Introduction

Leprosy is one of six diseases the World Health Organization (WHO) considers a major threat in developing countries and often results in severe, life-long disabilities and deformities due to delayed diagnosis (1). Notable epidemiological features of global leprosy today include a continued new case detection rate of approximately 225,000 per year and indirect evidence suggesting that millions of unreported cases linger undetected (2, 3, 4) (see Chapter 1.1). As a result of numerous endemic countries reaching the 2001 WHO elimination goal of 1 case per 10,000 population, leprosy control activities have become integrated into general health delivery networks. This integration has had a negative effect on the number of trained leprologists and laboratory technicians, leading to an increase in misdiagnosis and a failure to treat early and appropriately. Operational policies for diagnosis adopted by some countries, based on new case validation by a leprologist with extensive expertise in clinical leprosy diagnosis, could slow the initiation of treatment. It has been estimated that this type of policy for leprosy diagnosis in India has led to approximately 26% of suspect cases awaiting confirmation of diagnosis for up to 8 months after their initial primary health care visit (5).
Delayed or missed diagnosis are occurring in non-endemic areas as well due to increased migration and the reliance on health care systems in developed countries where clinicians rarely encounter leprosy cases and initial misdiagnosis is frequent (6, 7, 8, 9). Collectively, these developments demonstrate that the active transmission of *M. leprae* continues unabated in the face of an antibiotic-based leprosy elimination strategy. Clearly, more effort is needed to understand the relationship between transmission and the continuing reservoir of *M. leprae* in infected contacts, the environment, and individuals with subclinical leprosy.

**EARLY DIAGNOSIS**

The early detection of the *M. leprae* infection, followed by effective intervention, is considered a vital component of strategies aiming at reducing the transmission of bacteria. In this respect, the lack of tests for identifying individuals with asymptomatic *M. leprae* infection as well as subclinical, undiagnosed leprosy, likely the two major sources of unidentified transmission, not only causes delayed or missed diagnoses, but also hampers the reduction of transmission.

Currently available diagnostic tools often lack sufficient sensitivity and specificity to reach the goal of early detection. An important aim, therefore, is the development of improved diagnostic tools that are able to detect *M. leprae* infections before clinical manifestations occur, especially in household contacts (HHC) of leprosy patients, who constitute the group at the highest risk for developing leprosy. Moreover, such new diagnostic tools should distinguish an *M. leprae* infection from a routine BCG vaccination or an infection with other mycobacteria, such as *M. tuberculosis* and (nonpathogenic) environmental mycobacteria.

In order to identify those individuals at risk of developing the disease themselves or those who are able to transmit *M. leprae*, measurable risk factors for asymptomatic *M. leprae* infection and pre-clinical disease must be determined objectively. These factors can be used as tools for the early detection of leprosy or the prediction of progression to disease in infected individuals. Such tools or biomarkers are defined as characteristics that can be objectively measured and evaluated as indicators of a normal biological process, a pathogenic process, or a pharmacologic response to a therapeutic intervention. Biomarkers can be utilized for informed decision-making regarding who needs treatment at a pre-clinical stage, thereby enabling targeted interventions to reduce transmission.

**IMMUNITY TO *M. LEPRAE***

Leprosy represents an intriguing model of human immunoregulatory disease, since the interindividual variability in its clinical manifestations remarkably and closely parallels the ability of the host to mount an effective cellular mediated immune response to *M. leprae* (10, 11, 12). This variability is evidenced by the natural resistance to leprosy of most individuals exposed to
this mycobacterium, which is accompanied by a high (innate) cellular immune reactivity against \textit{M. leprae} (13). In addition, it is apparent from the immunological and clinical leprosy spectrum in those who progress to the disease despite there being minimal genetic variation between \textit{M. leprae} isolates reported. This spectrum ranges from tuberculoid (TT) leprosy to lepromatous (LL) leprosy (14) (see Chapter 2.1). TT 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 (15, 16); 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 patients unable to generate \textit{M. leprae} specific Th1 cell responses, instead producing high IgM titers to \textit{M. leprae} PGL-I with resulting disseminating, progressive infections. The borderline states of leprosy (BL, BB, and BT) are positioned in between and are rather unstable immunologically and prone to the occurrence of leprosy reactions, which are divided into type 1/reversal reactions (RR) and type 2/erythema nodosum leprosum (ENL) (see Chapter 2.2).

