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# Nanotechnology-Based Promising Therapeutic Approaches Against Protozoan

Alok Raghav, Suraiya Khanam Ansari, Amresh Kumar Singh, Prashant Tripathi, Saurabh Agarwal, Richa Giri, Syed Ghazanfar Ali, and Haris M. Khan

**Abstract**

Protozoan-borne diseases are major drivers of global morbidities and mortalities, especially in developing countries. These diseases pose a unique challenge to effective treatment strategies, thereby contributing to significant death. Current treatment approaches have low efficacy, high toxicity and side effects of therapeutic doses and therefore generate a need for alternative new treatment

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approaches. Nanotechnology-based drug delivery systems have evolved as a new and promising approach to therapy and prevention of protozoan-associated infectious diseases. The formulation of new drugs and drugs' carriers using nano-sized particles including exosomes, liposomes, solid lipid nanoparticles, dendrimers and nano-vaccines promises to overcome the limitations of low bioavailability, low toxicity, sub-therapeutic drug accumulation in microbial sanctuaries and reservoirs and low patient adherence due to drug-related toxicities and extended therapeutic regimens. Nanotechnology-based therapeutic approaches offer an important weapon in the fight against infectious protozoan diseases.

**Keywords**

Nanoparticles · Therapeutics · Protozoan disease · Nanopharmaceuticals · Exosomes · Liposomes · Nano-vaccines

## 1 Introduction

Protozoan parasites are a diverse group of unicellular eukaryotes affecting animals and humans worldwide. It has been seen that over one billion people are infected by the protozoan parasites around the globe. The most prevalent protozoan diseases in humans include African trypanosomiasis, Chagas disease, leishmaniasis, cryptosporidiosis and giardiasis, which possess health burdens, especially in underdeveloped and developing countries of tropical and subtropical origin. It is known that developing countries showed a significant presence of *Trypanosoma* and *Toxoplasma* protozoans that contribute to mortality, morbidity and economic burden [1–3]. Protozoan presents a vesicular diffuse nucleus with scattered chromatin. The protozoan vesicular nucleus consists of a central body, referred to as the endosome or karyosome. *Amebas* and *trypanosomes* are species that are devoid of such endosomes having nucleic material. However, *Apicomplexa* contains more than one nucleoli having DNA, while ciliates have micro- and macronucleus both with the homogeneous organization. Protozoan organelles perform the same function as the organs of higher animals, and their plasma membrane (PM) helps to perform locomotion functions through pseudopodia, flagella and cilia. In some protozoans including *trypanosomes* and *Giardia*, pellicle plays an important role in providing rigidity and shape.

Protozoan-borne infections were among the leading causes of morbidity and mortality around the world with a significant number of 58 million diarrhoeal cases diagnosed annually [4, 5]. Underreporting of actual protozoan infection cases contributes to misleading information about the actual burden of such cases [6, 7]. Moreover, intestinal protozoan causes a serious threat to human health that may contribute to diarrhoea, iron deficiency, malnutrition, impaired cognitive function and other mental health problems [8]. Some of the common intestinal protozoa showed transmission through the faecal-oral route from the infected person or animals, or it may also be transmitted through ingestion of contaminated food and

**Table 1** General features of protozoan infectious diseases. (Adopted from ref. 10 under Creative Commons Attribution License (CC BY))

| Disease | Some representative aetiological agents | Geographical localization | Clinical features |
|---|---|---|---|
| Malaria | *Plasmodium falciparum*, *P. vivax* | Over 100 countries in the tropic and subtropics | Fever, shivering, cough, respiratory distress, pain in the joints, headache, watery diarrhoea, vomiting, convulsions severe anaemia |
| African trypanosomiasis | *Trypanosoma brucei* | 36 countries in sub-Saharan Africa | Initial haemolytic phase (fever and joint pains followed by neurological disorder, somnolence) |
| Chagas disease | *Trypanosoma cruzi* | From Northern Mexico to south Argentina | Acute phase (fever and splenomegaly)Chronic phase (irreversible damage to the heart, oesophagus and colon) |
| Leishmaniasis | *Leishmania donovani*, *L. major*, *L. mexicana*, *L. braziliensis* | Over 88 countries in tropic and subtropics | Skin ulcers, mucocutaneous complications and visceral diseases (hepatosplenomegaly) |
| Toxoplasmosis | *Toxoplasma gondii* | Worldwide | Blindness and mental retardation can result in congenitally infected childrenImmunosuppressed patients can present more severe symptoms: splenomegaly, polymyositis, dermatomyositis, chorioretinitis, myocarditis, pneumonitis, hepatitis, encephalitis and multisystem organ failure |
| Trichomoniasis | *Trichomonas vaginalis* | Worldwide | Vaginal discharge, odour and oedema or erythema |
| Intestinal protozoan | *Giardia lamblia*, *Entamoeba histolytica*, *Cryptosporidium parvum*, *Cyclospora cayetanensis* | Worldwide | Haematuria, anaemia, impaired growth, renal, hepatic and spleen failure |

water. There are over 200 million people worldwide that are affected by diarrhoea caused by *Giardia intestinalis* [9]. Human faecal samples are usually reservoirs for several pathogenic species including *Blastocystis* spp., *Entamoeba histolytica*, *Giardia*, *Trypanosoma* and *Toxoplasma*. It is estimated that around 40 million people suffered from protozoan-related infection which accounts for 40,000 annual mortalities due to dysentery and liver abscesses [10]. Table 1 demonstrates the general features of some protozoan causing infectious diseases. Protozoan causing

