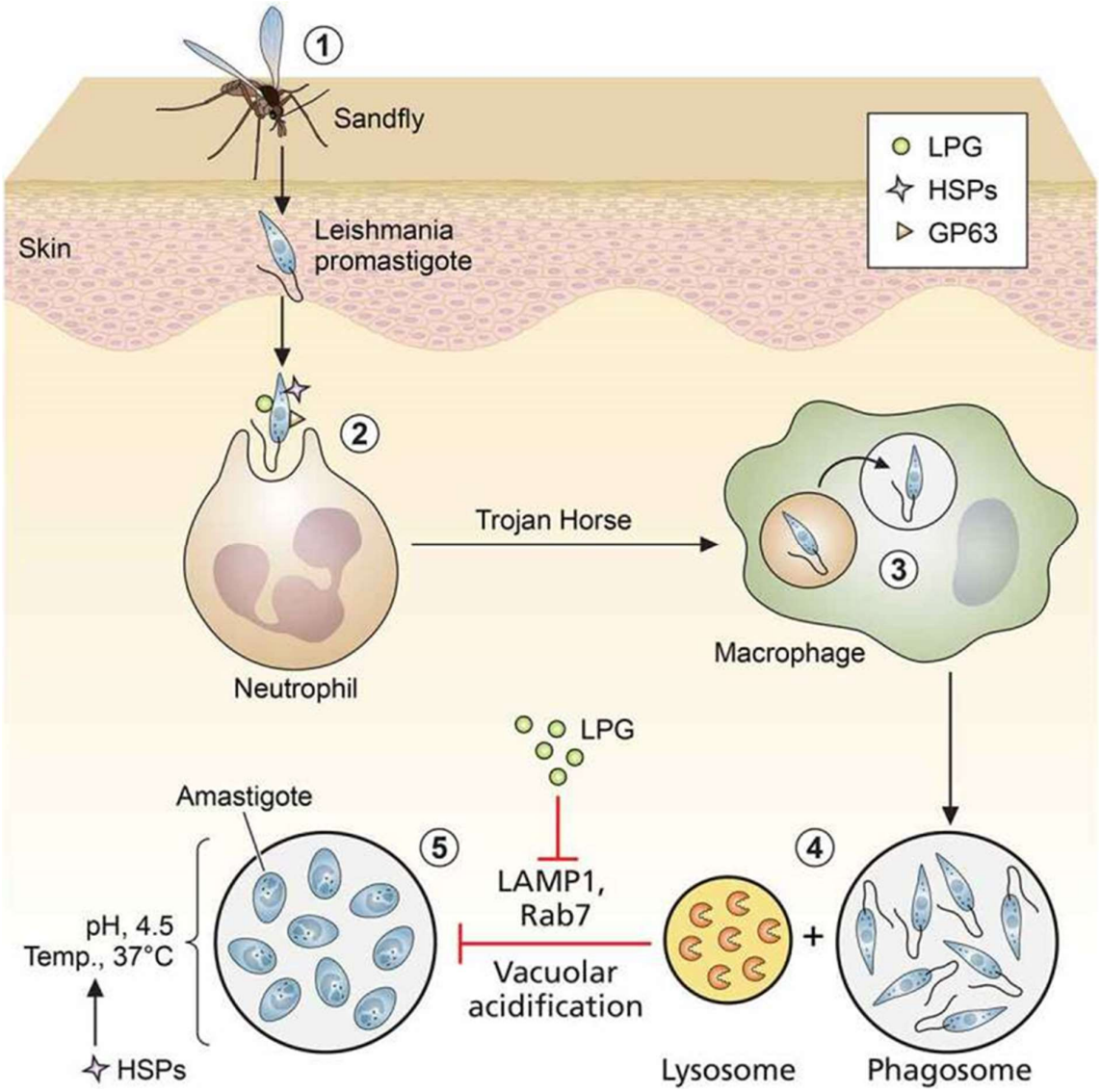
rijksuniversiteit groningen

[1] Department of Molecular Genetics, GBB, University of Groningen

April 2024

Aspects of pathogenesis, infection and lipid metabolism of Leishmania species

Rjimke H. Bottema[1] (S3753735), prof. dr. G.N. (Gert) Moll[1] (supervisor)



Contents

Abstract

Leishmania can be found in and in the vicinity of the majority of the world's (sub)tropical countries and is mostly transmitted via sandflies. There are around 20 different species of Leishmania that are known to infect humans. The parasite, which is transmitted via sandflies, is known to infect white blood cells. Cells that are able to sustain Leishmania infection are, amongst others, macrophages, inflammatory monocytes, neutrophils and dendritic cells. Other cell types have also been found to sustain Leishmania infection, which are endothelial and epithelial cells, fibroblasts, keratinocytes, chondrocytes and myoblasts.

The parasite occurs in two different forms. Inside the mammalian cell, it resides as an amastigote. Within the sandfly and during the initial infection, the parasite is in its promastigote form. The parasite replicates as an amastigote inside the cell and after cell rupture it infects other cells in the vicinity. The pathogenesis differs between species and can be categorised into three major types: visceral leishmaniasis, mucocutaneous leishmaniasis and cutaneous leishmaniasis.

In this essay, multiple aspects per species that are known to infect humans are described. In addition, I have discussed the pathogenesis of Leishmania together with currently available treatment options. Furthermore, the different mechanisms the promastigote form of Leishmania might use to infect the mammalian cell are discussed. Focussed sections discuss how the parasite enters the cell and from where the parasite gets its lipids. Enzymes have been described that are specifically involved in the biosynthesis of two of Leishmania's phospholipids: phosphatidylethanolamine and phosphatidylcholine. Next to *de novo* synthesis, host lipids can be salvaged/remodelled to provide suitable lipids to the parasite. The extent of *de novo* synthesis and remodelling of host lipids depends on the development phase of the parasite. Promastigotes mostly synthesise their required lipids, while amastigotes mostly salvage/remodel required lipids from the host. Target options for new treatments are discussed as well as the potential of filling knowledge gaps to yield the identification of new targets.

Aims

The goals of this essay were: 1) to assess the spread and dangers leishmaniasis poses and 2) to briefly consider and evaluate to what extent the change in climate might accommodate the spread of sandflies to the north. Such accommodation might increase the threat of this 'tropical' disease in Western countries. An additional goal was: 3) to briefly explain what types of pathogenesis different species of Leishmania can cause together with a (limited) list of current treatment options. Further goals were: 4) to ultimately go into depth about how the Leishmania parasite is able to enter the host cell and: 5) to investigate which types of cells can be infected by Leishmania. The last goal was: 6) to study how the parasite gets its lipids.

Introduction

Abundance

Leishmania parasites are the cause of a disease named leishmaniasis (*Parasites - Leishmaniasis*, 2023). Leishmania are trypanosomatid protozoans (Bard, 1989). This disease is found in parts of the tropics, subtropics and southern Europe (*Parasites - Leishmaniasis*, 2023). Currently, Leishmaniasis is among the top 10 neglected tropical diseases with more than 12 million people infected (*Leishmaniasis*, n.d.) and an estimated 700,000 to 1 million new cases annually (World Health Organization, 2023). The disease continues to be a major health problem in four eco-epidemiological regions of the world: the Americas, East Africa, North Africa and Western and Southeast Asia (*Leishmaniasis*, n.d.). The therapeutic tools available need to be replaced, since current treatment is ineffective, expensive, difficult to administer and induces numerous adverse effects (Basmaciyan & Casanova, 2019). On top of that, resistance to current treatments becomes increasingly worrying (Basmaciyan & Casanova, 2019).

Life cycle

Over 20 different species of the Leishmania parasite have been characterized (Mann et al., 2021). They are transmitted to animals and humans from approximately 70 different types of sandflies (Leishmaniasis, n.d.; Mann et al., 2021) that belong to the Phlebotomine subfamily of the family Psychodidae. The life cycle of the Leishmania species is dependent on both the sandfly and a vertebrate host (*Leishmaniasis*, n.d.). Figure 1 represents this life cycle using a human as a host.