The different outcomes of infection with leprosy are most likely caused by host defense mechanisms, which are dominated by the innate and adaptive immune responses (10, 11, 17) (see Chapter 6.1; Chapter 6.2). After recognition of the bacteria by Toll-like receptors (TLRs), NF-kB is activated, resulting in the upregulation of pro-inflammatory cytokines (GM-CSF, IL-1β, TNF-α, IP-10, IL-12) and chemokines inducing the migration and activation of antigen presenting cells (APCs) such as macrophages. Subsequently, APCs migrate to the lymphoid organs in order to present mycobacterial antigens to naïve T cells. Depending on different co-stimulatory or inhibitory molecules and cytokines, different T cells will develop, which may vary from CD4$^+$ Th1 or Th2, CTL, or Th17 cells. Finally, regulatory T cells (Treg), of which the first human variants were described in leprosy (18, 19), are believed to play a role in the \textit{M. leprae}-specific unresponsiveness encountered in LL patients (20, 21) (see Figure 1).

Host resistance to \textit{M. leprae} is associated with the emergence of a protective Th1-based response dominated by CD4$^+$ Th1 cells and characterized by the secretion of pro-inflammatory cytokines (17). Th1 cells produce IFN-γ and TNF-α, which synergize to activate microbicidal effector mechanisms in human macrophages. In LL and TT leprosy patients, imbalances in cytokine homeostasis in response to \textit{M. leprae} have been reported (22). LL patients secrete predominantly anti-inflammatory mediators such as IL-10, accompanied by the absence of IFN-γ and other Th1-associated cytokines, in response to \textit{M. leprae} antigens and mostly lack CD4$^+$ T cells in their lesions. Instead, Th2 T cells producing IL-4, IL-5, and IL-13 dominate in these patients, leading to the production of antibodies as well as IL-4-induced dysregulation of host defenses against \textit{M. leprae} (23). Conversely, CD4$^+$ Th1 cells predominate in lesions of TT leprosy and these patients produce exacerbated levels of pro-inflammatory cytokines (IFN-γ, IL-15). The cytokines include those produced by Th17 rather than Th1, possibly driven by strong innate immune activation resulting in the release of IL-1β and/or IL-6, TGF-β, and IL-23, all of which are involved in Th17 (24) induction. Since the outcome of the immune response to \textit{M. leprae} is determined by chemokines and cytokines that act as molecular signals for communication between the cells of the immune system, they are informative tools to predict either protection from or progression to the disease.
Immunodiagnostics

SEROLOGY-BASED

The diagnosis and classification of leprosy is largely based on clinical assessment, e.g., hypo-pigmented, anaesthetic skin patches and enlarged affected nerves, and on microscopic detection of acid-fast bacilli in skin slit smears or biopsies of suspected cases (see Chapter 2.1; Chapter 1.1). However, none of these diagnostic approaches are able to detect asymptomatic M. leprae infections. Besides the clinical approach, there are also diagnostic techniques that are based on immunological responses directed against M. leprae. In particular, several lateral flow-, dipstick-, and particle agglutination tests that incorporate the synthetic di- or trisaccharide epitope of phenolic glycolipid-I (PGL-I) have been used in field-based studies (25, 26, 27). The anti-PGL-I antibody (Ab) ELISA test is based on the detection of IgM antibodies against PGL-I, an M. leprae specific, dominant glycolipid component of the cell wall (see Chapter 5.1). Although this test is useful for...
the detection of most multibacillary (MB) patients (28, 29), as the antibody levels correlate well with the bacillary load, the detection of anti-PGL-I Ab has limited value in identifying PB leprosy patients (30). Moreover, in areas hyper-endemic for leprosy, more than 50% of young schoolchildren surveyed had positive anti-PGL-I responses (31). Still, the vast majority of individuals with a positive antibody titer will never develop leprosy (25), despite the fact that the presence of anti-PGL-I antibodies was determined to be a risk factor for the development of leprosy (32, 33).

