JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 1995, p. 45–49 Vol. 33, No. 1
0095-1137/95/$04.00+0

Detection of *Mycobacterium leprae* Infection by PCR

JESDAWAN WICHITWECHKARN,[1]* SOMJIT KARNJAN,[1] SIRIPHAN SHUNTAWUTTISETTEE,[1] CHALUAY SORNPRASIT,[2] KOWIT KAMPIRAPAP,[1] AND SOMCHAI PEERAPAKORN[1]

Raj-pracha-samasai Institute, Leprosy Division, Soi Bamrasnaradoon Hospital, Nonthaburi 11000,[1] and Bangkhaen Skin Clinic, Bangkok,[2] Thailand

Received 23 June 1994/Returned for modification 25 July 1994/Accepted 14 September 1994

PCR amplification of the 531-bp fragment of the *Mycobacterium leprae pra* gene in fresh biopsy and slit skin smear samples was evaluated for its usefulness in the detection of leprosy bacilli in patients in Thailand. In multibacillary patients, 87.1% (27 of 31) of biopsy specimens and 41.9% (13 of 31) of slit skin smear specimens were positive by PCR, whereas in paucibacillary patients, 36.4% (8 of 22) of biopsy specimens and 18.2% (4 of 22) of slit skin smear specimens yielded detectable PCR amplification. Compared with other diagnostic procedures, PCR showed a clear advantage over both microscopic examination of slit skin smears and serologic detection of anti-phenolic glycolipid 1 antibody, especially in paucibacillary patients when bacterial indexes were 0 and seropositivity was only 6.25%. PCR was also evaluated for its potential to help monitor bacterial clearance in some of these patients during chemotherapeutic treatment. The PCR results on slit skin smear samples at 1, 3, and 6 months of chemotherapy showed that the number of PCR-positive cases of both multibacillary and paucibacillary types decreased sequentially. The results of this study are encouraging. However, investigation of a larger number of clinical specimens with an improvement in PCR methods, especially on slit skin smears, needs to be done before PCR can be established as a diagnostic procedure for leprosy patients and subclinical cases or as a tool for drug assessment.

Leprosy, caused by *Mycobacterium leprae*, is still considered a major health problem in many developing countries. It is a chronic infectious disease of skin, nasal cavity, and peripheral nerves which eventually leads to disability, disfiguration, and socioeconomic problems (9, 11). There is no useful serologic test for the diagnosis of leprosy (6, 11). Early detection of the causative microorganisms is, therefore, the key element to early identification and treatment of patients and subclinical cases before the disease progresses and neural involvement occurs. These organisms are not cultivable on artificial media, and attempts to identify them by inoculating a susceptible animal such as the armadillo (13) and mouse footpads (3, 10, 14) have proved cumbersome and time-consuming. The routine bacteriologic diagnostic test, the demonstration of acid-fast bacilli in skin smears (25), is not sufficiently sensitive or specific. This study was undertaken to evaluate the use of PCR as a means of diagnosis of leprosy and its potential to help assess the bacterial load reduction in patients during the course of chemotherapeutic treatment.

MATERIALS AND METHODS

Specimen collection. Slit skin smears and punch biopsies were obtained according to standard procedures (25) from 53 untreated leprosy patients at three institutes under the Department of Communicable Disease Control, Thailand: two skin clinics in Bangkok and a leprosy hospital in Samutprakarn province. Specimens were collected between October 1992 and March 1994. These patients were of both the paucibacillary (PB; having a negative bacterial index [BI] but distinctive histopathological lesions diagnostic for leprosy) and the multibacillary (MB; having both histopathological lesions and a positive BI) types. The classification of patients was done clinically and histopathologically according to the Ridley-Jopling scale (19). Disease types included indeterminate, tuberculoid (TT), borderline tuberculoid (BT), mid-borderline, borderline lepromatous, and lepromatous (LL) leprosy. Additionally, the BT leprosy type was subdivided into BT(−) for negative bacterial index and BT(+) for positive bacterial index. All histopathological readings were done by the same pathologist throughout the study. Patients who were clinically and histopathologically diagnosed with leprosy of any type were included in this study. The patients were physically examined by physicians who determined the active lesions from which skin smears and biopsies would be taken. After the start of chemotherapy, additional slit skin smears were taken from previous lesions from all patients at 1, 3, and 6 months after treatment. The bacterial index (BI), which represents a quantitative estimate of the bacteria on the basis of counting acid-fast bacilli, was obtained by employing Ridley's logarithmic scale (17, 18) for each patient at every time point of specimen collection. The BI from each patient was reported as an average BI value determined from slit skin smears performed on six sites for MB patients and on three sites for PB patients. To date, complete data for follow-up study are only available for 28 of 53 cases.

