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Use of Protein Antigens for Early Serological Diagnosis of Leprosy▽

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Leprosy is a chronic and debilitating human disease caused by infection with the *Mycobacterium leprae* bacillus. Despite the marked reduction in the number of registered worldwide leprosy cases as a result of the widespread use of multidrug therapy, the number of new cases detected each year remains relatively stable. This indicates that *M. leprae* is still being transmitted and that, without earlier diagnosis, *M. leprae* infection will continue to pose a health problem. Current diagnostic techniques, based on the appearance of clinical symptoms or of immunoglobulin M (IgM) antibodies that recognize the bacterial phenolic glycolipid I, are unable to reliably identify early-stage leprosy. In this study we examine the ability of IgG within leprosy patient sera to bind several *M. leprae* protein antigens. As expected, multibacillary leprosy patients provided stronger responses than paucibacillary leprosy patients. We demonstrate that the geographic locations of the patients can influence the antigens they recognize but that ML0405 and ML2331 are recognized by sera from diverse regions (the Philippines, coastal and central Brazil, and Japan). A fusion construct of these two proteins (designated leprosy IDRI diagnostic 1 [LID-1]) retained the diagnostic activity of the component antigens. Upon testing against a panel of prospective sera from individuals who developed leprosy, we determined that LID-1 was capable of diagnosing leprosy 6 to 8 months before the onset of clinical symptoms. A serological diagnostic test capable of identifying and allowing treatment of early-stage leprosy could reduce transmission, prevent functional disabilities and stigmatizing deformities, and facilitate leprosy eradication.

Cases in which *Mycobacterium leprae* infection manifests to cause leprosy present as a bacteriologic, clinical, immunologic, and pathological spectrum ranging from the extremes observed in paucibacillary (PB) and multibacillary (MB) patients (21, 24). PB patients have one or a few skin lesions and a low or absent bacterial index (BI; a measure of the number of acid-fast bacilli in the dermis, expressed on a logarithmic scale) and demonstrate specific cell-mediated immunity against *M. leprae*, but they have low or absent titers of *M. leprae*-specific antibodies and a granulomatous dermatopathology. In marked contrast, MB patients have multiple symmetric skin lesions and a high BI and demonstrate high titers of anti-*M. leprae* antibodies but an absence of specific cell-mediated immunity and a dermatopathology largely devoid of functional lymphocytes (21). Despite the implementation of a WHO-directed eradication program over the last 20 years, the worldwide annual rate of new case detection for leprosy remains stable at approximately 300,000 (17, 18, 26, 27). Earlier and objective diagnosis of leprosy could interrupt transmission and, in the long term, help further reduce the number of new cases and facilitate eradication.

There is no single diagnostic laboratory test for leprosy, and diagnosis remains essentially clinical. Clinical diagnosis of leprosy is dependent upon recognition of disease symptoms and is therefore only possible once the disease has manifested. WHO experts have listed diagnostic criteria as one or more of the following: hypopigmented or reddish skin patches with definite loss of sensation; thickened peripheral nerves; acid-fast bacilli on skin smears/biopsy specimens (WHO Expert Committee on Leprosy, 1998). Pure neuritic leprosy forms, however, present with no skin lesion. Confounding WHO's implementation of a global leprosy eradication strategy is that the number of trained leprologists has diminished. This is inadvertently increasing the likelihood that a clinical diagnosis is delayed or even missed, especially in regions where leprosy has been controlled (1, 13, 16, 25).

The presence of serum immunoglobulin M (IgM) antibody to phenolic glycolipid I (PGL-I) correlates with BI in leprosy patients and has been used to support disease symptoms as a means to categorize leprosy patients. Enzyme-linked immunosorbent assay (ELISA) and rapid lateral flow test formats have been developed for the detection of anti-PGL-I antibody (3, 4, 8, 19, 22, 23, 28). In one study, a lateral flow assay correctly diagnosed 97.4% of MB patients, with a specificity of 86.2% (4). Patients toward the PB end of the leprosy spectrum have low or no BI, however, and the majority of these patients are not identified by PGL-I-based tests (4, 7, 19). In addition, false-positive results in areas of endemicity are relatively high (>10%) (4, 7, 19). Consequently, none of these PGL-I-based tests has been widely implemented in

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field situations. In addition, many studies have demonstrated that MB patients have high titers of *M. leprae*-specific antibodies but PB patients have low or absent titers. For these reasons, the potential for serological diagnosis of low-BI patients, such as PB patients or MB patients who are developing disease, has not been thoroughly pursued.

In a recent small-scale study, we demonstrated that the ML0405 and ML2331 proteins were recognized by sera from MB leprosy patients presenting with high BI (20). In the current study we demonstrate that ML0405 and ML2331 are diagnostically relevant antigens by analyzing a large panel of MB leprosy patient sera from a variety of leprosy-affected regions (the Philippines, central and coastal Brazil, and Japan). We also examine the ability of *M. leprae* protein antigens to diagnose low-BI leprosy (PB patients and early MB patients) and show here the diagnostic potential of ML0405, ML2331, and a newly discovered *M. leprae* antigen, ML1556c. Based on the results, we construct and evaluate a fusion protein comprising ML0405 and ML2331 (designated *l*eprosy *I*DRI *d*iagnostic 1 [LID-1]) and demonstrate that this construct can be used to serologically diagnose leprosy patients among presymptomatic individuals, that is, before a clinical diagnosis is possible. Moreover, ML1556c may be a valuable adduct to LID-1 for the diagnosis of PB leprosy.

MATERIALS AND METHODS

Subjects and samples. Sera were obtained from patients with leprosy (MB and PB) or tuberculosis (TB), healthy household contacts of MB leprosy patients (HHC), and endemic and nonendemic controls (EC and NEC). MB and PB leprosy patient sera used in this study were derived from recently diagnosed, previously untreated individuals who did not have signs of reversal reactions. Leprosy was classified in each case by bacterial, histological, and clinical observations carried out by qualified personnel, with the BI recorded at the time of diagnosis. HHC were defined as adults living in the same house as an MB index case for at least 6 months. TB patients were included to evaluate potential antigen cross-reactivity with other mycobacterial infection. Sera from TB patients were obtained after drawing blood from *Mycobacterium tuberculosis* sputum-positive, human immunodeficiency virus-negative individuals with clinically confirmed pulmonary TB who were undergoing treatment. Normal sera (EC and NEC) were obtained after blood draws from volunteers with no history of leprosy or TB infection. In all cases, drawing of blood was carried out with informed consent (with local institutional review board approval or local ethics committee approval in Brazil, Japan, the Philippines, Seattle, and St. Louis). The composition of each study population is summarized in Table 1.

In Cebu City, leprosy and TB patients were recruited at the Cebu skin clinic and Leonard Wood Memorial Research Center in Cebu City, Cebu (Philippines) from 2003 to 2006. Between 1985 and 1991, sera were collected prospectively from individuals who resided with MB patients (BI > 2) for at least 2 years and were free of leprosy as determined by clinical dermato-neurological examination at the inclusion point of the study. Some of these individuals developed MB leprosy as the study progressed, and these sera have previously been described (11).

In Goiânia, the state capital of Goiás State (western central Brazil), leprosy and TB patients were recruited at the main outpatient clinics of Centro de Referência em Diagnóstico e Terapêutica and Hospital Anuar Auad in 2006. PB leprosy patients were selected from a cohort of leprosy patients with a single skin lesion recruited at Brazilian sites of endemicity from 1999 to 2001, as previously described (9).

In Salvador, the state capital of Bahia State (northeast coastal Brazil), leprosy patients were recruited at Hospital Dom Rodrigo de Menezes in 2006.

In Japan, leprosy patients were recruited at the National Sanatorium Oshimaseishoen, Kagawa.

In St. Louis, sera were collected from U.S.-based individuals at a variety of times following *Mycobacterium bovis* BCG immunization.

All serum specimens were aliquoted and stored at −20°C or −80°C prior to assay.

TABLE 1. Study populations

Site	Sample categorization (total no.)	BI (mean)	Sex ratio[a]	Mean age (yr) (range)
Cebu City, Philippines	MB (17)	2.8	2.4	30 (18–55)
	PB (54)	0.5	0.4	31 (15–45)
	TB (6)		5	45 (35–53)
	EC (8)		1	26 (19–38)
	HHC (10)		0.4	38 (18–60)
Goiânia, Brazil	MB (28)	2.4	1.5	44 (19–81)
	PB (83)	0	0.4	33 (7–76)
	TB (26)		2.7	39 (17–66)
	EC (30)		0.1	20 (19–26)
	HHC (11)		0.5	28 (18–51)
Salvador, Brazil	MB (10)	NA[b]	3.5	35.1 (20–70)
	PB (6)	0	5	31.6 (12–42)
	HHC (11)		0.1	48.5 (25–57)
Kagawa, Japan	MB (30)	NA	NA	60 (48–79)
	PB (30)	0	NA	70 (55–90)
	EC (26)		NA	54 (48–62)

[a] Male/female ratio.
[b] NA, not available.

