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Chapter 9

Serology

Masanori Kai

The key to controlling Hansen's disease lies in curbing the number of new cases that has shown little decline over the years, requiring the stemming of disease through early detection and treatment. Although the advanced methods of late using molecular techniques to diagnose the disease are useful, the less costly and more convenient conventional serodiagnostic methods continue to be of value. Serological methods to detect specific antibodies against *Mycobacterium leprae* can be used for both early diagnosis, and for monitoring treatment effect in patients under treatment with antibiotics. Their use in the detection of relapse and for identifying patients at risk of developing type 1 or type 2 reactions are also under consideration. Furthermore, the seropositive rate in a given group or geographical area is believed to correspond to the infection rate by *M. leprae* in that group or region. Monitoring using serological methods can also determine the efficacy of the various countermeasures being taken for leprosy.

In this section, the serology of Hansen's disease is reviewed through description of the various antigens of *M. leprae*, and the methods used for their serodiagnosis.

9.1 INTRODUCTION

The current treatment for leprosy consists primarily of the multi-drug therapy (MDT) regimens recommended by the World Health Organization (WHO). The MDT has been effective in reducing the number of registered leprosy cases in the world to date. However, we have yet to see comparable reduction in the number of new cases of leprosy, and at present, the number of both registered cases and new cases has plateaued at 210,000 and 250,000 cases, respectively (WHO, 2009). As such, emphasis in the next stage of leprosy control is the identification and eradication of sources of infection, and the control of relapses and intractable cases through early detection and treatment.

Clinically, diagnosis of leprosy is based on histopathological detection of the acid-fast *M. leprae* bacilli in skin smears or biopsies, in patients suspected of Hansen's disease given clinical evidence of skin lesions with peripheral nerve damage or enlargement. Recent advances in diagnostic methods now allow for detection of *M. leprae* DNA sequences from biopsy samples (Donoghue *et al*, 2001; Phetsuksiri *et al*, 2006). However, it is difficult to incorporate such methods in developing countries that still have leprosy hot spots, because such methods require expensive machinery and materials, as well as skilled technicians. In the interest of leprosy control, early detection of patients in the stage before onset of clinical manifestations is desired. However, it is difficult to obtain consent to biopsies from asymptomatic persons, even when they are household contacts (HHC) of leprosy patients. In contrast, serological tests employing blood samples sharply reduce subject load increasing compliance to testing. Serological tests that detect antibodies against components of *M. leprae* can be utilized for both monitoring the effectiveness of drug treatment as well as early diagnosis of infection (Roche *et al*, 1993). In addition, the serological tests are applicable for the early diagnosis of relapse, and determination of patients at risk of developing type 1 or type 2 reactions during the course of therapy. However, despite these benefits, serological methods are still regarded as being no more than complementary, as they are unable to detect all types of leprosy under all conditions. Detection of specific antibodies is difficult in many paucibacillary type (PB) patients whose response to Hansen's disease is believed to be the result of mainly cell-mediated immunity. Various antigens have been reported as target candidates for serodiagnosis, including the glycolipids lipoarabinomannan (LAM) (Gelber *et al*, 1989) and phe-

nolic glycolipid-I (PGL-I) (Patil *et al*, 1990), and proteins with relative molecular masses of 10-kDa (Rojas *et al*, 1997), 15-kDa (Britton *et al*, 1988), 18-kDa (Mohanty *et al*, 2004), 25-kDa (Schorey *et al*, 1995), 27-kDa (Young *et al*, 1985), 28-kDa (Mohanty *et al*, 2004), 30-kDa (Filley *et al*, 1994), 35-kDa (Roshe *et al*, 1999), 36-kDa (Klatser *et al*, 1985), 45-kDa (Rinke-de-Wit *et al*, 1992), 48-kDa (Britton *et al*, 1988), 65-kDa (Meeker *et al*, 1989), and 70-kDa (Britton *et al*, 1988). However, further study on their specificity and reproducibility have reduced these possibilities to a few, such as PGL-I, and the 35-kDa and 45-kDa proteins that have been popularly used. As it now stands, serodiagnosis using these antigens allow for detection of 90-100% of patients in the active phase of the disease, but only 40-60% of those in the early phase (Sengupta, 1990).

This chapter provides an overview of leprosy serology with reference to Buchanan's review (Buchanan, 1994), incorporating some of the more recent applications and methods in serodiagnosis.

9.2 LIPID ANTIGENS

9.2.1 LAM

The *M. leprae* bacillus is surrounded by a coating of lipoarabinomannan (LAM), and many serological tests have been developed to detect antibodies against the LAM antigen (Gelber *et al*, 1989; Mwatha *et al*, 1988; Jayapal *et al*, 2001). Mwatha et al used a competitive inhibition assay employing an RI-labeled monoclonal antibody, ML34, which responds to an epitope on the LAM antigen. Gelber et al and Jayapal et al have measured antibodies to LAM directly in a microtiter enzyme-linked immunosorbent assay (ELISA). Serum antibodies in leprosy patients, especially multibacillary type (MB) patients can be detected well by either method. However, as LAM is not structurally or antigenically unique to *M. leprae*, the detection of antibodies to LAM can not rule out the possibility of infection by other LAM-carrying bacteria.

9.2.2 PGL-I

Apart from the LAM antigen, the cell surface of *M. leprae* is studded with characteristic phenolic glycolipids. The main component of these phenolic glycolipids is phenolic glycolipid-I (PGL-I) (Brennan & Barrow, 1980), which is characterized by a terminal trisaccharide unique to *M. leprae* consisting of three immunodominant saccharides—methylglucose, methylramnose, and methylramnose. Many serological studies have been conducted following discovery of this molecule, and various methods have been developed to measure specific antibodies against PGL-I, employing the whole molecule either alone (Cho *et al*, 1983), or within liposomes (Schwerer *et al*, 1989), in deacylated form (Young & Buchanan, 1983), or as synthetic forms of the terminal monosaccharide (Douglas *et al*, 1988), disaccharide (Cho *et al*, 1983; Petchlai *et al*, 1988), or trisaccharide (Izumi *et al*, 1990). Detection of antibody to PGL-I has for most part been by direct ELISA, although Izumi *et al* have developed a unique gelatin particle agglutination test (Izumi *et al*, 1990) (Fig.9.1).