Biopsy specimens were also collected from five patients with skin diseases other than leprosy for use as controls.

After clinical specimens were collected at each collaborating institute, they were kept frozen at −20°C for 1 to 2 days. They were kept on ice during messenger transport to the Sasakawa Research Building and were refrigerated at 4°C for a maximum of 2 days before DNA extraction and PCR were performed.

Five milliliters of blood was also withdrawn from randomly selected individuals from the same group of patients. The sera obtained were frozen at −20°C before analysis with the gelatin particle agglutination test (12) for the presence of anti-phenolic glycolipid 1 (PGL-1) antibody.

Extraction of DNA. The blades used for slit skin smears, along with the collected tissue, were placed in 1.5-ml sterile Eppendorf tubes. The DNA extraction was performed by adding 300 μl of lysis buffer containing 1 mg of proteinase K per ml and 0.05% Tween 20 in 100 mM Tris-HCl (pH 8.5). The samples were covered with mineral oil to prevent evaporation, incubated at 60°C for 18 h, and then heated at 97°C for 15 min to inactivate the proteinase K.

Skin biopsy specimens were incised to small pieces with sterile scissors. They were then homogenized by hand in a homogenizer with 1 ml of sterile distilled water for about 1 to 2 min until the suspensions turned turbid white. DNA extraction followed by heat inactivation of proteinase K was performed as described above.

PCR. Primers S13 and S62 (Synthetic Genetics, San Diego, Calif.) employed in PCR were those originally designed and used by Hartskeerl et al. (8); they specifically amplify the 531-bp fragment of the proline-rich antigen (*pra*) gene encoding the species-specific 36-kDa antigen of *M. leprae* (22). Purified *M. leprae* DNA was kindly provided by M. J. Colston as part of the United Nations Development Programme/World Bank/World Health Organization Special Programme for Research and Training in Tropical Diseases. The PCR amplifications were performed in 50-μl reaction volumes containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 3.5 mM $MgCl_2$, 600 μM (each) dATP, dCTP, dGTP, and dUTP (Boehringer, Mannheim, Germany), 200 ng each of primers S13 and S62, and 1.25 U of *Taq* DNA polymerase (Perkin-Elmer, Norwalk, Conn.). dUTP was used instead of dTTP as a precaution, but no carry over contamination was ever detected in our negative controls. The reaction mixtures were then covered with 40 μl of mineral oil (Sigma, St. Louis, Mo.). The sample added to the mixtures

* Corresponding author. Mailing address: Department of Biotechnology, Faculty of Industrial Technology, Silpakorn University, Nakorn Pathom 73000, Thailand. Phone/Fax: (6634) 253-918.

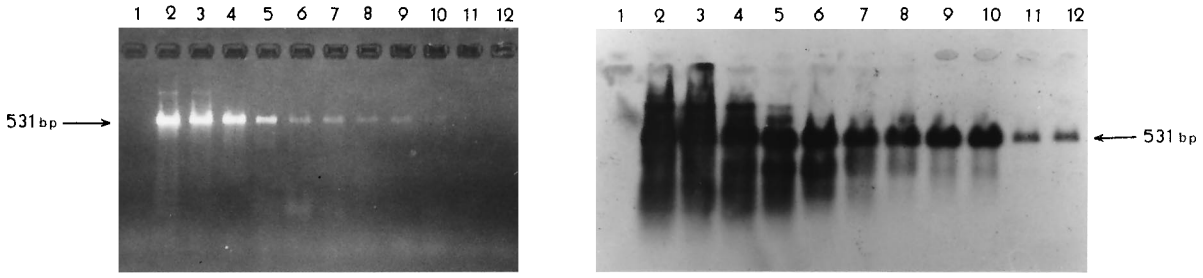

FIG. 1. Results of PCR amplification of the *M. leprae pra* gene in sequential dilutions of purified *M. leprae* DNA as detected by an agarose gel (left) and Southern blot hybridization (right). Lanes 2 to 12 are the PCR products of 42.5, 6.25, 0.625 ng, 62.5, 6.25, 0.625, 0.3125, 0.125 pg, 62.5, 6.25, and 3.125 fg of purified *M. leprae* DNA, respectively. Lane 1 is the negative control.

was 25 μl of either DNA extracts, purified *M. leprae* DNA, or sterile distilled water for clinical specimens, positive controls, or negative controls, respectively. The amplifications were carried out in a programmable temperature control system PC-700 (Astec, Fukuoka, Japan) as follows: an initial template denaturation step at 94°C for 10 min, followed by 32 cycles of 2-min denaturation at 94°C, 2-min primer annealing at 60°C, and 3-min extension at 72°C and then a final extension step at 72°C for 10 min.

