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Research Institute For Health Sciences (RIHES)
The Center For Public Health Research
Chiang Mai University
PO Box 80 CMU, Chiang Mai 50200, Thailand
Telephone: [053] 221966 • Facsimile: 66 53 221849

# FINAL REPORT

Covering Period: 01.07.88 to 30.06.92

Submitted to the Office of the Science Advisor
US Agency for International Development

**Development of an immunodiagnostic "spot" field test for acute reactions in leprosy**

**Project Number: 8.183**

Principal Investigator: Choti Theetranont, MD
Co-Principal Investigator: Lertlakana Bhoopat, MD

Grantee Institution: Research Institute for Health Sciences (RIHES)
The Center for Public Health Research
Chiang Mai University

Collaborator: David M. Scollard, MD, PhD
Department of Pathology
University of Hawaii

Project Number: 936-5542-G-00-8046-00

AID Project Officer: Jaroon Kamnuanta, PhD

Project Duration: 01.07.88 to 30.06.92

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**Executive Summary**

The work reported herein continued earlier research activities proposed and funded by the USAID (PSTC), namely, "Development of an immunoperoxidase test for early diagnosis of acute reactional states in leprosy (grant no. 936-5542-G-00-5044-00 & SCI Research Proposal 5.141, 22.04.85 to 30.11.87). Thus we will take this opportunity to summarize the work on both projects.

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. 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.

Reversal reactions (RR) and Erythema Nodosum Leprosum (ENL), the two different types (Type I and Type II respectively) of leprosy reactions, are major complications with high risks of serious long-term disability. The differences observed between reversal reactions and ENL can be conceptualized as representing fundamentally different dynamic events occurring in the lesions of patients.

In the work specifically undertaken in this project, we have examined cutaneous leprosy lesions *in vivo* using blisters induced by prolonged, gentle suction, providing unique direct access to the cutaneous compartment. 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 *M. leprae* phenolic glycolipid 1 and levels of soluble interleukin-2 receptor (Tac peptide).

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.

This project - and the previous one mentioned above, together with support for ancillary research activities received from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (WHO/TDR) from 1985 to 1990 - has considerably strengthened leprosy research work at Chiang Mai University. For example, an immunopathology laboratory has been established at RIHES with a strong link to the clinical facilities and laboratories in the Department of Pathology of the Faculty of Medicine, and the strengthening of both the human and physical resources in the RIHES immunopathology laboratory are being carried forward in newly funded research on the interaction between HIV and leprosy.

In addition, an important research technique, namely the development of a suction-induced skin blister as a means of collecting tissue fluid for a study of cell mediated immunity in leprosy, has been established, thanks to a technology transfer from the laboratory of our collaborator, Dr D M Scollard. Lastly, RIHES has strengthened its expertise in routine and/or specific histochemical and immunohistochemical staining of specimens obtained from patients in leprosy research projects.

**Objectives**

i) To measure the intracutaneous levels of *M. leprae* antibodies and of soluble interleukin-2 receptors (IL-2R) in leprosy patients at high risk for the development of Erythema Nodosum Leprosum (ENL) and "reversal reactions" (RR) respectively

ii) To improve methods of predicting and monitoring reactions by measuring *M. leprae* antibodies and soluble IL-2R in intradermal fluid obtained routinely in leprosy patients from slit-skin smears.

**Methods**

Acute leprosy reactions were studied in a population of 312 patients with active leprosy from September 1984 to September 192. Of these. 137 (44%) developed one or more episodes of either Type I or Type II reaction or both (5 patients).

During this period 176 new, untreated patients, first seen between September 1984 and December 1989, were followed until September 1992 (minimum follow up of 3.25 years) in a longitudinal study of the clinical epidemiology of reactions (Table 1). Of these, 83 patients (47%) developed one or more reactions; 43 developed type II (ENL) and 35 type I ("reversal") reactions; 5 had both (Figure 1).

