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NEWS and NOTES

This department furnishes information concerning institutions, organizations, and individuals engaged in work on leprosy and other mycobacterial diseases, and makes note of scientific meetings and other matters of interest.

Notice. Several extra copies of the old issues of *The International Journal of Leprosy* are available from the business office. Due to a shortage of storage space, some of these must be discarded soon. If you wish to obtain any of these back issues of the JOURNAL, please contact Dr. Paul Saunderson by e-mail: psaunderson@leprosy.org.

Notice. *The International Journal of Leprosy* is now available on-line by visiting our website at http://www.leprosy-ila.org./ This provides the most convenient access to the JOURNAL on-line. You can also renew your membership, or join if you are not already a member of the ILA. The JOURNAL will accept submissions electronically, as well.

Academic Meeting at Kalyan. The Indian Association of Leprologists— Maharashtra Branch in collaboration with Bombay Leprosy Project organized a seminar on "Leprosy—from a Practicing Dermatologists Point of View" on Sunday 22.06.2003 at Kalyan.

This seminar was organized for the members of the "Kalyan-Dombivli Dermatologists Club," a newly formed local academic association.

Issues such as the Role of standard Prednisolone in management of reactions, Role of newer drugs like Cyclosporin, Pentoxyphylline and other useful drugs like Thalidomide availability to needy patients in managing chronic/recurrent reactions, as an alternative line of treatment were discussed at length. Dr. R. Ganapati and Dr. V. V. Pai were the Resource Persons.

Discussion on methods of recording the past treatment details of patients in a "Treatment Graph" experimented and prepared by BLP was also demonstrated. The objective of such innovative exercise was a scientific study of the treatment details given to patients either referred or institute cases, helpful in deciding a rationale management.

Clinically interesting cases (staying in Kalyan area) with recurrent type II reaction put on Thalidomide were also demonstrated and discussed. The seminar was sponsored by M/s Jansen's Cilag Pharmaceuticals Ltd.

The Editorial office received the following letter from the Leonard Wood Memorial.

TO: Friends of the Leonard Wood Memorial
Subject: New Scientific Director

I am most happy to inform you that effective September 1, 2003, Dr. Robert Gelber will officially join the Leonard Wood Memorial as its new Scientific Director.

As you may know, Dr. Gelber has been working in leprosy, both as a clinician and researcher, particularly in the field of chemotherapy, for almost four decades. During this time, he has published well over 100 articles and written major chapters in prestigious textbooks. He comes to us from his position of Clinical Professor at the University of California, San Francisco and also Senior attending physician of the TB control program at San Francisco General Hospital.

We are delighted to have hired someone who is highly qualified, both in the field of leprosy and tuberculosis. He is excited and enthusiastic about this position and we look forward to a rich and rewarding association with Dr. Gelber.

Please join us in welcoming him.

Sincerely,
August Zinsser III
President
LWM Board of Trustees

ILA GLOBAL PROJECT ON THE HISTORY OF LEPROSY

ACADEMIC NETWORK MINUTES OF INAUGURAL MEETING, SORIA MORIA CONFERENCE CENTER, OSLO

Friday, 5 September 2003

Present: Jo Robertson (chair), Jaime Benchimol, Harriet Deacon, Deborah Emmitt, Mark Harrison, George Joseph, Sanjiv Kakar, Simonne Horwitz, Anwei Law, Laurinda Maciel, John Manton, Renisa Mawani, Yara Monteiro, Chandi Nanda, Diana Obregón, Shubha Pandya, Biswamoy Pati, David Scollard, Magali Romero Sá

Apologies: Bernardino Fantini

1. THE PROJECT AND ITS INTERESTS

Jo Robertson summarized the Project's activities to date. The last funding period of twenty months ended in May 2003, and the Project has now entered a bridging stage of funding, provided by the Sasakawa Foundation, until future funding of a further three years is assured.

The main aims of the Project are, firstly, to build an online database of archives on leprosy, held in numerous locations throughout the world, and secondly, to establish and maintain a network of researchers who are working on different aspects of the history of leprosy. This network is expected to be self-perpetuating, that is, the members will maintain contact amongst themselves once the Project has made them aware of each other's existence through its website and activities.

Oral History

Anwei Law explained the oral history component, which will begin once further funding is assured. Oral history "makes history more rounded," as it is related by the people actually involved. When making oral histories, it is important to include families, and both younger and older generations as this establishes continuity. Guidelines on making oral histories will be developed, and other expressions produced by people who have been affected by leprosy will be identified, such as poetry, artwork and music. The idea is to develop a network that is dedicated to making oral history, as there are not enough resources for Anwei Law and her team to carry out all the recording themselves. She pointed out that anyone over the age of seventy is a "fragile resource," so their identification and oral history will be a priority.

2. INDIVIDUAL RESEARCH AREAS

This item on the agenda was postponed, but Jo Robertson pointed out that many members' research interests are described on the Academic Network page of the Project website.