For the PCR sensitivity test, the purified *M. leprae* DNA was diluted with sterile distilled water to the required concentrations and 25 μl of each dilution sample was then added to the PCR mixture. The conditions for the amplification reactions were the same as given above. The correlation between the amount of DNA and the number of bacilli was based on the fact that approximately 5 fg of DNA is equivalent to one bacterium, given the size of the *M. leprae* genome of 2.2×10^9 Da (2).

The amplicons obtained (20 μl) were then analyzed by 1.5% (wt/vol) agarose gel electrophoresis (20) in buffer containing 0.089 M Tris-HCl (pH 8.0), 0.044 M boric acid, and 0.001 M EDTA (TBE buffer). Electrophoresis was performed at 70 V for 2 h. The DNA in the gel was stained with ethidium bromide and visualized by UV transillumination. Positive and negative controls were always included in every gel.

Southern blot and hybridization. The presence of the 531-bp fragment of the *pra* gene in agarose gels was further confirmed by Southern hybridization (20, 21), using a digoxigenin DNA labeling and detection kit (Boehringer). The agarose gel was soaked, with slow shaking, in 0.5 M NaOH–1 M NaCl for 30 min, rinsed in distilled water for 30 s, and then slowly shaken in 1 M Tris-HCl (pH 8.0)–0.6 M NaCl for 30 min. The DNA in the agarose gels was transferred onto the GeneScreen Plus nylon membrane (New England Nuclear, DuPont, Boston, Mass.) by capillary transfer (20). The membranes were air dried and prehybridized for 1 h in hybridization buffer containing 5× SSC (prepared from the stock of 20× SSC, which contained 3 M NaCl plus 0.3 M sodium citrate, pH 7.0), 1% blocking reagent (Boehringer), 0.1% *N*-lauroylsarcosine, sodium salt, and 0.02% sodium dodecyl sulfate. The hybridization was performed in hybridization buffer containing heat-denatured probe at 68°C for 16 h. The hybridized membrane was then washed, and *M. leprae*-specific DNA was detected colorimetrically by alkaline phosphatase-conjugated anti-digoxigenin Fab fragments and the color-forming substrates nitroblue tetrazolium and X-phosphate) according to the manufacturer's instructions (Boehringer). The probe used was a digoxigenin-labeled 286-bp fragment located within the 531-bp fragment of the *pra* gene. The probe was simultaneously synthesized and labeled by PCR as previously described (15), with some modifications. Briefly, two rounds of PCR were carried out, the first of which was simply the amplification of purified *M. leprae* DNA, using the procedure mentioned above. About 1 to 5 μl of the amplicon obtained from the first PCR was used as a DNA template for the second PCR, which was performed in a 50-μl reaction volume containing 200 μM (each) dATP, dCTP, and dGTP, 130 μM dTTP, 70 μM digoxigenin-11–dUTP (Boehringer), 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1 mM $MgCl_2$, 1.25 U of *Taq* DNA polymerase (Perkin-Elmer), and 200 ng of each primer. The primers used here were T3 and T4c inner primers of the 531-bp fragment of the *pra* gene, whose sequence positions are 286 bp apart (22). The amplification steps and conditions were the same as described above. The amplified 286-bp product obtained was purified by phenol-chloroform extraction, followed by ethanol precipitation (20). After being resuspended in 10 mM Tris-HCl (pH 8.0)–1 mM EDTA, it was either immediately used as a DNA probe or stored at −20°C until used.

Samples were only scored as positive for analysis if they were positive on the Southern blot. Any time a blot showed bands in the negative control or failed to demonstrate amplification in the positive control, results of that entire blot were not included in analysis.

All PCR tests, followed by Southern hybridization, were performed at Sasakawa Research Building with a frequency of about one run per week.

