

**Mycobacterial Research Lab,
Anandaban Hospital, Nepal
January - June 2006**

General introduction

The past few months have been frustrating in many ways: the significant political changes which took place in Nepal in the first half of the year are still working themselves out. Although we are pleased that there is less tension in country, the practical implications for the lab and the hospital of the changes which have taken place have been that there have been excessive delays in dealings with government departments. This is especially true with regard to the NHRC (Nepal Health Research Council- see below), and with Customs exemption arrangements for lab supplies. It is hoped that these problems will soon be resolved

Individual progress reports are below, but one interesting phenomenon which has been observed in the last few months has been the large number of relapse cases presenting to clinic: 7 clinically confirmed relapse cases have come in the first 6 months of the year, a much higher rate than usual. We plan to follow up these and a number of other patients who were highly smear positive (and therefore liable to relapse) in the next few months. The ability to perform the mouse foot pad assay adds an important dimension to these analyses: it is vital that TLM South Asia maintains this facility

Progress reports:

1. Skin test study:

This study, in collaboration with Prof. Patrick Brennan from Colorado State University, aims to test potential new skin tests in a leprosy endemic population. Parts A and B have previously been completed; we are awaiting permission to undertake part C, which in many ways will be the most interesting, as we will be testing household contacts of patients, as well as patients themselves. Because of concerns that some of these individuals may show ulceration when injected with the higher dose of the test antigens, Part C1 will involve testing on 20 individuals each from the following groups: household contacts, TT/ BT patients, BL/ LL patients and TB patients. A safety review will then take place: if any of the participants in part C1 show ulceration at either of the higher doses (10µg MLCwA or MLSA- LAM), this dose of antigen will be dropped from the remainder of the study

Funding from this study has allowed the purchase of a portable incubator, specifically to transport blood samples for use in the Quantiferon assay

As alluded to above, permission is still awaited from the NHRC; we are hopeful that all necessary permissions will be in place soon.

2. Methyl prednisolone study

This clinical study, in collaboration with Drs Diana Lockwood and Steve Walker, London School for Hygiene and Tropical Medicine, aims to study the use of high dose methyl prednisolone (MP) in the treatment of Type I reaction (TIR). A modified form of the severity scale employed in our previous study in TIR (see Trans R Soc Trop Med Hyg. 98, 602- 609 (2004)) is being used to assess the effectiveness of 3 days 1g of Methylprednisolone plus 20 weeks prednisolone, in comparison a 20 week prednisolone regimen.

A total of 60 patients will be recruited into this study: 30 will receive MP plus prednisolone, while 30 will receive prednisolone only. Due to the requirement that patients should have new nerve impairment at admission, recruitment has been slow, and 8 patients have so far been recruited for this study; they have been randomized as per the protocol. No adverse events have been observed. Dr Steve Walker and Dr Diana Lockwood, our collaborators on this project, plan to visit in September

3. Post- genomic diagnostics

This study, in collaboration with Drs Tom Ottenhoff and Annemiek Geluk, LUMC, the Netherlands, involves the drawing of blood from a total of 110 leprosy patients and controls. This will be incubated with identified antigens and peptides, and the IFN- γ responses to these will be measured. Funding through LUMC has allowed the purchase of an ELISA plate washer, which will be very useful in this and other studies. A number of such antigens have previously been studied by the LUMC group in patients in Brazil; the study in which we are involved will confirm and extend the findings of the Brazilian study.