**Table 2** Population affected by major protozoan disease around the world. (Adopted from ref. 10 under Creative Commons Attribution License (CC BY))

| Disease | Population at risk ($\times 10^6$) | Number of people infected ($\times 10^6$) | Number of death ($\times 10^3$) |
|---|---|---|---|
| Malaria | ~2000 | ~300 | ~1000–2000 |
| African trypanosomiasis | ~60 | ~0.3–0.5 | ~50 |
| Chagas disease | ~40 | ~17 | ~21 |
| Leishmaniasis | ~350 | ~2 | ~59 |
| Intestinal protozoans | >1000 | ~450 | ~40–100 |

diarrhoeal complications exhibit zoonotic, waterborne and foodborne transmission routes to humans. Amoebiasis and giardiasis account for an annual human infection of 280 and 50 million cases, respectively, with 2.5 million and 1 million deaths, respectively [11]. Intestinal protozoan infections rank second among the infectious disease-causing morbidities and mortalities after malaria [11]. The prevalence and distribution of the protozoan intestinal species vary from country to country depending on environmental, social and geographical factors [12]. Previously published study showed that conditions like age, host gender, poor hygiene, water, zoonotic contact, seasonal variation and geographical location also majorly contribute to the transmission of protozoan-related infectious diseases [10].

It has been observed that over 77 million dogs and 93 million cats in the US household alone showed the presence of protozoan infestation [13]. Protozoan diseases including Chagas and leishmaniasis are considered to be insidious with transmittance from asymptomatic animals that tend to transmit protozoan diseases. It has been considered that *Giardia duodenalis* and *Toxoplasma gondii* seem to be endemic to the United States with the highest prevalence in household animals [14, 15]. Moreover, *Leishmania* species and *Trypanosoma cruzi* are considered to be the low prevalent species in the United States [16, 17]. This significant reduction in the cases of protozoan infections in the United States is contributed to the good nutritional status of the population, sanitation and good hygiene; however, increasing diabetic patients and other co-morbidities demonstrated a higher risk of developing protozoan-associated infections [18, 19]. Table 2 demonstrated the population affected by major protozoan disease around the world [10].

These protozoan infections in developing countries cause significant morbidity and mortality annually worldwide. These increased cases of protozoan infections in such countries were contributed by several factors including increased immunocompromised patients' numbers, poor sanitation and hygiene and declined nutritional status in the last few decades. In patients with HIV infection, there is an increasing trend of protozoan infection due to immunosuppression. Due to very limited immunotherapies available in underdeveloped and developing countries, such protozoan infection severely affects health economics and poses an economic burden on countries. On the contrary, in the immunosuppressed population in developed nations around the world along with long-term antibiotics therapy, malignancies

showed increased episodes of protozoan-associated diseases [20]. The World Health Organization (WHO) observed that three protozoan-borne diseases have disability-adjusted life years (DALYs) in the millions [21]. WHO is now focused on tropical and subtropical regions of the globe along with temperate regions of North America and Asia Pacific region for the incidences and prevalence of protozoan diseases.

Despite the significant burden of protozoan-related diseases, limited treatment approaches are available for the treatment of protozoan-related diseases. Some of these available regimes exhibit toxicity due to bio-molecular resemblances among eukaryotic parasites and mammalian cells. Moreover, these drugs showed accumulation of their toxic derivate in the cells causing harm to them. A recent study reported the toxicity of anti-Chagas drugs benznidazole and nifurtimox due to enzymatic reduction of their nitro-groups [22]. Some of the other issues related to these drugs include parasitic resistance that develops due to their overdose, increased efflux and augmented drug metabolism [23]. Similar drug resistance mechanisms have been reported with *Leishmania* in which the $Sb^{III}$ (trivalent) reduced from its pentavalent form $Sb^{V}$ in the usage of drugs including miltefosine and amphotericin B [24]. In another classical example, the declining expression of the $Sb^{III}$ transporter AQP1 protein complex generates elevated resistance to antimonials [24]. Furthermore, elevated expression of protein complex ABCG-like transporter TcABCG1 in *T. cruzi* confers resistance to benznidazole [25]. Because of these associated limitations and concerns with the currently available drugs and treatment approaches, it is imperative to look after newer nanotechnology-based therapeutic approaches with possible low toxicity to human cells along with very high sensitivity and specificity against the protozoan parasites.

## 2 Current Treatment Regimes and Their Limitations

Nifurtimox and benznidazole are two commonly available drugs against protozoan infection especially *T. cruzi* infection [26]. However, nifurtimox has some associated limitations including hepatic and renal dysfunction along with neurological and gastrointestinal impairments in some cases. Similarly, benznidazole accounts for hypersensitivity reaction in protozoan-infected persons upon long-term treatment. Besides such associated complications, usage of these drugs induces the resistance that initiates hindrance in the treatment of the disease [27]. The previous clinical trial showed that nifurtimox-eflornithine combination therapy (NECT) produces a promising potential effect against protozoan infection [28]. Some more drug combinations include 2-piperazine-1-ylquinazoline-4-ylamine derivative and lapachol, UR-9825 and triazoles, allopurinol, bisphosphonate, miltefosine, N-methyl-piperazine-urea-F-h Fvinyl-sulfone-phenyl and semicarbazone scaffold, with their corresponding targets, i.e. cruzipain inhibitor, farnesyl pyrophosphate synthase and prenyl and N-myristoyl transferase inhibitors, that showed efficient treatment results in combating protozoan diseases [29, 30].

