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Isolation, Characterization, Molecular Cloning and Amplification of a Species-Specific *M. leprae* Antigen[1]

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Leprosy is an infectious disease caused by the acid-alcohol-resistant bacillus *Mycobacterium leprae.* Severe socioeconomic and psychological implications are associated with this disease ([22, 30]) which is a serious health problem in tropical and subtropical areas. Leprosy exhibits a wide spectrum of clinical forms and immunological manifestations, ranging from lepromatous to tuberculoid extremes. Lepromatous leprosy, in contrast to tuberculoid leprosy, presents a significantly suppressed cellular immune response to *M. leprae* and therefore the bacillus multiplies freely. Also, lepromatous patients present high circulating antibody titers against several *M. leprae* antigens ([1, 21]).

Although leprosy can be treated with chemotherapy, knowledge of the bacillus' individual components is a prerequisite in the search for molecules of potential immunoprophylactic or immunodiagnostic value ([17]). Because *M. leprae* cannot yet be cultured *in vitro,* recombinant DNA technology must be used to study its genome's molecular structure, as well as the function of specific genes. Many proteins with antigenic characteristics have been identified from the recombinant libraries constructed by Young, *et al.* ([33]) and Clark-Curtiss, *et al.* ([2]), some of them being unique to the *M. leprae* organism ([32]). Potential polymerase chain reaction (PCR)-based diagnostic methods have been developed, including the detection of genes coding for the 18-kDa, 36-kDa and 65-kDa proteins, 16 rRNA, and repetitive element (REP) sequences ([5, 7, 9, 15]).

With the aim of finding antigens having relevance to the immune response, the present study has focused on the search for genes and proteins exclusive to *M. leprae* using polyclonal sera obtained from patients with active, untreated lepromatous leprosy. Identification of a *M. leprae* chromosomal fragment isolated from a genomic library constructed with bacilli of human origin is reported here. This sequence also has partial homology to a hypothetical protein from the *M. tuberculosis* MTCY9C4 cosmid ([4]) and comprises an epitope which is recognized by both lepromatous leprosy and healthy tuberculosis contacts and acute tuberculosis patients' sera.

MATERIALS AND METHODS

Bacterial strains and vectors. The following mycobacterial strains were obtained from the ATCC and Trudeau Mycobacterial Collection (TMC): *M. africanum* (ATCC 25420), *M. avium* (TMC 25291), *M. bovis* (ATCC 19210), *M. bovis* BCG (ATCC 27291 Pasteur substrain), *M. chelonae* subsp. *abcessus* (ATCC 19977, 11758), *M. chelonae* subsp. *chelonae* (ATCC 35752), *M. diernhoferi* (TMC 2301), *M. flavescens* (ATCC 14744), *M. fortuitum* (ATCC 6841), *M. gastri* (TMC 1456), *M. gordonae* (ATCC 14470), *M. intracellulare* (TMC 13950), *M. kansasii* (TMC 1204), *M. marinum* (ATCC 927), *M. microti* (ATCC 35781), *M. nonchromogenicum* (TMC 19530), *M. peregrinum* (TMC 1547), *M. phlei* (ATCC 11758), *M. scrofulaceum* (TMC 19981), *M. simiae* (TMC 1595), *M. smegmatis* (ATCC 14468), *M. szulgai*

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(TMC 1201), *M. terrae* (TMC 1450), *M. triviale* (TMC 1453), *M. tuberculosis* (ATCC 27294 strain H37Rv), *M. tuberculosis* (ATCC 25177 strain H37Ra), *M. vaccae* (TMC 1526) and *M. xenopi* (TMC 1470). *Escherichia coli* strains Y1088, Y1089, Y1090 and JM101 and the λgt11 bacteriophage were obtained from Amersham, Amersham, U.K. The phagemid vector Bluescript M13 (+/–) was purchased from Stratagene, La Jolla, California, U.S.A.

***M. leprae* isolation.** Bacilli were isolated from lepromatous patients' lepromas following the methodology previously described by Clark-Curtiss, *et al.* [2].

Immunological analysis. On the basis of clinical and histopathological criteria, an outpatient from the Agua de Dio leprosy colony (Cundinamarca, Colombia) was identified. The patient was diagnosed as suffering from active lepromatous leprosy by routine bacteriological diagnostic tests, with the demonstration of acid-fast bacilli (AFB) in skin smears. The patient had not received multidrug therapy (MDT) at the time. Two grams (wet weight) of lepromas and a blood sample were obtained. The lepromas were frozen and transported for immediate processing. The polyclonal antileprosy antiserum was pre-adsorbed against *E. coli*-λgt11, as described by Snyder, *et al.* [23], and an immunoblot using *M. leprae* sonicate as antigen (leprosin) was carried out. Briefly, leprosin extract was separated by SDS-PAGE and transferred to a Hybond-N membrane (Amersham) as described by Towbin, *et al.* [26]. The membrane was cut into two strips, each of which was subsequently incubated with either antileprosy antiserum or a healthy subject's serum and detected with horseradish peroxidase-conjugated anti-human IgG (Promega Corporation, Madison, Wisconsin, U.S.A.) and H_2O_2-diaminobenzidine substrate (GIBCO BRL, Gaithersburg, Maryland, U.S.A.).