Figure 9.1 "Serodia-Leprae" is commercially available from Fujirebio Inc.,Japan, which was used MLPA (*M. leprae* particle agglutination) method originally developed by Izumi *et al.*

The PGL-I structure with its unique trisaccharide was analyzed in detail, and mixed synthetic saccharides (mono-, di-, or tri-saccharides) were engineered for laboratory use. As patients with Hansen's disease exhibit high antibody titers to PGL-I, this has been the most intensely studied of *M. leprae* antigens to date. The study of responses to PGL-I by antibody class has demonstrated that while IgG, IgA, and IgM antibodies can recognize PGL-I antigen, detection of IgM antibodies has become standard in analyses for PGL-I, given predominance of the IgM response (Praputpittaya *et al*, 1990). Comparative analyses between PGL-I and LAM (Jayapal *et al*, 2001; Sekar *et al*, 1993) have demonstrated that both PGL-I and LAM are capable of detecting high antibody responses in MB patients, but not in PB patients, and that sensitivity of PGL-I was slightly higher than that of LAM, supporting the popular use of PGL-I for serodiagnosis.

9.2.3 Other glycolipids

Cord factor—identified as one of the virulence factors in *M. tuberculosis*—is chemically a type of glycolipid, trehalose dimycolate (TDM). TDM is present in many mycobacteria, but the structure of one of its components—mycolic acid—is slightly different between species. Using this slight difference, TDM has been utilized as a serodiagnostic antigen (Wang *et al*, 1999), although until the recent extraction of TDM from *M. leprae*, the TDM used in ELISA tests for leprosy had been derived from *M. bovis* BCG. Recent progress in technology has enabled the detection, analysis, and extraction of *M. leprae* TDM, which is now regarded as a candidate antigen for the serodiagnosis of leprosy (Kai *et al*, 2007).

9.3 PROTEIN ANTIGENS

M. leprae has few surface-exposed proteins in contrast to other bacteria, but some proteins are known to be processed and presented on the surface of antigen-presenting cells (macrophage or dendritic cells), and specific antibodies have been detected to such processed proteins or secretory proteins. Leprosy patients infected with *M. leprae* produce antibodies against such protein antigens, although not at levels noted in response to PGL-I and LAM. The major protein antigens with possible serodiagnostic value reported to date are described below.

9.3.1 30-kDa protein

Three types of 30-kDa protein—85A, 85B, and 85C—are secretory or membrane-binding proteins known as the Antigen 85 complex. The homology rates between each of the three proteins exceed 80%. The 85A and 85B proteins have a fibronectin-binding domain similar to the fibronectin-binding protein (Thole *et al*, 1992). The 85B protein is involved in mycolic acid biosynthesis (Anderson et al, 2001), and the Antigen 85 complex comprised of these antigens reacts well with leprosy patient serum but not with TB patient serum (Filley *et al*, 1994).

9.3.2 35-kDa protein (MMP-I)

The 35-kDa protein is a membrane-binding protein known as MMP-I (major membrane protein-I), and is one of the most well-analyzed and reported antigens (Sinha *et al*, 1983) recognized as having serodiagnostic value approaching that of PGL-I (Roshe *et al*, 1999; Jayapal *et al*, 2001; Parkash *et al*, 2002).

9.3.3 MMP-II (22-kDa)

MMP-II (major membrane protein-II) was identified as one of the major proteins in *M. leprae* by Hunter *et al* (Hunter *et al*, 1990), also shown to be a mycobacterial bacterioferritin (Pessolani *et al*, 1994). MMP-II, with a molecular weight of 22-kDa, has been shown to induce both humoral and cellular immune response in leprosy patients (Ohyama *et al*, 2001), indicating possible serodiagnostic value. Maeda *et al* have identified, extracted, and cloned MMP-II protein as a cellular membrane protein that reacts with serum of PB patients (Maeda *et al*, 2007). Using purified MMP-II protein fused with maltose binding protein (MBP) (64-kDa), they demonstrated high reactivity with serum from Japanese leprosy patients, in terms of both sensitivity and specificity. In particular, MMP-II was found to react more strongly with PB patient serum compared to other *M. leprae* antigens. Good results were also obtained in a recent survey using MMP-II ELISA conducted in other countries where leprosy continues to be endemic (Kai *et al*, 2008; Hatta *et al*, 2009). Moreover, while comparison with PGL-I ELISA showed comparable sensitivity and specificity between MMP-II and PGL-I, slightly higher positive rates have been obtained with MMP-II ELISA in PB patients.

9.3.4 45-kDa protein (ML0411: serine-rich protein)

The 45-kDa serine-rich protein derived from *M. leprae* was reported as a *M. leprae*-specific protein by Rinke de Wit *et al* (Rinke de Wit *et al*, 1993), in spite of homology with that of *M. tuberculosis* (Rinke-de-Wit *et al*, 1992). Although sensitivity does not equal PGL-I, there have been reports of positive results being obtained in many samples negative by PGL-I ELISA (Thole *et al*, 1995), indicating possible utility as a serodiagnostic antigen.

9.3.5 CFP-10 and ESAT-6

Parkash *et al* analyzed the usefulness of *M. leprae* CFP-10 and ESAT-6 in independent experiments (Parkash *et al*, 2006a; Parkash *et al*, 2006b). The possible utility of these secretory proteins was confirmed, with 82-83% of MB patients and 18% of PB patients being seropositive for these antigens.