3. RESEARCH TOPICS OF INTEREST FROM THE POINT OF VIEW OF PEOPLE IN THE FIELD

David Scollard, as Editor of the *International Journal of Leprosy and Other Mycobacterial Diseases*, talked about the submission of articles by members of the network. The journal is a bio-science publication, but has always accepted the occasional article of historical interest. There is a commitment to support the history of leprosy, and submitted papers from social science disciplines will be reviewed by appropriate social scientists. Readers of the journal, mainly leprosy doctors and researchers around the world, must see the articles as useful. David Scollard invited members of the leprosy history network to submit articles of historic interest and to ask themselves how their submission is useful in the field, how it could help medical workers, scientists, and others.

4. RESEARCH OPPORTUNITIES

Mark Harrison explained how he intends to make the history of leprosy one of the main areas of research at the Wellcome Unit, Oxford, and will invite applications for this. He will be putting in research proposals to the Grants Committee—at least one large one, or maybe two smaller ones. In addition, individual research applications can also be made to the Wellcome Trust, from academics from the European Economic area. Wellcome collaborative grants make available a relatively small amount of money to develop specific research projects, in order to facilitate travel between the

two units for meetings and conferences, as well as the employment of researchers. Mark Harrison invited suggestions for collaborative projects. Biswamoy Pati asked whether Ph.D. students could apply, Mark replied that there was nothing in the rubric against it, and that all the basic details concerning this can be found on the Wellcome Unit website.

5. STRATEGIES FOR THE FUTURE OF THE NETWORK

Jo Robertson outlined three main strategies. Firstly, an electronic discussion forum will be set up, the importance of which was made clear by the recent pre-conference exchange of emails, mainly concerning use of terminology in historical articles. Secondly, suggestions on papers to be submitted to history of medicine conferences are welcome. Thirdly, possible collaborations among members of the network, which Jo Robertson left open for discussion.

Discussion

Diana Obregon—The electronic forum could be used to share bibliographic information as each academic is not necessarily aware of other publications in the field.

Sanjiv Kakar—It will also be useful for sharing information on conferences worldwide.

George Joseph—The submission of papers to conferences is normally more productive when in panels, rather than sending individual papers, which are often difficult for organizers to place in the program. He is in the early stages of planning a conference for late April or early May in the United States. The American Association for the History of Medicine (AAHM) will meet in Birmingham, Alabama, during the first week of May and in conjunction with the leprosy conference most likely to be held in New Orleans, Louisiana, it may be possible to arrange a visit to Carville at the same time. George Joseph suggested the circulation of the papers prior to the conference to allow a more advanced level of discussion.

John Manton—There is a problem with studying leprosy history in "tropical" Africa (i.e., Africa except South Africa). It would therefore be useful to underpin networks of Africa scholars and/or have a symposium in an African University. It would be difficult to get funding but it is important to do so.

Jo Robertson—If anyone knows of any other academics in the leprosy history field, let her know and she will establish communication with them.

Henk Menke—It may be useful to define one main historical problem as a group, and see how the research of each country relates to it.

Jo Robertson—This approach is important, and could be fulfilled by the research projects outlined by Mark Harrison. Also, on the academic network web page, there is information on members' publications and research interests. Academics need the freedom to go in the direction that their work leads them, and would prefer not to be pinned down to one particular research area.

George Joseph—Maybe three or four areas could be established to begin with but one would be too constraining.

Jo Robertson—We could look at the current areas being researched by the network and compile a list of core issues.

Jaime Benchimol—Agreed that it is too early to identify specific research areas. What would be valuable is an appraisal of what has been done in the field, e.g., leprosy and public health.

Sanjiv Kakar—Oral history is one dominant theme that we already have.

Jo Robertson—The politics of oral history are being handled carefully in the proposal for further funding, as there were problems with this area previously. We are trying to incorporate it once more.

Harriet Deacon—Oral history is a methodology, not an analytical approach. By pointing out that it is part of every history may be a convincing argument in its favor.

Anwei Law—The problem arises from not seeing oral history as a good source of history due to a lack of understanding of its use in different contexts.

Harriet Deacon—Asked whether the Project has to clear all methodologies with WHO.

Jo Robertson—Now that the Project is strong, we have received an email from WHO that is virtually contractual, stating that the website content must be cleared by WHO. This issue will go to the Steering Group to be debated. Jo Robertson listed the members of the Steering Group, as not

all the network members were aware of whom it comprised. There is now a page on the website with this information.

Chandi Nanda—Asked whether it is possible to identify some people who have been cured of leprosy, who can be brought in to the network.

Jo Robertson—This is an academic network. Anwei Law will develop a network of people to gather oral histories.

Henk Menke—The main group working for leprosy patients to date has been doctors and nurses. The historical method is relatively new. We need to make our work clear to those in the field, not only through publications but also meetings. If it is limited to historians, we may miss the important goal.

David Scollard—The last ILA Congress in Salvador, Brazil, is an example of how historians and present day medical workers have already come together. The history symposia during this Congress were very successful. There are people from all fields at this regular, international conference, so to have history also represented is very good. Hopefully we can find future ways in congresses to put forward particular historical problems and issues.

Jo Robertson—It was a very international gathering, and people who had had the disease were also responding.