Isolation of human and mycobacterial DNA. Human DNA was isolated from lymphocytes and healthy skin cells by previously described methods [14]. *M. leprae* DNA from human lepromas and other fast- and slow-growing mycobacterial DNAs were isolated as described previously [17].

M. leprae DNA isolated from armadillo tissues, used in the Southern blot analysis, were kindly provided by Dr. Patrick J. Brennan (Colorado State University, College of Medicine, Veterinary and Biomedical Sciences, Microbiology Department, Fort Collins, Colorado, U.S.A.).

Library construction and screening. An *M. leprae* genomic library was constructed in the λgt11 expression vector following previously described methodology [17]. The library was packaged in Gigapack Gold extracts (Stratagene) and amplified in *E. coli* Y1088. Antibody screening was done with a polyclonal antileprosy antiserum (1:50 dilution) as previously described [12], using horseradish alkaline-phosphatase conjugated anti-IgG (Promega) and NBT-BCIP as substrates (IMMUNO-Select Kit; GIBCO BRL).

Restriction mapping, DNA sequencing, and computer analysis. DNA was purified from recombinant phage as previously described by Manfioletti, *et al.* [13]. ML4-1 insert in the λgt11 bacteriophage transcription direction was determined using the *Kpn* I restriction enzyme (GIBCO BRL). The λgt11 : ML4-1 clone insert was excised after digestion with *Eco* RI, as recommended by manufacturer (GIBCO BRL), purified according to the Gene Clean II Kit protocol (Bio-101, Inc., La Jolla, California, U.S.A.) and cloned in phagemid Bluescript SK+ II (Strategene). The resulting construct was subsequently amplified in *E. coli* JM101; plasmid DNA was isolated using the maxi-prep methodology [18] and restriction mapping was done.

Unidirectional deletion generation was performed with the Erase-a-base system [18] in order to obtain the entire ML4-1 clone's sequence. Single- and double-strand sequencing [20] was done using a Sequenase V.2.0 kit (USB, Cleveland, Ohio, U.S.A.) and dye primers and dye terminators cycle sequencing, with Taq polymerase (Perkin-Elmer Applied Biosystems, Foster City, California, U.S.A.) on a 373 DNA sequencer (Perkin-Elmer Applied Biosystems). The reaction products were subjected to electrophoresis in 8% polyacrylamide gels, with 7M urea.

Computer-assisted analysis was performed using GENEPRO (Genetics Computer Group, University of Wisconsin Biotechnology Center, Madison, Wisconsin, U.S.A.), Staden [25] and Gene-Runner

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PCR primers and probe. The primer sequences used in the PCR amplification correspond to nucleotides 114 to 131 and 543 to 560. These were named LC1 and LC2 and the sequences are: GATCCTGAACGAGAGGGA and GATCGACATTGTCGGGAA, respectively. The oligonucleotides were synthesized in a Gene Assembler equipment (Pharmacia, Upsala, Sweden), and purified in a 20% polyacrylamide gel ([16]).

PCR analysis of the amplified products. Amplifications by PCR were performed as described elsewhere ([19]). Briefly, 50 µl reaction mixtures containing 100 ng of purified mycobacterial DNA, 125 µM dNTPs, 20 pmol of each primer, 5 µl 10× PCR buffer (100 mM Tris-HCl [pH 8.3], 500 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin) (Perkin-Elmer Cetus, Instruments, Norwalk, Connecticut, U.S.A.) and 5 U Taq polymerase were used. Each cycle was carried out for 1 min at 95°C, 1 min at 60°C and 2 min at 72°C for a total of 30 cycles. Samples were stored at 4°C in the thermal cycler until removed for further analysis.

Southern blot and hybridization. DNAs from different mycobacteria were digested with *Eco* RI enzyme and run on 1% agarose gel as described elsewhere ([24]). The DNAs were transferred onto Hybond-N membranes (Amersham) by the alkaline method and hybridized using the ML4-1 clone's 585 bp *Eco* RI-*Bam* HI fragment as probe, labeled with [^{32}P] dCTP by random priming, using the Megaprime DNA labeling system (Amersham) to a specific activity of 10^8 cpm/µg DNA. Hybridization was performed for 16 hr at 65°C in a solution containing 1× SSC (1× SSC is 150 mM NaCl and 15 mM sodium citrate, pH 7.0), 0.2% bovine serum albumin, 0.2% Ficoll, 0.2% polyvinylpyrrolidine (PVP), 0.5% SDS, and 20 µg/ml salmon sperm DNA. The filter was gradually washed at 65°C using SSC from 1× to 0.1× and 0.5% SDS. PCR amplification products with LC1 and LC2 primers were blotted and hybridized as described above.

