Review

Receptor-mediated phagocytosis of *Leishmania*: implications for intracellular survival

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The extracellular promastigote stage of *Leishmania* spp. is transmitted to mammals by a sand fly vector. *Leishmania* promastigotes ligate host macrophage receptors, triggering phagocytosis and subsequent internalization, a crucial step for survival. Parasites transform intracellularly to the amastigote stage. Many studies document different receptors detecting promastigotes and amastigotes, but the relative importance of each interaction is ill-defined. Recent studies suggest that the macrophage receptors utilized during phagocytosis impact the intracellular fate of the parasite. This review summarizes the receptors implicated in *Leishmania* phagocytosis over the past 30 years. It then proceeds to weigh the evidence for or against their potential roles in intracellular parasite trafficking.

Macrophage receptors implicated in *Leishmania* phagocytosis

More than 20 species of *Leishmania* cause symptomatic leishmaniasis in over 12 million people, with disease severity ranging from cutaneous ulcerations to fatal visceral infections [1]. *Leishmania* are found in several mammalian cells, but the majority of parasites reside in macrophages [2,3]. The promastigote stage of *Leishmania* ligates macrophage surface receptors that trigger phagocytosis. This is followed by parasite transformation to the obligate intracellular amastigote stage. Receptors reported to facilitate *Leishmania* internalization include the third complement receptor (CR3) (see Glossary), first complement receptor (CR1), mannose receptor (MR), Fc gamma receptors (FcγRs, in particular FcγRII-B2), and fibronectin receptors (FnRs) (Table 1) [4–8]. A definitive understanding of the roles of various receptors in parasite survival during natural infection has remained elusive [9,10]. The mechanism of macrophage entry reflects, in part, the dynamic nature of the parasite surface. The most abundant surface membrane components differ between the extracellular promastigote form found in insects and the intracellular amastigote form found in mammals [11]. Metacyclogenesis, the developmental process leading promastigotes to

Glossary

Amastigote: the aflagellated form of *Leishmania* spp. parasites that resides intracellularly within vertebrate host cells.
COS cells: an African green monkey kidney cell line, with the name derived from 'being CV-1 in origin', carrying portions of the SV40 genome.
DC-SIGN (dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin): a receptor for mannosylated glycoconjugates that is found on macrophages and mature dendritic cells. DC-SIGN has been implicated in pathogen recognition as well as leukocyte adhesion via attachment to intercellular adhesion molecules (ICAM)-2 and -3.
Fc gamma receptors: receptors on the surface of phagocytes, including macrophages and neutrophils, that bind to the Fc fragment of immunoglobulin G and stimulate the phagocytosis of particles attached to the antibodies.
Fibronectin receptors: integrins on the surface of a variety of cell types, such as phagocytes and fibroblasts, which bind to fibronectin, an abundant glycoprotein in the extracellular matrix that mediates tissue connectivity.
CR1 (first complement receptor): a receptor on the surface of phagocytes, including macrophages and neutrophils, that bind predominantly to C3b and C4b proteins of the complement cascade, facilitating clearance of particles opsonized by these proteins via phagocytosis.
GP63 (glycoprotein 63): the major metalloprotease that is abundantly expressed on the surface of virulent *Leishmania* promastigotes. When promastigotes are opsonized by C3b, GP63 cleaves this to iC3b, which facilitates promastigote recognition by CR3. GP63 is also called leishmanolysin, PSP (promastigote surface protease) or MSP (major surface protease).
Ligate/ligation: specific binding of receptors on the host cell with their appropriate antigen on the surface of pathogens.
Lipophosphoglycan (LPG): a *Leishmania* surface antigen that densely coats the surface of promastigotes. LPG is attached to the parasite surface by a glycoinositide anchor. A phosphodiester bond links to the anchor an extended polysaccharide backbone, 'capped' by terminal sugar residues that vary between different *Leishmania* spp.
Mannose receptor (MR): a C-type lectin carbohydrate binding protein that serves as a broad-range pattern recognition receptor via detection of mannose on the surface of pathogens. MR is predominantly expressed in macrophages but is also found on neutrophils and dendritic cells.
Metacyclic promastigotes: the virulent form of *Leishmania* promastigotes that arise following metacyclogenesis, a developmental process that occurs as the parasites move progressively from the midgut to the foregut and proboscis of the sand fly insect vector.
Opsonin: proteins or protein complexes that bind to antigens on the surface of foreign particles. Opsonins form part of the innate immune response of the host. Receptors on immune cells then bind to opsonins, resulting in indirect detection of pathogens.
Phagolysosome: a pathogen-containing compartment that develops within phagosomes following a series of fusion events with endosomal and lysosomal organelles. A fully mature phagolysosome is a hostile environment for intracellular organisms that are unfit to evade the parasitophorous vacuole.
Promastigote: the flagellated form of *Leishmania* that exists extracellularly in the sand fly insect vector.
Pseudopod: protrusions of the macrophage membrane that is the result of extensions of actin filaments near the edges of the cell.
Third complement receptor (CR3): an integrin on the surface of phagocytes, including macrophages and neutrophils that primarily detect iC3b during pathogen recognition. The role of CR3 is varied, and encompasses particle clearance, leukocyte adherence, and actin reassembly.
U937 cells: an immortalized monocyte-like cell line of human origin. U937 cells can be stimulated to differentiate

