INTRODUCTION
1. Introduction

*Mycobacterium tuberculosis* (*M. tb*), the acid-fast rod shaped bacteria is the etiological agent of tuberculosis in humans. The bacteria is also known as Koch’s bacilli after the name of it discoverer Robert Koch who isolated the tubercle bacillus in 1882 and later got Nobel prize for his discovery in 1905.

Although multiple organs are infected by *M. tb* it is typically a lung pathogen transmitted from patients to healthy individuals by their respiratory secretions. It enters the healthy individual by inhalation of droplet nuclei containing infectious organism. Once inside the host, it is encountered and phagocytized by the alveolar macrophages which carries it to the lymphatic system and bloodstream from where it can spread to multiple organs. Antigen Presenting Cells (APCs) like macrophages and dendritic cells (DCs) after phagocytizing the bacteria break it into small proteolytic fractions which then binds to the major histocompatibility complex MHC-II on their surface. This peptide-MHC complex (pMHC) thus formed ligands with the T cell receptor (TCR) which thereafter activates CD4⁺ T cells and subsequently initiates acquired immune response against mycobacterial infection. Consequently, activated CD4⁺ T cells produce several cytokines that are crucial for the activation of bactericidal mechanisms of the macrophages harboring the pathogen. During infection Tumor Necrotic Factor α (TNFα) is one of the earliest cytokine produced by macrophages, DCs and T cells. IFN-γ is another cytokine which plays the central role in prevention of mycobacterial infection. It is mainly produced by Th1 cells and Natural Killer cells (Lieberman, Hunter et al. 1990).

CD8⁺ T cells alongwith TCR γδ T cells also produce IFN-γ but in a relatively less amount (Flynn, Chan et al. 2001). Infected cells produce TNFα that has an autocrine activity and prime the cell’s (macrophages) microbicidal mechanisms together with IFN-γ. Both TNFα and IFN-γ are indispensible for the control of mycobacterial infections (Cooper, Adams et al. 2002). They partly do it, by spurring expression of inducible nitric oxide synthase (iNOS), which is expressed upon macrophage activation and further leads to the production of (Flesch, Kaufmann et al. 1990; Liew, Li et al. 1990) reactive nitrogen species (RNS) (MacMicking, Xie et al. 1997).

It has been well documented that the cellular response to *M. tb* is relatively slow as compared to responses to viral infection or fast-growing bacterial infections (Davenport, Belz et al. 2009). This might be due to immune-modulation of the infected and nearby cell by the *M. tb* which aids it to evade robust host immune response. The persistence of the mycobacterial infection is thought to be, in part, dependent
on the suppressive effect of regulatory T cells in order to avoid exacerbated pathology (Belkaid, Rouse et al. 2005). These are CD25+ T cells that express forkhead box P3 (Foxp3) transcription factor (Fontenot, Rasmussen et al. 2005) which play a potential role in mycobacterial subversion of host immune response. In previous study, an increased proportion of these suppressive regulatory T cell subset has been observed during the experimental mycobacterial infections (Kursar, Koch et al. 2007; Roque, Nobrega et al. 2007; Scott-Browne, Shafiani et al. 2007). Similar results have been documented in patients infected with M. \textit{tb} (Guyot-Revol, Innes et al. 2006; Ribeiro-Rodrigues, Resende Co et al. 2006; Chen, Zhou et al. 2007). In addition to culmination of robust innate and adaptive immune response several hypothesis have been put forward in the context of the ability of the bacteria to prevent its elimination from the host and persist within the host: i) Unique biochemical property of the mycobacterial cell wall render it impermeable to anti- microbial compounds produced by the host, ii) bacteria can actively e modulate its antigen presentation on the surface of APCs (Gercken, Pryjma et al. 1994; Pryjma, Baran et al. 1994; Boom, Canaday et al. 2003) and forestall the microbicidal function of the host cell (Rhoades and Ullrich 2000; Boom, Canaday et al. 2003) attributing its persistence within the host for lifetime; iii) mycobacteria enter into an inactive, non-dividing state \textit{in vivo} once their cell division is restricted by the cellular immune response (Rogerson, Jung et al. 2006; Peyron, Vaubourgeix et al. 2008). iv) It seems that the protective immune response against mycobacterial infections depends partly on the formation of organized inflammatory lesions containing macrophages, T cells, B cells, DCs, neutrophils, fibroblasts and extracellular matrix components termed granulomas (Ehlers, Benini et al. 1999; Mogues, Goodrich et al. 2001; Florido, Cooper et al. 2002, Cosma, Sherman et al. 2003). However the role of granuloma can be assessed differently. Granuloma plays a critical role in prevention of dissemination of the infection. It provides the microenvironment where the acquired and the innate immune systems of the host interact for effective macrophage activation.(Saunders, Frank et al. 2000; Ulrichs, Kaufmann et al. 2006). However, mycobacteria uses granuloma as a confined niche where it can safely persist within the macrophages walling off itself from host immune component, persisting lifetime and become active once the host immune system weakens.

