Studies of Human Leprosy Lesions In Situ Using Suction-Induced Blisters: Cell Changes with IgM Antibody to PGL-1 and Interleukin-2 Receptor in Clinical Subgroups of Erythema Nodosum Leprosum

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Leprosy is a disease with various clinical and histologic stages. Patients at the tuberculoid end mount a strong cell-mediated immune response, while those at the lepromatous pole are unresponsive to the antigens of *Mycobacterium leprae* which does not revert even after treatment.¹,² During the usually chronic course of leprosy approximately 50% of newly diagnosed leprosy patients in Thailand will experience one or more acute reactional episodes, usually within the first year or two after diagnosis. Fifty percent of the patients first seek medical treatment because of a reaction.³

A reactional state known as erythema nodosum leprosum (ENL) occurs in patients with large numbers of leprosy bacilli in their lesions.⁴ It is characterized by fever and malaise associated with crops of tender, erythematous, subcutaneous nodules and, histologically, the lesions show perivascular infiltration of polymorphonuclear cells along with histiocyte containing fragmented bacteria.⁵ It has been suggested that ENL is a clinical manifestation of the Athus phenomenon.⁶,⁷ This is supported by the presence of immunoglobulin⁷ and complement⁸ in the lesions and circulating immune complexes and complement degraded products.⁹ Serologic studies have indicated that the IgM antibody against *M. leprae* phenolic glycolipid antigens are most abundant and correlate best with the status of the disease activity.¹⁰ Some investigators have reported a decreased IgM antibody level in ENL while others found significant change of IgM antibody in these lesions.¹⁰-¹² Suppressor cells have been implicated in the pathogenesis of the ENL reaction.¹³ Complement levels were increased in the skin lesions while ENL lesions showed a decreased number of CD8+ (T-suppressor) cells and increased helper/suppressor ratio as compared to those in acute ENL and non-reactional leprosy; b) Tac peptide and IgM antibody to PGL-1 were elevated in the chronic ENL lesions; c) and systemic administration of corticosteroids appeared to cause a reduction in the intralesional CD4+ cell population and IgM antibody to PGL-1 but did not change CD8+ cell population and the levels of Tac peptide in the lesions. The elevated levels of Tac peptide were localized in the skin lesions while increased levels of IgM anti-PGL-1 seemed to be filtered from the peripheral blood. We conclude that spontaneous lymphocyte activation in situ, primarily of decreased CD8+ and relatively increased CD4+ cells, are important features of chronic, recurrent ENL reactions and may be an intermittent or cyclic phenomenon during the reaction. Understanding the mechanisms of these spontaneous changes in immunity in leprosy will enlarge our knowledge of reactions and of the underlying determinants of delayed type hypersensitivity and cell-mediated immunity in leprosy, which in turn will allow us to realize the potential for artificially manipulating these responses as proposed with vaccines or immunotherapy.

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reaction with some contradictions among investigators.\textsuperscript{13-16}

We have examined cutaneous leprosy lesion \textit{in vivo} using blisters induced by prolonged, gentle suction, providing unique direct access to the cutaneous compartment.\textsuperscript{3,17} Using this method, we have studied separate clinical subgroups of ENL and lepromatous lesions without ENL to determine the following immunologic parameters within the skin: subsets of T cells, IgM antibody to \textit{M. leprae} phenolic glycolipid 1 and levels of soluble interleukin-2 receptor (Tac peptide).

\textbf{MATERIALS AND METHODS}

\textbf{Patients:}

Fifty-seven (57) patients with ENL (37 LL and 20 BL), 61 patients with active lepromatous leprosy and 33 cured control patients were recruited from among inpatients at McKean Rehabilitation Center, Chiang Mai. The latter three groups have been described in the first and second part of this series of articles.\textsuperscript{3,17} These included 26 BL and 35 LL newly diagnosed and untreated patients. The cured controls had no evidence of reactivation. These included 9 former LL, 2 former BL, 1 former BB, 12 former BT and 5 former TT patients. A representative lesion was biopsied in all patients with active disease and patients were classified by clinical and pathologic criteria according to the 5 part scale of Ridley and Jopling.\textsuperscript{18} Only 11 of 57 patients with ENL were female; the age distribution was also similar to that of patients without ENL (median age 34 years, range 15 to 74 years).

