Chapter 1  INTRODUCTION

The two major mycobacterial diseases, tuberculosis and leprosy, continued to have considerable impact on human health worldwide even today. Tuberculosis is a major health problem throughout the world causing large number of deaths, more than that from any other single infectious disease (Raja 2004). The World Health Organization (WHO) declared tuberculosis (TB) a global health emergency in 1993 with good reason; sixteen years later, the scale of the problem still remains an urgent global challenge, with more than 9 million new cases and almost two million deaths each year (WHO report 2007). There were estimated 9.0 million new cases of TB including 5.7 million new smear positive cases in 2013, of which, 1.1 million (13%) were HIV positive. Asia (south east and western pacific) accounts for 56% of global cases. India ranked 1st based on estimated number of incident cases of all forms in 2013 (WHO report 2014).

Leprosy, another chronic infectious disease, caused by *Mycobacterium leprae*, has a wide distribution in the world. As on march 2014, Leprosy was eliminated as public health problem in India attaining the national prevalence rate of < 1: 10,000(0.68/10,000), but five states/UTs, Chattishgarh, Odisha, Chandigarh, Lakshadweep and Dadra&Nagar Haveli of India still reporting a prevalence rate between 1 and 2 per 10,000, above the elimination goal (NLEP 2014). Although significant progress has been made in controlling leprosy and reducing the burden of the disease worldwide, but a marginal global increase in prevalence and new case detection is observed in 2012 compared to 2011, so much still remains to be done to reduce the disease burden further (WHO 2013 report). Though the annual detection of new case continued to decline globally, the transmission is still continuing in the certain communities.

Everyone exposed to these mycobacterial species do not become infected, even after infection, only about 1/10 infected individuals become seriously ill. The factors determining an individual’s risk of infection and breakdown to active disease are multifactorial and involve host pathogen interactions and environmental conditions. One of the reasons behind this is thought to be the effective immune defense mechanisms of the individuals (Takiff 2007). In human, innate immune and adaptive immune systems, in combination, provide the defense mechanism against pathogenic invaders.
Phagocytosis is the mechanism by which cells of innate immune system directly engulf the pathogen and destruct the pathogen. In this process, cells of the immune system, such as monocytes, neutrophils, macrophages, mast cells and dendritic cells function as important effectors in the innate immune response. Among these cells, circulating monocytes are increasingly implicated as essential player in defense against a range of microbial pathogens. It also contributes to antimicrobial defense by supplying tissues with macrophage and dendritic cell precursors (Kubey et al. 2012, Paul 2012). Inflammatory monocytes respond to microbial stimuli by secreting cytokines and antimicrobial factors. It expresses CCR2 chemokine receptor and traffics to sites of microbial infection in response to MCP-1 secretion (Serbina et al. 2008). Chemokine mediated monocyte recruitment is pivotal for immune control of a variety of microbial infections.