9.3.6 10-kDa protein

The 10-kDa protein is known as heat shock protein GroES, that binds to form a chaperone with heat shock protein GroEL. The two heat shock proteins found in *M. leprae* and *M. tuberculosis* are almost identical, accounting for the cross-reactivity with TB patients (Young & Buchanan, 1983).

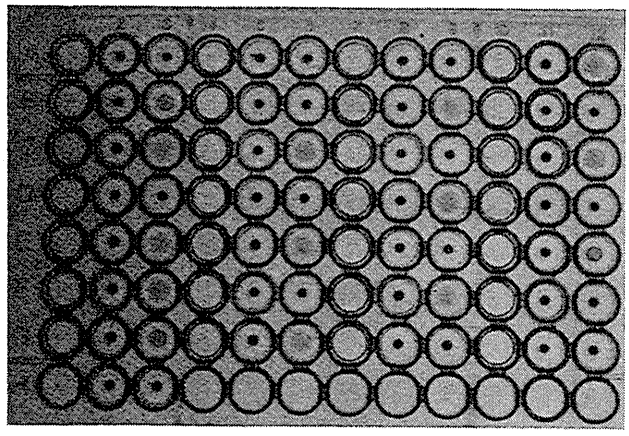
9.3.7 Pepetide antigens

An attempt to identify T cell epitopes on the 45-kDa serine-rich protein of *M. leprae* was conducted using 17 overlapped peptides synthesized to cover the entire protein, and analyzing its response in leprosy patients in Pakistan and healthy (non-exposed) controls in England (Brahmbhatt *et al*, 2002). T-cell recognition of some peptides in PB patients suggested possible diagnostic potential of such T-cell epitopes, although unfortunately, T cells from many TB patients also responded to the peptides. Many of these initially studied antigens are proteins found in abundance in the *M. leprae* bacillus, but utility was often limited by the cross-reactivity with other mycobacteria.

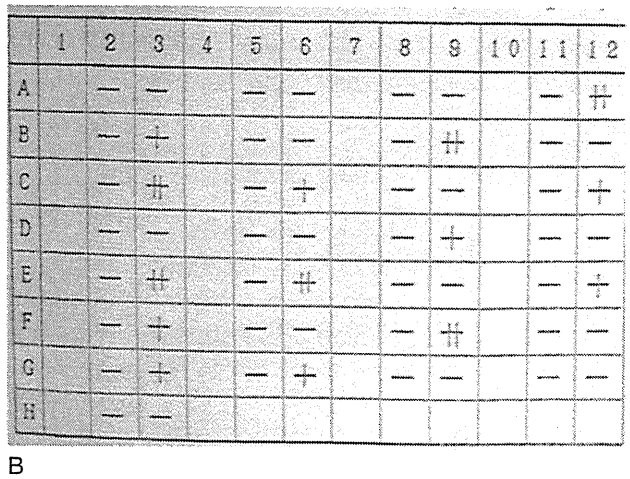
9.4 SEROLOGICAL METHODS

9.4.1 Advanced rapid serodiagnosis (ML-flow test)

Although methods such as radio-immunoassay and monoclonal antibody inhibition tests are available, the most common method in laboratory serodiagnosis is probably the ELISA. The ELISA is used to detect specific antibodies in subject serum, plasma, or whole blood, in microtiter plates coated with various antigens. As mentioned above, high sensitivity and specificity have been reported for various antigens specific to *M. leprae* as tested by the ELISA. However, ELISA testing requires skilled technicians and costly specialized equipment and facilities. Additionally, the test routinely requires a full day to obtain results. Such being the case, development of simpler methods with higher cost performance was desired for field use in endemic areas. Multiplex ligation-dependent probe amplification (MLPA) was applied to develop the first such simple agglutination test using PGL-I antigen (Izumi *et al*, 1990) (Fig. 9.1, 9.2). It was followed by development of a simple card test using 35-kDa antigen (Roche *et al*, 1999). These simple tests are no longer in wide circulation, being replaced by the dipstick test developed in 1998 by Buhrer *et al* as a simple method capable of producing results in 3 hours (Buhrer-Sekula *et al*, 1998), while showing more than 97% agreement with the ELISA test. More recently, the same group has developed the ML-flow test (lateral flow test for *M. leprae*) using the same principle, capable of detecting antibody with comparable reliability from a single drop of whole blood in just 10 minutes (Buhrer-Sekula *et al*, 2003) (Fig. 9.3). The plastic strip system used in the ML-flow test is the same as the used in diagnostic kits for TB, filariasis, and other such diseases, which is becoming a standard tool in the diagnosis of infectious diseases.



A



B

Figure 9.2 An example of MLPA qualitative assay (Serodia-Leprae kit).
A: Two-fold serum dilutions from 1:4, 1:8, 1:16 were made in 3 wells (for example: from A-1, -2, -3 to G-1, -2, -3). Buffer is added to H-1,-2,-3 as negative control. The PGL-I sensitized particles are added to lane 3, 6, 9, 12 and unsensitized particles are added to lane 2, 5, 8,11. The result is interpreted according to the instructions after incubation at room temperature for 2 hrs.
B: The agglutination pattern is determined as follows: +: positive, ++: strong positive, –: negative

9.4.2 Other methods and modifications

(1) Low temperature ELISA.

