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REVIEW

Biomarkers for Leprosy: would you prefer T (cells)?

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The need for rapid diagnostic tests that can be applied in non-expert settings may now be greater than ever before, due to changes in leprosy control programmes and the decrease in special expertise required for (early) diagnosis of leprosy. However, there is no test available that can detect asymptomatic *Mycobacterium leprae* infection or predict progression of infection to clinical disease.

The characteristics of the leprosy disease spectrum, in which both cellular and humoral immunity against *M. leprae* determine the outcome of infection, are pre-eminently suitable for development of tests that simultaneously detect biomarkers specific for both types of immune responses, thereby covering the complete immunological leprosy spectrum.

Since publication of the *M. leprae* genome, many research groups have investigated the potential of *M. leprae*-unique antigens in serologic as well as T cell assays. Despite the early promise of the genome towards identifying antigens that induce *M. leprae*-specific T cells, it took more than a decade before promising candidates were identified.

This review describes recent studies on *M. leprae*-specific T cell responses and discusses the application of measuring specific cellular mediated immune responses possibly combined with serologic responses in field-friendly tests for early diagnosis of *M. leprae* exposure and infection.

Introduction

Leprosy is a treatable infection caused by *Mycobacterium leprae* (*M. leprae*) that ranks as the second most pathogenic mycobacterial infectious disease after tuberculosis (TB). It particularly parasitizes Schwann cells and macrophages/ histiocytes in the dermis, leading to severe nerve damage and subsequent life-long disabilities and deformities, the hallmark of leprosy.[1,2]

Most individuals are naturally resistant to leprosy, but despite there being minimal genetic variation between *M. leprae* isolates reported, susceptible individuals can manifest completely different forms of the disease, ranging from strong cellular immunity in

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tuberculoid (TT) patients to predominantly humoral responses in lepromatous leprosy (LL), with unstable borderline states positioned in between.

Despite the spectacular decrease in global prevalence since 1982, leprosy remains a public health problem in 32 countries, mostly from the African, Asian and South American continents that cover 92% of all registered patients. Worldwide, 2 million people are estimated to be disabled by leprosy. Transmission of leprosy is sustained as evidenced by the hundreds of thousands of new cases of leprosy that keep being detected globally every year, with 228,474 new cases in 2010 amongst whom 20,472 were children.[3] This reservoir of *M. leprae* infected contacts and individuals with potential, subclinical leprosy, represents an important source of continued future transmission.

On top of that, in many countries leprosy control is now integrated into the general health delivery network and the number of trained leprologists and laboratory technicians has decreased, leading to an increase in misdiagnosis and a failure to treat early and appropriately. In line with this, a study conducted in India reported that inadequate monitoring of a policy of 'new case validation,' in which treatment was not initiated until primary diagnosis had been verified by a leprosy expert, may have led to approximately 26% of suspect cases awaiting confirmation of diagnosis 1–8 months after their initial primary health care visit.[4]

Thus, in order to allow informed decision making on who needs treatment at a preclinical or early clinical stage, diagnostic tests are required that measure (multiple) biomarkers that indicate *M. leprae* infection and/or that predict disease development in infected individuals. Such early detection of leprosy is considered an essential step to help prevent immunopathological sequellae occur and help disrupting *M. leprae* transmission and leprosy incidence. However, there are no biomarkers for detection of asymptomatic infection in leprosy yet, nor is it possible to accurately predict disease development in *M. leprae* exposed individuals using specific biomarkers.

Diagnosis of leprosy remains classically based on clinical manifestations as well as labour intensive and time consuming laboratory or histological evaluation. The scarcity of symptoms in early disease as well as the possible virtual lack of bacteria, resulting in suboptimal cell mediated immunity and antibody responses, further hampers diagnosis. Consequently, in order to assist in informed decision making on who needs treatment at a preclinical stage, new diagnostic tests for leprosy need to be highly specific as well as sensitive to be able to detect transit towards *M. leprae* infection.