Careful clinical histories and physical examinations were performed for evaluation and daily follow up of reactions. The time of onset and prior duration of the reaction were determined as clearly as possible and studies commenced within 24 hours of admission. ENL can be rapidly controlled with corticosteroid and/or thalidomide. The latter is considered to be the most effective drug for ENL, despite its teratogenicity.\textsuperscript{19} When treatment with corticosteroid and/or thalidomide was initiated before or during the study, the precise timing of medication was recorded with respect to the time of collection of each laboratory specimen.

\textbf{Blister Induction:}

After obtaining informed consent from each volunteer, four nearly identical blisters were induced by gentle, continuous suction as previously described\textsuperscript{17} directly over a representative lesion or, in inactive patients, in clinically healthy skin. No skin test antigen or other material was injected. The fluid filled blisters were covered by an inverted plastic cap to prevent breakage and each was aspirated only once. To insure that results from blister fluids are reproducible, paired blisters were aspirated from several of the inactive patients, each aspirate being handled and assayed individually.

\textbf{Handling of Blister Fluids:}

Each blister was sampled only once at 24, 30, 48, 72 or 96 hours after induction. (In reproducibility studies with paired blisters, some samples were taken at 6, 12 and 18 hours). Fluid was aspirated into a heparinized 1.0 cc tubercul cin syringe; after measuring the volume in the syringe, it was filtered through a 13 mm, 0.22 micro polyvinylidine difluoride filter ("Durapore", Millipore Corp., Bedford, Mass.) The cell free material was flushed through the filter with RPMI 1640 tissue culture medium, and the first 1.0 cc of filtrate was divided into aliquots and frozen at -70°C until assayed.

\textbf{Cell Staining and Counting:}

Cells on the membrane were immediately fixed briefly in formol acetone and washed in PBS, and then divided into several segments to be stained with different antibodies.\textsuperscript{3} Monoclonal antibodies Leu 3 or T4 and Leu 2 or T8 were employed to identify subpopulations of lymphocyte bearing CD3, CD4 and CD8 receptors respectively. An indirect immunoperoxidase method was used as described.\textsuperscript{17} Briefly, the secondary antibody was biotinylated sheep anti-mouse Fab' fragment, followed by a horse radish peroxidase-avidin-biotin complex (Vector Laboratories, Inc, Buringame, CA) with H$_2$O$_2$ and aminoethyl carbazole.

\textbf{Assay for PGL:}

IgM antibodies against synthetic disaccharide and trisaccharide antigens of \textit{M. leprae} phenolic glycolipid 1 conjugated to BSA (ND-O-BSA and NT-O-BSA, respectively) were used in a standard ELISA assay.\textsuperscript{20} Optical density at 492 nm, and OD = 0.11 was determined to be the cutoff for "positive" results in the assay.

\textbf{Assay for Tac Peptide:}

Tac peptide levels were determined using an ELISA employing monoclonal antibodies anti-Tac and 7G7/B6, recognizing different epitopes of IL-2R, as previously described.\textsuperscript{21} Briefly, 100 \mu l aliquots of three dilutions of each blister fluid were placed in wells of microtiter plates coated with a 4/1000 dilution of FITC-conjugated 7G7/B6, followed by alkaline phosphatase conjugated rabbit anti-FITC, and by the substrate, p-nitrophenyl phosphate (1 mg/ml; Sigma Chemical Co., St Louis, Missouri, USA), with thorough washing between each step. Absorbance was determined at 405 nm in an automated 96-well ELISA reader. Serial dilutions of a standardized IL-2 containing supernatant were used to define a reference curve, for which the undiluted supernatant was assigned a maximum value of 1000 units/ml. The units (U) of Tac peptide in the test samples were calculated from this reference curve.
Total Protein Measurements:

The Coomassie blue method was used to measure total protein in blister fluids and matched serum samples. All results were expressed as mg protein per ml fluid; for each blister, this was calculated by multiplying the test result by the dilution factor for that sample.