Antigen preparation. Based on the ML4-1 protein sequence, 32 nonoverlapping peptides, 20 residues in length, were chemically synthesized using the Solid Phase Multiple Peptide Synthesis Technique. Once synthesized, the peptides were extracted with 10% acetic acid and purified by HPLC. Peptides were divided into six groups of five peptides each and one group of two peptides only. A dilution of 10 µg/mL in PBS was prepared from each peptide group. The synthetic peptide groups' antigenic capacity was tested by ELISA using sera from leprosy patients (4 lepromatous leprosy), tuberculosis patients (6 acute, 6 under treatment, 3 post-treatment and 5 healthy contacts), malaria patients (2 *Plasmodium falciparum,* 5 *P. vivax* and 3 co-infected with *P. vivax* and *P. falciparum*), and sera from human newborn subjects (18 samples). The last group was used as a control group in order to determine the cut-off values in the ELISA.

Enzyme-linked immunoassay (ELISA). For the ELISA, 1 µg per well of each peptide group was added to the Micro-ELISA plates (Nunc, Roskilde, Denmark). After 1 hr of incubation at 37°C and a further overnight incubation of plates at 4°C, the wells were washed three times with a washing solution containing 0.2 g potassium chloride, 8 g sodium chloride, 0.2 g potassium monobasic phosphate, 1.15 g sodium dibasic phosphate and 0.5% Tween-20 per liter, pH 7.4. Then 200 µl 5% skim milk in washing solution (blocking solution) was added and the plates were incubated for 1 hr at 37°C. Wells were then washed three times and incubated for 1 hr at 37°C with sera diluted 1:200 or 1:400 (for peptide 221–240). The wells were subsequently washed five times with washing solution and incubated for 1 hr at 37°C with anti-human IgG peroxidase conjugate diluted 1:10,000 and reaction was developed with 100 µl of the chromogen substrate mixture (3-3′-5-5′ tetramethyl benzidine and hydrogen peroxide) being added per well. Fifteen min later the reaction was stopped with 100 µl 1 M phosphoric acid per well, and the absorbances were read at 450 nm.

RESULTS

Recognition by antileprosy antibodies. A leprosin protein extract was evaluated by immunoblot with both human antileprosy antisera and normal human serum. Three predominant bands of the *M. leprae* extract with molecular masses of 24, 29 and 47.5

kDa were strongly recognized in the Western blot analysis by the antileprosy antisera (Fig. 1).

***M. leprae* isolation, genomic library construction and screening.** Two grams of lepromas (wet weight) were analyzed and shown to contain AFB of +2 (1 to 10 bacilli in 10 observed fields) without cellular debris. DNA extraction yielded approximately 500 ng of *M. leprae* bacilli DNA/mg of leproma and 100 µg/mg from the other mycobacteria. A genomic library was constructed containing 10^5 pfu/µg DNA amplified in *E. coli* Y1088 to a final titer of 10^8 pfu/ml. The library comprised 86% recombinants as assessed by a β-galactosidase color assay, with insert sizes ranging from 1 to 7 Kbp.

Ten filters, representing a total of 20,000 plaques, were screened with the human antileprosy antiserum from the leprosy patient from whom lepromas were isolated. In the first round of screening two positive clones were detected. These remained positive through three further rounds of consecutive purification and were later determined to be identical. This clone, named λgt11:ML4-1, was analyzed by digestion with *Eco* RI and shown to contain an insert approximately 1.9 Kpb long.

Sequence analysis of the ML4-1 fragment. The ML4-1 clone restriction map allowed the identification of unique recognition sites for *Sal* I, *Kpm* I and *Bam* HI enzymes within the insert. The orientation in the λgt11 : ML4-1 clone was determined to be in the *Eco* RI → *Bam* HI direction. The ML4-1 fragment's complete sequence was obtained by a combined strategy of subcloning and re-ligations as described in the Materials and Methods section. The *M. leprae* fragment's sequence in the ML4-1 clone is shown in Figure 2. The 1932 bp sequence's G+C base composition was calculated to be 63.5%, and one important open reading frame (ORF) was found. This ORF seems to code for a protein of 644 amino acids (72.3 kDa) with a 61% identity (132/218) to an *M. tuberculosis* H37Rv cosmid nucleotide sequence MTCY 09C4 ([4]).

Southern blot and PCR. As shown in Figure 3, a positive 1932 bp signal was obtained in the *M. leprae* genome digested with the *Eco* RI enzyme but not in any of the other 24 mycobacterial genomes tested. Also, from PCR amplifications, using the LC1 and LC2 primers, it was possible to detect a specific 446 bp fragment from *M. leprae* DNA; no amplification product was observed using either human DNA or any of the other 23 mycobacterial genomic DNA samples (Fig. 4), indicating the exclusive presence of the 446 bp fragment in the *M. leprae* genome.