SEROLOGY-BASED ASSAYS AND SKIN TESTS

The existence of high titer IgM antibodies to phenolic glycolipid-I (PGL-I), an *M. leprae* specific cell-surface antigen,[5–7] has allowed the development of field-friendly, kit-based tests used for leprosy diagnostics. Although these are useful for detection of most multibacillary (MB) leprosy patients, they have limited value in identifying paucibacillary (PB) leprosy patients since the latter typically develop cellular rather than humoral immunity and 60–80% lack antibodies to PGL-I.[5] Notwithstanding the fact that among contacts of MB patients, those with anti-PGL-I antibodies have an estimated 8-fold increased risk of developing clinical symptoms than those without anti-PGL-I antibodies almost half of those individuals with antibodies against PGL-I never develop leprosy and many of those who develop leprosy do not have PGL-I antibodies, particularly PB patients.[8–11]

Alternative tests that measure cellular rather than humoral immunity, such as skin tests, have been developed in various forms.[12] The Mitsuda skin test, for example, evaluates the *in vivo* immune response against a standardized extract of inactivated *M. leprae* bacilli (lepromin) and is used for classification of leprosy. However, generally skin tests are not specific for *M. leprae* since, analogous to the tuberculin skin test (TST) which is commonly used for detection of infection with *M. tuberculosis*, they rely on the use of complex and often incompletely defined mixtures of *M. leprae* components, many of which cross-react immunologically with other mycobacteria. Thus, they do not represent adequate tools to measure *M. leprae* exposure or latent infection.[13,14] For leprosy, such cross reactivity is particularly undesirable in countries with high incidence rates of TB, routine BCG vaccination practice, and high levels of exposure to non-pathogenic environmental mycobacteria.

CMI-BASED TEST DEVELOPMENT: IDENTIFICATION OF INFORMATIVE T CELL RESPONSES

Leprosy has been one of the very first human diseases in which HLA-genes were shown to co-determine disease outcome. HLA molecules display highly allele-specific peptide binding capacity, thereby restricting antigen presentation to *M. leprae* reactive T cells, and controlling the magnitude of the ensuing immune response.[15,16] Furthermore, specific peptide-HLA class II complexes can also determine the quality of the immune response by selectively activating regulatory T cells.[17] All these factors are believed to contribute to leprosy disease susceptibility.

In attempts to develop assays based on cellular mediated immunity (CMI) particularly for the identification of subclinical leprosy, we first focused on *M. leprae* homologues of ESAT-6 and CFP-10 (ML0049 and ML0050) based on the success of the commercially available IFN-γ release assays (IGRAs) for specific diagnosis of *M. tuberculosis* infection[18,19] that exploit the selective expression of ESAT-6, CFP-10 and TB7.7 by *M. tuberculosis*. However, no such luck for leprosy researchers: despite limited sequence homology (36% and 40%, respectively) and no cross-reactivity at the serological level,[20,21] the *M. leprae* homologues of ESAT-6 and CFP-10 were also recognised by T cells from TB patients. In view of the high prevalence of TB in most leprosy endemic areas, these findings prohibited any diagnostic use of *M. leprae* ESAT-6 and CFP-10.[22,23]

Publication of the genome sequences of *M. leprae,*[24,25] preceded and followed by those of other mycobacteria (e.g. *M. tuberculosis*, *M. bovis*, *M. smegmatis*) provided an important incentive for several research groups to identify antigens unique to *M. leprae* which can probe T cell responses in an informative way[26–30] (reviewed in[31]). This resulted in the identification of *M. leprae* antigens that induced *in vitro* IFN-γ responses in *M. leprae* exposed/ infected individuals. In addition, T cell responses to some antigens also provided significant added value compared to humoral assays alone (anti-PGL-I antibody) for detecting *M. leprae* exposure/ infection in healthy household contacts (HHC) of MB leprosy patients in Brazil Brazilian population:[26,27] 71% of the exposed healthy individuals that would not have been detected using serology-based assays, were identifiable using T cell assays based on *M. leprae*-unique proteins (Fig. 1).[26]

In contrast to these early findings, similar analysis of these *M. leprae* antigens showed that, notwithstanding their *M. leprae*-unique sequence, these antigens also induced IFN-γ production in blood or PBMC from endemic controls (EC).[32] Since these EC lived in areas with pockets of high leprosy prevalence (e.g. Dhaka and Karachi), the observed cellular responses towards the *M. leprae*-unique proteins may, however, still indicate