Cloning and purification of target antigens. DNA encoding selected *M. leprae* proteins was PCR amplified from *M. leprae* Thai-53 genomic DNA using *Pfx* DNA polymerase (Invitrogen, Carlsbad, CA). PCR primers were designed to incorporate specific restriction enzyme sites 5′ and 3′ of the gene of interest and excluded in the target gene for directional cloning into the expression vector pET28a (Novagen, Madison, WI). After PCR amplification, purified PCR products were digested, ligated with vector DNA, and used to transform *Escherichia coli*, and individual clones were induced to produce recombinant proteins, as previously described (20). Recombinant proteins were quantified using the bicinchoninic acid protein assay (Pierce, Rockford, IL), and quality was assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The characteristics of each *M. leprae* protein evaluated are summarized in Table 2. The ML1556c protein was included because portions of the ML1556 protein were identified in four separate clones during serological expression screening with sera from PB leprosy patients (data not shown) (20). Recognition of the clones was derived from amino acids 58 to 256 of ML1556, which are only 47% identical to the *M. tuberculosis* protein Rv2839 (compared to 82% identity across the entire amino acid sequences of ML1556 and Rv2839).

Determining patient reactivity by ELISA. ELISAs were conducted independently at IDRI, Seattle, WA (Cebu and St. Louis sera); UFG, Goiânia, and UFB, Salvador, Brazil; and NIID, Tokyo, Japan. Polysorp 96-well plates (Nunc, Rochester, NY) were coated with 1 μg/ml recombinant protein or 200 ng/ml of natural disaccharide with octyl linkage (NDO), the synthetically derived B-cell epitope of PGL-I, conjugated to bovine serum albumin (NDO-BSA; kindly supplied by John Spencer, Colorado State University, under NIH contract N01 AI-25469), in bicarbonate buffer overnight at 4°C and blocked for 1 h at room temperature with phosphate-buffered saline–Tween with 1% BSA on a plate shaker. Serum diluted appropriately in 0.1% BSA was added to each well, and plates were incubated at room temperature for 2 h with shaking. Plates were washed with buffer only, and horseradish peroxidase-conjugated IgG or IgM (Rockland Immunochemicals, Gilbertsville, PA), diluted in 0.1% BSA, was added to each well and incubated at room temperature for 1 h with shaking. After washing, plates were developed with peroxidase color substrate (Kirkegaard & Perry Laboratories, Gaithersburg, MD), and the reaction was quenched by the addition of 1 N H_2SO_4. The optical density (OD) of each well was read at 450 nm. Positive responses were defined as an OD of >2× the mean OD of endemic control sera or an OD of >0.1, whichever was higher.

Statistics. *P* values were determined using Student's *t* test.

RESULTS

Recognition of *M. leprae* proteins by Filipino leprosy patient sera. The majority of MB leprosy patients are readily identified

TABLE 2. Main characteristics of *M. leprae* antigens tested[a]

Gene accession no.	Functional classification[b]	Protein type	Length (bp)	Product size (kDa)	% Identity[c] with:				
					M. tuberculosis H37Rv[d]	*M. bovis* AF2122/97[e]	*M. avium* 104[d]	*M. marinum* ATCC BAA-535[e]	*M. smegmatis* MC2 155[d]
ML0091	II.C.2	28-kDa antigen precursor	711	23.7	53	53	54	54	48
ML0405	V	Conserved hypothetical	765	25.3	62	62	None	NA	None
ML1633	II.C.2	Possible secreted hydrolase	1,608	57.0	25	25	35	81	62
ML2055	IV.A	Probable cell surface protein	864	29.5	72	72	69	73	54
ML2331	II.C.2	Possible secreted protein	771	26.5	80	80	77	80	67
ML2346	VI	Hypothetical	906	33.9	None	None	None	None	None
ML1556	II.A.6	Translation initiation factor	2,775	96.6	84	82		90	

[a] Annotations for gene accession number, functional classification, and protein type are according to the Sanger database.
[b] Functional classifications: II.C.2, surface polysaccharides, lipopolysaccharides, proteins, and antigens; V, conserved hypotheticals; IV.A, virulence; VI, unknowns; II.A.6, protein translation and modification.
[c] BLAST reports were performed in September 2006; tBLASTn was used for comparisons of proteins versus translated DNA. NA, not applicable.
[d] From http://www.tigr.org.
[e] From http://www.sanger.ac.uk/Projects.

by ELISA and lateral flow tests, which assess the capacity of patient IgM to bind *M. leprae* PGL-I or its synthetic analogue (NDO) conjugated to a carrier protein (BSA). In comparison with MB leprosy patients, PB leprosy patients have low or no anti-PGL-I responses and are more difficult to diagnose serologically. We therefore sought to determine whether PB sera recognized protein antigens, expanding our previous analyses and comparing the potential of NDO-BSA, ML0405, and ML2331 to diagnose leprosy, and found that the protein antigens have a similar profile for leprosy diagnosis as that for NDO-BSA; all three test antigens were readily detected by MB patient sera, by some PB patient sera, and by few, if any, EC, HHC, or TB sera (Fig. 1). Thus, similar to NDO-BSA, ML0405 and ML2331 demonstrate good potentials for the diagnosis of leprosy.

Recognition of MB leprosy patient sera with refined ML0405 antigen constructs. To learn more regarding the seroreactivity of ML0405 and enhance recombinant ML0405 expression for purification, we expressed a variety of ML0405 polypeptide fragments and determined whether Filipino MB leprosy patient sera had similar binding capacities to these fragments and to full-length (ML0405FL) protein. All constructs were able to bind MB patient sera (Fig. 2) ($P < 0.01$ for MB versus EC). The reactivity of a truncated form (ML0405Tr) of the protein was equivalent to the reactivity of ML0405FL ($P = 0.885$ for MB patient sera), whereas the reactivity of the protein construct lacking the predicted membrane-spanning region (ML0405Tm) declined slightly (Fig. 2) ($P = 0.047$ and 0.060 for Tm versus FL and Tr forms, respectively, for MB). These data indicate that the majority, if not all, of the B-cell epitopes recognized by antibodies in patient sera are retained and accessible in the truncated form of the protein. Further testing was conducted using either ML0405FL or ML0405Tr.

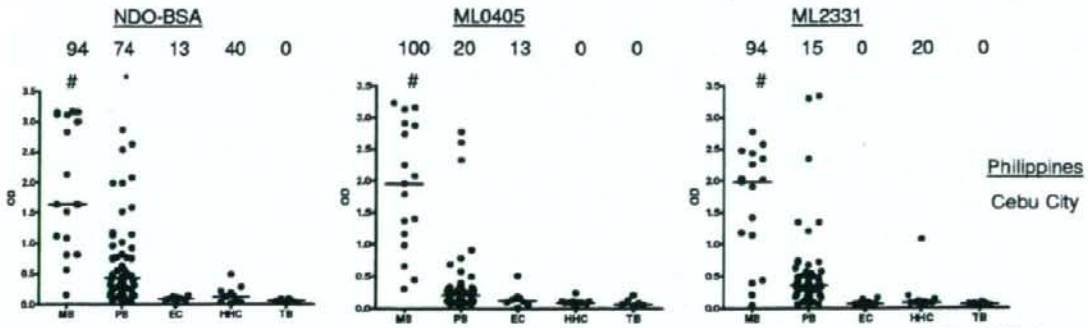

FIG. 1. Sera from Filipino leprosy patients react with recombinant *M. leprae* antigens. Sera from clinically diagnosed MB and PB leprosy patients, EC individuals, and HHC of MB leprosy patients were assessed against NDO-BSA, ML0405, and ML2331. NDO-BSA reactivity was assessed by IgM binding, and protein reactivity was assessed by IgG binding. Sera were from Cebu City, Philippines. Each point represents an individual serum sample, and the median is represented by the line. The number above each data set is the percent positive responses. *, $P < 0.05$; #, $P < 0.001$ versus EC.