Harriet Deacon—One of the dangers of classing us as a leprosy network limits the focus to that disease. However, it is useful to make comparisons with issues surrounding syphilis, AIDS and other diseases, to see how policies that develop around these matters relate to leprosy.

Jaime Benchimol—Emphasis also needs to be given to national studies; tuberculosis is an important comparison.

Anwei Law—This kind of study should not be limited to diseases, but human rights issues too.

Jo Robertson—Bernardino Fantini is developing a human rights program and wants to include leprosy.

David Scollard—Expressed a desire to publish the abstracts of the current conference in the *International Journal of Leprosy*. Members were asked to make any final adjustments to their abstracts, and send them to Jo Robertson as soon as possible.

INDEPENDENT EVALUATION OF THE GLOBAL ALLIANCE FOR THE ELIMINATION OF LEPROSY (GAEL)

June 13, 2003

Released by WHO 4 July 2003, the evaluation of the Global Alliance for the Elimination of Leprosy, GAEL, was drafted by an independent panel of six, led by Dr. Richard Skolnik. With the exception of Professor Michel Lechat, the evaluators are not part of the leprosy community. They based the evaluation on a review of literature, communications, documents and approximately 100 widely representational interviews.

Richard Skolnik (Team Leader), Florent Agueh, Judith Justice, Michel Lechat.

The George Washington University, The University of Louvain, and The University of California at San Francisco.

ABSTRACT

This is an independent evaluation of the Global Alliance for the Elimination of Leprosy. It assesses the extent to which the Alliance has contributed to the goal of eliminating leprosy as a public health problem. This evaluation was based on a review of literature, documents, communications, and almost 100 informant interviews.

The evaluation team believes that the Alliance has added important value to the goal of eliminating leprosy as a public health problem. It has mobilized political commitment, financial resources, and free drugs. It has helped to improve the management and reach of multi-drug therapy. It has energized a number of leprosy programs. During the course of the Alliance, 16 of 22 endemic countries have been deemed to have met the goal of elimination.

In addition, at the country level, the Alliance appears to be functioning well. Most countries are actively leading and coordinating their leprosy programs. Collaboration is good, with the World Health Organization (WHO) playing an advisory role and non-government organizations (NGOs) in-

volved in a range of leprosy efforts in conjunction with WHO and government.

Despite these important successes, the Alliance is not adding the value that it could add and this poses threats to country leprosy programs and to the reputations of collaborators on leprosy work. Relations among some collaborators at the global level are very bad. Concerned NGOs, physicians, and scientists have raised important questions to WHO about technical, operational, and strategic matters but they have not been resolved. In addition, some collaborators do not have a clear understanding of the aims of the Alliance, or a clear agreement on how the Alliance should be governed. There are also strong views among some collaborators that the Alliance is too embedded in WHO and that WHO has not been sufficiently consultative in its management of the Alliance.

This is already mid-2003, and the target date for elimination that was set by the World Health Assembly and extended by the Alliance is very close. There will continue to be significant numbers of leprosy patients after the goal of elimination has been achieved. In addition, there will also be needs at the global level for advocacy efforts, funds for leprosy activities, and exchanges of information and best practices among those working on leprosy. At the local level, all countries will need to lead their leprosy programs in sound ways. If these measures are not addressed effectively, some of the important gains on leprosy will be lost.

For these reasons, the panel believes that the Alliance must be rebuilt and refined immediately. Much of the global work of the Alliance would be convened and lead by the NGO and foundation movement. These activities would focus on ensuring effective advocacy, as needed, and promoting learning and input into country programs on technical, operational, and strategic issues. They would build on earlier work by the International Association of Anti-Leprosy Associations (ILEP), the International Leprosy Association (ILA), and the Sasakawa Memorial Health Foundation. They would include all who work with leprosy, including the private sector and groups of people affected by the disease.

If not already doing so, countries should organize their leadership around a country-level leprosy task force. WHO should play the advisory role to country programs, with effective use of input from other collaborators. The WHO should also convene a group of technical advisors, selected with the advice of others involved in leprosy, to carry out independent monitoring and evaluation of leprosy activities. The Technical Advisory Group (TAG) of WHO would have its membership strengthened, again with the advice of others.

It is also hoped that the Novartis Corporation, working with the Novartis Foundation for Sustainable Development, would continue to provide drugs and that the Sasakawa Memorial Health Foundation and the Nippon Foundation would continue to support technical cooperation and research, including through its important financial assistance.

The above approach would carry on from the work done effectively to date and would build on the comparative advantages of different actors engaged in leprosy efforts. It would also build on the unique role and commitment in leprosy work of NGOs. It would have clear and accountable roles for all actors and would be inclusive. It would also have to be based on open, transparent, and collegial relations, the lack of which would preclude any alliance from effectively supporting the important work on leprosy that will remain, even after 2005. Finally, these arrangements would help provide a sound transition to further leprosy control and rehabilitation efforts.

Obtained directly from the WHO web-site, http://www.who.int/lep/GlobalAlliance/evaluation.doc, at which the full report may be examined.

Notice. On 13–15 October 2003 a Workshop was held in Amsterdam on Leprosy Transmission and Diagnosis. During this workshop it was decided to co-ordinate research activities in this field.