Gelatin particle agglutination test. Antibody against *M. leprae*-specific PGL-1 in patient sera was detected by using the novel gelatin particle agglutination test, called MPLA, as described previously (12). The tests were done with the Serodia-Leprae, an *M. leprae*-specific antibody detection kit (Fujirebio Inc., Tokyo, Japan), according to the manufacturer's instructions. The cutoff value used here was the titer of 64.

RESULTS

The PCR protocol employed here was tested for sensitivity, using known amounts of purified *M. leprae* DNA as templates. Quantities of 42.5, 6.25, and 0.625 ng, 62.5, 6.25, 0.625, 0.3125, and 0.125 pg, and 62.5, 6.25, and 3.125 fg of purified *M. leprae* DNA template, equivalent to DNA contents of approximately 6.8×10^6, 10^6, 10^5, 10^4, 10^3, 10^2, 50, 20, 10, 1, and 0.5 *M. leprae* bacilli, respectively, were used in PCR reactions. The PCR results were determined by the presence or absence of the amplified 531-bp DNA band in an agarose gel and/or a Southern blot. As shown in Fig. 1, the intensity of the 531-bp DNA band was greatest with the highest amount of DNA template used and decreased sequentially with the decrease in the amount of template (lanes 2 to 12). The results were the same with the Southern blot. The bands for 62.5, 6.25, and 3.125 fg of DNA template (lanes 10, 11, and 12), which were either barely seen or undetectable in the original agarose gel, were clearly visible in the Southern blot. PCR was shown to amplify *M. leprae* DNA template in amounts as low as 3.125 fg (approximately equivalent to 0.5 bacillus). No band was detected for the negative control (lane 1) on either the agarose gel or the Southern blot.

DNA was extracted from fresh biopsies and slit skin smears of all clinical categories of untreated leprosy patients. An agarose gel of PCR amplifications of some of these clinical specimens and the subsequent Southern blot are shown in Fig. 2. The DNA extract from each clinical specimen was diluted twofold, and both the undiluted and the twofold-diluted DNA extracts were used as templates for PCR. The amplification products obtained from both dilutions were analyzed in parallel on an agarose gel. The 531-bp DNA bands were detected on the agarose gel for the positive control (lane 2) and for the biopsy and slit skin smear samples from a patient at month 0 (lanes 3, 4, 5, and 6), while no band was detected for the skin smear samples from a patient at month 1 (lanes 7 and 8). However, these two bands were clearly detected on the Southern blot. The negative control in lane 1 showed no detectable DNA band by either method. PCR tests followed by the Southern hybridization performed here almost always yielded definite results. Only 18 of approximately 1,000 tests performed were inconclusive and had to be repeated. The intensity of the 531-bp DNA bands obtained from the amplifications of the two-fold-diluted DNA samples was usually slightly greater than that of the undiluted ones from the same clinical specimens. It is possible that PCR-inhibiting impurities in the clinical samples account for this difference.

PCR results of various types of new leprosy patients before

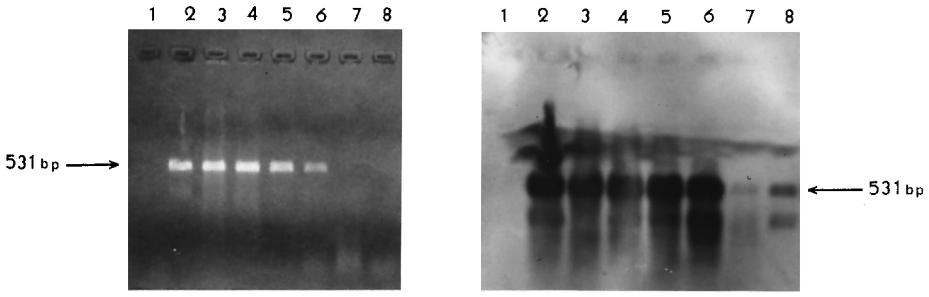

FIG. 2. Detection of the 531-bp DNA band resulting from the amplification of clinical specimens from leprosy patients. Lanes 3 and 4, 5 and 6, and 7 and 8 were the undiluted and twofold-diluted DNA extracts of a biopsy from an untreated leprosy patient, a slit skin smear from an untreated leprosy patient, and a slit skin smear from a leprosy patient treated for 1 month, respectively. Lanes 1 is the negative control. Lane 2 is the positive control. The agarose gel is shown on the left, and a Southern blot is shown on the right.