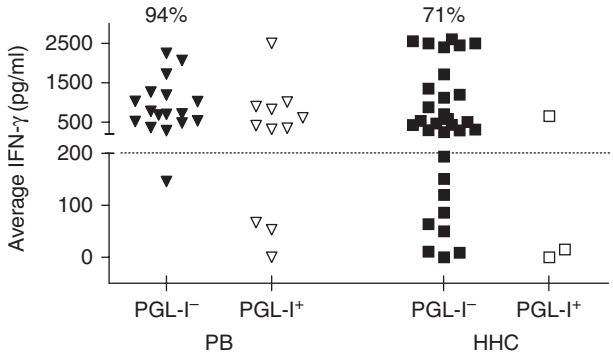

Figure 1. Added value of the use of CMI-based assays. Highest IFN-γ production induced by PBMC from Brazilian individuals against *M. leprae* unique recombinant proteins[26] is depicted for several groups in the context of their PGL-I seropositivity. Percentages of individuals without Ab against PGL-I, responding to *M. leprae* unique proteins, is indicated in the figure. PB: paucibacillary leprosy patients; HHC: healthy household contacts. PGL-I$^-$: individuals without anti-PGL-I Ab; PGL-I$^+$: individuals with anti-PGL-I Ab.

M. leprae-specificity. Moreover, it is of note that the *M. leprae*-specific IFN-γ responses detected in EC in areas hyperendemic for leprosy are consistent with earlier findings on the presence of *M. leprae* in nasal swabs of EC in Indonesia[33] indicating that a vast proportion of leprosy patients probably acquire *M. leprae* infection from unidentified infected individuals or subclinical leprosy cases in the community and not necessarily from diagnosed leprosy patients.

To identify biomarkers for field-applicable diagnostics, recent studies therefore included individuals from areas with distinct degrees of endemicity of leprosy (EC_{high} vs. EC_{low}) from different countries[34] or residing in distinct areas of the same city.[34,35] Importantly, these studies included EC_{high} with identical socioeconomic background as the majority of the leprosy patients. The results from both studies demonstrated that the extent of leprosy endemicity in a certain community (and thus likely *M. leprae* exposure) correlates with the IFN-γ production in response to certain selected *M. leprae*-unique proteins or peptides, but not to preparations of killed whole *M. leprae*. Thus, these defined *M. leprae*-unique antigens, particularly ML2478, can be applied in the context of various genetic backgrounds (African, Asian, and South American) as biomarker tools in IGRAs to measure *M. leprae* exposure.

IFN-γ OR ELSE. . .?

Although not a real immunological correlate of protection, the number of IFN-γ-releasing antigen-specific T cells and the amount of total IFN-γ released remain widely used as surrogate markers for the pro-inflammatory immune response against *M. leprae*[26,27,30,32,36,37] and *M. tuberculosis.*[38]

A pitfall of the use of IFN-γ for leprosy diagnosis in a leprosy endemic area, however, is that not only infected individuals but also individuals with adequate immunity against *M. leprae* produce substantial concentrations of IFN-γ in response to *M. leprae* antigens. In a similar fashion IFN-γ induced protein 10 (IP-10) was found to be a useful biomarker to differentiate between *M. leprae* exposure levels,[34,39] which corroborates the potential of this cytokine as a biomarker for *M. tuberculosis* exposure/ infection.[40,41]

Thus, despite discriminatory IFN-γ/IP-10 profiles observed between BL/LL and TT/BT,[26,27,36] and between EC_{high} and EC_{low},[34,35] no *M. leprae* proteins have been identified that can distinguish leprosy patients from EC based on IFN-γ when both groups are derived from the same leprosy hyperendemic area and have identical socioeconomic status. In addition, it remains a topic of debate whether increased IFN-γ production measured in whole blood assays (WBA) in response to *M. leprae* antigens indicates protection against infection or coincides with (progression to) disease.

