

LONDON
SCHOOL of
HYGIENE
& TROPICAL
MEDICINE



Dockrell, HM (2011) Keep the faith—leprosy still needs new diagnostic tools and laboratory research. *Leprosy review*, 82 (4). pp. 340-3. ISSN 0305-7518

Downloaded from: <http://researchonline.lshtm.ac.uk/20669/>

DOI:

Usage Guidelines

Please refer to usage guidelines at <http://researchonline.lshtm.ac.uk/policies.html> or alternatively contact researchonline@lshtm.ac.uk.

Available under license: Copyright the publishers

EDITORIAL

Keep the faith – leprosy still needs new diagnostic tools and laboratory research

HAZEL M. DOCKRELL

London School of Hygiene & Tropical Medicine, London, UK

Accepted for publication 02 November 2011

Leprosy is in danger of being abandoned by the scientific community, just at a time when real advances could be made in new molecular diagnostic assays. Although the number of cases on treatment is still falling, there are still too many countries where large numbers of new cases are being diagnosed.¹ It is likely that, as the lag period between exposure and development of disease can be so long, new cases will continue to present. There is a very real risk that as the focus on leprosy diminishes and as leprosy expertise is lost or diverted to other public health problems, new cases will not be diagnosed and so will develop irreversible nerve damage, as well as continuing the transmission chain.

There are a number of reasons why now is the time to focus on laboratory work on leprosy and on leprosy diagnostics, but three seem of greatest importance in late 2011. Firstly there has been extraordinary progress in new platform technologies that provide insight into disease pathogenesis and that can identify the distinct biosignatures of infection, or of protection against the development of disease. This is critical, as although *Mycobacterium leprae* itself can be identified in multibacillary lepromatous leprosy patients, a simple read-out such as interferon- γ (IFN- γ) produced in peripheral blood mononuclear cell cultures stimulated by *M. leprae* antigens cannot distinguish between a healthy leprosy contact and a paucibacillary tuberculoid leprosy patient. Techniques such as gene expression analysis using microarrays are identifying gene pathways and genes that are up-regulated or down-regulated in tuberculosis,^{2–5} and there are anecdotal reports that many of these genes validate in different groups and cohorts. The risk of obtaining too many false positives when analysing the expression of as many as 48,000 probes simultaneously is proving to be lower than expected, and the increased or decreased gene expression identified using microarrays can usually be validated by PCR. There are a number of ongoing multicentre studies and trials that are investigating the biosignatures associated with TB disease, following the modulation of such profiles with treatment, and monitoring TB contacts in longitudinal follow-up studies, to develop biosignatures that predict control of latent TB infection, or progression to clinical tuberculosis. Their findings will undoubtedly provide valuable insights into the immune response in leprosy.

Correspondence to: Hazel M. Dockrell, Department of Immunology and Infection, Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, Keppel Street, London WC1E 7HT, UK. (e-mail: Hazel.dockrell@lshtm.ac.uk)

Moreover, once a few key genes of interest can be identified, assays based on existing simple automated machines such as the GeneXpert assay for diagnosis of *M. tuberculosis* infection⁶ could be developed. Other multiplexing methodologies enable the analysis of 60–80 genes using reverse-transcriptase multiplex ligation-dependent probe amplification.⁷ Further new technologies for miniature ‘lab on a chip’ and lateral flow assays⁸ are progressing so fast that such assays would be feasible at point of care, in a rural clinic without access to a good laboratory.