chemotherapeutic treatment are shown in Table 1. In MB cases, 87.1% (27 of 31) of biopsy samples and 41.9% (13 of 31) of skin smear samples yielded positive PCR results, whereas in PB cases, only 36.4% (8 of 22) of biopsy samples and 18.2% (4 of 22) of skin smear samples were PCR positive. The number of cases for each clinical type of leprosy was small because the incidence of new leprosy patients is rapidly decreasing in Thailand. However, the data shown here still indicate a trend in which the proportion of PCR-positive cases decreased from the LL end to the indeterminate end of the leprosy clinical spectrum, as should be expected. The proportion of PCR-positive cases was always lower (about half or less) for slit skin smear samples than for biopsy samples in almost all clinical types of leprosy. The average BI for each clinical type was reported as a range. In biopsy samples of five patients with skin diseases other than leprosy, none gave positive result with PCR (data not shown).

Forty-one patients from this study have also been examined for the presence of anti-PGL-1 antibody in their sera. The PCR results for these patients before chemotherapeutic treatment were then correlated with the anti-PGL-1 antibody results. Table 2 shows the correlation between the PCR results and the detection of anti-PGL-1 antibody for MB and PB patients. In MB cases, 23 of 25 (92%) were PCR positive, while 17 of 25 (68%) were seropositive. Of 23 MB cases that showed positive PCR results, most (16 cases) were also positive for anti-PGL-1 antibody, while only 7 cases were antibody negative. In one MB case which had a demonstrable antibody level, organisms could not be detected by PCR in repeated attempts on the same DNA sample. This case was an LL type with a BI of 5. In PB cases, less than half (7 of 16, or 43.75%) yielded detectable 531-bp amplified DNA bands by PCR, while only 1 of 16 (6.25%) was seropositive for PGL-1. Of seven PCR-positive PB cases, only one showed a detectable level of anti-PGL-1 antibody. One of the six PCR-positive, antibody-negative PB cases was clinically classified in the indeterminate category, the category associated with very recent infection. None of the PCR-negative PB cases had detectable levels of anti-PGL-1 antibody.

Slit skin smear samples were collected from previous lesions in 28 patients (20 MB and 8 PB) at 1, 3, and 6 months after initiation of chemotherapeutic treatment. The PCR tests were performed on these samples to evaluate its potential to help assess the bacterial load reduction in MB and PB leprosy patients during the course of chemotherapeutic treatment. Table 3 shows that, for MB-type leprosy patients, the proportion of PCR-positive cases decreased sequentially with the length of time after treatment. The same was true for BT(−) leprosy, the only PB type that has been monitored for up to 6 months. The proportion of PCR-positive cases for BT(−) cases at month 0 were six of eight for biopsy samples and three of

TABLE 1. Detection of the *M. leprae pra* gene by PCR amplification of clinical samples from various types of leprosy patients before the start of chemotherapy[a]

Bacillary and clinical type	No. of cases PCR positive/no. studied		Avg BI range
	Biopsy	Skin smear	
MB			
LL	17/20	11/20	0.6–5.7
BL[b]	8/8	2/8	0.16–3.3
BB[c]	1/1	0/1	1.1
BT(+)	1/2	0/2	1.0–1.16
Total	27/31	13/31	
PB			
BT(−)	8/15	3/15	0
TT	0/5	0/5	0
Indeterminate	0/2	1/2	0
Total	8/22	4/22	

[a] Both biopsy specimens and slit skin smear samples were analyzed. The BI resulting from microscopic examination is included for comparison.
[b] BL, borderline lepromatous.
[c] BB, mid-borderline.

TABLE 2. Correlation between PCR amplification results and anti-PGL-1 antibody detection in untreated leprosy patients

Detection of anti-PGL-1 antibody	PCR result[a]		Total
	No. of cases positive	No. of cases negative	
MB type			
Positive	16	1	17
Negative	7	1	8
Total	23	2	25
PB type			
Positive	1	0	1
Negative	6	9	15
Total	7	9	16

[a] Cases were considered positive if either the biopsy or the slit skin smear sample was positive.

