Leprosy is a devastating human disease caused by infection with *Mycobacterium leprae* bacilli. The disease predominantly affects the skin, although during infection, significant nerve destruction leads to deformities of the hand, foot, face, and, in some cases, eye (1). The disease is represented by a clinical spectrum. Lepromatous leprosy/borderline lepromatous (LL/BL) patients represent one pole of the spectrum, demonstrating significant cell-mediated immunity. At the opposite pole, tuberculoid -specific antibodies and an absence of *M. leprae*-specific cell-mediated immunity. At the opposite pole, tuberculosis/borderline tuberculosis (TT/BT) patients demonstrate very low or absent BI and are designated paucibacillary. These individuals demonstrate significant *M. leprae*-specific cell-mediated immunity and very low or absent titers of *M. leprae*-specific antibodies.

Despite the success of multidrug therapy in reducing the number of registered leprosy cases worldwide, evidence suggests that *Mycobacterium leprae* continues to be transmitted. A serological diagnostic test capable of identifying and allowing treatment of early-stage disease could reduce transmission and prevent the onset of the disability, a common complication of the disease in later stages. Serological diagnosis based on antibody recognition of phenolic glycolipid I (PGL-I) cannot reliably identify individuals with lower bacterial indices (BI). One strategy that might improve this situation is the provision of highly specific serological antigens that may be combined with PGL-I to improve the sensitivity of diagnosis. Using serological expression cloning with a serum pool of untreated lepromatous leprosy (LL) patients, we identified 14 strongly reactive *M. leprae* proteins, 5 of which were previously unstudied. We present results suggesting that two of these proteins, ML0405 and ML2331, demonstrate the ability to specifically identify LL/borderline lepromatous (BL) patients on the basis of immunoglobulin G (IgG) reactivity. In a household contact study, LL index cases were identified on the basis of this reactivity, while household contacts of these patients demonstrated undetectable reactivity. At a serum dilution of 1:800, suitable to reduce background PGL-I IgM reactivity, two BL patients with a BI of <4 showed anti-human polyvalent immunoglobulin G, A, and M reactivity measured with a combination of ML0405, ML2331, and natural disaccharide O-linked human serum albumin (NDOHSA) (synthetic PGL-I) that was markedly higher than IgM reactivity to NDOHSA alone. We suggest that ML0405 and ML2331 may have utility in serological leprosy diagnosis.
of these antigens to investigate their potential for serologically diagnosing leprosy.

MATERIALS AND METHODS

Patients. Leprosy patient and household contact sera were obtained after drawing blood at the Leonard Wood Memorial Center for Leprosy Research, Cebu City, Philippines. All LL, BL, TT, and BT sera used in this study derived from recently diagnosed and untreated individuals. 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. Sera from tuberculosis patients were obtained after drawing blood from Mycobacterium tuberculosis sputum-positive Seattle-based individuals with clinically confirmed pulmonary tuberculosis (PT). Normal sera were obtained after drawing blood from Seattle-based volunteers with no history of leprosy or tuberculosis infection. In all cases, drawing of blood was carried out with informed consent with local institutional review board approval in Seattle and local ethics committee approval in the Philippines.