Permission to undertake this study is currently being sought, and it is hoped that participant recruitment can begin soon

4. IDEAL

Anandaban has become a vital part of the IDEAL (Initiative for Diagnostic and Epidemiological Assays for Leprosy) consortium. This group, funded by the Heiser Institute, has been instrumental in bringing together researchers active in lab based leprosy studies throughout the world. The group has two main arms: the epidemiological and the diagnostic. The major practical involvement of the MRL is in the latter, although we remain in close contact with developments in the epidemiological field. Anandaban is one of the field sites chosen to undertake the first phase of testing of diagnostic antigens newly identified through the IDEAL consortium. This will again take advantage of the extensive experience which our staff have of using the WBA to assess IFN- γ responses

As part of the IDEAL study, we will also be undertaking a study to compare two lateral flow tests, independently developed by our colleagues in the Netherlands and Korea. These tests, designed to detect antibodies against PGL-I, have great potential for utility in the field

Progress in this study has also been delayed due to permission from the NHRC not yet having been obtained

5. Genetic susceptibility to leprosy reactions:

This study, in collaboration with Dr Gilla Kaplan (New York) and Dr Tom Hawn (Seattle) has resumed following a period of quiescence. Over 1000 blood samples have previously been collected from leprosy patients (with and without reaction (Type I or Type II)), and also from suitable control individuals. The analysis, which involves the PCR amplification of a number of loci, including the upstream region of the TNF- α gene, and subsequent sequence analysis, has already begun to indicate that variability in the region being studied exists in the population. Testing of the hypothesis, which proposes an association between such variability and the propensity for the patient to undergo reaction, will require most of the samples to be analysed successfully. Approximately 400 samples have previously been sent to the US for sequence analysis; the remainder should be shipped very soon

6. PCR detection of Rifampicin resistance in *M. leprae*:

This project involves the comparison of novel PCR based techniques to detect rifampicin resistant *M. leprae*: the Line Probe Assay (LiPA) derived by Prof. Stuart Cole in the Pasteur Institute in Paris, and a multiplex "ARMS" PCR system developed in our laboratory. The results obtained are being compared to the current "gold standard" for measuring drug resistance in leprosy, the mouse footpad (MFP) assay).

Technical difficulties have meant that the interpretation of some of the results obtained have been difficult. Dr Nadine Honoré (Pasteur, Paris) and some of our other collaborators have been assisting us in providing sequence analysis of the samples. It appears that, if rifampicin resistance in *M leprae* is conferred by mutation in the rpoB gene, as is the case in *M tuberculosis*, the mutation may lie outside the 81bp “rifampicin resistance determining region” identified in TB. This has implications for assays such as the LiPA assay, which are designed to detect mutations this region

7. TLR-2:

A B. Sc. Student from Murdoch University, Australia, undertook a small study into the reported association between a mutation of the Toll like receptor 2 (TLR2) gene and lepromatous leprosy in a Korean population. Using DNA samples from patients which were available in the lab, this was studied in the Nepali population; care was taken to avoid including the pseudogene identified by an Indian group in our analysis. Interestingly, the preliminary results from this project indicate that the Arg753Gln mutation in this gene may be common in the Nepali population- a result which concurs with observations in other populations. We are seeking to confirm these results by sequencing

8. Triggers of Type I reaction

A new proposal has been developed in collaboration with Dr Annemiek Geluk, LUMC, and Lal Gadh Leprosy Hospital (NLT, Janakpur) to study the cytokine triggers which are involved in type I reaction. It is proposed that approximately 300 patients will be followed for one year, in anticipation that one third will develop T1R in that period. A blood sample will be taken at enrollment, and also when a patient develops reaction. Cytokine responses elicited in response to various *M leprae* antigens will be measured using the Luminex system, which allows the simultaneous detection of a number of cytokines in the same sample

An application for funding for this project is currently lodged with The Order of Malta Grants for Leprosy Research (“MAGRALEPRE”)

Publications

Three manuscripts have been submitted for publication; abstracts will also be submitted to the International Symposium on New Frontiers in Tuberculosis Research, taking place in Delhi (December 4- 6th), which Mr. Ranjit and Mr. Sapkota will attend.