Numerous broad-spectrum antibiotics such as synthetically derived tetracyclines (doxycycline) have been shown promising anti-malarial effects. This drug is a

partially efficacious causal prophylactic against liver stages of the malaria parasite [31, 32]. It was observed that doxycycline in combination with quinine and quinidine showed an effective treatment approach [33]. Similarly, several antibacterial sulphonamides, sulphadiazine and sulphadoxine, showed an anti-malarial effect in combination therapies to treat *T. gondii* and *P. falciparum* infections [34]. Another sulfone antibiotic dapsone showed promising treatment against leprosy and *P. carinii* pneumonia in AIDS patients [35]. In the year 1990, the Food and Drug Administration (FDA) approved the drug named eflornithine (Ornidyl®) for the treatment of African sleeping sickness [36]. Another well-known drug used for the treatment of *Plasmodium falciparum* and *P. vivax* malaria includes primaquine (an aminoquinoline) that showed efficacy [37]. It was observed that in 2–4% of patients, some side effects upon using primaquine were observed showing the presentation of headache, epigastric pain, nausea/vomiting, dizziness, anorexia, chromaturia and black urine (a possible symptom of haemolytic anaemia) [37]. The study also showed that 0.1% of treated patients showed black urine with a higher prevalence of an enzyme glucose-6-phosphate dehydrogenase deficiency [37].

Moreover, consumption of quinacrine showed severe gastrointestinal distress within the first 2 months along with significantly elevated liver function test values [38]. A randomized, double-blind, parallel, placebo-controlled trial of thiamine hydrochloride in *P. falciparum* malaria patients showed some presentation of diarrhoea and dizziness [39]. In another multicentred, double-blinded, non-inferiority clinical trial, combination of trimethoprim-sulfamethoxazole to trimethoprim-sulfamethoxazole plus doxycycline was given to the patients, and the trial reported that out of the total patients, three have reported the adverse effect of combination therapy [40].

Despite recent advances in pharmacological interventions, several drugs and antibiotics possess significant efficacy against protozoan. Moreover, many have associated side effects on human health along with drug and antibiotic resistance problems, which is a matter of big concern. With the advancement in nanotechnology and nanomedicine, these associated side effects can be overcome by nanocarriers that have shown promising results in combating the toxicity of drugs and resistance to antibiotics [41]. Table 3 demonstrated some limitations associated with current drug usage against protozoan infection. Many recent studies demonstrated the application of nanocarriers and nano-therapeutics for better management and efficient treatment ways to tackle infection and other diseases [42, 43]. The current chapter will provide the current advancement in the nanotechnology field for the development of nano-therapeutics in combating protozoan disease.

**Table 3** Limitations associated with current anti-protozoan disease drugs. (Adopted from ref. 10 under Creative Commons Attribution License (CC BY))

| Disease | Some currently used drugs | Limitations |
|---|---|---|
| Malaria | Chloroquine, 1945 | Resistance |
| | Artemisinin, 1994 | Compliance, cost, manufacture |
| African trypanosomiasis | Suramin, 1920 | Safety, not effective in late-stage diseases, injectable |
| | Pentamidine, 1939 | Safety, resistance, not effective in late-stage diseases, injectable |
| | Melarsoprol, 1949 | Safety, resistance, injectable |
| | Eflornithine, 1991 | Cost, injectable, only effective against *T. gambiense* |
| Chagas disease | Nifurtimox, 1970 | Safety, long treatment, compliance, activity limited to acute stage of disease |
| | Benznidazole, 1974 | Safety |
| Leishmaniasis | Pentamidine, 1939 | Safety, resistance, injectable |
| | Antimonials, 1950 | Safety, resistance, injectable |
| | Liposomal amphotericin B, 1990 | Cost, injectable |
| | Miltefosine, 2002 | Contraindicated in pregnancy |
| Toxoplasmosis | Sulphonamides, 1932 | Safety, only in combined therapy |
| | Pyrimethamine, 1951 | Safety, contraindicated in pregnancy |
| Trichomoniasis | Metronidazole, 1955 | Resistance |
| Intestinal protozoan | Metronidazole, 1955 | Resistance |
| | Diloxanide, 1956 | Resistance |

## 3 Nanotechnology-Based Promising Therapeutic Approaches for Combating Protozoan-Associated Diseases

The current advancement in the field of nanosciences and nanotechnology has opened up new horizons for effective and efficient treatment approaches against protozoan-associated diseases. Nanopharmaceutical agents of several physio-chemical characteristics contribute to enhancing the effectiveness of such molecules to be used in the treatment of protozoan disease. Nanocarriers belong to the colloidal drug carrier organization with a size range of $<$500 nm [44]. In the past few decades, nanocarriers for therapeutics as drug delivery were investigated well by several authors, due to their high surface area to volume ratio and potential to amend the basic structure, function, properties and bioactivity of drugs. Nanocarriers as drug delivery systems become a preferable choice nowadays due to their amended pharmacokinetics, negligible toxicities, significant biodistribution, controlled release and target-specific delivery [45, 46]. It was reported for these nanocarriers that their physicochemical characteristics including size, composition, shape, surface

