Challenges in immunodiagnostic tests for leprosy

Annemieke Geluk
Leiden University Medical Center (LUMC), Department of Infectious Diseases, Leiden, The Netherlands

Introduction: Despite the effectiveness of multidrug therapy, leprosy still represents a significant global health problem: transmission of *Mycobacterium leprae* (*M. leprae*) is not sufficiently reduced as witnessed by unavailing new case rates in leprosy-endemic countries. Early detection of *M. leprae* infection (before clinical manifestations occur) is vital to reduction of transmission. Current diagnosis relies on detection of clinical signs since there are no tests available to detect asymptomatic *M. leprae* infection or predict progression to leprosy.

Areas covered: Identification of risk factors (immunological or genetic biomarkers) for disease development and/or onset of leprosy reactions is imperative for efficient diagnosis. Tests simultaneously detecting biomarkers specific for cellular and humoral immunity are well suited for diagnosis of different clinical outcomes of leprosy. This review describes the challenges of discovery of biomarkers for *M. leprae* infection and their implementation in field-friendly tests.

Expert opinion: In view of the complicated nature of *M. leprae* infections, it is essential to invest in longitudinal studies allowing intra-individual comparison of immune and genetic biomarkers in various leprosy-endemic areas. Furthermore, the effect of co-infections on biomarker profiles should also be taken into account. Diagnostic tests based on such biomarkers can contribute significantly to early detection of leprosy (reactions) thus helping reduce nerve damage.

Keywords: biomarkers, cytokines, early diagnosis, field-friendly assays, leprosy, *M. leprae*, recombinant proteins

1. Introduction

Leprosy, also known as Hansen’s disease, is a chronic immune-pathogenic infectious disease that is infamous for its great disfiguring capacity and still represents a public health problem in many regions worldwide. Its etiologic agent, the intracellular *Mycobacterium leprae* (*M. leprae*), ranks as the second most pathogenic mycobacterial infectious disease after tuberculosis (TB). *M. leprae* has a predilection for dermal macrophages in the skin and Schwann cells of the neuronal sheath in the peripheral nervous system [1]. This latter tropism is a major cause of sensory loss and sensorimotor dysfunction often resulting in severe, life-long disabilities and deformities, the hallmark of leprosy [1-3]. Leprosy has afflicted humanity for ages and still affects over 200,000 new patients annually. It is strongly associated with poverty inflicting severe human suffering in communities and populations in underprivileged and under-resourced settings. Notwithstanding a spectacular decrease in global prevalence since 1982, it consistently remains a public health problem in 32 countries, mostly in Africa, Asia and South America [4]. In addition, treated patients, although not included in statistics anymore, still suffer from handicaps as well as social stigma.
Transmission of leprosy is sustained as evidenced by the quite static number of globally detected new cases: in 2010, 228,474 new cases, representing 625 individuals per day, were detected among whom 20,472 were children [4]. Understanding the mode of \textit{M. leprae} transmission has been complicated due to the long incubation time of leprosy as well as the lack of tests that can detect asymptomatic \textit{M. leprae} infection, a presumed major source of transmission, or predict possible progression of infection to clinical disease. Furthermore, in view of changes in leprosy control programs leading to a decrease in special expertise required for diagnosis of leprosy, the development of rapid diagnostic tests that detect \textit{M. leprae} infection currently represents an urgent topic on the leprosy research agenda.

This review discusses the quest for \textit{M. leprae}-specific immune responses that can be applied in field friendly diagnostic tests for leprosy.