In addition to IgM antibodies directed against \textit{M. leprae} antigens, IgG antibodies against a fusion protein of \textit{M. leprae} antigens ML0405 and ML2331 designated leprosy IDRI Diagnostic (LID)-1 have been studied (34, 35, 36, 37). Antibody responses towards LID-1 have been shown to be positive in over 95% of those at the lepromatous end of the spectrum (38). In a prospective study involving household contacts of MB patients whose serum antibody responses were analyzed retrospectively to four years prior to the clinical diagnosis of MB leprosy, 7 of 11 patients showed an IgG antibody response to LID-1 up to one year prior to developing clinical symptoms. These responses were strikingly more elevated and occurred much earlier than the increases in the anti-PGL-I IgM response in these same individuals (36). Nevertheless, the responses to LID-1 in PB individuals were rather weak, similar to the percentage of anti-PGL-I positivity, which is usually around 20–40% (36).

Serological responses based on IgG Ab against \textit{M. leprae} major membrane protein (MMP)-II provided slightly less diagnostic potential than IgM Ab against PGL-I for MB patients (88.1% vs. 94.9%), as estimated in a Chinese population (39). However, the number of PB patients testing positive increased significantly (61.1% vs. 38.9%) when using MMP-II. About a third of the leprosy patients’ contacts, 21 individuals, was positive for Ab against MMP-II, seven of whom developed leprosy in the following three years. However, healthy individuals from the same population were not included in this study.

The use of the anti-PGL-I assay as a screening procedure to identify those at high risk of developing leprosy in endemic populations is unlikely to be particularly useful in most leprosy control programs, since in previous follow-up studies most of the new cases emerged from the seronegative group in the population (40). The assay could, however, be applied for the epidemiological monitoring of changes in the intensity of infection with \textit{M. leprae} in a community and for the study of carefully defined groups of contacts during some phases of control programs (41, 42). More for information about the disease than for identification, anti-PGL-I antibody titers can be utilized to monitor efficient treatment by following the antibody clearance in patients. Similarly, following increases in titers may detect treatment failure or relapsing disease (43, 44, 45, 46, 47, 48, 49).

**CELLULAR-MEDIATED IMMUNE RESPONSES**

An alternative test based on immunity to \textit{M. leprae} antigens, the Mitsuda skin test, measures cellular rather than humoral immunity against lepromin (50, 51). Lepromin consists of a bacillary suspension standardized by the number of \textit{M. leprae} in the suspension. The reaction to lepromin is measured as induration in millimeters 3–4 weeks after intradermal inoculation and provides
information about the ability of an individual’s T cells to respond to *M. leprae* and the likelihood of granuloma formation in that individual. In contrast to the tuberculin skin test (TST), which measures cellular immunity to tuberculin (or PPD) and indicates previous infection with *M. tuberculosis*, the lepromin test does not detect prior *M. leprae* infection. Rather, its use lies in the determination of the type of immune response (as estimated by tissue injury) that would ensue upon infection with *M. leprae*. A negative Mitsuda reaction is generally seen in LL/BL patients, indicating the lack of a protective cellular response (52, 53).

Leprosin, or Rees’s antigen, consists of proteins extracted from *M. leprae* and has been used as a 48-hour skin test to determine the cell mediated immune status of individuals (54). In that respect, tests based on the use of leprosin bear a similarity to the TST. Since leprosin and tuberculin are incompletely defined mixtures of mycobacterial antigens, they are of limited diagnostic value due to their inherently high cross-reactivity with other mycobacteria, which results in a test with low specificity. For leprosy, such cross-reactivity is particularly problematic in countries with high incidence rates of TB, routine BCG vaccination, and high levels of exposure to non-pathogenic environmental mycobacteria. One possible approach to avoiding cross-reactivity is the development of tests using *M. leprae* antigens that can induce cellular immune responses specific to the *M. leprae* infection.

