The International Journal of Biochemistry & Cell Biology 55 (2014) 196–208

Contents lists available at ScienceDirect

The International Journal of Biochemistry & Cell Biology

journal homepage: www.elsevier.com/locate/biocel

Leishmania donovani activates SREBP2 to modulate macrophage membrane cholesterol and mitochondrial oxidants for establishment of infection

Madhuchhanda Mukherjee [1], Writoban Basu Ball [1], Pijush K. Das *

Infectious Diseases and Immunology Division, CSIR-Indian Institute of Chemical Biology, Kolkata, India

ARTICLE INFO

Article history:
Received 12 April 2014
Received in revised form 11 July 2014
Accepted 23 August 2014
Available online 16 September 2014

Keywords:
Visceral leishmaniasis
Cholesterol
HMGCR
SREBP2
Mitochondria

ABSTRACT

Establishment of infection by an intracellular pathogen depends on successful internalization with a concomitant neutralization of host defense machinery. *Leishmania donovani*, an intramacrophage pathogen, targets host SREBP2, a critical transcription factor, to regulate macrophage plasma membrane cholesterol and mitochondrial reactive oxygen species generation, favoring parasite invasion and persistence. *Leishmania* infection triggered membrane-raft reorientation-dependent Lyn-PI3K/Akt pathway activation which in turn deactivated GSK3β to stabilize nuclear SREBP2. Moreover, cells perceiving less available intracellular cholesterol due to its sequestration at the plasma membrane resulted in the deregulation of the ER-residing SCAP-SREBP2-Insig circuit thereby assisting increased nuclear translocation of SREBP2. Both increased nuclear transport and stabilization of SREBP2 caused HMGCR-catalyzed cholesterol biosynthesis-mediated plasma membrane cholesterol enrichment leading to decreased membrane-fluidity and plausibly assisting delay in phagosomal acidification. Parasite survival ensuing entry was further ensured by SREBP2-dependent trasnscriptional up-regulation of UCP2, which suppressed mitochondrial ROS generation, one of the primary microbicidal molecules in macrophages recognized for its efficacy against *Leishmania*. Functional knock-down of SREBP2 both *in vitro* and *in vivo* was associated with reduction in macrophage plasma membrane cholesterol, increased ROS production and lower parasite survival. To our knowledge, this study, for the first time, reveals that *Leishmania* exploits macrophage cholesterol-dependent SREBP2 circuit to facilitate its entry and survival within the host.

1. Introduction

Macrophages play host to the protozoan parasite *Leishmania donovani*, causative agent of fatal visceral leishmaniasis. The dimorphic parasite has a motile promastigote form and a non-motile amastigote form, respectively. The promastigotes colonize the lumen of the digestive tract of sandflies, where they multiply and differentiate into metacyclic promastigotes, which are infectious for mammals. Metacyclic promastigotes are inoculated into the dermis of mammals during the bloodmeal of infected sandflies. The promastigotes, which do not invade host cells actively, are phagocytosed by macrophages where they transform into amastigotes within membrane-bound organelles of the endocytic pathway progressively acquiring late endosomal/lysosomal characteristics (Courret et al., 2002). The evasion of macrophage microbicidal mechanisms is necessary for the promastigotes to survive and differentiate into amastigotes in parasitophorous vacuoles which normally starts in the hours following phagocytosis and takes at least 24–72 h (Ruhland et al., 2007). Understanding the strategies developed by the parasite from the initial stages of interaction with the host leading to the creation of a congenial niche may help in identifying novel therapeutic targets.

The route of initial entry influences how host cells respond to intracellular pathogens (Rosenberger et al., 2000). Cellular

Abbreviations: PM, plasma membrane; ER, endoplasmic reticulum; SREBP2, sterol regulatory element binding protein 2; SCAP, SREBP cleavage activating protein; HMGCR, 3-hydroxy-3-methyl glutaryl CoA reductase; UCP2, uncoupling protein; ROS, reactive oxygen species; bHLH-Zip, basic helix–loop–helix leucine zipper; MβCD, methyl β cyclodextrin; DAPI, 6-diamidino-2-phenylindole; ORO, oil red O; LDLr, low density lipoprotein receptor.

