leprae* is not entirely understood, it is thought that long-term exposure of the respiratory system to airborne droplets is the main route of infection.[34,35] *M. leprae* is not very virulent, meaning that most people affected with leprosy are non-infectious, probably because the bacilli remain within the infected cells. Multibacillary patients, however, excrete *M. leprae* from their nasal mucosa and skin.[36] Close and repeated contact with these patients is also a source of transmission. Upon MDT treatment, however, the patients rapidly lose infectivity.

Even if infected, a long incubation period is required before clinical manifestation. The long incubation period of leprosy was demonstrated by an SNP analysis of an *M. leprae* genome derived from one of four spontaneous leprosy cases in chimpanzees. The chimpanzee was infected with *M. leprae* during infancy in West Africa, but the pathogenic signs of leprosy did not appear for at least 30 years.[30]

Mycobacterium leprae primarily infects histiocytes (or tissue macrophages) in the dermis and Schwann cells in the peripheral nerves. The unique tropism for peripheral nerves can lead to deformities even after the pathogen is successfully treated. The outcome of infection and clinical manifestation depend on the cellular immunity of the host, which is the first line of defense against *M. leprae* infection. There is a relationship between clinical manifestation and cytokine profiles within the skin lesions. T-helper cell (Th)1 cytokines, such as interleukin (IL)-2 and γ-interferon, play important roles in cellular immune responses in paucibacillary leprosy. Th2 cytokines, including IL-4, IL-5 and IL-10, augment humoral immune responses and predominate in multibacillary leprosy. Thus, there is an inverse correlation in the cytokine profiles that create the basis of paucibacillary and multibacillary leprosy.

Mycobacterium leprae should be recognized by the innate immune system and phagocytized by host macrophages. Toll-like receptor (TLR)2, in conjunction with TLR1, recognizes the cell wall lipids of *M. leprae* and subsequently activates innate immune responses.[37,38] However, some bacilli escape this initial attack of innate immunity and successfully parasitize the phagosome of macrophages. CORO1A, an actin-binding scaffold protein in the cell membrane of host cells, inhibits the phagosome/lysosome fusion, thereby helping the pathogen escape digestion.[38–40]

Figure 1. Only half of the *Mycobacterium leprae* genome contains functional genes. The percentage of functional genes, pseudogenes and non-coding regions are illustrated for *M. leprae* and *Mycobacterium tuberculosis* genomes.

M. leprae
(3.3 Mbp)
50% 26% 24%

M. tuberculosis
(4.4 Mbp)
91% 9%

Genes Pseudogenes Non-coding regions

2

Mycobacterium leprae parasitization of macrophages occurs in a foamy or enlarged phagosome filled with lipids.[40,41] Because it is aerobic, it may survive in a granuloma environment with a relatively low oxygen tension gradient using lipids and fatty acids as carbon sources.[42] *M. leprae* creates a lipid-rich phagosome environment that is favorable for its survival.[43] Adipose differentiation-related protein (ADRP) and perilipin expression, which contribute to lipid intake, significantly increase following *M. leprae* infection. Infection also has a pronounced effect on Schwann cell lipid homeostasis via regulation of lipid droplet biogenesis and traffic, which favors *M. leprae* intracellular survival.[44]

It was long thought that leprosy might have a strong host genetic component. With the use of gene expression profiling, gene expression patterns associated with host immune response in lesions of human leprosy have been clarified.[45] Genes belonging to the leukocyte immunoglobulin-like receptor (LIR) family were significantly upregulated in lesions of lepromatous patients suffering from the disseminated form of the infection.[45] A genome-wide search for loci affecting the susceptibility to leprosy mapped a susceptibility locus to chromosome 6q25-q26.[46] There is a close relationship between leprosy susceptibility and SNP in the genes encoding tumor necrosis factor (TNF)-α and IL-10.[47]

CLINICAL FEATURES

Leprosy is a systemic disease that primarily affects the skin, nerves and eyes. *M. leprae* infection induces diverse clinical manifestations depending on the host immune responses. Paucibacillary leprosy is a milder disease characterized by few (≤5) hypopigmented, anesthetic skin lesions. The multibacillary form is associated with multiple (>5) skin lesions, nodules, plaques, thickened dermis or skin infiltration, and in some instances, involvement of the nasal mucosa, resulting in nasal congestion and epistaxis. The involvement of certain peripheral nerves may also be noted. In most cases of both paucibacillary and multibacillary disease, the diagnosis is straightforward. However, the small proportion of suspected cases that do not exhibit anesthetic patches require examination by a specialist to find other cardinal signs of the disease, including nerve involvement and a positive laboratory test for acid-fast bacilli.