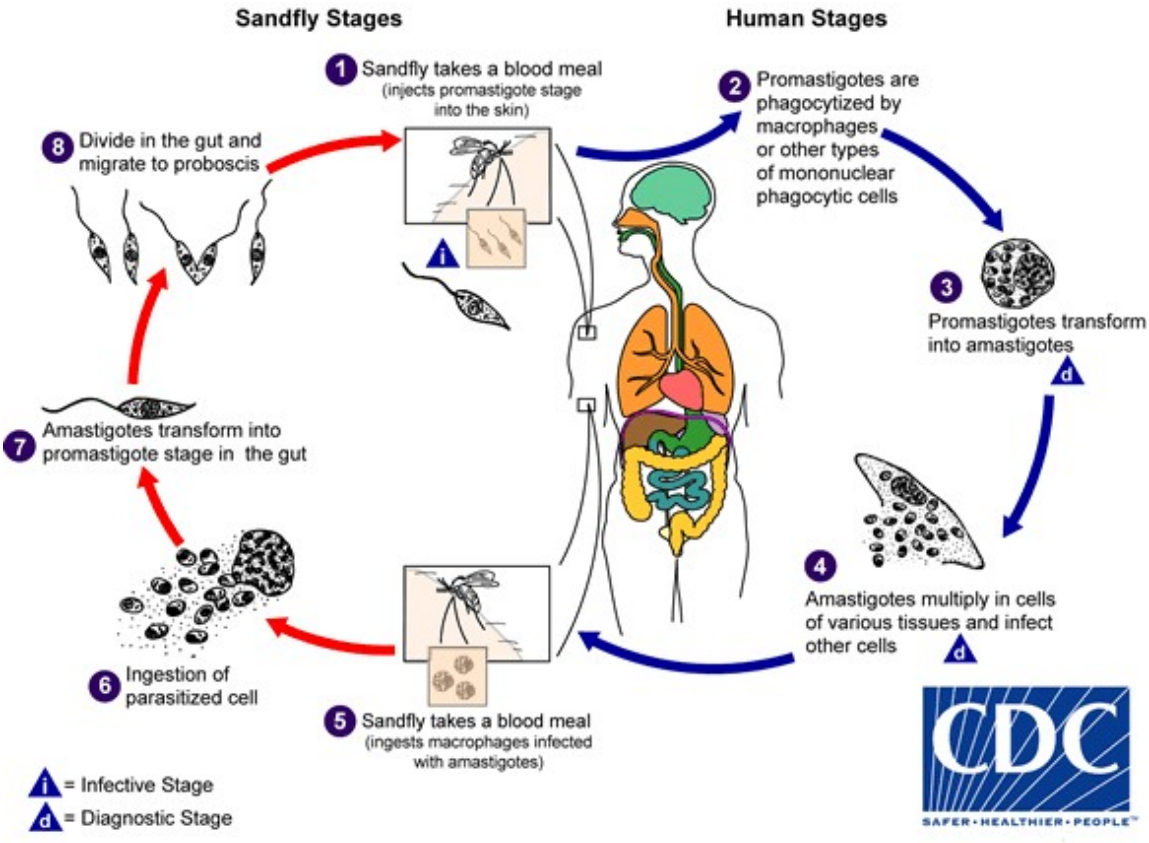


Figure 1*: Life cycle of the Leishmania parasite in its vector and the host.*
*1) Leishmaniasis is transmitted by the bite of infected female phlebotomine sandflies. The sandflies inject the infective stage (i.e., promastigotes) from their proboscis during blood meals. 2) Promastigotes that reach the puncture wound are phagocytized by macrophages and other types of mononuclear phagocytic cells. 3) Promastigotes transform in these cells into the tissue stage of the parasite (amastigotes). 4) The amastigotes multiply by simple division and proceed to infect other mononuclear phagocytic cells. (5&6) Sandflies become infected by ingesting infected cells during blood meals. 7) In sandflies, amastigotes transform into promastigotes in the gut (in the hindgut for leishmanial organisms in the Viannia subgenus; in the midgut for organisms in the Leishmania subgenus). 8) The promastigotes divide in the gut and migrate to the proboscis. Figure taken from (*Leishmaniasis*, 2017).*

The female Phlebotomine species of the sandfly, mostly active from dusk till dawn, transmits the Leishmaniasis parasite to a wide range of vertebrate and avian host species (Mann et al., 2021; *Phlebotomine Sand Flies - Factsheet for Experts*, 2020). Leishmania species have two phases in their life cycle: promastigote and amastigote (Mann et al., 2021). The promastigote form has a flagellum, which allows for mobility in the sandfly's intestines (Mann et al., 2021). When the sandfly takes blood from a vertebrate host, the promastigote form is injected into the skin and is phagocytosed by mononuclear cells of the vertebrate host (Mann et al., 2021). Once phagocytosed, the parasite transforms into the amastigote form (Mann et al., 2021). The amastigotes can travel via the blood or lymph system to cause mucosal and visceral disease (Mann et al., 2021). The transmission of Leishmania species from non-human mammals, mostly dogs, rodents, marsupials, monkeys and edentates, back to the sandfly can even occur when the mammal doesn't show any signs of symptoms or disease (Mann et al., 2021). However, in the same article written in 2021, it was still thought that transmission from a human back to the sandfly is predominantly driven by symptomatic infection and post-kala-azar dermal leishmaniasis (Mann et al., 2021). It was still the assumption that asymptomatic human cases do not infect sandflies (Mann et al., 2021). This assumption has been refuted in more recent reviews. Leishmania is now said to persist and accumulate in the uninflamed skin of the host while still being infectious for sandfly vectors (Valigurová & Kolářová, 2023).

Sandflies

Sandflies do not have difficult standards in terms of habitat requirements and also do not have an aquatic stage in their life cycle (*Phlebotomine Sand Flies - Factsheet for Experts*, 2020). Therefore they are able to survive almost everywhere. However, humidity is still an important factor for survival, in combination with temperatures above 15.6 °C for at least three months of the year (Cecílio et al., 2022; *Phlebotomine Sand Flies - Factsheet for Experts*, 2020). This corresponds to the greatest portion of the world ranging from latitude 50° N to latitude 40° S (Cecílio et al., 2022). Figure 2 represents the global distribution of sandflies. Sandflies are absent in New Zealand and the Pacific Islands (Cecílio et al., 2022).

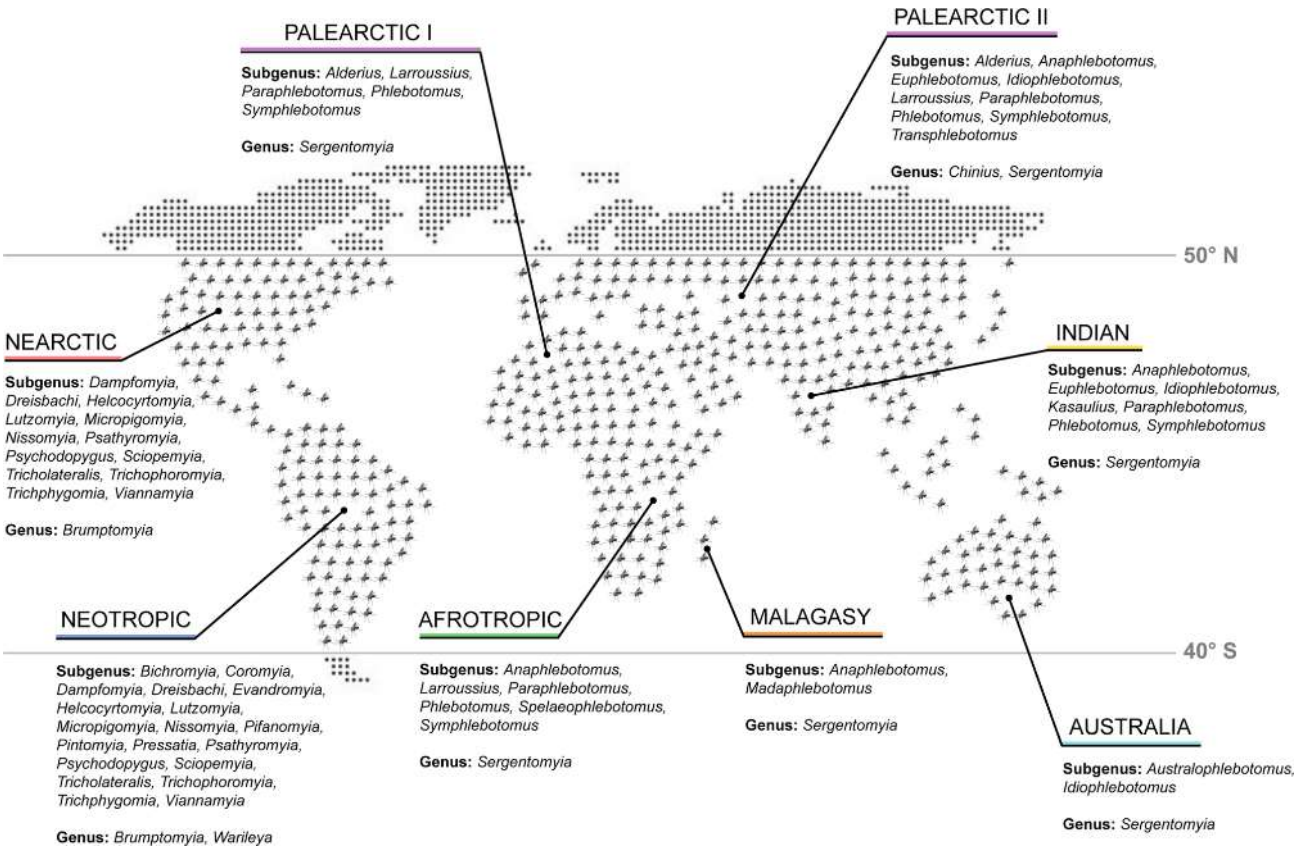


***Figure 2**: The global distribution of sandflies.*
The distribution extends between latitude 50° N and latitude 40° S (roughly marked by grey horizontal lines), excluding New Zealand and the Pacific Islands. The relevant sandfly genera/subspecies are listed based on their presence in defined zoogeographical regions depicted in the picture. Caption and figure adapted from Cecílio et al., 2022.