TABLE 3. PCR amplification results in clinical samples from MB leprosy patients during the course of chemotherapeutic treatment[a]

Leprosy type	No. of cases PCR positive/no. tested at mo:				
	0[b]		<1 (skin smear)[c]	<3 (skin smear)[c]	6 (skin smear)[c]
	Biopsy	Skin smear			
LL	15/16	9/16	6/16	4/16	3/16
BL	4/4	2/4	2/4	0/4	0/4
Total	19/20	11/20	8/20	4/20	3/20

[a] Results from PB patients are reported in the text.
[b] Before the start of chemotherapy.
[c] One, 3, and 6 months after the start of chemotherapeutic treatment.

eight for slit skin smear samples. The proportion then decreased to one, one, and none of eight for slit skin smear samples at months 1, 3, and 6, respectively. The largest decreases occurred after the first month of drug treatment in LL and BT(−) cases.

DISCUSSION

Several studies have reported successes in using PCR to detect *M. leprae* specifically and sensitively in tissue samples (1, 5, 8, 16, 23, 24, 26). In an attempt to evaluate the use of PCR methodology in the early diagnosis of leprosy in Thailand, PCR amplifications of biopsy and slit skin smear samples from leprosy patients were compared with other routine diagnostic procedures. Primers S13 and S62 have been used to amplify a portion of the *M. leprae pra* gene, the gene coding for an *M. leprae*-specific 36-kDa protein antigen which is rich in the amino acid proline (8, 22). The amplification has been reported to be specific for *M. leprae* and to have a detection limit of approximately one bacterium (8). In our hands, PCR amplified purified *M. leprae* DNA at a level as low as 0.5 bacillus equivalent. Even though a reduction in sensitivity would be expected when PCR is performed on clinical samples, possibly due to the presence of PCR inhibitors, we think that this is a reliable system for early detection of *M. leprae* organisms in leprosy patients and possibly in subclinical cases.

The number of PCR-positive cases is higher in MB than in PB patients. As many as 87.1% of MB cases were PCR positive, while only 36.4% of the PB cases were detected by PCR. This should be expected since MB leprosy has a higher bacterial load than PB leprosy. However, considering that the PB type carries so few *M. leprae* organisms that none of these cases could be detected by microscopic examination (BI = 0), the 36.4% PCR positivity becomes more meaningful, clearly showing an advantage over microscopic examination.

Slit skin smear samples always yielded approximately half as many PCR-positive cases as did biopsy samples. This might be because some skin smear samples were contaminated with impurities that inhibited PCR. Moreover, the amount of tissue available for DNA extraction was always less than that in biopsy samples. None of five TT cases studied showed a detectable 531-bp DNA band in repeated tests. It is worth noting that three of these cases, which were clinically determined to be TT leprosy patients, had histopathological readings characteristic of nonleprosy patients. Some of the five TT cases, along with four LL cases that gave negative PCR results, might have been treated with antileprotic drugs from other clinics before coming in as new patients. It is not uncommon for patients to be reluctant to disclose information about prior treatment. Another explanation for the negative PCR results might be the intrinsic error within PCR itself. Yoon et al. (26) and de Wit et al. (5) have reported their failure to detect *M. leprae* by PCR from certain biopsy specimens of leprosy patients with BIs as high as 5. The fact that one of two indeterminate cases studied yielded positive results with PCR is very encouraging, since the indeterminate type is the earliest type to appear in the clinical spectrum of leprosy.

The PCR method was correlated with serological detection of antibody directed against PGL-1 antigen for untreated leprosy patients. The PGL-1 antigen is immunologically specific to *M. leprae*, and its structure has been well characterized (7). Detection of antibody against this antigen has been tried for serodiagnosis of leprosy with good results in LL patients. However, the response is disappointingly low in TT patients and contacts (11). In this study, PCR showed a clear advantage over the serologic detection of anti-PGL-1 antibody. However, the one MB case that was PCR negative but positive for anti-PGL-1 antibody detection (clinically and histopathologically diagnosed as the LL type with a BI of 5) was a good example of PCR failing to detect *M. leprae* while other routine diagnostic methods showed positive results. The advantage of PCR over the serological method was most striking in PB patients, in whom only 6.25% were seropositive. The indeterminate case did not show a detectable anti-PGL-1 antibody level but was readily detected by PCR. These data make PCR a very promising tool for early diagnosis of leprosy.