Parkash et al have reported that both sensitivity and specificity of PGL-I ELISA could be en-

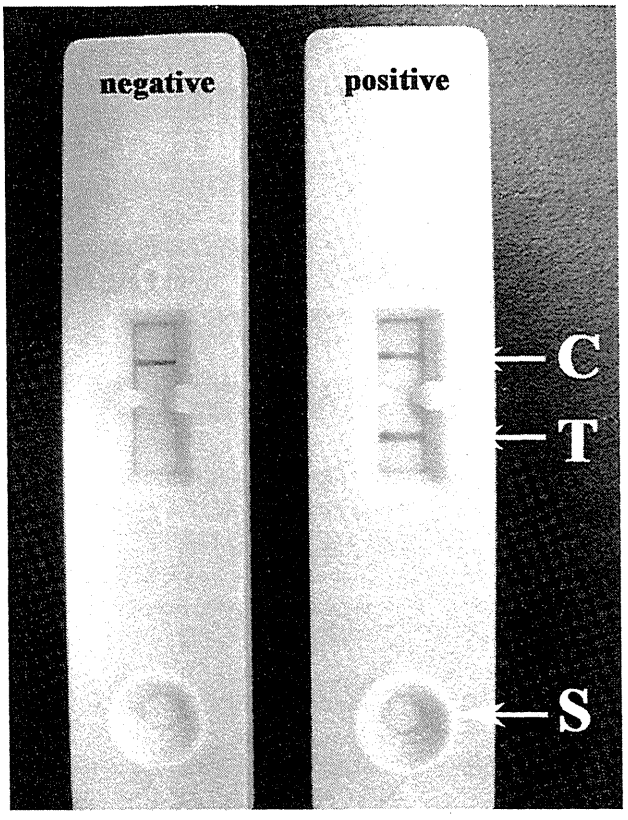


Figure 9.3 The latest serodiagnostic kit, ML flow test. Sample (serum or blood) is put into the lower circle (S) and the reaction buffer is add to the same circle. After 10 min the result is visualized as lines in the upper windows. Single band at position C indicates negative for anti-PGL-I antibodies. Double bands at position C and T indicate positive.
*The kit was purchased from KIT Biomedical Research in Netherland.

hanced by carrying out the test at low temperatures (Parkash *et al*, 2007a). Positive results could be obtained in 50-70% of PB patients using this modification. However, two drawbacks have been noted. First, this effect was not evident in ELISA using another protein antigen, 45-kDa, indicating this is not a general improvement applicable to all ELISAs. Secondly, the low temperature treatment requires an additional 24 hours to produce results, making it an even more time-consuming procedure.

(2) Double usage.

Parkash *et al* also tested the use of two antigens—45kDa protein and PGL-I—in low temperature ELISA (Parkash *et al*, 2007b). A positive rate of 100% in MB patients and 76% in PB patients was achieved with this double usage, more than making up for the aforementioned disadvantages, awaiting further study and evaluation by other researchers to confirm these findings.

(3) CMI (cell mediated immunity) method

The CMI method was adapted from its original use in diagnosing tuberculosis, in a new attempt for the early diagnosis of patients in early stage PB disease, in whom cell mediated immunity is the primary response (Ferrara *et al*, 2006)). The CMI method measures γ-IFN produced by T cells stimulated by specific bacterial antigens to determine the presence of infection. Geluk *et al* first demonstrated utility of the CMI method in leprosy diagnosis using various *M. leprae* antigens (Geluk *et al*, 2005). In their report, 90% of PB patients who were PGL-I ELISA negative, and 70% of household contacts (HHC) of leprosy patients were positive by CMI, while positive readings in healthy controls amounted to only about 7%. Effective use of this method is visualized in combination with other conventional forms of serodiagnosis such as PGL-I ELISA.

Recently, several other antigens of *M. leprae* were analysed and reported by members of the IDEAL Consortium (Geluk *et al* 2009).

9.5 SERODIAGNOSIS AND THE CLINICAL STATE

Reports of serodiagnosis using PGL-I have indicated that while it is possible to identify 75-100% of MB patients, positive results could be obtained in only 15-40% of patients with PB disease (Oskam *et al*, 2003). This means that it is not possible to diagnose all types of leprosy with a single serodiagnostic test using any of the antigens studied to date. However, the ability to detect most all cases of MB disease is of definite value. Use of the serological test in combination with clinical information such as the number of skin lesions has also been proposed as a useful method for proper determination of the type of disease, for accurate diagnosis and selection of optimum treatment. In terms of treatment, Buhrer *et al* have come up with concrete suggestions regarding interpretation of serodiagnostic results, recommending application of MB treatment for all seropositive patients even when less than six lesions are noted to reduce the number of patients who exhibit insufficient response to therapy (Buhrer *et al*, 2000).

With regard to the correlation between treatment course and serodiagnostic findings, antibody titers generally decrease with progress of treatment, although this is not a constant feature, being largely dependent upon the individual. Patients sometimes remain seropositive for many years after treatment (Gelber *et al*, 1989), a possible cause of such persistency being the continuing presence of dead or dormant bacteria inside the body (Meeker *et al*,

1990).

Fine has expressed negative views regarding utility of serological tests for either early detection of disease or monitoring of drug effect (Fine, 1989). Reporting that many healthy seropositive individuals become seronegative in subsequent follow-up without developing leprosy, Fine questioned the utility of serodiagnosis for large-scale screening in low incidence areas.

As such, given the correlation between decrease in antibody titers and bacillary index noted through serial follow-up in individual patients, serological testing is believed to have greater utility as a complementary method for monitoring progress in individual patients rather than as a tool for mass screening.

9.6 SERODIAGNOSIS IN THE FIELD

In addition to the utility of serodiagnosis for early detection and treatment of Hansen's disease, its validity in identifying subclinical patients who may be possible sources of infection among the house-hold contacts of leprosy patients has been studied in the interest of decreasing the incidence of new cases (Kai *et al*, 2004). However, correct interpretation of results from such surveys is difficult. Comparison of serological test results between healthy individuals and HHC in a given area shows a general tendency of higher seropositivity of the HHC group. However, this tendency does not necessarily hold true for all regions, for example, there have been reports of no difference in positive rates between the general population and HHC in prevalent areas, where as much as 1.7-3.1% of the general population has been reported seropositive for *M. leprae*.