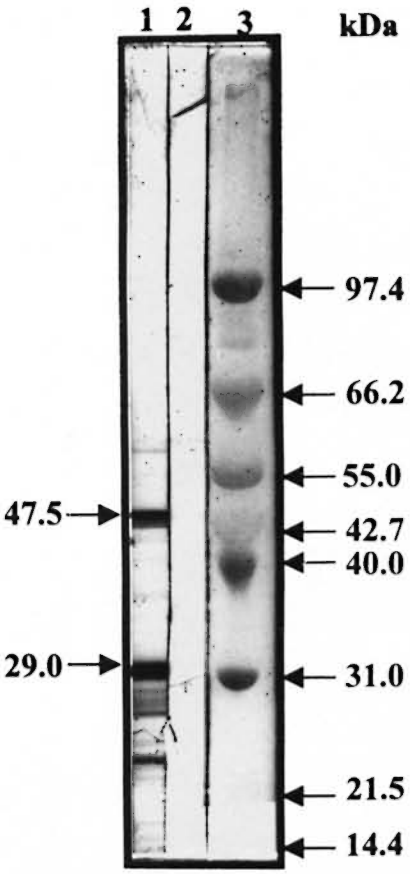

FIG. 1. Antileprosy antiserum and normal serum reactivity against an *M. leprae* leprosin extract in a Western blot assay. Leprosin extract (100 µg per lane) was diluted in Laemmli buffer containing 2.5% β-mercaptoethanol, boiled for 5 min at 100°C and loaded onto a 7.5% SDS-polyacrylamide gel. Proteins were then transferred to a nitrocellulose membrane which was cut into strips and incubated in a 1:50 dilution with either the antileprosy antiserum (lane 1) or healthy subject (lane 2) sera. An anti-human IgG horseradish-peroxidase conjugate was used as a second antibody. Molecular weight markers are indicated.

ELISA peptide assays. All synthetic peptides were tested by groups and later on

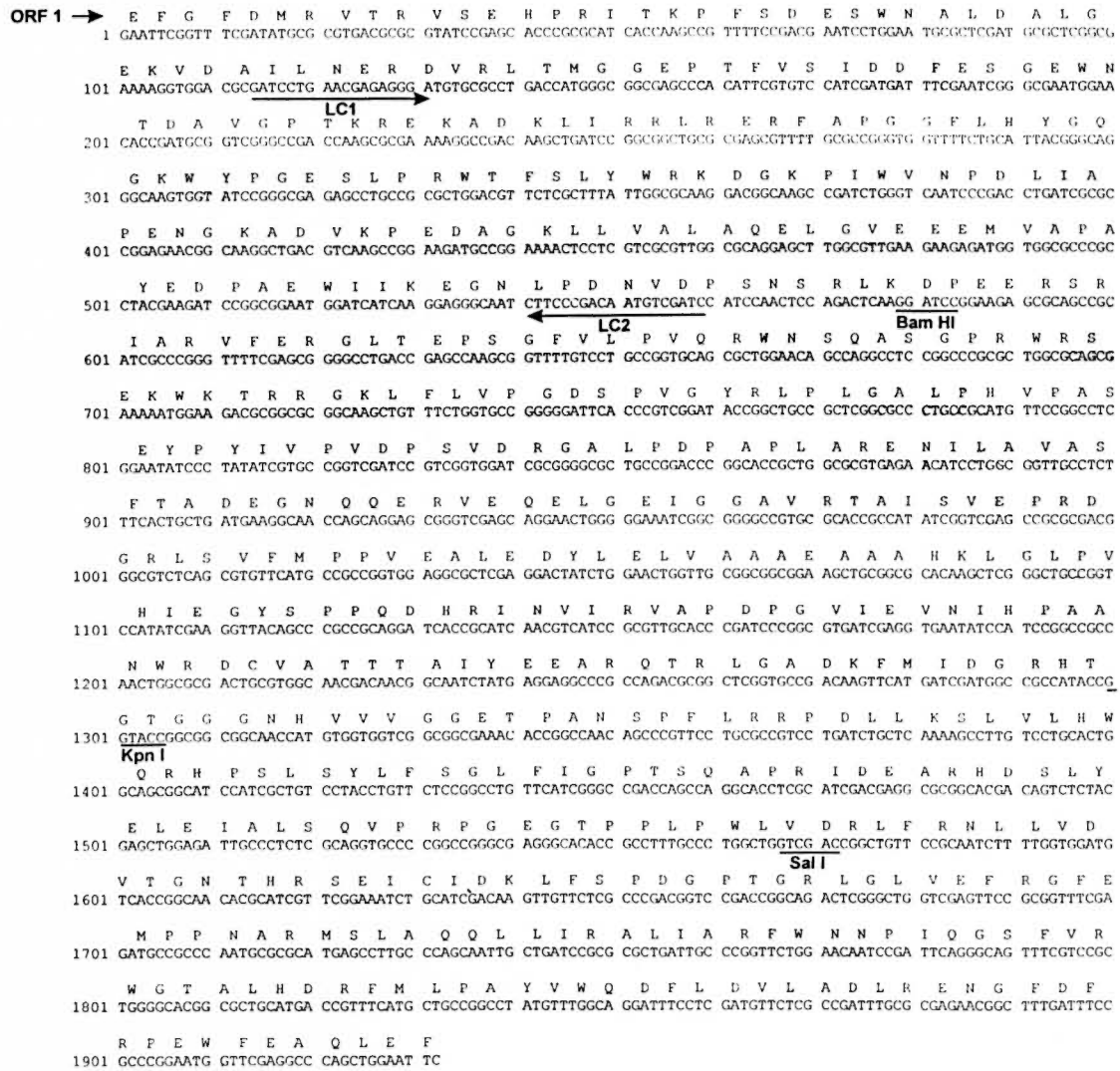

FIG. 2. Nucleotide sequence of ML4-1 genomic fragment and amino-acid sequences deduced from main ORF. ORF1 and the *Bam* HI, *Kpn* I and *Sal* I restriction sites are shown. Localization of LC1 and LC2 primers are indicated.