2. Leprosy diagnosis

2.1 Leprosy spectrum

The characteristic interindividual variability in clinical manifestations of leprosy closely parallels the hosts’ abilities to mount effective immune responses to \textit{M. leprae}. This is evidenced by the natural resistance to leprosy in most infected individuals, which is accompanied by high cellular immune reactivity against \textit{M. leprae}. In addition, this is also clear from the immunological and clinical leprosy spectrum [5] in those who progress to disease despite there being minimal genetic variation between \textit{M. leprae} isolates reported. This spectrum ranges from tuberculoid (TT/ BT) or paucibacillary (PB) leprosy to lepromatous (LL/BL) or multibacillary (MB) leprosy. TT/ BT patients in general show high cellular responses to \textit{M. leprae} antigens injected in the skin as well as in \textit{in vitro} T-cell assays [6,7], have low antibody titers to \textit{M. leprae} antigens and develop localized granulomatous disease with often few if any detectable bacilli in their lesions. At the opposite pole of the spectrum are LL/BL patients, with a characteristic inability to generate \textit{M. leprae}-specific Th1 (T-helper 1) cell responses, high IgM titers to \textit{M. leprae} PGL-I and with disseminating progressive infection. The borderline states of leprosy are positioned in between and are immunologically rather unstable and prone to the occurrence of leprosy reactions, reversal reactions (RR; type 1) and erythema nodosum leprosum (ENL; type 2).

The different outcomes of infection in leprosy are most likely caused by host defense mechanisms, which are still not completely understood in terms of their pathogenic effects [2,3]. Important questions thus arise including determining which genetic and/or environmental factors dictate these very different outcomes of \textit{M. leprae} infection and whether biomarker profiles can be derived that predict the type of leprosy and/or the occurrence of leprosy reactions [8].

2.2 Genetics of leprosy

The host genetic background is an important risk factor for leprosy susceptibility as determined in population and twin studies [9]. In this respect, some genes control susceptibility \textit{per se}, whereas other genes control clinical manifestation of the disease. Also, genetic variants of \textit{TLR1} [10] and \textit{TLR2} [11] genes were yet again reported to determine the occurrence of leprosy type 1 reactions [8].

Strong associations have been found between leprosy and HLA genes (HLA-DRB1 and HLA-DQA) [9,12-15], HLA-linked genes (\textit{TAP}, \textit{MICA} and \textit{MICB}) as well as genes located in the HLA region (\textit{TNFA}). Furthermore, linkage and association studies have implicated variants of \textit{PARK2} [16], \textit{LTA} [17], \textit{IL-10} [18], \textit{VDR} [19], chromosomes 10p13 [20] and 17q22 [21] in conferring leprosy susceptibility.

A recent genome-wide study in China [22] showed that 93 SNPs (single nucleotide polymorphisms) have a significant association with leprosy: testing of these SNPs in more than 3000 leprosy patients and nearly 6000 controls implicated genes like \textit{CCDC12}, \textit{C13orf31}, \textit{NOD2}, \textit{TNFSF15}, \textit{RIP2K} and the \textit{HLA-DR-DQ} locus as leprosy susceptibility genes. Interestingly, this study also demonstrated the apparent genetic link between susceptibility to leprosy and Crohn’s disease, since several genes (\textit{NOD2}, \textit{TNFSF15} and \textit{C13orf31}) confer susceptibility to both diseases.

Human transcriptome arrays, on the other hand, can also offer cutting-edge tools to identify and assess gene expression profiles for leprosy subtypes providing more insight into the mechanism of \textit{M. leprae} infection [23].

In order to apply gene expression profiles to field-friendly diagnostic tests for leprosy, functional protein association networks, using disease-specific gene expression profiles as biomarkers, need to identify compounds that can be stably detected in blood or urine. Of note in this respect is that the leprosy susceptibility region 17q2 is known to carry susceptibility gene(s) for intra-macrophage pathogens including candidates MCP-1 and MIP-1\textbeta which encode proteins that were shown to be abundantly produced by immune cells of \textit{M. leprae}-infected individuals [24]. Future studies at endemic sites worldwide will focus on new biomarkers derived from such gene expression profiles for leprosy as well as leprosy reactions.