A consortium supported by the WHO/TDR Special Program therefore now issues a call for interest for partners to engage in this comprehensive research program to apply modern developments in the molecular typing of *M. leprae* and specific

antigen/epitope definition to field studies towards better understanding of the epidemiology and transmission of leprosy, and the improved diagnosis of leprosy infection. The purpose of this call is to recruit partners to participate in working groups on:

Assays for molecular epidemiology
Immunology-based diagnostic assays
Field studies related to transmission and diagnosis

The purpose of the working groups is to (i) raise funds to advance the necessary basic and operational research; and (ii) set policies, proposals, protocols, under the umbrella of the consortium.

If you are interested in participating, please submit a letter of interest to the Interim Steering Committee of the consortium briefly stating the extent of your interest in these areas, your experience and your association with leprosy field studies. A standard form and more information is available at: *http://www.kit.nl/biomedical_research/htm/leprosy_research_consortium.asp*

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Calendar of Meetings and Events

Day	mm/yy	Location	Details	Contact	e-mail
9–13	12/3/2003	Valencia	ILEP Working Session and General Assembly	ILEP Secretariat	ilep@ilep.org,uk
19–22	12/3/2003	Chattisgarh	National Conference on Elimination of Leprosy	Dr. S.K. Noordeen	vinodkoomar@rediffmail.com

AMERICAN SOCIETY FOR MICROBIOLOGY DIVISION U SYMPOSIUM

Washington Covention Center
Washington D.C.
18–22 May 2003

The 103rd General Meeting of the American Society for Microbiology was held at the Washington Convention Center in Washington D.C., U.S.A., on May 18–22, 2003. At this meeting, Division U (Mycobacteriology) sponsored a symposium entitled, "Advances in Leprosy Research 2003 and Beyond: Following in Shepard's FootPads." This symposium featured a distinguished list of speakers covering a range of topics which emphasized the rapid gains in knowledge since the sequencing of the *Mycobacterium leprae* genome. This symposium also featured the prestigious Division U lecture, which is awarded each year to a member of Division U whose outstanding achievements have contributed to advances in mycobacteriology.

The conveners of the symposium were Drs. James L. Krahenbuhl and Diana L. Williams, both of the National Hansen's Disease Programs Laboratory, Baton Rouge, LA, U.S.A. After a brief introduction by Dr. Krahenbuhl, the symposium commenced with this year's Division U lecturer, Dr. Warwick J. Britton from the University of Sydney, Sydney, Australia, who presented an overview of his research on leprosy and tuberculosis vaccines. His lecture was followed by a presentation by Dr.

Williams on her work in defining a partial *M. leprae* transcriptome. Dr. Patrick J. Brennan, Colorado State University, Fort Collins, Colorado, U.S.A., presented his work on the genes encoding *M. leprae* cell wall components. Last, Dr. Thomas P. Gillis, National Hansen's Disease Programs Laboratory, discussed his research on the use of bioinformatics to search for potential vaccine and skin test antigen candidates.

This informative and timely symposium was attended by over 350 meeting participants and each presentation stimulated thoughtful discussion. In an effort to share the proceedings of this symposium with the readership of the *International Journal of Leprosy* who were unable to attend the meeting, the four lecturers have here provided a overview of their presentations.

—Linda Adams
Chair, Division U (Mycobacteria)
American Society for Microbiology

ABSTRACTS

Britton, W. J. On the vaccine trail: from Leprosy to Tuberculosis.

The control of leprosy has improved markedly since the introduction of multidrug therapy with dramatic falls in the prevalence of leprosy patients receiving antimicrobial therapy during the 1990's. Despite this widespread implementation of MDT, the incidence of leprosy as measured by case detection rate has not yet fallen in major endemic countries. This justifies continuing research to understand the transmission, host response in protective immunity against *Mycobacterium leprae*. Immunization to improve host response against *M. leprae* infection will also be an important component in the long term control of leprosy.

Current leprosy vaccines. The antituberculosis vaccine, *Mycobacterium bovis* bacille Calmette-guerin (BCG) has partial efficacy against clinical leprosy. In four randomized clinical trials, BCG stimulated a degree of vaccine efficacy (VE) ranging from 34% in India to 80% in Uganda. The prospective vaccine study in Malawi demonstrated a 52% VE for BCG, with a further 50% reduction in clinical leprosy following repeat BCG immunization ([1]). In addition, 9 case control studies have shown that past BCG immunization, as indicated by the presence of BCG a scar, was associated with approximately 50% reduction in clinical leprosy (range 20 to 81%). The impact of widespread BCG implementation on leprosy control is difficult to quantitate, however, it is probable that BCG is one factor which has contributed to the decline in leprosy in some countries. The recent South India vaccine trial provided further evidence of the effectiveness of vaccines against clinical leprosy ([2]). In this study the addition of heat-killed *M. leprae* to BCG resulted in improvement in the vaccine efficacy from 34 to 61%. This is contrast to earlier studies in Malawi and Venezuela, which showed no benefit from the addition of heat-killed *M. leprae* to BCG ([1]). An additional finding was that the cultivatable bacillus ICRC, probably a member of the *M. avium-intracellulare* family, also conferred significant protection (VE, 65%) when given as a dead mycobacterium. This provides further evidence that immunization with heterologous mycobacteria protects against *M. leprae*.

