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Original article

Immuno-informatics based approaches to identify CD8+ T cell epitopes within the *Leishmania donovani* 3-ectonucleotidase in cured visceral leishmaniasis subjects

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Abstract

Leishmaniases are vector-borne diseases for which no vaccine exists. These diseases are caused by the *Leishmania* species complex. Activation of the $CD8^+$ T cell is crucial for protection against intracellular pathogens, and peptide antigens are attractive strategies for the precise activation of $CD8^+$ T in vaccine development against intracellular infections. The traditional approach to mine the epitopes is an arduous task. However, with the advent of immunoinformatics, *in silico* epitope prediction tools are available to expedite epitope identification. In this study, we employ different immunoinformatics tools to predict $CD8^+$ T cell specific 9 mer epitopes presented by HLA-A*02 and HLA-B40 within the highly conserved 3′-ectonucleotidase of *Leishmania donovani*. We identify five promiscuous epitopes, which have no homologs in humans, theoretically cover 85% of the world's population and are highly conserved (100%) among *Leishmania* species. Presentation of selected peptides was confirmed by T2 cell line based HLA-stabilization assay, and three of them were found to be strong binders. The *in vitro* peptide stimulation of peripheral blood mononuclear cells (PBMC) from cured $HLA\text{-}A02^+$ visceral leishmaniasis (VL) subjects produced significantly higher IFN-γ, IL-2 and IL-12 compared to no peptide control healthy subjects. Further, $CD8^+$ cells from treated VL subjects produced significantly higher intracellular IFN-γ, lymphocyte proliferation and cytotoxic activity against selected peptides from the PBMCs of treated $HLA\text{-}A02^+$ VL subjects. Thus, the $CD8^+$ T cell specific epitopes shown in this study will speed up the development of polytope vaccines for leishmaniasis.

Keywords: Vaccine; Epitope; Immunoinformatics; 3′-ectonucleotidase; $CD8^+$ T cells

1. Introduction

Leishmaniases are a heterogeneous group of diseases caused by the parasites of the *Leishmania* species. Visceral leishmaniasis (VL) caused by *Leishmania donovani* is the most severe form of leishmaniasis in the Indian subcontinent. It is fatal if untreated [1]. The present control measures rely on chemotherapy and vector control. However, chemotherapies

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for the treatment of leishmaniasis are fraught with high cost, toxicity and widespread drug resistance [2,3]. Vector control is equally daunting, as sandflies are flexible in adapting to diverse environmental conditions [4]. Therefore, in addition to new chemotherapy regimens, an efficient prophylactic vaccine is desperately needed.

The adaptive immune system comprises of two arms. One arm is responsible for the humoural immune response while the other is responsible for the cell mediated immune response. Humoural immunity has historically not been considered to be of much importance in *Leishmania* infection [5,6]. On the other hand, both $CD4^+$ and $CD8^+$ T cells are required for successful recovery from leishmaniasis. IFN-γ production by $CD4^+$ and $CD8^+$ T cells skews Th2 response at the very beginning to Th1 [7]. Muller et al. demonstrated an elevated IFN-γ production at the reinfection of immune mice to *Leishmania major* due to $CD8^+$ T cells [8,9]. $CD8^+$ T cells play a crucial role in the secretion of pro-inflammatory cytokines, the recruitment of T cells at the inflammatory site and the formation and maintenance of granulomas [10–12]. $CD8^+$ T cell depleted mice failed to control *leishmania* infection and showed impaired granulomas formation [13–15]. Conversely, the adoptive transfer of $CD8^+$ T cells resulted in a 90% reduction in the splenic parasite burden [16]. Indeed, a 50-fold increase in the influx of antigen specific $CD8^+$ T cells was observed in the spleen and lymph nodes during the resolution of disease in *Leishmania* infected BALB/c mice [9,10]. These studies conclusively establish that $CD8^+$ T cells not only participate in primary response to *leishmania* infection, but they are also major mediators of resistance upon reinfection [14].