However, the geographical distribution of sandflies used to be below 45° N and, due to climate change, has gradually shifted northward in the last few decades to just above 50° N (Chalghaf et al., 2018). Given the current trend of global warming, it is estimated that temperatures will further rise (Lindsey & Dahlman, 2024). It was already predicted in 2008 that Phlebotomine sandflies will invade Central Europe (north of the Alps) (Aspöck et al., 2008). In 2018 the potential distribution of Leishmania vectors in the Mediterranean basin was modelled (Chalghaf et al., 2018). That research was prompted by the fact that the presence of Phlebotomine sandflies had already been recorded in several parts of Germany and Belgium (Chalghaf et al., 2018). In central Europe, cases of autochthone Leishmania infections have been recorded in regions that were previously Leishmaniasis-free (Chalghaf et al., 2018). This means that the prediction of the article published in 2008 appears to be confirmed thus far. Chalghaf et al., 2018 took four different sandfly species of the genus Phlebotomus (Phlebotomus sergenti, Phlebotomus ariasi, Phlebotomus alexandri, Phlebotomus papatasi) into account and modelled the possible spread taking two possible climate change scenarios in mind (a

positive and a negative scenario). In all instances, these species are prospected to gain new areas northward that are currently not yet suitable for vectors' survival (Chalghaf et al., 2018).

These data lead to the conclusion that, without finding new treatment options, Leishmaniasis will ultimately not just be a neglected tropical disease anymore (*Parasites - Leishmaniasis*, 2023), but a neglected general disease.

Pathogenesis

There are many different species of Leishmania distributed over the world. The pathogenesis of around 20 of these species has been described. The progression of the diseases can be categorised into three main clinical forms. In Table 1 (pages 8-9), 20 different Leishmania species are described with their corresponding pathogenesis form of leishmaniasis. Table 2 (page 10) describes the current treatment options available for each category of Leishmaniasis and the advantages and disadvantages of each treatment.

Visceral Leishmaniasis

The most serious form -fatal without treatment- is visceral leishmaniasis. Herein the parasite usually infects the spleen, the liver and the bone marrow (Al-Khalaifah, 2022; World Health Organization, 2023). Months to years later, after treatment of visceral leishmaniasis, post-kala-azar dermal leishmaniasis can occur (Ngan & Wootton, 2017). This is a cutaneous form of leishmaniasis which most commonly affects the face, trunk (torso) and extremities (Ngan & Wootton, 2017). The lesions are either indurated papules and nodules or areas of macular hypopigmentation (Ngan & Wootton, 2017). The majority of cases occur in Sudan (up to 50%), but lesions in 80% of the patients will resolve spontaneously within a year (Ngan & Wootton, 2017). The second largest majority of the cases occur in India (up to 10%). The spontaneous resolution rate is much lower here, hence systemic therapy is necessarily used earlier (Ngan & Wootton, 2017).

Cutaneous Leishmaniasis

The most common form is cutaneous leishmaniasis (World Health Organization, 2023). This form usually causes skin ulcers (World Health Organization, 2023). These start with a small skin lesion of around 1 cm which increases in size (Al-Khalaifah, 2022). In most cases, when healing occurs, there is 100% immunity against re-infection (Al-Khalaifah, 2022). But in some cases, failure in cell-mediated immunity causes 1) leishmaniasis diffusa (Al-Khalaifah, 2022). Leishmaniasis diffusa covers most of the skin surface comparable with lepromatous leprosy (Al-Khalaifah, 2022). There are two other distinctions within the cutaneous form: 1a) the diffuse cutaneous leishmaniasis and 1b) disseminated cutaneous leishmaniasis. Diffuse cutaneous leishmaniasis results from an anergic response to the infection due to reduced cell-mediated immunity (Ngan & Wootton, 2017). After the primary cutaneous leishmaniasis lesion, non-ulcerative nodules and plaques can develop and may extend over the whole body (Ngan & Wootton, 2017). There is an absence of ulcers and also an absence of mucosal involvement (Machado et al., 2019). Disseminated cutaneous leishmaniasis is characterised by the presence of more than 10 polymorphic cutaneous lesions (acneiform papules, inflammatory papules, superficial nodules, ulcers) that are distributed over more than two non-contiguous parts of the body (Machado et al., 2019). The nasal mucosa is also affected by up to 53% (Machado et al., 2019). The second form of cutaneous leishmaniasis, 2) Leishmaniasis recidivans, also termed lupoid leishmaniasis, is a rare cutaneous form that occurs in patients with a good cellular immune response (Ngan & Wootton, 2017).

Mucocutaneous Leishmaniasis

The third form of the disease is mucocutaneous leishmaniasis which causes permanent destruction of the mucous membrane in the mouth, nose and throat cavities (Al-Khalaifah, 2022).

***Table 1**: 20 different species of Leishmania and their clinical features, reservoir, transmission and region described in literature.*
Natural progression if possible is described for each species and the estimated annual worldwide incidence. Legend: CL cutaneous leishmaniasis; DCL diffuse cutaneous leishmaniasis; DsCL disseminated cutaneous leishmaniasis; LR leishmaniasis recidivans; MCL mucocutaneous leishmaniasis; PKDL post-kala-azar dermal leishmaniasis; VL visceral leishmaniasis. Notes: # L. colombiensis has been included in the genus Endotrypanum. This table is assembled based on references from the following sources: (Cecílio et al., 2022; Pratlong et al., 2015; Yao, 2019).

***Leishmania* species**	**Clinical form**	**Main clinical features**	**Natural progression**	**Risk groups**	**Main reservoir**	**Transmission**	**High-burden countries or regions**	**Estimated annual worldwide incidence**
Leishmania (Leishmania) aethiopica	CL, DCL, DsCL, and oronasal CL	Localized cutaneous nodular lesions; occasionally oronasal; rarely ulcerates	Self-healing, except for DCL, within 2–5 years	Limited evidence; adolescents	Hyraxes	Rural zoonotic	Ethiopia and Kenya	20,000–40,000 CL
Leishmania (Leishmania) amazonensis	CL, DCL, and DsCL	Ulcerating lesions, single or multiple	Not well described	No well-defined risk groups	Opossums rodents	Sylvatic zoonotic	South America	Limited number of cases, included in the 187,200–300,000 total cases of New World CL
Leishmania (Leishmania) donovani	VL and PKDL	Persistent fever, splenomegaly, weight loss, and anaemia in VL; multiple painless macular, papular, or nodular lesions in PKDL	VL is fatal within 2 years if untreated; PKDL lesions self-heal in up to 85% of cases in Africa but rarely in Asia	Predominantly adolescents and young adults for VL; young children in Sudan and no clearly established risk factors for PKDL	Humans	Epidemic anthroponotic	India, Bangladesh, Ethiopia, Sudan, and South Sudan	50,000–90,000 VL cases; unknown number of PKDL cases
Leishmania (Leishmania) infantum	VL and CL	Persistent fever and splenomegaly in VL; typically single nodules and minimal inflammation in CL	VL is fatal within 2 years if untreated; CL lesions self-heal within 1 year conferring individual immunity	Children under 5 years and immunocompromised adults for VL; older children and young adults for CL	Dogs rodents rabbits hares foxes opossums humans	Peridomestic zoonotic	China, southern Europe, Brazil, and South America for VL and CL; Central America for CL	6200–12,000 cases of Old World VL and 4500–6800 cases of New World VL; unknown number of CL cases
Leishmania (Leishmania) major	CL	Rapid necrosis, multiple wet sores, and severe inflammation	Self-healing in >50% of cases within 2–8 months; multiple lesions slow to heal, and severe scarring	No well-defined risk groups	Rodents	Rural zoonotic	Iran, Saudi Arabia, north Africa, the Middle East, central Asia, and west Africa	230,000–430,000 CL
Leishmania (Leishmania) mexicana	CL, DCL, and DsCL	Ulcerating lesions, single or multiple	Often self-healing within 3–4 months	No well-defined risk groups	Rodents marsupials	Sylvatic zoonotic	South America	Limited number of cases, included in the 187,200–300,000 total cases of New World CL
Leishmania (Leishmania) tropica	CL, LR, and rarely VL	Ulcerating dry lesions, painless, and frequently multiple	CL lesions often self-heal within 1 year	No well-defined risk groups	Humans hyraxes	Urban anthroponotic	Eastern Mediterranean, the Middle East, and northeastern and southern Africa	200,000–400 000 CL