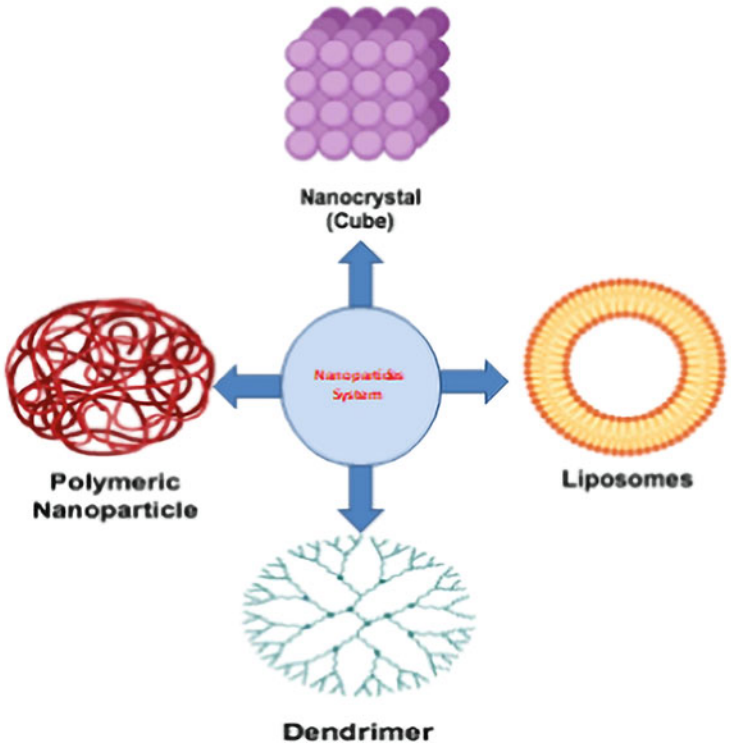


**Fig. 1** Structures showing representation of nanoparticle system

moieties, functional moieties and other targeting moieties can be tuned for increasing their effectiveness [47]. Figure 1 showed structures showing representation of nanoparticle system.

## 3.1 Nanopharmaceuticals

Nanopharmaceuticals including nanocarriers, nano-rods, quantum dots and nano-emulsions play an important role in the delivery of the desired and targeted drug against protozoan-borne disease. Structural and physical characteristics of the nanocarriers contribute to controlling their functions and targeting protozoan diseases effectively [48]. Particle dimension, morphology, net surface charge, mechanisms and patterns of biodistribution determine the performances and effectiveness of the nanomaterials [49]. For effective treatment against protozoan disease, the nanoparticles possessing drugs must exhibit high distribution volume and prolonged circulation time within the body [50].

A previously published paper showed that anionic nanoparticles exhibit a long circulation time in the body compared to cationic particles [51]. On the contrary,

**Table 4** List of pre-clinical studies that implicated the use of nanoparticle-based therapeutic approach against Chagas disease. (Adopted from ref. 29 under Creative Commons Attribution License (CC BY))

| No. | Nanomaterial | Composition | Active agent | Preparation method | Size (nm) |
|---|---|---|---|---|---|
| 1. | Liposomes | pH-sensitive liposomes | ETZ | Extrusion | 379 |
| | | | Amphotericin B | Not indicated | NI |
| | | | Stearylamine | Hydration | |
| 2. | Polymeric nanoparticles | (a) Poly-ε-caprolactone | Ursolic acid | Nanoprecipitation | 172.2 |
| | | (b) PLA-PEG | Bis-triazole D0870 | Simple emulsification | 100–200 |
| | | (c) NC-PCL-PLA-PEG | LYC | Nanoprecipitation | 105.3 |
| | | (d) PCL-PLA-PEG | LYC | Nanoprecipitation | 100–250 |
| | | (e) RSNO | Nitric oxide | Ionotropic gelation | 270–500 |
| | | (f) SEDDSs | RAV | Self-emulsifying | 100–250 |
| | | (g) PACA | Nifurtimox | Emulsion polymerization | ≤200 |
| | | | Allopurinol | | |
| | | (h) Multiparticulate benznidazole polymers | BNZ | Nanoprecipitation and freeze-drying | 233 |
| 3. | Solid lipid nanoparticles | $H_2$bdtc-SLNs | S-Benzyldithiocarbazate | High-pressure homogenization and microemulsion | 127.4 |
| 4. | Mesoporous silica nanoparticles | Mesoporous silica nanoparticle and chitosan coating | BNZ | Hydration | 3.3 |
| 5. | Nano-emulsions | Sulphonamides | Clove oil | Emulsification | 35–100 |
| | | | Ursolic acid | | 57.3 |
| | | | BNZ | | 241.6 |
| 6. | Quantum dots | – | CdTe | Colloidal chemistry | NI |

some studies observed that neutral nanoparticles or nanoparticles with partially negative charges showed long circulation time in the body due to negative charges on the cell membranes [52, 53]. Similarly, nanoparticles with a size range up to 200 nm showed prolonged circulation time and an efficient approach for the treatment of diseases. Numerous studies quoted the utility of nanoparticles and their modifications in the structure and function demonstrated a promising approach against trypanosome disease with enhanced efficacy with the carrier drugs [48, 54]. Table 4 showed the list of pre-clinical studies that implicated the use of nanoparticle-based therapeutics approach against Chagas disease [29].

## 3.2 Exosomes

Extracellular vesicles or exosomes are secreted, naturally occurring nanoparticles having endosomal origin that are released by several cell types within the size range of 30–150 nm having cup-shaped morphology usually [55]. These exosomes contain multiple biological materials inside it including proteins (heat shock proteins, cell adhesion proteins, tetraspanin membrane proteins, cell signalling proteins, transcription proteins and trafficking membrane fusion proteins), nucleic acids (non-coding RNAs such as micro-RNA, small nuclear RNA, small nucleolar RNA, long non-coding RNA, PIWI-interacting RNA, rRNA and tRNA) and lipids (phosphatidylserine (PS), phosphatidic acid, cholesterol, sphingomyelin (SM), arachidonic acid and other fatty acids, prostaglandins and leukotrienes) [56].