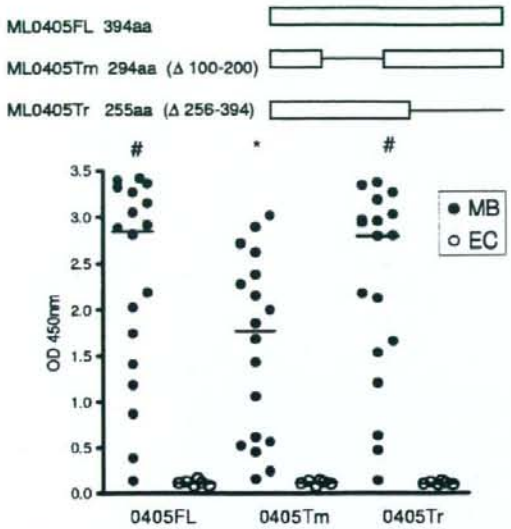

FIG. 2. ML0405 constructs react with MB leprosy patient sera. Different ML0405 constructs were created and expressed as recombinant proteins. The schematic diagram shows the sequence alignment of each of these constructs, with the deleted regions indicated by the line. Each construct was tested for IgG reactivity by ELISA with individual Filipino MB leprosy patient sera (n = 18) or EC sera (n = 6). *, $P < 0.05$; #, $P < 0.001$ versus EC.

Diagnosis of Filipino PB leprosy patients with *M. leprae* proteins. We then went on to more closely investigate the potential of *M. leprae* antigens for diagnosing PB leprosy. Sera from Filipino patients clinically diagnosed with PB leprosy and with a low BI were tested for reactivity with potential diagnostic *M. leprae* antigens (ML0405Tr, ML2331, ML1556c, and NDO-BSA). NDO-BSA was capable of identifying 57% (26 of 46) of these Filipino PB leprosy patients, but a substantial number of samples provided weak positive responses (Fig. 3). ML0405 and ML2331 also reacted with sera from some PB patients (Fig. 3A and B). Most of these Filipino sera that reacted with these proteins also demonstrated strong NDO-BSA responses, however, and so the added benefit of using these antigens for leprosy diagnosis within the Filipino population appeared minimal. In contrast, 4 of 20 sera that were weak positive/negative by NDO-BSA ELISA testing demonstrated strong reactivity to ML1556c (Fig. 3C). This result suggests that ML1556c may be useful as an adjunct to PGL-I testing, or other tests, to improve the sensitivity and clarity of leprosy diagnosis.

To test the specificity of ML1556c as a leprosy diagnostic reagent, we directly compared the reactivities of ML1556c with sera from PB leprosy patients, MB leprosy patients, TB patients, EC, and HHC of MB leprosy patients located in Cebu City, Philippines (Fig. 3D). Positive responses were observed in five of eight additional PB leprosy sera tested, with three of the sera yielding strong responses that could provide a clear diagnosis. Positive responses to ML1556c were also observed in two of seven MB leprosy sera tested in this experiment.

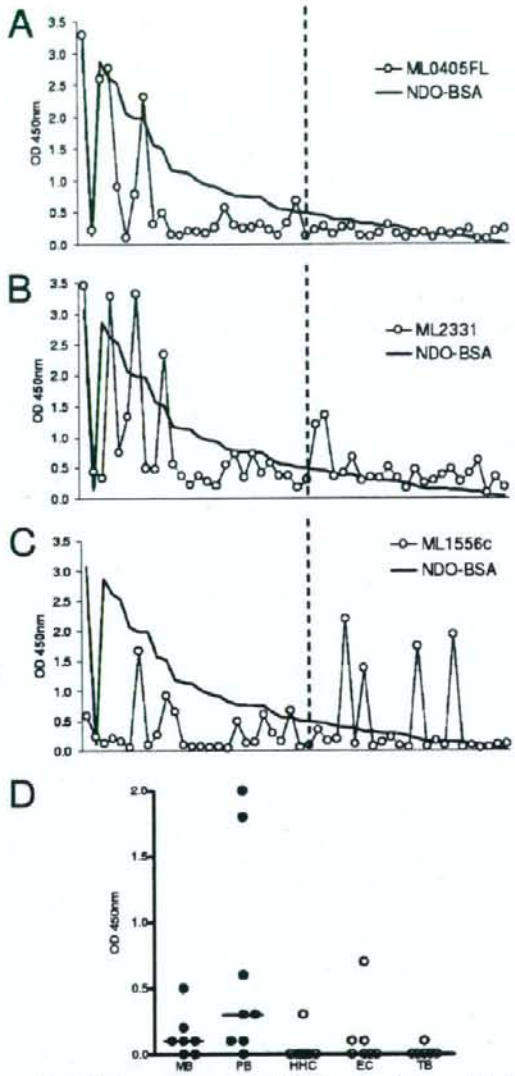

FIG. 3. *M. leprae* proteins react with PB leprosy patient sera. (A to C) Antibody reactivities of sera from a pool of clinically diagnosed MB leprosy patients, from a pool of negative control individuals, and from 46 clinically diagnosed PB leprosy patients were assessed against NDO-BSA and ML0405 (A), ML2331 (B), and ML1556c (C). NDO-BSA reactivity was assessed by IgM binding and, for reference, is shown in each plot. Recombinant protein reactivity was assessed by IgG binding. The first open circle represents the value obtained for pooled MB sera, while the next open circle represents the reactivity of pooled EC sera; individual PB sera are then arranged along the x axis according to their responsiveness versus NDO-BSA. The dashed line indicates the point at which diagnosis by NDO-BSA reactivity becomes unclear. ML1556c reacts with PB leprosy patient sera. (D) IgG reactivities of ML1556c with a small panel of individual sera from EC, leprosy patients (MB and PB), and TB patients were determined by ELISA using samples from Cebu City, Philippines. Each point represents an individual serum sample, and the median is represented by the line.

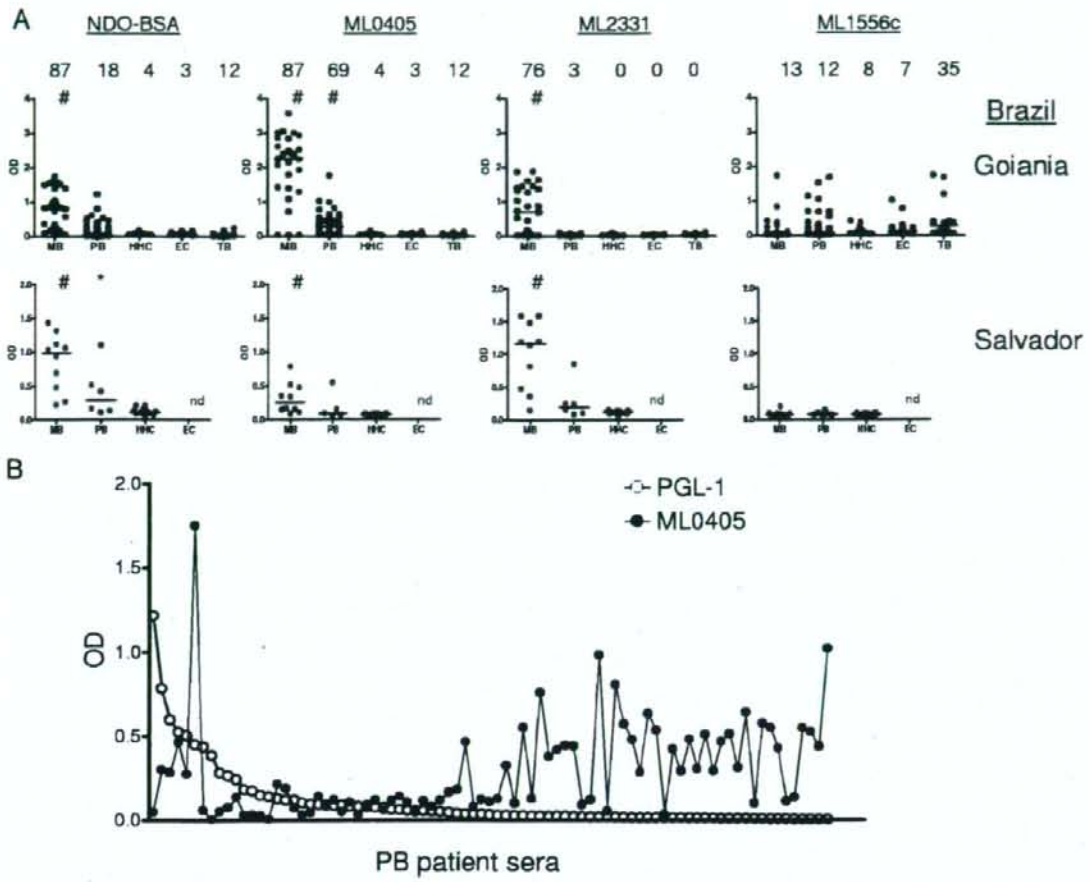

FIG. 4. Sera from Brazilian leprosy patients react with recombinant *M. leprae* antigens. Sera from clinically diagnosed MB and PB leprosy patients, EC individuals, and HHC of MB leprosy patients were assessed against NDO-BSA, ML0405, ML2331, and ML1556c. NDO-BSA reactivity was assessed by IgM binding, and protein reactivity was assessed by IgG binding. Sera were from Goiânia and Salvador (see Table 1). (A) Each point represents an individual serum sample, and the median is represented by the line. The number above each data set is the percent positive responses. *, $P < 0.05$; #, $P < 0.001$ versus EC. (B) To demonstrate complementarity, the individual PB sera from Goiânia are arranged along the *x* axis according to their responsiveness versus NDO-BSA and overlaid with the response of each serum to ML0405.