Leishmania (Leishmania) venezuelensis	CL	Ulcerating lesions	Not well described	No well-defined risk groups	Unknown	Zoonotic	Venezuela	Limited number of cases, included in the 187,200–300,000 total cases of New World CL
Leishmania (Mundinia) martiniquensis	CL and VL	Not well described	Not well described	No well-defined risk groups	Unknown	Likely sylvatic zoonotic	Martinique, Thailand, Central Europe, USA	Limited number of cases
Leishmania (Mundinia) orientalis	CL and VL	Not well described	Not well described	No well-defined risk groups	Unknown	Likely sylvatic zoonotic	Thailand	Limited number of cases
Leishmania (Viannia) braziliensis	CL, MCL, DCL, and LR	Ulcerating lesions can progress to mucocutaneous form; local lymph nodes are palpable before and early on in the onset of the lesions	Might self-heal within 6 months; 2·5% of cases progress to MCL	No well-defined risk groups	Dogs humans rodents horses	Sylvatic zoonotic	South America	Majority of the 187 200–300 000 total cases of New World CL
Leishmania (Viannia) guyanensis	CL, DsCL, and MCL	Ulcerating lesions, single or multiple that can progress to mucocutaneous form; palpable lymph nodes	Might self-heal within 6 months	No well-defined risk groups	Opossums sloths anteaters	Sylvatic zoonotic	South America	Limited number of cases, included in the 187,200–300,000 total cases of New World CL
Leishmania (Viannia) lainsoni	CL	Ulcerating lesions, single or multiple	Not well described	No well-defined risk groups	Rodents porcupines	Sylvatic zoonotic	Brazil, Bolivia, Peru, Ecuador	Limited number of cases, included in the 187,200–300,000 total cases of New World CL
Leishmania (Viannia) lindenbergi	CL	Ulcerating lesions	Not well described	No well-defined risk groups	Unknown	Zoonotic	Brazil	Limited number of cases, included in the 187,200–300,000 total cases of New World CL
Leishmania (Viannia) naiffi	CL	Ulcerating lesions, single and small	Not well described	No well-defined risk groups	Rodents Anteaters	Sylvatic zoonotic	Brazil, French Guyana	Limited number of cases, included in the 187,200–300,000 total cases of New World CL
Leishmania (Viannia) panamensis	CL, MCL and DCL	Ulcerating lesions, single or multiple that can progress to mucocutaneous form	Not well described	No well-defined risk groups	Rodents dogs?	Sylvatic zoonotic	Central and South America	Limited number of cases, included in the 187,200–300,000 total cases of New World CL
Leishmania (Viannia) peruviana	CL	Ulcerating lesions, single or multiple	Not well described	No well-defined risk groups	Unknown dogs?	Zoonotic	Peru, Bolivia	Limited number of cases, included in the 187,200–300,000 total cases of New World CL
Leishmania (Viannia) shawi	CL	Ulcerating lesions	Not well described	No well-defined risk groups	Rodents sloths	Sylvatic zoonotic	Brazil	Limited number of cases, included in the 187,200–300,000 total cases of New World CL
Leishmania colombiensis #	CL and VL	Not well described	Not well described	No well-defined risk groups	Sloths	Sylvatic zoonotic	Colombia	Limited number of cases
Leishmania waltoni	DCL	Producing multiple nodular skin lesions.	Long-term evolution without spontaneous cure.	Resistant to treatment by antimonials and amphotericin B.	Hutias? Solenodon? Human	Sylvatic Zoonotic	Dominican Republic	Limited number of cases

***Table* 2**: Current Treatments Against Leishmaniasis.
Administration routes, and therapeutic characteristics including use against which manifestation of leishmaniasis as of 2023. Table adapted from Pacheco-Fernandez et al., 2023 and Pradhan et al., 2022.

Treatment	Admin route	Type of Therapy	Therapeutic activity	Active against	Advantages	Disadvantages
Pentavalent antimonials (SSG, MA)	IM, IV parenteral	Chemotherapy	Promotes macrophage activity against parasites, promotes oxidative stress within parasites, inhibits trypanothione reductase leading to parasite death	VL, CL, MCL	Relatively low cost in shortened regimens, generic versions available	Severe side effects: cardiotoxicity, pancreatitis; nephrotoxicity; hepatotoxicity. Efficacy against MCL is variable, parasite resistance, costly, lengthy regimen, contraindications in multiple patient cohorts
Amphotericin B	IV	Chemotherapy	Binds to parasitic ergosterol, promotes oxidative stress	VL, CL, MCL	Primary resistance is not common;	Requires hospitalization for administration; nephrotoxicity; heat; instability; injection-related reactions; hypokalaemia
L-AmB	Parenteral, IL, IV	Chemotherapy	Binds to parasitic ergosterol, promotes oxidative stress	VL, CL, MCL	Good for use when antimonial resistance is relevant, less toxic than AmB, good for use in HIV patients, regimen can be short	Lengthy regimen, availability, costly, chills and rigours during injection; mild nephrotoxicity
Pentamidine	Parenteral, IM	Chemotherapy	Inhibits parasitic mitochondrial activity, blocks active transport function	VL, CL	Good for use when antimonial resistance is relevant, short course needed	Hyperglycaemia, hypotension, tachycardia, electrocardiographic changes, risk of parasite resistance, efficacy in immunodeficient populations varies, varied efficacy depending on Leishmania species
Miltefosine	Oral	Chemotherapy	Damages parasite mitochondrial membrane, decreases cytochrome-c oxidase activity, prompts apoptotic cell death	VL, CL, MCL	Good for use when antimonial resistance is relevant	Contraindicated during pregnancy, renal and liver toxicity, relatively lengthy regimen, parasite resistance, efficacy varies (including in immunodeficient populations)
Paromomycin	Parenteral, topical, IM	Chemotherapy	Binds to parasitic ribosomes to inhibit normal protein synthesis, damages membranes	VL, CL, limited efficacy against MCL	Low cost, can treat co-infections	Renal, ear and liver toxicity, relatively lengthy regimen, varied efficacy according to geographical area, potential for resistance
Antifungal azoles (Fluconazole, ketoconazole, itraconazole)	Oral, topical	Chemotherapy	Leishmanicidal activity against promastigote stages, inhibits growth	CL, VL	Safe	Costly, variable efficacy, lengthy regimen
Thermotherapy (Infrared light, lasers, radiofrequency waves)	Local	Thermotherapy	Directly targets infection site to decrease parasite replication, collagen contraction may play a role, enhances elimination by macrophages	CL	No toxicity or severe side effects, battery-operated, regimen can be quick, lesion size specific	Costly, burns possible, local anaesthesia may be needed, scarring
Cryotherapy (Liquid nitrogen, carbon dioxide solids)	Local	Cryotherapy	Directly targets infected tissue, exact leishmanicidal activity unknown	CL	No toxicity or severe side effects, regimen can be quick, low-cost	Painful, burns possible, risk of secondary infections in treated tissue, risk of scarring, risk of depigmentation

Leishmania can infect different types of cells

Now we know that different species can cause different trajectories of disease. But to understand the trajectory better on a cellular level, which cells can the parasite infect? The Leishmania parasite is mostly known to inhabit professional phagocytes, macrophages and monocytes (macrophages, inflammatory monocytes, neutrophils and dendritic cells) (Valigurová & Kolářová, 2023).

But other cell types, that used to be regarded as non-canonical host cells, could also be victims of the Leishmania parasites. The presence of Leishmania major transcripts has been shown to be associated with multiple (noncanonical) cell types (endothelial cells, epithelial cells, fibroblasts, keratinocytes, chondrocytes and myoblasts) at the site of infection (Valigurová & Kolářová, 2023). But how would the parasite be able to enter the different host cells?

Host cell entry

Passive host cell entry is the widely accepted mode of Leishmania internalisation for both promastigotes and amastigotes (Ueno & Wilson, 2012; Valigurová & Kolářová, 2023). The idea used to be that Leishmania was completely dependent on the phagocytic activity of the host cell by deliberate binding of host cell opsonins (Valigurová & Kolářová, 2023). The passive entry into the professional phagocytes is generally considered to be receptor-mediated phagocytosis (Valigurová & Kolářová, 2023). Phagocytosis, however, is only one of several processes by which a cell is able to take up extracellular materials. Endocytosis can be divided into four basic categories: 1) actin-mediated endocytosis (including phagocytosis and micropinocytosis, 2) caveolin-dependent endocytosis, 3) clathrin-dependent endocytosis and 4) clathrin-caveolin-independent endocytic pathways (Valigurová & Kolářová, 2023). It turns out that Leishmania is more likely to be internalised by caveolin-dependent endocytosis and clathrin-dependent endocytosis does not appear to play a role in the uptake of Leishmania promastigotes (Valigurová & Kolářová, 2023). Figure 3 shows three accepted models of Leishmania promastigote entry into the host cell. The three models of host cell entries shown are: 1) actin-dependent phagocytosis, 2) caveolin-mediated endocytosis and 3) lysosome-triggered endocytosis (Valigurová & Kolářová, 2023). The phagocytic process is associated more with dedicated phagocytes, but it turns out that almost all cells in the human body are capable of phagocytosis and caveolin-dependent endocytosis (Valigurová & Kolářová, 2023).