**Immunodiagnostic Tools**

**M. LEPRAE ANTIGENS**

To develop field-friendly assays based on cellular mediated immune (CMI) responses, an approach similar to that used for the commercially available IFN-γ release assays (IGRAs) for specific diagnosis of *M. tuberculosis* infection (55, 56) was used for leprosy diagnosis. Since the Quantiferon-TB assays exploit *M. tuberculosis*-specific antigens ESAT-6 (Rv3875), CFP-10 (Rv3874), and TB7.7 (Rv2654), the *M. leprae* homologs of ESAT-6 and CFP-10 (ML0049 and ML0050) were assayed as leprosy-specific diagnostic tools. Despite limited sequence homology (36% and 40%, respectively) and no cross-reactivity at the serological level (57, 58), the *M. leprae* homologues of ESAT-6 and CFP-10 were recognized by T cells from *M. tuberculosis* infected individuals. Accordingly, cross-reactions with these assays limit the diagnostic potential of ESAT-6 and CFP-10 in leprosy endemic areas with a high prevalence of tuberculosis (59, 60). Similar data were reported for the *M. leprae*-specific 45 kDa serine rich antigen (ML0411), which was also recognized by TB patients, probably due to sequence homology with Rv2108 (61).

The completion of the *M. leprae* and *M. tuberculosis* genome sequences (62, 63) allowed post-genomic approaches in which comparative analyses of annotated mycobacterial genomes were used to select putative open reading frames that were found only in the *M. leprae* genome and lacked homologues in any of the mycobacterial databases available at that time (61, 64, 65, 66,
Bioinformatic analyses of these *M. leprae*-unique sequences identified several (hypothetical) antigens that were subsequently analyzed for their ability to induce *in vitro* T cell responses in *M. leprae* infected individuals, specifically.

Since IFN-γ is a stable cytokine, it has been widely used as a surrogate marker for pro-inflammatory immunity against mycobacteria (74). More recently, IFN-γ induced protein 10 (IP-10) has been shown to be a useful biomarker for diagnosis of the *M. tuberculosis* infection as well (75). Unlike IFN-γ, IP-10 also can be used in HIV infected patients, since it is not affected by low CD4 counts in TB patients with HIV (76).

In *vitro* T cell stimulation assays predominantly assessing IFN-γ production were used to test the potential candidate immunodiagnostic antigens identified by the bioinformatic analysis. The antigens were tested for their potential as diagnostic tools for humans (65, 68, 69, 70, 71, 73, 77) as well as in animal models for leprosy in mice and armadillos (78, 79). Surprisingly, despite being selected for their exclusive presence in *M. leprae*, so as to avoid T cell cross-reactivity with BCG-vaccinated or *M. tuberculosis* infected individuals, it was found that most of these *M. leprae*-unique proteins induced IFN-γ in healthy control individuals (EC) from the same leprosy-endemic areas (70, 73, 80). However, since these EC were living in areas with pockets of high leprosy prevalence, the observed cellular responses towards the *M. leprae*-unique proteins may have indicated *M. leprae*-specificity but could be irrelevant to the pathogenic cellular immunity that leads to leprosy. In contrast, the production of IFN-γ as well as IP-10 in response to *M. leprae*-unique proteins or peptides was able to differentiate between EC groups drawn from areas with different levels of leprosy prevalence. This latter approach allowed the identification of distinct degrees of *M. leprae* exposure and, thereby, estimations of the risk of infection and, potentially, subsequent transmission (80, 81, 82, 83).

**EXPOSURE, INFECTION, AND DISEASE**

As stated above, discriminatory IFN-γ and IP-10 profiles are observed between different types of leprosy (69, 70, 71) and between EC<sub>high</sub> and EC<sub>low</sub> (80, 81). Regardless, no *M. leprae* proteins have been identified that can distinguish TT/BT patients from EC or HHC based on IFN-γ or IP-10 production when both groups are drawn from the same leprosy hyper-endemic area and have the identical socioeconomic status. For efficient diagnosis, it is thus imperative to identify new host markers (immunological- or genetic biomarkers) that can be used to discriminate between *M. leprae* exposure, *M. leprae* infection, and disease. This goal requires a clear consensus with respect to the different stages that can occur after encountering *M. leprae*.