General comments:

Dr Murdo and Dr Rachel will be leaving TLM at the end of their next term- probably mid-2008; plans to replace Dr Murdo have been discussed with Mr. Daniel (TLM South Asia Director), Ms Nash (TLM International) and Prof Britton, as well as the local management

We are grateful for the ongoing support received from Leprosy Mission Nepal, TLM South Asia and TLM International. Among other things, their support has allowed the purchase of a gel documentation system and two new gel systems this year.

One of the ongoing frustrations for the lab has been the lack of internet access. We are very pleased that the proposal to install a satellite internet connection has been approved by Leprosy Mission Nepal, TLM South Asia and TLM International, and that TLM Scotland have agreed to fund this project. We hope that this system can be installed in the second half of the year

**Dr Murdo Macdonald,
Head, Mycobacterial Research Laboratory
March 17, 2007**

Project title	Aim	Collaborators	Target population
Skin test study	New diagnostics	Prof Patrick Brennan, Colorado State University, Fort Collins, Colorado 80523-1682 Patrick.Brennan@colostate.edu	Healthy Non-contacts 190 Household/ professional Contacts 140 Leprosy patients 147 Tuberculosis patients 48
Methyl prednisolone	Treatment of T1R	Dr Diana Lockwood Dr Steve Walker London School of Hygiene & Tropical Medicine, London WC1E 7HT diana.lockwood@lshtm.ac.uk drstevewalker@hotmail.com	60 Type I reaction patients
Post- genomic diagnostics	New diagnostics	Dr Annemiek Geluk Prof Tom Ottenhoff Leiden University Medical Center, 2333 ZA Leiden The Netherlands t.h.m.ottenhoff@lumc.nl A.Geluk@lumc.nl	Leprosy patients: 20 PB 20 MB 20 reactions patients Controls: 20 healthy household contacts of MB patients 20 endemic healthy controls 10 TB patients
IDEAL	New diagnostics/ epidemiology	Dr Linda Oskam, KIT (Royal Tropical Institute), 1105 AZ Amsterdam, The Netherlands l.oskam@kit.nl Dr Hazel Dockrell London School of Hygiene & Tropical Medicine, h.dockrell@lshtm.ac.uk	A. T cell responses Leprosy patients: 10 TT/ BT 10 BL/ LL Controls: 10 healthy household contacts 10 endemic healthy controls 10 TB patients B. Antibody responses 40 MB patients 40 PB patients 20 BB patients 50 Household contacts of MB patients 50 Endemic controls

Genetic susceptibility to reactions	Type I reaction	<p>Dr Gilla Kaplan, The Public Health Research Institute, Newark, New Jersey 07103-3535 kaplan@phri.org</p> <p>Dr Tom Hawn University of Washington, Seattle, Washington thawn@u.washington.edu</p>	800 leprosy patients with and without reaction 300 non- leprosy endemic controls
Rifampicin resistance in <i>M. leprae</i>	Drug resistance	<p>Dr Nadine Honoré Institut Pasteur, Paris, France. nhonore@pasteur.fr</p> <p>Prof Stuart Cole, Institut Pasteur, Paris, France. stcole@pasteur.fr</p>	All available MFP proven Rifampicin resistant strains of <i>M. leprae</i> , plus appropriate control (Rif sensitive) samples
TLR-2 mutation	Genetic susceptibility	<p>Prof Warwick Britton University of Sydney, NSW, 2006, Australia wbritton@med.usyd.edu.au</p> <p>Prof. Dr. med. Ralf R. Schumann Charité-Universitätsmedizin, 10117 Berlin, Germany ralf.schumann@charite.de</p>	120 leprosy patients 100 non- leprosy endemic controls

Triggers of Type I reaction	Type I reaction	Dr Annemiek Geluk Leiden University Medical Center, 2333 ZA Leiden The Netherlands A.Geluk@lumc.nl Lal Gadh Leprosy Hospital Lal Gadh, Janakpur Nepal damberlgh@mail.com.np dscoll1@lsu.edu	300 leprosy patients
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