General Introduction
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Leprosy, an infectious disease caused by *Mycobacterium leprae*, is considered as two conjoined diseases characterized by chronic mycobacterial infection in the first phase and peripheral neuropathy in the second phase. During the first phase, an extraordinary range of cellular immune responses are elicited by the system to fight against the infection. The second phase is initiated as a result of both the bacterial infection and the accompanying immunological responses elicited by the host [Scollard *et al.*, 2006a]. The disease mainly affects skin and peripheral nerves. The neural complications associated with *M. leprae* infection leads to debilitating physical disabilities thereby affecting the physical, social and psychological well-being of the patients.

Leprosy is a complex disease with diverse clinical and histopathological manifestations. Ridley and Jopling classified leprosy based on the clinical, immunological and histopathological criteria into five forms – tuberculoid (TT), borderline tuberculoid (BT), mid borderline (BB), borderline lepromatous (BL) and lepromatous (LL). Tuberculoid leprosy results in a positive lepromin skin test and is characterized by a few skin lesions with low or nil bacteria in the macrophages. The TT patient responds to the infection with a strong cell mediated immune response and a weak antibody mediated immune response. In contrast, lepromatous leprosy results in a negative lepromin skin test and is characterized by a large number of skin lesions with the number of bacteria per gram of tissue reaching up to $10^{12}$. A high titer for anti-*M. leprae* antibodies is seen in LL patients, but their cell mediated immune response is modest or absent. The intermediate forms are immunologically unstable and it can progress or recede to either end of the spectrum [Ridley and Jopling, 1966]. During the course of treated or untreated leprosy, the patient can be presented with acute inflammatory complications called leprosy reactions. Two major types of leprosy reactions called Type 1 or reversal reactions (RR) and Type 2 or erythema nodosum leprosum (ENL) can be seen in leprosy patients. Type 1 reactions generally occur in BT, BB and BL cases and Type 2 reactions occur in BL and LL cases. Approximately 30-50% of leprosy patients develop reactions [Scollard
For therapeutic purposes, leprosy is mainly divided into two groups based on the number of skin lesions present - paucibacillary (PB) and multibacillary (MB) [WHO, 1982]. Less than or equal to five lesions are seen in PB cases (TT and BT) whereas MB (BB, BL and LL) is presented with greater than five lesions.

In the early 1990s, World Health Organization (WHO) implemented multidrug therapy (MDT) for the treatment of leprosy with the goal of eliminating the disease as a global health problem by the year 2000. Reduction in the prevalence to less than one case per 10000 populations was considered as the target for achieving global leprosy elimination. The registered cases of leprosy had gone down from about 5.4 million in 1985 to about 200000 new cases detected in 2010 and the global prevalence rate per 10000 had been reduced from 21.1 to 0.37 [WHO, 2010a; 2011]. The prevalence of leprosy in India was considerably very high and there were 57.6 cases per 10000 populations before the implementation of MDT as per 1981 estimates. As a result of the rigorous leprosy eradication campaign, India achieved the goal of national level elimination of leprosy as a public health problem by the end of 2005 when the prevalence rate stood at 0.95 cases per 10000 populations. As on March 2011, the national prevalence rate is reported to be 0.69 cases per 10000 populations with 32 out of 35 states/union territories in India achieving the goal of elimination [NLEP, 2011; 2012].

Leprosy continues to be an important global health concern as it can cause disabilities and deformities in the affected individuals even beyond the cure of infection [WHO, 2010b]. Despite the reduction in global prevalence rate, highly endemic pockets still remain in certain areas of Angola, Brazil, Central African Republic, Democratic Republic of Congo, India, Madagascar, Mozambique, Nepal, and the United Republic of Tanzania [WHO, 2012a]. This indicates that prevention of active transmission is yet to be achieved. Inadequate knowledge on the mode of transmission of the disease and the complications in identifying how many M. leprae infected individuals actually develop the clinical disease are the major hindrances in preventing active transmission and reducing the prevalence further [Rodrigues and Lockwood, 2011]. The incubation period of this disease can vary from 3 to 5 years and the affected individual may self-cure or develop PB or MB leprosy [Mira, 2006]. Diagnosis of the disease in the subclinical stages is important to initiate the treatment
in the early stages. The delay in diagnosis of leprosy can have serious consequences such as increased risk of irreparable nerve damage leading to permanent disabilities. Although clinical diagnosis of leprosy is generally straightforward, there is no good point-of-care test available to confirm leprosy. Generally, clinical diagnosis of leprosy is based on the presence of skin lesions, anesthetic patches and enlarged nerves in the patients [WHO, 1998]. Detection of subclinical leprosy based on these criteria is impractical since there is no clinical clue to look for the presence of bacilli or pathological changes in this stage. This makes detection of the disease in an early stage, a key challenge.

