Transmission of Leprosy: A Study of Skin and Nasal Secretions of Household Contacts of Leprosy Patients Using PCR

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Abstract. It is generally held that dissemination of Mycobacterium leprae is from nasal mucosa and not through the skin of infected patients. In this study, we evaluated M. leprae in the unbroken skin and nasal secretions of multibacillary (MB) leprosy patients and their contacts. Specimens were examined by direct microscopy and polymerase chain reaction (PCR) for M. leprae DNA. Results showed that 60% of untreated MB leprosy patients examined histologically had acid-fast bacilli in the keratin layer. By PCR studies it was found that 80% of the patients had M. leprae DNA in skin washings and 60% had M. leprae DNA on swabs obtained from the nasal mucosa. Ninety-three contacts of the untreated MB cases were also tested for exposure to M. leprae by analyzing skin washings and nasal secretions by PCR. PCR analysis showed significant skin (17% positive) and nasal mucosal (4%) exposure in contacts before instituting treatment of the index cases. After 2 months of treating the index cases, all contacts tested were negative for M. leprae DNA. These data suggested that both skin and nasal epithelia of untreated MB leprosy patients contribute to the shedding of M. leprae into the environment and contacts of untreated MB cases are at risk for contact with M. leprae through both the nasal mucosa and exposed surfaces of their skin.

INTRODUCTION

The estimated global prevalence of leprosy has been greatly reduced as a result of the multidrug therapy (MDT) program advocated by the World Health Organization (WHO) and its implementation with the help of governmental and non-governmental organizations. Although >14 million cases have been cured with MDT since 1985, new case detection rates have decreased only marginally over the same time period.1 Therefore, the global use of MDT seems to have had only minimal, if any, effect on transmission of the disease,2 and an adequate explanation for this situation is lacking.3

It has been shown by experimental studies using nude mice that abraded skin and nasal mucosa are routes of transmission in mice and may be operative in humans.4 In human studies, nasal carriage of Mycobacterium leprae has been shown in 5–8% of household contacts of leprosy patients using polymerase chain reaction (PCR) methodology to detect M. leprae DNA.5,6 However, no invasive lesion has been reported in these contacts nor has the persistence of this condition been shown,7 making the case for infection through the nasal route associative at best. Accordingly, there is no conclusive proof for the nasal mucosal route of infection in humans.

Using conventional techniques for staining acid-fast bacilli (AFB) in skin, Chatterjee8 has shown 5.8% of contacts of leprosy patients carry AFB in their skin. Also, Abraham and others9 showed that, when sites of single leprosy skin lesions from children were superimposed with areas associated with skin abrasions and scars from age- and sex-matched children from the same environment, a statistically significant correlation was observed between the leprosy skin lesions and skin abrasions and scars. The authors concluded that these data supported skin as a potential route of infection for M. leprae.

The argument has been put forward that M. leprae—positive nasal secretions may represent transient contamination from

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Patients and household contacts. The St Thomas Hospital and Leprosy Center at Chettupattu, India, manages a leprosy control program covering 450,000 people and an outpatient clinic where patients with leprosy and other dermatologic complaints are treated. From among these patients were recruited newly diagnosed, untreated MB patients (nine lepromatous leprosy [LL] and one borderline leprosy [BL]) with bacterial indices of 3+ or greater, MB patients (six LL, four BL) treated for 1 year, and 101 household contacts of 43 untreated MB patients for the study. MB patients consisted of both LL and BL patients. All contacts studied were carefully examined clinically for signs and symptoms of leprosy. Informed consent was obtained from all human adult participants, and approval of the project was obtained from both the Louisiana State University and St Thomas Hospital institutional review boards.

