Expression and characterization of a parasite-specific antigen on macrophages after infection with *Leishmania donovani*

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**Abstract**

A rabbit polyclonal antibody to crude soluble antigen of *Leishmania donovani* promastigotes recognized a determinant expressed on the surface membrane of mouse peritoneal macrophages and human monocyte derived macrophages infected *in vitro*. The determinant was recognized on infected macrophage surface only when F(ab')₂ fragments of anti-leishmanial antiserum was employed in immunofluorescence. F(ab')₂ fragments of human patient sera also could recognize the determinant. The expression of this antigen was not stage-specific for the parasite. Immunochromical analyses revealed this antigen to be of 51 kDa protein. Specific leaching of membrane proteins by trypsin showed three bands of expressed antigens of 26, 11 and 10 kDa, which in all likelihood might be arising from the 51 kDa antigen. The antigen was not expressed until 12 h of post infection, reached a maximum level at 24 h and thereafter attained a steady state level as studied upto 96 h of post infection. This type of antigen might have a great potential in immunodiagnostics and site-specific drug targeting. (Mol Cell Biochem 132: 1-6, 1994)

**Key words:** macrophage, *Leishmania donovani*, parasite antigen, leishmaniasis, membrane

**Introduction**

Visceral leishmaniasis is a widespread parasitic disease throughout much of the third world. The disease is caused by the protozoan, *Leishmania donovani*, an obligate intracellular parasite in man that resides and multiplies within macrophages of reticuloendothelial system [1, 2]. The drugs currently in use against leishmaniasis are highly toxic and often lead to severe toxic side reactions [3, 4]. We were trying for a drug targeting approach to eliminate the parasites specifically from macrophages without affecting other cell types of the host. For this purpose we exploited the mannosyl receptor, a high affinity recycling receptor known to bind and internalize intravenously infused glycoproteins with terminal mannose residues. This receptor system is found to be present exclusively on the surface of mature macrophages [5]. Methotrexate, an anticancer drug which affects most of the cells, was chosen for establishing the principle of receptor mediated drug targeting. The exclusive presence of mannose receptors on macrophage surface together with the obligatory localization of
Leishmania parasites within macrophages formed the basis of our drug delivery using the neoglycoprotein, mannosyl bovine serum albumin as the carrier for methotrexate. The neoglycoprotein-drug conjugate was highly efficient in suppressing parasite burden in experimental visceral leishmaniasis [6]. Although this mannose receptor mediated approach looks very promising, still it suffers from the limitation that drug would be directed not only to infected macrophages but also to normal macrophages thereby causing some toxicity. A better alternative approach would be the exploitation of parasite specific antigen that might express on infected macrophages for exclusive targeting to infected cells. A few preliminary reports are available indicating the possible expression of Leishmania antigens on the surface of infected macrophages [7, 8]. Leishmania braziliensis panamensis major surface glycoconjugate was not expressed on the surface of murine peritoneal macrophages infected in vitro, until 6 h of post infection [9]. Diminished cellular immune responses to Leishmania antigens are negative prognostic signs in leishmaniasis. Recent efforts have turned towards the identification of leishmanial antigens recognized by T cells in human leishmaniasis. Heterogeneous T cell responses of patients was demonstrated in various disease states to specific Leishmania antigen [10, 11]. The obligatory intracellular localization of Leishmania parasites within the macrophages suggests a possibility of expression of parasite specific antigen on infected macrophage surface, which may serve as modulator of host immune response. We report here on the identification and characterization of such a parasite specific antigen expressed on infected macrophage surface in vitro.

Materials and methods

Parasites

Leishmania donovani strain AG83 (MHOM/IN/1983/AG83) was isolated from Indian patients with Kala-azar. Promastigotes were cultured at 22°C in medium 199 (GIBCO Laboratories, Grand Island, New York) with Hanks salts containing HEPES (12 mM), L-glutamine (20 mM), 20% heat inactivated fetal calf serum, 50 U/ml penicillin and 50 μg/ml streptomycin. Amastigotes were maintained in BALB/c mice by intravenous passage every 6 weeks. Amastigotes were purified by homogenizing the infected spleen and passing through Percoll gradient according to Meade et al. [12]. Crude soluble antigen (CSA) was prepared from promastigotes by freeze-thawing the cell suspension (5 × 10⁶ cells/ml) for 3–5 times and sonicating for 5 × 45 sec at 20 k cycles/min in an ice bath. The contents were centrifuged at 10,000 g for 20 min and the supernatant was used as crude soluble antigen. Protein was estimated according to Lowry et al. [13].

Macrophage culture and infection

Macrophages were collected by peritoneal lavage from mice (BALB/c, 20–25 g) given intraperitoneal injections of 0.5 ml (4%) of thioglycollate broth 5 days before harvest and were isolated and cultured as described earlier [14]. Promastigotes or amastigotes were used to infect cultures of adherent macrophages at a ratio of 10 parasites per macrophage. Infection was permitted to proceed for 4 h at 37°C after which all extracellular organism that failed to parasitize the macrophages were eliminated by thorough washing with phosphate buffered saline (PBS). After 24 h post infection, slides were washed with media, rinsed with cold water and then used as a substrate in Indirect Immunofluorescent Antibody Test (IFAT).

Production of antisera against crude soluble antigen

An adult New Zealand rabbit was immunized with crude soluble extract of L. donovani AG83 promastigotes for the production of a polyclonal antisera. Immunization protocol consisted of two subcutaneous injections of 2.0 mg protein emulsified with an equal volume of complete Freund’s adjuvant given 5 days apart. This is followed by two subcutaneous injections of the same amount of protein with incomplete Freund’s adjuvant on 12th and 22nd day of the first injection. Rabbit was bled prior to immunization for control preimmunized serum.

Separation of F(ab')₂ fragment

Peripheral blood of Kala-azar patient was supplied by Dr. K.K. Mallick, School of Tropical Medicine, Calcutta. Serum was separated and dialyzed against 0.1 M phosphate buffer, pH 7.2 at 4°C for 24 h. IgG fractions were separated from both the human serum and rabbit antisera against CSA by absorbing onto DEAE cellulose