PCR has also been evaluated here for its potential to assess the bacterial clearance in leprosy patients during the course of chemotherapeutic treatment. Only slit skin smear samples were used for months 1, 3, and 6 in the follow-up study because it was impractical to perform biopsy, which is rather painful and possibly scar forming, on patients at every visit. Slit skin smears were to be taken from these patients at this schedule as a routine practice already. Even though the number of cases studied was small and the number of PCR-positive cases on slit skin smear at each time point was not very high, we can still see a trend in which the PCR-positive cases decreased sequentially with time after drug treatment in all leprosy types studied. This is a positive sign that PCR might be helpful in assessing bacterial load reduction during the course of drug treatment. This can be valuable to physicians since PCR is known to detect potentially viable microorganisms, as reported by Woods and Cole (24), while microscopic examination of leprosy bacilli cannot always differentiate reliably between live and dead bacilli. It can also give an indication of patient compliance, of the presence of drug-resistant organisms or persisters, or of relapsed cases in which the number of viable bacilli has increased again after an initial decrease during the course of treatment.

Even though the results shown here are encouraging enough to indicate that PCR has a potential to be used as a tool for the early diagnosis of leprosy and for assessment of bacterial clearance during chemotherapy, a larger-scale study with an improved PCR method still needs to be pursued. Since slit skin smears seem to be an easy and practical way to obtain samples from patients and people at risk in field work, PCR methodology on this type of specimen needs further improvement. Reduction of the required DNA extract volume to be added to the PCR reaction mixture (4), enhanced purification of DNA extracts to remove PCR-inhibiting impurities, or further optimization of the reaction itself may all contribute to such improvement.

Because of its expense and technical challenge, PCR will probably never replace conventional diagnostic methods such as histopathological and clinical examinations. Rather, PCR may be useful as a complementary tool in the diagnosis of

certain doubtful cases when conventional methods are not conclusive. PCR may also be useful in epidemiologic studies to determine the distribution of *M. leprae* in various populations. Since one of the most important strategies to control a disease like leprosy that has no vaccine is to detect the causative microorganisms, all clinical, histopathological, and PCR tests will complement each other to help achieve the ultimate goal of the leprosy control program, which aims to eradicate leprosy in the near future.

de Wit et al. (4) successfully used PCR on occupational contacts and leprosy-endemic and -nonendemic controls. It would be of great value to further extend the use of PCR to detect leprosy bacilli in subclinically infected individuals such as close family contacts to assist in studying and controlling the spread and transmission of *M. leprae* organisms.

ACKNOWLEDGMENTS

We gratefully acknowledge Paul R. Klatser and Gabrielle M. E. van der Vliet for helpful discussions during this study. We thank the directors and the working staffs of Phra-pradaeng Hospital, Bangkhaen Skin Clinic, and Bangkok Skin Clinic for collaborating on providing clinical specimens from patients. We thank Maeya Ngamying for providing data on the *M. leprae* particle agglutination test. Skillful technical assistance from Chaturong Pakpitcharoen deserves special acknowledgment. We thank V. Ann Stewart for helpful critical reading of the manuscript.

This work was supported by the Sasakawa Memorial Health Foundation, Japan.