Calculation of a Tac Peptide/Protein Index:

To differentiate the local production of IgM anti-PGL-I and Tac peptide in the skin from IgM anti-PGL-I and Tac peptide which entered a blister by filtration from the plasma, we calculated a "blister IgM index" and a "blister Tac index" modeled after the cerebrospinal fluid immunoglobulin index widely used to identify local production of IgG in cerebrospinal fluid. From the Tac peptide (U/ml) and total protein (mg/ml) concentrations in blister fluids and matched sera, the blister Tac protein ratio was divided by the serum Tac/protein ratio as follows:

Blister Tac Index = \frac{\text{Tac Peptide/Blister} \times \text{Protein/Serum}}{\text{Protein/Blister} \times \text{Tac Peptide/Serum}}

Statistical Analysis:

Differences in ELISA values between paired blister samples taken from each subject within a given group were analyzed by a paired Student's t-test. Parametric (Student's t-test) and non-parametric (Wilcoxon) tests were used to compare ranked data from different groups of patients. Correlation coefficients were calculated according to the method of Pearson.

RESULTS

Clinical Groups

The details of each group are shown in Table 1.

Group 1.

Lepromatous leprosy patients without reaction (BL and LL). Of 61 patients who did not have reaction at the time of examination, 35 patients were classified as lepromatous (LL) and 26 as borderline (BL) leprosy patients. Most of these were receiving anti-M.leprae treatment at the time of study. The age groups of these patients was 18-71 years with a median age of 36 years.

Group 2.

Acute erythema nodosum leprosum. Nineteen (19) out of 57 lepromatous patients with ENL were classified as an acute group according to the pattern of reaction, with an abrupt onset, subsiding within seven to ten days after steroid treatment. No further episodes of reaction were observed during follow up of one to two years. The age range of this group was 15-74 years with a median age of 32 years.

Group 3.

Chronic or recurrent erythema nodosum leprosum. Of 57 lepromatous patients with ENL, 38 presented with intermittently waxing and waning episodes of ENL. Reac-

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Table 1. Age Distribution and Steroid Responsiveness by Clinical Groups in BL/LL, Acute and Chronic ENL.

<table>
<thead>
<tr>
<th>Clinical Group</th>
<th>No. of Blister Studies</th>
<th>No. of Cases</th>
<th>Age Range (Yrs)</th>
<th>Median Age (Yrs)</th>
<th>Response to Steroids</th>
<th>Relapse When Steroid Stopped of Tapered Off</th>
<th>Duration of MDT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lepromatous</td>
<td>BL 26</td>
<td>26</td>
<td>18-71</td>
<td>36</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LL 35</td>
<td>35</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute ENL</td>
<td>BL 7</td>
<td>7</td>
<td>15-74</td>
<td>32</td>
<td>Yes</td>
<td>No 60%</td>
<td>40%</td>
</tr>
<tr>
<td></td>
<td>LL 12</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic ENL</td>
<td>BL 33</td>
<td>13</td>
<td>15-54</td>
<td>34</td>
<td>Yes</td>
<td>Yes 68.75%</td>
<td>31.25%</td>
</tr>
<tr>
<td></td>
<td>LL 60</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*No role of steroids in this group.
tions flared up in a few days when corticosteroids were discontinued or tapered off. Eight (8) patients in this group started with the reactional pattern of acute ENL and then developed recurrent episodes of ENL within one to seven months after the last dose of steroid. Their laboratory results were similar to those of group three (chronic ENL) and have been included in this group. Their age range was similar to that of the previous groups with a median age of 34 years. No significant differences in the median age were observed among the groups.