On the other hand, Douglas *et al* have demonstrated that seropositive HHC in a region of the Philippines are at increased risk of developing leprosy (Douglas *et al*, 1988). This suggests the possibility that serological testing may have practical utility in identifying high-risk individuals among the contacts of leprosy patients, and furthermore, allow for prophylactic treatment of seropositive HHCs, heralding a new dimension in leprosy control.

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Asian Skin and Skin Diseases

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Hansen's Disease in Asia

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Introduction

Hansen's disease (Leprosy), a chronic infectious disorder caused by the rod-shaped bacillus *Mycobacterium leprae* (*M. leprae*), primarily affects the skin and peripheral nerves[1]. Without treatment, it can be progressive and result in permanent damage to the affected areas. Patients also suffer from social stigma due to the skin lesions and deformities of the face, extremities, eyes, and other regions in advanced cases. Effective therapy using dapsone (4,4-diaminodiaphenylsulfone, DDS) was first introduced in the 1940s, followed by the introduction of multidrug therapy (MDT) in the 1980s. While the use of these drugs has significantly improved care and prognosis, the nerve damage and deformities cannot be recovered when treatment is delayed. Although the World Health Organization (WHO), community health organizations, and non-governmental organizations (NGOs) have worked to control and eliminate Hansen's disease, it is still a major public health and societal problem in many regions, including Asia. Therefore, monitoring and prevention programs must be maintained in these countries.

Bacteriology and Genomics of *M. leprae*

M. leprae is an obligate intracellular parasite that measures 0.3-0.5×4.0-7.0 μm and multiplies very slowly, with an approximate generation time of 12 to 14 days. The bacterium can remain viable for several days *ex vivo*, but cannot be cultured in artificial media. However, it multiplies extensively in the footpads of nude mice, nine-banded armadillos, and, to a limited extent, in the footpads of normal mice. It grows best around 30°C, and hence prefers the cooler areas of the human body. The cell wall is highly complex and contains proteins, phenolic glycolipid (PGL), arabinoglycan, peptideglycan, and mycolic acid, the latter possibly being responsible for its acid-fastness.

The *M. leprae* genome was completely sequenced in 2001[2]. The most striking feature of the genome is the extensive deletion and inactivation of genes, referred to as gene degradation: only 49.5% of the genome contains protein-coding genes, and 27% contains recognizable pseudogenes[2,3]. In particular, genes encoding various enzymes have been replaced by pseudogenes; which suggests limited metabolic activity in *M. leprae*. This genomic feature might correspond to its unique bacteriological characteristics such as exceptionally slow growth and failure to multiply in synthetic media.

Immunology of Hansen's Disease

Infection with *M. leprae* induces diverse clinical features corresponding to the ability of the host to mount an immune response. Hansen's disease presents as two types: polar tuberculoid and polar lepromatous, with subtypes ranging between these two polar forms. Cell-mediated immunity is the first line of defence against *M. leprae* infection. Therefore, the outcome of infection depends how the host responds to the pathogen-the magnitude of cell-mediated immunity determines the extent of the disease.

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Early pioneering works revealed an apparent relationship between the dominant cytokine profiles and the clinical presentation of Hansen's disease. Interleukin 2 (IL-2) and interferon gamma (IFN-γ) were the prevalent cytokines in tuberculoid lesions, whereas IL-4, IL-5 and IL-10 were characteristic of lepromatous lesions. The Th1-Th2 dichotomy is a central determinant of the type of host defence: the T helper type 1 (Th1) subset characterized by IL-2 and IFN-γ preferentially elicits cell-mediated immunity, whereas Th2 cells that produce IL-4, 5, and 10 augment humoral immunity. Both the classic reciprocal relationship between antibody production and cell-mediated immunity and resistance or susceptibility to *M. leprae* can be explained by different T-cell subset patterns of cytokine production.

Recognition of *M. leprae* by Toll-like receptors (TLRs) and subsequent activation of innate immune responses may also play an important role in the pathogenesis of leprosy[4]. *M. leprae* activates TLR2 and TLR1, which are found on the surface of Schwann cells, especially in tuberculoid patients. Although this cell-mediated immune defense is most active in mild forms of the disease, it might also be responsible for the activation of apoptosis genes and, consequently, the hastened onset of nerve damage found in persons with mild disease.

Disease Spectrum

According to the Ridley-Jopling classification system, which is based on the individual *M. leprae*-specific immunological status, the disease can be classified into six categories based on dermatological, neurological, and histopathological findings: indeterminate (I), tuberculoid (TT), borderline tuberculoid (BT), mid-borderline (BB), borderline lepromatous (BL), and lepromatous (LL). Most newly diagnosed patients, however, live in developing countries where sufficient medical resources are not available. Since the Ridley-Jopling classification system is not practical in these countries, the WHO established a simplified classification system that consists of just two categories-paucibacillary (PB) and multibacillary (MB), on the basis of clinical manifestations and slit skin smear results. PB leprosy