ML1556c did not react with any of the Filipino TB patient sera tested, was recognized by only one of eight HHC sera, and reacted with only one of six EC sera. Negative results were obtained upon further testing involving another 45 TB sera and 23 NEC sera (data not shown). Taken together, these results generated from sera from the Philippines suggested the utility of ML1556c to improve the diagnosis of PB leprosy.

Identification of leprosy patients in Brazil. We also examined the ability of recombinant *M. leprae* antigens to identify leprosy patients located around Goiânia, Brazil, and Salvador, Brazil. Within the clinically diagnosed leprosy population, PGL-I/NDO-BSA was capable of identifying 87% (33 of 38) of the MB patients (Fig. 4). In agreement with the results obtained by analysis of Filipino leprosy patient sera, ML0405 and ML2331 reacted with large proportions of Brazilian MB patient sera (87% [33 of 38] and 76% [29 of 38], respectively), and ML1556c reacted with only some MB patient sera (13%, 5 of 38) (Fig. 4). In Goiânia, positive responses were also observed against antigens ML0091 (71%, 20 of 28), ML1633 (32%, 9 of 28), ML2055 (75%, 21 of 28), and ML2346 (29%, 8 of 28) (data not shown). The clarity of MB leprosy diagnosis (strength of signal in positive samples versus negative samples) in Goiânia was greater when using ML0405 rather than NDO-BSA, but in Salvador it was greater when using ML2331 rather than NDO-BSA.

We also determined if these antigens were recognized by Brazilian PB patient sera. PGL-I/NDO-BSA was capable of identifying only 20% (18 of 89) of the PB patients, a level not appreciably higher than the proportion of positive responses observed with TB patients (12%, 3 of 26) (Fig. 4A). An IgG

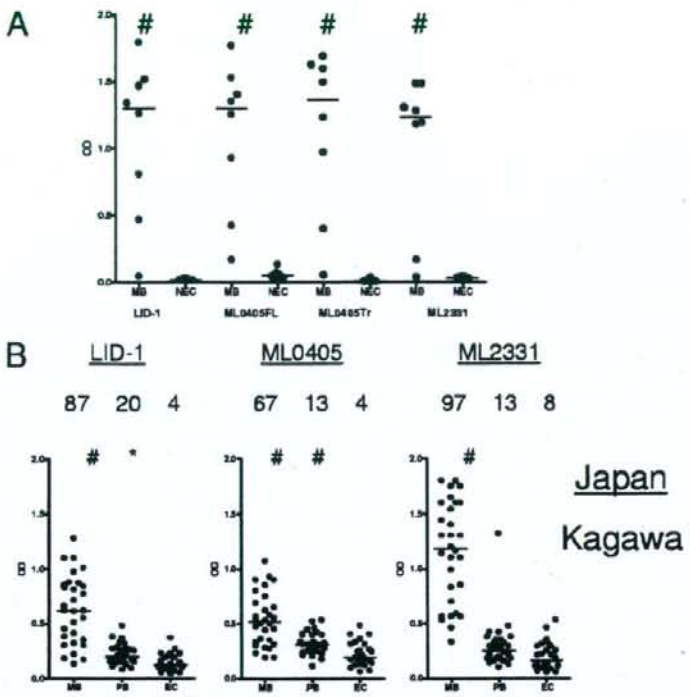

FIG. 5. LID-1 retains reactivity with leprosy patient sera. (A) LID-1 (a fusion construct of ML0405 and ML2331), ML0405FL, ML0405Tr, and ML2331 reactivities were assessed by IgG binding in an ELISA with eight MB leprosy patient serum samples from Salvador and eight NEC serum samples. (B) Sera from clinically diagnosed Japanese MB and PB leprosy patients, and Japanese EC individuals, were assessed for IgG reactivities with LID-1, ML0405, and ML2331. Each point represents an individual serum sample, and the median is represented by the line. The number above each data set is the percent positive responses. *, $P < 0.05$; #, $P < 0.001$ versus EC.

reactivity that permitted serologic diagnosis of an increased number of PB leprosy patients was observed for ML0405 (69%, 61 of 89), but responses to ML2331 were very weak, with very few positives (3%, 3 of 89) (Fig. 4A). The antigens ML0091 (6%, 5 of 83), ML1633 (17%, 14 of 83), ML2055 (13%, 11 of 83), and ML2346 (27%, 22 of 83) were recognized by some PB patient sera, but responses were generally weak (data not shown). Many of the PB patient sera that did not react with PGL-1 had a strong reactivity with ML0405 (Fig. 4B). ML1556c was recognized by only a minor subset of PB leprosy patient sera (12%, 11 of 89) and Brazilian EC individuals (6.7%, 2 of 30), but ML1556c reactivity was detected in a substantial number of Brazilian TB patients (35%, 9 of 26). These data indicate only a minor number of positive results in the Brazilian population if ML1556c is used for leprosy diagnosis, with a further complication of false-positive diagnosis in TB patients. Antigen ML0405, however, did not react with significant numbers of EC sera (3.3%, 1 of 30) or TB sera (12%, 3 of 26) (Fig. 4A). These results indicate that ML0405 can recognize some PB leprosy patients in the Brazilian population and could be used to augment leprosy diagnosis with PGL-1.

Construction of a fusion construct of ML0405-ML2331 (LID-1). Having extended our earlier observation that the single antigens ML0405 and ML2331 have the potential to diagnose leprosy (20), and given the observations that ML0405 appeared better for diagnosis in Goiânia and Cebu City but ML2331 appeared better for diagnosis in Salvador, we constructed a single fusion molecule incorporating both proteins. ML0405Tr was expressed at the C terminus of the molecule and ML2331 in the N terminus. Following recombinant expression, we validated the reactivity of the construct by assaying LID-1 versus a small panel of sera from Salvador that had bound each single component. These sera readily detected LID-1, ML0405FL, ML0405Tr, and ML2331 (Fig. 5A). Importantly, construction of the fusion protein did not introduce false-positive results with NEC sera (Fig. 5A).

We further extended our examination of sera from different geographic locations by assessing sera from Japanese leprosy patients for reactivity with ML0405, ML2331, and LID-1. Positive response were observed with MB patient sera (67% [20 of 30] for ML0405, 97% [29 of 30] for ML2331, and 87% [26 of 30] for LID-1) and PB patient sera (13% [4 of 30] for ML0405, 13% [4 of 30] for ML2331, and 20% [6 of 30] for LID-1), with few responses in EC sera (4% [1 of 26] for ML0405, 8% [2 of 26] for ML2331, and 4% [1 of 26] for LID-1) (Fig. 5B). Taken together, these data indicate that LID-1 is useful as a diagnostic antigen for leprosy.

