

Review articles

Immunosuppression during *Leishmania donovani* infection: a potential target for the development of therapy

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ABSTRACT. Dysfunction of T-helper 1 mediated immune responses is a hallmark of the progression of visceral leishmaniosis (VL). Several factors such as altered antigen presentation, and abnormalities in MHC/HLA, antigen processing, and T cell receptor recognition regulate the onset of immunosuppression. Recent investigations on VL patients suggest that susceptibility to visceral leishmaniosis is genetically determined and varies between populations in different geographical locations. Emerging evidence also indicates the importance of the role played by myeloid derived suppressor cells in progressive VL. This study provides a mechanistic view of means to target the signaling mechanisms of immunosuppression to determine potential therapeutic interventions.

Key words: *Leishmania donovani*, targeting immunosuppression mechanism

Introduction

Immunosuppression is a common reason for a fatal outcome in visceral leishmaniosis (VL). The mechanism of onset is diverse, and its severity during disease progression depends on the genetically determined susceptibility of a particular population, which can vary according to geographical location.

The hemoflagellate kinetoplastidae parasite *Leishmania donovani* is the causative organism of VL. During a blood meal, the female *Phlebotomus* sandfly introduces flagellated promastigotes into the bloodstream. These motile promastigotes are engulfed by skin macrophages, dendritic cells and converted to aflagellated amastigotes, which grow in the phagolysosomal vacuole in an intracellular manner [1–5]. The patients die if left untreated.

Several leishmanial antigens including protease glycoprotein 63 (gp63), 50–55 kDa proteins, 20–28 kDa proteins, 30–45 kDa proteins have been identified in both patient sera and animal models [6–8]. The amastigote-derived virulent A2 protein has gained importance in the aspect of diagnosis and found to be immunogenic in both murine models and patients [9,10].

Preventive strategy of visceral leishmaniosis

The preventive strategy of visceral leishmaniosis in tropical regions is based on four programs: (a) firstly, vector control [11], (b) secondly, the development of drugs as suitable alternative of stibanate [12], (c) thirdly, immunotherapy [13–16] and (d) fourthly, choice of proper vaccination strategy [17,18].

Particular attention is being paid to the molecular determinants of host-parasite interactions during the entry, engulfment, and propagation of parasites in infected individuals. An effective blockade in any one of the steps can prevent parasite infection. Observations of canine VL suggest that a vaccination approach using various leishmanial and DNA-derived synthetic epitope antigens may be a promising way to prevent *Leishmania donovani* infection [19,20].

A new approach to developing a treatment strategy to overcome anergic immune responses is through the successful implementation of immunotherapy. The discovery of novel compounds with both, immunostimulatory and leishmaniacidal properties with minimum or no side effects, is necessary for drug development. Clinical vaccination trials have been performed by various laboratories with variable degrees of success in preventing *Leishmania* infection [21–24]. The following sections discuss the mechanisms of immunosuppression which take place during *Leishmania donovani* infection in human animal models from the perspective of effective prevention of fatality in visceral leishmaniosis.

Effect of parasite load on dysfunction of immune response

Leishmania donovani infection in hosts depends on the successful entry of the parasite into macrophages [4,5,25]. During a sandfly bite, flagellated promastigotes enter macrophages via receptor-mediated endocytosis and the amastigotes multiply in the phagolysosome compartment of the macrophages [25,26]. The mannose, fucose receptor binding protein and CR3 complement receptor play active role in the internalization of the promastigotes [27]. The CR3 has been found to bind to the ArgGlyAsp tripeptide sequence of the gp63 surface protease of *Leishmania* promastigotes [28]. Inhibition of the function of these receptors in murine cell culture was found to prevent internalization of promastigotes in the macrophage. Hence, one possibility for preventing *Leishmania* infection to develop a synthetic vaccine and/or therapy with siRNA or microRNA construct intended for selective knockdown of CR3, mannose receptor binding protein. Alternatively, a live attenuated and avirulent *Leishmania* parasite may be developed for successful immunization purposes. Gamma-irradiated attenuated promastigotes have been found to potentially induce efficient immune responses and reduce parasite burden in a golden hamster model of *Leishmania donovani* infection (S. Dasgupta, A.C. Ghose, unpublished observation). The third approach is to generate bioengineered promastigotes with modified gp63.