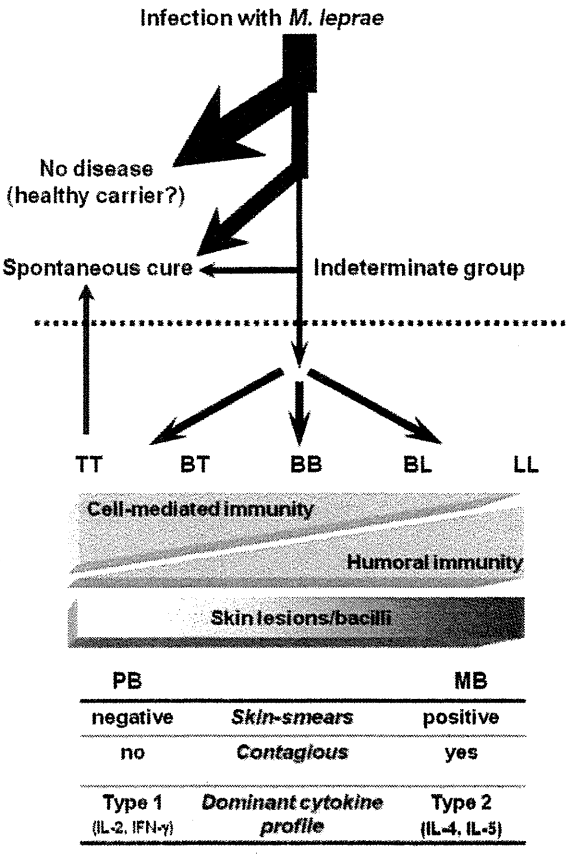

Fig. 1. Flowchart for leprosy diagnosis and classification.

is defined as five or fewer skin lesions with no bacilli in slit skin smears. MB leprosy cases have six or more lesions and may be slit skin smear positive. As for the correlation between the two classification systems, I, TT, and part of BT are generally equivalent to PB disease, while part of BT, BB, BL, and LL correspond to MB disease. Fig. 1 shows the flowchart for diagnosis and classification cited from an atlas designed for use in areas of endemicity. The procedure is simple and clear, so that patients can be diagnosed and classified without sophisticated medical facilities or staff.

Laboratory Tests and Diagnostic Procedures

Biopsies from skin or nerves of lesions are stained with hematoxylin and eosin for histological evaluation and Fite-Faraco for detection of acid-fast bacilli (Table 1). The ba-

cillus is also identified in acid-fast stains of slit skin smears taken from lesions and cooler areas of the skin, such as the ear-lobes, elbows, and knees. If acid-fast bacteria are found in slit skin smears, the patients are diagnosed with MB leprosy. Negative smears are classified as PB leprosy.

Laboratories are now encouraged to use the polymerase-chain reaction (PCR) to detect *M. leprae* DNA in infected tissues[5]. Gene variants that confer resistance to rifampicin (RFP), dapsone, and quinolone are also detectable by PCR.

Diagnosis of Hansen's disease is most commonly based

Table 3. Diagnosis and Treatment in Selected Asian Countries and Brazil

Country	Diagnosis	Classification	Treatment	Duration of treatment (MB)	Duration of treatment (PB)	Monthly supervised treatment
China	Skin lesion, Nerve, *M. leprae* (smear, pathol), Pathology	MB/PB Ridley-Jopling	WHO-MDT	2 years	6 mo	Yes
Indonesia	Skin lesion, Nerve, *M. leprae* (smear), +/-Pathology	MB/PB Ridley-Jopling	WHO-MDT	1 year	6 mo	Yes
Japan	Skin lesion, Nerve, *M. leprae* (smear, pathol, PCR), Pathology	MB/PB Ridley-Jopling	WHO-MDT	1-3 years	6 mo	No
Malaysia	Skin lesion, Nerve, *M. leprae* (smear, pathol), Pathology	MB/PB Ridley-Jopling	See below*	See below*	12 mo	Yes
Myanmar	Skin lesion, Nerve, *M. leprae* (smear), +/-Pathology	MB/PB	WHO-MDT	1 year	6 mo	Yes
Philippines	Skin lesion, Nerve, *M. leprae* (smear)	MB/PB	WHO-MDT	1 year	6 mo	Yes
Republic of Korea	Skin lesion, Nerve, *M. leprae* (smear, pathol, PCR), Pathology	MB/PB Ridley-Jopling	WHO-MDT	Until BI = 0	24 mo	
Thailand	Skin lesion, Nerve, *M. leprae* (smear, +/-pathol), +/-Pathology	MB/PB Ridley-Jopling	WHO-MDT	2 years	6 mo	Yes
Viet Nam	Skin lesion, Nerve, *M. leprae* (smear, pathol), Pathology	MB/PB Ridley-Jopling	WHO-MDT	1 year	6 mo	Yes
Brazil	Skin lesion, Nerve, *M. leprae* (smear), +/-Pathology	MB/PB Ridley-Jopling	WHO-MDT	1 year	6 mo	Yes

*Augmented Sungai Buloh Regime (Malaysia)
PB
Monthly
RFP 600 mg
CLF 300 mg
Daily
Dapsone 100 mg
CLF 100 mg
Surveillance 5 years
MB
Intensive daily till MI= 0
RFP 600 mg
CLF 100 mg
Dapsone 100 mg
then
Monthly (3 years)
RFP 600 mg
CLF 300 mg
Daily
CLF 100 mg
Dapsone 100 mg
Surveillance 10 years

on the clinical signs and symptoms (Table 1 and 2). They are easily observed and elicited by any health worker after a short period of training. In practice, the majority of persons with such complaints self-report to a health center. Only in rare instances is there a need to use laboratory and other investigative procedures to confirm a diagnosis of leprosy. Therefore, an individual should be diagnosed with leprosy if he or she shows ONE of the following cardinal signs: 1) skin lesions consistent with leprosy with definite sensory loss, with or without thickened nerves; or 2) positive slit skin smears. In developed countries, for example in Japan, both the WHO and Ridley-Jopling classifications are generally used, and in-depth examination is preferentially attempted using histopathological and molecular biological tests. We suggest a first diagnosis as PB or MB, and classification by Ridley-Jopling only if the facilities and reagents are available for histopathology and molecular testing.

Peripheral Nerve Damage and the Lepra Reaction

How does *M. leprae* invade the peripheral nerves? *M. leprae* has an extreme predilection for the Schwann cells that surround the peripheral nerve axons. A recent study demonstrated that the species-specific PGL of *M. leprae* triggers uptake into Schwann cells by creating a complex with laminin-2. The *M. leprae*/laminin-α2 complexes then bind α/β dystroglycan complexes expressed on the Schwann cell surface.