2.3 Serology-based laboratory assays for leprosy control

The basis of leprosy control has been case detection and treatment since the introduction of dapsone in the 1950s. However, the methods of case detection have in most areas gradually changed from active population-based and school surveys to more passive approaches based on awareness and voluntary reporting [25,26] thereby reducing the possibilities of early detection of subclinical cases and the spread of infection [27,28]. 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.
Challenges in immunodiagnostic tests for leprosy

There are no sensitive tests available to detect leprosy or *M. leprae* infection. In addition, the dearth of longitudinal studies has also prevented the development of tests that allow prediction of leprosy. Thus, current diagnosis still relies on clinical observations combined with time-consuming invasive tests to confirm acid-fast bacilli in slit skin smears or immunopathological changes in biopsies of skin lesions for determining the presence of the disease and its classification [5].

The presence in sera of IgM antibodies against phenolic glycolipid-I (PGL-I), an *M. leprae*-specific cell-surface antigen [29,30], has shown potential for classification of leprosy patients as well as identification of the risk of relapse and of developing disease in healthy household controls (HHC) [31]. This has resulted in development of field-friendly, kit-based tests used for leprosy diagnostics [31-34]. Although PGL-I-based serodiagnostic tests are positive in nearly all leprosy patients with high bacillary loads, most PB leprosy patients do not develop detectable antibody levels against PGL-I, almost 50% of those with positive anti-PGL-I IgM responses never develop leprosy and many of those who develop leprosy do not have PGL-I antibodies [35-39]. Besides serology for PGL-I, the diagnostic potential of other antigens such as LAM (lipoarabinomannan) and secreted proteins such as Ag85 (ML2028) and CFP-10 (ML0050) has been tested in serologic assays as well [40,41]. In addition, IgG antibody responses directed against *M. leprae*-specific recombinant proteins can be used as supplemental tools for diagnostic serology for leprosy: similar to the IgM response against PGL-I, these IgG responses generally correlate with the bacterial load and hence are detected mostly in MB patients [42]. Moreover, retrospective analysis [43] showed detection of antibody responses to a fusion protein of ML0405 and ML2331 (designated LID-1) [44,45] in leprosy patients a year in advance of disease symptoms [45]. Nevertheless, IgG responses to the *M. leprae* proteins tested are weak or absent in PB patients [24] nor is it possible to predict the occurrence of leprosy reactions using antibody titers against *M. leprae* antigens [46,47]. Thus, the characteristics of the leprosy disease spectrum, in which both cellular and humoral immunity against *M. leprae* determine the outcome of infection, necessitate a diagnostic test simultaneously detecting biomarkers specific for both types of immune responses.

2.4 Cellular immunity to *M. leprae*

Protective immune responses against *M. leprae* are based on CD4+ Th1 cells [48], characterized by the secretion of the innate and adaptive cytokines lymphotixin-αβ, IL-12p70 and IFN-γ and (moderate levels) other pro-inflammatory cytokines such as TNF which synergize to activate microbicidal effector mechanisms in human macrophages [49]. CD4+ Th1 cells also predominate in lesions of patients with TT/ BT leprosy, whereas they are reduced in lesions of BL/LL patients [2,50] who secrete predominantly anti-inflammatory mediators such as IL-10, accompanied by the absence of IFN-γ and other Th1-associated cytokines in response to *M. leprae* antigens. Other pro-inflammatory cytokines, including those that may well be produced by Th17 rather than Th1, involve IL-1β, IL-6, TGF-β and IL-23 (Figure 1) and are produced by leprosy patients during reactions as well [51,52]. Finally, regulatory T cells (Treg) [53], the first of which in humans were described in leprosy [54,55], are believed to play a role in the *M. leprae*-specific unresponsiveness encountered in LL patients.