Patients commonly present with weakness or numbness as the result of a peripheral-nerve lesion, or a burn or ulcer in an anesthetic hand or foot. In typical multibacillary leprosy, diffuse infiltration of the skin is evident. There may be many lesions that are not hypoaesthetic, while only a few hypopigmented lesions with reduced sensation are seen in paucibacillary patients. Careful inspection of the entire body is important. The great auricular nerve, ulnar nerve, median nerve, radial-cutaneous nerve, posterior tibial nerve and lateral popliteal nerve are frequently involved with enlargement, with or without tenderness, and standard regional patterns of sensory and motor loss.[48] Neuritic leprosy in India and Nepal is characterized by asymmetrical involvement of peripheral nerve trunks without visible skin lesions.[49–51]

The Ridley–Jopling classification system,[52] based on the *M. leprae*-specific immunological resistance status of the host, is clinically relevant and widely used, although the WHO only distinguishes between paucibacillary and multibacillary for simplicity of use in endemic countries. Ridley–Jopling divided the disease into six categories based on dermatological, neurological and histopathological findings: indeterminate (I), tuberculoid (TT), borderline tuberculoid (BT), mid-borderline (BB), borderline lepromatous (BL) and lepromatous (LL) (Fig. 2). TT leprosy can be associated with rapid and severe nerve damage, whereas LL is associated with chronicity and long-term complications. Borderline disease is unstable and can be complicated by lepra reactions as described in the "Lepra Reactions" section.
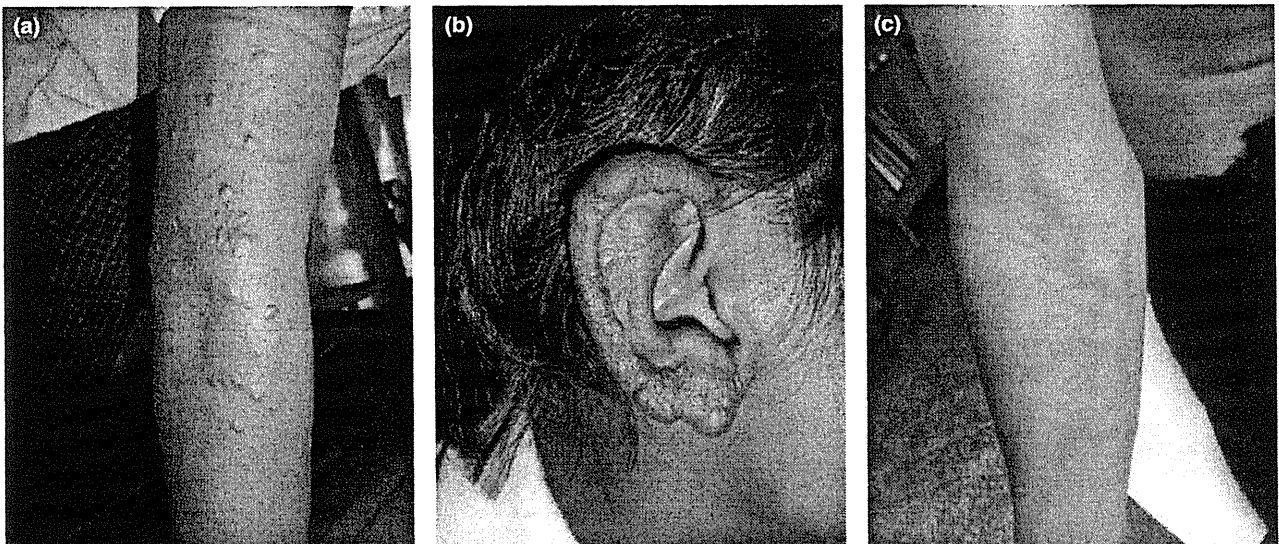

Figure 2. Typical dermatological views of leprosy patients. A multibacillary case (lepromatous) showing multiple nodules in the arms (a) and ears (b), and a paucibacillary case (borderline tuberculoid) with large erythema annulare, with discoloration in the middle of the lesion accompanied by loss of sensation (c).

DIAGNOSIS AND LABORATORY TESTS

Leprosy exerts systemic effects in addition to skin lesions, which is evident in the infiltration of bacilli into the nasal mucosa, bones and other organs of multibacillary patients.[53] Severe skeletal lesions, the hallmark of lepromatous leprosy, have been observed in excavated skeletal remains,[54–58] and *M. leprae* DNA has been isolated from such lesions (Fig. 3).[59] Eye damage is frequently seen in multibacillary patients resulting from both nerve damage and direct bacillary invasion.[60] Typically, lagophthalmos is caused by involvement of the zygomatic and temporal branches of the facial nerve. Other facial nerve damage, such as involvement of the ophthalmic branch of the trigeminal nerve, causes anesthesia of the cornea and conjunctiva, resulting in dryness and the risk of ulceration.