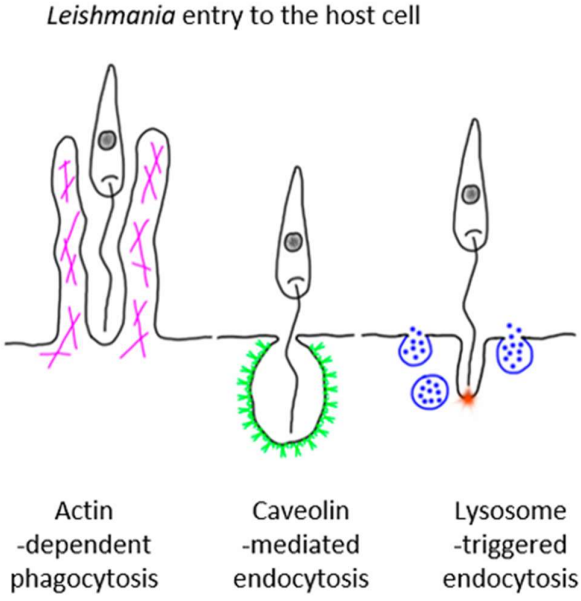


***Figure 3**: Visualization of the three accepted models of Leishmania promastigote entry. Magenta: actin-dependent phagocytosis. Green: caveolin-mediated endocytosis. Blue: lysosome-triggered endocytosis. Figure taken from (Valigurová & Kolářová, 2023).*

Actin-dependent phagocytosis

It used to be the idea that Leishmania uptake of both promastigotes and amastigotes was completely dependent on receptor-mediated phagocytosis (Ueno & Wilson, 2012; Valigurová & Kolářová, 2023).

Receptor-mediated phagocytosis belongs to the actin-mediated endocytosis pathway (Valigurová & Kolářová, 2023). It turns out, however, that the Leishmania promastigote can also be internalised by caveolin-dependent endocytosis (figure 3) (Valigurová & Kolářová, 2023). In the case of amastigotes, it can also be endocytosed with the caveolin-independent pathway (Valigurová & Kolářová, 2023; third option in figure 3).

Caveolin-dependent phagocytosis

Caveolins, necessary for caveolin-dependent endocytosis, are integral membrane proteins that preferably oligomerise in cholesterol-rich lipid rafts to induce membrane invagination (Valigurová & Kolářová, 2023). Through this type of endocytosis, the uptake of extracellular material can also be mediated by specific receptors (Valigurová & Kolářová, 2023). Caveolin-coated phagosomes also have another advantage. It has been noted that caveolin-coated phagosomes showed delayed fusion with lysosomes, giving the promastigote time to transform into the amastigote (Valigurová & Kolářová, 2023). As a resulting advantage, amastigotes are better adapted to survive in acidified phagolysosomes (Valigurová & Kolářová, 2023).

Lysosome triggered uptake

Uptake of avirulent, serum opsonised promastigotes and amastigotes appears to be caveolin-independent (Valigurová & Kolářová, 2023). This in turn shows no delay in phagosome fusion with lysosomes (Valigurová & Kolářová, 2023). This lack of delay is only fatal for promastigotes, since, as has been established before, amastigotes are already adapted to the phagolysosomal environment (Valigurová & Kolářová, 2023).

Active entrance

Leishmania parasites seem to also be able to enter host cells actively (Figure 4).

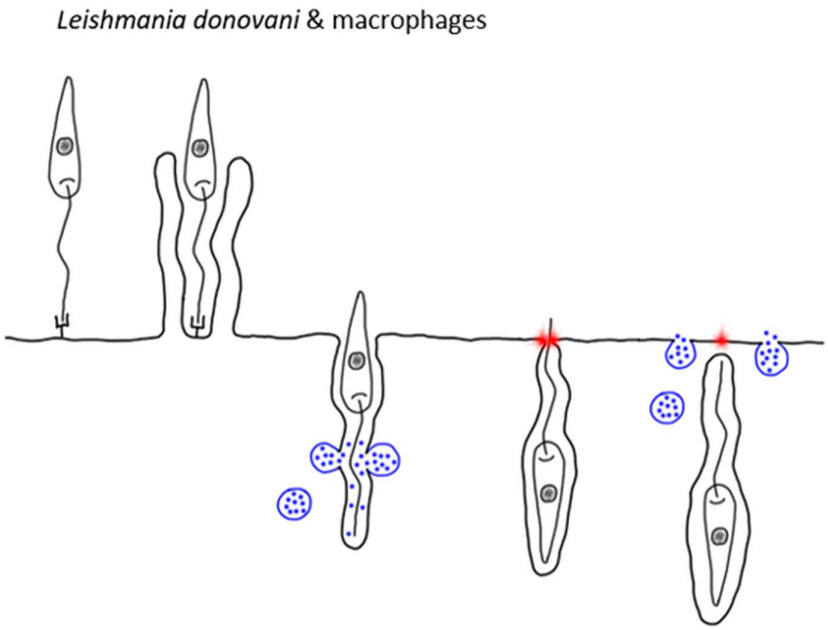


***Figure 4**: Leishmania donovani promastigote enters the macrophage presumably via receptor-ligand mediated pathway. This leads to the formation of pseudopodia starting from the flagella and extends towards the cell body. During internalisation, lysosomes fuse with the phagosome in formation. The flagellar motility damages the host membrane. Due to damage, lysosomes are exocytosed. Figure taken from (Valigurová & Kolářová, 2023).*

This is seen at least for *Leishmania donovani* (Valigurová & Kolářová, 2023). In the promastigote state, the parasite has a flagella, which is used to actively participate in phagocytic uptake by the macrophage (Valigurová & Kolářová, 2023). *Leishmania donovani* metacyclic promastigotes preferably enter primary bone-marrow-derived murine macrophages via the flagellar tip (Valigurová & Kolářová,

2023). During the internalisation, lysosomes fuse with the forming phagosome and just before completing internalisation, the internalized promastigote reorients the flagellar tip towards the macrophage plasma membrane and in some cases, it sticks out of the host cell (Valigurová & Kolářová, 2023). The flagellar motility causes damage to the plasma membrane, causing Ca^{2+}-dependent recruitment of host lysosome exocytosis and increased endocytosis of the damaged plasma membrane together with the Leishmania promastigote (Valigurová & Kolářová, 2023). Once completely internalised, the promastigote loses its motility and the phagolysosome is located close to the cell nucleus (Valigurová & Kolářová, 2023).

Spread

Leishmania parasites first infect phagocytes, such as neutrophils and tissue-resident macrophages (Gupta et al., 2022; Vellozo et al., 2021). The promastigotes appear to parasitise these first host cells without replicating (Valigurová & Kolářová, 2023). Within a few days, the parasite infects other myeloid cells, for example, inflammatory monocytes, monocyte-derived dendritic cells and eosinophils at the infection site (Valigurová & Kolářová, 2023; Vellozo et al., 2021). The increased migration of infected dendritic cells could help the parasite spread further into the body (Valigurová & Kolářová, 2023). During the chronic phase of infection, neutrophils infected with amastigotes are able to support Leishmania multiplication, however, not the transformation from promastigote to amastigote (Valigurová & Kolářová, 2023). It is not yet completely clear when and how the parasite enters non-canonical host cells. However, a mechanism is explained on how the Leishmania parasite is transmitted from the neutrophils to the macrophages.

Trojan horse mechanism: transfer of parasite from neutrophil to macrophage

The Leishmania parasite is transferred from the neutrophil to the macrophage using the 'Trojan Horse' mode of transfer (Figure 5) (Gupta et al., 2022).

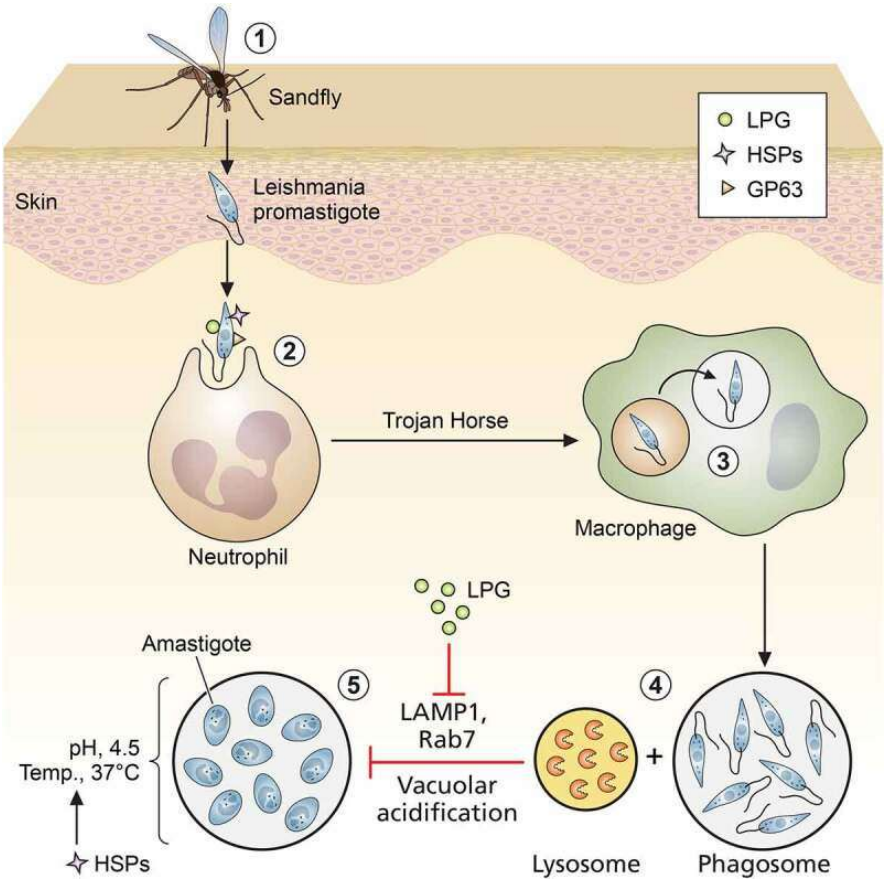


***Figure 5**: Entry of the Leishmania parasite and the trans-differentiation. 1) Entry of promastigotes through skin via bite of sandfly. 2) Uptake of promastigotes by neutrophils. 3) Safe transport of promastigotes from neutrophils to macrophages by "Trojan Horse" mechanism. 4) Formation of parasitophorous vacuoles (PVs) by prevention of phago-lysosomal fusion. 5) trans-differentiation of promastigotes to amastigotes inside PVs (LPG: lipophosphoglycan; HSPs: heat shock proteins; and GPI: glycosyl phosphatidyl inositol). Image and caption taken from (Gupta et al., 2022).*