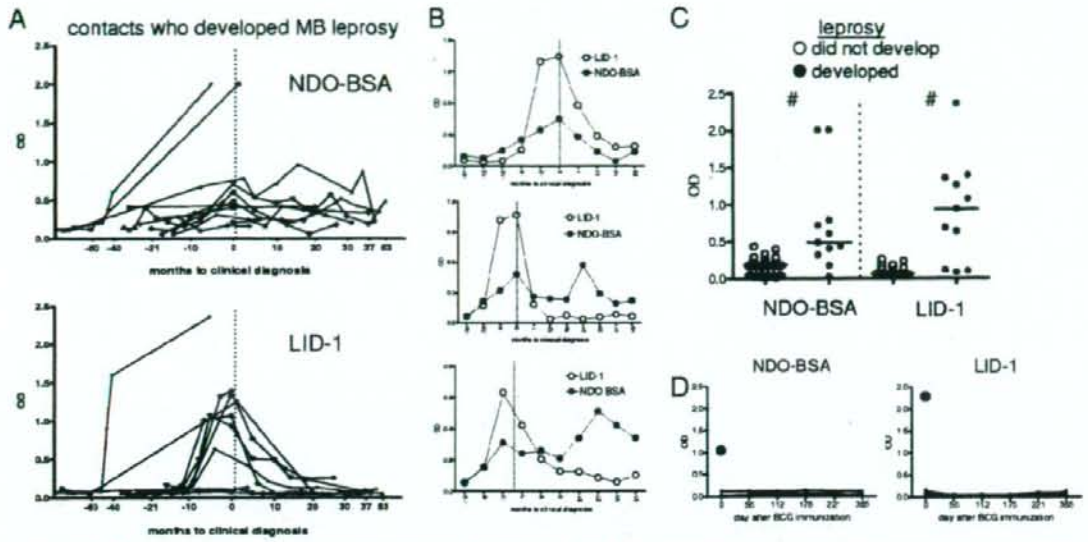

FIG. 6. LID-1 reactivity can diagnose leprosy before clinical symptoms. (A) LID-1 and NDO-BSA reactivities within sera from a prospective study conducted in Cebu City, Philippines, were assessed by either IgG or IgM binding in an ELISA. Sera were collected at a variety of times prior to the clinical diagnosis of MB leprosy in 11 patients and at a variety of times after the commencement of treatment. (B) Representative plots for individual patients are shown. (C) Sera were collected from 57 household contacts that did not develop clinical leprosy and were compared with single serum samples from each individual contact that developed leprosy (serum samples were collected within 3 months of clinical diagnosis). #, $P < 0.001$. (D) LID-1 and NDO-BSA reactivities within sera from a prospective study using 10 U.S.-based individuals who were immunized with BCG were assessed. Sera were collected at regular intervals following BCG immunization. The solid circle at day zero designates the reactivity of a leprosy patient serum sample that was included as a positive control.

LID-1 reactivity can diagnose leprosy before clinical symptoms. Having demonstrated that the LID-1 fusion molecule retained the ability to diagnose leprosy patients but lacked responses to EC sera, we obtained sera from a prospective study conducted in Cebu City, Philippines, between 1985 and 1991 (11). In that study, household contacts of leprosy patients were monitored over a prolonged period of time, and some developed clinical MB leprosy. In sera from the individuals who developed MB leprosy, as previously reported, anti-PGL-I levels increased before leprosy was diagnosed by clinical exam (Fig. 6A). Our data also indicate that anti-LID-1 antibody levels began to increase markedly as soon as 1 year prior to clinical diagnosis (Fig. 6A). For many of the patients (7 of 11, 64%) the increase in the anti-LID-1 IgG response was strikingly more obvious than the increase in the anti-PGL-I IgM response (Fig. 6B). Those patients that developed clinical leprosy had anti-PGL-1 antibody levels not dissimilar to many individuals who did not develop leprosy (Fig. 6C). The difference in anti-LID-1 antibody levels was much clearer, with a much larger differentiation between the positive responses of patients who developed leprosy compared with the extremely low levels of anti-LID-1 antibody in individuals who did not develop leprosy (Fig. 6C). Taken together, these data indicate that LID-1 is capable of providing an early serological diagnosis of leprosy.

LID-1 does not react with sera from individuals recently exposed to BCG. To examine in detail if leprosy diagnosis could be complicated by exposure to or infection with other mycobacteria, we also examined sera collected longitudinally from 10 U.S.-based individuals who were immunized with BCG. None of these BCG-immunized individuals developed positive serological responses against LID-1 or NDO-BSA (Fig. 6D). These data indicate that LID-1 can provide a clear diagnosis of *M. leprae* infection prior to the onset of signs that permit clinical leprosy diagnosis and that LID-1-based diagnostic tests could be used to expedite leprosy treatment.

DISCUSSION

Current diagnosis of leprosy is based on the appearance of clinical signs, and it is well established that the earlier a patient is identified the better their response to treatment. In addition, MB leprosy patient household contacts have a higher risk of developing clinical leprosy than contacts of PB leprosy patients (10, 12). This has been attributed to increased shedding and spreading of viable bacteria by MB leprosy patients (2). Accurate and early detection of *M. leprae*-infected individuals will open the possibility of earlier treatment that could both prevent disability and significantly reduce leprosy transmission.

We have evaluated the serological responses to a variety of *M. leprae* protein antigens in an attempt to discover antigens that can improve diagnosis of leprosy by detecting patients with a low BI (PB leprosy patients or early MB leprosy patients). We demonstrated that (i) ML0405 and ML2331 can be used to diagnose MB leprosy patients independently of geographic location; (ii) ML1556c can recognize some PB patients (al-

though it is recognized by some TB sera as well); (iii) ML0405 and ML2331 can be used for diagnosis of some PB patients; (iv) a fusion construct of ML0405 and ML2331 (LID-1) retains diagnostic capability; and (v) LID-1 can provide a clear leprosy diagnosis before the onset of clinical symptoms. These findings will improve both leprosy diagnosis and patient care.

One approach for the early detection of *M. leprae* infection is through serological diagnosis. We have conducted screening to identify *M. leprae* antigens that have not previously been described, and we then evaluated the diagnostic potential of these antigens with leprosy patient sera. In this study, the diagnostic potential of select antigens was assessed in clinically disparate leprosy patient groups, ranging from MB patients who presented with large bacterial burdens and large skin lesions to PB patients who presented with low or absent bacterial burdens and a few, small skin lesions. As expected, MB leprosy patients were easier to identify by serological assays and typically yielded higher responses than PB patients. Unexpectedly, close examination of patients with a low BI from the Philippines indicated that some patients exhibited strong responses against the ML1556c protein. The responses of Filipino PB patients to ML1556c were often greater than those of MB patients. These results suggested the utility of this protein either as an adjunct to antigens that could identify MB patients to provide a cross-spectrum leprosy diagnosis or as a stand-alone protein for PB leprosy diagnosis. An objective and differential diagnosis of MB or PB leprosy could lead to better treatment of patients by guiding the multidrug therapy regimen provided to them.

We also analyzed the diagnostic potential of each antigen within geographically disparate groups of patients, from the Philippines and two sites in Brazil. In the Brazilian (Goiânia) PB leprosy patient group, ML1556c provided only a few positive responses; this dampened the enthusiasm for ML1556c to be a widely used diagnostic or prognostic leprosy antigen. Of interest, many PB leprosy patients in Brazil (both Goiânia and Salvador) could be diagnosed by ML0405 reactivity, and several PB patients (Salvador) could be diagnosed with ML2331 reactivity. It is unclear if the differences in the responses of patients from different geographic locations are related to differences in *M. leprae* strains or to regional variations in host genetics. These possibilities might be addressed by analysis of patient sera on fragments of ML1556c or by a survey of anti-ML1556c antibody on lysates of different *M. leprae* strains. Regardless, the observed differences indicate the importance of examining antigen-specific responses in several regions when considering their ability to diagnose leprosy globally.

Given that the ML0405Tr and ML2331 proteins could provide diagnosis of leprosy, we made a fusion protein (LID-1) of these individual components. After ensuring the fusion protein retained reactivity against leprosy sera from Salvador, Brazil, we tested the antigens against sera from Japan. As with results obtained using sera from Brazil, Japanese MB leprosy patient sera reacted as strongly with the fusion LID-1 as with the ML0405 and ML2331 components. In addition, some Japanese PB leprosy patient serum antibodies recognized these antigens.

Studies have argued that the presence of anti-PGL-I antibodies is an indicator of leprosy development, but this has been debated (5, 6, 14, 15). Many contacts of leprosy patients have anti-PGL-I antibodies but do not develop disease, limiting the capacity of PGL-I-based assays to predict disease development. Indeed, PGL-I-based tests are typically marketed as a support reagent to confirm clinical diagnosis and aid leprosy classification but are not recommended for use as a stand-alone for diagnosis (19). The differential in responses of sera from contacts that developed leprosy compared with contacts that did not develop leprosy was much greater for LID-1 than PGL-1. We demonstrated that LID-1 is capable of providing an early serological diagnosis of MB leprosy. A clear and early diagnosis was achieved in 7 of 11 contacts of leprosy patients who themselves went on to develop clinical leprosy. For the small panel of sera tested, the time benefit of a LID-1-based diagnosis over a clinical-based diagnosis was 6 to 8 months. Thus, screening for LID-1-reactive antibodies, either in the general population or within more focused at-risk populations, could significantly expedite treatment of leprosy patients and, also, affect transmission rates by reducing the number of individuals who develop large bacterial burdens. As another benefit, antibody levels against LID-1 dropped following the implementation of drug treatment in these individuals, and thus the reduction and disappearance of antibodies against LID-1 may be a useful measure of multidrug therapy efficacy.

We are currently evaluating additional antigens, diagnostic formats, and different geographic sources of patient sera with the objective of early and simple identification of leprosy patients regardless of incidence locality.

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Clinicopathologic challenge

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What is your diagnosis?