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Table 1. Receptors for *Leishmania* phagocytosis

Receptor, host cell type[a]	Cooperation[b]	Life stages[c]	Binding mechanism[d]	Functional consequences[e]
CR3 [6,11,31,34,35,42] **(CD11b/CD18)** *Primary MΦ*: BMM, MDM, MPM *Cell lines*: J774A.1, THP-1, COS + CR3, 293 + CR3 *Other*: PMN	**CR1** [44] *L. major* **MR** [10,34] *L. donovani* *L. i. chagasi* **FnRs** [49,55,56] *L. amazonensis* **FcγRs** [6] *L. mexicana*	*Avirulent PM*: *L. amazonensis* *L. i. chagasi* *L. donovani* *L. infantum* *L. major* *L. Mexicana* *Metacyclic PM*: *L. i. chagasi* *L. infantum* *L. major* *Amastigote*: *L. amazonensis* *L. donovani* *L. major* *L. mexicana*	*Direct*: Unknown ligand binds alternate lectin-like binding site of CR3 (*L. infantum*) [4,7,34] PSA-2 binds CD11b subunit (*L. infantum*) [35] *iC3b-mediated*: Opsonized C3b from serum is converted by CR1 and factor I into iC3b, a CR3 α-chain ligand (all spp.) [4,31,33,34,44]	CR3-mediated phagocytosis recruits Rho GTPase for cytoskeletal rearrangement [67] Ligation of CR3 by iC3b inhibits IFN-γ mediated proinflammatory signaling, downregulating H_2O_2 and IL-12 production [69] PVs formed after CR3-mediated uptake are tight fitting [2] Phagolysosome maturation is delayed. LAMP-1 and cathepsin-D accumulation is impaired [10,28]
CR1 [5,44] **(CD35)** *Primary MΦ*: MDM *Cell lines*: CHO + CR1	**CR3** [44] *L. major*	*Avirulent PM*: *L. amazonensis* *L. major* *Metacyclic PM*: *L. major*	*C3b-mediated*: Opsonized C3 from serum is cleaved by GP63 into C3b, the natural ligand for CR1 (*L. major*) [24]	CR1 likely functions to enhance CR3 ligation [44,71] CR1 signaling by *Leishmania* spp. may not trigger respiratory burst [5]
MR [4,9,10,34] **(CD206)** *Primary MΦ*: BMM, MDM, MPM	**CR3** [10,34] *L. donovani*	*Avirulent PM*: *L. i. chagasi* *L. donovani*	*Direct*: Mannan-capped LPG backbone and side chains possibly bind the CRD4 and CRD5 domains of MR (*L. chagasi*, *L. donovani*) [26,27,78] Virulent parasites avoid MR ligation (*L. chagasi*, *L. donovani*, *L. major*) [9,10,48]	Signaling depends on MΦ activation state [78,79,83] MR can trigger synthesis of TNF-α, O_2^- and lysosomal enzymes [80–82] Lysosomal fusion can be prevented by inhibiting tyrosine kinase Hck recruitment [84] MR inhibits IL-12 production [85]
FnRs [8,49,50] **(CD49d.CD29, CD49e/CD29, CD41/CD61)** *Primary MΦ*: MDM, MPM, Human monocytes *Cell lines*: CHO + $α_4$ subunit	**CR3** [49,55,56] *L. amazonensis* *L. major* *L. infantum*	*Avirulent PM*: *L. amazonensis* *L. major* *L. infantum* *Amastigotes*: *L. amazonensis*	*Direct*: $α_4$ subunit of 'fibroblast-origin' FnR binds to a non-RGDS motif of GP63 (*L. amazonensis*) [49,52] *Fibronectin-mediated*: Nonspecific opsonization by serum fibronectin allows FnRs to detect RGDS and EILDV motifs (*L. amazonensis*) [49,87]	Internalization via FnRs requires intact β1 subunit [49] Activation of CR3 by FnR is required for CR3-bound particle ingestion [38] FnR-mediated phagocytosis generates O_2^- [51] PM can shed membrane bound to fibronectin to evade intracellular lysis [50]
FcγRs [6,11,60–62] **(CD64, CD32, CD16)** *Primary MΦ*: BMM *Dendritic cells*: MDDC *Cell lines*: COS + FcγRII-B2	**CR3** [6] *L. mexicana*	*Amastigotes*: *L. amazonensis* *L. major* *L. Mexicana* *L. pifanoi*	*IgG-mediated*: Circulating IgG following initial infection bind to amastigotes (*L. major*) [6,11,60] FcγRI, II, and III bind to amastigotes opsonized by IgG (*L. amazonensis*, *L. mexicana*, *L. pifanoi*) [11,61]	FcγR-mediated phagocytosis recruits Rac GTPase for cytoskeletal rearrangement Rac activates NADPH oxidase PVs formed after FcγR-mediated uptake are spacious [67]
DC-SIGN [64,65] **(CD209)** *Dendritic cells*: MDDC *Cell lines*: K562 + DC-SIGN	Unknown	*Avirulent PM*: *L. donovani* *L. infantum* *L. pifanoi* *Metacyclic PM*: *L. infantum* *L. pifanoi* *Amastigotes*: *L. infantum* *L. pifanoi*	*Direct*: Unknown ligand binds to an unknown region of DC-SIGN [64] Binding is independent of LPG (*L. donovani*, *L. infantum*, *L. pifanoi*) [65]	DC-SIGN-mediated uptake into immature MDDCs does not stimulate maturation [66]

