GENERAL INTRODUCTION
Visceral leishmaniasis (VL) or kala-azar, a disseminated infection of the lymphoreticular system, is caused by the protozoan parasite(s) *Leishmania donovani* and/or *L. infantum/chagasi*. An estimate of 0.2-0.4 million global VL cases are reported each year with more than 90% of them occurring in India, Bangladesh, Sudan, South Sudan, Brazil and Ethiopia (Alvar et al. 2012). *L. donovani* infections can lead to clinical manifestations such as persistent low-grade fever, hepatosplenomegaly, cachexia, pancytopenia, and hypergammaglobulinemia or may remain clinically asymptomatic depending on the immune status of the host. Active VL is characterized by suppression of cell mediated immunity (CMI), which is apparent from the unresponsiveness of patients to different delayed type hypersensitivity (DTH) tests (Leishmanin skin test or Montenegro test) as well as the defective lymphoproliferative response of the peripheral blood mononuclear cells (Asad et al. 2015). Recovery from infection following an effective chemotherapy, on the other hand, is associated with a strong cell-mediated DTH response (Ejazi and Ali 2013). Thus, a favorable CMI in response to appropriate treatment marks the successful cure.

Regulatory T cells (Treg cells) are considered as a multitalented master of immune regulation that promotes bystander suppression of effector T cells and infectious tolerance through secretion of regulatory cytokines (Tang and Bluestone 2008). In infectious diseases, IL-10, TGFβ and other immunosuppressive cytokines are secreted from various regulatory cell populations. These cytokines not only suppress the protective immune response but are actively involved in differentiation of induced Treg cells. These cytokines attenuate the antimicrobial activities of macrophage leading to increased probability of parasite survival (Asad and Ali, 2014). IL-12, which is also known as cytotoxic lymphocytes maturation factor, is a central immunoregulator of initiation and maintenance of Th1 response. IL-12 driven IFN\(\gamma\) dominated Th1 response promotes healing and parasite clearance. IFN\(\gamma\), TNF\(\alpha\) and IL-12 act as host response, induce microbial activity against promastigotes and amastigotes of *L. donovani*. These cytokines synergistically generate protective immune responses against VL and their dominancy marks cure of the disease. During active disease, these cytokines are downregulated by elevated IL-10 and TGFβ (Adhikari et al. 2012). The mechanism of immunosuppression during *Leishmania*
infection is, however, still poorly understood. Increased mRNA levels of IFNγ in both liver and spleen of the infected subjects (Nylen et al. 2007) emphasizes a mixed Th1/Th2 response during VL, which was unconventional to other infectious diseases. IL-35 which is a member of the IL-12 family and a heterodimer comprised of Ebi3 (IL-27β) and IL12a/p35 (IL-12β), is secreted by Treg cells and is required for maximal Treg function in vitro and in vivo. IL-35 was shown to inhibit the proliferation of mouse T effector (Teff) cells in vitro (Collison et al. 2010). Nevertheless, its effects on leishmaniasis have not been investigated. Therefore, a comprehensive study of immune cells profile at the site of *Leishmania* infection, and their modulation is immensely important. In the present study, attempts are made to identify the regulation of immune responses of the T cells and the major Treg cell populations in the *L. donovani* infected BALB/c mice. BALB/c mice infected with *L. donovani* strain Ag83 shows a progressive visceral disease mimicking human VL. Here we have studied the involvement of different subsets of CD4⁺ and CD8⁺ T cells and the immunoprotective and immunosuppressive cytokine profile of these cells at different time points of infection with the progression of the disease in mice. Moreover, we studied the suppressive activity of Treg cells on immunoprotective responses by co-culturing CD4⁺ T cells with Treg cells. Further, we used blocking/neutralizing antibodies against Treg cells as well as immunosuppressive cytokines secreted by Treg cells to establish the role played by these cytokines in creating suppressive milieu during the disease progression.

Recent research suggests Th17 as an additive to Th1 response. In the absence of Th1 response, Th17 shows some protection against leishmaniasis through an unconventional pathway for activating effector T cell responses {Asad and Ali, 2014}, but to what extent this is true in controlling VL remains unanswered. Involvement of TGFβ in both Th17 cell priming and development of Treg could also be looked upon as an important issue. It is intriguing to note that Th17 cells have close developmental links with CD4⁺FOXP3⁺ Tregs. FOXP3 and RORyt can directly interact via a DNA-independent mechanism, and during Th17 cell development FOXP3 is transiently expressed (Zhou et al. 2008). The function of Th17 and Treg cells in VL, including their trafficking and mechanism of action by way of secreting cytokines, are needed to be established for better understanding and control of the disease. Here we have
studied the profile of Th17 cells and their cytokines during the progression of murine VL and their role as an additive to Th1 response for protection against the disease.

In the absence of a successful vaccine, chemotherapy is the mainstay to combat the disease. Amphotericin B is a proficient anti-leishmanial drug, especially when pentavalent antimonials, the first line treatment against leishmaniasis are globally challenged by the emergence of resistant strains. But AmB treatment is associated with nephrotoxicity, hepatotoxicity and hypokalemia. Due to this, liposomal formulations of AmB have been introduced which are however very costly. Thus, we evaluated a new ergosterol-rich liposomal amphotericin B formulation, KALSOMETM10 for its toxicity, efficacy as well as its immunomodulatory role which is a prerequisite for long time protection. This drug is expected to not only reduce the treatment cost but improve safety and provide life-long protection.