The initiative for diagnostic and epidemiological assays for leprosy (IDEAL), taken under Tropical diseases research program of WHO, gathers all the authoritative leprosy research groups in the world to develop diagnostic tests for detection of leprosy at an early stage, even before the disease is manifested [Aseffa et al., 2005]. IDEAL consortium is mainly focused on identifying field-friendly diagnostic tests and recommends serology and T-cell based diagnostic assays employing M. leprae specific proteins and peptides for the detection of subclinical leprosy. Serological approaches are the best non-invasive methods for diagnosing a disease. The serological tests for diagnosis of leprosy are based on detection of antibodies to M. leprae antigens such as phenolic glycolipid -1 (PGL-1) [Burgess et al., 1988], lipoarabinomannan (LAM) [Levis et al., 1987], 35 kDa antigen [Triccas et al., 1996], 45 kDa antigen [Parkash et al., 2006a], early secretory antigenic target-6 (ESAT-6) [Parkash et al., 2007], culture filtrate protein-10 (CFP-10) [Parkash et al., 2006b] in the patient sera. These tests are specific for M. leprae infection and were found to be reproducible in laboratories across the world. The greatest drawback of these tests was the poor sensitivity as they failed to give a confident seropositivity for paucibacillary leprosy in many instances [Parkash et al., 1995], thus making their application in diagnosis of subclinical leprosy in high risk populations obscure [Sinha et al., 2004]. Other commonly used diagnostic methods for leprosy such as histopathological examination (detection of acid fast bacilli in the skin smear) and molecular-type assays (detection of M. leprae DNA by PCR) will not be applicable to detect subclinical leprosy, since these depend on the presence of detectable number of bacilli in the sample.
An alternative approach to diagnose a disease is to look for the disease specific changes in the patients [Hoffmann, 2006]. The disease specific changes can usually be identified as variations in the protein profile of biological fluids such as plasma/serum. Plasma/serum is of substantial clinical relevance since it contains tens of thousands of proteins along with their modified forms which carry out or regulate most biological processes. Hence, the proteome profile of plasma/serum can reflect the physiological and pathophysiological state of an organism [Ahrens et al., 2010]. The changes in plasma/serum profile can be satisfactorily studied using high resolution protein separation methods such as two dimensional gel electrophoresis (2D PAGE) and two dimensional difference gel electrophoresis (2D DIGE) [Issaq and Veenstra, 2008]. DIGE technology provides adequate sensitivity, reproducibility and tolerance to a wide dynamic range for the analysis of complex samples such as plasma/serum to exploit this in quantification of differentially expressed proteins [Marouga et al., 2005]. Identification of the differentially expressed proteins can be achieved by mass spectrometry. Integration of two dimensional separation, quantification of differential expression using suitable image analysis softwares and identification of differentially expressed proteins by mass spectrometry have revolutionized the field of disease proteomics to promote better understanding of the disease process, develop new biomarkers for diagnosis at the early stages and to accelerate drug development.

Alterations in the serum proteome profile in response to *M. leprae* infection had been demonstrated in LL and ENL patients using proteomic approaches [Gupta et al., 2007; 2010]. Identification of these host responses during the early stages of infection will aid or complement the diagnosis of infection. Host-pathogen interactions during the early stages of infection could leave an imprint in the plasma/serum proteome of the host and this strategy could be applied to identify screening markers for *M. leprae* infection during the subclinical stages. One of the major hindrances in studying the host-responses to early stages of *M. leprae* infection is identification of subclinical infection in humans. The disease has a long window period and hence the onset of infection might not be predicted accurately. Moreover, there will not be any clinically detectable signs of infection such as skin lesions, enlarged nerves or anesthetic patches in this subclinical stage.
Hypothesis-driven experimentation can be carried out using animal models where the pre- and post-symptomatic phases can be monitored accurately after initiating experimental infection [Bousette et al., 2008]. In this study mice, experimentally inoculated with *M. leprae* in the footpads, were used as model systems to study the host-responses in subclinical leprosy. Even though *M. leprae* infection in mice does not lead to multispectral clinical leprosy as seen in humans, evidences suggest that the bacteria remain virulent in mice [Williams et al., 2004]. This suggests that mice may elicit a generalized response to the presence of virulent bacteria in the footpads. Analysis of plasma proteome of the *M. leprae* infected mice using 2D PAGE and 2D DIGE and comparison with uninfected control samples demonstrated that the infection of *M. leprae* in the footpads of mice indeed altered the plasma proteome profile of the host. The differentially expressed proteins were quantified using ImageMaster™ 2D Platinum and DeCyder™ 2D image analysis softwares. The proteins showing significant changes in the expression levels were identified employing MALDI-TOF and LC-MS/MS mass spectrometry. The association of these proteins to the disease state was studied. The expression pattern of proteins showing the highest level of variation during *M. leprae* infection was validated using antibody based methods such as immunoblotting and ELISA.