individually. Peptides belonging to group three showed a marked pattern difference between distinct sera tested, as seen in The Table. Peptide corresponding to position 221–240 was predominantly recognized either by all lepromatous leprosy patients or healthy tuberculosis contact sera examined. Surprisingly, this peptide had a cut off point higher than the rest of the peptides, even working at a serum dilution of 1:400. Also, a Chow and Fasman analysis showed that this peptide has a predominant alpha helix and a hydrophilic pattern, indicating its potential antigenic capacity.

DISCUSSION

The identification and characterization of *M. leprae* protective antigenic determinants is important for two reasons. First, it is relevant for the identification of species-specific sequences for the development of improved diagnostic methods and, secondly, for the ultimately more important goal of developing an effective vaccine ([30]). In this study, a recombinant DNA expression library with *M. leprae* DNA isolated from human lepromas was constructed, and it was screened using human serum from a

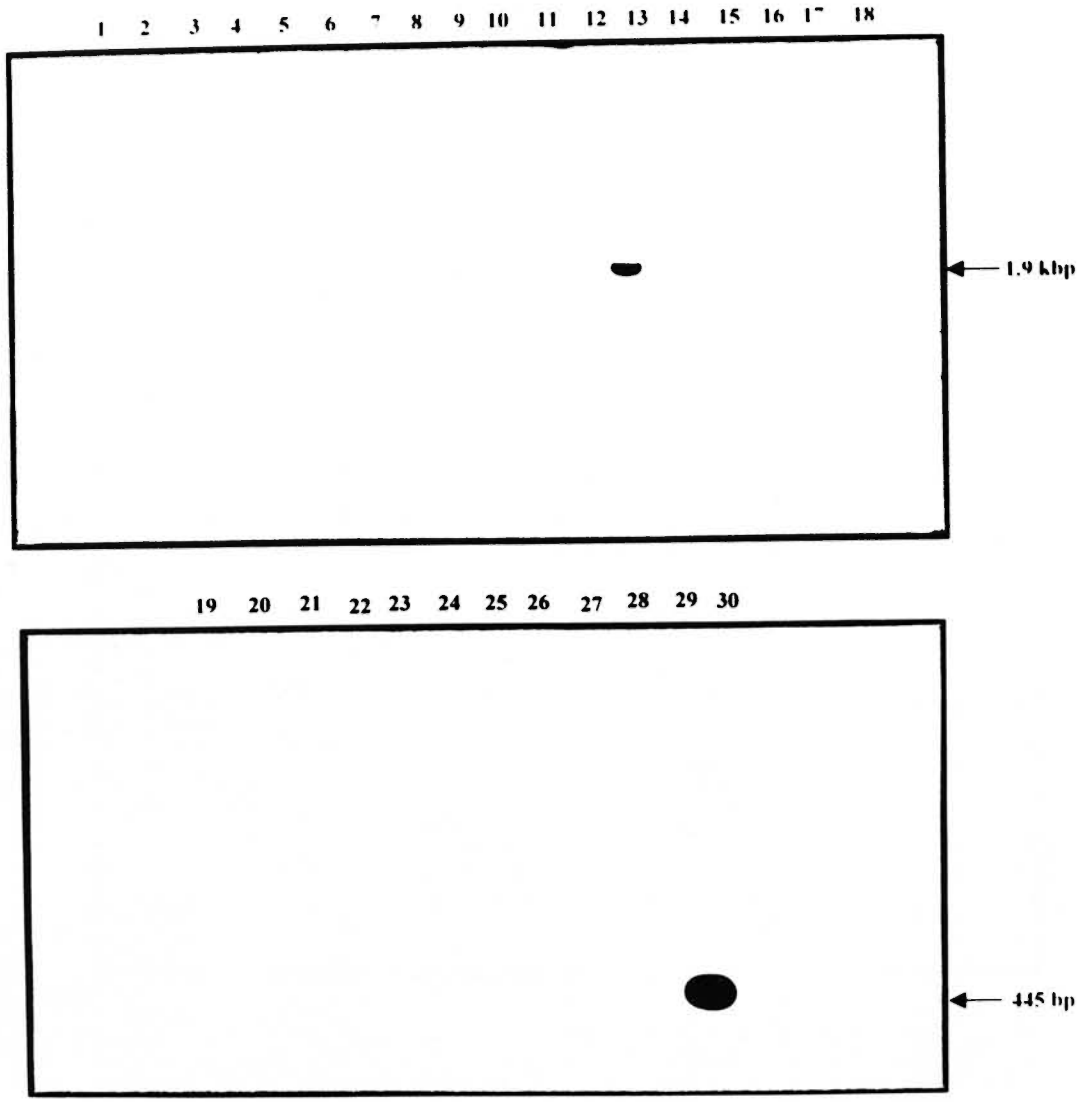

FIG. 3. Southern hybridization using ML4-1 *Eco* RI-*Bam* HI 585 bp fragment as a probe. Chromosomal DNAs from different mycobacteria were digested with *Eco* RI and hybridized with ML4-1 *Eco* RI-*Bam* HI 585 bp probe. Lanes: 1) *M. avium*, 2) *M. bovis*, 3) *M. bovis* BCG, 4) *M. chelonae* subsp. *abs.*, 5) *M. chelonae* subsp. *che.*, 6) *M. diernhoferi*, 7) *M. flavescens*, 8) *M. fortuitum*, 9) MWM, 10) *M. intracellulare*, 12) *M. kansasii*, 13) *M. leprae*, 14) *M. marinum*, 15) *M. nonchromogenicum*, 16) *M. peregrinum*, 17) *M. phlei*, 18) *M. scrofulaceum*, 19) *M. simiae*, 20) *M. smegmatis*, 21) *M. szulgai*, 22) *M. terrae*, 23) *M. triviale*, 24) MWM, 25) *M. tuberculosis* H37Rv, 26) *M. vaccae*, 27) *M. xenopi*, 28) human lymphocytes, 29) human skin, 20) positive control (446 bp PCR product).

lepromatous leprosy patient. A λgt11 recombinant clone was subsequently isolated and sequenced, having a 1.932 bp with one main open reading frame coding for a 72.3-kDa protein. Also, three predominant bands of the *M. leprae* extract with molecular masses of 24, 29 and 47.5 kDa were strongly recognized in the Western blot analysis by the antileprosy antisera which could be breakdown products of the putative 72-kDa protein (Fig. 1).

The human-derived recombinant DNA library described in this article may help to answer some of the many outstanding questions regarding *M. leprae* infection, such as the role of polymorphism in drug resis-