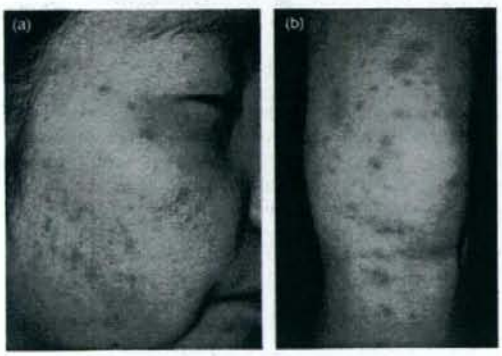

Figure 1 Note erythematous, nodular lesions on the face, limbs, and trunk and plaques on the trunk; (a) face; (b) right lower extremity

Figure 3 Histologic examination of her liver sample reveals vascular degeneration with multiplication of nuclei in the liver cells (hematoxylin and eosin stain, ×80)

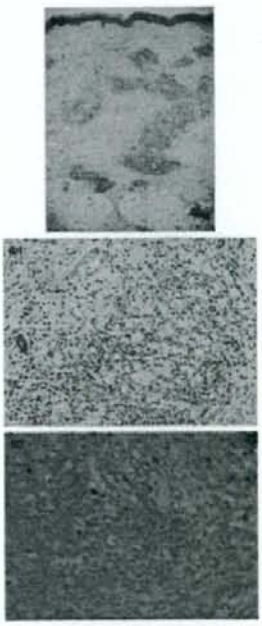

Figure 2 (a) Macrophage granuloma formation in the dermis interspersed with dense aggregates of neutrophils (hematoxylin and eosin stain, ×20); (b) Foamy macrophages with an intense neutrophilic infiltrate (hematoxylin and eosin stain, ×100) (c) Numerous, fragmented, acid-fast bacilli (AFB) in the foamy macrophage granuloma formation (Fite stain, ×200)

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History

A 43-year-old woman visited with multiple, uncountable, evanescent, and painful skin lesions on the extremities, face, and trunk of 8 years' duration (Fig. 1(a,b)).

Diagnosis

Erythema nodosum leprosum with hepatic involvement.

Discussion

Leprosy, an achronic granulomatous multisystem disease caused by *Mycobacterium leprae*, primarily affects the skin and peripheral nervous system and presents with two types of granulomatous lesions: macrophagic (lepromatous leprosy) and epithelioid (tuberculoid leprosy). Lepra reactions, which occur during this disease, are acute episodes that are divided into the type-1 lepra reaction (occurring in borderline disease) and the type-2 lepra reaction (erythema nodosum leprosum [ENL] occurring in the spectrum of lepromatous disease).[1] ENL is an immunological reaction seen in patients with lepromatous leprosy. It is a serious complication affecting 10% of lepromatous multibacillary leprosy patients. Skin reactions in ENL are erythematous nodules associated with arthralgia, fever, iritis, malaise, and neuritis.

Classically, ENL presents as crops of tender, erythematous papules, plaques, or nodules that are evanescent and last only 7–10 days. Visceral manifestations can include hepatosplenomegaly, nephritis, orchitis, and pleuritis. We report on a Japanese female patient with lepromatous leprosy who had recurrent episodes of ENL and presented with hepatic involvement. In our patient, the histopathologic alterations found in hepatic biopsy were similar to those described in the literature.[2,3] The patient showed remarkable improvement when administered thalidomide to treat her ENL with hepatic damage, indicating that thalidomide is an effective medication for ENL refractory to conventional therapies. Based on our results, we strongly suggest that thalidomide be indicated for serious ENL.

Thalidomide was developed in the 1950s as a sedative drug but was withdrawn in 1961 because of its teratogenic effects. Recently, this agent has been recognized as a potent immune response-modifying drug.[4–7] Thalidomide is being successfully used in other disorders with inflammatory and immunologic bases. Although thalidomide is generally considered to be a last resort when all other viable therapies fail because of its associated birth defects and irreversible peripheral neuritis, thalidomide represents a useful therapeutic option for ENL. Sheskin *et al.*[8] reported improvement in lepromatous leprosy patients treated with thalidomide for ENL. Similarly, our patient received thalidomide for active lepromatous leprosy with ENL and responded well. During the clinical course of leprosy, the drug is effective against ENL, acute inflammation affecting skin and nerves. Therefore, we believe that in appropriately selected patients, thalidomide can be an extremely efficacious medication.

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2006年における世界のハンセン病の現況について

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キーワード：世界保健機関、多剤併用療法、ハンセン病、ハンセン病制圧、有病率

世界のハンセン病の疫学は各国の保健担当の部署から世界保健機関（WHO）に報告される。報告されたデータはWHOによってまとめられ、速報的に週間疫学記録（weekly epidemiological record）に掲載される。今回、2006年初頭のデータが報告された。それによると、1,000例以上の新患発生のある国々が世界で17カ国あり、新規患者数が増加傾向にある国も存在することが示された。早期発見と無料薬剤の安定的供給を継続するとともに、医療サービスの統合化が重要であることなどが述べられている。

世界保健機関（WHO）発行の週刊疫学記録（weekly epidemiological record）、2006年8月11日号（No.32, 81：309-316, 2006）（http://www.who.int/wer）に掲載された「世界のハンセン病状況、2006年」（global leprosy situation,2006）についてWHOの許可を受け、日本語訳を行った。

世界のハンセン病の現況をWHO事務局別、各国別で表示してある。各国の報告は国情により内容の信頼性に温度差があるものの、概略はこの報告で伺うことができる。この報告を参考にして、ハンセン病の世界の現況を把握するとともに、我々日本人として行うことができる国際協力に関して考察を頂ければ幸いである。

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世界のハンセン病の現況：2006

ハンセン病問題のさらなる減少と制圧活動維持のための世界戦略案（企画期間：2006－2010）は2000－2005年のWHO戦略的ハンセン病制圧プランから発展したものである。この新たな方策は流行国において、有病率が低くなるにつれハンセン病に罹患した人々に対してサービスを提供する際に起こりうる問題と取り組むために開発された。今後も、公平に、手頃な価格で、そして身近な場所で、良質な診断と多剤併用療法（MDT）による治療が受けられるようにすることが重要であることは変わらない。この戦略は、全ての協力者たちにより広く支持され、認められたものである。関係者の努力によって、ハンセン病制圧による成果が維持され、今後の疾病問題のさらなる減少を確実にすることが期待される。ハンセン病を根絶する戦略を開始するにあたって、費用対効果が期待できる新規の方策が必要となることは明白である。現在はそれらの方策を利用できない状態に

ある。

WHOは、まだ制圧目標を達成していない流行国が数年以内に目標を達成するよう努力を続ける。また、ハンセン病制圧活動を一般保健サービスに統合するための全ての流行国の努力を支援し、特にサービスの質の向上、多剤併用療法（MDT）の無料提供、信頼に足る登録方法の促進、統合プログラムの一貫としての能力向上や権限の強化、地域社会での認識の向上、そして流行国におけるハンセン病状況の監視、指導、及び評価の向上に重点をおく。

データの由来

世界統計値は、アフリカ地域の36、南北アメリカ地域の22、南東アジア地域の11、東地中海地域の20、そして西太平洋地域の26を含む115の国や地域から収集したものである。表1は、登録患者数、新規患者数、新規多菌型（MB）患者数、新規女性患者数、新規小児患者数、新規患者の中の第2級障害者（G2D）数、及びその年に報告された再発患者数の各国のデータをWHO地域ごとに表している。比率は国連人口部の2005年人口データに基づいて計算された。

表1　2006年初頭におけるWHO地域（ヨーロッパ地域を除く）と国・地域のハンセン病状況

WHO地域と国・地域	登録患者数[a]	新規患者数[b]	新規MB患者数[c]	新規女性患者数	新規小児患者数	新規G2D患者数[d]	再発患者数[e]
アフリカ地域							
アルジェリア	0	0	0	0	0	0	0
アンゴラ	1,449	1,877	1,426	865	168	183	0
ベナン	306	397	238	173	43	86	0
ボツワナ	6	5	3	2	0	1	0
ブルキナファソ	882	872	628	NR	45	121	0
ブルンジ	509	293	234	67	25	62	0
カメルーン	537	537	412	NR	63	27	NR[f]
カーボベルデ	15	2	2	0	0	0	0
中央アフリカ	315	252	158	151	30	41	2
チャド	786	367	275	77	14	52	0
コモロ	72	133	30	60	52	4	0
コンゴ共和国	215	207	149	88	19	35	0
コンゴ民主共和国	9,785	10,737	5,963		1,308	1,051	2
エチオピア	5,277	4,698	4,157	1,473	324	589	261
エリトリア	8	8	5	5	0	1	4
カボン	45	30	28	10	1	5	2
ガーナ	762	803	624	392	87	25	0
ギニア	712	980	642	NR	88	108	NR
ギニアビサウ	80	64	45	28	5	12	0
ケニア	180	158	146	74	5	16	13
レソト	12	7	7	3	1	0	2
リベリア	292	267	170	NR	79	NR	1
マダガスカル	2,094	2,709	2,021	658	394	246	NR
マリ	484	537	369	NR	NR	NR	0
モーリタニア	64	115	64	0	0	22	NR
モザンビーク	4,889	5,371	3,364	NR	563	445	45