Subunit vaccines against leprosy. The potential of subunit vaccines against leprosy was raised by the early studies of Gelber and Brennan, which showed that crude *M. leprae* cell wall fractions and native cell wall-derived proteins protected against *M. leprae* footpad infection in mice. More recently, Ngamying and colleagues showed that *M. leprae* cytosol and membrane fractions protected against mouse footpad infection, but the cell wall skeleton of mycolyl-AG peptidoglycan from *M. leprae* was not protective ([3]). Therefore, protein components are essential for effective subunit vaccines. The trail to determine which *M. leprae* protein antigens induce effective immunity dates back to studies with monoclonal antibody-defined proteins in the 1980's. These included the *M. leprae* GroEs, GroEl and 70 kDa heat shock proteins, widely shared with other mycobacteria, and the *M. leprae* 18 kD protein, which contains *M. leprae*-specific T cell epitopes but has a homologue in *M. avium*. Subsequently, native proteins were purified from *M. leprae*, including the cytoplasmic 10 kDa GroEs protein and the membrane-associated proteins MMPI and MMPII. Fractions of *M. leprae* containing GroEs

and MMPI stimulated some protective effect in the mouse footpad model. More recently, a bioinformatics approach has been employed to define *M. leprae* homologues of *M. tuberculosis* antigens known to induce protective immunity against tuberculosis, such as the secreted proteins, Antigen 85B and ESAT-6.

Our own group has focused on the *M. leprae* 35 kDa (MMPI) as a subunit vaccine against leprosy. This protein was first recognized by *M. leprae*-specific monoclonal antibodies to conformational determinants on the protein, which are also the dominant epitopes for human leprosy sera. We cloned the gene for the *M. leprae* 35 kDa protein ([4]) and found it had no homologues in *M. tuberculosis* or BCG, but one was present in *M. avium* with 94% amino acid identity ([5]). Recombinant 35 kDa protein expressed in the rapidly growing *M. smegmatis* forms highly immunogenic multimers of >900 kDa. This protein is recognized across the leprosy spectrum, so that paucibacillary leprosy patients and contacts of leprosy patients develop a strong T cell response with low levels of antibody and multi-bacillary patients demonstrate a strong antibody response and weak T cell response ([4]). Further, the protein elicits delayed type hypersensitivity (DTH) in *M. leprae* sensitized guinea pigs.

DNA vaccines expressing the *M. leprae* or *M. avium* 35 kDa proteins induced protective immunity against *M. leprae* and *M. avium* infection in mice, which was equivalent to BCG in both cases ([6, 7]). This was accompanied by strong specific Interferon (IFN)-γ T cell responses, as well as high titre antibody responses to the conformational determinant on the protein. This establishes that immunization with a single antigen can be effective against experimental leprosy infection. Major antigens shared with *M. tuberculosis* and BCG may also induce protection when used as a subunit vaccines. For example, immunization with the *M. tuberculosis* antigen 85B as a DNA vaccine induced heterologous protection against *M. leprae* footpad infection in Swiss albino mice. ([8]).

Improving subunit vaccines against mycobacterial infections. Although subunit vaccines against leprosy infection show an equivalent protection to BCG in a mouse model, single protein or DNA vaccines against tuberculosis infection have generally been less effective than BCG in mice (9). Therefore, we and others have been examining ways of increasing the protective efficacy of subunit vaccines against mycobacterial infections. A number of cytokines were compared as adjuvants for DNA immunization and plasmid IL-12 was the most effective. Co-immunization with DNA-85B and plasmid IL-12 resulted in a rise in IFN-γ and T cell responses, a fall in specific antibody responses with increased protection against *M. tuberculosis* infection ([10]). Co-immunization with DNA-35 and plasmid IL-12 produced significantly greater protection against virulent *M. avium* infection than BCG ([11]). IL-12 was more effective than IL-18, another Th1-promoting cytokine, at improving DNA vaccine efficacy against mycobacterial infections ([12]).

We have also examined the interactions of subunit vaccines and BCG against *M. tuberculosis* infection. Priming with a DNA vaccine expressing the *M. tuberculosis* antigen 85B, followed by boosting with BCG, significantly improved the effective efficacy against *M. tuberculosis* to a level greater than that achieved with BCG alone ([10]). This may be due to focusing of the immune response against a dominant secreted antigen of *M. tuberculosis*. These findings demonstrate that protection against mycobacterial infections in experimental models is not limited to that achieved with BCG alone.