Secondly, after many false starts, leprosy researchers are close to having leprosy antigens with which to probe the immune response in an informative way. The early promise of the *M. leprae* genome⁹ was not fulfilled in terms of delivering *M. leprae*-specific antigens in the initial research, and a number of studies have failed to identify *M. leprae*-specific antigens or to confirm their specificity.^{10–13} However, helped by improvements to the sensitivity and specificity of these assays such as by combining peptides or adding cytokines,^{14–15} some promising candidates are being identified.^{16–18} The tuberculosis field was very lucky to identify ESAT-6 and CFP-10 as (relatively) *M. tuberculosis*-specific antigens, which could be exploited in interferon-gamma release assays,¹⁹ but unfortunately there are T cell cross-reactivities with the *M. leprae* ESAT-6 antigen which prevent the *M. leprae* homologue of being useful as a diagnostic antigen.²⁰ So far it has been difficult to identify *M. leprae* antigens that are more sensitive and specific than phenolic glycolipid-I (PGL-I) for use in serological assays for leprosy, although some antigens of interest are being identified;^{17,21} this may be less surprising as the tuberculosis field has also failed to develop specific antibody tests for clinical tuberculosis.²² In terms of cross-reactivity, it is still not quite clear whether *M. leprae* is just a closer cousin to many environmental mycobacteria and thus many *M. leprae*-antigens pick up cross-reactive T cell responses (there is no convincing evidence that *M. leprae* can be found in the environment²³), or whether high proportions of those living in highly leprosy-endemic areas have been exposed to *M. leprae*. A number of studies have picked up nasal carriage of *M. leprae* by PCR in healthy individuals living in leprosy-endemic areas so exposure to *M. leprae* may be much more common than was thought, although such carriage may be temporary^{24–26} and it is not clear if it is associated with detectable peripheral blood T cell recognition of *M. leprae* antigens.

The third reason for optimism is the huge progress being made in the tuberculosis vaccine field, from which leprosy research can benefit. There are new TB vaccines in development²⁷ many of which might also protect against leprosy, just as BCG does. And the associated search for correlates of protection should also provide the leprosy field with useful new knowledge, as discussed above.

There are further examples where the leprosy field can learn from tuberculosis, despite the fact that *M. leprae* has a down-sized genome compared to *M. tuberculosis*, and a predilection for nerves.^{1,9} However it is a member of the same family and so a lot can be learnt from work on *M. tuberculosis*. The importance of TNF- α in tuberculosis was shown by the progression to clinical tuberculosis when patients with latent tuberculosis infection were given monoclonal antibody therapy to block TNF- α , and there has been a recent description of a patient who developed leprosy when given the same therapy.²⁸ Latent *M. tuberculosis* infection is now recognised to be a more active process than a lazy deep sleep, with the associated expression of particular genes²⁹ and there may be lessons for leprosy as more is learnt about the *M. tuberculosis* dosR-related, enduring hypoxia response and resuscitation-related genes. We are also learning more about how *M. tuberculosis* subverts the immune system, interfering with normal macrophage function, antigen

presentation and T cell immunity.^{30–33} Despite its slow growth rate, *M. leprae* is also a pathogen that blocks the development of effective immune responses at least partly due to the effects of phenolic glycolipid-I,^{34,35} and that controls its intracellular environment. Otherwise, how could it multiply to produce the numbers of *M. leprae* found in lepromatous leprosy patients?

Leprosy has been privileged to have highly dedicated clinical and nursing staff who have devoted their lives, sometimes at great cost, to helping leprosy patients. The leprosy field also has a small band of dedicated researchers, who have refused the seductive call of tuberculosis as a research area. Although a lot of progress has been made in leprosy research, we still need to identify what are the best antigens to use to dissect the immune response to *M. leprae*, exploiting our knowledge of its genome sequence. Combining these specific antigens in a simple test that will discriminate between *M. leprae* exposure and *M. leprae* disease (or likely progression to disease) has been the target of much recent work. These diagnostic tools are now close and could also be used to identify the patterns of genes and immunological components that are responsible for the control of *M. leprae* growth in the majority of those exposed to infection. The leprosy research community should be supported to exploit the huge wealth of knowledge and expertise in the tuberculosis field and apply it to leprosy. Such tools are needed to effectively interrupt the transmission of leprosy.³⁶ These studies need to be funded and carried out now, while there are still experienced leprosy clinicians and field staff, as well as skilled laboratory scientists. The laboratory research community must not give up now, just when there is a real prospect of imminent breakthroughs and new tools for leprosy, that are derived from so much underpinning research from not only leprosy but also tuberculosis.

Acknowledgements

HMD is a member of the IDEAL Leprosy Consortium (Initiative for Diagnostic and Epidemiological Assays for Leprosy).

