Progress in Developing a Rapid Diagnostic Test for the Early Detection of Leprosy

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Elimination of leprosy as a public health problem

- A WHO study group recommended multidrug therapy (MDT) in 1981. Since 1985, over 14 million leprosy patients have been cured using MDT.

- Resolution by the World Health Assembly in 1991 to reduce prevalence rate to less than 1 case per 10,000 individuals by the year 2000 worldwide.

- Target prevalence was achieved in 113 out of 122 countries by 2005.

- However, the prevalence rate in 2004 was exceeded in 9 countries, and new case detection remained obstinately high despite years of MDT.

# Clinical Spectrum of Leprosy

<table>
<thead>
<tr>
<th>Bacterial load</th>
<th>TT</th>
<th>BT</th>
<th>BB</th>
<th>BL</th>
<th>LL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incubation time</td>
<td>(3 - 5 years)</td>
<td></td>
<td></td>
<td>(8 - 10 years)</td>
<td></td>
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<tr>
<td>Cell mediated immunity</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Skin lesions</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Nerve damage</td>
<td>1-2</td>
<td>1-3</td>
<td>2-3</td>
<td>2-3</td>
<td>1-3</td>
</tr>
</tbody>
</table>

- **Tuberculoid** (TT/BT): No test available
- **Lepromatous** (BL/LL): PGL-I Ab test

Strategy for identifying and testing novel proteins of *M. leprae*

1. Identify unique or low homology genes from *M. leprae* genome database
2. Clone the gene by PCR, transform *E. coli* to express protein
3. Purify recombinant His-tagged proteins by affinity chromatography
4. Immunize mice to produce polyclonal Ab reagents
5. Test IFN-γ responses of PBMC from leprosy patients
6. Identify existence of protein in native fractions of *M. leprae*
7. Determine DTH responses in sensitized guinea pigs
8. Show existence of protein by immunohistology of *M. leprae* in nude mouse footpad
Theoretical 2-DE of 1,601 predicted *M. leprae* proteins with a current total of 256 (16% of the proteome) of these identified by us and others (in red).

Marques, M. A. M., et al., manuscript in preparation
How to best select *M. leprae* proteins that are leprosy-specific?

Identifying Ag unique to *M. leprae* may lead to improved tools to measure leprosy-specific T cell responses

*M. leprae* unique sequences, possibly including some of the 142 hypothetical unknowns from class VI proteins listed in the Sanger site, many of which have no homologs in any of the current mycobacterial databases.
Recombinant antigens and peptides that were tested

- Recombinant hypothetical unknown proteins ML0008, ML0126, ML1057, and ML2567
- 34 peptides from 13 different hypothetical unknowns, including 18 from the four hypothetical unknowns above
- 24 peptides from other proteins of interest, some with known homologues in TB to test for cross-reactive responses in TB patients or other groups

Groups tested using an *in vitro* IFN-γ assay to determine responses to *M. leprae* antigens

PBMC or whole blood cultured *in vitro* with native, recombinant proteins, or peptides

- PB = paucibacillary (T cell response to leprosy Ag intact)
- MB = multibacillary (depressed T cell responses to leprosy Ag)
- HC = household contacts (T cell responses intact, as good or better than PB)
- EC = healthy endemic control, low exposure to leprosy
- TB = TB group, measures cross-reactive responses to leprosy Ag
- NEC = non-endemic control (CSU), background responses to leprosy Ag

Measure cell-mediated response to antigens by cytokine analysis

IFN-γ ELISA
IFN-γ responses of PBMC unstimulated or stimulated with control Ag

IFN-γ levels (pg/ml)

M. leprae

SEB

Unstimulated
IFN-γ responses of PBMC to ML0126 and five ML0126 peptides
IFN-γ responses of ten paucibacillary leprosy patients towards recombinant proteins and peptides

|        | ML0008 | 37 | 38 | 39 | 40 | 41 | ML0126 | 42 | 43 | 44 | 45 | 80 | 81 | ML0394 | 46 | 47 | 48 | 49 | 50 | 51 | 52 | ML1057 | 49 | 50 | 51 | 52 | 53 | 54 | 55 | ML2567 |
|--------|--------|----|----|----|----|----|--------|----|----|----|----|----|----|--------|----|----|----|----|----|----|----|--------|----|----|----|----|----|----|----|----|--------|
| PB1    |        |    |    |    |    |    |        |    |    |    |    |    |    |        |    |    |    |    |    |    |    |        |    |    |    |    |    |    |    |    |        |
| PB2    |        |    |    |    |    |    |        |    |    |    |    |    |    |        |    |    |    |    |    |    |    |        |    |    |    |    |    |    |    |    |        |
| PB3    |        |    |    |    |    |    |        |    |    |    |    |    |    |        |    |    |    |    |    |    |    |        |    |    |    |    |    |    |    |    |        |
| PB4    |        |    |    |    |    |    |        |    |    |    |    |    |    |        |    |    |    |    |    |    |    |        |    |    |    |    |    |    |    |    |        |
| PB5    |        |    |    |    |    |    |        |    |    |    |    |    |    |        |    |    |    |    |    |    |    |        |    |    |    |    |    |    |    |    |        |
| PB6    |        |    |    |    |    |    |        |    |    |    |    |    |    |        |    |    |    |    |    |    |    |        |    |    |    |    |    |    |    |    |        |
| PB7    |        |    |    |    |    |    |        |    |    |    |    |    |    |        |    |    |    |    |    |    |    |        |    |    |    |    |    |    |    |    |        |
| PB8    |        |    |    |    |    |    |        |    |    |    |    |    |    |        |    |    |    |    |    |    |    |        |    |    |    |    |    |    |    |    |        |
| PB9    |        |    |    |    |    |    |        |    |    |    |    |    |    |        |    |    |    |    |    |    |    |        |    |    |    |    |    |    |    |    |        |
| PB10   |        |    |    |    |    |    |        |    |    |    |    |    |    |        |    |    |    |    |    |    |    |        |    |    |    |    |    |    |    |    |        |

Key:
- < 100 pg/ml
- 100-299 pg/ml
- 300-499 pg/ml
- 500-999 pg/ml
- > 1000 pg/ml
IFN-γ responses of ten TB patients towards recombinant *M. leprae* proteins and peptides
M. leprae unique candidate Ag:
ML1990

IFN-γ (pg/ml)

Controls | MB | PB/Rx | HC | TB

13/26 | 30/47

Data provided by Annemieke Geluk, Ph.D., Leiden University, Amsterdam
Preliminary peptide data of Brazilian leprosy patients (PB/Rx)

Data provided by Annemieke Geluk, Ph.D., Leiden University, Amsterdam
Responses of groups to recombinant proteins

Clinical leprosy diagnosis:
- MB (n = 19)
- PB/Rx (n = 28)
- HC (n = 34)
- neg controls (n = 30)

PGL-I serology:
- 0% 100%
- 61% 39%
- 91% 9%
- 100% 0%

T cell response to *M. leprae* Ag ML0576, ML1989, ML1990, ML2283 or ML2567:
- 6% 94%
- 29% 71%
- 93% 7%

\[ p \leq 0.001 \]
\[ p \leq 0.002 \]

**IDEAL Consortium**: Initiative for Diagnostic and Epidemiological Assays for Leprosy

- **May 2005.** Awarded a grant of $600,000 from the Heiser Program.

- **Summer 2006:**

  Evaluate the responses of leprosy patients and controls towards 5 recombinant proteins and 27 peptides at five separate sites (Brazil, Pakistan, Bangladesh, Nepal, and Ethiopia).
Collaborators

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