M. leprae library construction. M. leprae genomic library construction was carried out using the ZAP Express EcoRI predigested vector kit and Gigapack cloning kit according to the manufacturer’s instructions (Stratagene, La Jolla, CA). M. leprae total DNA was recovered from genomic DNA inserts (20 M leprae DNA encoding partial or full-length sequences of M. leprae genomic DNA insert size. Serological expression screening. Serological expression screening was carried out with pooled sera from five untreated LL/BL patients as previously described (20). Briefly, 20,000 PFU of library phage in LB top agar were plated per each 30-cm LB agar plate and incubated at 37°C until plaque formation was visible (after approximately 3 h). Nitrocellulose filters soaked previously in 10 mM IPTG (isopropyl-β-D-thiogalactoside) were then placed onto plates and incubated overnight at 4°C. Nitrocellulose filters were removed from top agar and blocked in phosphate-buffered saline buffer containing 0.1% (vol/vol) Tween 20 (PBST) and 1% bovine serum albumin (BSA). Subsequently, nitrocellulose filters were incubated in PBST containing a 1:100 dilution of LL/BL pooled sera at room temperature with gentle shaking for 1 h, washed in PBST three times (10 min each), and incubated for 1 h with a 1:5,000 dilution of alkaline phosphatase (AP)-conjugated anti-human polyvalent immunoglobulins (IgGAM). Membranes were washed in 0.1% Tris—HCl buffer overnight at 4°C. Membranes were blocked in phosphate-buffered saline buffer containing 5% (wt/vol) nonfat dried milk, washed in 0.1% Tween 20, and incubated in 1% BSA in PBST containing a 1:5,000 dilution of monoclonal anti-His antibody (Novagen) for 1 h. Membranes were washed three times in PBST and incubated with a 1:5,000 dilution of alkaline phosphatase (AP)-conjugated anti-mouse IgG or AP-conjugated anti-human IgG (Sigma, St. Louis, MO). Membranes were washed three times in PBST and incubated with a 1:10,000 dilution of alkaline phosphatase (AP)-conjugated anti-mouse IgG or AP-conjugated anti-human IgG (Sigma, St. Louis, MO). Membranes were washed three times in PBST and incubated with a 1:10,000 dilution of alkaline phosphatase (AP)-conjugated anti-mouse IgG or AP-conjugated anti-human IgG (Sigma, St. Louis, MO). Membranes were washed three times in PBST and incubated with a 1:10,000 dilution of alkaline phosphatase (AP)-conjugated anti-mouse IgG or AP-conjugated anti-human IgG (Sigma, St. Louis, MO). Membranes were washed three times in PBST and incubated with a 1:10,000 dilution of alkaline phosphatase (AP)-conjugated anti-mouse IgG or AP-conjugated anti-human IgG (Sigma, St. Louis, MO). Membranes were washed three times in PBST and incubated with a 1:10,000 dilution of alkaline phosphatase (AP)-conjugated anti-mouse IgG or AP-conjugated anti-human IgG (Sigma, St. Louis, MO). Statistical analysis. All statistical analyses were carried out using a two-tailed Student’s t test (two-sample equal variance [homoscedastic]).

RESULTS

Serological expression cloning of LL/BL patients reveals recognition of predominantly membrane-bound and secreted protein sequences. An M. leprae expression library was constructed using the ZAP Express system. To assess library quality, 20 random clones were excised and digested with restriction enzymes. The clones contained an average insert size of 1.4 kb of M. leprae genomic DNA (data not shown). A total of 200,000 PFU were subsequently screened with a serum pool from five untreated LL/BL patients. A serum dilution of 1:100 was selected for screening, as this titer gave the most easily identifiable positive signal relative to nonspecific reactivity in our experiments. After selection of positive plaques, excision of phage DNA, and sequencing of M. leprae genomic inserts, a number of clones encoding partial or full-length sequences of a discrete number of proteins were identified. The identities of 14 proteins, deriving from 38 distinct clones, are shown in Table 1. Two proteins were identified from single clones, and the remaining 12 proteins were identified from multiple clones. Five proteins were previously uncharacterized as serologically reactive. ML1213 shows conservation of an M. tuberculosis membrane protein, Rv1565c. ML2331 shares Pfam motifs with amida-se-3, an N-acetylmuramoyl-l-alanine amidase, and ML1812
shares a Pfam motif with p60, both belonging to a superfamily of surface-expressed proteins of pathogenic bacteria (27). ML0405 and ML0568 show homology to \textit{M. tuberculosis} proteins Rv3616c and Rv1435c, respectively, and have unknown functions. Both sequences were analyzed with PSORTb v.2, a computer algorithm that predicts gram-positive bacterial protein subcellular location (9). This algorithm identified five transmembrane helices located within the ML0405 protein sequence, giving a high probability of membrane location. The algorithm was unable to predict a subcellular location for ML0568.