In general, exposure is defined as the contact of a potential host with a harmful agent, colonization as the invasion of the host by germs without signs or symptoms, and infection as the invasion of a host’s bodily tissues by a disease-causing organism. Infection generally results in the multiplication of the invading germ and the reaction of host tissues to these organisms. Infection may lead to disease, in which case an abnormal, pathological condition that affects part or all of
the host can be observed. Subsequently, the types of immunity associated with these stages (i.e., stage-specific biomarkers) need to be unambiguously identified (see Figure 2).

**FIG 2** Schematic overview of the stages of *M. leprae* infection and their respective biomarker profiles, modified for biomarkers and for leprosy from (127).

If the host is capable of eliminating *M. leprae* without the priming of adaptive immunity (T cells or B-cells), the cytokines and chemokines measured in the blood reflect innate immune responses (stage 1) caused by, for example, natural killer cells, macrophages, or neutrophils. If a combination of specific T cells and innate immune cells cause elimination of *M. leprae* bacilli, additional blood biomarkers for adaptive immunity may be found in the blood as well as tissues (stage 2). Alternatively, if *M. leprae* is replicating and invading the host without causing clinical symptoms, biomarkers for early infection need to be identified (stage 3). These biomarkers will be both innate and adaptive, in which 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 throughout the host’s body (BL/LL) or causing harmful T-cell responses resulting in lesions (TT/BT), pathogenic immune responses predominate and the chronic battlefield between host and pathogen ends in favor of *M. leprae*. At this stage, the analysis of host blood biomarkers represents the result of pathogenic as well as protective immune responses that are present in leprosy patients (stage 4), a consequence of either vigorous T cell responses in TT/BT or suppressive T cells in LL/BL patients (19, 22). In stage 4, similar to stages 2 and 3, biomarkers produced by innate immune responses will also be present, as is the case in TB (84, 85). Disease-specific biomarkers likely can be deduced by the comparison of cellular immune markers, such as cytokines and chemokines, but also by genetic markers (86) in leprosy patients, their contacts, and endemic and nonendemic healthy individuals. Biomarker studies including leprosy patients and HHC, but lacking different EC groups (87), are less likely to identify markers for protective innate immunity (stage 1) such
as chemokines secreted by activated macrophages (88, 89). These studies are unlikely to identify markers since the HHC are frequently and intensely exposed to *M. leprae* and, therefore, produce similar cytokines and chemokines as those found for TT/BT patients (80, 87).

Finally, infections with HIV (90, 91, 92, 93, 94), Th2 inducing parasites (95), and also diabetes (96) will produce biomarker profiles of *M. leprae*-affected individuals, thereby complicating diagnosis. The differential effects of co-infections on host biomarkers for an *M. leprae* infection and leprosy will require more elaborate studies as well.

**HOST PROFILES**

Although IFN-$\gamma$ production is used as a surrogate marker of Th1-induced protection, it remains uncertain whether the production of this cytokine in response to *M. leprae* antigens correlates with either protection against infection or (progression to) disease. Therefore, other analytes, measurable in serum such as cytokines and chemokines, need to be investigated as potential biomarkers. For example, using whole blood assays, IL-1$\beta$, CCL4, and MCP-1 could, in contrast to IFN-$\gamma$, discriminate between patients (leprosy and TB) and healthy EC living in the same endemic areas in Bangladesh and possibly reflect differences between *M. leprae* exposure and pathogenic immunity (80).

Despite the obvious differences between leprosy patients and HHC on one hand and EC on the other hand, no significant differences were observed between HHC and BT/TT. While some HHC displayed biomarker profiles similar to patients, others seemed to be more like EC. To identify biomarkers in HHC that are specific for (pre-clinical) disease, longitudinal analyses need to be conducted allowing the intra-individual comparison of immune profiles of the cyto-/chemokine responses.

Given that host immunity and immuno-pathogenicity in response to *M. leprae* involves complex interactions between a variety of cells expressing different effector and regulatory molecules, it is rather unlikely that only a single cytokine or chemokine is linearly correlated to protection or to disease. In view of this low likelihood, it is essential to increase the biomarker potential of single cytokines or other markers by using specific combinations of them, also referred to as a biomarker profile. In such a profile, all single markers may not be able to diagnose each patient but, if selected in such a way that each marker is independent of the others in the profile, better performance is expected (97).