Sample acquisition. Skin. For MB leprosy cases, a defined area (312 cm²) of the skin from the posterior surface of both upper arms (3 cm × 12 cm × 2 = 72 cm²) and the back of both

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sides of the chest (12 cm × 10 cm × 2 = 240 cm²) was washed as follows. In a 50-mL beaker, 20 mL of sterile saline was taken. With sterile gauze dipped in the saline, the areas of the skin defined above were stroked 20 times, taking care not to spill any of the fluid from the swab. The skin washings from the beaker were transferred to two 10-mL centrifuge tubes and were centrifuged for 25 minutes at 8,000g. From the sediment, direct smears were prepared on slides, fixed, stained for AFB, and examined by light microscopy at ×100 magnification. The remainder of the sediments was fixed in 70% ethyl alcohol for PCR study. For contacts, a similar strategy of skin washings was performed except that the swabs were taken from the posterior aspect of the forearms of the contacts between the elbows and the wrists of both arms and combined into one sample per person.

Nasal secretions. Secretions were collected from both nostrils of each contact or index case using dry, sterile dacron swabs by gently swabbing of the outer nares. Each swab tip was placed into a 1.5-mL microfuge tube containing 1.0 mL of freshly prepared sputolysin (CalBiochem; EMD Biosciences, San Diego, CA). After the addition of Tween 20 to a final concentration of 0.05%, the tubes were mixed by vortexing and allowed to stand at room temperature for 15 minutes. Fluid was expressed from the swabs, and the particulate fraction was collected by centrifugation at 10,000g for 10 minutes. The supernatant fluid was decanted, and the pellet was resuspended in 100 μL of sterile deionized water. An aliquot was spotted on a slide and stained for bacterial enumeration, and the remainder was extracted for DNA as described below. As a control for false-positive PCR reactions, 100 nasal samples were obtained from student volunteers attending the LSU School of Veterinary Medicine. The samples were processed as described above and run blindly along with the patient and contact samples.

Rehydrated sediments obtained from skin and nasal washings were extracted for DNA after digestion with proteinase K (1 mg/mL) dissolved in 10 mmol/L Tris-HCl, 1 mmol/L EDTA, and 150 mmol/L NaCl, pH 8.0 (TE) buffer. Equal volumes of the proteinase K (PK) digest and phenol/chloroform/isoamyl alcohol (24:1:1) were mixed, and the resultant aqueous phase was removed, and the DNA was precipitated at −20°C after adding two volumes of chilled absolute ethanol after increasing the final NaCl concentration to 100 mmol/L. The precipitate was washed once with chilled 70% ethanol, dried at room temperature, and resuspended in 30 μL of TE. Ten microliters of the DNA extract (neat or diluted 1:5 in TE) was added to the PCR buffer and primer mix, including AmpliTaq, as per instructions of the manufacturer (Applied Biosystems, Foster City, CA). PCR conditions and primers used to amplify M. leprae DNA were described previously.27,10

Slot blotting with a digoxigenin-labeled probe specific for the M. leprae PCR amplicon was used to monitor the presence of M. leprae in clinical samples. Briefly, after treating PCR products in denaturant (50 mmol/L NaOH, 150 mmol/L NaCl) for 10 minutes, the samples were transferred to a nylon membrane in a slot blot apparatus. The membrane was air dried, heated at 80°C for 2 hours, and hybridized with the digoxigenin probe, and samples containing M. leprae DNA were detected by chemiluminescence using the Lumigene PPD detection system as described by the manufacturer (Boehringer Ingelheim Pharmaceuticals, Ridgefield, CT). Biopsies of the skin were also obtained from the back of the chest in seven patients and the back of the upper arm in six patients to further evaluate the location of bacilli in the skin.

Statistics. The SAS statistical package (version 9.1.3; SAS Institute, Cary, NC) was used to analyze the data in a series of McNemar tests of agreement and calculated k coefficients for repeated-measures two-way tables. Comparisons were made both within and between nasal and skin washing treatments across time points. All comparisons were considered significant at P ≤ 0.05.