Since IFN-γ is a stable cytokine, it is widely used as a surrogate marker for pro-inflammatory immunity against mycobacteria [56]. More recently, IFN-γ-induced protein 10 (IP-10) has been shown to be a useful biomarker for diagnosis of *Mycobacterium tuberculosis* infection as well [57] and can also be used in HIV-infected patients since, unlike IFN-γ, IP-10 is not affected by low CD4 counts in TB patients with HIV [58].

More than a decade ago, the *M. leprae* genome sequence was unraveled by Cole *et al.* [59]. Since the pre-genomic era had not led to identification of *M. leprae* antigens applicable in IGRA (IFN-γ release assay) for leprosy diagnostics similar to the (QuantiFERON-TB-Gold-In-Tube®) [60-62], this catalyzed the identification of genes encoding *M. leprae*-unique (hypothetical) antigens representing potential candidate immunodiagnostic tools for leprosy.

Therefore, *in vitro* T-cell stimulation assays, predominantly assessing IFN-γ production were used to test such antigens for their potential as diagnostic tools for humans [63-69] as well as in animal models for leprosy in mice and in armadillo [70,71] (reviewed in [41]). Besides being selected for their exclusive presence in *M. leprae* to avoid T-cell cross-reactivity with BCG-vaccinated or *M. tuberculosis*-infected individuals, most of these *M. leprae*-unique proteins induced IFN-γ responses in endemic control (EC) individuals [24,67,68]. However, since these EC were living in areas with pockets of high leprosy prevalence, the observed cellular responses toward the *M. leprae*-unique proteins may still have indicated *M. leprae* specificity but could be irrelevant to pathogenic cellular immunity that leads to leprosy. By contrast, production of IFN-γ as well as IP-10 in response to *M. leprae* proteins or peptides was able to differentiate between EC groups derived from areas with different leprosy prevalence. This allowed identification of distinct degrees of *M. leprae* exposure and thereby the risk of infection and subsequent transmission [72-74].

In summary, despite discriminatory IFN-γ/IP-10 profiles observed between different types of leprosy [64,65,68], and between EC*high* and EC*low* [24,72], no *M. leprae* proteins have been identified yet that can distinguish TT/ BT patients from EC based on IFN-γ/IP-10 production when both groups are derived from the same leprosy hyperendemic area and have identical socioeconomic status.

2.5 Exposure, early infection and disease: new biomarkers

Infection is the invasion of a host organism’s bodily tissues by disease-causing organisms, their multiplication and the reaction of host tissues to these organisms. On the other hand,
exposure is defined as contact of an organism with a harmful agent. In order to establish appropriate biomarkers for leprosy, it is important to define the different stages of *M. leprae* infection and the type of immunity associated with these stages as clearly as possible (Figure 2).

Since it remains a topic of debate whether increased IFN-γ production measured in response to *M. leprae* (-unique) antigens correlates either with protection against infection or with (progression to) disease, other analytes, measurable in serum such as cytokines and chemokines, need to be investigated as potential biomarkers. In this respect, it is rather unlikely that only a single cytokine or chemokine is linearly correlated to protection or to disease, since host immunity and immunopathogenicity in response to *M. leprae* involves complex interactions between a variety of cells expressing different effector and regulatory molecules.

If the host is capable of eliminating *M. leprae* without priming of adaptive immunity (T or B cells), cytokines and chemokines measured in WBA (whole blood assay) reflect innate immune responses (stage 1) caused by, for example, natural killer (NK) cells, macrophages or neutrophils. In case a combination of specific T cells and innate immune cells cause elimination of *M. leprae* bacilli, WBA can additionally identify biomarkers for adaptive immunity (stage 2). Alternatively, if *M. leprae* is replicating and invading the host without causing clinical symptoms (yet), biomarkers for early infection would be detected (stage 3). These consist of innate as well as adaptive biomarkers while the latter differ in quality or quantity from those detected in stage 2 where they lead to clearance of infection. Finally, when *M. leprae* is disseminating the host’s body (BL/LL) or causes harmful T-cell responses resulting in lesions (TT/BT), pathogenic immune responses predominate and the chronic battle field between host and pathogen ends in favor of *M. leprae*. At this stage, WBA detect the result of pathogenic as well as protective immune responses as present in leprosy patients (stage 4), being either caused by vigorous T-cell responses in TT/BT or suppressive T cells in LL/BL patients [55,75]. In stage 4, similar to stage 2 and 3, biomarkers produced by innate immune responses will also be present as is the case in TB [76,77]. By comparison of cellular immune markers such as cytokines and chemokines and also genetic markers [78] in leprosy patients, their contacts and endemic and non-endemic healthy individuals, disease-specific biomarkers can likely be deduced.