A diagnosis of leprosy is made based on cardinal signs such as hypopigmented or reddish patches with definite loss of sensation, thickened peripheral nerves and acid-fast bacilli in slit-skin smears or biopsy materials.[61,62] Smear and biopsy samples are subjected to acid-fast staining in addition to conventional histopathological diagnosis in order to demonstrate the presence of mycobacterium; however, bacilli are not usually detected in paucibacillary cases. The presence of neural inflammation is a histological characteristic of leprosy that can differentiate it from other granulomatous disorders. The polymerase chain reaction (PCR) is a sensitive method for the detection of *M. leprae* DNA that is widely used for differential diagnosis in advanced countries, although it cannot determine if viable organisms are present because DNA can persist long after microorganisms are dead.[15,30,59,63] Serum antibodies against *M. leprae* phenolic glycolipid-I (PGL-I) are found in multibacillary patients and some household contacts, although its specificity is relatively low.[30,64–66] Non-endemic countries do not usually consider leprosy during the differential diagnosis of skin lesions; however, it should be considered in a case of peripheral neuropathy or persistent skin lesions if patients are from endemic countries. Late diagnosis leads to continued transmission and increased risk of disability.[67,68]
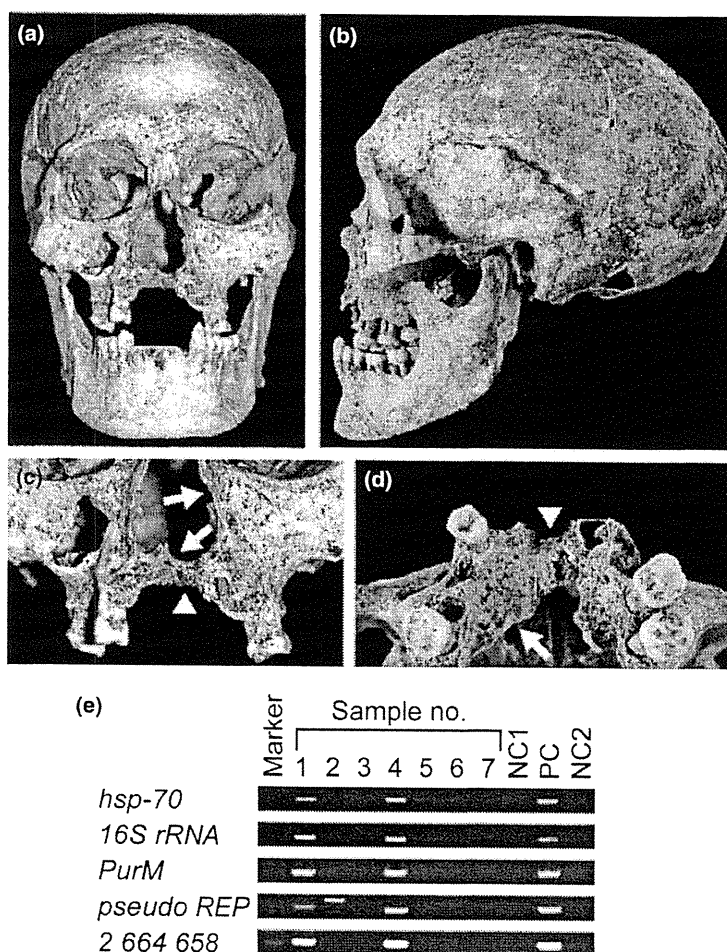

Figure 3. Skeletal lesions of leprosy and isolation of lesion-associated *Mycobacterium leprae* DNA.[59] Frontal view (a) and left side view (b) of archaeological skeletal remains showing erosive deformity of the nasal aperture and disappearance of the anterior nasal spine (arrows) and severe atrophy of the alveolar bone in the maxilla/palatal process with loss of anterior teeth (arrowheads) in panels (c) and (d). Polymerase chain reaction detection of *M. leprae* DNA from skeletal samples (samples 1–4). Samples 5–7 were taken from other skeletons found in the same cemetery, which had no leprous changes as a negative control. *M. leprae* DNA was detected in sample 1 (maxillary palate) and 4 (fibula) (e).