Overall ascertainment of this cohort was 45% (176/388) of all new, untreated patients registered at the McKean Rehabilitation Center (the major leprosarium in the north of Thailand). The study was biased toward multibacillary patients (LL-BB), due to the greater likelihood of admission in this group and a reluctance of out-patients to agree to the laboratory studies. Thus, ascertainment of multibacillary patients was 78%, but only 33% for BT patients. (TT patients was excluded from this evaluation since they are not thought to be at risk for either reaction, and although a few were enrolled, they were not actively sought during the study). The incidence and risk of Type I reactions in BT patients is therefore probably underestimated in this study.

Distribution of patients in this cohort by classification is shown in Table 1. The Distribution shows the preponderance of multibacillary patients well described in this population, in addition to the bias in this study.

The distribution of reactions among all patients in this study is shown in Table 2.

The incidence of reaction in the cohort of new patients is shown in Table 3. Thirty-four percent (34%) of patients "at risk" for ENL (i.e., multibacillary patients) developed ENL, and 229% of patients at risk for reversal reactions (i.e., borderline patients, BL-BB-BT) developed this reaction.

The WHO recommended multi-drug treatment (MDT) appears to have had little effect on the occurrence of reactions (Table 5) when contemporary patients who have reactions on admission (i.e., before treatment) are compared to patients experiencing reactions only after starting treatment. No difference is seen in these 2 groups in patients only after starting MDT and in 11% of patients on admission. There is no evidence from these data to indicate that the treatment *per se* initiates or precipitates ENL, but also no evidence that the treatment "protects" patients from ENL.

The recurrence of these two reactions follows very different patterns. Patients tend to have only one, sometimes two Type I reactions, while ENL typically recurs more than four times.

The strongest asscoaition with development of a reaction was found in evaluating the age of onset of leprosy of partients who develop ENL (not the age of the patient at the tiome of the reaction) (Table 4). Those whose first symptom occurred during adolsecence had a much higher risk than patients whose onset of leprosy symptoms was later. This is highly significant when assessed as a single variable (P <0.0000?). No such correlations are seen with Type I reactions.

The various time points in the course of a patient's illness were compiled as follows:

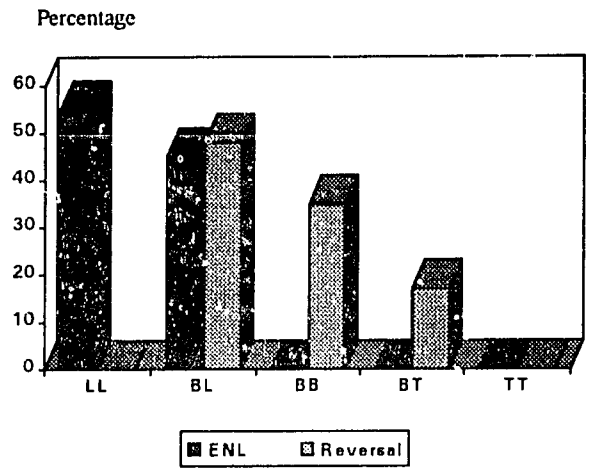
| | | | | | | |
|---|---|---|---|---|---|---|
| Age at onset of leprosy | + Duration of symptoms | = AGE of patient on presentation; MDT started | + Duration of MDT | = Age at first reaction |

These points in time (and intervals) can be considered as possible independent variables; but thety are also related numerically, as AGE is the sum of Age Onset + Duration of Symptoms, and so forth.

**Figure 1**

**Incidence of Reactions in Leprosy**

**1986 to 1992**




Source: McKean Rehabilitation Center: Cohort N = 176

**Table 1**

**Distribution of New Patients by Class**

| Class | Male | Female | Total | (‰) | M:F |
|---|---|---|---|---|---|
| LL | 43 | 12 | 55 | (31) | 3.6 |
| BL | 46 | 18 | 64 | (36) | 2.6 |
| BB | 10 | 6 | 16 | (9) | 1.7 |
| BT | 25 | 6 | 31 | (18) | 4.2 |
| TT | 8 | 1 | 9 | (5) | 8.0 |
| Indeterminate | - | 1 | 1 | | |
| Total | 132 | 44 | 176 | | 3.0 |

**Table 2**

**Ascertainment (9/84 - 12/89)**

| | Total Patients | ‰ "MB" | ‰ |
|---|---|---|---|
| Study Group | 176 | 76 | 24 |
| McKean | 388 | 44 | 50 |
| Ascertainment | 45% | 78% | 19%[1] |

[1] Total does not equal 100% because of indeterminate and unclassified patients in McKean database.