Exosomes play an important role in the protection against several protozoan diseases. A previously published pre-clinical study showed that the production of immunoglobulin G (IgG) antibodies and the recognition of *P. yoelii*-infected RBCs in response to vaccination along with secreted exosomes significantly reduce the parasitic load and are also helpful in increased survival of reticulocytosis [57]. In another related study, the authors demonstrated the protective effect of dendritic cell (DC)-derived exosomes against *Leishmania* infection [58]. Aline and their coworkers revealed the protective effect of exosomes derived from DCs against *T. gondii* infection due to activation of protective humoral immune response and JNK signalling against the pathogen [59]. Moreover, proteases' component present in the exosomes could be promising targets to control sleeping sickness [60].

## 3.3 Liposomes

Liposomes are lipid bilayer spherical, closed entities having phospholipids within them in the size range of 5–200 nm with almost 4 mm thickness [61]. Liposomes comprise amphiphilic phospholipids having a hydrophobic tail and hydrophilic head with self-sealing characteristics in an aqueous medium. A recent study quoted several studies published in the past on the delivery of antibiotics, drugs, genes, antifungal agents, anti-inflammatory agents and anti-tumour agents using liposomes [62]. A previous study suggests that drug encapsulation within the liposomes alters

its pharmacokinetics and efficacy along with attenuation of toxic effects [63]. A diverse range of cargo including ribosomes, proteins, antibodies, drugs, adjuvants and nucleic acids can be transported through liposomes for therapeutics purposes [64]. In one study, the authors fabricated liposomes loaded with benznidazole (BNZ) and observed its significant accumulation within the mice liver but also observed that there was no significant clearance of parasitic load levels in *T. cruzi*-infected mice [65]. Liposome showed several advantages over the convention drug delivery approach which are but are not limited to increased drug delivery, protection of the desired drug of delivery with harsh environmental factors, avoiding early degradation of encapsulated drugs and reduced systemic toxicity along with efficient treatment strategy.

## 3.4 Solid Lipid Nanoparticles (SLNs)

SLNs are a class of nanoparticles that are comprised of a solid and a liquid lipid mixture within which the lipid moieties showed a solid state at room and body temperature. This system is comparable to more stable and also provides controlled release of several drugs. SLNs showed fair biocompatibility, stability, efficiency in drug encapsulation, biodegradability, raised bioavailability and controlled release within the scope of large-scale production [66–68]. A previously published study showed protection against Chagas disease using 5-hydroxy-3-methyl-5-phenyl-pyrazoline-1-(S-benzyl dithiocarbazate) (H2bdtc) system of class S-dithiocarbazates, although with low solubility [69]. In continuation with this concept, another study fabricated H2bdtc-loaded SLNs that showed efficiency reduction in parasitemia at 100× lower concentration with benznidazole [70]. H2bdtc-SLN system overcomes the lower solubility issues that arouse in an aqueous medium, so increased accessibility against the protozoan parasites is there. Homogeneous mixing of drugs with lipid matrix is also possible using the SLN system as a therapeutic approach. Moreover, rapid elimination of SLNs by reticule endothelial cells, encapsulation of hydrophilic and ionic drugs and also controlling the rate of release of drugs from the SLNs are some of the limitations that restrain them from showing the effectiveness of these nanocarriers against protozoan diseases. SLNs are a nanocarrier system that offers several advantages over the classical system of nano-emulsion, liposomes and other polymeric nanoparticles.

## 3.5 Dendrimers

Dendrimers are designated for specially designed macromolecules with their arms originating from the central core [71]. Their existence came from naturally occurring components including sugars, amino acids and nucleic acids. Their sequential method of synthesis helps us to adjust the molecules with desired branching pattern, desired length and desired molecular weight having peripheral groups. Dendrimers allow drug delivery systems with variable shapes, branching, molecular weight and

dispersion having an average diameter of 1.5–14.5 nm [72]. A dendrimer molecule consists of the branched layer having reiterating units with numerous active terminal ends and an initiator core. Flexibility on the dendrimer design offers desired shape, size, branching, length and surface functionality. Drugs can be loaded into the cavities in these dendrimers via hydrogen bonds, hydrophobic interactions and chemical linkages at every generation to form dendrimer-drug conjugates. A previously published study quoted these dendrimers have been extensively used in the field of biomedicine, magnetic resonance imaging (MRI), vaccines, anticancer drugs and antiviral applications [73].

### 3.6 Polymeric Nanoparticles

Polymeric nanoparticles (PNPs) are solid colloidal particles that can be encapsulated and adsorbed onto the constituent polymer matrix. Previous studies reported increased bioavailability using these nanocarrier PNPs [74, 75]. There are several natural and synthetic polymers that can be used to fabricate PNPs including poly (lactide-co-glycolide) (PLGA), polylactide (PLA), polyglycolide (PGA), polycaprolactone (PCL), poly(D,L-lactide), chitosan and PLGA-polyethylene glycol (PEG) [76]. Authors have reported that these PNP polymers have fair biodegradability and biocompatibility that have been approved by the US Food and Drug Administration (FDA) [77]. In another study, authors have performed in vitro studies using these polymeric poly(alkyl cyanoacrylate) nanoparticles loaded with nifurtimox that showed efficient anti-epimastigote activity [78]. Another study reported the role of these PNPs against trypanosomal infection [79]. Authors have previously reported that a self-emulsifying drug delivery system showed effective drug delivery against *T. cruzi* using epimastigote and amastigote [80]. Lychnopholide (LYC)-loaded poly-ε-caprolactone (PCL) and poly(lactic acid)-polyethylene glycol (PLA-PEG) polymer-fabricated nanoparticles showed an effective protective effect against trypanosomal infection [81]. It can be evident from several studies that polymeric nanoparticles can be used as effective drug delivery agents for treating protozoan disease.