4

TREATMENTS

The implementation of MDT for leprosy treatment has been successful over the past three decades. The WHO has designed two easy-to-use blister pack medication kits for paucibacillary and multibacillary patients. The kits contain enough medication for 28 days and are supplied at no cost to registered patients. The treatment for paucibacillary patients include daily doses of 100 mg DDS and a monthly dose of 600 mg rifampicin (RFP) over a 6-month period. Multibacillary patients are administrated 100 mg DDS and 50 mg clofazimine (CLF) once a day in addition to monthly administration of 600 mg RFP and 300 mg CLF for 12 months. Treatment is usually automatically terminated at the end of the proscribed regimen because, in public health terms, it is reasonable to conclude that infectiousness is unlikely after starting MDT (Fig. 4).[69] Many countries, however, prefer longer treatments, especially for
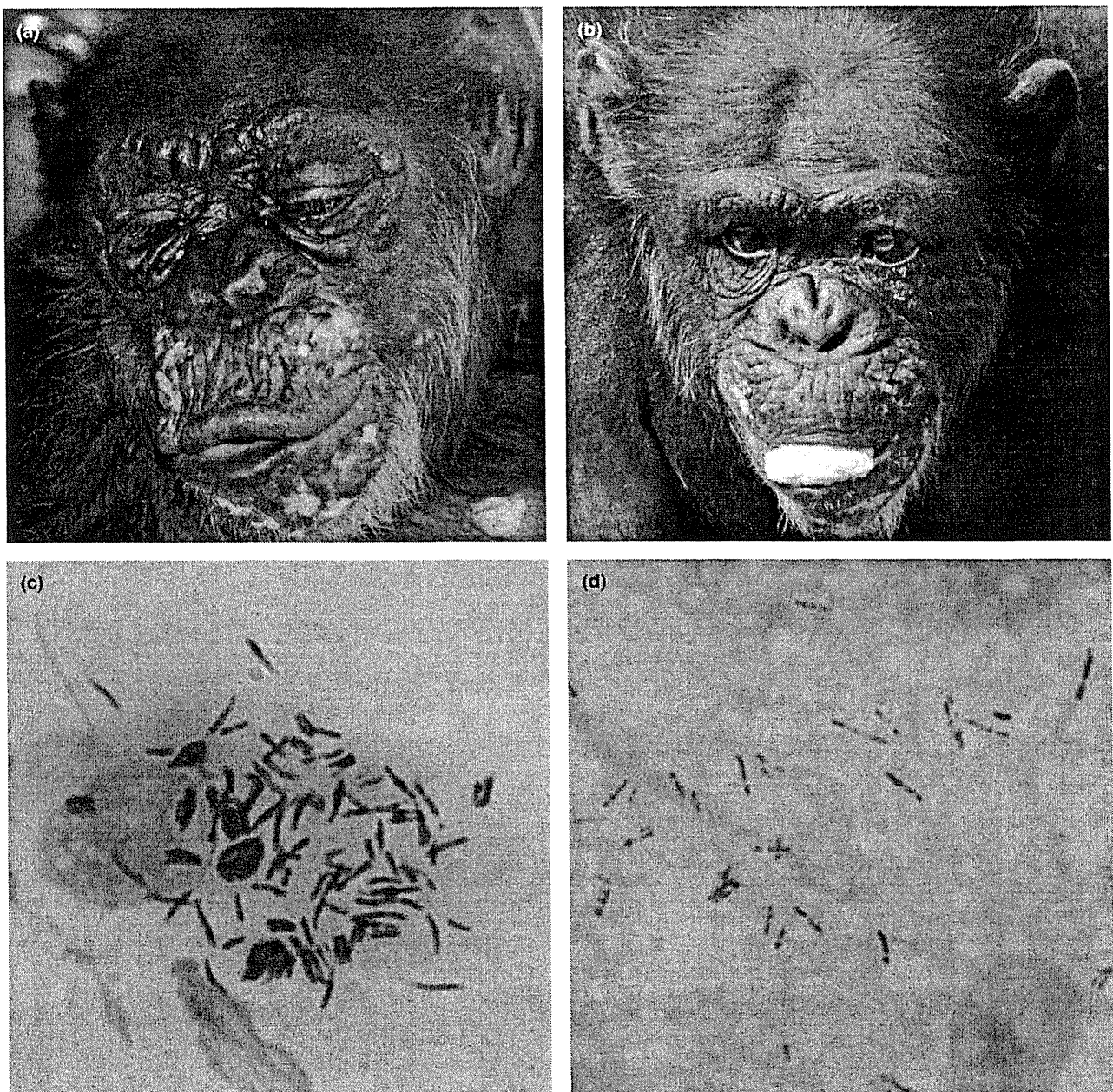

Figure 4. Female chimpanzee at leprosy diagnosis (a) and 3 months after the initiation of multidrug therapy (MDT), showing significant improvement of facial lesions (b).[30] Intact *Mycobacterium leprae* bacilli before treatment (c) fragmented and showed a granular staining pattern 6 months after MDT (d).

