Clinical aspects of nerve damage in leprosy
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Chapter 7

Risk Factors for Type-1 Reactions in Borderline Leprosy Patients

From: Risk Factors for Type-1 Reactions in Borderline Leprosy Patients
Paul W. Roche, Wim J. Theuvenet, and Warwick J. Britton
SUMMARY

Type-1 or reversal reactions are the major cause of nerve damage and disability in leprosy. We wished to determine whether there were any clinical or laboratory markers that identified patients with an increased risk of type-1 reaction. 42 (31%) of 136 Nepalese borderline leprosy patients (97 male, 39 female; age range 7-73 years) had a type-1 reaction during the first 2 years of multidrug therapy. Before therapy, 41 (98%) of the 42 patients were seropositive for antibodies to one of three mycobacterial antigens. Seropositivity for IgM antiphenoxyglycolipid-1 (PGL-1) antibodies, but not IgG anti-lipoarabinomannan or anti-M. leprae 35 kDa protein antibodies, was significantly associated with subsequent manifestation of a type-1 reaction (p<0.001). The concentration of IgM anti-PGL-1 antibodies in serum was significantly higher in patients in whom a type-1 reaction developed. The risk attributable to anti-PG1-A seropositivity was independent of leprosy class, skin smear positivity, and the presence of other anti-M leprae antibodies (adjusted odds ratio = 8.7, p<0.001). In the 87 patients who had a lepromin test, anti-PGL-1 seropositivity and lepromin reactivity were significant independent risk factors for subsequent reaction. 78% of patients with positive lepromin reactivity and IgM anti-PGL-1 antibodies had type-1 reactions. Patients with these risk factors should be carefully monitored during antimicrobial therapy to permit early initiation of anti-inflammatory treatment thus minimising permanent nerve damage and resultant disability.

INTRODUCTION

Leprosy remains a significant cause of morbidity in countries where it is endemic. The development of effective multi-drug therapy (MDT) is a major advance in treatment and raises the possibility of long-term control of the disease. However, nerve damage can occur despite adequate antimicrobial therapy, largely because of the complication known as type-1 or reversal reactions. These are episodes of increased inflammatory activity in nerves, skin lesions, or both, and the resulting nerve damage is responsible for muscle imbalance and anaesthesia leading to increased deformity. Type-1 reactions occur in patients with borderline leprosy rather than in those with immunologically stable polar tuberculoid or polar lepromatous leprosy. During the first 6-12 months of therapy with dapsone alone, 50% of borderline leprosy patients have type-1 reactions. In a field study of MDT, 25% of Ethiopian borderline leprosy patients developed a reversal reaction. In addition, nerve damage can occur without overt neuritis despite adequate MDT, probably because of low-grade inflammation within the perineural sheath.

Type-1 reactions have been associated with an increase in the cellular immune response to mycobacterial antigens, but the reasons for fluctuations in immune responsiveness and the factors predisposing to reactions are not well understood. We have monitored 136 newly diagnosed borderline leprosy patients who were commencing MDT to determine whether
any clinical or laboratory markers found at the time of diagnosis identified patients at increased risk of developing type-1 reactions.

**PATIENTS AND METHODS**

**Patients**
The study population comprised 136 self-referred borderline leprosy patients presenting to Anandaban Leprosy Hospital. The patients had not been treated previously or had active disease after defaulting from prior monotherapy for more than 1 year. Leprosy was diagnosed on clinical and bacteriological grounds and confirmed by histopathology in a minority of cases. The duration and extent of disease were estimated at the time of initial examination. Extent of disease was assessed by the number of involved nerves (defined as unequivocal enlargement or loss of function) and affected skin patches and body areas. Patients were followed-up for an average of 21 months (range 7-35 months), including visits following release from treatment. Patients were seen at monthly intervals during the first 6-12 months and thereafter at a maximum interval of 2 months. 121 patients received multibacillary MDT and 15 paucibacillary MDT.

Blood for serological testing was taken before starting MDT.

Type-1 reaction was defined as an acute neuritis that presented with the tender enlargement of a peripheral nerve trunk associated with partial or complete loss of motor or sensory function. This was accompanied in most patients by swelling and erythema in skin patches. Cell-mediated immunity was assessed by lepromin reactivity in 87 patients. Lepromin A (3 x 10^6 bacilli in 100 μl; supplied by the Immunology of Leprosy [IMMLEP] programme of the World Health Organisation) was injected intradermally on the volar aspect of the forearm and the degree of induration at 3-4 weeks was measured. Induration of greater than or equal to 3 mm was considered positive. The mean bacteriological index was calculated from slit skin smears taken from four sites including, one clinical lesion.

