Serological detection of leprosy employing *Mycobacterium leprae* derived serine-rich 45 kDa, ESAT-6, CFP-10 and PGL-I: a compilation of data from studies in Indian populations

OM PARKASH

*Department of Immunology, National JALMA Institute for Leprosy and Other Mycobacterial Diseases, TajGanj, Agra-1, India*

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Summary This article is a compilation of our findings recorded in the recent past where we have investigated the serological performance of *Mycobacterium leprae* antigens like-serine-rich 45 kDa protein (45 kD), early secretory antigenic target-6 (ESAT-6), culture filtrate protein-10 (CFP-10) and phenolic glycolipid-I (PGL-I) for detection (employing antibody detecting ELISA) of leprosy patients, particularly those belonging to the paucibacillary (PB) group. All of these antigens were capable of detecting, by themselves the majority (82–100%) of multibacillary (MB) patients. However, with respect to PB patients, only 18–47% (i.e. less than half) of the cases could be detected. Based on the results of serological assays for each of the four antigens separately a combinatorial approach was performed for these antigens, which increased the sensitivity for detection of PB patients to 73%, giving 36% improvement over conventional PGL-I based ELISA. Thus, the multi-antigenic serological approach is worthwhile for its establishment for detection of leprosy patients. Since ESAT-6 and CFP-10 are secreted proteins by nature, antibodies against them are worth exploring for detection of early infections and for monitoring of treatment efficiency. Nevertheless, efforts towards identification of more new antigens with serological potential are still desirable in order to further improve the detection rate of leprosy.

Introduction

Routinely, leprosy is diagnosed based on clinical criteria recommended by World Health Organization. According to this, people with one or more of the following characteristic symptoms are considered leprosy patients: hypopigmented or reddened skin lesion(s) with definite loss of sensation and/or involvement of the peripheral nerves and skin smear positivity for acid-fast bacilli. Unfortunately, in a significant number of cases even...
an experienced physician fails to diagnose leprosy due to inherent difficulties in clinical
diagnosis. Moreover, integration of leprosy with general health services has resulted
in non-availability of sufficiently experienced health workers to diagnose leprosy. Therefore, development of diagnostic test to complement the clinical diagnosis still remains a research priority.

Bacteriologically, leprosy has been grouped as highly bacillated [smear positive for acid
fast bacilli; Mycobacterium leprae (M. leprae)] infectious form called multibacillary (MB) and
less infectious form having a low load of M. leprae [smear negative for acid fast bacilli; M. leprae] called paucibacillary (PB). Further, antibody response is known to correlate with
the bacterial load in leprosy patients. Generally, assays based on the detection of specific
antibodies in serum are considered attractive, as they are simple, cost effective and easily
implementable in conditions faced in developing countries. In this context, phenolic
glycolipid-I (PGL-I) based antibody detecting assay has remained the most widely studied
serological tool for detection of M. leprae infection (as evidenced by several other papers in
this issue). Paucibacillary (PB) patients are a group in which, generally, the sensitivity for
detection of anti- M. leprae specific antibodies remains low i.e. 15–40%. At our laboratory
we have investigated, in the recent past, the serodiagnostic potential of serine-rich 45kDa
protein (45kDa; ML0411), early secreted antigenic target-6 (ESAT-6; ML0049), culture
filtrate protein–10 (CFP-10; ML0050) and phenolic glycolipid–I (PGL-I) antigens. Below, a brief review is provided describing serological data obtained with these four M. leprae antigens.

45 KDA ANTIGEN

In 1993, Vegas-Lopez et al. isolated and sequenced a gene encoding for a serine rich 45kDa
protein. The gene was expressed to produce a beta-galactosidase fusion protein. Using this
fusion protein, 78% of serum samples from MB patients and 68% of serum samples from PB
leprosy patients could be detected, indicating that 45kDa protein is a major M. leprae
antigen. In contrast, all serum samples from endemic controls were negative, while 26% of
the serum samples from tuberculosis (TB) patients were weakly positive. In southern
hybridization experiments the DNA from Mycobacterium tuberculosis and atypical myco-
bacteria failed to hybridize with the 45kDa antigen encoding DNA probe which highlighted
the specificity of the gene and thereby of its corresponding protein. Further, western blotting
analysis in the same investigation showed that M. leprae 45kDa protein was frequently
recognised by antibodies from leprosy patients. This seroreactivity was specific since
antibodies could not be detected in sera of TB patients. Summarised, these findings indicate
that 45kDa based assays are likely M. leprae-specific and warrant further exploration.