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5

multibacillary cases. Although there has been little standard monitoring of clinical outcomes and relapse rates, accurate diagnosis of relapse requires clinical, bacteriological and histopathological evidence.[70]

Rifampicin is an effective bactericidal agent against *M. leprae*. Within a few days of administrating a single 600-mg dose to multibacillary patients, the bacilli are no longer viable when inoculated into mouse footpads.[71] DDS is bacteriostatic or weakly bactericidal against *M. leprae* and was the mainstay leprosy treatment for many years until widespread resistant strains appeared. CLF binds preferentially to mycobacterial DNA and exerts a slow bactericidal effect on *M. leprae* by inhibiting mycobacterial growth. Skin discoloration ranging from red to black, is one of the most troublesome side-effects of CLF, although the pigmentation fades slowly in most cases after withdrawal. A characteristic ichthyosis is also some times evident. Other effective chemotherapeutic agents against *M. leprae* include ofloxacin (OFLX), minocycline (MINO), levofloxacin (LVFX), sparfloxacin (SPFX), moxifloxacin (MFLX) and clarithromycin (CAM).[72]

As with most chemotherapies, drug-resistant strains are becoming a problem in leprosy, which is a potential threat to the success of current leprosy control efforts. Dapsone resistance is associated with missense mutations in the *folP1* gene encoding dihydropteroate synthase.[73,74] Resistance to RFP is induced by a mutation in *rpoB*, which encodes DNA-dependent RNA polymerase subunit-b.[75] PCR analysis can provide a simple assessment for possible susceptibility to these drugs.[73,74]

LEPRA REACTIONS

Lepra reactions (or reactional states) are acute inflammatory complications that occur in treated or untreated leprosy and often present as medical emergencies. There are two major clinical types of lepra reactions that affect 30–50% of all leprosy patients.[76–78] Severe inflammation associated with these reactions results in nerve injury accompanied by subsequent loss of sensation, paralysis and deformity. The different types of reactions appear to have different underlying immunological mechanisms; however, the factors that initiate them are unknown.

Reversal reactions (type 1 reactions) manifest as erythema and edema of dermal lesions and tender peripheral nerves with rapid loss of nerve function. It generally occurs during the first several months of treatment, and occasionally after MDT is completed.[79,80] Treatment is aimed at controlling acute inflammation, easing pain,
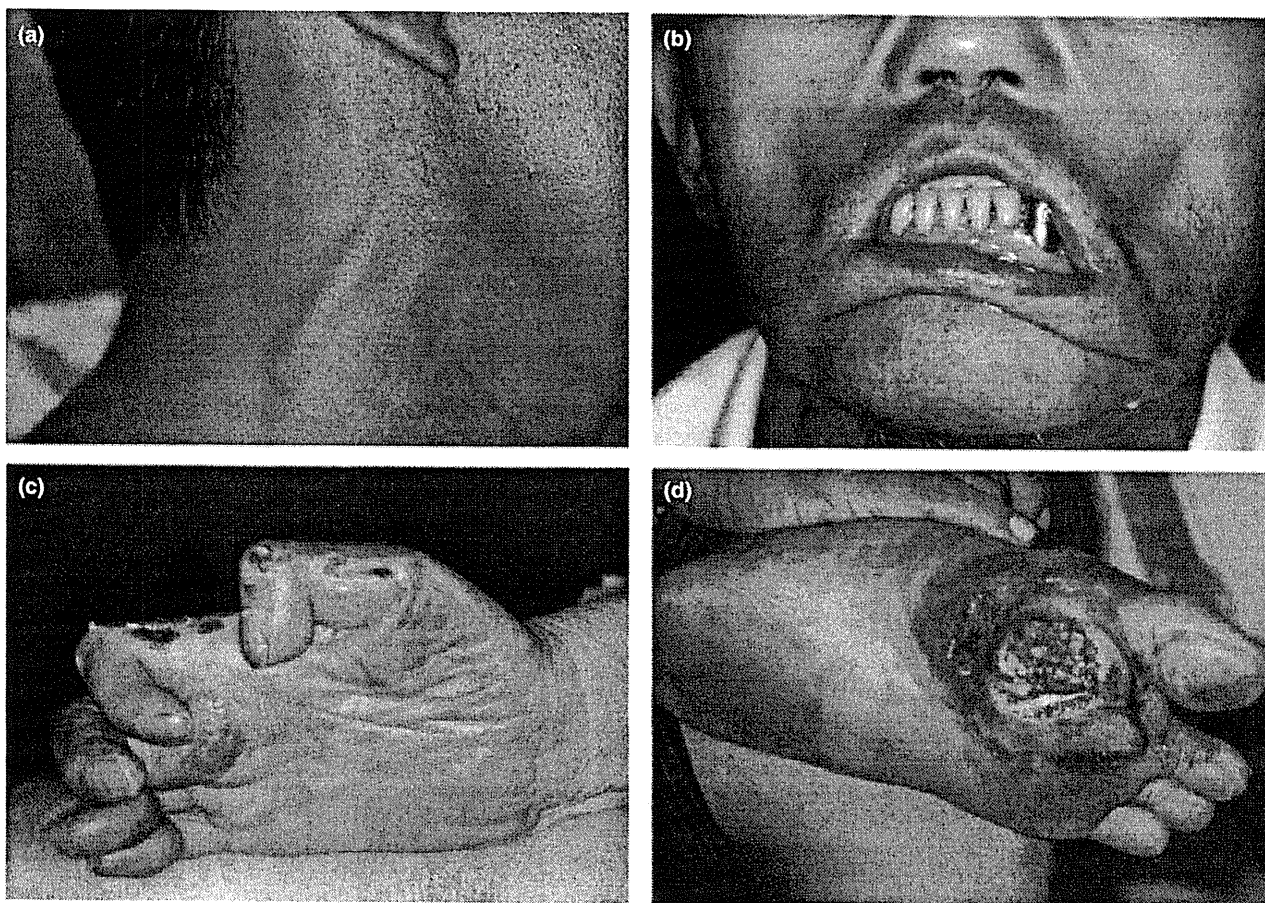