Since cytokines modulate each other’s effects, their ratios can in fact be more informative than single cytokine values in order to discriminate between different stages of mycobacterial infection. For example, the IFN-$\gamma$/IL-10 ratio has been found to correlate with tuberculosis (TB) severity (98) and combinations of cytokine responses are indicative of active versus latent TB (99). For leprosy, IP-10/IL-10 ratios in unstimulated plasma differed significantly between patients and EC in a small Ethiopian cohort (100), indicating the feasibility of identifying *M. leprae* infection in endemic areas. Moreover, the onset of type 1 leprosy reactions is associated with an increase in
ratios of pro-inflammatory cytokines versus IL-10 (7), indicating the potential of measuring cytokine ratios for these reactions as well.

In summary, since immunity against *M. leprae* matches the clinical manifestations after infection, it is essential to perform longitudinal studies comparing these immunological relationships. Since the majority of those exposed to *M. leprae* develop a protective immune response against the bacterium, such follow-up studies need to be large-scale efforts to allow identification of critical host-derived immune biomarker profiles as risk factors in exposed populations in leprosy-endemic areas.

**TRANSCRIPTOMIC HOST PROFILES**

Besides host immune profiles, human transcriptome-arrays offer cutting-edge tools for identifying gene expression profiles for leprosy (101). Using a transcriptomic assay dual color Reverse Transcription Multiplex Ligation-dependent Probe Amplification (dcRT-MLPA), a biomarker profile composed of several genes was identified for infection in a follow-up study of TB contacts (102). This dcRT-MLPA platform was also used to identify genetic markers associated with leprosy reactions (12). RNA expression profiles revealed that IFN-induced genes, (V)EGF, and genes associated with cytotoxic T-cell responses (GNLY, GZMA/B, PRF1) were upregulated during T1R, whereas expression of T-cell regulation-associated genes were decreased.

Recently in an African TB cohort (South Africa and Malawi) including HIV+ individuals, a 27-gene transcript signature was identified that discriminated between TB patients and latently *M. tuberculosis*-infected individuals (LTBI). Moreover, the same study showed that a transcript signature including 44 genes could specifically distinguish TB patients from patients with other respiratory diseases (103). These data show the promise of transcriptomic host profiles for diagnosis as well. Combined with functional protein association networks, transcriptomic expression profiles can be used to identify proteins discernible in blood for application as biomarkers in field-friendly assays.

**METABOLIC HOST PROFILES**

Comprehensive approaches to identifying metabolic variations associated with disease are rapidly evolving. The field of metabolomics aims to characterize the concentration (changes) of small molecules occurring in bio-fluids and has contributed to knowledge of various diseases as well as biomarkers of disease (9, 10). As key experimental technologies, mass spectrometry and nuclear magnetic resonance allow the assessment of a cell’s or tissue’s metabolic activity or state, which is subject to environmental stresses. Although this field remains largely unexamined for leprosy, some recent reports showed the utility of metabolomics for the identification of potential contributors to disease pathology in the sera of leprosy patients (104, 105). Thus, the identification of metabolic host profiles may facilitate the development of more targeted treatments for leprosy patients and their contacts.
HOST BIOMARKERS FOR LEPROSY REACTIONS

Leprosy often coincides with acute, inflammatory episodes due to augmented anti-mycobacterial host immunity. These reactions represent the major cause of leprosy-related permanent neuropathy and disability. Up to 50% of leprosy patients experience a leprosy reaction at least once, yet no laboratory test is available that allows early diagnosis and treatment that would help prevent nerve damage. Two types of skin- and nerve-damaging reactions are recognized: type 1 reversal reactions (T1Rs) and type 2 reactions (T2Rs), or ENL (see Chapter 2.2). T1Rs are considered a delayed hypersensitivity reaction with the characteristic infiltrations of skin and nerve lesions by CD4+ T-cells producing IFN-γ and TNF-α (106).