Immune response to all pathogens, at least a part, dependent on cytokines, regulate all cells of the immune system. Cytokines are the messengers for the immune system, which take signals to the immune cells to respond against infectious individuals. Chemokines are superfamily of specialized cytokines, that directly and differentially chemoattract specific subsets of leukocytes (Serbina et al. 2008). The immune response against mycobacteria is mounted in a complex process. It has been shown that chemokines participate in protective and immunopathologic host response during tuberculosis (Jo et al. 2003). The hallmark of mycobacterium induced pathology is characterized by an inflammatory response culminating in the formation of granuloma. Thus, production of chemokines is essential for the recruitment of inflammatory cells at the site of infection and the formation and maintenance of granuloma (Flynn and Chan 2001). The role of chemokines in tuberculosis and leprosy are gradually being unraveled. MCP-1 (monocytes chemoattractant protein-1) is a monocyte specific chemotactic factor produced by wide variety of cell types, including monocytes, fibroblasts, vascular endothelial cells and smooth muscle cells (Rot and Andrian 2004). MCP-1/CCL2 is the most potent chemoattractant and activator of monocytes, attracts CD4+ and γδ T cells. It is a central component of the granulomatous response (Taub et al. 1995). The essential role of CCL2 is further elucidated by murine studies where it is shown to play a role in protection against tuberculosis (Lu et al. 1998 and Rutledge 1995). Apart recruiting
monocytes to the site of infection, CCL2 has further role in Th cell polarization during mycobacterial infection. It recruits both Th1 and Th2 type of cells but higher CCL2 levels specifically favours Th2 type of response by inducing the production of several Th2 cytokines (Chensue et al 1995, Handel and Domaille 1996). It was reported that functional promoter polymorphism in MCP-1 distal regulatory region, located at position -2518 (A/G), is associated with increased susceptibility to pulmonary tuberculosis (Flores-Villanueva et al 2005), while -362G/C polymorphism in MCP-1 promoter region provides protection against M.tb infection (Thye et al 2009). Several genetic associations of MCP-1 gene polymorphisms with susceptibility or protection has been reported. Subjects with MCP-1 -2518G allele were shown to be at increased risk of clinical TB in study populations from Mexico and Korea, Peru, Zambia, Tunisia compared to subjects carrying -2518A allele (Flores-Villanueva et al 2005, Ganachari et al 2010, Buïjtels et al 2008, Selma et al 2011). While no effect of MCP-1 -2518A/G polymorphism were observed on TB susceptibility or resistance in Canadian, South African, Russian, Brazilian, Gambians and Argentineans (Larcombe et al 2008, Moller et al 2009, Jameson et al 2004, Erwards et al 2012), contradictory results were reported in populations of Ghana and Morocco, where -2518A/G polymorphism shows association with protection against TB (Thye et al 2009, Arji et al 2012). In a report on sahariya tribe a population of central India, showed no association of -2518A/G and -362G/C polymorphism with TB (Mishra et al 2012). Study has been reported on MCP-1 gene polymorphism in HIV & TB patients from south Indian population, where they found no association (Alagarasu et al 2009). Another study done on south Indian population also showed no association of -2518A/G polymorphism with TB (Singh et al 2013). Two meta analysis reports conclude that -2518A/G polymorphism of MCP-1 gene showed significant association with susceptibility to TB and Asians and Hispanics are more prone to the disease (Feng et al 2011, Zhang et al 2012). Beside TB, several studies conducted in different populations showed that -2518 (A/G) polymorphism has association with several other diseases, like psoriasis, type2 diabetes, leishmaniasis, myocardial infarction, renal carcinoma, hypertension, crohn’s etc. (Wang et al 2007, Jeon et al 2013, Ahluwalia et al 2009, Bucova et al 2009, Ramasawmy et al 2010, Liu et al 2013, Mahgoub et al 2011, Palmeri et al 2010). These reports suggest that among other factors, host genetic factor
could have a significant role in patients towards the drug response and disease progression. Several studies have been made to find out the association of different gene polymorphisms with tuberculosis in different populations but the results have been contradictory and not sufficient to reach to a final conclusion. To establish a strong correlation it is essential to study different populations of the same region or of other region, so that a clear understanding on the possible role of these polymorphisms in disease susceptibility or resistance can be revealed. Thus a case control based study is required to know if SNPs in MCP-1 gene have role. It is also interesting to know the effect of these SNPs on the expression of other chemokine genes present in the same chromosomal region and play an important role in immune response against tuberculosis in association with other immune cells. The role of MCP-1 in leprosy is not well understood and MCP-1 gene polymorphism is unexplored, but increased expression of MCP-1 has been detected in both skin lesions and serum of lepromatous leprosy patients (Wook et al 2002, Kirkaldy et al 2003). Thus any association between MCP-1 gene polymorphism and these mycobacterial diseases would provide insight into clinically important immune mechanisms that influence disease susceptibility, or as factors for differential disease progression.