ESAT-6 AND CFP-10

Recent publications on the genome sequences of M. tuberculosis and M. leprae have
provided unprecedented opportunities to identify M. leprae unique proteins. In this process,
homologues of M. tuberculosis derived ESAT-6 (Rv3874) and CFP-10 (Rv3875) have also
been identified in M. leprae. M. leprae ESAT-6 and CFP-10 encoding genes (ML0049 and
ML0050 respectively) are located one after the other in the same operon, homologous to their
organisation in the M. tuberculosis genome. The sequence identity of M. leprae ESAT-6
and CFP-10 with their M. tuberculosis homologues is 36% and 40%, respectively.
At the T cell level both *M. leprae* proteins (ESAT-6; ML0049 and CFP-10; ML0050) have been documented to be potent antigens to stimulate T cell dependent IFN-\(\gamma\) production in a large proportion of individuals exposed to *M. leprae*.\(^{13,14}\) However, there is a significant cross-reactivity (at the level of IFN-\(\gamma\) production) between *M. leprae* ESAT-6 and CFP-10 as well as those of *M. tuberculosis* ESAT-6 and CFP-10.\(^{13,14}\) In contrast, Spencer et al.\(^{15,16}\) reported on the B cell stimulating potential of both of these *M. leprae* antigens. We considered these as encouraging findings to give us a lead towards pursuing the investigations on the potential of recombinant *M. leprae* ESAT-6 and CFP-10 for serodiagnosis of leprosy.

**PHENOLIC GLYCOLIPID-I**

In 1981, the identification and characterisation of *M. leprae* specific glycolipid, phenolic glycolipid-I was reported, for the first time, by Hunter et al.\(^{17}\) The oligosaccharide segment of this molecule is a species specific terminal trisaccharide: 3,6-di-\(O\)-methyl-\(\beta\)-D-glucopyranosyl-(1 \(\rightarrow\) 4)-2,3-di-\(O\)-methyl-\(\alpha\)-L-rhamnopyranosyl-(1 \(\rightarrow\) 2)-3-\(O\)-methyl-\(\alpha\)-L-rhamnopyranose. Payne et al. reported its antigenic nature in 1982.\(^{18}\) Both di- and trisaccharide parts of the molecule are principal antigenic determinants of PGL-I.\(^{19,20}\) As *M. leprae* cannot be cultured *in-vitro*, production of sufficient quantities of PGL-I from *M. leprae* was problematic. To overcome this problem, semi-synthetic derivatives were produced by linking the terminal di or trisaccharide part of PGL-I to a protein carrier such as bovine serum albumin (BSA) or human serum albumin (HSA), resulting in natural disaccharide linked to BSA/ HSA via an octyl linker (ND-O-BSA/HSA) and natural trisaccharide linked to BSA/ HSA via a phenyl linker (NT-P-BSA/HSA).\(^{21}\) Over the years, using these semi-synthetic derivatives various types of serological assays for detection of *M. leprae* infection have been designed.\(^{5}\) In our studies, we have included ND-O-HSA (an analogue of PGL-I) to compare the performance of 45 kDa or ESAT-6 or CFP-10 protein with that of PGL-I based assays. In addition, we explored the use of PGL-I in combination with these other three proteins, with the aim to improve the detection rate of leprosy.

**PERFORMANCE OF ANTIGENS FOR DETECTION OF LEPROSY PATIENTS**

In our studies, recombinant proteins derived from serine-rich 45 kDa antigen of *M. leprae*,\(^{6}\) ESAT-6\(^{7}\) and CFP-10\(^{8}\) demonstrated to be serologically useful antigens. Regarding these three antigens, our reports are in line with several findings indicating that the 45-kDa\(^{9,10,22,23}\) ESAT-6\(^{15,22–24}\) and CFP-10\(^{16,22–25}\) are potent B-cell stimulating antigens and that their serological behaviours are highly specific for leprosy. Among these antigens, the performance of the serine-rich 45 kDa antigen was found to be the most impressive (sensitivity: 100% in MB and 47-4% in PB; specificity: 100%),\(^{6}\) whereas that of phenolic glycolipid-I (PGL-I, the most widely used antigen for serological studies in leprosy) was slightly lower (sensitivity: 94-4% in MB and 36-8% in PB; specificity: 96%).\(^{6}\) The performances of ESAT-6 (sensitivity: 82-4% in MB and 19-4% in PB; specificity: 100%),\(^{7}\) and CFP-10 (sensitivity: 83-3% in MB and 18-4% in PB; specificity: 98%)\(^{8}\) were less sensitive, but nonetheless very specific. Thus, all of these antigens (45 kDa, ESAT-6 and CFP-10) were highly efficient for detection of MB patients, although they failed to detect the majority (53–82%) of PB patients (sensitivity varied from 18%–47%). Nevertheless, combination of results obtained with PGL-I and those with 45 kDa or ESAT-6 or CFP-10 further improved the detection rate: all three antigens showed improved detection capacity when combined with PGL-I (45 kDa + PGL-I,
ESAT-6 + PGL-I and CFP-10 + PGL-I). With respect to PB patients the performance was most efficient and statistically significant (sensitivity: 60.5%; specificity: 96%) when the combined results of 45 kDa + PGL-I were used resulting in an improvement of 23.7% over PGL-I alone.6