Leishmania donovani promastigote and amastigote surface glycoprotein gp63 gene has been isolated and cloned for characterization [29–31]. The developmental importance of *Leishmania* surface protease gp63 mRNA expression patterns and glycolipids has been suggested by different investigators in promastigotes and amastigotes [32,33]. During infection, gp63 was found to interact with fibronectin-like receptors [34]. The gp63 glycoprotein deserves special consideration as it prevents AP1 and NF-κB activation [35,36]. The studies thus underline the importance of this glycoprotein in designing a preventive strategy.

Further evidence suggests that *L donovani* infection induces increased production of ceramide in macrophages [36,37]. Knapp and English [38] reported that, the accumulation of ceramide is involved in expression of inducible nitric oxide synthase (iNOS) and tumor necrosis factor in murine macrophages (RAW 264.7) following stimulation with prototype inflammatory agent lipopolysaccharide (LPS) *in vitro*. Recent research indicates that amastigote protein A2 may be important in the prevention of the multiplication and spread of intracellular amastigotes *in vivo*: immunization with A2 protein and/or inhibition of the expression of A2 protein has been found to restrict internalization of the parasites. The A2 protein thus has promise to take part in preparation of vaccine for *L. donovani* infection [9,10].

Dysfunctional antigen presentation

The inability of antigen presenting cells to process the *Leishmania* antigen, and the presentation of the processed antigen with HLA/MHCII to TCR generates nonfunctional T cell response during progressive illness [4,39]. *Leishmania donovani* infection is associated with a loss of antigen-specific cell-mediated immunity (CMI), which has been demonstrated as a failure to respond to leishmanial crude soluble antigen by peripheral blood mononuclear cells in lymphocyte proliferation experiments [40,41]. The anergy of CMI response in immunocompetent hosts during progression of disease is specific to the *Leishmania* antigen [40,42–44] and is of a generalized nature [45–47].

Suppression of the T_H1-mediated immune response has been found in the altered cytokine milieu. The progression of visceral leishmaniosis is associated with a decrease in IFN-γ expression and increased IL10 expression [48,49]. A decrease in delayed type hypersensitivity reaction (DTH) in response to intradermal injection of *Leishmania* antigen indicates a stage of anergy to *Leishmania* antigen. However, such DTH reaction is positive

when PPD antigen is used under similar condition to VL patients. The nature of immune response has a correlation ship with degree of parasitemia and shows significantly suppressed under severe parasite load in patients during progression of illness. Thus, the observed phenomena suggest that, an effect of susceptibility pattern shifts *Leishmania* antigen specific immune responses to generalized antigen independent immunosuppression [42,43,50].

In laboratory, experimentally-induced intracellular parasitemia via intravenous inoculation has been found to depend on the H-2^a and H-2^b phenotype in inbred mouse model. The Balb/c mice are genetically susceptible to *L. donovani* infection. The C57BL/6, C57BL/10, DBA mice are resistant to *Leishmania donovani* infection [51,52]. The inbred mice with defined genetic backgrounds are valuable tools in explaining the mechanism of expression of genetic determinants controlling susceptibility. Golden hamster is a susceptible rodent model which mimics the progression of *L. donovani* infection in susceptible humans [53,54].

In humans, susceptibility-determining genes include Kaza1 (ID 387582), HLA DRB1 (ID 3123), HLA-DQA1 (ID 3117), IL 10 (ID 3586), CRP (ID 1401 C-reactive protein) and the Fork head box transcriptional regulator (FOXP3) (ID: 50943). Dominant expression of these genes alters antigen presentation by macrophages and APCs, and shifts the balance from inflammatory T_H1 response to T_H2 responses. Cluster of genes and regulator proteins are the determinants of the severity of disease and the nature of immune suppression during infection [55–57].