Since host immunity and immuno-pathogenicity in response to *M. leprae* involves complex interactions between a variety of cells expressing different effector and regulatory molecules, assessment of multiple rather than single biomarkers may be more representative of the immune status of the host and may identify patterns predisposing to leprosy. Based on this starting point recent studies have assessed the concentrations of various cytokines and chemokines, besides IFN-γ, by multiplexing technology. Both studies analysed 24 h WBA stimulated with *M. leprae* antigens. One study[42] that was conducted among leprosy patients (TT/BT and BL/LL) and HHC in Central-Western Brazil, did not identify cytokines among the 14 (eotaxin, IFN-γ, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-15, IL-17A, IL-23, IL-31, IP-10, and TNF-α) tested that had improved diagnostic value. Instead, it confirmed IFN-γ as an indicator of antigen-specific cellular immune responses of TT/BT patients and most HHC. Furthermore, the same antigens promoted IL-4 production in blood of BL/LL patients. However, since EC were not included in this study it is not clear whether any of these biomarkers would differentiate healthy controls from leprosy patients or HHC. The other study analysed TT/BT leprosy patients, HHC and EC in areas (hyper)endemic for leprosy in Bangladesh, Brazil and Ethiopia[34] or EC from an area not endemic (anymore) for leprosy (South-Korea). Using a different panel of 18 analytes (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13, IL-17A, IFN-γ, IP-10, G-CSF, GM-CSF, MCP-1, MIG, MIP-1β and TNF), the latter study identified cellular host biomarkers (MCP-1, MIP-1β and IL-1β) that were significantly increased in leprosy patients and (partly in) HHC compared to EC in one endemic area (Fig. 2).

Analysis of ROC (receiver operating characteristics) for MCP-1, MIP-1β and IL-1β as biomarker tools for TT/BT leprosy patients vs. EC in Bangladesh provided AUC (areas under the curve) ranging from 0.89 (IL-1β) to 0.94 (MIP-1β), indicating good to excellent discrimination between these groups. Combining these three biomarkers enhanced the diagnostic ability even more and resulted in an AUC value of 0.99.[34] Thus, these chemokines could have value for diagnosing early *M. leprae* infection and monitoring the response to MDT.

Despite the obvious differences between leprosy patients/HHC on one hand and EC on the other hand, no significant differences were observed between HHC and BT/TT in both studies, although some HHC displayed biomarker profiles similar to patients whereas others seemed to be more like EC. It is therefore of great importance to invest in longitudinal follow-up studies. Since the majority of those exposed to *M. leprae* develop a protective immune response against the bacterium, such large-scale longitudinal follow-up studies, allowing intra-individual comparison of immune profiles in healthy controls from leprosy-endemic areas worldwide, will be essential to evaluate these biomarkers as tools for prediction of pathogenic immune responses to *M. leprae*.

In addition to multiplex cytokine/chemokine analysis, the availability of human transcriptome-arrays offers cutting-edge tools to identify and assess gene expression profiles for leprosy. For TB for example recent microarray studies, highlighted a potential role for type I interferon-ab signaling pathways.[43] In addition, a biomarker profile composed of several genes was identified for infection in a follow-up study of TB contacts using Reverse