* Corresponding author: Dr. Pijush K. Das, Infectious Diseases and Immunology Division, CSIR-Indian Institute of Chemical Biology, 4 Raja S.C. Mullick Road, Kolkata 700032, India. Tel.: +91 33 2414 0921; fax: +91 33 2473 5197.
E-mail address: pijushdas@iicb.res.in (P.K. Das).

[1] These authors contributed equally to work.

http://dx.doi.org/10.1016/j.biocel.2014.08.019
1357-2725/

invasion by classical and non-classical microorganisms into professional and non-professional phagocytes involve cholesterol-sphingolipid-enriched highly dynamic membrane microdomains or rafts to compartmentalize and segregate cellular processes (Fernandes et al., 2007), thereby plausibly interfering with the host cellular activities. HIV-1 utilizes rafts for nearly all of the crucial events in its lifecycle ranging from its entry to budding, subversion of host-cell signaling and immune evasion (Mañes et al., 2003). Bacteria such as *Mycobacterium* spp., *Salmonella typhimurium*, *Shigella flexneri*, *Brucella* spp. and *Legionella pneumophila* exploit host rafts to generate phagosomes that allow them to survive inside phagocytic cells (Gatfield and Pieters, 2000; Watarai et al., 2002, 2001). Protozoa exploit rafts for their intracellular survival and/or to modulate the host response. *Toxoplasma gondii* and *Plasmodium falciparum*, two obligate intracellular parasites enter their respective target cells using an active penetration mechanism leading to the formation of parasitophorous vacuoles (Mañes et al., 2003; Aikawa et al., 1978; Suss-Toby et al., 1996). *Theileria parva*, a protozoan parasite constitutively activate raft-resident Src-family kinases to transform bovine T and B cells, resulting in uncontrolled proliferation and spreading to different tissues (Dobbelaere et al., 2000). Specific GPI-anchored proteins can be isolated from rafts that are derived from *Trypanosoma* and *Leishmania* (Mañes et al., 2003). In short, rafts have been implicated in numerous protein–protein and lipid–protein interactions at the cell surface due to their capacity to incorporate or exclude proteins selectively and their ability to coalesce into larger domains (Mañes et al., 2003). Entry *via* lipid rafts can avoid lysosomal fusion and therefore allow pathogen survival and parasites might modulate signaling pathways, including lipid-raft-associated protein kinases (Vieira et al., 2010). But, the functional relevance of rafts in protozoan pathogenesis remains obscure. Studies involving various cholesterol manipulating agents have pointed to the specific requirement of macrophage plasma membrane (PM) cholesterol-enriched rafts in efficient attachment and entry of *Leishmania* promastigotes (Pucadyil et al., 2004). A few recent conflicting reports exist regarding the status of macrophage cholesterol level following *Leishmania* infection. Persistent *Leishmania* infection induces reduction in PM cholesterol-mediated raft disruption which on restoration leads to reduced parasite survival (Sen et al., 2011). On the other side, work from some laboratories demonstrated that *Leishmania* infection is associated with significant increase in cellular cholesterol (Osorio et al., 2009; Rabhi et al., 2012) and neutral lipid content (Lecoeur et al., 2013). Inconsistencies in the host cell cholesterol content in the course of *Leishmania* infection prompted us to investigate the underlying regulatory mechanisms.