Although $CD8^+$ T cells are main effector cells in controlling *L. donovani* infection, less was known about the cognate interaction between infected macrophages and $CD8^+$ T cells [17]. A seminal study by Beattie et al. (2010) demonstrated that *leishmania* infected macrophages directly present a parasite-derived peptide to $CD8^+$ T cells [18]. Recently, Duarte et al. (2015) showed that macrophages loaded with *leishmania* parasite peptide antigens undergo $CD8^+$ T cell mediated lysis in the spleens of mice [19]. These *in vivo* cognate interactions between $CD8^+$ T cells and infected macrophages have fuelled interest in the potential for the immunoprophylactic or immuno-therapeutic expansion of $CD8^+$ T cells as a means of disease control.

During the past decades, many antigens have been used effectively to generate protective immunity in various strains of mice with different degrees of protection [20–22]. Despite a huge number of research studies, there still is no effective prophylaxis vaccination against human leishmaniasis. Traditional vaccination with whole inactivated or live attenuated microbes can be unsafe and might induce the Th2 response. On the other hand, vaccination with whole cell lysate or a combination of proteins may induce unwanted autoimmune responses [6]. Therefore, identification of specific immunogens rather than crude antigens is highly desirable for vaccine success.

$CD8^+$ T lymphocytes are generally activated by epitopes derived from intracellular pathogens. Unlike B cell epitopes, activation of the cytotoxic T lymphocyte (CTL) response does not depend on the structural conformation of the peptide, as a simple linear 9 mer is able to elicit a cytotoxic response [23]. In this context, an epitope-based vaccine makes a prudent approach, because peptide epitopes represent the minimal immunogenic part of the protein that evokes the precise immune response and the purity of the peptide antigen can be monitored easily. Here, we have selected the *L. donovani* 3′-ectonucleotidase because (a) this enzyme is abundantly expressed on the outer surface of the parasite [24], (b) expression is constitutive throughout the parasite life cycle with higher expression in the amastigotes stage [25], (c) it is indispensable for both stages of the parasite, (d) it is one of the virulent factor of pathogenesis [26,27] and (e) it is conserved among the *Leishmania* species and is highly non-self to the mammalian host [28,29]. Therefore, the 3′-ectonucleotidase of *L. donovani* is presumed to be a potential vaccine candidate for the development of a *Leishmania* vaccine.

The conventional method of peptide mapping is an arduous, time consuming and costly task, as it requires laborious assay for each peptide. With the advent of computers and informatics, parallel developments in molecular biology and whole genome sequencing prelude the bioinformatics analysis of microbial genome/proteome data for the *in silico* prediction of vaccine targets [30,31]. In recent years, both the preventive and therapeutic based vaccine concept has drawn considerable focus in the field of leishmaniasis. Epitope-based vaccines have been successfully tested for viral diseases and other diseases. Many peptide-based vaccines are in now clinical trials and a few peptide-based vaccines have been approved for human usage [32–34]. However, an acute dearth of such epitope candidates for leishmaniasis remains. In this study, we predict three potential peptide epitopes of the *L. donovani* 3′-ectonucleotidase protein through the bioinformatics approach and validate the selected peptide epitopes *in vitro* for their protective immune response.

2. Material and methods

2.1. Clinical samples and diagnosis

Twenty-five cured VL participants (16 HLA-A*02 positive and 9 HLA-A*02 negative) of both sexes were selected for this study. The participants were aged 13–45 at the time of the study and came from endemic areas of the Bihar state of India. All of the active VL patients presented characteristic signs and symptoms of leishmaniasis, and diagnosis was confirmed by the presence of amastigotes in Giemsa stained bone marrow aspirates and by positive serology (direct agglutination test). The active VL subjects were treated with a 10 mg/kg body weight single dose of Ambisome and were followed up for six months to two years after treatment.

In addition, 15 healthy donors from both sexes who were aged 23–45 years and had no sign or symptoms of leishmaniasis were sampled. The non-endemic healthy participants had no apparent history of VL and tested negative in the rK39 serological test. Recommendations outlined in the Helsinki Declaration were followed, and ethical approval was obtained