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# Integrity of the Actin Cytoskeleton of Host Macrophages is Essential for *Leishmania donovani* Infection

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ABSTRACT

Visceral leishmaniasis is a vector-borne disease caused by an obligate intracellular protozoan parasite *Leishmania donovani*. The molecular mechanism involved in internalization of *Leishmania* is poorly understood. The entry of *Leishmania* involves interaction with the plasma membrane of host cells. We have previously demonstrated the requirement of host membrane cholesterol in the binding and internalization of *L. donovani* into macrophages. In the present work, we explored the role of the host actin cytoskeleton in leishmanial infection. We observed a dose-dependent reduction in the attachment of *Leishmania* promastigotes to host macrophages upon destabilization of the actin cytoskeleton by cytochalasin D. This is accompanied by a concomitant reduction in the intracellular amastigote load. We utilized a recently developed high resolution microscopy-based method to quantitate cellular F-actin content upon treatment with cytochalasin D. A striking feature of our results is that binding of *Leishmania* promastigotes and intracellular amastigote load show close correlation with cellular F-actin level. Importantly, the binding of *Escherichia coli* remained invariant upon actin destabilization of host cells, thereby implying specific involvement of the actin cytoskeleton in *Leishmania* infection. To the best of our knowledge, these novel results constitute the first comprehensive demonstration on the specific role of the host actin cytoskeleton in *Leishmania* infection. Our results could be significant in developing future therapeutic strategies to tackle leishmaniasis.



## 1. Introduction

Leishmaniasis is a vector-borne disease, caused by various species of the genus *Leishmania*, which are obligate intracellular protozoan parasites. Leishmaniasis causes substantial public health problems, especially in tropics, subtropics and the Mediterranean basin, and is usually fatal if left untreated [1–4]. Leishmaniasis threatens about 350 million men, women and children in 98 countries around the world. As many as 12 million people are believed to be currently infected, with about 1–2 million estimated new cases occurring every year [5,6]. In socioeconomic terms, leishmaniasis is often associated with poverty [7] and is believed to be one of the most neglected diseases due to limited funding available for diagnosis, treatment and control [8]. According to available estimates, leishmaniasis is considered to be second in mortality and fourth in morbidity among all tropical diseases [9]. Based on clinical syndromes, leishmaniasis is classified into four major types: cutaneous, muco-cutaneous, visceral (also known as *kala-azar*) and post-*kala-azar* dermal leishmaniasis. Among these, visceral leishmaniasis (VL) is fatal in the absence of treatment [3]. The current worldwide increase in leishmaniasis to epidemic proportions, and the emergence of VL as an important opportunistic infection among people infected with HIV-1 [10] have given rise to an urgency to provide treatment for leishmaniasis.

Leishmaniasis is transmitted by the bite of the infected female sandfly (*Phlebotomus* spp.) while taking a bloodmeal from a host [11]. The lifecycle of *Leishmania* has two distinct forms: an extracellular promastigote flagellar form found in the mid-gut of sandflies, and an intracellular amastigote form that resides in phagolysosomes of host macrophages. After entering the bloodstream, promastigotes are internalized by dendritic cells and macrophages, and subsequently transform into amastigotes by losing their flagella [3,12]. The entry of promastigotes into host macrophages involves multiple host–parasite interactions such as recognition of specific ligands on the parasite cell surface by receptors on the macrophage cell surface [4]. Studies aimed at understanding the molecular mechanisms of entry of *Leishmania* into host cells have led to the identification of a number of candidate receptors facilitating multiple routes of entry, thereby highlighting the redundancy in the entry process [2,13,14]. These include membrane

*Abbreviations:* CD, cytochalasin D; DMSO, dimethyl sulfoxide; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide; PE, phycoerythrin; VL, visceral leishmaniasis

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receptors on the host macrophage cell surface such as the mannose-fucose receptor, receptor for advanced glycosylation end products, the fibronectin receptor, the Fc receptor and complement receptors such as CR1 and CR3. Due to the large variety of receptors responsible for parasite entry into host macrophages, no panacea is available for the treatment of leishmaniasis.

The entry of intracellular parasites such as *Leishmania* involves interaction of the parasite with the plasma membrane of host cells. In this context, we were the first to demonstrate the requirement of host membrane cholesterol in the binding and internalization of *Leishmania donovani* into macrophages using complementary approaches [12,15–20]. Membrane cholesterol has also been shown to be necessary for the entry of *Leishmania chagasi* into host macrophages [21]. Interestingly, depletion of plasma membrane cholesterol has recently been reported to result in possible reorganization of the cortical actin cytoskeleton [22–27]. With the overall goal of delineating plasma membrane components of host macrophages responsible for the entry of *Leishmania* and arriving at a comprehensive molecular mechanism of parasite entry, in this work, we have explored the role of the actin cytoskeleton in parasite entry. Our results show that destabilization of the actin cytoskeleton of host macrophages results in a dose-dependent reduction in the attachment of *Leishmania* promastigotes, along with a concomitant reduction in the intracellular amastigote load. Importantly, we demonstrate here that *Leishmania* infection is strongly correlated with cellular F-actin level. To the best of our knowledge, these novel results constitute the first comprehensive demonstration on the specific role of the host actin cytoskeleton in *Leishmania* infection.