### 3.7 Quantum Dots

Quantum dots (QDs) are nanoscale semiconducting entities that transport electrons when illuminated with UV light so that they can emit light of various colours making them a potential candidate for drug delivery and diagnosis [48]. In one of the previously published studies, authors have demonstrated the side effects of using these QDs both in vitro and in vivo and also reported potential human health risks [82]. Vieira and their coworkers used cadmium telluride QDs against *T. cruzi* epimastigotes and found that its high dose is very effective against *T. cruzi* growth mediated through its DNA damage and blister formation in plasma membranes along with mitochondrial swelling [83]. A previously published study showed that QDs

are more photostable than fluorescein isothiocyanate for *Cryptosporidium parvum* [84]. Tokumasu and their coworkers used QDs against *Plasmodium falciparum* infection that attenuates innate host defence [85]. QDs can also be useful in the treatment of protozoan infection effectively by combating associated limitations of conventional drug delivery approaches.

### 3.8 Nano-vaccines

A wide range of nano-vaccines against protozoan diseases has been tested in pre-clinical studies which exploit whole parasites or purified recombinant proteins into the viral vectors forming vaccines. In one of the recently published studies, the authors have subcloned the two *T. cruzi* antigens TcG2 and TcG4 and evaluated the nano-vaccine for prophylactic protection against repeat *T. cruzi* infections in the C57BL/6 mice model [86]. Authors have reported that nano-vaccine is significantly eliciting early activation and generation of IFN-γ by $CD4^+$ T effector/effector memory ($T_{EM}$) cells along with expression of cytolytic perforin (PFN) and granzyme B (GZB) molecules by $CD4^+$ and $CD8^+$ $T_{EM}$ subsets demonstrating parasitic control at 10 days and offers protective T cell immunity against repeat *T. cruzi* infections [86].

Another study used the Tc24 protein present in the flagella of *T. cruzi* and used this protein as a potent antigen target to protect the host against *T. cruzi* infection [87]. The same study used PLGA nanoparticle-based vaccine for protecting against Chagas disease [87]. A previous study used influenza virosome-based vaccine containing blood-stage *Plasmodium falciparum* cysteine-rich protective antigen (PfCyRPA) on their surface against malaria [88].

## 4 Conclusion

The nanotechnological advancement for drug delivery and vaccine development using nanoparticle-based approaches offers a very potent treatment method against protozoan disease. This study focused on the evidence collected over the use of nanotechnology-based strategies for delivering desired drugs with enhanced efficiency, reduced toxicity and increased biocompatibility and bioavailability. Nanotechnology nowadays offers several advantages over conventional drug delivery methods with some limitations of an economic burden especially for developing and underdeveloped nations. More pre-clinical and clinical studies are required to overcome such limitations and make these interventions into the main streamline the society. The current situation needs joint efforts of governmental organizations, private agencies and the pharmaceutical sector to develop and implement nanomedical strategies for efficient anti-protozoan treatment.

## References


1. Creek DJ, Barret MP. Determination of antiprotozoal drug mechanisms by metabolomics approaches. Parasitology. 2014;141:83–92.
2. Adeyemi OS, Sulaiman FA. Biochemical and morphological changes in Trypanosoma brucei brucei-infected rats treated with homidium chloride and diminazene aceturate. J Basic Clin Physiol Pharmacol. 2012;23:179–83.
3. Adeyemi OS, Murata Y, Sugi T, et al. Inorganic nanoparticles caused death of Toxoplasma gondii through alteration of redox status and mitochondrial membrane potential. Int J Nanomed. 2017;12:1647–61.
4. Thompson RCA, Ash A. Molecular epidemiology of Giardia and Cryptosporidium infections. Infect Genet Evol. 2016;40:315–23.
5. Putignani L, Menichella D. Global distribution, public health and clinical impact of the protozoan pathogen cryptosporidium. Interdiscip Perspect Infect Dis. 2010;2010:753512. https://doi.org/10.1155/2010/753512.
6. Kosek M, Bern C, Guerrant RL. The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. Bull World Health Organ. 2003;81:197–204.
7. WHO. Prevention and control of schistosomiasis and soil-transmitted helminthiasis: first report of the joint WHO expert committees. WHO Tech Rep Ser. 2002;912:1–57.
8. Checkley W, Buckley G, Gilman RH, et al. Multi-country analysis of the effects of diarrhoea on childhood stunting. Int J Epidemiol. 2008;37:816–30.
9. Groudan K, Gupta K, Chalhoub J, et al. Diagnosed incidentally by duodenal biopsy. J Investig Med High Impact Case Rep. 2021;9:23247096211001649. https://doi.org/10.1177/23247096211001649.
10. Monzote L, Siddiq A. Drug development to protozoan diseases. Open Med Chem J. 2011;5:1–3. https://doi.org/10.2174/1874104501105010001.
11. Berhe B, Mardu F, Tesfay K, et al. More than half prevalence of protozoan parasitic infections among diarrheic outpatients in Eastern Tigrai, Ethiopia, 2019; a cross-sectional study. Infect Drug Resist. 2020;13:27–34. https://doi.org/10.2147/IDR.S238493.
12. Raso G, Utzinger J, Silué KD, et al. Disparities in parasitic infections, perceived ill health and access to health care among poorer and less poor schoolchildren of rural Côte d'Ivoire. Tropical Med Int Health. 2005;10:42–57. https://doi.org/10.1111/j.1365-3156.2004.01352.x.
13. APPA. APPA national pet owners survey 2011–2012. Washington, DC: APPA; 2012. http://www.americanpetproducts.org/press_industrytrends.asp. (Accessed on 11 April 2022)
14. Ballweber LR, Xiao L, Bowman DD, et al. Giardiasis in dogs and cats: update on epidemiology and public health significance. Trends Parasitol. 2010;26:180–9.
15. Jones JL, Dargelas V, Roberts J, et al. Risk factors for toxoplasma gondii infection in the United States. Clin Infect Dis. 2009;49:878–84.
16. Barr SC. Canine chagas' disease (American trypanosomiasis) in North America. Vet Clin North Am Small Anim Pract. 2009;39:1055–64.
17. Petersen CA. Leishmaniasis, an emerging disease found in companion animals in the United States. Top Companion Anim Med. 2009;24:182–8.
18. Chuang YM, Ho YC, Chang HT, et al. Disseminated cryptococcosis in HIV-uninfected patients. Eur J Clin Microbiol. 2008;27:307–10.
19. Cribier BJ, Bakshi R. Terbinafine in the treatment of onychomycosis: a review of its efficacy in high-risk populations and in patients with nondermatophyte infections. Br J Dermatol. 2004;150:414–20.
20. Cox FE. History of human parasitology. Clin Microbiol Rev. 2002;15(4):595–612. https://doi.org/10.1128/CMR.15.4.595-612.2002. Erratum in: Clin Microbiol Rev. 2003 Jan;16(1):174
21. Andrews KT, Fisher G, Skinner-Adams TS. Drug repurposing and human parasitic protozoan diseases. Int J Parasitol Drugs Drug Resist. 2014;4(2):95–111. https://doi.org/10.1016/j.ijpddr.2014.02.002.