Hansen's disease results in a wide range of impairments, the most important of which is damage to the peripheral nerves. Peripheral nerve damage causes loss of sensory, motor, and autonomic nerve function to the affected region, leading in turn to deformity, secondary deformity resulting from repeated trauma to the skin, and the inability to perform daily living. The consequences of nerve damage can impact the quality of life of those affected by the disease and also generate social stigma. Prevention of nerve damage and management of impairments are important components of any Hansen's disease program. Rehabilitation should be fully integrated within existing community-based programs in developing countries on an equal basis with other disabilities.

Most of the severe nerve destruction in Hansen's disease takes place during the lepra reaction, which consists of the reversal reaction (RR; type 1 reaction) and erythema nodosum leprosum (ENL; type 2 reaction). In RR, the level of cell-mediated immunity against *M. leprae* is suddenly el-

Table 2. Who Treats New Patients of Hansen's Disease in Selected Asian Countries and Brazil

Country	Dermatologist	Hansen's disease specialist	General physician	Registered nurse	Basic health medical staff	Basic health nonmedical staff
China	○	○	×	×	×	×
Indonesia	○	×	○	○1)	○1)	×
Japan	○	×	×	×	×	×
Malaysia	○	×	×	×	×	×
Myanmar	×	○	×	×	○	×
Philippines	○	○	○		○	○
Republic of Korea	○	○	×	×	×	×
Thailand	○2)	×	○	×	×	×
Viet Nam	△3)	△3)	×	△4)	△4)	×
Brazil	○	○	○	×5)	×5)	×6)

○ : yes, × : no and/or no staff
1) : yes (○), but confirmed with district administrator for leprosy
2) : sometimes when GP in field can't diagnosis & symptom is not clear
3) : confirm diagnosis
4) : supply MDT after diagnosis
5) : yes (○) if family health program is implemented
6) : yes (○) if remote area

evated, resulting in a severe inflammatory response in the areas of the skin and nerves affected by the disease. Acute inflammation in RR can destroy nerves and result in paralysis that can be permanent if not treated promptly and adequately. Clinically detectable neural involvement occurs in approximately 10% of PB and 40% of MB patients. Prompt and adequate treatment of the lepra reaction with anti-leprosy chemotherapy is the key to preventing irreversible nerve damage and disabilities. In the unfortunate case that permanent impairment occurs, patients should be given a course of rehabilitation.

Treatment and Control Strategy

Leprosy control has three major strategic components: Early detection, adequate treatment, and care to prevent disabilities and provide rehabilitation. Since the disease is caused by an infection, treatment with antibiotics plays a pivotal role in managing newly diagnosed patients. There are several effective chemotherapeutic agents against *M. leprae*. Dapsone, RFP, clofazimine (CLF), ofloxacin (OFLX), and minocycline (MINO) are components of the MDT regimen recommended by WHO (Table 1) and are commonly used by clinicians. Levofloxacin (LVFX), sparfloxacin (SPFX), moxifloxacin (MFLX), and clarithromycin (CAM) are also effective against *M. leprae*[6]. Following classification according to the flowchart, PB patients receive 600 mg RFP monthly, supervised, and 100 mg dapsone daily, unsupervised, for 6 months. MB cases are treated a 12-month period with 600 mg RFP and 300 mg CLF monthly, supervised, and 100 mg dapsone and 50 mg CLF daily. WHO has designed blister pack medication kits for

Table 2. Global Hansen's Disease Situation by Main Asian and Endemic Countries during 2009 (WHO)

Country	No. of new cases detected, 2009	No. of new cases of MB	No. of new female cases	No. of new cases less than 15 y/o	No. of new cases with G2 disabilities
India	133,717	64,782	47,361	13,331	4,117
Indonesia	17,260	14,227	6,887	2,073	1,812
Bangradesh	5,239	2,247	2,128	366	542
Nepal	4,394	2,216	1,479	282	178
Myanmar	3,147	2,189	1,106	165	468
Philippines	1,795	1,706	387	120	87
Sri Lanka	1,875	893	816	186	119
China	1,597	1,347	511	39	364
Viet Nam	413	295	114	12	80
Thailand	300	215	108	11	41
Cambodia	351	244	105	27	35
Malaysia	187	138	54	12	8
Timor	160	119	53	13	8
Lao PDR	101	73	18	2	14
Singapore	8	2	2	0	0
Republic of Korea	5	5	4	0	3
Hong Kong	5	2	1	0	0
Japan	2	1	1	0	0
Brazil	37,610	21,414	16,865	2,669	2,436
DR Congo	5,062	3,001	2,450	594	509
Nigeria	4,219	3,733	1,772	409	494
Ethiopia	4,417	3,909	287	302	408
Tanzania	2,654	2,138	1,068	260	292
Global total	244,796	139,125	89,538	22,485	14,320

PB and MB. Each easy-to use kit contains enough medication for 28 days.

RFP is an exceptionally potent bactericidal agent against *M. leprae*. Dapsone is bacteriostatic or weakly bactericidal against *M. leprae* and was the mainstay treatment for leprosy for many years until widespread resistant strains appeared. CLF binds preferentially to mycobacterial DNA and exerts a slow bactericidal effect on *M. leprae* by inhibiting mycobacterial growth. CLF also produces anti-inflammatory properties in the control of ENL. Combination therapy has become essential in reducing or preventing the development of resistance. Direct observation or supervision of RFP treatment is very important in avoiding the development of drug resistance. Patients take dapsone (and CLF) for the other 27 days, and health workers must ensure a regular and uninterrupted supply every day.