Challenges for new anti-leprosy vaccines. Although considerable progress has been made, there remain uncertainties in the understanding the complex host immunological response to mycobacteria and this influences the development of more effective anti-leprosy and anti-tuberculosis vaccines. First, the factors which determine the immunological dominance of antigens are still not resolved. Factors which may affect this include the quantity of mycobacterial protein and the timing of exposure. For example, the GroES protein is the major cytoplasmic protein in armadillo-derived *M. leprae*, and is a dominant antigen in host response to *M. leprae* ([13]). Secreted proteins, including antigen A85 complex, may be the first antigens encountered and appear to stimulate protective immunity against a number of mycobacteria ([8, 10]). There may

be intrinsic properties to certain proteins, such as the multimeric form of the 35 kDa *M. leprae* protein, which contribute to their persistence in the phago-lysosome and so their antigenicity.

Second, the importance of species specificity in determining the protective efficacy of individual proteins is unclear. In fact, species-specific proteins may not be the optimal vaccine candidates, although they have obvious importance as a diagnostic reagents. Recent studies by Black and colleagues in Malawi ([14]) have demonstrated apparent cross-reactivity between the major antigens of *M. leprae*, including the 35kDa and 18kDa proteins, and environmental mycobacterial species isolated in Malawi. These antigens may stimulate pre-existing T cell responses in infected subjects in of endemic regions and this may blunt the apparent effectiveness of mycobacterial vaccines in that environment.

Third, the relative contribution of different T cell subsets to protective immunity may vary between different species of mycobacteria. Both CD4 and CD8 T cells appear to contribute to effective immunity against *M. tuberculosis*, although the exact role of CD8 T cells inducing protection against *M. leprae* infection has not been established.

Fourth, understanding the factors controlling of T cell memory, particularly in CD4 T cells is incomplete and this has major implications for subunit vaccines against mycobacterial infections. Although protein and DNA subunit vaccines can stimulate short term protective immunity against tuberculosis and leprosy in animal models, their ability to stimulate long term protection is yet to be determined. These subunit vaccines may prove most useful in boosting immunity established by BCG or other viable vaccines.

The second major challenge for new anti-leprosy vaccines are limitations of the models for testing protective efficacy. The dynamic range of the mouse footpad infection model is low, and it is difficult to measure increased effective efficacy above that achieved with BCG. The lack of immunological reagents and complexibility of the armadillo model mean that it is not currently applicable to testing vaccines. Further, the length of time for testing individual vaccines, currently 9 to 12 months, restricts the rate of progress. In addition, it is important to confirm that the vaccine antigens induce protective immunity in mice with differing genetic backgrounds to confirm their applicability in human populations.

The third and most important challenge for introduction of new anti-leprosy vaccines is the capacity to test these in endemic regions. Will it be possible to conduct another major human leprosy vaccine trial on the scale of the recent Indian vaccine study? This would require sufficient number of new cases and the extensive infrastructure required for such a study. Another approach would be to include leprosy in future tuberculosis vaccine trials. A number of candidate TB vaccines are moving into Phase I and Phase II clinical trials. If these are to be used in leprosy endemic countries, it is be important that they have an effective anti-leprosy component, either because the antigens are shared between *M. tuberculosis* and *M. leprae,* or *M. leprae*-related components are added to the vaccine. This will require the design and conduct of the vaccine trials to measure the effects on leprosy as well as tuberculosis in leprosy-endemic regions.

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Williams, D. L. Establishing the Transcriptome of *Mycobacterium leprae*: A Minimal Gene Set for Survival.

The genome of *Mycobacterium leprae* has been completely sequenced and annotated. Approximately, 1604 open reading frames, encoding potentially functional proteins, and 1104 inactivated genes (pseudogenes) have been identified. However, the minimum gene set required for intracellular growth and survival (transcriptome) has not yet been defined. To address this, we have initiated studies to determine the potential transcriptome using RT-PCR and cross-species DNA microarray analysis using a comprehensive *M. tuberculosis* array using a commercially available oligonucleotide set (Operon Technologies, Alameda, CA) as a prelude to evaluating global gene expression using an *M. leprae* cDNA array, which is not currently available. For RT-PCR, RNA was obtained from two geographically distinct strains of *M. leprae* and cDNA was produced by reverse-transcription using random priming. Gene transcripts of interest were amplified from cDNA using PCR with primer sets flanking gene fragments of several potentially functional families of *M. leprae*. PCRs were initially characterized using DNA from *M. leprae* T-53 resultant PCR fragments were analyzed by gel electrophoresis. Cross-species DNA microarray analysis was accomplished using 5 ug total RNA from T-53 and 4089 and labeled with either Cy3 or Cy5 fluorochromes using RT. The labeled cDNAs will be hybridized to the slides, the slides will be washed and scanned using an Axon Scanner. The intensities of the two dyes at each spot will be quantified using the GenePix software package. Results of RT-PCR and cross-species microarray experiments demonstrated that genes encoding a variety of enzymes were transcribed in both strains. These include enzymes involved with folic acid synthesis, iron utilization, cofactor biosynthesis, gluconeogenesis, glycolysis, glyoxylate bypass, those associated with beta oxidation of fatty acids, degradation of phosphorous compounds, degradation of DNA, detoxification and virulence associated proteins, synthesis of mycolic acids, modification and maturation of ribosomes, synthesis of RNA, stress proteins, proteins of the SecA-dependent secretion pathway and 25 proteins containing secretion motifs or, and several proteins with unknown

functions. These data have provided us with the first insight into the transcriptome of *M. leprae* and further demonstrated the homogeneity of this species. It is anticipated that this analysis will help to identify a larger set of functional genes in *M. leprae* which will potentially help us to understand the minimal requirements for growth and replication of this pathogen. This information may lead to the identification of new drug targets, skin test antigens, and to identify factors that allow this pathogen to evade the immune system and destroy peripheral nerves.