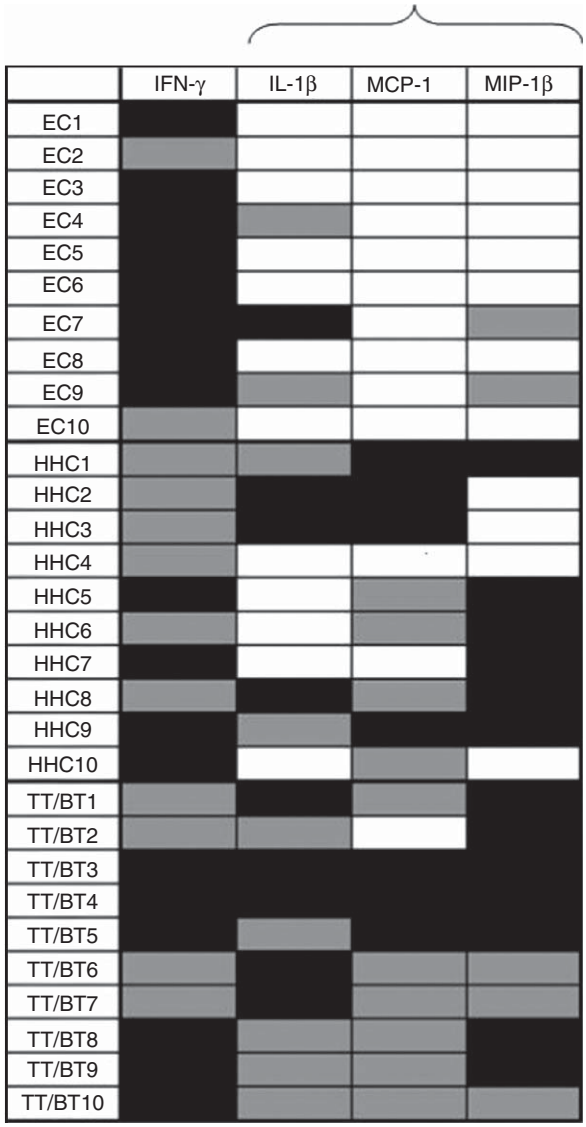

Figure 2. Heat map of cytokine production in response to *M. leprae* in EC (n=10), HHC (n=10) and TT/BT (n = 10) from a region hyperendemic for leprosy. All groups showed high levels of IFN-γ. Production of IL-1β, MCP-1 and MIP-1β was minimal for EC and increased for TT/BT and in most HHC; White squares indicate <10-fold the background response; grey squares indicate 10–100 fold the background response; black squares indicate >100-fold the background response. MIP-1β: macrophage inflammatory protein-1β; MCP-1: monocyte chemotactic protein-1.

Transcription Multiplex Ligation-dependent Probe Amplification (RT-MLPA) assays.[44] Such studies are currently also performed for leprosy as well as leprosy reactions in several leprosy endemic sites worldwide. Combined with functional protein association networks, transcriptomic expression profiles can be used to identify proteins demonstrably in blood that subsequently can be applied as biomarkers in field-friendly assays.

FIELD-FRIENDLY ASSAY DEVELOPMENT FOR LEPROSY

Despite the predilection of *M. leprae* for nerve cells and skin in lower temperature body parts,[45] recruitment and activation of immune cells into the lesional sites is largely influenced by levels of various cytokines and chemokines in circulation. As discussed above, indications of the disease status, such as high anti-*M. leprae* antibody levels, can be found systemically.[5] It is thus conceivable that cytokines and chemokines, which may ultimately find their way into the circulation, may provide indications of impending disease or protective immunity.

Leprosy endemic areas often lack sophisticated laboratories, necessitating development of a field-friendly diagnostic test. Since WBA are much simpler and faster than conventional assays using peripheral blood mononuclear cells (PBMC), they have been successfully applied in commercially available IFN-γ release assays (IGRAs) for diagnosis of TB (QuantiFERON-TB-Gold-In-Tube®).[18] In this respect, 24 h WBA could provide a useful immunodiagnostic assay format for leprosy as well.

Accurate diagnosis of *M. leprae* exposure/infection requires testing for cellular and humoral response markers to cover the complete immunological leprosy spectrum. WBA combined with *M. leprae* antigens can induce a 'fingerprint' of Th1 or Th2 cytokines that may, combined with detection of anti-PGL-I antibodies, be used to specify disease type in the leprosy spectrum. Recently, we reported the development of a robust, quantitative and user-friendly lateral flow assay for detection of IFN-γ based on up-converting phosphor technology (UCP-LF).[46] To integrate cellular and humoral response testing in one assay, we next developed UCP-LF assay for IL-10 and anti-PGL-I Ab as well.[47] The assay format demonstrated its potential for simultaneous detection of multiple cytokines, as well as combinations of cytokines and antibodies in one sample on a single LF strip. Although development of such a test still requires strong efforts, this multiplex format could eventually have significant advantages for classification of leprosy status and measuring/monitoring the effects of intervention (therapeutic drugs, vaccines) since it allows simultaneous detection of biomarkers characteristic for the poles of the leprosy disease spectrum responses.[46,47] For example, using *M. leprae* antigen-stimulated WBA, UCP-LF assays could be applied in poorly equipped laboratories to estimate levels of *M. leprae* exposure, by measuring both cellular, Th1 (IFN-γ) and Th2 (IL-10), as well as humoral, anti-PGL-I IgM antibodies, responses.

Besides measurements of cytokine levels *per se*, determination of ratios can be informative as well. Since both pro- and anti-inflammatory cytokines play a role in protection from and pathogenesis of mycobacterial diseases, their balance rather than their actual levels may control a possible clinical outcome. In this respect the IFN-γ/ IL-10 ratio was shown to correlate with TB cure and severity.[48–52] For leprosy the pro-inflammatory cytokine IFN-γ provides protection against mycobacteria, the anti-inflammatory cytokine IL-10, preferentially expressed in lepromatous lesions and abundantly produced by type 2 macrophages,[53,54] has been shown to be associated with dampening Th1 cells and exerts a suppressive role by inducing an inhibitory effect on cell-mediated immune responses towards mycobacteria.[54–56] These data emphasise the need of a diagnostic test with the ability for simultaneous analysis of multiple cellular biomarkers.

