

# Leprosy - New Opportunities In Basic Science

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The decade between initiation of the *Mycobacterium leprae* genome project and its final publication [1] witnessed a progressive decline in interest in leprosy research. This was caused by the perception that multidrug therapy provided a sufficient solution to the clinical problems of leprosy, the fact that *Mycobacterium tuberculosis* presented a more tractable experimental system than *M. leprae* for exploitation of the emerging techniques of mycobacterial genetics [2], and disappointing results with the heat-killed *M. leprae* vaccine developed under the IMMLEP programme [3]. To take advantage of new opportunities arising from the genome sequence, there is a need to rebuild momentum in the leprosy research community. This can be achieved within a framework that combines research on the fundamental biology of leprosy alongside research that addresses practical aspects of leprosy control.

## Fundamental biology of leprosy

Study of the genetic make-up of *M. leprae* is an important component of the general investigation of evolution of human pathogens and is of particular relevance in the context of comparison with the closely related *M. tuberculosis*. Investigation of the dramatic neurological and immunological pathologies of leprosy provides a unique perspective that may increase understanding of normal human physiology.

## Evolution of microbial genomes

The explosion of information from microbial genome sequencing projects has had a major impact in the field of evolutionary biology [4]. There is an active interest in trying to understand how genetic exchange and genome reorganization have contributed to the evolution of different pathogenic strategies [5]. *M. leprae* provides an interesting example as an evolutionary snapshot of a genome in transition [1]. By comparison with *M. tuberculosis*, a quarter of the genome has already been deleted, and the large number of pseudogenes are thought to represent intermediates on the way to gene loss. *M. leprae* has undergone a process of adenine thymine enrichment that has been observed in several obligate pathogens [6]. This may reflect loss of DNA repair enzymes that are required for correction of spontaneous cytosine to uracil deamination.

To pursue research in this area, there is a need to collect information about genetic variation amongst *M. leprae* isolates; experience with *M. tuberculosis* indicates that deletions and single nucleotide polymorphisms are likely to be particularly informative as evolutionary markers [7]. Strategies to approach this would include detailed analysis of chromosomal DNA from one or more additional isolates prepared by armadillo passage. Partial coverage by shotgun sequencing would be sufficient to identify candidate polymorphisms. This could be complemented by PCR-based analysis of target loci in clinical samples to determine the extent of strain diversity at a population level. In addition to

fundamental information about pathogenic mechanisms, investigation of *M. leprae* in an evolutionary context may provide some indication of when it adapted to human pathogenesis [8], and may allow us to determine whether it is now on an inevitable pathway to extinction. An interesting aspect of such studies involves examination of *M. leprae* DNA preserved in archaeological samples [9]. This allows analysis of long-term changes in population structure, and could address the question of whether present day isolates of *M. leprae* differ from those that were prevalent in medieval Europe.

## Host-pathogen interactions

Cellular microbiology – combining the tools of cell biology and microbiology – is at the forefront of current efforts to explore the host-pathogen interactions [10]. In addition to its direct application to understanding pathogenesis, this field has generated useful insights into the fundamental biology of signal transduction and cytoskeletal organization in mammalian cells. The neural predilection of *M. leprae* offers an opportunity for a novel perspective on the organization and physiology of human peripheral nerves, and this has been exploited in a recent series of papers implicating phenolic glycolipid in neurotropism [11]. There is considerable scope to extend these studies to investigate intracellular compartmentalization of *M. leprae* in Schwann cells and other cell types. It would be interesting to determine why *M. leprae* is found in multiple cell types during infection, for example, in contrast to the apparently restricted cell tropism of *M. tuberculosis*.

A central component of research on host-pathogen interactions involves analysis of changes in pathogen gene expression associated with adaptation to an appropriate in vivo phenotype. The technical problems associated with direct analysis of in vivo phenotypes have been a central feature of leprosy research. Experience in isolating *M. leprae* from infected tissues and analysis of protein and lipid profiles may provide a useful model for parallel studies with other bacterial pathogens. Reciprocally, increased interest in the application of molecular approaches to histopathology may provide ways of examining leprosy lesions. Microarray-based whole genome expression profiling can be applied to characterization of the *M. leprae* transcriptome. This will depend on development of techniques for amplification of mycobacterial mRNA [12].

## Immunology

There is considerable current interest in analysis of initial recognition of pathogens by innate immune mechanisms, and translation into signals that alert and direct the adaptive response. This is mediated in part by the family of toll-like receptors on macrophages and dendritic cells, and includes release of a series of proinflammatory cytokines [13]. *M. leprae* can accumulate in tissues to much higher levels than *M. tuberculosis*, and appears to present a less potent proinflammatory stimulus. Comparison of responses to the two pathogens, together with knowledge of surface components, may be useful in identifying molecular determinants regulating innate immune recognition. Understanding of inflammatory responses to *M. leprae* is of particular importance in the context of leprosy reactions.

The specific absence of a Th1 response to *M. leprae* antigens in lepromatous leprosy represents a remarkable model for studying immune regulation. Evidence from analysis of leprosy lesions suggests that this may in part reflect polarization of the immune response towards a Th2 phenotype [14], but other mechanisms of tolerance remain to be investigated. It is not clear whether Th1 anergy is a predisposing factor for lepromatous disease, or is a subsequent consequence of prolonged exposure to antigen. There is a current renewal of interest amongst basic immunologists in understanding mechanisms of peripheral tolerance and the role of regulatory T cell subsets [15]; lepromatous leprosy would seem to provide an interesting challenge in this context. Findings from leprosy can be expected to generate insights into the less polarized immune changes underlying reactivation tuberculosis. While the immune response in infected humans should be the main target for research, experiments in

genetically manipulated mouse strains may assist in dissecting fundamental aspects of immunity to *M. leprae* [16].