Patrick J. Brennan and Varalakshmi D. Vissa. Maintenance of Genes for *Mycobacterium leprae* Cell Wall Synthesis.

Sequencing of the *Mycobacterium leprae* genome by S.T. Cole, *et al.* (http://www.nature.com/nature/v409/n6823/fig_tab/4091007aO_F1.html) was a momentous event, comparable to the introduction of MDT (multiple drug therapy) in the 1980's. Initial analysis indicated a genome size of about 3.3 megabases, a G/C content of 57.8%, only 1604 protein genes, 1116 pseudogenes, and hence a protein coding capacity of 49.5% (these latter figures are to be compared to a size of about 4.4 megabases for the *Mycobacterium tuberculosis* H37Rv genome, a G/C content of 65.6%, 3959 protein genes, only about 6 pseudogenes, and thus a protein coding capacity of 90.8%). (These data are being constantly revised in light of more recent and ongoing analysis of bacterial genomes.) Thus, the *M. leprae* genome has undergone reductive evolution, becoming trapped and crippled. In light of such a paucity of protein coding genes, it is worthwhile to examine the cell wall of *M. leprae* from both the perspectives of known biochemical information and *in silico* analysis. This was the purpose of this review.

For instance, we have known from the early chemical analysis of the cell wall peptidoglycan of *M. leprae* by P. Draper, *et al.*, that this essential component of all eubacteria is intact and comparable to that of *M. tuberculosis* and other bacteria. Indeed, *M. leprae* apparently retains the full *mur* operon (*ftsZ, ftsQ, murC, murG, ftsW, murD, murX, murF, murE*) and other related genes (e.g., *murA, murB, ponA1, ponA2*). Polyprenyl-phosphates are the membrane carrier lipids for many aspects of cell wall synthesis, such as arabinose, arabinogalactan, peptidoglycan, and, as expected, *M. leprae* contains the majority of genes encoding enzymes of the non-mevalonate pathway for polyprenyl-phosphate synthesis (e.g., *dxsI* and *ispC–G*). It is devoid of the *dxsII* of *M. tuberculosis*, which helped in deciding that *dxsI* is the functional gene for deoxylulose-5-phosphate synthase. The entire array of genes required for rhamnose synthesis (*rmlB, rmlC, rmlD, rmlA*) are present in the *M. leprae* genome, as expected, since rhamnose is a component of the key diglycosyl-phosphoryl unit joining the mycolyl-arabinogalactan complex to peptidoglycan. Most of the known genes responsible for the synthesis of mannose, the PIMs (phosphatidylinositol mannosides), LM (lipomannan), and LAM (lipoarabinomannan), are present in *M. leprae*, such as *pmmA*, the Rv3256c homolog, *pmi, manB*, the Rv2609c homolog, *pimA*, Rv2611c homolog, and *pgsA*. However *pimB* is apparently missing, which requires new thinking on the mechanism of synthesis of PIMs/LM/LAM; we do know from chemical analysis that all three types of products are present in *M. leprae*. Likewise, most of the genes for mycolic acid synthesis, modification, and deposition are present in the *M. leprae* genome, such as *fasI, fabD, acpM, kasA, kasB, accD6, mabA, inhA, umaA2, mmaA4, mmaA1, fbpA, fbpB, fbpC*, and *fbpC2*. However, as noted initially by Cole, *et al.*, *umaA1, mmaA3*, and *mmaA2* are missing as whole genes, and this absence is exactly in accord with the absence of methoxymycolates in *M. leprae* as reported by several workers in the 1980's.

The *pks* (polyketide synthase)-like genes of *Mycobacterium tuberculosis* responsible for the synthesis of the phthiocerol, phenolphthiocerol, and methyl branched fatty acids of DIM (dimycocerosyl phthiocerol) and the phenolic glycolipids (PGL) are receiving considerable current attention, since these products have been implicated in disease processes. In the case of *M. tuberculosis*, it has been demonstrated that disruption of the *pks10* and *pks7* genes which are clustered with *pks8*,

pks17, pks9 and *pks11* resulted in mutants deficient in the synthesis of DIM. Similarly, the *pks12* gene has also been implicated in synthesis of DIM. However, a careful analysis of the *M. leprae* genome indicates that *pks10, pks7* and *pks12* are pseudogenes; and *pks 8, 17, 9,* and *11* are absent.

