Identification of Serological Biomarkers of Infection, Disease Progression and Treatment Efficacy for Leprosy

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## 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>(8 - 10 years)</td>
<td></td>
<td></td>
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<tr>
<td>Cell mediated immunity</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>

Skin lesions:
- **Light (1)**: Mild
- **Moderate (2)**: Moderate
- **Severe (3)**: Severe

Nerve damage:
- **Light (1)**: Mild
- **Moderate (2)**: Moderate
- **Severe (3)**: Severe

Tuberculoid (TT/BT) and Lepromatous (BL/LL) types of leprosy.
Serological assays: Primary issues

PGL-I antibody detection in patients and household contacts

- PGL-I antibody is detected in virtually all lepromatous patients but only about 40% of tuberculoid patients are positive.

- Contacts who are positive for Ab to PGL-I have a >8-fold risk of developing the disease, but half who develop disease do not have Ab to PGL-I, particularly PB patients.

- Other proteins or target antigens have been examined that can predict infection or disease progression (LID-1, Ag85B).

- Developing biomarkers that can predict those most at risk of coming down with disease is key to reducing leprosy transmission.
Outline of Study

• The reactivity of 12 recombinant proteins with lepromatous and tuberculoid patient sera was examined. All sera obtained from subjects from the Philippines.

• The temporal antibody titers of lepromatous patients was assessed during MDT therapy and for a total of two years after diagnosis by serial bleeds every 3 months.

• 51 household contacts were bled every 3 months and their antibody titers checked for at least two years to determine if there were any increases associated with onset of disease symptoms.

• An ELISA assay was used to determine the titer of the sera against the *M. leprae* antigens ML2028 (Ag85B), PGL-I, and LAM. Western blot was used to determine the reactivity to ML2028, LID-1 and the native cytosolic protein fraction.
LID-I (fusion of ML0405 and ML2331) as a potential leprosy diagnostic

Variable responses of MB and PB patients to recombinant protein antigens

1. LID-1 (IDRI)
2. ML2055 modD
3. ML0286 fba
4. ML2028 Ag85B
5. ML2038 bfr
6. ML0050 CFP-10
7. ML0380 GroES
8. MLSA cytosol

MB-10

MB-11

MB-12

PB-4

PB-6
Western blot patterns of 51 HC baseline sera to LID-1, Ag85, and native *M. leprae* MLSA

Lane 1, LID-1; lane 2, Ag85B; lane 3, native MLSA cytosolic protein
Increases in antibody titer over time during a 15 month period in a household contact

Lane 1, Ag85B; lane 2, native *M. leprae* cytosolic protein

Effect of MDT treatment on the antibody titer towards *M. leprae* antigens

Temporal antibody responses in leprosy patient MB-2 from the time of diagnosis (baseline, classified as LL) to 24 months after beginning MDT therapy against *M. leprae* antigens as determined by Western blot. The BI at diagnosis was 3.5+, reduced to 2.5+ after completion of 1 year MDT. Reversal reactions at 1 month, 9 months, and 12 months, treated with steroids each time.

Lane 1, LID-1; lane 2, ML2055; lane 3, ML2028; lane 4, CFP-10; lane 5, MLSA native cytosolic protein.
Effect of MDT treatment on the antibody titer towards *M. leprae* antigens

Temporal antibody responses in leprosy patient MB-3 from the time of diagnosis (baseline, classified as BL) to 24 months after beginning MDT therapy against *M. leprae* antigens as determined by Western blot. The BI at diagnosis was 3.2+, reduced to 1.33+ after completion of 1 year MDT. Treated with low dose steroids the first few months while on MDT for neuritis, tapered off by 6 months. Dramatic improvement and clearance of lesions within the first few months.

Lane 1, LID-1; lane 2, ML2055; lane 3, ML2028; lane 4, CFP-10; lane 5, MLSA native cytosolic protein.
Conclusions and future directions

• MDT therapy causes a reduction in the titer of antibody responses over time, with a more rapid clearance of antibody towards protein antigens, and a more gradual lowering of antibody to PGL-I and LAM, suggesting that these antigens either persist or are degraded more slowly than proteins.

• A higher initial BI generally elicits a higher titer to all antigens, although responses to specific recombinant proteins are variable and patient specific.

• In healthy HC of MB index cases, positive antibody responses against both LID-1 and Ag85B are likely biomarkers of infection. Increases in the titers against both PGL-I and LAM over time may indicate active disease with an increase in the BI.

• During the first 15 months of monitoring the 51 HC, one individual progressed to active disease. This translates to 2% overall, a rate very similar to a larger Brazilian study of HC over a 5 year period. We will continue to follow this group to determine if others will come down with disease.
Collaborators and funding

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