The duration of multiple drug therapy showed no correlation with acute or chronic groups of ENL as shown by approximately the same percentages among each group (Table 1).

The severity of reaction was classified into three groups: (a) mild = ENL nodules < 15 without systemic involvement; (b) moderate = generalized ENL nodules without systemic involvement; (c) severe = generalized ENL nodules with systemic involvement, e.g., orchitis, uveitis or pustular eruption. The percentages of acute and chronic ENL patients which fell into mild (61.9% vs. 61.1%), moderate (23.8% vs. 24.4%) and severe (14.29% vs. 13.33%) groups were not significantly different.

**Lymphocyte Subsets in Lesions with ENL**

All patients studied during acute and chronic recurrent ENL in this series were classified BL or LL and the results have therefore been compared to lesions in 37 BL and LL patients without ENL. The T-helper suppressor ratio (CD4+ : CD8+) was higher in lesions with chronic, recurrent ENL than in lesions with acute ENL, median values being 2.05 and 1.3 respectively (p = 0.1718). This ratio was also higher in lesions with chronic ENL compared to lesions without ENL, median values being 2.05 and 1.2 respectively (p = 0.0340) (Fig. 1). The absolute count of CD4+ cells showed no difference in non-reactional lesions compared to acute or chronic ENL (Fig. 2). Significantly, the absolute count of CD8+ cells was lower in lesions with chronic ENL than in lesions with acute ENL (p = 0.0292) and with uncomplicated lepromatous leprosy (p = 0.1772) (Fig. 3).

**Correlation of CD4+ lymphocyte with Effects of Thalidomide and/or Prednisone**

Analyses of the absolute count of CD4+ lymphocytes were performed on blister of 9 acute and 10 chronic ENL lesions prior to thalidomide and/or prednisone treatment compared to the results on 24 episodes of ENL with prednisone and thalidomide. Prior to these treatments we found no difference in the absolute count of CD4 among uncomplicated, acute and chronic ENL, as stated above. Interestingly, after prednisone treatment the absolute count of CD4 was lower in chronic ENL than untreated lesions (p = 0.05) while the absolute count of CD4 was not significantly different in the groups of chronic ENL with thalidomide and thalidomide plus prednisone than in untreated lesions (p = 0.9431 and p = 0.7210 respectively). Among
Fig. 2 Absolute numbers of CD4+ cells in lesions of non-reacting LL/BL, acute and chronic ENL. Lymphocytes were collected and stained as described (Fig. 1 and text). Number of CD4+ cells per ml were calculated from the total number counted and the volume of blister fluid recovered. Each point represents one blister; bars indicate median values.

Fig. 3 Absolute numbers of CD8+ cells in lesions of non-reacting LL/BL, acute and chronic ENL. Staining and counting were performed as described. Each point represents one blister; bars indicate median values.
Fig. 4 Absolute numbers of CD4$^+$ lymphocytes in chronic ENL with prednisone and/or thalidomide treatment. Each point represents one blister; bars indicate median values. CD4$^+$ lymphocytes in chronic ENL lesions after prednisone treatment was lower than those prior to treatment ($p = 0.05$). $P =$ prednisone; $T =$ thalidomide.

Fig. 5 Absolute numbers of CD8$^+$ lymphocytes in chronic ENL with prednisone and/or thalidomide treatment. Each point represents results from one blister; bars indicate results from one blister. Absolute numbers of CD8$^+$ lymphocytes in chronic ENL prior to and after treatment showed no statistical difference.
Fig. 6  Cutaneous Tac peptide levels in skin blisters from cured, non-reacting LL/BL, reacting acute and chronic ENL. Results shown are from samples aspirated 48 hrs after blister induction. "Cured" refers to fully treated, inactive patients. Each point represents one blister; bars indicate median levels.