## 2. Materials and methods

### 2.1. Materials

$MgCl_2$, $CaCl_2$, cytochalasin D (CD), antibiotic antimycotic solution, gentamicin sulfate, IMDM (Iscove's Modified Dulbecco's Medium), M-199 (Medium-199), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide), FITC (Fluorescein isothiocyanate) and Giemsa stain were obtained from Sigma (St. Louis, MO). Fetal calf serum (FCS) was purchased from Gibco/Life Technologies (Grand Island, NY), PE (phycoerythrin) rat anti-mouse CD14 antibody was obtained from BD Biosciences (Franklin Lakes, NJ) and Alexa Fluor 546 phalloidin was obtained from Molecular Probes/Invitrogen (Eugene, OR). All other chemicals used were of the highest available purity. Water was purified through a Millipore (Bedford, MA) Milli-Q system and used throughout.

### 2.2. Methods

#### 2.2.1. Cell culture

Murine macrophage cell line J774A.1 (American Type Culture Collection) was maintained as described previously [15,28] with some modifications. Cells were maintained in IMDM medium supplemented with 2.4 g/l sodium bicarbonate, 10% heat-inactivated FCS, and antibiotic antimycotic (100 U/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml amphotericin B) solution in a humidified atmosphere with 5% $CO_2$ at 37 °C.

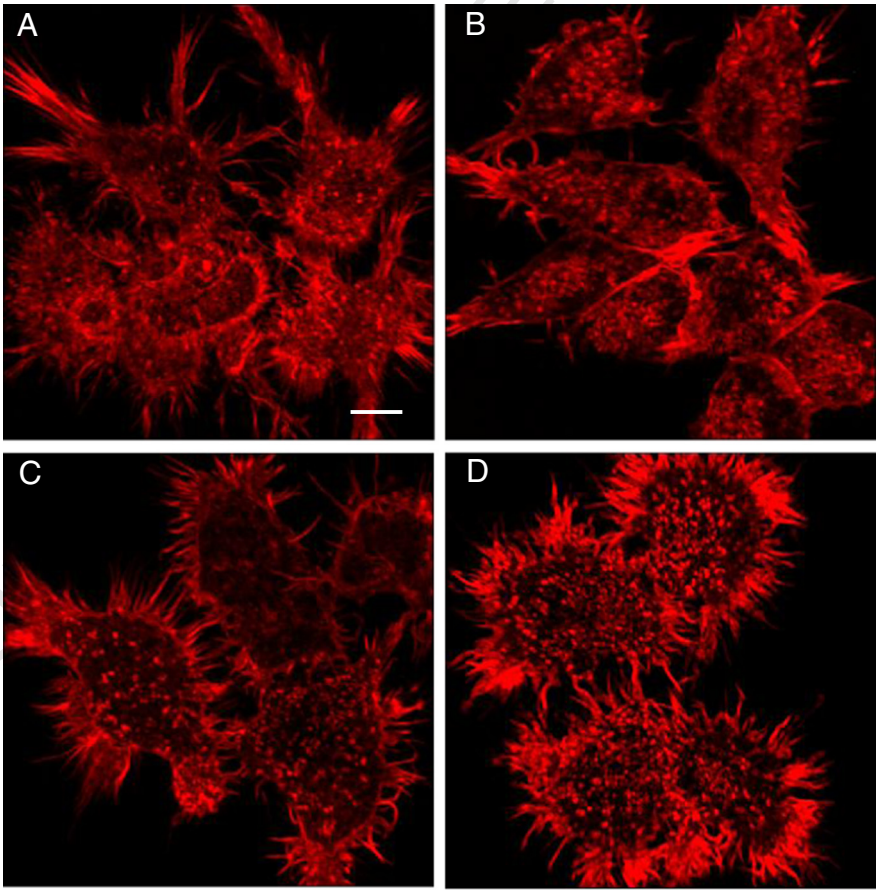


**Fig. 1.** Organization of the actin cytoskeleton in J774A.1 macrophages treated with increasing concentrations of cytochalasin D (CD). The actin cytoskeleton was stained with Alexa Fluor 546 phalloidin. Projections of 11 sections from the base of the coverslip (~3.5 μm from the base into the cell) are shown. Panel A shows representative projected image for control macrophages, and panels B–D show corresponding images for macrophages treated with 2.5, 5 and 10 μM CD, respectively. Loss of F-actin filaments and formation of F-actin aggregates can be observed upon treatment with increasing concentrations of CD. The scale bar represents 10 μm. See Materials and methods for other details.

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