Further analysis of the results indicated a serological heterogeneity against various antigens (PGL-I, 45 kDa, ESAT-6 and CFP-10).26 The heterogeneity among MB patients was lower (29.4%) in comparison to that (69.7%) in PB patients. Prompted by this differential serological response, we analysed the results in a combined fashion considering the results of all four antigens together. Interestingly, the sensitivity for detection of PB patients rose to 73% which represented a significant (21–54% improvement in sensitivity with 94% specificity) when compared to either PGL-I or ESAT-6 or CFP-10 based assays. However, there was 21% improvement in detection rate over that of 45 kDa antigen. From all these observations, it appears that multi-antigenic testing for detection of antibodies could help in detecting larger number of leprosy patients.

In our studies, there were some cases when antibodies were not detectable despite the obvious presence of M. leprae inside the host.6–7 This could be due to situations where antibodies may not be freely available due to antigen antibody complex formation and due to genetic make-up of some individuals where antibody response against a particular antigen may be absent or it may be too weak for detection.

PERSPECTIVES

Since 1985 the global leprosy programme has remained appreciably successful recording treatment of more than 14 million leprosy patients resulting in more than 90% fall in global case-load. However, approximately 250,000 new cases of leprosy are still recorded annually indicating that the chain of transmission has not been broken and that interruption of leprosy transmission is one of the main challenges for leprosy control programmes. In terms of case load: India, Brazil, Indonesia, Bangladesh, Democratic Republic of the Congo, Ethiopia, Nepal and Nigeria top the list respectively.27

With respect to India, though this country has achieved the goal of elimination (i.e. prevalence rate of less than one case per 10,000 populations) of leprosy as a public health problem at national level in December 2005, higher prevalence (about 1–2/10,000 as against the national prevalence rate (0.71/10,000) as documented on 1st April 2009) of leprosy is reported in some areas.28 Moreover, considering newly detected cases (133,717) and existence of registered cases (87,190) of leprosy, India holds more than 50% of the global leprosy burden (new case detection = 244,796; registered prevalence = 211,903).27 These numbers reflect that leprosy actually will remain a public health problem in India for several more years to come. This highlights the need for continued commitment towards control of the disease and to leprosy research in endemic countries including India. The current strategy for combating leprosy is based on the early detection and treatment of patients to halt transmission. Hence, there is need of a simple, sensitive, specific and cost effective test for diagnosis of leprosy. Since serological assays are easy and affordable, they may prove to be potential alternatives for the conditions prevalent in most developing countries. By adopting the various approaches outlined above, we could detect, specifically, 21–54% more of the PB patients. Hence, the strategies reported by us are worthwhile evaluating before their incorporation into the diagnostic algorithm for patients who have clinical signs suggestive of active leprosy.
ESAT-6 and CFP-10, being secreted proteins, may be produced in the early active phase of infection that in turn may give rise to early antibody responses. Consequently, serological assay(s) developed using M. leprae ESAT-6 and CFP-10 may be of use towards early detection of M. leprae infection and thereby in management and control of leprosy. Further, ESAT-6 and CFP-10 would be produced only by metabolically active viable Mycobacterium leprae growing inside the host. Therefore, detecting antibodies against ESAT-6 and CFP-10 may provide a better alternative marker for monitoring the anti-leprosy treatment. Considering this hypothesis, it would be interesting to evaluate ESAT-6 and CFP-10 based serology for monitoring chemotherapy in seropositive (mostly multibacillary) leprosy patients. However, a need for identification of other potential antigens is still felt in search of development of more sensitive serological assays.

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References


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