In most patients the presence of dead bacilli in the skin and other tissues does not cause any problem, and the dead organisms are gradually cleared by the phagocytic system of the body. However, in a small proportion of patients, the antigens from dead bacilli can provoke immunological reactions, such as the lepra reaction.

In most Asian countries, MDT was introduced by the WHO in 1981 and rapidly became the standard regimen. Ensuring a free supply of drugs for MDT, and an effective distribution system, is essential in all endemic countries. However, the duration of treatment differs in each country (Table 1) and some countries extend therapy beyond the WHO guidelines. The most critical activity is patient education at the end of treatment regarding the signs and symptoms of relapse and the importance of reporting immediately to the nearest health center when such problems arise.

Epidemiology

Recent epidemiological studies indicate that transmission of the leprosy bacillus is mainly mediated by airborne droplets through the respiratory system, in which the nose plays a central role. It is believed that there is widespread subclinical transmission of *M. leprae* with transient infection of the nose in endemic areas, although most of these cases do not develop clinical disease. The incubation period is unusually long for a bacterial disease: generally five to seven years, but can be as long as 40 to 60 years, especially in developed countries. The peak age of onset is young adulthood, usually 20-30 years of age; the disease is rarely seen in children less than five years old. Elderly new patients are registered in developed countries.

WHO publishes an annual report on the worldwide incidence of leprosy, including the number of new cases, prevalence and disabilities (Table 3)[7]. Many new cases are detected in countries such as India, Indonesia, Nepal, and other Asian countries, but the number of new cases detected annually is gradually declining in many countries, including those in the Asian region. Very few new leprosy patients are registered in developed countries, and most of those cases are among immigrants from countries where the disease is still endemic. Diagnostic methods, laboratory techniques, and treatment of Hansen's disease under WHO guidance have been adopted in developing countries. Dr. Saikawa investigated the association between Hansen's disease incidence and socio-economic factors such as gross national product (GNP), personal expenditure on housing, and the number of persons per household in Okinawa, Japan and Taiwan, and concluded that the improvement in socio-economic conditions greatly contributes to the reduction of Hansen's disease in the community[8]. Therefore, Hansen's disease is referred to as a "poverty-related disease". The proportion of children under the age of 15 among newly detected cases would be a good indicator of the situation in a country/region. Similarly, the proportion of cases with grade 2 disabilities and visible disabilities among newly detected cases is a reflection of early detection and treatment.

History of Hansen's Disease

Hansen's disease was recognized and described in the old literature of India, China, and Japan. The term "leprosy" originates from the Latin word "lepros," which means defilement. Throughout history, it has been feared as an incurable disease that causes severe deformities and disabilities, and its victims have suffered both from the disease

itself and from public discrimination. Hansen's disease was considered a divine punishment in the Old Testament and as a karmic disease in Buddhism.

In many countries, patients were isolated in villages far from human dwellings[9], and in socioeconomically improved countries patients were quarantined in leprosaria. Even after dapsone was found to be effective against Hansen's disease in the 1940s, such situations did not change significantly. The memory of the history of isolation is fading, and there is a danger that history will be repeated. A lack of proper understanding and the unabated propagation of traditional myths and beliefs about the disease have led to a build-up of negative social attitudes that culminate in social discrimination and stigma against persons affected by leprosy (PAL) and their families. While discrimination refers to the unjust or prejudicial treatment of people, especially on the grounds of being affected by leprosy, stigma is an ugly "act of labeling, rejection or unexplained fear of PAL."

The history of Hansen's disease in Asia can be broadly divided into three areas: 1. The presence of leprosy prevention law and the admittance of leprosy patients into leprosaria (e.g., Japan[10]). 2. The presence of leprosy prevention law and the admittance of leprosy patients to leprosaria in the pre-sulfone era. However, the leprosy policy changed to the settlement village movement in the post-sulfone era (e.g., Korea). 3. No leprosy prevention law and the government launch of an intensive program of leprosy control in consultation with WHO (Myanmar and many other countries).

Hansen's disease has been recently recognized in societies due to the civil liberty movement of NGOs and the WHO, but the prejudice against Hansen's disease persists.

Patients in Asia

The diagnosis and treatment of leprosy is relatively easy using the WHO-MDT regimen (Tables 1 and 2). Successful treatment with WHO-MDT can completely clear patients of the *M. leprae* pathogen. However, clinically cured patients continue to suffer from the deformities caused by delayed treatment. Once the diagnosis of Hansen's disease is made, patients are often ostracized by their neighbors and societies.

Asian governments are engaged in eliminating Hansen's disease with the aid of the Sasakawa Memorial Health Foundation, the International Federation of Anti-leprosy Association (ILEP), the Japan International Cooperation Agency (JICA), the WHO, and other GOs and NGOs. The governments promote further elimination of the disease, and use the slogan "Treat Early; Prevent Disability" to emphasize that minimization of leprosy complications is vital in developing countries. Since many patients continue to experience complications following MDT, preventing the worsening of complications, self-care, occupational training, and rehabilitation are steadily conducted.

In addition to preventing complications, the governments make every possible effort to dispel prejudice against leprosy. Stigmatization and ostracization, such as occurs in Japan or Korea, are common in many developing countries. There are villages inhabited by leprosy patients alone in the mountainous districts or remote rural areas. Since the introduction of MDT, the stigma has lessened considerably; however, some people do not know about MDT and believe that leprosy is still an incurable disease.

Hansen's Disease and Dermatologists

Since leprosy is not a skin disorder, patients are sent to a leprologist or leprosaria following a diagnosis of Hansen's disease by a dermatologist. Leprologists and healthcare workers are the main caregivers for leprosy patients in developing countries. However, a few dermatologists do offer treatment in developed countries because of the low number of patients. Therefore, dermatologists should make careful preparations and be able to recognize the disease's clinical manifestations because they could encounter a patient with Hansen's disease at any time.