**Immunological assays**

IgM anti-phenolic-glycolipid-1 (PGL-1) antibodies were measured by enzyme linked immunosorbant assay (ELISA), with disaccharide bovine serum albumin (provided by the IMMLEP programme) at a concentration of 250 ng/ml as the glucoconjugate and serum diluted 1 in 300. Samples with an absorbance greater than 0.199 (mean absorbance plus 3 SD of serum from 91 healthy Nepali control subjects) were considered positive. Known positive and negative control sera were included in each assay. Variation between assays was less than 10%.

IgG anti-lipoarabinomannan (LAM) antibodies were measured by ELISA, with *Mycobacterium tuberculosis* LAM (provided by Dr P. J. Brennan) at a concentration of 1μg/ml as the antigen and serum diluted 1 in 1000. Samples with an absorbance greater than 0.419 (mean absorbance plus 3 SD of serum from 100 healthy controls) were considered positive.
Table 1. Number of patients with a type-1 reaction and number positive or negative for antibodies or skin smear out of 136 Borderline Leprosy Patients.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>No. with type-1 reaction/ Total no. positive (%)</th>
<th>No. with type-1 reaction/ Total no. negative (%)</th>
<th>Unadjusted OR (95% CI)</th>
<th>Adjusted by multivariant analysis OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-PGL-1 antibody</td>
<td>38/83 (46)</td>
<td>4/53 (7.5)</td>
<td>10.3* (2.3-26.7)</td>
<td>8.7* (2.5-30.5)</td>
</tr>
<tr>
<td>Anti-LAM antibody</td>
<td>27/70 (39)</td>
<td>15/66 (23)</td>
<td>2.1 (1.0-4.4)</td>
<td>1.0 (0.4-2.5)</td>
</tr>
<tr>
<td>Anti 35 kDa antibody</td>
<td>30/80 (38)</td>
<td>12/56 (21)</td>
<td>2.2 (1.0-4.6)</td>
<td>0.9 (0.3-2.5)</td>
</tr>
<tr>
<td>Skin smear</td>
<td>30/70 (43)</td>
<td>12/66 (18)</td>
<td>3.4** (1.5-7.1)</td>
<td>1.7 (0.5-5.6)</td>
</tr>
</tbody>
</table>

* p < 0.001  
** p < 0.05

Table 2. Number of patients with a type-1 reaction and number positive or negative for antibodies, skin smear, or lepromin reactivity out of 87 Borderline Leprosy Patients tested for Lepromin Reactivity

<table>
<thead>
<tr>
<th>Antibody</th>
<th>No. with type-1 reaction/ Total no. positive (%)</th>
<th>No. with type-1 reaction/ Total no. negative (%)</th>
<th>Unadjusted OR (95% CI)</th>
<th>Adjusted by multivariant analysis OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-PGL-1 antibody</td>
<td>29/51 (51)</td>
<td>3/36 (8)</td>
<td>10.4* (2.9-34.0)</td>
<td>28.3** (5.0-162)</td>
</tr>
<tr>
<td>Anti-LAM antibody</td>
<td>19/46 (41)</td>
<td>10/41 (24)</td>
<td>2.2 (0.8-5.3)</td>
<td>0.7 (0.2-2.7)</td>
</tr>
<tr>
<td>Anti 35 kDa antibody</td>
<td>18/45 (40)</td>
<td>11/42 (26)</td>
<td>1.9 (0.7-4.5)</td>
<td>1.1 (0.3-4.0)</td>
</tr>
<tr>
<td>Skin smear</td>
<td>17/38 (45)</td>
<td>12/49 (24)</td>
<td>2.5 (1.0-6.0)</td>
<td>2.8 (0.6-12.0)</td>
</tr>
<tr>
<td>Lepromin</td>
<td>17/46 (37)</td>
<td>12/41 (29)</td>
<td>1.4 (0.6-3.4)</td>
<td>11.2*** (2.3-53.6)</td>
</tr>
</tbody>
</table>

* p < 0.005  
** p < 0.01  
*** p < 0.0005

Antibodies to the M lepraespecific 35 kDa protein were detected by a monoclonal antibody (MLO4) inhibition ELISA. Serum samples were considered positive if they caused 50% inhibition of binding of MLO4 to M leprae sonicate at a titre greater than 10. 56 control sera were all negative.