Cholesterol in PM is maintained at a constant level through a feedback regulatory circuit that senses the level of cholesterol in cell membranes and modulates the transcription of genes encoding the enzymes involved in cholesterol metabolism. Studies have shown that a change in the concentration of free cholesterol or that of phospholipids in the PM of cells affects the rate of cholesterol biosynthesis in the membranes of the endoplasmic reticulum (ER) (Radhakrishnan and McConnell, 2000). In addition, the total cholesterol concentration in the ER membrane depends sharply on the free cholesterol concentration in the PM (Lange et al., 1999). Cholesterol can originate from *de novo* biosynthesis by 3-hydroxy-3-methyl glutaryl CoA reductase (HMGCR) or through hydrolysis of cholesteryl esters primarily taken up by low density lipoprotein receptor (LDLr) (Simons and Ikonen, 2000). The regulatory system revolves around a family of ER membrane-bound bHLH-Zip containing transcription factor called SREBP2 (Brown and Goldstein, 1997). SREBP2, synthesized as a 125-kDa precursor protein, is escorted by SCAP from the ER to Golgi, where SREBP2 is proteolytically cleaved by proteases into a mature form of 65 kDa. This translocates to the nucleus and binds to the sterol regulatory element, triggering the transcription of target genes (Brown and Goldstein, 1997). At increased level of cellular cholesterol, the SCAP-SREBP complex is sequestered in ER by the insulin-induced gene product, Insig-1 (Yang et al., 2002). SREBP2 is the major transcription factor of HMGCR, the rate limiting enzyme of cholesterol biosynthesis (Horton et al., 2002). Apart from regulating cholesterol homeostasis, a rather dubious role of SREBP2 in regulating the pro and anti-inflammatory immune response generation has recently been reported (Horie et al., 2013). Recently, we showed that *L. donovani* infection differentially modulates SREBP2, Sp1 and USF1 transcription factors to activate transcription of mitochondrial inner membrane uncoupling protein 2 (UCP2) and suppress mitochondrial oxidative burst (Basu Ball et al., 2014). In the present work, we thought it worthwhile to explore whether *L. donovani* targets macrophage SREBP2 to facilitate establishment of infection and identify the events opted by the parasite to activate SREBP2 from the initial stages of infection.

2. Methods

2.1. Ethics statement

The present study was carried out in agreement with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol used for animal experiments was approved by the Committee on the Ethics of Animal Experiments of Indian Institute of Chemical Biology (Permit Number: 147-1999). All experiments confirmed to the National Regulatory Guidelines issued by CPSEA (Committee for the Purpose of Supervision of Experiments on Animals), Ministry of Environment and Forest, Government of India.

2.2. Reagents and chemicals

All antibodies were from Santa Cruz Biotechnology (Dallas, TX, USA), Cell Signaling Technology (Danvers, MA, USA) and Merck-Millipore (Darmstadt, Germany). Cholesterol, MβCD, mevastatin, ketoconazole, 6-diamidino-2-phenylindole (DAPI), filipin, Oil red O (ORO), 6-dodecanoyl-2-dimethylaminonapthalene (laurdan), Giemsa stain, radio-immunoprecipitation assay (RIPA) buffer and iodixanol (OptiPrep Density Gradient Medium) were purchased from Sigma Aldrich (St. Louis, MO, USA). Cholera Toxin Subunit B (Recombinant)-Alexa Fluor 488 Conjugate (CTxB-AF488), Prolong Gold antifade reagent and Platinum *Taq* DNA Polymerase High Fidelity were from Molecular probes, Life Technologies (Carlsbad, CA, USA). Oligo dT mRNA primer, deoxynucleotides, M-MuLV reverse transcriptase and *Taq* DNA polymerase were procured from New England Biolabs (Ipswich, MA, USA).

2.3. Animals and parasites

The pathogenic promastigotes of *L. donovani* strain MHOM/IN/1983/AG83, isolated from an Indian patient with kala-azar, was maintained in 4–6 week old inbred BALB/c mice by intravenous passage every 6 weeks. *L .donovani* promastigotes were obtained by allowing isolated splenic amastigotes to transform in parasite growth medium for 72 h at 22 °C. The growth medium consisted of medium 199 (M199) (Invitrogen, Life Technologies, Carlsbad, CA, USA) supplemented with 10% (v/v) heat-inactivated FCS (HI-FCS) (Das et al., 2001).

2.4. Cell culture, in vitro and in vivo infection

Macrophages were collected by peritoneal lavage from mice (BALB/c; 20–25 g) and were cultured as described earlier (Das et al., 1986). The culture medium consisted of RPMI 1640 supplemented