One promising new way to effectively treat tuberculosis is to supplement the current anti-TB drug treatment with a new strategy that could induce a strong immune response with the potential for developing long-lasting immunity against re-infection by M. \textit{tb}.

Although host mounts a robust immune response against M. \textit{tb}, it can anyhow evade host immune system and can persist within lifelong. Th1 mediated immunity is the key player in preventing mycobacterial infection, as opposed to Th2 mediated, because of the fact that gene-deleted mice incapable of making IFN-\(\gamma\) are unable to inhibit M. \textit{tb} growth in their lungs and other organs (Cooper, Dalton et al. 1993;
Flynn, Chan et al. 1993). Type 1 T helper (Th1) cells prevent host body from intracellular bacteria and some viruses and characteristically produce IFN-γ, IL-2, and tumour necrosis factor β (TNF-β). These cytokines further activate macrophages and are responsible for cell-mediated immunity. On the other hand, Th2 cells produce IL-4, IL-5, IL-10, and IL-13, which are responsible for strong antibody production, activation of eosinophil, and inhibition of several macrophage functions. Failure of hosts to resolve *M. tb* infection is eventually assessed by the inability of immune cells to attain mycobactericidal function as insufficient Th1 cells are generated which fail to activate macrophages to a mycobactericidal state. The reduction in number of thus activated T cells might be due to inhibition of their proliferation in response to *M. tb* infection. Suppressive effect of some cells which are used by the bacteria to persist even in presence of robust host immune response. Th1 cell have been reported to be suppressed by cells like regulatory T cell (Treg) and MSCs (Thornton, Shevach et al. 1998; Bartholomew, Sturgeon et al. 2002; Di Nicola, Carlo-Stella et al. 2002). Gr-1CD115 Myeloid Suppressor Cells, is another population of cell that not only suppress T-cell proliferation *in vitro*, but also induce the development of Foxp3* T regulatory cells (Treg) *in vivo*, which are suppressive (Huang, Pan et al. 2006). Veto cells is yet another subset of suppressor cells that are passively recognized by autoreactive cytotoxic T cells and eliminate them (Miller et al. 1980). T cells are also documented to be suppressed by suppressor monocyte. They have been reported to do so by tryptophan catabolism after human hematopoietic stem-cell transplantation (Hainz, Obexer et al. 2005). But on characterization, we observed that there was immense recruitment of MSCs to the organ infected with *M. tb* and hence could be responsible for persistence of *M. tb*.

MSCs are multipotent precursors to many mesodermal cell lineages in vertebrates, generally resides in bone marrow. MSCs have a characteristic ability to differentiate *in vitro* and *in vivo* into adipogenic, chondrogenic, and osteogenic lineage (Alhadlaq, Mao et al. 2004) and are the focus of multiple clinical applications. Due to their growth and differentiation characteristics, MSCs are currently used as a therapeutic in treatment of various skeletal tissue defects in animals, as well as osteogenesis imperfecta in children (Horwitz, Prockop et al. 1999). Characteristics such as migration, stable long-term transduction (Lee, Kohn et al. 2004), and the fact that allogenic MSCs can be tolerated make them potential candidate to be used in regenerative medicine (Song, Webb et al. 2006). MSCs have been useful in treatment of several clinical disorders like arthritis therapy (Chen, Tuan et al. 2008), cardiac repair (Hare, Chaparro et al. 2008; Nesselmann, Ma et al. 2008), hematopoietic transplantation (Ball, Bernardo et al. 2008), intervertebral disk repair (Crevensten, Walsh et al. 2004), kidney regeneration (Hopkins, Li et al. 2009), skin wound healing (Fu, Fang et al. 2006). Several possible therapeutic functions are attributed to MSCs. First, easy accessibility, ease in handling, high expansion and differentiation potential make them suitable therapeutic candidate for treatment of diseases caused by physical and chemical
(Di Nicola, Carlo-Stella et al. 2002). Krampera et al. further demonstrated that the suppressive effect of murine MSCs required MSC-T cell contact and is also dependent on antigen-specific responses (Krampera, Glennie et al. 2003).