Statistical methods
The significance of differences in rates of seropositivity was tested with the chi-square statistic and Yates correction. Chi-square contingency tables were used to calculate the odds ratios (ORs) and 95% CIs for unadjusted differences in the prevalence of type-1 reactions. Adjusted ORs and 95% CIs were calculated by multivariant analysis to assess the significance of individual factors. Tests for colinearity between factors were done by multiple
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regression analysis. Differences in the range of antibody levels were assessed by the Mann-Whitney test.

RESULTS

There were 97 male and 39 female patients, with an age range from 7 to 73 years. 42 patients (31%) had type-1 reactions during the study period. Reactions occurred from the time of presentation to 19 months after the start of therapy, and developed in 37 patients (88%) within the first 6 months of therapy. Type-1 reactions were significantly more common in patients classified as mid-borderline (BB) and borderline lepromatous (BL) leprosy compared with those classified as borderline tuberculoid (BT) leprosy (6 of 13 [46%], 0.05 < p < 0.1, and 24 of 62 [39%], p < 0.05. vs 12 of 61 [20%]). Leprosy classification was not an independent risk factor for the type-1 reaction when paucibacillary BT patients were compared with multibacillary BB and BL patients (12 of 61 [20%] vs 30 of 75 [40%]; OR=0.4 [95% CI=0.2-0.8], adjusted OR= 1.2 [95% CI = 0.4-3.8]).

All but one of the patients who had a type-1 reaction were initially seropositive for one of the three antibodies tested. Type-1 reactions developed in a significantly greater proportion of anti-PGL-1-antibody-positive patients than seronegative patients (p<0.001). When individual risk factors were assessed by multivariate analysis, anti-PGL-1 antibodies were associated with a significant increase in the risk of a type-1 reaction (adjusted OR = 8.7, p < 0.001) (table 1). There was no significant association between risk of a type-1 reaction and positivity for anti-LAM or anti-35 kDa antibodies (table 1), or between risk of a type-1 reaction and a patient’s age or sex (data not shown). Patients with a positive skin smear had an apparent increased risk of reaction but this was not independent of PGL-1 seropositivity or leprosy class (adjusted OR = 1.7) (table 1). The relation between skin smear positivity and anti-PGL-1 seropositivity has been reported previously.13 Analysis of the different patient categories revealed that PGL-1 seropositivity was an independent risk factor in BT patients (OR= 10-0, 95% CI = 8.4-11-6, p < 0.005) and BB/BL patients (OR = 7-6, 95% CI = 0-8-67-9, 0.05 < p < 0. 1).

Of the 87 patients skin tested with lepromin, 46 were positive and 41 negative. 17 (37%) lepromin-positive and 12 (29%) lepromin-negative patients had a type-1 reaction. Multivariate analysis of individual risk factors in patients tested for lepromin showed that PGL-1 seropositivity (OR=28.3, p<0.0005) and to a lesser extent lepromin reactivity (OR=11.2, p<0.005) were independently associated with increased risk of type-1 reactions (table 2). The effect of lepromin positivity was only apparent in the multivariate analysis. It was not due to a difference in the rates of type-1 reaction in the lepromin-tested group (33%) and the whole cohort (31%). Patients with anti-PGL-1 seropositivity and positive lepromin reactivity had a high rate of type-1 reactions. 78% of patients with both risk factors developed type-1 reactions (table 3).
The mean absorbance of serum samples tested for anti-PGL1 antibody was significantly higher (p < 0.01) in patients who subsequently manifested type-1 reactions (A$_{492}$ = 0.758) than those who did not (A$_{492}$ = 0.457) (figure top next page). This effect was most apparent in BT patients (A$_{492}$ = 0.674 in patients with subsequent reactions and 0.185 in those without reactions; p<0.01). The mean absorbance of serum samples tested for anti-LAM antibody was also significantly higher in patients who developed type-1 reactions than in those who did not (A$_{492}$ = 0.866 vs 0.564; p<0.01), and there was a similar, though non-significant, trend for anti-35 kDa antibodies (mean titres causing 50% inhibition= 33 vs 13). The three patient subgroups were not significantly different in the mean absorbance of 50% inhibition titre of serum samples tested for anti-LAM and anti-35kDa antibodies.

Mean initial antibody levels in borderline leprosy patients who did or did not develop a type-1 reaction.

Bars represent mean (SE) absorbance at 492 nm (anti-PGL-1 and anti-LAM) or mean titre causing 50% inhibition (anti-35 kDa) in patients who did (N) or did not (S) have a type-1 reaction.