RESULTS

Histopathology of the skin using the modified Fite stain from the untreated MB leprosy patients showed that 6 of 10 had AFB in the keratin layer of the skin and hair follicles (Figure 1). Direct smears of skin and nasal washings of the untreated MB patients showed that 9 of 10 and 7 of 10 samples, respectively, were positive for AFB (Table 1). PCR results from these same specimens were concordant, except for one specimen in each sampling group, confirming the AFB observed was M. leprae. Skin washings proved to be slightly more reliable than nasal washings for detecting M. leprae DNA in these patients before treatment; however, the comparison did not reach statistical significance. Nasal secretion samples from all 100 non-endemic controls tested negative by PCR. These results indicated that both anatomic sites

![Figure 1. Photomicrograph showing the epidermis of skin of a lepromatous leprosy patient containing several acid-fast bacilli (arrows) in the keratin layer (Fite stain). This figure appears in color at www.ajtmh.org.](image-url)
from untreated MB cases may contribute to the dissemination of *M. leprae* and, therefore, to transmitting leprosy.

Duration of treatment had little effect on detection of bacilli for the first 3 months in the skin and nasal secretions, whether monitoring AFB or *M. leprae* DNA by PCR (Table 1). However, follow-up studies of MB patients treated for 1 year with MDT indicated that the majority of patients tested negative for *M. leprae* both in skin washings and nasal secretions (Table 2). This was true for direct microscopic observation and PCR of skin washings and nasal secretions at both the 1- and 2-year follow-up testing (Table 2). These same patients remained BI positive when analyzed by routine skin-slit scrapings and biopsy after 1 year of MDT (data not shown).

Contacts of the untreated MB cases were also tested for exposure by analyzing skin washings and nasal secretions by PCR. PCR analysis showed 16 of 93 (17%) household contacts had skin exposure to *M. leprae* before instituting treatment of the index cases (Table 3). The same group of contacts showed 4% positivity by PCR in their nasal secretions. None of the PCR-positive contacts tested positive for *M. leprae* DNA at both anatomic sites. After 1 month of MDT of index cases, 1 of 93 (1%) contacts tested positive for *M. leprae* DNA by PCR from skin washings, whereas 6 of 93 (6%) contacts tested positive in nasal secretion specimens. Two of the six PCR-positive (nasal secretions) contacts tested positive in both their pretreatment and 1-month post-treatment testing, but both were negative at their 2-month post-treatment testing.

After 2 months of MDT of index cases, nasal secretion and skin washing specimens from 26 contacts plus the 7 contacts that had tested positive at 1 month post-treatment were examined. PCR results were negative for all 33 contacts tested.

### DISCUSSION

In an innovative study of the skin of leprosy patients, Pedley12 concluded that the number of *M. leprae* discharged from intact skin of lepromatous patients was negligible and, therefore, nasal secretions were the major source of infection. This study used a technique referred to as composite skin contact smears, in which a glass slide was pressed repeatedly against the skin lesion of lepromatous patients and, after staining, examined microscopically for AFB. From 34 slides prepared from 11 patients, only 20 AFB were observed, leading to the conclusion that few bacilli are discharged from the intact skin of a lepromatous patient. More recently, Hosokawa13 reported a histopathology study of the skin of lepromatous leprosy and concluded that, whereas viable bacilli could be excreted from non-ulcerating skin lesions, the possibility seemed to be small. These studies coupled with recent PCR studies6,7,14 monitoring *M. leprae* in nasal secretions of contacts have reinforced the hypothesis that leprosy transmission is primarily through the nasal route. We undertook this study to compare the potential for *M. leprae* excretion in nasal secretions and from the skin of MB patients with an alternative procedure for sampling skin. We also compared the exposure of skin and nasal mucosa to *M. leprae* in contacts of untreated MB patients in an attempt to establish the degree and duration of exposure to *M. leprae* after implementation of MDT in the index cases.

Sites selected for sampling the skin of MB index cases were chosen with the idea that the upper arms and the back would provide a general measure of skin-associated *M. leprae* in disseminated leprosy and be less likely to be contaminated with *M. leprae* from exogenous sources through contact with the external environment. In contrast, household contacts were monitored for exposure to *M. leprae* by sampling exposed areas of the skin. Accordingly, contacts were sampled by preparing washings obtained from the area of each arm between the elbow and the wrist.