Inhibition of T cell proliferation by MSCs seems to be dependent not only on the release of soluble factors but also requires cell to cell contact (Di Nicola, Carlo-Stella et al. 2002; Rasmusson, Ringden et al. 2003; Tse, Pendleton et al. 2003; Augello, Tasso et al. 2005; Zappia, Casazza et al. 2005). Soluble factors that are responsible for the observed T-cell suppression by MSCs, are either constitutively produced by MSCs or released following cross-talk with target cells. These include Transforming growth factor-β (TGF-β), hepatocyte growth factor, indoleamine 2,3-dioxygenase (IDO), and prostaglandin E2 (PGE2) (Di Nicola, Carlo-Stella et al. 2002; Krampera, Glennie et al. 2003; Le Blanc, Tammik et al. 2003). Another potent soluble factor candidate for T-cell suppression is nitric oxide (Huang, Pan et al.) because it is known to inhibit T-cell proliferation (Albina, Abate et al. 1991; Lejeune, Lagadec et al. 1994; Young, Wright et al. 1996; Bingisser, Tilbrook et al. 1998; Bobe, Benihoud et al. 1999; Medot-Pirenne, Heilman et al. 1999; Angulo, de las Heras et al. 2000; Mazzoni, Bronte et al. 2002). Previous studies have indicated that NO produced by MSCs is one of the major mediators of T-cell suppression. They claimed that MSCs produce NO in a dose-dependent manner in response to CD4+ or CD8+ T cells and NO is involved in the suppression of Stat 5 phosphorylation in T cells thereafter reduction in T cells proliferation. Furthermore, these author demonstrated that inhibiting nitric oxide synthase (NOS) restored T cells proliferation (Sato, Ozaki et al. 2007). The efficacious immunomodulatory effect of MSCs redirected the attention of scientists away from the multipotentiality of MSCs towards their possible regulatory effects on immune cells, which paved the way for the characterization of the broad immunoregulatory activities of MSCs and thereafter launching them as potent candidate in therapeutics.

In light of these facts, the present body of work is focused towards the immunomodulatory activities of MSCs in tuberculosis disease model. The long lifespan and homing ability of MSCs are attractive targets in the context of therapeutic strategies directed against infectious diseases and metastatic tumors. Although tradional medicines are indispensible, use of stem cell as therapy may complement the available drug regimens for effective and long lasting treatment of otherwise incurable diseases. We report here that, in both murine experimental tuberculosis and tuberculosis patients, T-cell mediated immune response was suppressed in the early stage of infection. The hyporesponsiveness was associated with a reduced number of Mycobacterium-specific CD4+ T cells. We also observed that there was huge recruitment of MSCs in secondary lymphoid organs in and around the M. tb containing granuloma in both human patients and murine models. Furthermore, the immune suppression caused by so recruited MSCs contributed to exacerbation of bacterial count only during initial phase of infection due to immune suppression but later
this effect is diminished as the disease progresses. This finding is consistent with the notion that MSCs assist the host in establishing equilibrium between the pathogenic microorganism and the immune response. Our results show the preferential absence of any such immune suppression in Peripheral Lymphoid Organs hence the absence of global immune suppression.

Overall our study indicates that the therapeutic effect of MSCs can result from its antiproliferative and anti-inflammatory properties. The immunosuppressive activity of MSCs could be instrumental for induction of peripheral tolerance after MSCs administration. It might be due to its capacity to ‘freeze’ or ‘wall off’ immunocompetent cells through the inhibition of cell division, thereby preventing their responsiveness to antigenic triggers while maintaining them in a quiescent state. Therefore the principle focus of the study is to lay the groundwork for MSCs role in evading host immune response and persistence of pathogen as one important facet of host-pathogen relationships.