REFERENCES

1. **Arnoldi, J., C. Schluter, M. Duchrow, L. Hubner, M. Ernst, A. Teske, H.-D. Flad, J. Gerdes, and E. C. Bottger.** 1992. Species-specific assessment of *Mycobacterium leprae* in skin biopsies by *in situ* hybridization and polymerase chain reaction. Lab. Invest. **66:**618–623.
2. **Clark-Curtiss, J. E., W. R. Jacobs, M. A. Docherty, L. R. Rithcie, and R. Curtiss III.** 1985. Molecular analysis of DNA and construction of genomic libraries of *Mycobacterium leprae*. J. Bacteriol. **161:**1093–1102.
3. **Colston, M. J., and G. R. F. Hilson.** 1976. Growth of *Mycobacterium leprae* and *M. marinum* in congenitally athymic (nude) mice. Nature (London) **262:**399–401.
4. **de Wit, M. Y. L., J. T. Douglas, J. McFadden, and P. R. Klatser.** 1993. Polymerase chain reaction for detection of *Mycobacterium leprae* in nasal swab specimens. J. Clin. Microbiol. **31:**502–506.
5. **de Wit, M. Y. L., W. R. Faber, S. R. Krieg, J. T. Douglas, S. B. Lucas, N. Montreewasuwat, S. R. Pattyn, R. Hussian, J. M. Ponnighaus, R. A. Hartskeerl, and P. R. Klatser.** 1991. Application of a polymerase chain reaction for the detection of *Mycobacterium leprae* in skin tissue. J. Clin. Microbiol. **29:**906–910.
6. **Fine, P. E. M., J. M. Ponnighaus, P. Burgess, J. A. Clarkson, and C. C. Draper.** 1988. Seroepidemiological studies of leprosy in Northern Malawi based on an enzyme-linked immunosorbent assay using synthetic glycoconjugate antigen. Int. J. Lepr. **56:**243–254.
7. **Gaylord, H., and P. J. Brennan.** 1987. Leprosy and the leprosy bacillus: recent developments in characterization of antigens and immunology of the disease. Annu. Rev. Microbiol. **41:**645–675.
8. **Hartskeerl, R. A., M. Y. L. de Wit, and P. R. Klatser.** 1989. Polymerase chain reaction for the detection of *Mycobacterium leprae*. J. Gen. Microbiol. **135:**2357–2364.
9. **Hastings, R. C.** 1985. Leprosy. Churchill Livingston, London.
10. **Hastings, R. C., S. P. K. Chehl, M. J. Morales, E. J. Shannon, and W. F. Kirchheimer.** 1980. Multiplication of acid-fast bacilli in nude mice inoculated with armadillo-derived *M. leprae*. Int. J. Lepr. **48:**490–491.
11. **Hastings, R. C., T. P. Gillis, J. L. Krahenbuhl, and S. G. Franzblau.** 1988. Leprosy. Clin. Microbiol. Rev. **1:**330–348.
12. **Izumi, S., T. Fujiwara, M. Ikeda, Y. Nishimura, K. Sugiyama, and K. Kawatsu.** 1990. Novel gelatin particle agglutination test for serodiagnosis of leprosy in the field. J. Clin. Microbiol. **28:**525–529.
13. **Kirchheimer, W. F., and E. E. Storrs.** 1971. Attempts to establish the armadillo (Dasypus novemcinctus Linn.) as a model for the study of leprosy. Int. J. Lepr. **39:**693–703.
14. **Kohsaka, K., T. Mori, and T. Ito.** 1976. Lepromatoid lesion developed in nude mouse inoculated with *Mycobacterium leprae*. Int. J. Lepr. **44:**540.
15. **Lion, T., and O. A. Hass.** 1990. Nonradioactive labeling of probe with digoxigenin by polymerase chain reaction. Anal. Biochem. **188:**335–337.
16. **Plikaytis, B. B., R. H. Gelber, and T. M. Shinnick.** 1990. Rapid and sensitive detection of *Mycobacterium leprae* using a nested primer gene amplification assay. J. Clin. Microbiol. **28:**1913–1917.
17. **Ridley, D. S.** 1958. Therapeutic trials in leprosy using serial biopsies. Lepr. Rev. **29:**45–52.
18. **Ridley, D. S.** 1964. Bacterial indices, p. 620–622. *In* R. G. Cochrane and T. F. Davey (ed.), Leprosy in theory and practice. The Williams & Wilkins Co., Baltimore.
19. **Ridley, D. S., and W. H. Jopling.** 1966. Classification of leprosy according to immunity—a five group system. Int. J. Lepr. **34:**255–273.
20. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning, a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
21. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98:**503–517.
22. **van der Vliet, G. M. E., C. J. Hermans, and P. R. Klatser.** 1993. Simple colorimetric microtiter plate hybridization assay for the detection of amplified *Mycobacterium leprae* DNA. J. Clin. Microbiol. **31:**665–670.
23. **William, D. L., T. P. Gillis, R. Booth, D. Looker, and J. Watson.** 1990. The use of a specific DNA probe and polymerase chain reaction for the detection of *Mycobacterium leprae*. J. Infect. Dis. **162:**193–200.
24. **Woods, S. A., and S. T. Cole.** 1989. A rapid method for the detection of potentially viable *Mycobacterium leprae* in human biopsies: a novel application of PCR. FEMS Microbiol. Lett. **65:**305–310.
25. **World Health Organization.** 1987. Laboratory techniques for leprosy. World Health Organization, Geneva.
26. **Yoon, K.-H., S.-N. Cho, M.-K. Lee, R. M. Abalos, R. V. Cellona, T. T. Fajardo, Jr., L. S. Guido, E. C. Dela Cruz, G. P. Walsh, and J.-D. Kim.** 1993. Evaluation of polymerase chain reaction amplification of *Mycobacterium leprae*-specific repetitive sequence in biopsy specimens from leprosy patients. J. Clin. Microbiol. **31:**895–899.