Our results showing AFB and PCR detection of *M. leprae* DNA in 80% of skin washings and 60% of nasal secretions from untreated MB leprosy cases suggests that both anatomic sites serve as potential sources for transmission of leprosy. These results support earlier findings regarding the presence of *M. leprae* in nasal secretions of both index cases and their contacts. In addition, our results extend the concept of exposure of contacts through the skin and argue against earlier assertions that the number of *M. leprae* discharged from intact skin of MB patients is negligible. Although our study tested neither the viability nor the absolute number of *M. leprae* in the skin washings, it has been documented numerous times that viable organisms can be recovered from skin biopsies and expanded in rodent foot pads routinely.15–17 To our knowledge similar viability studies have not been reported using *M. leprae* harvested from nasal secretions. However, a granulomatous lesion in the nasal mucosa of some leprosy patients is not an uncommon clinical finding and is likely the site of nasal dispersion of viable *M. leprae*.

Earlier studies have shown that PCR positivity associated with *M. leprae* from skin biopsies from patients receiving successful anti-leprosy therapy was reduced significantly as early as 2 months after initiating therapy.11,18 Similarly, in our study, successful treatment of MB patients for 1 year showed reductions in PCR reactivity from both sites sampled, suggesting that neither nasal secretions nor skin are likely portals of exit for *M. leprae* from patients receiving appropriate therapy. Earlier time points, ranging from 1 to 3 months, showed minor reductions in PCR positivity rates from both skin washings and nasal secretions. Because viability of *M. leprae* from the skin drops precipitously after three pulses of rifampin19 or six doses of MDT,20 it is likely that the positivity recorded by PCR in the specimens, taken during the first few months after initiation of treatment, is caused by residual

### Table 2

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<th>Treatment period</th>
<th>Skin washings (PCR)</th>
<th>Nasal washings (PCR)</th>
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<td></td>
<td>Direct smear</td>
<td>PCR</td>
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<tr>
<td>One year</td>
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<td>Two years</td>
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ND, not done.
undegraded DNA from dead \textit{M. leprae} and not viable \textit{M. leprae}. It has been shown repeatedly that macrophages in MB leprosy patients are not able to clear \textit{M. leprae} efficiently from lesions because dead organisms and their fragments persist for a period even after treatment is completed.

The data also showed that \textit{M. leprae} was present in a small percentage of samples taken from both nasal mucosa (4%) and skin (17%) of contacts of untreated MB leprosy cases. The presence of \textit{M. leprae} from skin washings, which we define as skin exposure, was approximately four times greater than nasal mucosa exposure. Although the PCR positivity rates of the two anatomic sites were not different when compared statistically, the finding supports a role for skin and nasal exposure in transmission of leprosy. Further supporting the role of skin in transmission are reports by Sehgal and others\textsuperscript{21–23} and Abraham and others,\textsuperscript{9} documenting cases of leprosy that developed in the skin at sites of trauma.

Treatment of index cases with MDT for 1 month greatly reduced skin exposure in contacts but had little effect on the low level of exposure through the nose as judged by PCR positivity of nasal secretions. This finding could be interpreted to suggest the establishment of a nasal carriage state in these contacts as has been proposed earlier.\textsuperscript{2} However, only two of the six positive nasal secretion specimens tested positive at two time points and all six contact’s specimens were negative on the third testing, which coincided with 2 months of treatment of the index cases.

Our results add to the basic understanding of how \textit{M. leprae} is shed from index cases and further defines the risk of exposure to contacts. Both nasal secretions and skin from untreated MB cases of leprosy are capable of shedding \textit{M. leprae} to the environment, which may be deposited on either or both the nasal and skin epithelia of contacts with the potential for initiating infection. What remains unclear is whether one or both sites are critical in establishing a productive infection. It is likely that under the appropriate circumstances either site is capable of sustaining an initial infection, but definitive evidence of primary infection will require better immunologic tests for monitoring early infection with \textit{M. leprae}.

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