The remaining nine proteins had previously been described as serologically reactive. ML2028 (Ag85b), ML2655 (Ag85c),

![Image](image_url)

**FIG. 1.** (A to C) Reactivity of LL/BL, TT/BT, and NEC sera to selected full-length recombinant proteins by ELISA using protein A. (A) ML0405; (B) ML2331; (C) ML2055. Results are shown as mean ODs ± standard deviations. Closed circles, LL/BL sera \((n = 5)\); closed squares, TT/BT sera \((n = 6)\); open squares, normal sera \((n = 6)\).
and ML0097 (Ag85a) are members of the Ag85 complex and are relatively well conserved among mycobacteria and dominant antigens in both leprosy and tuberculosis (17, 24). Two clones contained sequences of both ML0049 and ML0050 encoding the \emph{M. leprae} ESAT-6 and CFP-10 homologues, respectively. These represent the only two sequences derived from an \emph{M. leprae} genomic region-homologous RD-1 in \emph{M. tuberculosis}, which is deleted from BCG vaccine strains. CFP-10 has previously been demonstrated to be serologically reactive in leprosy patients (31). ML0091 was identified as serologically reactive in a previous screen using a lambda-gt11 \emph{M. leprae} DNA expression library (3). ML2055 is a surface-expressed protein that binds laminin-2 on Schwann cells to facilitate nerve invasion (30). GroEL2, a 60-kDa chaperonin 2 (ML0317), and DnaK, a 70-kDa heat shock protein (ML2496), are well established as immunodominant antigens during infection (22, 34). Taken together, these data indicate that the majority of proteins identified in this screen were putatively membrane bound or secreted (11 of 14 proteins).

ML0405, ML2331, and ML2055 are serologically recognized by LL/BL but not TT/BT patients. His-tagged recombinant derivatives of selected proteins were expressed, purified, and screened for reactivity with pooled LL/BL sera and nonendemic control (NEC) sera by ELISA at a serum dilution of 1:200. ML0405, ML2055, and ML2331 all showed the highest signal-to-background serological reactivity as recombinant proteins (Table 1). The recombinant proteins were further analyzed for reactivity by titrating ELISA of individual LL/BL sera pooled for expression cloning \( (n = 5) \), sera from a group of TT/BT patients \( (n = 6) \), and NEC sera \( (n = 8) \) (Fig. 1). ML0405 and ML2331 showed marked reactivity to LL/BL sera, with reactivity clearly detectable at a serum dilution of 1:1,600. The reactivity of ML2055 titrated out earlier than that observed for ML0405 and ML2331, with reactivity close to background levels at a serum dilution of 1:800. Low or absent reactivity to TT/BT or NEC sera was observed in all cases. Western blotting was performed to ensure that reactivity was specific to the purified His-tagged recombinant protein. Anti-His antibody
and pooled LL/BL sera were identified by all three antigens, confirming that the serological reactivity observed is directed to the purified His-tagged recombinant protein in each case (Fig. 2).

Individual LL/BL patients but not pulmonary tuberculosis patients show serological reactivity to ML2055, ML2331, and ML0405. ML0405, ML2055, and ML2331 demonstrate significant homology to M. tuberculosis proteins (60 to 80%) (Table 1), and significant serological cross-reactivity with pulmonary TB patients could preclude their use as serological diagnostic reagents for leprosy. We screened individual pulmonary TB patient sera (n = 10) and NEC sera (n = 8) for reactivity to recombinant ML0405, ML2055, and ML2331 by ELISA. Of the 10 pulmonary TB patient sera screened, 6 were serologically reactive by ELISA to either TbF10, an M. tuberculosis fusion protein (12), or Rv3875 (Esat-6) (data not shown). In addition, we expanded our analysis of LL/BL patients with 24 individuals. All 24 LL/BL sera failed to recognize the M. tuberculosis antigen Rv3874 (CFP-10) when tested by ELISA (data not shown). Strikingly, ML2055, ML0405, and ML2331 all showed significantly higher reactivity to LL/BL patient sera than to pulmonary TB patient sera (P < 0.0005 in all cases) (Fig. 3). In each case, reactivity of pulmonary TB patient sera was not significantly different from that of NEC sera for ML2055, ML0405, and ML2331 (P > 0.1 in all cases).