References

- ¹ Rodrigues LC, Lockwood DNJ. Leprosy now: epidemiology, progress, challenges, and research gaps. *Lancet Infect Dis*, 2011; **11**: 464–470.
- ² Jacobsen M, Repsilber D, Gutschmidt A *et al*. Candidate biomarkers for discrimination between infection and disease caused by *Mycobacterium tuberculosis*. *J Mol Med (Berl)*, 2007; **85**: 613–621.
- ³ Maertzdorf J, Repsilber D, Parida SK *et al*. Human gene expression profiles of susceptibility and resistance in tuberculosis. *Genes Immun*, 2011; **12**: 15–22.
- ⁴ Berry MPR, Graham CM, McNab FW *et al*. An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. *Nature*, 2010; **466**: 973–977.
- ⁵ Sutherland JS, Hill PC, Adetifa IM *et al*. Identification of probable early-onset biomarkers for tuberculosis disease progression. *PLoS One*, 2011; **6**: e25230.
- ⁶ Helb D, Jones M, Story E *et al*. Rapid detection of *Mycobacterium tuberculosis* and rifampin resistance by use of on-demand, near-patient technology. *J Clin Microbiol*, 2010; **48**: 229–237.
- ⁷ Joosten SA, Goeman JJ, Sutherland JS *et al*. Identification of biomarkers for tuberculosis disease using a novel dual-color RT-MLPA assay. *Genes Immun*, 2011 Sep 29; doi: 10.1038/gene.2011.64. [Epub ahead of print].
- ⁸ Corstjens PL, de Dood CJ, van der Ploeg-van Schip JJ *et al*. Lateral flow assay for simultaneous detection of cellular- and humoral immune responses. *Clin Biochem*, 2011; **44**: 1241–1246.
- ⁹ Cole ST, Eiglmeier K, Parkhill J *et al*. Massive gene decay in the leprosy bacillus. *Nature*, 2001; **409**: 1007–1011.
- ¹⁰ Dockrell HM, Brahmabhatt S, Robertson BD *et al*. A postgenomic approach to identification of *Mycobacterium leprae*-specific peptides as T-cell reagents. *Infect Immun*, 2000; **68**: 5846–5855.