Fig. 7  Tac peptide levels in chronic ENL with prednisone and/or thalidomide treatment. Each point represents Tac peptide level measured in one blister; bars indicate median values. Tac peptide levels in chronic ENL prior to and after treatment showed no statistical difference.
Fig. 8  Cutaneous IgM anti-PGL-1 levels in skin blisters from cured, non-reacting LL/BL and BT/TT. Results shown are from samples aspirated 48 hrs after blister induction. "Cured" refers to fully treated, inactive patients; each point represents IgM anti-PGL-1 level (optical density at 492 nm) in one blister; bars indicate median levels.

Fig. 9  Cutaneous IgM anti-PGL-1 levels in lesions with non-reacting LL/BL, new cases of ENL (under MDT treatment for less than one year) and old cases of ENL (under MDT treatment for more than one year). Each point represents one blister; bars indicate median levels. IgM anti-PGL-1 levels in new ENL patients were higher than those in old ENL cases (p = 0.0011).
groups of ENL with prednisone and/or thalidomide, the absolute count of CD4 was lower in ENL with prednisone than ENL with thalidomide and as compared to ENL with thalidomide plus prednisone (p = 0.0572 and p = 0.1031 respectively) (Fig. 4). We also found a decrease in the absolute count of CD4 in acute ENL after prednisone treatment (data not shown).

**Correlation of CD8⁺ Lymphocyte Effects of Thalidomide and/or Prednisone**

Similarly, we found that treatment with prednisone and/or thalidomide did not change the absolute count of CD8⁺ lymphocytes in acute or chronic ENL, as compared to the untreated lesions. Among groups of chronic ENL with prednisone and/or thalidomide there are also no significant differences in percentages of CD8 (Fig. 5).

**Correlation of Tac Peptide with Effects of Thalidomide and/or Prednisone**

We have studied effects of thalidomide and/or prednisone only in the chronic group of ENL which showed elevated levels of Tac peptide prior to treatment. We found an insignificant reduction in Tac peptide levels after treatment with prednisone and/or thalidomide (Fig. 6, 7).

**Cutaneous Tac Peptide Levels in Active and Inactive Leprosy**

In blisters induced on the skin of cured, inactive patients, median levels of Tac peptide was 684 U/ml (Fig. 6). No statistically significant difference was observed between immunopathologic types of leprosy at any time studied. Tac peptide levels remained relatively constant over the study period in most patients. When elevations were observed, they tended to occur 48-72 hours after treatment.
### Table 2. Tac Peptide Index Protein and Tac Peptide in Blister Fluids and Sera of Chronic ENL Prior to Steroid and/or Thalidomide Treatment.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Type</th>
<th>Reaction</th>
<th>Tac Peptide Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Serum (U/ml)</td>
</tr>
<tr>
<td>20/5/30</td>
<td>LL</td>
<td>ENL</td>
<td>684</td>
</tr>
<tr>
<td>38/1/24</td>
<td>LL</td>
<td>ENL</td>
<td>—</td>
</tr>
<tr>
<td>56/1/30</td>
<td>BL</td>
<td>ENL</td>
<td>—</td>
</tr>
<tr>
<td>63/1/30</td>
<td>BL</td>
<td>ENL</td>
<td>1578</td>
</tr>
<tr>
<td>63/2/30</td>
<td>BL</td>
<td>ENL</td>
<td>2173</td>
</tr>
<tr>
<td>67/2/48</td>
<td>LL</td>
<td>ENL</td>
<td>724</td>
</tr>
<tr>
<td>93/1/30</td>
<td>LL</td>
<td>ENL</td>
<td>1829</td>
</tr>
<tr>
<td>67/3/30</td>
<td>LL</td>
<td>ENL</td>
<td>807</td>
</tr>
<tr>
<td>56/3/30</td>
<td>BL</td>
<td>ENL</td>
<td>618</td>
</tr>
<tr>
<td>56/3/96</td>
<td>BL</td>
<td>ENL</td>
<td>535</td>
</tr>
<tr>
<td>93/3/30</td>
<td>LL</td>
<td>ENL</td>
<td>1335</td>
</tr>
<tr>
<td>38/5/30</td>
<td>LL</td>
<td>ENL</td>
<td>1505</td>
</tr>
<tr>
<td>56/4/30</td>
<td>BL</td>
<td>ENL</td>
<td>1746</td>
</tr>
</tbody>
</table>