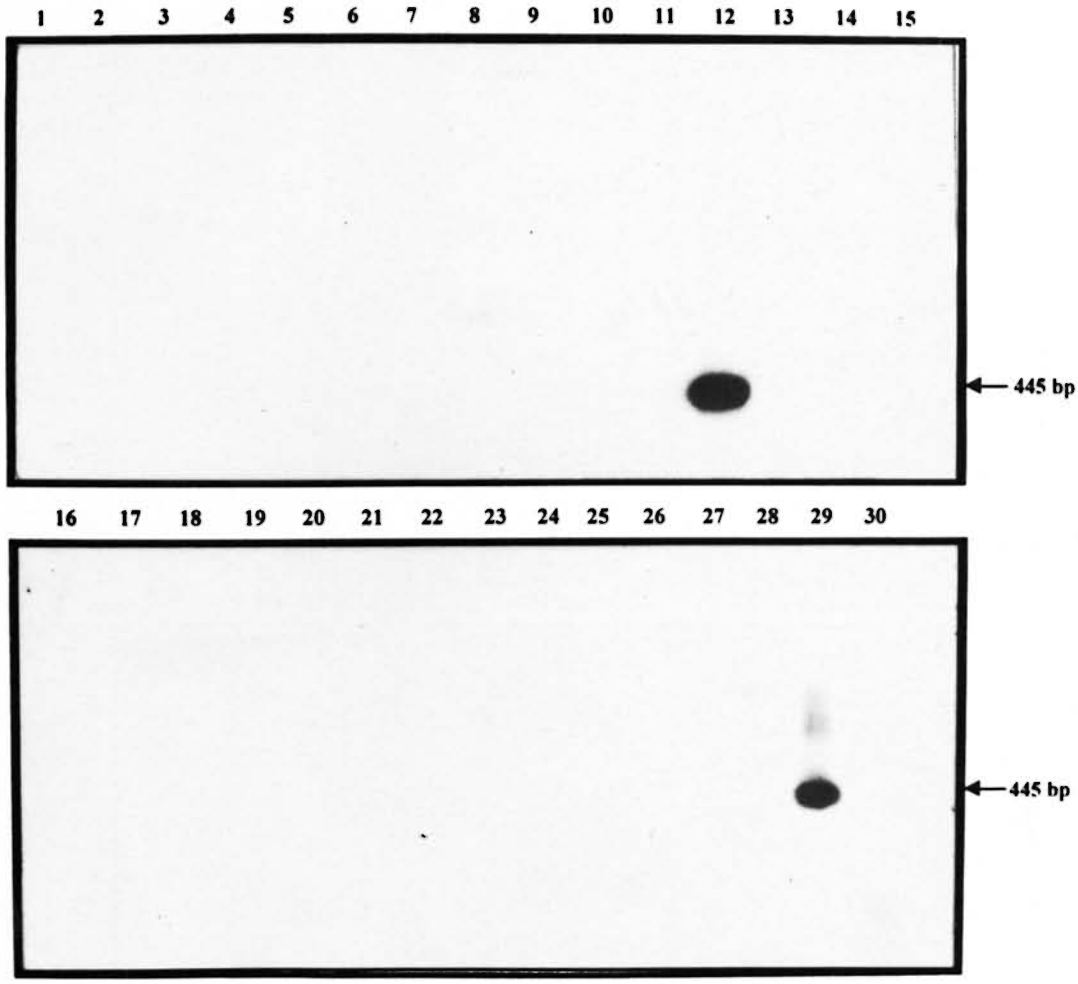

FIG. 4. Hybridization of PCR amplification products using LC1 and LC2 primers. Lanes: 1) *M. africanum,* 2) *M. avium,* 3) *M. bovis,* 4) *M. bovis* BCG, 5) *M. chelonae,* 6) *M. diernhoferi,* 7) *M. fortuitum,* 8) MWM, 9) *M. gastri,* 10) *M. intracellulare,* 11) *M. kansasii,* 12) *M. leprae,* 13) *M. marinum,* 14) *M. microti,* 15) *M. nonchromegenicum,* 16) *M. peregrinum,* 17) *M. phlei,* 18) *M. scrofulaceum,* 19) *M. smegmatis,* 20) *M. szulgai,* 21) *M. terrae,* 22) *M. triviale,* 23) MWM, 24) *M. tuberculosis* H37Rv, 25) *M. tuberculosis* H37Ra, 26) *M. xenopi,* 27) human lymphocytes DNA, 28) human skin DNA, 29) positive control (pBS:ML4-1) and 30) negative control (without DNA).

tance. Polymorphism has been found in different geographical and host isolates' chromosomal DNA ([3]), but all recombinant DNA clones isolated to date have been obtained from genomic libraries constructed with *M. leprae* grown in armadillo ([2, 33]). The level of polymorphism present in human-derived isolates is, therefore, completely unknown and the use of recombinant libraries derived from leprosy patients would allow the detection of alterations at the genomic level associated with resistance and, in addition, could provide probes for drug-resistance markers.

Synthetic peptides derived from the entire sequence reveal an important epitope (amino acid position 221–240) recognized by sera obtained from lepromatous leprosy, from healthy contacts and from acute tuberculosis patients. By comparison, 6 out of 20 amino acids are identical in the putative *M. tuberculosis* protein from the MTCY9C4

THE TABLE. *ELISA from group 3 individual peptides.*[a]

Peptide	No. positive sera tested for each patient group[a]						
	A	B	C	D	E	F	G
201–220	0/4	0/5	0/6	0/6	0/3	0/5	0.125
221–240	4/4	1/5	3/6	1/6	0/3	4/5	0.244
241–260	1/4	0/5	0/6	0/6	0/3	0/5	0.149
261–280	1/4	1/5	0/6	1/6	0/3	0/5	0.089
281–300	1/4	1/5	0/6	0/6	0/3	0/5	0.115