ナイジェリア	4,544	5,024	4,346	2,799	471	586	490
ルワンダ	38	61	40	33	6	13	11
サントメプリンシペ	0	0	0	0	0	0	0
セネガル	427	356	263	139	42	64	21
セイシェル	2	1	1	0	0	0	1
シエラレオネ	526	777	354	164	27	53	0
タンザニア	4,190	4,237	2,863	1,815	322	407	47
トーゴ	166	188	141	80	16	32	2
ウガンダ	607	552	379	308	70	50	3
ザンビア	554	192	153	NR	37	NR	0
合計	**40,830**	**42,814**	**29,700**	**9,464**	**4,305**	**4,337**	**907**
南北アメリカ地域							
アルゼンチン	775	484	348	198	5	8	11
ブラジル	27,313	38,410	19,515	17,796	3,259	1,890	1,433
ボリビア	NR	114	41	47	11	2	1
チリ	NR	1	0	1	0	0	0
コロンビア	1,017	585	402	0	19	57	35
コスタリカ	33	11	8	6	0	3	0
キューバ	226	208	173	93	8	7	5
ドミニカ	301	155	99	78	25	5	2
エクアドル	178	116	73	41	0	0	3
エルサルバドル	10	6	4	3	0	4	0
グアテマラ	6	3	3	0	0	0	0
ガイアナ	NR	28	19	13	5	4	NR
ジャマイカ	19	9	6	2	1	3	2
メキシコ	776	289	218	108	8	32	43
パナマ	9	3	2	0	0	0	1
パラグアイ	735	480	373	187	19	38	0
ペルー	26	26	19	112	2	1	55
セントルシア	NR	5	3	2	0	0	NR
スリナム	37	42	22	12	7	3	0
トリニダード・トバゴ	46	31	15	11	7	1	2
ウルグアイ	8	6	6	2	0	2	2
ベネズエラ	1,389	768	496	261	56	47	32
合計	**32,904**	**41,780**	**21,845**	**18,873**	**3,432**	**2,107**	**1,627**
南東アジア地域							
バングラデシュ	6,198	7,882	3,018	3,316	807	650	NR
ブータン	36	15	15	1	0	2	NR
朝鮮民主主義人民共和国	0	0	0	0	0	0	NR
インド	95,150	161,457	73,149	53,083	16,112	3,015	NR
インドネシア	21,537	19,695	15,639	NR	1,790	1,722	NR
モルディブ	10	15	1	6	4	0	NR
ミャンマー	2,679	3,571	2,175		226	346	0

ネパール	4,921	6,150	3,369	1,910	333	227	NR
スリランカ	1,296	1,924	803	808	202	109	4
タイ	1,306	638	414	234	32	77	NR
東ティモール	289	288	212	61	15	61	NR
合計	**133,422**	**201,635**	**98,795**	**59,419**	**19,521**	**6,209**	**4**
東地中海地域							
アフガニスタン	29	31	23	11	0	8	0
バーレーン	0	0	0	0	0	0	0
ジブチ	6	0	0	0	0	0	0
エジプト	2,118	1,134	991	401	88	30	5
イラク	NR	0	0	0	0	0	0
イラン	NR	79	44	37	5	23	0
ヨルダン	0	0	0	0	0	0	0
クウェート	NR	6	4	1	0	0	0
レバノン	14	0	0	0	0	0	0
モロッコ	300	43	27	18	7	7	0
オマーン	7	5	2	2	0	0	0
パキスタン	969	551	451	214	40	110	26
ヨルダン川西岸及びガザ地区	0	0	0	0	0	0	0
サウジアラビア	10	30	18	10	0	4	0
ソマリア	138	62	46	25	11	8	0
スーダン	NR	782	720	220	32	86	0
シリア・アラブ共和国	7	7	3	1	0	2	0
チュニジア	3	1	1	1	0	0	2
アラブ首長国連邦	NR	7	NR	NR	1	NR	NR
イエメン	423	395	231	113	43	57	11
合計	**4,024**	**3,133**	**2,561**	**1,054**	**227**	**335**	**44**
西太平洋地域							
ブルネイ	1	1	0	1	0	0	0
カンボジア	348	429	299	121	39	62	0
中国	3,171	1,658	1,477	511	35	353	168
香港	32	4	4	3	0	0	3
クック諸島	0	0	0	0	0	0	0
フィジー	5	4	3	0	1	0	1
フランス領ポリネシア	16	10	5	4	0	0	2
グアム	9	6	6	1	1	0	0
日本	NR	6	5	1	0	0	0
キリバス	19	34	11	18	11	0	0
大韓民国	420	15	15	9	1	4	5
ラオス	140	143	109	50	8	20	0
マレーシア	NR	263	177	82	16	8	12
モンゴル	0	0	0	0	0	0	0
ミクロネシア連邦	158	260	77	94	84	2	3

ニウエ	0	0	0	0	0	0	0
パラオ	2	2	2	2	0	0	0
パプアニューギニア	536	381	204	134	109	55	0
フィリピン	3,096	3,130	2,951	NR	159	46	5
サモア	5	7	7	0	2	2	0
シンガポール	25	13	7	NR	NR	NR	0
ソロモン諸島	21	25	16	12	7	0	0
トンガ	0	0	0	0	0	0	0
ツバル	0	0	0	0	0	0	0
バヌアツ	0	0	0	0	0	0	0
ベトナム	642	746	452	269	47	121	2
合計	**8,646**	**7,137**	**5,827**	**1,312**	**520**	**673**	**201**

[a] 2006 年初頭の登録患者数
[b] 2005 年の新規患者数
[c] 多菌型：MB
[d] 新規患者における第 2 級障害者 (G2D) 数
[e] 2005 年に発生した再発患者数
[f] NR= 未報告

ハンセン病問題

表 2 で示されるように、2006 年初頭に登録された世界の登録患者数は 219,826 人であった。2005 年に報告された新規患者数は 296,499 人であった。世界的に新規患者数は急激な減少を続けている。2004 年に報告された新規患者数に比べると、2005 年の報告数は 110,000 人（27%）以上低下した。

表 2　2006 年当初の WHO 地域におけるハンセン病状況（ヨーロッパ地域を除く）

WHO 地域と国・地域	2006 年初頭登録患者数（人口 10,000 人あたりの有病率）	2005 年の新規患者数（人口 100,000 人あたりの発見率）
アフリカ地域	40,830(0.56)	42,814(5.92)
南北アメリカ地域	32,904(0.39)	41,780(4.98)
南東アジア地域	133,422(0.81)	201,635(12.17)
東地中海地域	4,024(0.09)	3,133(0.67)
西太平洋地域	8,646(0.05)	7,137(0.41)
合計	**219,826**	**296,499**

表3は、2001年以来世界の新規患者数が減少していることを表す。アフリカ地域では、2005年の新規患者数が2004年に比べて8.7%の減少を報告した。同様に南北アメリカ地域で20.1%、南東アジア地域で32.5%、そして東地中海地域で7.6%であった。しかし西太平洋地域では、同期間内に14.8%増加している。

表3　2001～2005年のWHO地域でのハンセン病新規患者数の動向（ヨーロッパ地域を除く）

	新規患者数				
WHO地域	**2001**	**2002**	**2003**	**2004**	**2005**
アフリカ地域	39,612	48,248	47,006	46,918	42,814
南北アメリカ地域	42,830	39,939	52,435	52,662	41,780
南東アジア地域	668,658	520,632	405,147	298,603	201,635
東地中海地域	4,758	4,665	3,940	3,392	3,133
西太平洋地域	7,404	7,154	6,190	6,216	7,137
合計	**763,262**	**620,638**	**514,718**	**407,791**	**296,499**

表4は制圧目標を達成していない主要な6流行国でのハンセン病状況を示している。これらの6ヶ国は、2005年の世界の新規患者数の23%と、2006年初頭の世界の登録患者数の24%を占める。6カ国とはブラジル、コンゴ民主共和国、マダガスカル、モザンビーク、ネパールとタンザニアである。