Household contacts of LL patients do not show serological reactivity to ML2331 and ML0405. Reactivity of ML2055, ML2331, and ML0405 was tested in a household contact study within an area where leprosy is endemic. NDOHSA was employed as a control. Six families containing a single LL patient (BI of >5 at diagnosis) and at least four family members living in the same dwelling were selected. Serological reactivity of each LL patient serum sample and household contact serum sample to NDOHSA, ML2055, ML2331, and ML0405 was evaluated by IgG and IgM ELISA at a serum dilution of 1:200 (Fig. 4). All LL index cases demonstrated IgM antibodies to NDOHSA, and 7.1% of household contacts (2 of 28) showed IgM reactivity above the arbitrary cutoff value. All index cases showed the presence of IgG antibodies to ML0405 and ML2331, and 50% (3 of 6) showed the presence of IgG antibodies to ML2055. None of the household contact sera demonstrated IgM reactivity to ML0405, ML2331, or ML2055 measured above the cutoff. One of the six index case sera showed elevated IgM reactivity to ML0405 and ML2331, while the remainder showed IgM reactivity not significantly different from that of household contact serum or NEC serum. Thus, at least in
the families tested, individuals with LL could be discriminated from household contacts by IgG reactivity to ML2331 or ML0405. These proteins could represent useful diagnostic tools in areas where leprosy is endemic.

ML0405 and ML2331 show the potential to complement PGL-I in the serological diagnosis of BL leprosy. At serum dilutions of 1:800, all household contacts we tested showed IgM reactivity to NDOHSA, ML0405, and ML2331 that measured below the cutoff (OD of <0.2) (data not shown). All index cases in this study had a BI of 5, and since patients with a BI below 4 often demonstrate a lack of antibody to PGL-I, we examined whether ML0405 and ML2331 would aid serological diagnosis at this dilution with BL patients with a BI of <4. Ten sera were selected from patients with a BI between 3 and 5 and screened for IgM reactivity to NDOHSA and IgG reactivity to ML0405 or ML2331 by ELISA (Fig. 5A). At a serum dilution of 1:800, the patient with lowest BI (3.0) showed significant IgG reactivity to ML0405 but relatively low IgM reactivity to NDOHSA. In fact, the two patients with the lowest BI in this study showed IgGAM reactivity to a combination of all three antigens that was markedly higher than IgM reactivity to NDOHSA alone (Fig. 5B).

**FIG. 5.** Potential utility of ML0405 and ML2331 in the serological diagnosis of BL patients. (A) ELISA of BL patients with a BI between 3 and 4.17 (n = 6) and LL patients with a BI of >5 at a serum dilution of 1:800. At this serum dilution, all household contact and NEC sera used in this study were tested at an OD of <0.2 (data not shown). At a lower BI, reactivity to NDOHSA (IgM) was reduced, and in one case (BI, 3.0), serum reactivity was markedly higher in response to ML0405 (IgG). (B) ELISA at the same serum dilution using combinations of antigens. In the patients with the lowest BI, a measured combination of all three antigens (IgGAM) improved signal compared to NDOHSA alone (IgM). An arbitrary cutoff for reactivity of an OD of 0.2 is indicated by a horizontal line.
DISCUSSION