[a]Surface receptors that ligate *Leishmania* are written in bold font. The host cell type on which the receptors are expressed are divided into primary macrophages (primary MΦ), neutrophils (PMNs), dendritic cells, and cell lines. Cell lines that have undergone transfection to express receptors are designated 'cell line name + receptor name'.

[b]Receptors that have been shown to cooperate with the receptor mentioned in the left column in 'coligating' *Leishmania* are written in bold font. Parasite species demonstrated to 'coligate' these receptors are listed below.

[c]*Leishmania* life stages that ligate each receptor are separated into nonmetacyclic promastigotes (avirulent PM), metacyclic promastigotes (metacyclic PM), and amastigotes.

[d]Direct or opsonin-mediated mechanisms of binding to receptors are briefly outlined, with the parasite species studied listed in parentheses.

[e]Immunological consequences of the host cell entry via each receptor are briefly outlined.Abbreviations: AM, amastigote; BMM, bone marrow macrophage; CR1, first complement receptor; CR3, third complement receptor; CRD, carbohydrate recognition domain; DC-SIGN, dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin; FcγRs, Fc gamma receptors; FnRs, fibronectin receptors; IgG, immunoglobulin G; LAMP-1, lysosome associated membrane protein-1; LPG, lipophosphoglycan; MDDC, monocyte-derived dendritic cell; MDM, monocyte-derived macrophage; MPM, murine peritoneal macrophage; MR, mannose receptor; MΦ, macrophage; PM, promastigote; PMN, neutrophil; PV, parasitophorous vacuole.