## Host genetics

Twin studies demonstrate that there is an important genetic element in susceptibility to mycobacterial disease [17]. Considerable effort has been invested in searching for genetic determinants of susceptibility to tuberculosis in humans and in animal models. Few clearcut results have been obtained; it is likely that multiple loci contribute to susceptibility. For leprosy, the presence of well-established clinical phenotypes may offer an advantage in facilitating selection of tightly defined cohorts for genetic screens [18,19].

## Supporting leprosy control

In spite of the success of multidrug therapy over the last decade, there has been no obvious decline in the number of new cases of leprosy reported from high incidence countries [20]. This raises the question as to whether passive case finding and treatment is sufficient to interrupt leprosy transmission, or whether additional intervention tools are required. The *M. leprae* genome offers a range of research opportunities of direct relevance to current control strategies.

## Drugs and drug resistance

The emergence of multidrug resistant organisms has had a major impact on tuberculosis control. Although dapson resistance was widespread in the pre-MDT era, rifampicin resistance has not yet presented a major problem for leprosy control. Molecular genetic tests for mutations associated with rifampicin resistance provide a convenient surveillance tool to monitor this situation [21]. From a similar perspective, it would be of interest to identify the genetic basis of *M. leprae* resistance to other drugs.

While it is unlikely that novel drugs will be developed specifically for treatment of leprosy, it is important to have the ability to test whether newly developed antibiotics have any potential application in improved leprosy treatment. Assays based on measurement of transcriptional activity, and perhaps the use of reporter phage constructs [22], provide possible avenues for exploitation of genetic tools in development of novel tests to assess the effects of drugs on *M. leprae*.

## Transmission

Monitoring transmission of *M. leprae* may provide a powerful tool for assessment of the impact of leprosy control strategies, since reduced transmission may precede a reduction in disease incidence by years or even decades. In principle, transmission patterns can be analysed by studying immune responses in infected individuals, or by analysing the population structure of the pathogen.

Mycobacterial infection can be detected by antigen-specific immune responses manifest by delayed type hypersensitivity, T cell proliferation, or cytokine release. Antibody production represents an alternative readout, though this is likely to require presence of a higher concentration of antigen and may therefore be less sensitive. The most convenient current technologies involve measurement of interferon- $\gamma$  production by exposure of peripheral blood T cells to synthetic peptide antigens. The readout involves detection of soluble cytokine by enzyme linked immunosorbent assay (ELISA), or enumeration of cytokine-producing T cells by ELISpot. In the tuberculosis field, considerable enthusiasm has been generated by an ELISpot assay based on the ESAT6 antigen [23]. Comparative genomics offers a powerful solution to the prolonged search for *M. leprae*-specific antigens for use in

such an assay. Simple bioinformatic tools can be used to screen the limited panel of *M. leprae*-specific open reading frames to identify peptides that include appropriate major histocompatibility complex binding motifs and lack cross-reactive homologues [24]. Development of a specific test for *M. leprae* infection represents a very feasible short-term goal.

Molecular epidemiology has taken on an important role in tuberculosis research, with the use of strain typing to confirm reactivation disease [25], to distinguish reinfection from relapse (26), and to estimate the prevalence of disease due to recent transmission [27]. When disease arises predominantly as a consequence of recent transmission, the resulting isolates tend to share the same genetic features. When disease results from reactivation of some earlier infection, isolates are more likely to be genetically diverse. It is anticipated that the molecular epidemiology of leprosy will follow a similar pattern and that strain typing would therefore help clarify the relative importance of recent transmission within an endemic community. Several polymorphisms have been described for strain typing of *M. leprae* [28-30]. The number of copies of a non-coding triplet repeat sequence shows considerable variation between clinical isolates (30). A diverse range of triplet repeat patterns was found in a panel of isolates obtained from the same single leprosy clinic at Hyderabad in India (SK Young, GM Taylor, unpublished), suggesting that this polymorphism may be useful for localized epidemiological mapping. Tools for the molecular epidemiology of leprosy will be generated by the fundamental research on evolutionary biology of *M. leprae* described above, and will open up exciting new opportunities for practical application.

## **Nerve damage**

The need for new tools to assist in prevention of nerve damage during leprosy treatment has consistently been viewed as a high priority in leprosy research. Basic research on neurotropism and on immunology clearly have potential relevance in this area, which is discussed in detail in an accompanying working paper.

## **Vaccines**

The search for an effective prophylactic vaccine provided a central theme for TDR-sponsored leprosy research in the 1980s. An important finding from the resulting clinical trials was that BCG consistently confers protection against leprosy, and that this protection is boosted by a second BCG vaccination [31]. Both of these findings appear to be in contrast to experiences with the use of BCG against tuberculosis, and the repeat BCG result tends to contradict the prevailing thought that BCG is ineffective in individuals with pre-existing mycobacterial immunity [32-33]. A second mycobacterium, the ICRC bacillus, was also shown to elicit a protective effect, in this case after delivery as a killed vaccine [3]. In terms of leprosy control, it would seem attractive to include repeat BCG (or an alternative vaccine) as part of any targeted strategy to reduce leprosy in high incidence areas. From a research perspective, it would be interesting to try and understand the nature of the immune response that is boosted by repeat BCG and that confers protection against *M. leprae*.

## **Summary**

Leprosy offers an interlinked series of research topics ranging from fundamental biology to practical application. To exploit these opportunities, there is a need to bring about some restoration of the extent and momentum of the leprosy research community. TDR can play a role in this by publicizing research opportunities, coordinating meetings of basic and applied scientists working on leprosy and related topics, and supplying seed money to encourage new investigators to take an interest in leprosy.

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