Further, it has been shown that -2518 A/G polymorphism is responsible for overproduction of MCP-1 protein, which makes host susceptible for many diseases, including tuberculosis. Several transcription factors have been demonstrated to bind differentially to this -2518A/G MCP-1 polymorphisms, including PARP-1, IRF-1, Prep1/Pbx complexes, STAT-1 (Gonzalez et al 2002, Mummidi et al 2009, Wright et al 2008, Nyquist et al 2010) in different cell sources and in response to different stimulants in order to explain the increased CCL2 expression levels associated with -2518G allele. But still more direct evidences is needed to explore the mechanism behind. Therefore, it is important to investigate the mechanism responsible for the overexpression/reducedexpression of the protein in response to mycobacterial antigens. Out of various possible mechanisms, we intend to focus on whether this polymorphic site is a binding site for any nuclear factor, which regulates the production of MCP-1 protein resulting from the polymorphism. It has been reported that IFN-\( \gamma \) regulates and induce different chemokines production, including MCP-1 and MCP-3, in different in vitro/in vivo
studies (Mohammed et al 1998, Sauty et al 1999). IFN-γ is a key cytokine produced by Th1 cells in response to mycobacterial infection and involved in further production of inflammatory cytokines and chemokines (Flynn and Chan 2001). Thus, the investigation on the role of IFN-γ in the production of MCP-1 in polymorphic and non polymorphic (wild-type) individuals is important in order to understand further the susceptibility/protective mechanisms. While MCP-1 has a key role in both innate and adaptive immunity and polymorphism in MCP-1 gene is reported to have effect on these responses, it is most important to explore how these polymorphisms exert their effect on disease progression. The present study is aimed at explore the association of -2518 A/G and -362G/C promoter polymorphism with different groups of tuberculosis and leprosy patients and to demonstrate its association, if any, with the development of disease. As MCP-1 is reported to favour the Th2 response, it is also proposed to examine the effect of polymorphisms on the immunological response of the host against mycobacterial antigens in view of to the production of various Th1 cytokines, such as IL-12, TNF-α, IFN-γ, TGF-β, which are mainly involved in host immune response against M.tb. It is also proposed to reveal the expression of chemokine genes, MIP-1α, RANTES and IL-8, present in the same gene cluster of MCP-1 and reported to have a role in mycobacterial infections.

It is also proposed to explore the mechanism of overproduction/reduced production of MCP-1 protein due to -2518A/G polymorphism in reference to the binding of transcription factors at polymorphic site, being responsible for transcription of the gene, if any. The transcription factor bound to the oligonucleotides containing polymorphic site will then be identified by MALDI-TOF. Further, involvement of IFN-γ in the production of MCP-1 in polymorphic and wild type individuals will be investigated. The outcome of these studies may be helpful in understanding the differential immune response exhibited by tuberculosis and leprosy patients and healthy individuals. Thus, understanding the host parasite interaction at the level of crucial immune mechanism would be helpful in designing the immunotherapeutic research programme in different populations or individuals with different genetic characteristic.
Objectives:

1. To detect single nucleotide polymorphisms in the regulatory region of \textit{MCP-1} gene (-2518A/G and -362G/C) in tuberculosis and leprosy patients and healthy contacts.

2. To analyze the association of these SNPs with mycobacterial diseases (tuberculosis and leprosy).

3. To analyse of functional relevance of -2518A/G SNP with reference to immune response by -
   (A) Comparing the production of cytokines IL-12, TNF-\( \alpha \), IFN-\( \gamma \), TGF-\( \beta \) in response to mycobacterial antigens in polymorphic and wild type individuals.
   (B) Evaluation and comparison of expression of chemokine genes, MIP-1\( \alpha \), RANTES and IL-8 in polymorphic and wild type individuals.
   (C) Examination of the effect of rIFN-\( \gamma \) on the production of MCP-1 in polymorphic and wild type individuals, and
   (D) To explore the mechanism of up regulation or down regulation of MCP-1 in individuals with mutant allele with reference to transcription factors.