CONCLUDING REMARKS

Field-friendly diagnostic tests based on detection of T cell responses as well as humoral responses specific for *M. leprae* would be beneficial to leprosy control programs and case management (Fig. 3).

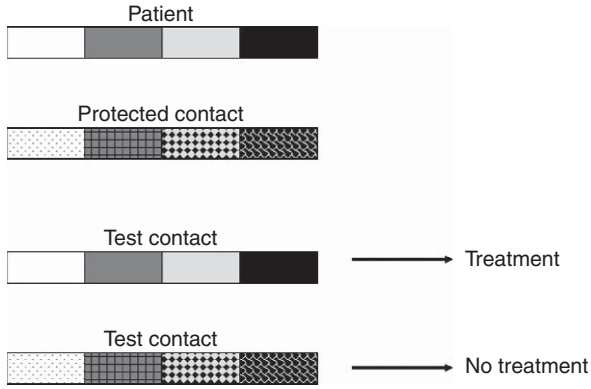

Figure 3. Simplified schematic representation of potential future decision making for treatment of leprosy patients' contacts based on measurement of biomarker profiles. Each rectangle represents a biomarker to be measured in diagnostic field tests; plain rectangles are phenotypes specific for patients whereas dashed/striped rectangles are phenotypes observed in protected healthy contacts of leprosy patients.

Recent studies measuring specific T cell responses have shown that defined *M. leprae*-unique antigens can be applied in the context of various genetic backgrounds as biomarker tools in IGRAs to measure *M. leprae* exposure. Additional analyses show that other cellular markers can potentially distinguish pathogenic immune responses from those induced during asymptomatic exposure to *M. leprae.* These data could be applied in order to identify those most at risk of developing disease. Future research efforts should thus continue to aim for the development and eventual application in control programmes of field-friendly diagnostic tests for detection of *M. leprae* infection and early leprosy in order prevent leprosy disability and further transmission by otherwise undiagnosed and untreated index cases.

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References

[1] Scollard DM, Adams LB, Gillis TP *et al.* The continuing challenges of leprosy. *Clin Microbiol Rev*, 2006; **19**: 338–381.
[2] Rodrigues LC, Lockwood DN. Leprosy now: epidemiology, progress, challenges, and research gaps. *Lancet Infect Dis*, 2011; **11**: 464–470.
[3] Leprosy update, 2011. *Wkly Epidemiol Rec*, 2011; 86: 389–399.
[4] Siddiqui MR, Velidi NR, Pati S *et al.* Integration of leprosy elimination into primary health care in orissa, India. *PLoS ONE*, 2009; **4**: e8351.
[5] Oskam L, Slim E, Buhrer-Sekula S. Serology: recent developments, strengths, limitations and prospects: a state of the art overview. *Lepr Rev*, 2003; **74**: 196–205.