- ¹¹ Geluk A, Klein MR, Franken KL *et al.* Postgenomic approach to identify novel *Mycobacterium leprae* antigens with potential to improve immunodiagnosis of infection. *Infect Immun*, 2005; **73**: 5636–5644.
- ¹² Spencer JS, Dockrell HM, Kim HJ *et al.* Identification of specific proteins and peptides in *Mycobacterium leprae* suitable for the selective diagnosis of leprosy. *J. Immunol*, 2005; **175**: 7930–7938.
- ¹³ Araújo R, Honoré N, Banu S *et al.* Towards an immunodiagnostic test for leprosy. *Microbes Infect*, 2006; **8**: 2270–2276.
- ¹⁴ Geluk A, van der Ploeg-van Schip JJ, van Meijgaarden KE *et al.* Enhancing sensitivity of detection of immune responses to *Mycobacterium leprae* peptides in whole-blood assays. *Clin Vaccine Immunol*, 2010; **17**: 993–1004.
- ¹⁵ Geluk A, van der Ploeg J, Teles RO *et al.* Rational combination of peptides derived from different *Mycobacterium leprae* proteins improves sensitivity for immunodiagnosis of *M. leprae* infection. *Clin Vaccine Immunol*, 2008; **15**: 522–533.
- ¹⁶ Geluk A, Spencer JS, Bobosha K *et al.* From genome-based in silico predictions to *ex vivo* verification of leprosy diagnosis. *Clin Vaccine Immunol*, 2009; **16**: 352–359.
- ¹⁷ Duthie MS, Truman RW, Goto W *et al.* Insight toward early diagnosis of leprosy through analysis of the developing antibody responses of *Mycobacterium leprae*-infected armadillos. *Clin Vaccine Immunol*, 2011; **18**: 254–259.
- ¹⁸ Sampaio LH, Stefani MM, Oliveira RM *et al.* Immunologically reactive *M. leprae* antigens with relevance to diagnosis and vaccine development. *BMC Infect Dis*, 2011; **11**: 26.
- ¹⁹ Lalvani A, Pareek MA. 100 year update on diagnosis of tuberculosis infection. *Br Med Bull*, 2010; **93**: 69–84.
- ²⁰ Geluk A, van Meijgaarden KE, Franken KL *et al.* Identification and characterization of the ESAT-6 homologue of *Mycobacterium leprae* and T cell cross-reactivity with *Mycobacterium tuberculosis*. *Infect Immun*, 2002; **70**: 2544–2548.
- ²¹ Spencer JS, Kim HJ, Wheat WH *et al.* Analysis of antibody responses to *Mycobacterium leprae* phenolic glycolipid I, lipoarabinomannan, and recombinant proteins to define disease subtype-specific antigenic profiles in leprosy. *Clin Vaccine Immunol*, 2011; **18**: 260–267.
- ²² Steingart KR, Flores LL, Dendukuri N *et al.* Commercial serological tests for the diagnosis of active pulmonary and extrapulmonary tuberculosis: an updated systematic review and meta-analysis. *PLoS Med*, 2011 Aug; **8**(8): e1001062.
- ²³ Truman R, Fine PE. ‘Environmental’ sources of *Mycobacterium leprae*: issues and evidence. *Lepr Rev*, 2010; **81**: 89–95.
- ²⁴ van Beers SM, Izumi S, Madjid B *et al.* An epidemiological study of leprosy infection by serology and polymerase chain reaction. *Int J Lepr Other Mycobact Dis*, 1994; **62**: 1–9.
- ²⁵ Hatta M, van Beers SM, Madjid B *et al.* Distribution and persistence of *Mycobacterium leprae* nasal carriage among a population in which leprosy is endemic in Indonesia. *Trans R Soc Trop Med Hyg*, 1995; **89**: 381–385.
- ²⁶ Beyene D, Aseffa A, Harboe M *et al.* Nasal carriage of *Mycobacterium leprae* DNA in healthy individuals in Lega Robi village, Ethiopia. *Epidemiol Infect*, 2003; **131**: 841–848.
- ²⁷ Kaufmann SH. Fact and fiction in tuberculosis vaccine research: 10 years later. *Lancet Infect Dis*, 2011; **11**: 633–640.
- ²⁸ Teixeira FM, Vasconcelos LM, Rola Cde A *et al.* Secondary leprosy infection in a patient with psoriasis during treatment with infliximab. *J Clin Rheumatol*, 2011; **17**: 269–271.
- ²⁹ Barry CE 3rd, Boshoff HI, Dartois V. The spectrum of latent tuberculosis: rethinking the biology and intervention strategies. *Nat Rev Microbiol*, 2009; **7**: 845–855.
- ³⁰ Gupta D, Sharma S, Singhal J *et al.* Suppression of TLR2-induced IL-12, reactive oxygen species, and inducible nitric oxide synthase expression by *Mycobacterium tuberculosis* antigens expressed inside macrophages during the course of infection. *J Immunol*, 2010; **184**: 5444–5455.
- ³¹ Carow B, Ye X, Gavier-Widén D *et al.* Silencing suppressor of cytokine signaling-1 (SOCS1) in macrophages improves *Mycobacterium tuberculosis* control in an interferon-gamma (IFN-gamma)-dependent manner. *J Biol Chem*, 2011; **286**: 26873–26887.
- ³² Rajaram MV, Ni B, Morris JD *et al.* *Mycobacterium tuberculosis* lipomannan blocks TNF biosynthesis by regulating macrophage MAPK-activated protein kinase 2 (MK2) and microRNA miR-125b. *Proc Natl Acad Sci U S A*, 2011; **108**: 17408–17413.
- ³³ Russell DG. *Mycobacterium tuberculosis* and the intimate discourse of a chronic infection. *Immunol Rev*, 2011; **240**: 252–268.
- ³⁴ Mehra V, Brennan PJ, Rada E *et al.* Lymphocyte suppression in leprosy induced by unique *M. leprae* glycolipid. *Nature*, 1984; **308**: 194–196.
- ³⁵ Manca C, Peixoto B, Malaga W *et al.* Modulation of the cytokine response in human monocytes by *Mycobacterium leprae* phenolic glycolipid-1. *J Interferon Cytokine Res*, 2011 Oct 7; [Epub ahead of print].
- ³⁶ Fischer EA, de Vlas SJ, Habbema JD, Richardus JH. The long term effect of current and new interventions on the new case detection of leprosy: a modelling study. *PLoS Negl Trop Dis*, 2011; **5**: e1330; [Epub 2011 Sep 20].