**Table 3**

**Distribution of Reactions, All Patients**

**By Sex and Class**

| | Males | Females | Total |
|---|---|---|---|
| LL + ENL | 33 | 7 | 40 |
| BL + ENL | 12 | 6 | 18 |
| Sub-Total | 45 | 13 | 58 |
| (LL-)BL + RR | 18 | 12 | 30 |
| BB + RR | 8 | 3 | 11 |
| RT + RR | 4 | 1 | 5 |
| Sub-Total | 30 | 16 | 46 |
| LL-BL + Both Reactions | 4 | 1 | 5 |

**Table 4**

**Incidence of Reactions in New Patients**

**(entered 9/84 - 12/89; followed through 9/92)**

| Class & Reaction | Males | Females | Reaction Total | Class Total | ‰ |
|---|---|---|---|---|---|
| LL + ENL | 22 | 5 | 27 | 55 | 49% |
| BL + ENL | 19 | 4 | 13 | 64 | 20% |
| Sub-Total[2] | 41 | 9 | 40 | 119 | 34% |
| BL + RR | 14 | 9 | 23 | 64 | 36% |
| BB + RR | 2 | 3 | 5 | 16 | 31% |
| BT + RR | 3 | 1 | 4 | 31 | 13% |
| Sub-Total[3] | 19 | 13 | 32 | 111 | 29% |

[2] ENL in MB patients.

[3] RR in borderline patients.

**Table 5**

**Risk of Reactions According to Treatment**

| | Onset of Pre-MDT | | | Onset Only After MDT | | |
|---|---|---|---|---|---|---|
| Reaction | No Prior DDS | Prior DDS | Total | No Prior DDS | Prior DDS | Total |
| ENL | 8 | 5 | 13 | 20 | 7 | 27 |
| Total at Risk (LL - BL) | | | 119 = 11% | | | (119-13)[4] = 25% |
| RR | 18 | 4 | 22 | 14 | 6 | 20 |
| Total at Risk (BL,BB, BT) | | | 111 = 20% | | | (111-22) = 22% |

**Table 6**

**Risk of ENL by Age at Onset of Leprosy**

| Age at Onset Lep (decade) | ENL + | ENL - | Total at Risk | % ENL + |
|---|---|---|---|---|
| < 10 | 0 | 3 | 3 | 0 |
| 10 - 19 | 11 | 6 | 3 | 65% |
| 20 - 29 | 12 | 25 | 17 | 32% |
| 30 - 39 | 10 | 14 | 37 | 42% |
| 40 - 49 | 4 | 12 | 24 | 25% |
| 50 - 59 | 3 | 10 | 16 | 23% |
| > 59 | 0 | 9 | 9 | 0 |

[4] Individuals in "pre-MDT" and "on MDT" groups were mutually exclsuive. Those with reaction "pre-MDT", i.e., on admission, could not be counted in the "on MDT" group, and were therefore subtracted from the total at risk to provide the denominator.

**Table 7**

**Total Protein (O.D) in Slit Skin Smears Among Different Leprosy Groups**

| Groups of Leprosy Patients | Number Studied | Median |
|---|---|---|
| Inactive (BT-TT) | 32 | .13 |
| Inactive (BL-LL) | 52 | .16 |
| Active (BT-TT) with reaction | 22 | .17 |
| Active (BT-LL) with reaction | 68 | .21 |
| Acute ENL | 5 | .14* |
| Chronic ENL | 20 | .15 |
| RR (BL-LL) | 9 | .28* |

* = $p < 0.05$
Acute = patient experiences only one episode of ENL
Chronic = patient experiences > one episode of ENL

**Table 8**

**IgM (O.D.) in Slit Skin Smears Among Different Leprosy Patients**

| Groups of Leprosy Patients | Number in Study | Median |
|---|---|---|
| Inactive (BT-TT) | 11 | .001 |
| Inactive (BL-LL) | 4 | 0 |
| Active (BT-TT) with reaction | 7 | .003 |
| Active (BT-LL) with reaction | 19 | .007* |
| Acute ENL | 4 | .29* |
| Chronic ENL | 4 | .13* |
| RR (BL-LL) | 3 | .004 |