Identification of biomarker profiles is influenced especially by the inclusion of EC and the leprosy prevalence of the area where they reside [24,72]. Biomarker studies including leprosy patients and HHC but lacking different EC groups [79] are less likely to identify markers for protective innate immunity (stage 1) such as chemokines secreted by activated macrophages [80,81], since recent studies show that HHC, who are frequently and intensely exposed to *M. leprae*, produce similar cytokines and chemokines as found for TT/BT patients [24,79]. By contrast, inclusion of EC from leprosy-endemic and non-endemic areas allowed identification of cellular host biomarkers (macrophage inflammatory protein-1β (MIP-1β), monocyte chemotactic protein-1 (MCP-1) and IL-1β) that were significantly increased in leprosy patients compared with EC in an endemic area [24].

Although IL-1β, MCP-1 and MIP-1β are secreted by innate cells like monocytes, macrophages and dendritic cells (DCs), cytokine secretion, antigen-presenting capacity and

---

**Figure 1.** Schematic representation of CD4+ T cells (possibly) involved in the leprosy disease spectrum. Transcription factors (T-bet, GATA3, RORγt and FoxP3) for all T-cell types are indicated.

APC: Antigen-presenting cell; aPGL-I: Anti-PGL-I IgM antibodies.
expression of cell surface molecules of these cells are influenced by mycobacterial infection [82,83]. Recent interest in the way adaptive and innate immunity may influence each other has even brought about the concept of ‘memory’ within the innate immune system [84-86]. MCP-1 has been well known as an effective promoter of macrophage infiltration in various inflammatory models of disease and host defense against several pathogens and is absolutely required for the formation of granulomas [87], which in return are associated with strong T-cell immunity. In addition, MCP-1 is considered an accurate indicator of inflammation and is under investigation as a therapeutic target for various inflammatory diseases, for example, atherosclerosis, and diabetes-associated kidney disease.

Thus, differential expression of MCP-1 between TT/BT leprosy patients and EC [24] could be due to the inflammation caused by *M. leprae* infection as present in patients. In *in vitro* knockout experiments [88], changes in the MCP-1 production were found to be controlled by expression of the protein product of PARK2 [16], a leprosy susceptibility candidate gene, thereby indicating the link between MCP-1 levels and *M. leprae* infection [24].

MIP-1β is secreted not only by cells of the innate system but has been shown to be secreted by T cells [89] as well. In general, the synthesis and release of MIPs require cell activation by various agents such as TNF-α, IFN-γ and IL-16/β [90]. It is therefore reasonable to speculate that differences in MIP-1β reflect differences in mycobacteria-specific T-cell recall responses between EC and leprosy patients in general.

IL-1β released from activated DCs is responsible for inducing differentiation of Th1 cells [91]. It has been demonstrated that IL-1β is crucial in the *Leishmania* disease mouse model: susceptible BALB/c mice produce significantly less IL-1β than resistant C57BL/6 strain. IL-1β also plays a major role in host resistance to *M. tuberculosis* [92] and is involved in the TLR2/1-induced vitamin D antimicrobial pathway leading to induction of the antimicrobial peptide defensin β4A [23]. Thus, differential expression of IL-1β by DCs may regulate development of characteristic helper T-cell responses (Th1 vs Th2) in leprosy where clinical outcome depends predominantly on the balance between Th1 versus Th2 responses and reduced expression of the IL-1β gene was reported for lesions of LL patients [23] who typically lack good cellular response. Therefore, differences in IL-1β secretion, although an innate response, are likely to exist and can demonstrate an association with early exposure and subclinical infection with *M. leprae*.