表4　WHOの制圧目標未達成国のハンセン病状況

国名	登録患者数[a]			新規患者数[b]		
	2004年初頭	2005年初頭	2006年初頭	2003年	2004年	2005年
ブラジル	79,908	30,693	27,313	49,206	49,384	38,410
	(4.6)	(1.7)	(1.5)	(28.6)	(26.9)	(20.6)
コンゴ民主共和国	6,891	10,530	9,785	7,165	11,781	10,737
	(1.3)	(1.9)	(1.7)	(13.5)	(21.1)	(18.7)
マダガスカル	5,514	4,610	2,094	5,104	3,710	2,709
	(3.4)	(2.5)	(1.1)	(31.1)	(20.5)	(14.6)
モザンビーク	6,810	4,692	4,889	5,907	4,266	5,371
	(3.4)	(2.4)	(2.5)	(29.4)	(22.0)	(27.1)
ネパール[c]	7,549	4,699	4,921	8,046	6,958	6,150
	(3.1)	(1.8)	(1.8)	(32.9)	(26.2)	(22.7)
タンザニア	5,420	4,777	4,190	5,279	5,190	4,237
	(1.6)	(1.3)	(1.1)	(15.4)	(13.8)	(11.1)
合計	**112,092**	**60,001**	**53,192**	**80,707**	**81,289**	**67,614**

[a] 数値は患者数（人口10,000あたりの有病率）
[b] 数値は新規患者数（人口100,000あたりの新患発見率）
[c] 2004年11月中旬から2005年11月中旬にかけての報告

表5は、2005年に1,000人以上の新規患者数が報告された17カ国を示している。これら17カ国で世界で発見された新規患者数の94%をも占めている。2002年以降、コンゴ民主共和国、インドネシア、およびフィリピンでの新規患者数は増加する傾向にある。

表5　2005年の新規患者数が1,000人以上であった17国の年 の比較

	新規患者数				
国名	**1993**	**2002**	**2003**	**2004**	**2005**
アンゴラ	339	4,272	2,933	2,109	1,877
インド	456,000	473,658	367,143	260,063	161,457
インドネシア	12,638	12,377	14,641	16,549	19,695
エジプト	1,042	1,318	1,412	1,216	1,134
エチオピア	4,090	4,632	5,193	4,787	4,698
コンゴ民主共和国	3,927	5,037	7,165	11,781	10,737
スリランカ	944	2,214	1,925	1,995	1,924
タンザニア	2,731	6,497	5,279	5,190	4,237
中国	3,755	1,646	1,404	1,499	1,658
ナイジェリア	4,381	5,078	4,799	5,276	5,024
ネパール	6,152	13,830	8,046	6,958	6,150
バングラデシュ	6,943	9,844	8,712	8,242	7,882
フィリピン	3,442	2,479	2,397	2,254	3,130
ブラジル	34,235	38,365	49,206	49,384	38,410
マダガスカル	740	5,482	5,104	3,710	2,709
ミャンマー	12,018	7,386	3,808	3,748	3,571
モザンビーク	1,930	5,830	5,907	4,266	5,371
合計	**555,307**	**599,945**	**495,074**	**389,027**	**279,664**
(世界合計に占める割合)	**(94%)**	**(97%)**	**(96%)**	**(95%)**	**(94%)**
世界合計	**590,933**	**620,638**	**514,718**	**407,791**	**296,499**

各WHO地域の国々の新規患者数の詳細は表6に示してある。これらすべての地域の国々では、新規患者の中に多菌型ハンセン病者が多数を占めることが報告された。アフリカ地域では、多菌型ハンセン病患者の割合はコモロの23%からケニアの92%、南北アメリカ大陸地域ではその範囲はボリビアの36%からキューバの83%であった。東地中海地域では、イエメンの58%からスーダンの92%、南東アジア地域はバングラデシュの38%からインドネシアの79%であり、また、西太平洋地域はミクロネシア連邦の30%からフィリピンの94%であった。

表6　WHO 地域ごとの新規患者の詳細（100 人以上の新規患者報告あった国に関してのみのデータ）

（単位：%）

WHO 地域	新規患者			
	多菌型ハンセン病の比率が最も多い国と少ない国	女性患者の比率が最も多い国と少ない国	小児患者の比率が最も多い国と少ない国	第２度障害者の比率が最も多い国と少ない国
アフリカ地域	コモロ：22.6	チャド：21	ケニア：3.2	コモロ：3
	ケニア：92.4	中央アフリカ：59.9	コモロ：39.1	ベナン：21.7
南北アメリカ地域	ボリビア：36.0	ベネズエラ：34	アルゼンチン：1	アルゼンチン：1.7
	キューバ：83.2	ドミニカ：50.3	ドミニカ：16	メキシコ：11.1
南東アジア地域	バングラデシュ：38.3	東ティモール：21.2	タイ：5.0	インド：1.9
	インドネシア：79.4	バングラデシュ：42.1	スリランカ：10.5	東ティモール：21.2
東地中海地域	イエメン：58.5	スーダン：28.1	スーダン：4.1	エジプト：2.7
	スーダン：92.1	パキスタン：38.8	イエメン：10.9	パキスタン：20
西太平洋地域	ミクロネシア連邦：29.6	カンボジア：28.2	中国：2.1	ミクロネシア連邦:0.8
	フィリピン：94.3	ミクロネシア連邦：36.2	ミクロネシア連邦：32.3	中国：21.3

アフリカ地域で新規患者の女性の割合は、チャドの 21% から中央アフリカ共和国の 60% の範囲であった。南北アメリカ地域の女性の割合は、ベネズエラの 34% からドミニカ共和国の 50%、南東アジア地域では東ティモールの 21% からバングラデシュの 42%。東地中海地域ではスーダンの 28% からパキスタンの 39%、西太平洋地域ではカンボジアの 28% からミクロネシア連邦の 36% であった。

このような幅広いばらつきは、特にアフリカ地域・南北アメリカ地域・西太平洋地域における新規患者に占める小児の割合でもみられる。アフリカ地域で新規患者に占める小児の割合は、ケニアの３% からコモロの 39%、南北アメリカ地域ではアルゼンチンの１% からドミニカ共和国の 16%、西太平洋地域では中国の 2.1% からミクロネシア連邦の 32% であった。しかし、南東アジアと東地中海地域における新規患者の小児の割合はそれぞれタイの 5%からスリランカの 11%、スーダンの 4% からイエメンの 11%と、そのばらつきは小さかった。

同様に、新規患者中の第２級障害者の割合は、全ての地域でばらつきが大きかった。アフリカ地域ではコモロの３% からベナン 22%、南北アメリカ地域ではアルゼンチンの２% からキシコの 11%、南東アジア地域ではインドの２% から東ティモールの 21%、西太平洋地域ではミクロネシア連邦の１% から中国の 21% であった。

結　論

早期発見と多剤併用療法（MDT）による治療が、引き続きハンセン病制圧戦略の土台となる。プライマリーケア健康スタッフが、患者の家の近くの保健医療施設で、容易にハンセン病サービスを提供できる、統合されたアプローチが今後も主要な戦略であり続ける。またこれにより将来におけるサービスの継続は確たるものになる。新規患者数がさらに減少するにつれて、紹介センターの役割の重要性は高まる。紹介センターは特に、末端レベルの保健医療施設における正確な患者の診断とその管理を支えるための重要な役割を果たす。ここで課題となるのは、こういった施設を各地域で他の病気に関する紹介サービスも同様に提供する統合システムとして確立させることである。また、各個人にとってこれらのサービスが身近で利用しやすく、手頃な価格であり、効果的であることを保障することである。

WHO は、ハンセン病の影響を受ける地域でハンセン病制圧サービスを維持するために、パートナーシップを促進、強化し続ける。国家プログラムと

協力し、WHOは、流行国においてハンセン病制圧サービスを維持するための国家計画を展開するため、技術サポートと援助を提供し続ける。これは、国内で働く様々なパートナーの密接な協力により実行される。今までと同様に、ハンセン病が流行している国々にはMDT薬剤は無料で供給される。

世界的な新規患者数の減少はこれからも続くことが期待されている。新たに発見された患者の中の第2度障害者の割合、小児の割合、また女性の割合は、世界戦略の一部として綿密に監視していく。これら新規患者に関する統計は、制圧活動の進行や質、また治療完了率および治癒率を評価する主な指標として活用されるからである。これまでの制圧戦略による成果がさらに整理統合され、末端レベルにおける良質なサービスの維持が重視されるなかで、流行国の疾病問題はさらに減少されることが期待される。

本論文は、平成18年度国際医療協力研究委託費「開発途上国で有効なハンセン病の診断、治療、障害予防に関する研究」の分担研究「開発途上国における偏見・差別の解消に向けた研究」の補助金を受けた。