Figure 5. Leprosy with peripheral nerve damage. Swelling of the great auricular nerve (a), facial nerve paralysis (b), dropped wrist, clawed fingers with stiff joints due to ulnar and median nerve damage (c), and foot ulceration due to loss of sensation (d).

6

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reversing nerve and eye damage, and reassuring the patient. Standard courses of corticosteroids have been used to treat patients for several weeks to months. Erythema nodosum leprosum (ENL or type 2 reactions) occurs in lepromatous and borderline lepromatous patients with higher bacterial loads in their lesions.[81] ENL can begin during the first or second year of treatment. Patients are febrile with skin nodules accompanied by iritis, neuritis, lymphadenitis, orchiitis, bone pain, dactylitis, arthritis, and proteinuria that is difficult to treat.[82] CLF has an anti-inflammatory effect on ENL, and thalidomide is better than steroids in controlling ENL, although thalidomide is not available in many countries because of its teratogenic effects.[83] The use of monoclonal antibodies or inhibitors of TNF-α, as used in rheumatoid arthritis, Crohn's disease and psoriasis, seems to be a logical choice for treatment, but more evidence is needed.[84]

DISABILITY AND STIGMA

Leprosy is a leading cause of permanent physical disability among communicable diseases. The disease and its associated deformities have been responsible for social stigmatization and discrimination against patients and their families in many societies. If unchecked, the disease gradually spreads over the entire body, attacks the soft tissue of the nose and throat, impairs vision and damages the nervous system. The morbidity and disability associated with leprosy are secondary to nerve damage (Fig. 5). Ultimately, the extremities become deformed and paralyzed, and may fall off after repeated but unperceived injuries. Therefore, timely diagnosis and treatment of the patient, before nerve damage has occurred, is extremely important in preventing disabilities. Management of lepra reactions and neuritis is also effective in preventing or minimizing the development of further disabilities.

The occurrence of leprosy in families has led to the misinterpretation that the disease is hereditary. The progressive symptoms and sometimes lethal secondary infections probably led to the assumption that patients are beyond medical support and that death is inevitable. In many societies, public stigmatization and exclusion coexist, and in some countries, the stigma is promoted by legislation against leprosy patients.[85] The accumulation of misnomers and misunderstandings have triggered unreasonable reactions in people, which have been difficult to overcome.

Self-awareness is crucial if the patient is to minimize damage. Treatment and/or surgical management, including reconstructions, should be provided for ulcers, and it is important that the patient understand the need for daily self-care and inspection for trauma.[86,87] Protective footwear and other tools are available to help patients improve their abilities and quality of life.[88] Community-based rehabilitation programs and other socioeconomic rehabilitation are required to support patients and families.[89]

CONCLUSIONS AND FUTURE PERSPECTIVES

Leprosy has affected humans for millennia. However, the MDT regimen recommended by the WHO has had a significant impact in reducing the global burden of leprosy, and research activities have led to increased knowledge of M. leprae genomic structure and host responses. Health-care workers and researchers should continue to support the intensive implementation of the elimination strategy and address issues related to the detection of M. leprae-infected individuals as a matter of urgency. Sustained quality patient care that is equitably distributed, affordable and easily accessible is still needed. A goal of the WHO is to bring institutional and management changes that strengthen the operational capacity of leprosy control programs. Improvement is needed in efforts to provide appropriate information to societies, dermatologists and patients. M. leprae is a very unique microorganism. It is expected that basic research for leprosy can be sustained.