In summary, although seemingly counterintuitive, it is plausible that innate immune responses differ between patients and EC in the same, highly endemic area as a result of *M. leprae*-specific adaptive immunity.

Besides 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 IFN-ab signaling pathways [77]. In addition, a biomarker profile composed of several genes was identified for infection in a follow-up study of TB contacts using reverse transcription multiplex ligation-dependent probe amplification (RT-MLPA) assays [78]. Such studies are currently performed for leprosy as well as leprosy reactions in several endemic sites worldwide. Combined with functional protein association networks, transcriptomic expression profiles [93] can be used to identify proteins discernibly in blood that may subsequently be applied as biomarkers in field-friendly assays.
2.6 New assays and their challenges

The integration of biomarker profiles into multiplexed diagnostic tools remains another important challenge: leprosy-endemic areas are often short of sophisticated laboratories, necessitating development of field-friendly diagnostic tests. Due to their simple and fast nature, like the IGRA-based commercially available assays for diagnosis of TB [61], WBA are much more convenient resource-poor settings than conventional assays using peripheral blood mononuclear cells (PBMC). On top of that, detection of anti-PGL-I antibodies is typically performed in serum and not influenced by incubation with *M. leprae* (-derived antigens), which conveniently accommodates for combined analysis of cellular and humoral markers in one sample after 24 h [94].

Recently, quantitative and user-friendly lateral flow assays for detection of IFN-γ, IL-10 and anti-PGL-I IgM were reported based on up-converting phosphor technology (UCP-LF) [94,95]. Notably, this format also allowed simultaneous quantification of cellular and humoral responses in one sample. Although development of such a test will require intense evaluation in large-scale studies before routine application at field sites, this multiplex format could eventually have significant advantages for classification of leprosy status and monitoring the effects of interventions like multidrug therapy (MDT) or vaccines.

3. Expert opinion

Diagnosis and prognosis as well as drug monitoring remain urgent challenges in the leprosy field. The lack of laboratory tests that allow detection of asymptomatic *M. leprae* infection, allegedly a principal source of transmission, as well as tests for prediction of progression of infection to clinical disease, urges us to develop new diagnostic tests for leprosy. In the light of the changed focus in the leprosy control programs as well as for case management, user-friendly diagnostic tests based on simultaneous detection of biomarkers characteristic for Th1, Th2 as well as anti-PGL-I IgM responses would be beneficial to early detection of leprosy and possibly help reduce serious nerve damage.

Considering the complex nature of the immune response against *M. leprae*, development of diagnostic tools for leprosy requires identification of biomarker profiles characteristic for different stages of *M. leprae* infection (Figure 2). However, the stages of infection as well as their concomitant biomarker profiles can overlap appreciably causing, for example, both protective and pathogenic immune responses to be detected in one sample. Consequently, it is exigent to identify thresholds for biomarker profiles above which a certain infection stage and clinical status prevails.

An elementary factor in future biomarker studies therefore is to include the proper control groups to become aware of responses that are due to exposure to *M. leprae* and not necessarily reflect pathogenic responses due to *M. leprae* infection [24,72-74]. Thus, EC with a different social/economic status than that of leprosy patients should only be studied in conjunction with EC derived from the same community as the leprosy patients.

This kind of approach has resulted in identification of *M. leprae*-unique proteins that, combined with IFN-γ or IP-10 as read-out, can be applied as biomarker tools to measure *M. leprae* exposure [24,72]. For the other site of the leprosy spectrum, additional *M. leprae* antigens, not necessarily unique for *M. leprae*, were identified that induce serological responses specific for MB patients [43,96].