When patients receive prompt and proper diagnosis and treatment, recovery from inflammatory nerve damage is more likely and the risks of permanent disability are significantly reduced (107). Unfortunately, in endemic contexts, leprosy reactions are frequently misdiagnosed due to a decrease in specialized expertise within integrated health services, and patients can experience significant delays in diagnosis (108). Consequently, if diagnosis and treatment is delayed beyond six months of the first symptoms, the neuropathy is likely to be permanent (109). Therefore, reliable, field-compatible or laboratory tests for the early detection or prediction of leprosy reactions could make significant differences in clinical outcomes. A major obstacle to developing such tests is the lack of dependable, specific biomarkers for leprosy reactions across endemic populations.

Substantial evidence points to increased numbers of CD4+ T-cells in skin lesions; high levels of IL-2 receptors, TNF-α, IL-6, IP-10, and IL17F in sera; and increased gene expression of pro-inflammatory cytokines during reactions (110, 111, 112, 113, 114). Consequently, it is conceivable that cytokines, chemokines, antibodies, and also metabolites, which can be measured in body fluids, may prognosticate reactional episodes. Improved knowledge of the relevant biomarker profiles, especially those that are specifically induced by unique *M. leprae* antigens, will help to accurately identify patients who are developing leprosy reactions.

Immunodiagnostic tests

Due to changes in leprosy control programs and the decrease in the specialized expertise required for the early and accurate diagnosis of leprosy, the need for rapid tests that can be applied in non-expert settings to detect asymptomatic *M. leprae* infection or predict progression to leprosy may be greater than ever before.

A novel rapid diagnostic test, the NDO-LID® test (115), was recently developed for the diagnosis of MB leprosy based on the complementary detection of antibodies against a novel protein-glycolipid conjugate. NDO-LID® is an immunochromatographic test that requires small amounts of serum or whole blood. Like the other serological tests for leprosy, it detects MB patients and, potentially, HHC at a higher risk of developing MB leprosy.
The characteristics of the leprosy disease spectrum, in which the outcome of an \textit{M. lepraee} infection ranges from the strong Th1 immunity in tuberculoid leprosy to the high antibody titers to \textit{M. leprae} with Th2 cytokine responses in lepromatous leprosy, are pre-eminently suitable for tests that simultaneously detect biomarkers specific for both types of immune responses. Tests of this nature could provide thorough monitoring of the complete immunological leprosy spectrum.

Although ELISA techniques, as used in IGRAs, are more widely applied than before, they still require laboratory facilities that are not available at all health centers in leprosy-endemic areas. Lateral flow assays (LFAs) are simple, immunochromatographic assays that detect the presence of target analytes in samples without the need for specialized and costly equipment. Combinations of LFAs with up-converting phosphor (UCP) reporter technology are useful for the detection of a variety of analytes, e.g., drugs of abuse (116); protein and polysaccharide antigens from pathogens like \textit{Schistosoma} and \textit{Brucella} (117, 118); bacterial and viral nucleic acids (119, 120); and antibodies against \textit{M. tuberculosis}, HIV, hepatitis virus, and \textit{Yersinia pestis} (121, 122, 123). The phosphorescent reporter utilized in UCP-LFAs is excited with infrared light to generate visible light, a process called ‘up-conversion’. UCP-based assays are highly sensitive, since up-conversion does not occur in nature, avoiding interference by auto-fluorescence of other assay components. Importantly, UCP-LF test strips can be stored as permanent records, allowing for re-analysis in a reference laboratory.

To accommodate ELISAs to field-applicable tests for leprosy and TB diagnosis or for the monitoring of vaccine and treatment efficacy, LF assays based on up-converting phosphor (UCP-LFAs) were developed for the detection of IFN-\(\gamma\), IP-10 (Th1), and IL-10 (Treg) as well as antibodies against the \textit{M. leprae}-specific PGL-I (100, 124, 125, 126). Simultaneous measurement of pro- and anti-inflammatory cellular as well as humoral immunity to \textit{M. leprae} can be a useful tool in leprosy control programs for the classification of leprosy. It also can allow early diagnosis of leprosy or leprosy reactions, leading to timely treatment and reduced transmission. However, since there is no gold standard for detecting an asymptomatic \textit{M. leprae} infection, biomarker signatures for the early detection of leprosy need to be identified in the near future.

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