The parasite resides as a promastigote in the polymorphonuclear neutrophils (PMNs) and the PMNs form apoptotic bodies (Gupta et al., 2022). The apoptotic bodies are used by the promastigotes to safely enter the macrophage via phagocytosis (Gupta et al., 2022). Lipophosphoglycans (LPG) are crucial in this transfer mechanism by assisting the entry of Leishmania promastigotes via binding with complement receptor CR3 and integrin receptor P150/95 (Gupta et al., 2022). LPGs are the dominant structural component of the Leishmanian surface glycocalyx and LPG is synthesized in promastigotes (Gupta et al., 2022). Inside the macrophage, the parasite prevents fusion of the phagosome and lysosome to limit the change of the microenvironment. Here the parasite will trans-differentiate from pH-sensitive promastigotes to pH-resistant amastigotes (Gupta et al., 2022). A remaining open question is: how does the parasite change from promastigote to amastigote and in reverse? LPG is absent in amastigotes, but LPG predesigns an immune-suppressed situation during parasite entry so that the parasite can easily survive and proliferate within (Gupta et al., 2022). LPG also has a list of other inhibitory and protective activities for some species of Leishmania (as depicted in Figure 5), making it crucial for these species for virulence and intracellular survival (Gupta et al., 2022). LPG is absent in amastigotes, however, LPG predesigns an immune-suppressed situation, enabling the parasite to easily survive and proliferate within (Gupta et al., 2022). Amastigotes replicate inside the phagocyte until they rupture the cell and infect other tissues (McGwire & Satoskar, 2014).

Fibroblasts

One of the more studied non-canonical host cell does provide some insight into how the Leishmania parasites spread in the body while increasing the chance of being engulfed by the vector during blood feeding, and also provides some understanding of latent leishmaniasis (Valigurová & Kolářová, 2023). Fibroblasts can migrate within the skin tissue and have a relatively long lifespan with low leishmanicidal activity (Valigurová & Kolářová, 2023). However, the parasite is able to transform from promastigote to amastigote in the fibroblasts and even replicate (Valigurová & Kolářová, 2023). Since fibroblasts are migratory, this could mean that fibroblasts could be another factor helping the spread of Leishmania into yet uninfected parts of the body.

Fibroblasts may also serve as an ideal reservoir host cell in latent cutaneous leishmaniasis and adipocytes may also play this role in visceral leishmaniasis (Valigurová & Kolářová, 2023). It has also been shown that Leishmania parasites are not dormant during latent leishmaniasis, but are continuously replicating while being under the tight immune control of macrophage-derived nitric oxide (Valigurová & Kolářová, 2023). Since Leishmania is able to persist and accumulate in the uninflamed skin of the host while still being infectious for sandfly vectors, it is important to also keep in mind that not only cured patients are potential reservoirs but also other hosts and/or asymptomatic humans (Valigurová & Kolářová, 2023).

Where does the parasite get its lipids from?

Once in the cell, the parasite still needs to be able to maintain itself. The bigger question is, how does the parasite get nutrients to maintain and even replicate? The focus in this respect in this essay lies on lipids. As described in the introduction, Leishmania parasites have two forms: the promastigote, extracellularly in the sandfly gut, and the amastigote, intracellularly in a vertebrate host. Promastigotes replicate fast and acquire most of their lipids via *de novo* synthesis (Basu et al., 2023; Zhang, 2021). Amastigotes are slowly growing and rely mostly on the uptake and remodelling of host lipids (Basu et al., 2023; Zhang, 2021). The switch of *de novo* synthesis to salvage reflects the transition of Leishmania from promastigote to amastigotes, as can be seen in Figure 6 (Zhang, 2021).

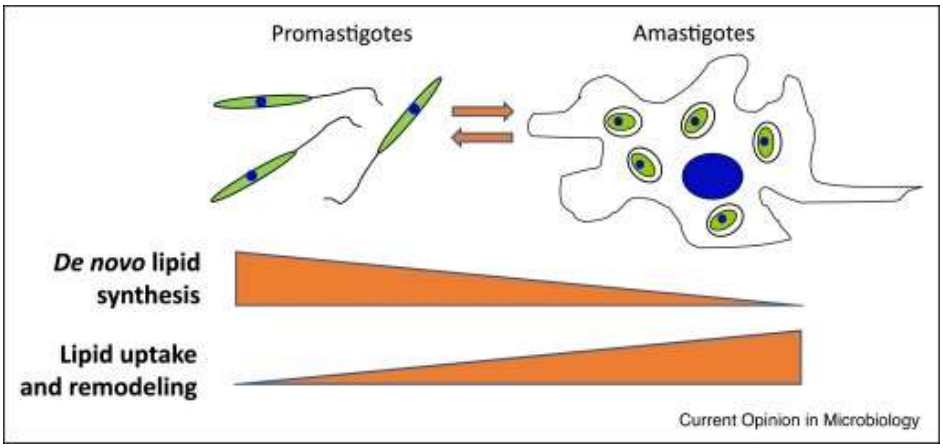


***Figure 6**: Reflection of the switch of lipid origin and Leishmania parasite form. Picture taken from* (Zhang, 2021)

Non-essential *de novo* pathways

The switch is based on the following findings. Loss of cholinephosphate cytidylyltransferase (CPCT) doesn't affect promastigote replication or their virulence (Zhang, 2021). CPCT is responsible for catalysing choline-P to CDP-choline and without this, *L. major* cannot incorporate choline into phosphatidylcholine (PC) (Zhang, 2021). There must be, however, some other way by which they accomplish this since promastigotes without the transferase still have similar levels of PC compared to the wild type (Zhang, 2021). Figure 7 demonstrates that CPCT is not the only pathway responsible for the cell's production of PC. Phosphatidylethanolamine (PE) can be methylated into PC via phosphatidylethanolamine N-methyltransferase (PEMT) (Zhang, 2021). Another route of compensating for the loss of PC is via salvaging/remodelling of exogenous lipids (Zhang, 2021). Promastigotes without CPCT showed reduced proliferation under serum-free or ethanolamine-restricting conditions (Zhang, 2021).

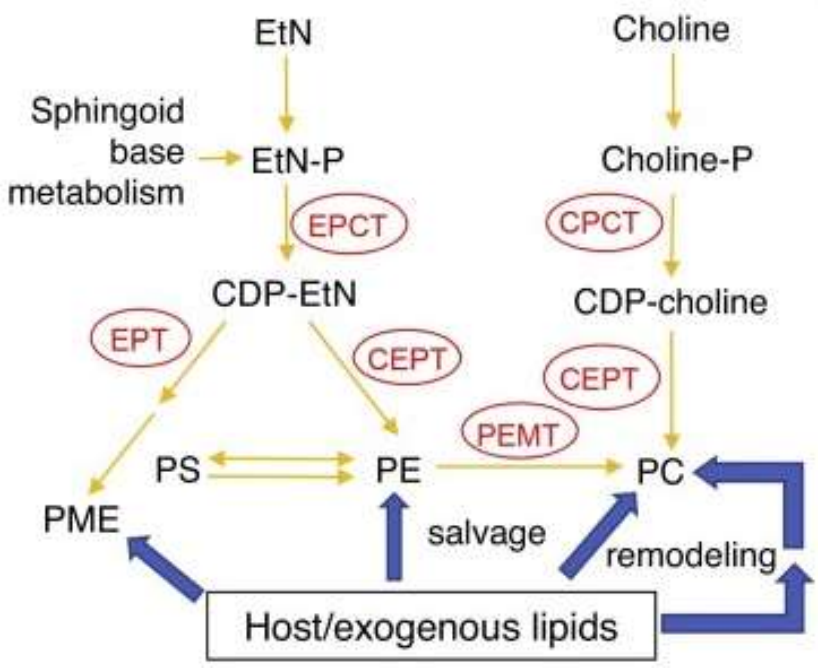


***Figure 7**: Pathways of lipid production of Leishmania.*
EtN: Ethanolamine; EtN-P: ethanolamine phosphate; Choline-P: Choline phosphate; EPCT: ethanolaminephosphate cytidylyltransferase; CPCT: cholinephosphate cytidylyltransferase; CDP-EtN: cytidine diphosphate ethanolamine; CDP-choline: cytidine diphosphate-choline; EPT: ethanolamine phosphotransferase; CEPT: choline ethanolamine phosphotransferase; PEMT: phosphatidylethanolamine N-methyltransferase; PS: phosphatidylserine; PE: phosphatidylethanolamine; PC: phosphatidylcholine; PME: phosphatidylmonomethylethanolamine. Picture taken from (Zhang, 2021).