### Table 3. IgM Anti−PGL−1 Index Protein and IgM Anti−PGL−1 in Blister Fluids and Serum.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Type</th>
<th>Reaction</th>
<th>IgM Anti−M. leprae Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Serum (OD492)</td>
</tr>
<tr>
<td>295/1/30</td>
<td>LL</td>
<td>non</td>
<td>0.517</td>
</tr>
<tr>
<td>296/1/30</td>
<td>BL</td>
<td>non</td>
<td>0.247</td>
</tr>
<tr>
<td>309/1/30</td>
<td>BL</td>
<td>non</td>
<td>0.050</td>
</tr>
<tr>
<td>314/1/30</td>
<td>BL</td>
<td>non</td>
<td>0.187</td>
</tr>
<tr>
<td>329/1/30</td>
<td>BL</td>
<td>non</td>
<td>0.088</td>
</tr>
<tr>
<td>332/1/96</td>
<td>LL</td>
<td>non</td>
<td>0.773</td>
</tr>
<tr>
<td>346/1/96</td>
<td>LL</td>
<td>non</td>
<td>0.134</td>
</tr>
<tr>
<td>347/1/96</td>
<td>BL</td>
<td>non</td>
<td>1.872</td>
</tr>
<tr>
<td>359/1/96</td>
<td>BL</td>
<td>non</td>
<td>0.027</td>
</tr>
<tr>
<td>358/1/30</td>
<td>BL</td>
<td>non</td>
<td>0.113</td>
</tr>
<tr>
<td>365/1/48</td>
<td>LL</td>
<td>non</td>
<td>1.501</td>
</tr>
<tr>
<td>226/2/30</td>
<td>LL</td>
<td>ENL,A</td>
<td>0.670</td>
</tr>
<tr>
<td>226/2/72</td>
<td>LL</td>
<td>ENL,A</td>
<td>0.695</td>
</tr>
<tr>
<td>307/1/30</td>
<td>LL</td>
<td>ENL,C</td>
<td>1.775</td>
</tr>
<tr>
<td>378/2/48</td>
<td>LL</td>
<td>ENL,C</td>
<td>0.340</td>
</tr>
<tr>
<td>378/2/72</td>
<td>LL</td>
<td>ENL,C</td>
<td>0.343</td>
</tr>
<tr>
<td>283/5/48</td>
<td>LL</td>
<td>ENL,C</td>
<td>0.063</td>
</tr>
<tr>
<td>283/5/48</td>
<td>LL</td>
<td>ENL,C</td>
<td>0.063</td>
</tr>
</tbody>
</table>

A = Acute Reaction.
C = Chronic ENL.
blisters over active lesions, primarily reflecting higher values in a small number of BT patients (data not shown).

**Cutaneous Tac Peptide Levels in Lesions of ENL**

The levels of Tac peptide in 13 episodes from chronic ENL were higher (median = 2590 U/ml) than those seen in uncomplicated lesions (p = 0.0235) while the levels of Tac peptide in acute ENL revealed insignificant difference compared to non-reactions (Fig. 6). The results obtained by using the "index" calculation (Table 2) confirm that Tac peptide was actually increased within skin lesions of chronic ENL compared to the blood of the same patients. Analyses showed no correlation between the low or high levels of Tac peptide and the patients' age, sex, classification, treatment or duration of active leprosy.

**Cutaneous IgM anti-PGL-1 Levels in Active and Inactive Leprosy**

In blisters induced on the skin of cured, inactive patients, median levels of IgM anti-PGL-1 varied from 0.0013 (OD492) (Fig. 8). No significant difference was observed between these individuals who had recovered from different types of leprosy.