[6] 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.
[7] Spencer JS, Brennan PJ. The role of Mycobacterium leprae phenolic glycolipid I (PGL-I) in serodiagnosis and in the pathogenesis of leprosy. *Lepr Rev*, 2011; **82**: 344–357.
[8] van Beers SM, Hatta M, Klatser PR. Patient contact is the major determinant in incident leprosy: implications for future control. *Int J Lepr Other Mycobact Dis*, 1999; **67**: 119–128.
[9] Douglas JT, Cellona RV, Fajardo TT, Jr *et al.* Prospective study of serological conversion as a risk factor for development of leprosy among household contacts. *Clin Diagn Lab Immunol*, 2004; **11**: 897–900.
[10] Moet FJ, Oskam L, Faber R *et al.* A study on transmission and a trial of chemoprophylaxis in contacts of leprosy patients: design, methodology and recruitment findings of COLEP. *Lepr Rev*, 2004; **75**: 376–388.
[11] Goulart IM, Goulart LR. Leprosy: diagnostic and control challenges for a worldwide disease. *Arch Dermatol Res*, 2008; **300**: 269–290.
[12] Brennan PJ. Skin test development in leprosy: progress with first-generation skin test antigens, and an approach to the second generation. *Lepr Rev*, 2000; **71**: S50–S54.
[13] Maeda SM, Rotta O, Michalany NS *et al.* Comparison between anti-PGL-I serology and Mitsuda reaction: clinical reading, microscopic findings and immunohistochemical analysis. *Lepr Rev*, 2003; **74**: 263–274.
[14] Roberts PP, Dockrell HM, McAdam KP. Evidence that the Mitsuda reaction to Mycobacterium leprae can be mediated by lymphocytes responsive to Mycobacterium tuberculosis. *Clin Exp Immunol*, 1988; **72**: 390–393.
[15] Ottenhoff TH, Elferink DG, Hermans J, de Vries RR. HLA class II restriction repertoire of antigen-specific T cells. I. The main restriction determinants for antigen presentation are associated with HLA-D/DR and not with DP and DQ. *Hum Immunol*, 1985; **13**: 105–116.
[16] Ottenhoff TH, Neuteboom S, Elferink DG, de Vries RR. Molecular localization and polymorphism of HLA class II restriction determinants defined by Mycobacterium leprae-reactive helper T cell clones from leprosy patients. *J Exp Med*, 1986; **164**: 1923–1939.
[17] Mutis T, Cornelisse YE, Datema G *et al.* Definition of a human suppressor T-cell epitope. *Proc Natl Acad Sci U S A*, 1994; **91**: 9456–9460.
[18] Ferrara G, Losi M, D'Amico R *et al.* Use in routine clinical practice of two commercial blood tests for diagnosis of infection with Mycobacterium tuberculosis: a prospective study. *Lancet*, 2006; **367**: 1328–1334.
[19] Pai M, Kalantri S, Dheda K. New tools and emerging technologies for the diagnosis of tuberculosis: part I. Latent tuberculosis. *Expert Rev Mol Diagn*, 2006; **6**: 413–422.
[20] Spencer JS, Marques MA, Lima MC *et al.* Antigenic specificity of the Mycobacterium leprae homologue of ESAT-6. *Infect Immun*, 2002; **70**: 1010–1013.
[21] Spencer JS, Kim HJ, Marques AM *et al.* Comparative analysis of B- and T-cell epitopes of Mycobacterium leprae and Mycobacterium tuberculosis culture filtrate protein 10. *Infect Immun*, 2004; **72**: 3161–3170.
[22] 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.
[23] Geluk A, van Meijgaarden KE, Franken KL *et al.* Immunological crossreactivity of the Mycobacterium leprae CFP-10 with its homologue in Mycobacterium tuberculosis. *Scand J Immunol*, 2004; **59**: 66–70.
[24] Cole ST, Eiglmeier K, Parkhill J *et al.* Massive gene decay in the leprosy bacillus. *Nature*, 2001; **409**: 1007–1011.
[25] Monot M, Honore N, Garnier T *et al.* Comparative genomic and phylogeographic analysis of *Mycobacterium leprae*. *Nat Genet*, 2009; **41**: 1282–1289.
[26] 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.
[27] 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.
[28] Geluk A, 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.
[29] Araoz R, Honore N, Cho S *et al.* Antigen discovery: a postgenomic approach to leprosy diagnosis. *Infect Immun*, 2006; **74**: 175–182.
[30] Dockrell HM, Brahmbhatt 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.
[31] Geluk A, Duthie MS, Spencer JS. Postgenomic Mycobacterium leprae antigens for cellular and serological diagnosis of M. leprae exposure, infection and leprosy disease. *Lepr Rev*, 2011; **82**: 402–421.
[32] 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.
[33] 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.
[34] Geluk A, Bobosha K, van der Ploeg-van Schip JJ *et al.* New biomarkers with relevance to leprosy diagnosis applicable in areas hyperendemic for leprosy. *J Immunol*, 2012; **188**: 4782–4791.