22. Bermudez J, Davies C, Simonazzi A, et al. Current drug therapy and pharmaceutical challenges for Chagas disease. Acta Trop. 2016;156:1–16. https://doi.org/10.1016/j.actatropica.2015.12.017.
23. Yasinzai M, Khan M, Nadhman, et al. Drug resistance in leishmaniasis: current drug-delivery systems and future perspectives. Future Med Chem. 2013;5:1877–88. https://doi.org/10.4155/fmc.13.143.
24. Ponte-Sucre A, Gamarro F, Dujardin, et al. Drug resistance and treatment failure in leishmaniasis: a 21st century challenge. PLoS Negl Trop Dis. 2017;11:e0006052. https://doi.org/10.1371/journal.pntd.0006052.
25. Zingales B, Araujo RG, Moreno, et al. A novel ABCG-like transporter of *Trypanosoma cruzi* is involved in natural resistance to benznidazole. Mem Inst Oswaldo Cruz. 2015;110:433–44. https://doi.org/10.1590/0074-02760140407.
26. Gurtler RE. Combining residual insecticide spraying campaigns with targeted detection and specific chemotherapy for Trypanosoma cruzi infection in children. PLoS Negl Trop Dis. 2007;1:e168. https://doi.org/10.1371/journal.pntd.0000168.
27. Campos MC, Leon LL, Taylor, et al. Benznidazole-resistance in Trypanosoma cruzi: evidence that distinct mechanisms can act in concert. Mol Biochem Parasitol. 2014;193:17–9. https://doi.org/10.1016/j.molbiopara.2014.01.002.
28. Kansiime F, Adibaku S, Wamboga, et al. A multicentre, randomised, non-inferiority clinical trial comparing a nifurtimox-eflornithine combination to standard eflornithine monotherapy for late stage Trypanosoma brucei gambiense human African trypanosomiasis in Uganda. Parasit Vectors. 2018;11:105.
29. Choudhury SD. Nano-medicines a hope for chagas disease! Front Mol Biosci. 2021;8:655435. https://doi.org/10.3389/fmolb.2021.655435.
30. Dupouy-Camet J. Les antiprotozoaires: des médicaments orphelins en médecine humaine? [new drugs for the treatment of human parasitic protozoa]. Parassitologia. 2004;46(1–2):81–4. French
31. Clyde DF, Miller RM, DuPont HL, et al. Antimalarial effects of tetracyclines in man. J Trop Med Hyg. 1971;74(11):238–42.
32. Rieckmann KH, Powell RD, McNamara JV, et al. Effects of tetracycline against chloroquine-resistant and chloroquine-sensitive Plasmodium falciparum. Am J Trop Med Hyg. 1971;20(6):811–5.
33. Tan KR, Magill AJ, Parise ME, Arguin PM. Doxycycline for malaria chemoprophylaxis and treatment: report from the CDC expert meeting on malaria chemoprophylaxis. Am J Trop Med Hyg. 2011;84(4):517.
34. Guerina NG, Hsu HW, Meissner HC, et al. Neonatal serologic screening and early treatment for congenital Toxoplasma gondii infection. N Engl J Med. 1994;330(26):1858–63.
35. Wolf R, Tuzun B, Tuzun Y. Dapsone: unapproved uses or indications. Clin Dermatol. 2000 Jan 1;18(1):37–53.
36. Kennedy PG. The continuing problem of human African trypanosomiasis (sleeping sickness). Ann Neurol. 2008;64(2):116–26.
37. Thurston S, Hite GL, Petry AN, Ray SD. Antiprotozoal Drugs. Side Eff Drugs Annu. 2015;37:321–7. https://doi.org/10.1016/bs.seda.2015.08.008.
38. Geschwind MD, Kuo AL, Wong KS, et al. Quinacrine treatment trial for sporadic Creutzfeldt-Jakob disease. Neurology. 2013;81(23):2015–23. https://doi.org/10.1212/WNL.0b013e3182a9f3b4.
39. Mayxay M, Khanthavong M, Cox L, et al. Thiamin supplementation does not reduce the frequency of adverse events after anti-malarial therapy among patients with falciparum malaria in southern Laos. Malar J. 2014;13:275. https://doi.org/10.1186/1475-2875-13-275.
40. Chetchotisakd P, Chierakul W, Chaowagul W, et al. Trimethoprim-sulfamethoxazole versus trimethoprim-sulfamethoxazole plus doxycycline as oral eradicative treatment for melioidosis (MERTH): a multicentre, double-blind, non-inferiority, randomised controlled trial. Lancet. 2014;383(9919):807–14. https://doi.org/10.1016/S0140-6736(13)61951-0.