In blisters induced directly over active lesions (without any reaction), the levels of IgM anti-PGL-1 in BL/LL patients varied from 0.00-1.245 while the levels on BT/TT patients varied from 0.0-0.130 (OD492). The median level of anti-PG IgM in BL/LL lesions was significantly higher than in BT/TT (p < 0.0001) and inactive lesions (p < 0.0001).

There was no tendency for peak levels of IgM in any certain period of time after blister induction. No correlation has been found between the low or high levels of anti-PG IgM and the patients' age and sex but they do correlate with classification as stated above.

**Cutaneous IgM anti-PGL-1 Levels in Lesions in ENL**

In 11 episodes of acute and 18 episodes of chronic ENL, the levels of anti-PG IgM in blisters over the chronic reacting lesions were higher (median OD = 0.5875) than those seen in acute ENL (median = 0.1700) (p = 0.0032) and those seen in non-ENL (median = 0.0071) (Fig. 10). There were no peak levels of anti-PG IgM observed at any period of time after blister induction. By using "index" calculation (Table 3) we found no increase in local production of IgM.

The low or high levels of anti-PG IgM were inversely correlated with duration of treatment. We found higher anti-PG IgM levels in new patients under MDT treatment less than one year than old patients who were under MDT treatment for more than one year (p = 0.0011) (Fig. 9).

**Correlation of anti-PG IgM with Effects of Thalidomide and/or Prednisone**

Preliminary data concerning the effects of thalidomide and/or prednisone treatment on acute and chronic ENL are presented in Fig. 10. Treatment with thalidomide and/or prednisone did not appreciably alter the anti-PG IgM levels in acute ENL. In contrast the level of anti-PG IgM in chronic ENL was significantly lowered by prednisone (p = 0.0000) or thalidomide (p = 0.0046) than those seen in chronic ENL without prednisone or thalidomide treatment.

**DISCUSSION**

ENL occurs in lepromatous leprosy, and although its precise nature is still debatable, several factors are known to precipitate the conditions. Several facets of the immunological basis of the type II (ENL) reaction, in particular, remain unknown. We sought to evaluate cell-mediated immune responses in ENL studying changes in T cell subsets, Tac peptide and correlating changes in IgM antibody against Mycobacterium leprae derived phenolic glycolipid 1 (PGL-1).

Using monoclonal antibodies to identify suppressor and helper T cells, Bach, Wallach, and Modlin found reduced helper, increased suppressor T cell percentages with reduced helper/suppressor ratio in lepromatous patients, whereas in patients with ENL, they observed increased helper/suppressor ratio. Van Voorhis and Rea found no difference between helper and suppressor cell percentages and helper/suppressor ratio in ENL patients and other lepromatous patients. Thus there are contradictory reports on the number of helper and suppressor T phenotypes. No reports however have studied separated clinical subgroups of ENL.

Our results on blister studies in non-reactional lepromatous and lepromatous patients with acute ENL revealed no difference in the helper/suppressor ratio, but the ratio did increase in chronic recurrent subgroups of ENL (p < 0.05). Besides an increasing the helper/suppressor ratio, we also found that the absolute count of suppressor T cells was lower in chronic, recurrent ENL than in acute ENL (p < 0.02) or in non-reactional leprosy (p = 0.17). Although the absolute count of CD4 was not altered in chronic ENL patients, an elevated T helper/suppressor ratio with decreased CD8+ cells indicated elevation of helper activity in chronic ENL.

We found anti-PG IgM levels increased in blister fluid from the tuberculoid (TT) to the lepromatous (LL) pole of the spectrum. This contrasts with other reports concerning serum levels which do not show antibody in tuberculoid patients. New patients with ENL (under MDT treatment < 1 year) had significantly higher levels of anti-PG IgM in blister fluid than non-ENL (p = 0.0256).
and old patients with ENL under MDT treatment (p = 0.0011).
The increased levels of anti-PG IgM in those cases correlated with the bacterial index. In the group of new ENL patients that later were classified into acute and chronic ENL, higher levels of anti-PG IgM were found in blister fluids of chronic ENL than in those with acute ENL (p = 0.0032) and in non-ENL patients (p = 0.0071) with comparable BI and duration of MDT treatment. There was no correlation of severity of reaction with acute or chronic ENL. Regarding anti-PG antibodies, we have no proof that they are implicated in the pathogenesis of ENL. This antigen is a technically useful marker, but our data only shows an association and implies no causation.