Multidrug therapy for treatment of early or subclinical leprosy could have a significant impact on both the prevention of nerve damage within infected individuals and the transmission of the *M. leprae* bacillus to uninfected individuals. The presence of anti-PGL-I antibodies is not a reliable marker for the diagnosis of subclinical leprosy, since approximately 10% of an endemic population demonstrate PGL-I antibody, and the majority of these individuals (~90%) do not progress to *M. leprae* infection (25). We carried out serological expression cloning and identified a total of 14 antigens that demonstrated serological reactivity to untreated MB leprosy patients. Five of these proteins are newly identified antigens. Of the antigens tested, ML0405, ML2331, and ML2055 demonstrated the highest serological reactivity for LL/BL sera compared to NEC sera. Importantly, pulmonary TB patients demonstrated low serological reactivity to these antigens in the form of purified recombinant proteins. However, TT/DT patients failed to show significant serological activity in response to these antigens, and diagnostic utility would therefore appear to be relevant only to patients with MB disease. Since MB patients represent a significant source of transmission, subclinical diagnosis and treatment of MB disease are likely to significantly benefit public health programs.

In a household contact study within an area where leprosy is endemic, using a serum dilution of 1:200, index cases showed significant levels of serum IgG that bound ML0405 and ML2331, whereas household contacts showed low or absent serological reactivity. The index cases all possessed IgM antibody that bound NDOHSA, but two household contacts showed IgM levels that measured above the arbitrary cutoff set in this experiment. At a serum dilution of 1:800, all household contacts demonstrated IgM levels against NDOHSA that were below the reactivity cutoff. At this serum dilution, in two cases of leprosy with a bacterial index below 4 (BI of 3 and 3.4), patients demonstrated markedly higher levels of antibody reactivity against a combination of ML0405, ML2331, and NDOHSA compared to IgM that was reactive to NDOHSA alone. Individuals with BI of this magnitude do not typically require serological diagnosis and are not representative of the early stage of MB disease. Despite this, these findings suggest that the sensitivity of serological diagnosis of leprosy can be improved by combining NDOHSA with additional antigens, and the impact of the addition of ML0405 and ML2331 to NDOHSA for the diagnosis of leprosy would be a worthwhile undertaking in a much larger study. Subsequent studies employing ML2331 and ML0405 might encompass the retrospective analysis of sera from household contacts that progressed to MB disease, similar to the analyses reported previously by Douglas et al. using PGL-I (6). In contrast to the IgM response that is elicited by NDOHSA, it is noteworthy that MB patient antibody directed to these antigens is primarily of the IgG subclass. Since the generation of specific IgG responses typically requires T-cell help, it is likely that at certain stages of infection, MB patients demonstrate specific T-cell reactivity to these antigens. Thus, the screening of strongly IgG-reactive antigens for T-cell reactivity may also represent a diagnostic rationale to detect early-stage MB leprosy.

The functions of ML0405 and ML2331 during human infection are unknown. However, recent data demonstrated that the *M. tuberculosis* homologue of ML0405, Rv3616c, is a virulence factor and is required for the secretion of Esat-6 and CFP-10 by *M. tuberculosis* (8, 29). Since ML0405 is recognized immunologically during leprosy, we speculate that ML0405 may play a similar role during *M. leprae* infection.

Taken together, our data indicate that further research on the roles of ML0405 and ML2331 is warranted and may lead to improved diagnosis of leprosy.

ACKNOWLEDGMENTS

This work was funded by the American Leprosy Missions. We wish to thank Raymond L. Houghton for advice on serological diagnosis of pulmonary tuberculosis and Malcolm S. Guthrie for critical evaluation of the manuscript.