Ethanolamine phosphotransferase (CEPT) is essential since CEPT-null promastigotes were unsuccessful to obtain (Zhang, 2021). This means that the salvage pathway is not enough for promastigotes to produce enough PC to support parasite proliferation (Zhang, 2021). However, amastigotes are able to lose the episomal copy of CEPT, meaning that amastigotes acquire the majority of PC through salvage and remodelling (Zhang, 2021). This does make sense in regards to doubling time. Promastigotes are able to double in about 6-8 hours in culture and 10-12 hours within the sandfly whereas, in contrast, the estimated doubling time of amastigotes is 60 hours (Zhang, 2021).

The same pattern of *de novo* synthesis versus remodelling, essentially previously depicted in Figure 6, has been observed in ethanolamine phosphotransferase (EPT)-null mutants and palmitoyltransferase subunit 2 (spt2)-null (sphingolipid synthesis) mutants (Zhang, 2021). Even though spt2 is not present in the amastigotes, they still have normal levels of IPC (inositolphosphoryl-ceramides) that are not synthesized by the host (Zhang, 2021). This means that they acquire sphingolipids from the host and carry out head group remodelling to convert them into Inositol phosphorylceramide (IPC) (Zhang, 2021). Amastigotes taking lipids from the host is only further proved by host cells showing enhanced cholesterol uptake and triacylglycerol synthesis and in turn forming lipid droplets near or within parasitophorous vacuoles (Zhang, 2021). Not only are lipids scavenged into membrane components, but they might also be utilized as an energy source via fatty acid β-oxidation (Zhang, 2021).

Essential *de novo* pathways

However, not all *de novo* synthesis pathways can be replaced with salvage and remodelling. In the case of ethanolaminephosphate cytidylyltransferase (EPCT), this transferase is essential in both promastigotes and amastigotes (Basu et al., 2023). This means that amastigotes still need some capacity for PE *de novo* synthesis. Elevated EPCT expression has shown overall altered PE synthesis and compromises the parasite's tolerance to adverse conditions and hinders the growth of intracellular amastigotes (Basu et al., 2023). A similar case can be made for sterol 14-α-demethylase (C14DM) and sterol methyltransferase (SMT) (Mukherjee et al., 2019, 2020; Zhang, 2021). They are crucial for the production of ergosterol and other ergostane-based sterols and without them, the growth and development of both the promastigote and the amastigote are seriously hampered (Zhang, 2021).

Discussion

To briefly summarize, Leishmania is able to persist and accumulate in the uninflamed skin of the host, while still being infectious for sandfly vectors. Therefore it is important to also keep in mind that not only cured patients are potential reservoirs, but also other hosts and/or asymptomatic humans (Valigurová & Kolářová, 2023). This means that tourists and other unsuspected hosts from endemic areas could bring the parasite to yet non-endemic areas. Especially when keeping in mind that the sandfly habitat is expanding due to climate change. No vaccine against Leishmania is available. Current treatments lack efficacy, are harsh and have several severe side effects. Taken together, these combined concerning facts urge for the discovery of new treatments.

Research addressing unsolved questions may unveil new targets for treatments. Current research still focuses mostly on how the promastigote enters the cell, but treatment-wise, this is only important for initial infection. A few aspects are known about how the amastigote enters the host cell, but detailed information is lacking. Researching more on how the amastigote enters other host cells might give greater insight into new stage-dependent treatment possibilities when the patient is already suffering from leishmania infection. Another topic of research might be on how the promastigote changes to amastigote within the host cell. Treatment based on this could be used to counter early infection and possibly prevent further infection and spread within the human body. Understanding the switch from amastigote to promastigote might eventually enable the permanent blocking of this process while

the parasite is still in the host before it can be taken up in the sandfly again. This might contribute to prevent Leishmania from becoming endemic in new countries. One last suggestion for new research, with both fundamental and applied perspectives, would be directed at understanding more how promastigotes and amastigotes enter non-canonical host cells.

Research in the past did already show some possible targets that could help eliminate or prevent Leishmania infection. Lipophosphoglycans (LPG), for instance, is the dominant structural component of the Leishmania promastigote surface glycocalyx and is said to have many functions during initial Leishmania infection. Ideally, a highly effective (inexpensive) vaccine could be developed using LPG to immunize the body against Leishmania. This has already been tested in 2014 and 2017 showing great potential (Allahverdiyev et al., 2017; Martínez Salazar et al., 2014). A vaccine like this would be great in preventing the disease trajectory of Leishmania.

Sterol 14-α-demethylase (C14DM), sterol methyltransferase (SMT) and ethanolaminephosphate cytidylyltransferase (EPCT) are crucial for the Leishmania parasite in both the promastigote and the amastigote form due to being indispensable in the lipid production pathway. Given this information, these enzymes could be used as a drug target against Leishmania.

C14DM is an enzyme that is found in all biological kingdoms (Lepesheva & Waterman, 2007) and is already being used as a primary drug target for several microbial infections in both animals and plants (Lepesheva & Waterman, 2007). But this target can also be used against protozoa and therefore Leishmania. Selective inhibition of the C14DM from protozoa is possible due to minor structural differences between the enzymes of protozoa and humans (I. Lepesheva & R. Waterman, 2011), in combination with the fact that human C14DM is highly resistant to inhibition compared to protozoan and fungal C14DM (Friggeri et al., 2019).

SMT is a far easier target. SMT is an enzyme not present in humans (Zhou et al., 2006) but it is present in other pathogenic organisms (Kidane et al., 2017; Zhou et al., 2006). This means there is less chance for off-target toxicity. Since the SMT enzyme is also present in other pathogenic organisms, the drug could not only work for Leishmania, but also other diseases.

EPCT is a group of transferases, just like C14DM, EPCT is an enzyme present throughout different biological kingdoms. It is regarded as Ect1 in budding yeast, PCYT2 in animals and PECT1 in plants (InterPro, n.d.). The difference to C14DM is that not a lot of research has yet been done comparing the EPCT from humans to protozoa. Neither is it used yet as a target in other protozoan treatments. This means a lot more research needs to be done for EPCT to find out if it is a viable drug target against Leishmania.

If a drug could be made to selectively hinder or even block the use of these enzymes in Leishmania, there could be opportunities for developing new treatments even for stages at which the disease has already progressed.

Leishmania is a fascinating organism. This essay clearly demonstrates the need for further fundamental and applied research to address unsolved basic questions and to (subsequently) develop new treatments for a disease that in all likelihood will soon impose an increasingly global threat.

References

Al-Khalaifah, H. S. (2022). Major Molecular Factors Related to Leishmania Pathogenicity. In *Frontiers in Immunology* (Vol. 13). Frontiers Media S.A. https://doi.org/10.3389/fimmu.2022.847797

Allahverdiyev, A. M., Cakir Koc, R., Bagirova, M., Elcicek, S., Baydar, S. Y., Oztel, O. N., Abamor, E. S., Ates, S. C., Topuzogullari, M., Isoglu Dincer, S., & Akdeste, Z. (2017). A new approach for development of vaccine against visceral leishmaniasis: Lipophosphoglycan and polyacrylic acid

conjugates. *Asian Pacific Journal of Tropical Medicine*, *10*(9), 877–886. https://doi.org/10.1016/j.apjtm.2017.09.001

Aspöck, H., Gerersdorfer, T., Formayer, H., & Walochnik, J. (2008). Sandflies and sandfly-borne infections of humans in Central Europe in the light of climate change. *Wiener Klinische Wochenschrift*, *120*(S4), 24–29. https://doi.org/10.1007/s00508-008-1072-8

Bard, E. (1989). Molecular biology of Leishmania. *Biochemistry and Cell Biology = Biochimie et Biologie Cellulaire*, *67*(9), 516–524. https://doi.org/10.1139/o89-083

Basmaciyan, L., & Casanova, M. (2019). La mort cellulaire chez Leishmania. In *Parasite (Paris, France)* (Vol. 26, p. 71). NLM (Medline). https://doi.org/10.1051/parasite/2019071

Basu, S., Pawlowic, M. C., Hsu, F. F., Thomas, G., & Zhang, K. (2023). Ethanolaminephosphate cytidylyltransferase is essential for survival, lipid homeostasis and stress tolerance in Leishmania major. *PLoS Pathogens*, *19*(7). https://doi.org/10.1371/journal.ppat.1011112