Since the majority of those exposed to *M. leprae* develop a protective immune response against the bacterium, a second elementary factor in studying biomarkers for leprosy concerns HHC, a group of individuals that is in particular at high risk of developing leprosy due to their frequent and intense exposure to *M. leprae*. In case there are no significant differences in biomarker profiles observed between HHC and BT/TT [24,79], the biomarker profile of HHC who are frequently and intensely exposed to *M. leprae*, requires longitudinal follow-up to identify predictive biomarkers. Heterogeneous responses within an HHC group [24] could indicate that some individuals in this group may induce pathogenic immune responses similar to BT/PT patients. Therefore, investments in 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 which biomarkers correlate with progression to disease and may be used as predictive biomarkers.

Longitudinal analysis not only is essential for the identification of biomarkers for (type of) leprosy but also for the identification of immune or genetic biomarker profiles that predict the occurrence of leprosy reactions [47,97,98]. Especially this aspect of leprosy calls for longitudinal intra-individual comparison since baseline levels may vary between patients [100]. Several studies are currently underway to analyze biomarker for leprosy reactions.

Finally, infections with HIV [100-104], Th2-inducing parasites [105] but also diabetes [106] will effect biomarker profiles of *M. leprae* affected individuals, thereby complicating diagnosis. Thus, more elaborate studies on the differential effects of co-infections in leprosy ought to have attention as well. Considering the similarities in the challenges that are faced in the TB research [78,93,107-109], identification of new genetic as well as immune biomarkers would benefit from integrated efforts of the leprosy and TB research communities.

Acknowledgement

The author apologizes to all colleagues whose work could not be discussed due to space limitations, and particularly thanks the IDEAL (Initiative for Diagnostic and Epidemiological Assays for Leprosy) members for their contributions and T Ottenhoff for stimulating discussions. The text represents the author’s view and does not necessarily represent a position of the funding agencies that will not be liable for information or views expressed herein. LUMC is part of the IDEAL Consortium.
Declarations of interest

The author has received grants from the Netherlands Leprosy Relief Foundation (NLR), the Turing Foundation, the Q.M. Gastmann-Wichers Foundation, the Order of Malta-Grants-for-Leprosy-Research (Magraprele) and EDCTP (European and Developing Countries Clinical Trials Partnership) through a project entitled AE-TBC under Grant Agreement N° IP_09_32040.

Bibliography

Papers of special note have been highlighted as either of interest (●) or of considerable interest (★★) to readers.

5. Ridley DS, Jopling WH. Classification of leprosy according to immunity. A five-group system. Int J Lepr Other Mycobact Dis 1966;34:255-73
8. Early work on the correlation between cell mediated immunity and the different clinical forms of leprosy.
   e. Describes two SNPs on the PARK2/ PACRG genes to be associated with leprosy susceptibility per se.
15. Describes the HLA genes as leprosy genetic risk factors.
23. Smith C, Richardus JH. Leprosy strategy is about control, not eradication. Lancet 2008;371:969-70
28. 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;82:344-57


Challenges in immunodiagnostic tests for leprosy


First lateral flow test detecting cellular and humoral immune responses against M. leprae.


96. Duthie MS, Hay MN, Rada EM, et al. Specific IgG antibody responses may be used to monitor leprosy treatment efficacy and as recurrence prognostic markers. Eur J Clin Microbiol Infect Dis 2011;30(10):1257-65


HIV/M. leprae co-infections.


Helminth/M. leprae coinfection.


Affiliation
Annemieke Geluk
Leiden University Medical Center (LUMC), Department of Infectious Diseases, PO Box 9600, 2300 RC Leiden, The Netherlands
Tel: +31 71 526 1974; Fax: +31 71 526 6758; E-mail: a.geluk@lumc.nl