REFERENCES

1. Britton, W. J., and D. N. Lockwood. 2004. Leprosy. Lancet 363:1209–1219.
2. Sharma, U., S. P. Glaaz, and C. J. Rothenb. 2004. Mutually dependent secretion of proteins required for mycobacterial virulence. Proc. Natl. Acad. Sci. USA 101:10676–10681.
3. Gardy, J. L., M. R. Laird, F. Chen, S. Ren, C. J. Walsh, M. Ester, and F. S. Brinkman. 2005. PSORTb v. 2.0: expanded prediction of bacterial protein subcellular localization and insights gained from comparative proteome analysis. Bioinformatics 21:617–623.
4. Hatta, M., S. M. van Beers, B. Madjid, A. Djinmadi, M. Y. de Wit, and P. R. Klatser. 1995. Distribution and persistence of Mycobacterium leprae nasal carriage among a population in which leprosy is endemic in Indonesia. Trans. R. Soc. Trop. Med. Hyg. 89:381–385.
5. Homer, M. J., M. J. Lodes, L. D. Reynolds, Y. Zhang, J. F. Douglass, P. D. McNeill, R. L. Houghton, and D. H. Persing. 2003. Identification and characterization of putative secreted antigens from *Babesia microti*. J. Clin. Microbiol. 41:723–729.
6. Houghton, R. L., M. J. Lodes, D. C. Dillon, L. D. Reynolds, C. H. Day, P. D. McNeill, R. C. Hendrickson, Y. A. Skeiky, D. P. Sampaio, R. Badaro, K. P. Lyashchenko, and S. G. Reed. 2002. Use of multiepitope polypeptides in serodiagnosis of active tuberculosis. Clin. Diagn. Lab. Immunol. 9:863–891.
7. Husson, R. N., and R. A. Young. 1987. Genes for the major protein antigens of Mycobacterium tuberculosis: the etiologic agents of tuberculosis and leprosy share an immunodominant antigen. Proc. Natl. Acad. Sci. USA 84:1679–1683.
8. Klatser, P. R. 2000. Use of a Mycobacterium leprae lipoprotein to classify patients with leprosy. Lepr. Rev. 71:676–772.
9. Kumar, S., K. D. Moudgil, A. H. Band, P. R. Narayanan, S. K. Gupta, A. K. Sharma, and G. P. Talwar. 1986. A dot enzyme immunoassay for detection of IgM antibodies against phenolic glycolipid-I in sera from leprosy patients. Lepr. Rev. 57:185–189.
10. Laal, S., Y. D. Sharma, H. K. Prasad, A. Murtaza, S. Singh, S. Tangri, R. S. Misra, and I. Nath. 1991. Recombinant fusion protein identified by lepromatous sera mimics native Mycobacterium leprae in T-cell responses across the leprosy spectrum. Proc. Natl. Acad. Sci. USA 88:1054–1058.
11. Lawton, P. M. N. Nation, J. L. Rubenstein, F. Rivier, A. Drowart, J. P. Van Voren, J. Millan, and K. Huygen. 1993. T-cell stimulation with purified mycobacterial antigens in patients and healthy subjects infected with Myco-
18. Lockwood, D. N. 2002. Leprosy elimination—a virtual phenomenon or a reality? BMJ 324:1516–1518.

19. Lodes, M. J., Y. Cong, C. O. Elson, R. Mohamath, C. J. Landers, S. R. Targan, M. Fort, and R. M. Hershberg. 2004. Bacterial flagellin is a dominant antigen in Crohn disease. J. Clin. Investig. 113:1296–1306.

20. Lodes, M. J., D. C. Dillon, R. L. Houghton, and Y. A. Skeiky. 2004. Expression cloning. Methods Mol. Med. 94:91–106.

21. Lodes, M. J., D. C. Dillon, R. Mohamath, C. H. Day, D. R. Benson, L. D. Reynolds, P. McNeil, D. P. Sampaio, Y. A. Skeiky, R. Badaro, D. H. Persing, S. G. Reed, and R. L. Houghton. 2001. Serological expression cloning and immunological evaluation of MTB48, a novel Mycobacterium tuberculosis antigen. J. Clin. Microbiol. 39:2485–2493.