* = $p < 0.05$

**Table 9**

**Percentage of $CD_8$+ Cells in Slit Skin Smears Among Different Leprosy Groups**

| Groups of Leprosy Patients | Number Studied | Median |
|---|---|---|
| Inactive (BT-TT) | 7 | 5 |
| Inactive (BL-LL) | 10 | 5 |
| Active (BT-TT) with reaction | 16 | 4 |
| Active (BT-LL) with reaction | 57 | 6* |
| Acute ENL | 4 | 11 |
| Chronic ENL | 11 | 38* |
| RR. (BL-LL) | 5 | 9 |

* = P >0.05

**Results**

The accomplishments of these two projects can best be demonstrated by listing the publications and current manuscripts which have arisen from the work:

1. Kenney RT, Rangdaeng S and Scollard DM. Skin Blister immunocytology: A new method to quantify cellular kinetics in vivo. J Immunol Methods 1987;97:101-110.

2. Scollard DM, Rangdaeng S, Suriyanon V, Chang P H, Theetranont C, Makonkawkeyoon S, Arnold R and Smith T. Increased numbers of epidermal IL-2 receptor-bearing cells characterize leprosy reversal reactions. Lab Invest 1987;56: 70 A.

3. Bhoopat L, Thamprasert K, Suriyanon V, Smith T, Scollard DM and Theetranont C. Evolution of in situ immune responses in clinical subgroups of erythema nodosum leprosum reactions of leprosy. Chiang Mai Med Bull 1988;27:331-341.

4. Scollard DM. A review of immunopathologic studies of human leprosy in situ. Hawaii Med Journal 1988;47(2):12-17.

5. Scollard DM, Wagner DK, and Nelson DL. Release of soluble interleukin-2 receoptors (Tac peptide) in vivo during human immune responses to tuberculin. Clin Immunol Immunopathol 1988;46:450-455.

6. Theetranont C, Scollard DM, Bhoopat L, Rangdaeng S, Suriyanon V, Thamprasert K, Makornkawkeyoon S and Smith T. Development of an immunoperoxidase test for early diagnosis of acute reactional state in leprosy. Chiang Mai Medical Bulletin 1988;27(2):127-151.

7. Bhoopat L, Thamprasert K, Suriyanon V, Smith T, Scollard DM and Theetranont C. Evolution of in situ immune responses in clinical subgroups of erythema nodosum leprosum reactions of leprosy. Chiang Mai Med Bull 1988;27:331-341.

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11. Scollard DM. Inside the skin: The local immune and inflammatory milieu in leprosy. Am J Trop Med Hyg 1991, 44(3):17-23 (supplement).

12. Scollard DM, Suriyanon V, Bhoopat L, Wagner DK, Smith T, Thamprasert K, Nelson DL and Theetranont C. Studies of human leprosy lesions in situ using suction-induced blisters. 2. Cell changes and soluble interleukin-2 receptors (Tac peptides) in reversal reactions. Int J Lep 1990;58:469-479.

13. Theetranont C, Bhoopat L, Scollard D M and Smith T. In situ antibodu level determination of skin fluids in human leprosy. Chiang Mai Med Bull 1991;30:141-151.

14. Bhoopat L, Scollard DM, Theetranont C, Chiewchanvit S, Nelson DL, and Utaipat U. 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. Asian Pacific Journal of Allergy and Immunology 1991;9:107-119.

15. Scollard DM, Bhoopat L, Kestens L, Vanham G, Douglas JT and Moad J. Immune complexes and antibody in blisters over human leprosy lesions with or without Erythema Nodosum Leprosum (ENL). Clin Immunol Immunopath 1992;230-236.

16. Scollard DM, Smith T, Bhoopat L, Theetranont C, Rangdaeng S, and Morens D. Clinical epidemiology of reactions in leprosy. 1993 (submitted for publication).

17. Utaipat U, Scollard D, Pattanapanyasat K, Bhoopat L and Theetranont C. FACS analysis of CD4+ T cell subsets in reacting and non-reacting leprosy skin lesions. 1993 (Submitted for publication).

END