[a]Patient groups: A = Lepromatous leprosy; B = malaria; C = acute tuberculosis; D = tuberculosis under treatment; E = post-treatment tuberculosis; F = healthy contacts of tuberculosis; G = cut-off point.

cosmid, where two tryptophan, two arginine, one glycine and one serine are preserved and could be implicated in the cross-reactivity between these mycobacterial species. Since hydrophilicity has been correlated with antigenicity ([11]), it is also possible that this region is the recognition site for the antileprosy antiserum antibodies. On the other hand, leprosy diagnosis is based on clinical and histopathological criteria, which means that it could be delayed by 2 or 3 years' post-infection. The low multiplication rate of the bacilli permits the routine identification of acid-fast bacilli only in late post-infection stages. Moreover, IgG antibody presence against phenolic glycolipid-I (PGL-I) has been shown to have low sensitivity ([27]). Thus, it is necessary to develop methodologies for routine diagnosis to facilitate the initiation of chemotherapy and immunotherapy during the disease's early stages. The use of PCR for infectious diseases' diagnosis is a powerful tool ([6]), and many tests for the specific PCR identification of *M. leprae* exist ([10, 27–29, 31]). A new *M. leprae* species-specific antigen is identified in this article through the use of PCR and hybridization studies using different mycobacterial strains. Although some nucleotide sequence homology with an *M. tuberculosis* cosmid was observed, there was not a positive signal either by Southern blot or PCR analysis, probably due to the stringency conditions used in both experiments and because the 61% nucleotide sequence homology is located in the 3′ end of the ML4-1 clone and the sequence analyzed in the present study is located in the 5′ end. Therefore, this unique genomic fragment could be proposed as a future candidate in the development of a potentially specific diagnostic method for the detection of *M. leprae* infection.