22. McKenzie, K. R., E. Adams, W. J. Britton, R. J. Garsia, and A. Basten. 1991. Sequence and immunogenicity of the 70-kDa heat shock protein of Mycobacterium leprae. J. Immunol. 147:312–319.

23. Mustafa, A. S., A. Deggerdal, K. E. Lundin, R. M. Meloen, T. M. Shinnick, and F. Oftung. 1994. An HLA-DRw3 restricted T-cell epitope from a novel Mycobacterium leprae protein antigen important to the human memory T-cell repertoire against M. leprae. Infect. Immun. 62:5595–5602.

24. Naito, M., S. Izumi, and T. Yamada. 1998. Two-dimensional electrophoretic analysis of humoral responses to culture filtrate of Mycobacterium bovis BCG in patients with leprosy and tuberculosis. Int. J. Lepr. Other Mycobact. Dis. 66:208–213.

25. Osakam, L. E., Slim, and S. Buhre-Sekula. 2003. Serology: recent developments, strengths, limitations and prospects: a state of the art overview. Lepr. Rev. 74:196–205.

26. Ramaprasad, P., A. Fernando, S. Madhale, J. R. Rao, V. K. Edward, P. D. Lodes, P. R. Klatser, M. Y. de Wit, W. C. Smith, and I. A. Cree. 1997. Transmission and protection in leprosy: indications of the role of mucosal immunity. Lepr. Rev. 68:301–315.

27. Rigden, D. J., M. J. Jedrzejas, and M. Y. Galperin. 2003. Amidase domains from bacterial and phage autolysins define a family of gamma-D,L-glutamate-specific amidohydrolases. Trends Biochem. Sci. 28:230–234.

28. Roche, P. W., S. S. Failbus, W. J. Britton, and R. Cole. 1999. Rapid method for diagnosis of leprosy by measurements of antibodies to the M. leprae 35-kDa protein: comparison with PGL-I antibodies detected by ELISA and “dipstick” methods. Int. J. Lepr. Other Mycobact. Dis. 67:279–286.

29. Sassetti, C. M., and E. J. Rubin. 2003. Genetic requirements for mycobacterial survival during infection. Proc. Natl. Acad. Sci. USA 100:12989–12994.

30. Shimozji, Y., V. Ng, K. Matsumura, V. A. Fischetti, and A. Rambukkana. 1999. A 21-kDa surface protein of Mycobacterium leprae binds peripheral nerve laminin-2 and mediates Schwann cell invasion. Proc. Natl. Acad. Sci. USA 96:9857–9862.

31. Spencer, J. S., H. J. Kim, A. M. Marques, M. Gonzalez-Juarrero, M. C. Lima, V. D. Vissa, R. W. Truman, M. L. Gennaro, S. N. Cho, S. T. Cole, and P. J. Brennan. 2004. Comparative analysis of B- and T-cell epitopes of Mycobacterium leprae and Mycobacterium tuberculosis culture filtrate protein 10. Infect. Immun. 72:3161–3170.

32. van Beers, S. M., M. Hatta, and P. R. Klatser. 1999. Patient contact is the major determinant in incident leprosy: implications for future control. Int. J. Lepr. Other Mycobact. Dis. 67:119–128.

33. World Health Organization. 2002. Leprosy. Global situation. Wkly. Epidemiol. Rec. 77:1–8.

34. Young, D., R. Lathigra, R. Hendrix, D. Sweetser, and R. A. Young. 1988. Stress proteins are immune targets in leprosy and tuberculosis. Proc. Natl. Acad. Sci. USA 85:4267–4270.

35. Young, R. A., V. Mehra, D. Sweetser, T. Buchanan, J. Clark-Curtiss, R. W. Davis, and B. R. Bloom. 1985. Genes for the major protein antigens of the leprosy parasite Mycobacterium leprae. Nature 316:450–452.