[35] Martins MV, Guimaraes MM, Spencer JS *et al.* Pathogen-Specific Epitopes as Epidemiological Tools for Defining the Magnitude of *Mycobacterium leprae* Transmission in Areas Endemic for Leprosy. *PLoS Negl Trop Dis*, 2012; **6**: e1616.
[36] 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.
[37] Araoz R, Honore N, Banu S *et al.* Towards an immunodiagnostic test for leprosy. *Microbes Infect*, 2006; **8**: 2270–2276.
[38] Wallis RS, Pai M, Menzies D *et al.* Biomarkers and diagnostics for tuberculosis: progress, needs, and translation into practice. *Lancet*, 2010; **375**: 1920–1937.
[39] Bobosha K, van der Ploeg-van Schip JJ, Esquenazi DA *et al.* Peptides Derived from *Mycobacterium leprae* ML1601c Discriminate between Leprosy Patients and Healthy Endemic Controls. *J Trop Med*, 2012; **2012**: 132049.
[40] Ruhwald M, Bjerregaard-Andersen M, Rabna P *et al.* IP-10, MCP-1, MCP-2, MCP-3, and IL-1RA hold promise as biomarkers for infection with M. tuberculosis in a whole blood based T-cell assay. *BMC Res Notes*, 2009; **2**: 19.
[41] Ruhwald M, Dominguez J, Latorre I *et al.* A multicentre evaluation of the accuracy and performance of IP-10 for the diagnosis of infection with M. tuberculosis. *Tuberculosis (Edinb)*, 2011; **91**: 260–267.
[42] Sampaio LH, Sousa AL, Barcelos MC *et al.* Evaluation of various cytokines elicited during antigen-specific recall as potential risk indicators for the differential development of leprosy. *Eur J Clin Microbiol Infect Dis*, 2012; **31**: 1443–1451.
[43] Berry MP, Graham CM, McNab FW *et al.* An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. *Nature*, 2010; **466**: 973–977.
[44] Joosten SA, Goeman JJ, Sutherland JS *et al.* Identification of biomarkers for tuberculosis disease using a novel dual-color RT-MLPA assay. *Genes Immun*, 2012; **13**: 71–82.
[45] Montoya D, Modlin RL. Learning from leprosy: insight into the human innate immune response. *Adv Immunol*, 2010; **105**: 1–24.
[46] Corstjens PL, Zuiderwijk M, Tanke HJ *et al.* A user-friendly, highly sensitive assay to detect the IFN-gamma secretion by T cells. *Clin Biochem*, 2008; **41**: 440–444.
[47] 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.
[48] Sahiratmadja E, Alisjahbana B, de BT *et al.* Dynamic changes in pro- and anti-inflammatory cytokine profiles and gamma interferon receptor signaling integrity correlate with tuberculosis disease activity and response to curative treatment. *Infect Immun*, 2007; **75**: 820–829.
[49] Hirsch CS, Toossi Z, Othieno C *et al.* Depressed T-cell interferon-gamma responses in pulmonary tuberculosis: analysis of underlying mechanisms and modulation with therapy. *J Infect Dis*, 1999; **180**: 2069–2073.
[50] Hussain R, Kaleem A, Shahid F *et al.* Cytokine profiles using whole-blood assays can discriminate between tuberculosis patients and healthy endemic controls in a BCG-vaccinated population. *J Immunol Methods*, 2002; **264**: 95–108.
[51] Ellner JJ. Immunoregulation in TB: observations and implications. *Clin Transl Sci*, 2010; **3**: 23–28.
[52] Hasan Z, Jamil B, Khan J *et al.* Relationship between circulating levels of IFN-gamma, IL-10, CXCL9 and CCL2 in pulmonary and extrapulmonary tuberculosis is dependent on disease severity. *Scand J Immunol*, 2009; **69**: 259–267.
[53] Verreck FA, de BT, Langenberg DM *et al.* Phenotypic and functional profiling of human proinflammatory type-1 and anti-inflammatory type-2 macrophages in response to microbial antigens and IFN-gamma- and CD40L-mediated costimulation. *J Leukoc Biol*, 2006; **79**: 285–293.
[54] Jamil B, Shahid F, Hasan Z *et al.* Interferon gamma/IL10 ratio defines the disease severity in pulmonary and extra pulmonary tuberculosis. *Tuberculosis (Edinb)*, 2007; **87**: 279–287.
[55] Misra N, Selvakumar M, Singh S *et al.* Monocyte derived IL 10 and PGE2 are associated with the absence of Th 1 cells and in vitro T cell suppression in lepromatous leprosy. *Immunol Lett*, 1995; **48**: 123–128.
[56] Lima MC, Pereira GM, Rumjanek FD *et al.* Immunological cytokine correlates of protective immunity and pathogenesis in leprosy. *Scand J Immunol*, 2000; **51**: 419–428.