Cecílio, P., Cordeiro-da-Silva, A., & Oliveira, F. (2022). Sand flies: Basic information on the vectors of leishmaniasis and their interactions with Leishmania parasites. In *Communications Biology* (Vol. 5, Issue 1). Nature Research. https://doi.org/10.1038/s42003-022-03240-z

Chalghaf, B., Chemkhi, J., Mayala, B., Harrabi, M., Benie, G. B., Michael, E., & Ben Salah, A. (2018). Ecological niche modeling predicting the potential distribution of Leishmania vectors in the Mediterranean basin: impact of climate change. *Parasites & Vectors*, *11*(1), 461. https://doi.org/10.1186/s13071-018-3019-x

Friggeri, L., Hargrove, T. Y., Wawrzak, Z., Guengerich, F. P., & Lepesheva, G. I. (2019). Validation of Human Sterol 14α-Demethylase (CYP51) Druggability: Structure-Guided Design, Synthesis, and Evaluation of Stoichiometric, Functionally Irreversible Inhibitors. *Journal of Medicinal Chemistry*, *62*(22), 10391–10401. https://doi.org/10.1021/acs.jmedchem.9b01485

Gupta, A. K., Das, S., Kamran, M., Ejazi, S. A., & Ali, N. (2022). The pathogenicity and virulence of Leishmania - interplay of virulence factors with host defenses. *Virulence*, *13*(1), 903–935. https://doi.org/10.1080/21505594.2022.2074130

I. Lepesheva, G., & R. Waterman, M. (2011). Sterol 14alpha-Demethylase (CYP51) as a Therapeutic Target for Human Trypanosomiasis and Leishmaniasis. *Current Topics in Medicinal Chemistry*, *11*(16), 2060–2071. https://doi.org/10.2174/156802611796575902

InterPro. (n.d.). *Ethanolamine-phosphate cytidylyltransferase*. Retrieved April 23, 2024, from https://www.ebi.ac.uk/interpro/entry/InterPro/IPR044608/

Kidane, M. E., Vanderloop, B. H., Zhou, W., Thomas, C. D., Ramos, E., Singha, U., Chaudhuri, M., & Nes, W. D. (2017). Sterol methyltransferase a target for anti-amoeba therapy: towards transition state analog and suicide substrate drug design. *Journal of Lipid Research*, *58*(12), 2310–2323. https://doi.org/10.1194/jlr.M079418

Leishmaniasis. (n.d.). Pan American Health Organization. Retrieved February 5, 2024, from https://www.paho.org/en/topics/leishmaniasis#:~:text=Visceral%20leishmaniasis,-Key%20facts&text=Globally%2C%20leishmaniasis%20is%20among%20the,CL%20and%20VL%2C%20are%20endemic.

Leishmaniasis. (2017, December 14). Centers for Disease Control and Prevention. https://www.cdc.gov/dpdx/leishmaniasis/index.html

Lepesheva, G. I., & Waterman, M. R. (2007). Sterol 14α-demethylase cytochrome P450 (CYP51), a P450 in all biological kingdoms. *Biochimica et Biophysica Acta (BBA) - General Subjects*, *1770*(3), 467–477. https://doi.org/10.1016/j.bbagen.2006.07.018

Lindsey, R., & Dahlman, L. (2024, January 18). *Climate change: global temperature*. NOAA Climate.Gov. https://www.climate.gov/news-features/understanding-climate/climate-change-global-temperature

Machado, G. U., Prates, F. V., & Machado, P. R. L. (2019). Disseminated leishmaniasis: clinical, pathogenic, and therapeutic aspects. *Anais Brasileiros de Dermatologia*, *94*(1), 9–16. https://doi.org/10.1590/abd1806-4841.20198775

Mann, S., Frasca, K., Scherrer, S., Henao-Martínez, A. F., Newman, S., Ramanan, P., & Suarez, J. A. (2021). A Review of Leishmaniasis: Current Knowledge and Future Directions. *Current Tropical Medicine Reports*, *8*, 121–132. https://doi.org/10.1007/s40475-021-00232-7/Published

Martínez Salazar, M. B., Delgado Domínguez, J., Silva Estrada, J., González Bonilla, C., & Becker, I. (2014). Vaccination with Leishmania mexicana LPG induces PD-1 in CD8+ and PD-L2 in macrophages thereby suppressing the immune response: A model to assess vaccine efficacy. *Vaccine*, *32*(11), 1259–1265. https://doi.org/10.1016/j.vaccine.2014.01.016

McGwire, B. S., & Satoskar, A. R. (2014). Leishmaniasis: clinical syndromes and treatment. *QJM*, *107*(1), 7–14. https://doi.org/10.1093/qjmed/hct116

Mukherjee, S., Moitra, S., Xu, W., Hernandez, V., & Zhang, K. (2020). Sterol 14-α-demethylase is vital for mitochondrial functions and stress tolerance in Leishmania major. *PLOS Pathogens*, *16*(8), e1008810. https://doi.org/10.1371/journal.ppat.1008810

Mukherjee, S., Xu, W., Hsu, F., Patel, J., Huang, J., & Zhang, K. (2019). Sterol methyltransferase is required for optimal mitochondrial function and virulence in *Leishmania major*. *Molecular Microbiology*, *111*(1), 65–81. https://doi.org/10.1111/mmi.14139

Ngan, V., & Wootton, C. (2017, January). *Leishmaniasis*. DermNet. https://dermnetnz.org/topics/leishmaniasis

Pacheco-Fernandez, T., Markle, H., Verma, C., Huston, R., Gannavaram, S., Nakhasi, H. L., & Satoskar, A. R. (2023). Field-Deployable Treatments For Leishmaniasis: Intrinsic Challenges, Recent Developments and Next Steps. *Research and Reports in Tropical Medicine*, *Volume 14*, 61–85. https://doi.org/10.2147/RRTM.S392606

Parasites - Leishmaniasis. (2023, July 1). Centers for Disease Control and Prevention. https://www.cdc.gov/parasites/leishmaniasis/index.html#:~:text=Leishmaniasis%20is%20a%20parasitic%20disease,bite%20of%20phlebotomine%20sand%20flies.

Phlebotomine sand flies - Factsheet for experts. (2020, July 15). European Centre for Disease Prevention and Control. https://www.ecdc.europa.eu/en/disease-vectors/facts/phlebotomine-sand-flies

Pradhan, S., Schwartz, R. A., Patil, A., Grabbe, S., & Goldust, M. (2022). Treatment options for leishmaniasis. *Clinical and Experimental Dermatology*, *47*(3), 516–521. https://doi.org/10.1111/ced.14919

Pratlong, F., Floeter-Winter, L., Ishikawa, E., El Baidouri, F., Ravel, C., Dedet, J.-P., & Shaw, J. (2015). Characterization of Leishmania (Leishmania) waltoni n.sp. (Kinetoplastida: Trypanosomatidae), the Parasite Responsible for Diffuse Cutaneous Leishmaniasis in the Dominican Republic. *The*

American Journal of Tropical Medicine and Hygiene, *93*(3), 552–558. https://doi.org/10.4269/ajtmh.14-0774

Ueno, N., & Wilson, M. E. (2012). Receptor-mediated phagocytosis of Leishmania: implications for intracellular survival. *Trends in Parasitology*, *28*(8), 335–344. https://doi.org/10.1016/j.pt.2012.05.002

Valigurová, A., & Kolářová, I. (2023). Unrevealing the Mystery of Latent Leishmaniasis: What Cells Can Host Leishmania? In *Pathogens* (Vol. 12, Issue 2). MDPI. https://doi.org/10.3390/pathogens12020246

Vellozo, N. S., Rigoni, T. S., & Lopes, M. F. (2021). New Therapeutic Tools to Shape Monocyte Functional Phenotypes in Leishmaniasis. *Frontiers in Immunology*, *12*. https://doi.org/10.3389/fimmu.2021.704429

World Health Organization. (2023, January 12). *Leishmaniasis*. World Health Organization. https://www.who.int/news-room/fact-sheets/detail/leishmaniasis#:~:text=An%20estimated%20700%20000%20to,will%20eventually%20develop%20the%20disease.

Yao, C. (2019). Leishmania spp. and leishmaniasis on the Caribbean islands. *Transactions of The Royal Society of Tropical Medicine and Hygiene*. https://doi.org/10.1093/trstmh/trz076

Zhang, K. (2021). Balancing de novo synthesis and salvage of lipids by Leishmania amastigotes. In *Current Opinion in Microbiology* (Vol. 63, pp. 98–103). Elsevier Ltd. https://doi.org/10.1016/j.mib.2021.07.004

Zhou, W., Lepesheva, G. I., Waterman, M. R., & Nes, W. D. (2006). Mechanistic Analysis of a Multiple Product Sterol Methyltransferase Implicated in Ergosterol Biosynthesis in Trypanosoma brucei. *Journal of Biological Chemistry*, *281*(10), 6290–6296. https://doi.org/10.1074/jbc.M511749200