An analysis of the intact *pks* genes of *M. leprae* from a different perspective, i.e., from the perspective of identification of the genes responsible for the synthesis of PGL-I, yields interesting information. The entire array of genes attributed to the synthesis of phthiocerol (*ppsA–E*) is located between ML2357 and ML2353. At a different location (ML0139) is the *mas* gene responsible for mycocerosic acid synthesis. The *pks1* and *pks15* of *M. tuberculosis* genes are fused as one gene in the *M. bovis* genome, which has been demonstrated to be involved in the synthesis of phenolphthiocerol, most likely a precursor of the *M. bovis* specific PGL. The *M. leprae* genome also contains the fused gene in concordance with the elaboration of its phenolic glycolipids. Elongation occurs with the *ppsA–E* cluster. Associated with *ppsA–E* are the genes *fadD26* and *drrA–C* implicated in the attachment of mycocerosic acid and phthiocerol followed by transport through the membrane. Putative glycosyltransferases and methyltransferases possibly involved in the synthesis of the trisaccharide segment of PGL-I can be located in one cluster (ML0125-ML0130).

We have previously commented on the "cell wall gene cluster" of *M. leprae* characterized by the presence of *embA–C* implicated in arabinan synthesis, the *fbp* genes responsible for mycolic acid deposition, and *glf* and *glfT* involved in D-galactofuranose and D-galactan synthesis. A detailed comparison of gene arrangements in this cluster in the *M. leprae* and *M. tuberculosis* genomes demonstrates the absence of several genes (Rv3784-3788) from the *M. leprae* genome, one of which is a putative glycosyltransferase (Rv3786c). Additionally, a dedicated analysis of putative glycosyltransferases over the entire genome of *M. leprae* versus *M. tuberculosis* shows that several orthologs (e.g., Rv1212c, Rv0539, and Rv2957) representative of the glycosyltransferase families 1 and 2 are missing from *M. leprae*. Likewise, a cluster of at least 8 glycosyltransferases in the Rv1500 to Rv1526 region of the *M. tuberculosis* genome is missing from *M. leprae*. This evidence supports the chemical evidence for "stunted" or "truncated" versions of some polysaccharides, such as LAM, in *M. leprae*.

Thus, an analysis of the genome of *M. leprae* versus those of *M. tuberculosis, M. bovis,* and other *Mycobacterium* spp. supports the chemical analytical evidence of an intact but minimal cell wall in *M. leprae*.

Some relevant publications:

Vissa, V.D., and P.J. Brennan. Impact of *the Mycobacterium leprae* genome sequence on leprosy research. In: *Genomics of GC Rich Gram-Positive Bacteria*, Wymondham, U.K.: Caister Academic Press, 2002. pp. 85-–118.

Crick, D.C., P.J. Brennan, and M.R. McNeil. The cell wall of *Mycobacterium tuberculosis.* In: *Tuberculosis. Second Edition* Philadelphia: Lippincott Williams & Wilkins, 2004. pp. 115–134.

Gillis, T. The Use of Bioinformatics in Leprosy Research.

Bioinformatics encompasses all aspects of biological information acquisition and analysis, and combines the tools of computer science and biology with the aim of understanding biological significance. The combination of enhanced sequencing and computing power has allowed for unprecedented advances in assimilating huge amounts of raw data and the initiation of meaningful molecular modeling. Bioinformatic approaches are particularly attractive for aiding studies of *M. leprae* since many conventional biological tools are unavailable to investigators working with nonculturable agents.

Three major areas of bioinformatics (genomics, proteomics, and transcriptional profiling) have had and should continue to have an impact on our understanding of *M. leprae* and the disease it causes. Genomics has provided a working genetic blueprint for *M. leprae* allowing for comparisons with other mycobacterial genomes to assess *M. leprae*'s basic physiological capabilities, potential virulence factors and establish

molecular markers for drug resistance and strain variation. Newly developed strain identification markers using simple repeated DNA sequences should provide tools to investigate transmission patterns of leprosy and may help define risk factors involved in reinfection versus relapse of disease. Algorithms for predicting open reading frames and for categorizing location and function of *M. leprae* proteins have initiated basic studies on secreted proteins as well as proteins involved in nerve invasion and *M. leprae*-specific proteins potentially useful for detecting exposure to the leprosy bacillus through skin testing or serological testing.

Proteomic studies of the leprosy bacillus have been hampered by low protein yields from purified bacilli derived from infected animals, but have established a baseline profile of highly expressed proteins from *M. leprae*. Combining transcription profiling of the leprosy bacillus with proteomic studies may provide new insights into proteins previously lost during purification from infected host tissues and provide new antigens useful for studying immune responses during infection. In addition, newly discovered proteins can be tested for their ability to induce protective immunity and may constitute a new group of proteins with vaccine or diagnostic potential. Transcriptional profiling may allow investigators to study *M. leprae*'s gene expression at different stages of growth as well. For example, gene expression in growth-permissive cells, such as the macrophage and Schwann cell could differ and, therefore, may reveal aspects of *M. leprae*'s unique tissue tropism.

Functional genomics is the integration of predictive bioinformatics with validation through experimental biological analysis. This part of the equation remains a major challenge to workers in the leprosy field. Surrogate genetics to study *M. leprae* genes in cultivable mycobacteria as well as new approaches for "knocking in" genes to *M. leprae* are areas in need of research and development. Both approaches will benefit from bioinformatics and should continue to further our understanding of the leprosy bacillus in particular, and the host-parasite relationship in general.