*p < 0.01 compared with patients who did not have a type-1 reaction.

Table 3. Relationship between Lepromin Reactivity, Seropositivity to PGL-1, and the Incidence of Type-1 reactions in 87 Borderline Leprosy patients tested for Lepromin Reactivity.

<table>
<thead>
<tr>
<th></th>
<th>Lepromin positive (N = 46)</th>
<th>Lepromin negative (N = 41)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anti-PGL-1 positive (N = 18)</td>
<td>Anti-PGL-1 negative (N = 28)</td>
</tr>
<tr>
<td>No. (% ) of patients with a type-1 reaction</td>
<td>14* (78)</td>
<td>3 (28)</td>
</tr>
</tbody>
</table>

*p <0.01 compared with proportion of lepromin-positive anti-PGL-1 negative and lepromin-negative anti-PGL-1- negative patients with a type-1 reaction.
DISCUSSION

In our patients multivariant analysis of the clinical, bacteriological and serological parameters of new borderline leprosy patients showed that the presence of anti-PGL-1 antibodies and lepromin positivity were significant risk factors for manifestation of type-1 reactions. The presence of other anti-leprosy antibodies and skin smear positivity were not useful predictors of reaction, nor were leprosy class, age, and sex. The risk attributable to PGL-1 seropositivity was greater among BT patients than BB/BL patients. Concentrations of anti-PGL-1 antibody (as indicated by absorbance) were significantly higher in those patients with subsequent type-1 reaction.

If type-1 reactions are caused by delayed-type hypersensitivity reactions directed against persisting mycobacterial antigens, why are they associated with anti-PGL-1 antibodies? In patients with multibacillary and paucibacillary leprosy there is a strong correlation between seropositivity and bacterial load and the clinical extent of the disease. In particular, increasing neural involvement is associated with and-M. leprae antibodies in patients with BT and primary neuritic leprosy. Although anti-M. leprae antibodies may not be involved directly in the pathogenesis of type-1 reactions, antibodies may be a marker of significant antigen load in nerves, which predisposes to type-1 reaction in the presence of an active T-cell response to M. leprae. Lepromin positivity is evidence of M. leprae-specific cellular reactivity, and patients who were both lepromin positive and IgM anti-PGL-1 seropositive had a greatly increased risk of type-1 reaction.

Leprosy patients mount a humoral response to a range of protein and carbohydrate antigens. The 35 kDa protein and PGL-1 are recognised by M. leprae-specific antibodies while LAM is recognised by a crossreactive antimycobacterial response. PGL-1 is a major secretory product of M. leprae and there is a strong correlation between the viability of M. leprae and PGL-1 production. PGL-1 is readily detectable in the sera of untreated multibacillary patients and it elicits a dominant IgM antibody response in contrast with the IgG response to LAM and 35 kDa protein antigens. Concentrations of PGL-1 fall rapidly with antimicrobial therapy as do concentrations of IgM anti-PGL-1 antibodies, but at a slower rate. While PGL-1 is not itself a T-cell antigen other antigens released from degraded bacilli may be the target of cellular immune responses during the type-1 reaction. It is significant that most reactions occur while antigen release is at its greatest during the first 6 months of therapy.

Although the factors that initiate type-1 reactions in individual patients are uncertain, the combination of PGL-1 seropositivity and lepromin positivity identifies a group at increased risk. Such patients should be carefully monitored during the first 6 months of therapy to detect clinical signs of type-1 reaction. This may lead to early treatment of reactional episodes with a resultant reduction in nerve damage and disability. The frequency of type-1 reactions in borderline leprosy patients appears sufficiently high to warrant a clinical trial to determine whether prophylactic therapy with and-inflammatory drugs can prevent the development of overt or silent neuritis during antimicrobial treatment.
ACKNOWLEDGEMENT

We thank the staff of Anandaban Leprosy Hospital for their cooperation, Dr. R.J.W. Rees for the provision of *Mycobacterium leprae* sonicate through the IMMLEP component of the WHO/UNDP/World Bank Tropical Diseases Research Programme, Dr. J.Ivanyi for providing ML04 conjugate, and Dr. P.J. Brennan for providing LAM (prepared under NIH contract A1-52582). The Mycobacterial Research Laboratory is fully supported by The Leprosy Mission International. We also thank Ms. Jenny Peat of the Department of Medicine, Sydney University, for assistance with statistical analysis.

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