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Chimpanzees used for medical research shed light on the pathoetiology of leprosy

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Leprosy is a chronic infectious disorder caused by *Mycobacterium leprae*, which mainly affects skin and peripheral nerves. It is classified as either paucibacillary or multibacillary based upon clinical manifestations and slit-skin smear results. It is speculated that leprosy develops after a long latency period following *M. leprae* infection. However, the actual time of infection and the duration of latency have never been proven in human patients. To date, four cases of spontaneous leprosy have been reported in chimpanzees who were caught in West Africa in infancy and used for medical research in the USA and Japan. One of these chimpanzees was extensively studied in Japan, and single-nucleotide polymorphism analysis for the *M. leprae* genome was conducted. This analysis revealed that the chimpanzee was infected with *M. leprae* during infancy in West Africa and the pathognomonic signs of leprosy appeared after at least 30 years of incubation. Analysis of leprosy in chimpanzees can contribute not only to medical research but also to the understanding of the pathoetiology of leprosy.

Leprosy is caused by a chronic infection with *Mycobacterium leprae* and has afflicted humans for millennia. Leprosy is a systemic disease that primarily affects the skin, nerves and eyes. The diverse clinical manifestations of the disease are produced by variations in host immune responses [1]. *M. leprae* is an obligate intracellular parasite that cannot be cultivated *in vitro*. The inability to cultivate *in vitro* and the lack of animal models have been major disadvantages for leprosy research. The genome sequence of *M. leprae* has revealed that only half of the small genome contains protein-coding genes, while the remainder consists of pseudogenes and noncoding regions [2,3]. However, analyses have demonstrated that some of these pseudogenes and noncoding regions are highly expressed at the RNA level. In clinical samples, these RNAs show varying expression patterns among patients, which suggests they have yet unknown functions [4–7]. The analysis of single-nucleotide polymorphisms (SNPs) revealed four primitive subtypes of *M. leprae*, but the number is increasing as the analysis progresses [8–10].

Multibacillary (MB) patients excrete bacilli from their nasal mucosa and skin [11], making close and repeated contact with these patients, directly and/or indirectly, a potential source of transmission. It is believed that clinical manifestations are only apparent after many years of incubation [12,13]. Although serum antibodies against phenolic glycolipid (PGL)-I have been widely evaluated in diagnosis and community surveys, there have been some arguments for their specificity [14–16]. Therefore, there is no definitive method that can be used to prove the existence of subclinical infection in humans.

The *M. leprae* bacterium was first described in modern literature in 1873, prior to the first description of *Mycobacterium tuberculosis* in 1882. Nevertheless, despite the passing of more than 130 years since its discovery, methods for *in vitro* cultivation of *M. leprae* have still not been established, and there is no effective animal model for the human disease. Therefore, the processes of infection, dormancy and disease activation of *M. leprae* remain unclear.

Since primates are humans' closest relatives, studying human diseases in primates is sometimes helpful when trying to understand the nature of diseases. Naturally acquired leprosy cases have been reported in mangabey monkeys and cynomologus macaques [17–20]. Among primates, the chimpanzee is considered to be an anthropoid as it is genetically known to be very similar to humans. In fact, the genetic difference between humans and chimpanzees is only 1.23% of genomic DNA [21]. For this reason, many infectious diseases that humans acquire are infectious in chimpanzees as well. Many of those infectious diseases are contagious from humans to chimpanzees and *vice versa*. For example, HIV, HBV

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and HCV are infectious diseases that are common in both humans and chimpanzees [22-26]. With this genetic similarity in mind, and keeping ethical considerations of using chimpanzees as subjects for animal research in mind, it can be argued that it is unfortunate for chimpanzees to be used as experimental animal models because they are so very similar to humans.

Cases of naturally acquired leprosy in chimpanzees

There have only been four reports of leprosy in chimpanzees in the literature (TABLE 1). All four chimpanzees were brought from Africa when they were infants for the purpose of being used for medical studies. The first three cases of leprosy were diagnosed solely by clinical and pathological evaluation, and *M. leprae* DNA was not identified.