The increased levels of anti-PG IgM in chronic ENL might reflect bacillary persistence or the slow elimination of the insoluble glycolipid antigen, so called "antigenic persistence". The reasons for bacillary persistence are currently poorly understood. Subpopulations of Schwann cells or macrophages may harbor antibiotic-sensitive bacilli, although in selected individuals antibiotic distribution pattern or other mechanisms such as genetics, may be involved. Existing methods for monitoring bacillary persistence in leprosy are cumbersome. The slow elimination of the glycolipid antigen may require introducing the term "antigenic persistence" as well as "bacillary persistence". An elevated anti-PG IgM could indicate both in some instances and only antigenic persistence in others.

ENL has been attributed to a possible Arthus hypersensitivity reaction, induced by immune complexes. The demonstration of IgM, but not IgG in ENL skin biopsies as well as the inhibition of IgM synthesis by thalidomide and also steroid as shown in our study, the effective treatment for ENL, suggest that IgM may play a major role in the pathogenesis of ENL. Our serial blister studies of lepromatous patients who developed ENL during the course of study showed significantly enhanced cellular immune responses by increasing Tac peptide in the lesions of chronic ENL as compared to non-reactional and acute ENL lesions (p = 0.0235 and p = 0.0317 respectively). Modlin et al. have also shown an increase in putative interleukin 2 producing cells during ENL by immunocytochemistry. The expression of IL-2R in cell membranes in vitro is accompanied by the release into the culture supernatant of soluble Tac peptide. The findings are consistent with the hypothesis that T-cell function and probably T cell products as well as immune complexes may be involved in the pathogenesis of ENL. The observation that the total protein filtered into blisters from non-reacting and reacting lesions is very similar, while blister Tac peptide levels in chronic ENL are much higher than circulating levels, provides strong evidence of the local, cutaneous origin of the Tac peptide found in blisters. Conversely, the elevated levels of IgM anti-PGL-1 both in blisters and sera of chronic ENL seemed to represent simple filtration of the antibodies from the peripheral blood, as examined in more detail in a companion study (Scollard DM, et al, Unpublished data).

The findings of significant decrease of anti-PG IgM but insignificant reduction of Tac peptide levels after prednisone treatment could explain why the patients with chronic ENL had recurrent episodes of ENL when corticosteroids were tapered or stopped. Inhibition of anti-PG IgM production after steroid with or without thalidomide in our result might only reflect suppression of the inflammatory process in ENL (manifestations), without effective correction of the immunologic basis of ENL including T cell activation. T cell products may keep triggering B cell function with IgM and/or immune complex production. Uyemura et al. reported a beneficial therapeutic effect of cyclosporine A in ENL associated with increased T suppressor cells in lesions, which is consistent with our findings and hypotheses.

This report has expanded the information concerning sequential changes of immune status from lepromatous state to acute and chronic, recurrent ENL in leprosy patients and provides us with an understanding of the pathogenesis of observed differences in cell mediated and humoral immune responses that exist in leprosy and its reactions. These findings will also be helpful in guiding the therapy of chronic, recurrent ENL which is the major problem of leprosy reactions.

The non-invasive blister technique might be useful in monitoring immune status and treatment of patients with ENL reactions. Moreover, it might be feasible to adapt these methods and findings to fluids obtained from skin smears, already routinely collected in leprosy treatment programs worldwide. Leprosy health care workers would then be able to obtain a great deal more information in a simple manner about the patients' disease and prognosis from samples already obtained.

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