The experimental evidences in an *in vitro* hamster model of *L. donovani* infection suggest that, adherent macrophage-like cells play a critical role in the immunosuppression process [46,52,58]. Successful removal of these cells restores the proliferation ability of lymphocytes. The observations may lead towards suppressor cells and provide a clue for therapeutic interventions and vaccination.

The impact of myeloid derived suppressor cells (MDSCs) on immunosuppression

Progression of *Leishmania donovani* infection is associated with reversible immunosuppression [40,46,50,59] in an animal model and immunocompetent hosts. The extent of immunosuppression has been determined by a gradual decrease in lymphocyte proliferation index in *in vitro* and *in vivo* cell culture based on Delayed Type Hypersensitivity responses (DTH). The findings do not, however, provide any detail of antigen-specific T cell immune response *in vivo* and *in vitro* during the progression of infection. Recent evidence suggests that a decrease in T_H1 response together with lower IFN-γ expression is associated with parasitemia. An increase in interleukin 10 expression has been demonstrated during progression of disease with immunosuppression [60]. The investigations also indicate dysfunction of antigen presentation due to changes in the HLADR/MHCII genes regulating susceptibility patterns in visceral leishmaniosis [55,56,61].

The observations on immunosuppression in *Leishmania* infection suggest the presence of myeloid-derived cells in suppression of T cell response. These myeloid derived suppressor cells (MDSCs) have morphological similarity with granulocyte monocyte progenitor cells expressing granulocyte monocyte markers CD11b (Mac1) and Gr1 [62]. Accumulation of MDSCs in the spleen confers immunosuppression and anergy of T_H1 cells via a mechanism not yet completely understood. However, MDSCs are found as a mixed population: one set is granulocytic while the other is monocyte derived with $Gr1^{hi}CD11b^{hi}$ F4/80 ^{int} marker expression. These cells release nitric oxide (NO) and suppress T cell-mediated immune response [63].

The regulation of MDSC-mediated immunosuppression in VL by the generation of specific inflammatory immune responses is significant for therapeutic point of view. Role of nitric oxide (NO) and NO-bound protein complex nitrotyrosine has been found very critical in MDSC mode of action and inflammatory responses. The Modolell et al. [64] suggest that arginase enzyme may be involved in the depletion of L-Arginine in nonhealing cutaneous leishmaniosis caused by *L. major*. Recently, Abebe et al. [65] have suggested that, an increase in arginase enzyme may serve as a marker for VL patients in Ethiopia: the elevated level of arginase in the peripheral blood circulation of VL patients decreased following successful treatment.

This observation is important for two reasons: firstly, the findings highlight the intrinsic susceptibility of the Ethiopian population through generation of MDSCs, and secondly, it indicates that, sustained activation of macrophage/monocyte system is required with inflammatory responses

which destroys intracellular amastigotes. Both of the findings, provide an indication of the susceptibility patterns of patients towards parasite infection. However, little is known on mode of interactions between MDSCs, professional antigen presenting cells (APCs) and T cells in the induction of the immunosuppression process.

Conclusion and future perspectives

The onset of immunosuppression is a critical event during the progression of visceral leishmaniosis in a susceptible population. Therefore, identification of the mechanism of antigen presentation and the mode of action of myeloid derived suppressor cells (MDSCs) are two important aspects whose understanding is needed for therapeutic interventions of the disease.

In addition to the conventional drug stibanate, immunotherapy and vaccination approaches deserve special attention in geographically-distinct populations. The choice of vaccination using anti-CR3, mannose binding protein (MBP)-specific neutralizing monoclonal antibodies has promise in the prevention of *L. donovani* infection. The design of specific siRNA (silencer RNA) or micro RNA profiles for CR3 and mannose binding protein is an approach for development of a synthetic vaccination strategy for the prevention of promastigote entry into macrophages during sandfly bite in endemic zone populations. In the aspects, further research is necessary to prevent progression of visceral leishmaniosis.

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