SUMMARY

A polyclonal serum sample from a lepromatous leprosy (LL) patient, which presented a specific recognition pattern for leprosin, was used to screen a *Mycobacterium leprae* genomic library constructed with DNA isolated from human lepromas. One clone, designated ML4-1, which expressed a specific antigenic determinant of *M. leprae* as part of a β-galactosidase fusion protein, was isolated. The 1.932 bp *M. leprae*-derived genomic fragment was sequenced, and it had an incomplete open-reading frame shown to code for a 644 amino-acid polypeptide (72.3 kDa). Some partial nucleotide homology to the *M. tuberculosis* MTCY9C4 cosmid and the *M. leprae* B1913 cosmid were found. Southern blot assays using the 584 bp *Eco* RI-*Bam* HI fragment excised from the ML4-1 clone revealed that this sequence is present only in the *M. leprae* genome and not in the 24 different mycobacterial DNA tested. Two oligonucleotides based on the genomic sequence were also synthesized and used as amplifiers for a polymerase chain reaction (PCR) test, giving a positive signal exclusively in *M. leprae* DNA. Furthermore, 32 sequential synthetic peptides, 20 amino-acids long, spanning the entire protein corresponding to the hypothetical ML4-1 clone sequence, were synthesized and evaluated by ELISA. A peptide included in the 221–240 region was significantly recognized by either lepromatous leprosy or healthy tuberculosis contact patient sera. Thus, PCR amplification of this fragment,

along with the recognition of its protein sequence by leprosy patient sera, could be a useful tool for a potential diagnostic method in the detection of *M. leprae* infection in the future.

RESUMEN

Se utilizó un suero policlonal de un paciente con lepra lepromatosa (LL) con un patrón de reconocimiento especifico de la leprosina, para analizar una biblioteca genómica de *Mycobacterium leprae* construida con el DNA aislado de lepromas humanos. Se aisló una clona, designada ML4-1, la cual expresó un determinante antigénico específico de *M. leprae* como parte de una proteina de fusión con beta-galactosidasa. El fragmento genómico secuenciado de 1.932 pares de bases (pb) de *M. leprae* tuvo un marco de lectura abierto incompleto que codificó para un polipéptido de 644 aminoácidos (72.3 kDa) y mostró homologias parciales con las secuencias de los cósmidos MTCY9C4 de *M. tuberculosis* y B1913 de *M. leprae*. Los análisis de Southern blot usando el fragmento de 584 pb *Eco* RI-*Bam* HI obtenido de la clona ML4-1 reveló que esta secuencia sólo está presente en el genoma de *M. leprae* y no en el DNA de 24 diferentes micobacterias probadas. También se sintetizaron dos oligonucleótidos basados en la secuencia genómica y se usaron como iniciadores en la prueba de la reacción en cadena de la polimerasa (PCR). Sólo se obtuvo señal positiva con el DNA de *M. leprae*. Además, se sintetizaron 32 péptidos sintéticos secuenciales de 20 aminoácidos de logitud, que abarcaron la proteina completa correspondiente a la secuencia hipotética de la clona ML4-1. Estos péptidos fueron evaluados por ELISA. Un péptido incluido en la región 221–240 fue reconocido de manera significativa por los sueros de pacientes con lepra lepromatosa o tuberculosis per no por los sueros de contactos sanos. Asi, la amplificación por PCR de este fragmento y el reconocimiento de su secuencia proteica por los sueros de los pacientes con lepra, podrian servir como herramientas de diagnóstico para detectar la infección por *M. leprae* en el futuro.

RÉSUMÉ

Un échantillon de sérum polyclonal provenant d'un patient souffrant de lèpre lépromateuse et qui exhibait une reaction spécifique à la léprosine, fut utilisé pour tester une bibliothèque génomique construite à partir d'ADN provenant de lépromes humains. Il fut isolé un clone, appelé ML4-1, qui exprimait un déterminant antigénique spécifique de *M. leprae* fusionné à une protéine de (β-galactosidase. Le fragment de génome dérivé de *M. leprae*, mesurant 1932 paires de bases, fut séquencé: il montrait un cadre de lecture ouvert incomplet codant un polypeptide de 644 acides aminés (72.3 kilodaltons). Une homologie partielle de séquence fut trouvée avec le cosmide MTCY9C4 dérivé de *M. tuberculosis* et le cosmide B1913 dérivé de *M. leprae*. Une analyse par Southern blot, utilisant un fragment de ML4-1 mesurant 584 paires de bases, obtenu par digestion avec les enzymes de restriction Eco R1et Bam H1, a démontré que cette séquence n'était rencontrée que dans le génome de *M. leprae* et non pas dans l'ADN de 24 autres mycobactéries testées. Deux oligonucléotides furent également synthétisés et utilisés pour amplifier le fragment ML4-1, par la réaction de polymérase en chaîne (PCR), ne donnant un résultat positif qu'en présence d'ADN de *M. leprae*. De plus, 32 oligopeptides artificiels de 20 acides aminés de long, qui mis bout à bout représentent la protéine hypothétique correspondant à la séquence du clone ML4-1, furent synthétisés et évalués par ELISA. Un peptide, inclu dans la région 221-240 de la protéine hypothétique, fut significativement reconnu par les sérums issus de patients lépromateux ou des personnes en contact avec des patients tuberculeux. Ainsi, l'amplification par PCR de ce fragment génomique et la reconnaissance par le sérum de patients atteints de lèpre de sa fraction protéique correspondante, pourrait être un outils intéressant et une méthode diagnostique potentielle pour la future détection de l'infection par *M. leprae*.

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