The first reported case was a male chimpanzee who was captured in Sierra Leone, West Africa [20,27-29]. He was one of eight chimpanzees being used for experiments on bovine leukemia virus infections. After 2 months of virus infection in 1975, a leprosy-like skin lesion (macular rash) appeared and was followed by a progressive maculopapular rash with a crust that covered his abdomen and the medial aspect of his thighs. The other seven chimpanzees used for the same experiment did not develop gross or microscopic lesions. After that, his entire trunk and limbs were covered with a rash and nodular thickening of the ear margins appeared. Lepromatous leprosy was diagnosed due to acid-fast bacteria that were identified in biopsy specimens of the granulomas. The infection progressed with appearance of nodular lesions in the lower lip, nostrils, nasal septum, eyebrows, carpus and scrotum. No specific treatment for leprosy was administered. The chimpanzee died inadvertently following sedation with phencyclidine and ketamine 33 months after the first appearance of the lesions. A necropsy was performed 2 h after death [29]. Marked atrophy of skeletal muscles, alopecia and diffuse thickening of the skin of the hands, feet and digits were observed as gross lesions. Microscopically, there were diffuse and multifocal infiltrations of foamy histiocytes with acid-fast bacilli in the dermis, nasal mucosa, epiglottis, lung interstitium and parenchyma, liver, spleen, kidneys, lymphnodes, peripheral nerves, both eyes (especially the sclera, cornea, ciliary body and iris) and testicular tunics.

In 1989, the second and third chimpanzees were diagnosed with leprosy after they had both been held in research facilities in the USA for many years [30-33]. The second case was a male who was brought from Africa when he was approximately 2-years-old [31]. The chimpanzee began self-mutilating his digits at 7 years. At 9 years, he had a positive reaction to an intradermal tuberculin test and was treated with antibiotics. However, after several examinations, it was revealed that there was no evidence of tuberculosis. At 13 years, his tuberculin test results again showed positive, but no evidence of active tuberculosis was found. At 18 years, he developed nodular and papular eruptions of his eyelids, face, ear margins, lips, distal portion of the penis and scrotum. Histologic evaluation of the cutaneous lesions revealed granulomatous dermatitis consisting predominantly of foamy histiocytes containing acid-fast bacilli. A diagnosis of borderline leprosy was made on the bases of clinical and histopathological findings and bacterial indices. Retrospective evaluations of tissue sections of an amputated finger revealed leprosy with neural involvement. Serum PGL-I antibodies were above baseline. A multidrug treatment (MDT) regimen was started as recommended by the WHO study group for human patients, and was continued for 4 years [34]. A total of 6 months after the start of treatment, a severe leprae reaction developed with the subject manifesting pain and marked impairment of locomotion. Although treatment with prednisone and aspirin restored quadrupedal locomotion and some climbing activity, he sustained permanent neurologic and musculoskeletal dysfunction.

The third case was a male chimpanzee who was imported to the USA when he was approximately 3 years old [31-33]. He had three episodes of ulcerative gingivitis of unknown origin. When he was 26-years-old, he initially developed a persistent clear nasal discharge and chronic areas of epidermal erosions, which were followed by a development of coalescent nodules in the skin of the supraorbital area, lips, chin, ear and scrotum. He was diagnosed as having subpolar lepromatous to borderline leprosy by histological examination. Intracellular aggregates of acid-fast bacilli were found in the liver histiocytes. Antibodies against lipoarabinomannan (LAM) and PGL-I were markedly elevated. He was treated with MDT, but died suddenly 1.5 years later when recovering from anesthesia with ketamine. Necropsy revealed that the immediate cause of death was heart failure secondary to acute, severe myocardial necrosis and hemorrhage.

Gormus *et al.* measured serum anti-PGL-I and anti-LAM antibodies in 160 chimpanzees housed in two research facilities in the USA [30].

Case number	Place of birth	Sex	Name	Place of diagnosis	Age of onset (years)	Locations of affected lesions	Histological diagnosis	Acid-fast bacilli	Anti-PGL-I	*M. leprae* DNA	Treatment	Purpose of import	Remarks	Ref.
1	Sierra Leone, West Africa	Male	Unknown	USA	5–7	Nostrils, nasal septum, nares, lower lip, eyebrows, scrotum and carpus	Lepromatous type leprosy	Yes	NT	NT	None	Bovine leukemia virus innoculation	Died 33 months after diagnosis	[20,27–29]
2	Africa	Male	Kevin	USA	7 or 18	Eyelids, face, ear margins, lips, hands, feet, arms, legs, penis and scrotum	Borderline lepromatous leprosy	Yes	Positive	NT	MDT	Not known	Self-multilation of digits, hyperglobulinemia	[30,31]
3	Africa	Male	Brian	USA	28	Multiple skin lesions	Lepromatous type leprosy	Yes	Positive	NT	MDT	Isoniazid pharmacology study, administration of SV40 peptide	Ulcerative gingivitis of unknown origin. Died from heart failure after anesthesia	[30–33]
4	Sierra Leone, West Africa	Female	Haruna	Japan	31	Eyebrows, lips, abdomen, forearms and crus	Lepromatous type leprosy	Yes	Positive	Positive	MDT	Hepatitis research	Symptoms developed after 30-year incubation period	[35]

Table 1. Reported leprosy cases in wild-born chimpanzees.

MDT: Multidrug treatment; NT: Not tested; PGL: Phenolic glycolipid.