41. Scire J, Hozé N, Uecker H. Aggressive or moderate drug therapy for infectious diseases? Trade-offs between different treatment goals at the individual and population levels. PLoS Comput Biol. 2019;15(8):e1007223. https://doi.org/10.1371/journal.pcbi.1007223.
42. Hauck TS, Giri S, Gao Y, Chan WC. Nanotechnology diagnostics for infectious diseases prevalent in developing countries. Adv Drug Deliv Rev. 2010;62:438–48. https://doi.org/10.1016/j.addr.2009.11.015.
43. Barratt GM. Therapeutic applications of colloidal drug carriers. Pharm Sci Technol Today. 2000;3:163–71. https://doi.org/10.1016/s1461-5347(00)00255-8.
44. Neubert RH. Potentials of new nanocarriers for dermal and transdermal drug delivery. Eur J Pharm Biopharm. 2011;77(1):1–2. https://doi.org/10.1016/j.ejpb.2010.11.003.
45. Mishra B, Patel BB, Tiwari S. Colloidal nanocarriers: a review on formulation technology, types and applications toward targeted drug delivery. Nanomedicine. 2010;1:9–24. https://doi.org/10.1016/j.nano.2009.04.008.
46. How CW, Rasedee A, Manickam S, et al. Tamoxifen-loaded nanostructured lipid carrier as a drug delivery system: characterization, stability assessment and cytotoxicity. Colloids Surf B Biointerfaces. 2013;112:393–9. https://doi.org/10.1016/j.colsurfb.2013.08.009.
47. Sun T, Zhang YS, Pang B, et al. Engineered nanoparticles for drug delivery in cancer therapy. Angew Chem Int Ed Engl. 2014;53(46):12320–64. https://doi.org/10.1002/anie.201403036.
48. Quijia Quezada C, Azevedo CS, Charneau, et al. Advances in nanocarriers as drug delivery systems in Chagas disease. Int J Nanomedicine. 2019;14:6407–24.
49. Nel AE, Madler L, Velegol D, et al. Understanding biophysicochemical interactions at the nano-bio interface. Nat Mater. 2009;8:543–57. https://doi.org/10.1038/nmat2442.
50. Morilla MJ, Romero EL. Nanomedicines against Chagas disease: an update on therapeutics, prophylaxis and diagnosis. Nanomedicine. 2015;10:465–81. https://doi.org/10.2217/nnm.14.185.
51. Arvizo RR, Miranda OR, Moyano, et al. Modulating pharmacokinetics, tumor uptake and biodistribution by engineered nanoparticles. PLoS One. 2011;6:e24374. https://doi.org/10.1371/journal.pone.0024374.
52. Gratton SE, Ropp PA, Pohlhaus, et al. The effect of particle design on cellular internalization pathways. Proc Natl Acad Sci U S A. 2008;105:11613–8. https://doi.org/10.1073/pnas.0801763105.
53. Osaka T, Nakanishi T, Shanmugam S, et al. Effect of surface charge of magnetite nanoparticles on their internalization into breast cancer and umbilical vein endothelial cells. Coll Surf B Biointerfaces. 2009;71:325–30. https://doi.org/10.1016/j.colsurfb.2009.03.004.
54. Ferreira LG, Oliveira D, et al. Advances and progress in chagas disease drug discovery. Curr Top Med Chem. 2016;16:2290–302. https://doi.org/10.2174/1568026616666160413124902.
55. Raghav A, Jeong GB. A systematic review on the modifications of extracellular vesicles: a revolutionized tool of nano-biotechnology. J Nanobiotechnol. 2021;19:459. https://doi.org/10.1186/s12951-021-01219-2.
56. Raghav A, Khan ZA, Upadhayay VK. Mesenchymal stem cell-derived exosomes exhibit promising potential for treating SARS-CoV-2-infected patients. Cell. 2021;10(3):587. https://doi.org/10.3390/cells10030587.
57. Martin-Jaular L, Nakayasu ES, Ferrer M. Exosomes from Plasmodium yoelii-infected reticulocytes protect mice from lethal infections. PLoS One. 2011;6:e26588.
58. Schnitzer JK, Berzel S, Fajardo-Moser M, et al. Fragments of antigen-loaded dendritic cells (DC) and DC-derived exosomes induce protective immunity against Leishmania major. Vaccine. 2010;28:5785–93.
59. Beauvillain C, Ruiz S, Guiton R, et al. A vaccine based on exosomes secreted by a dendritic cell line confers protection against T. gondii infection in syngeneic and allogeneic mice. Microbes Infect. 2007;9:1614–22.
60. Geiger A, Hirtz C, Becue T, et al. Exocytosis and protein secretion in Trypanosoma. BMC Microbiol. 2010;10:20.

61. Harendra S, Vipulanandan C. Production and characterization of liposome systems for pharmaceutical applications, vipulanandan center for innovative grouting material and technology (CIGMAT). Houston: Department of Civil and Environmental Engineering University of Houston; 2006. p. 77202–4003.
62. Andra VVSN, Pammi, et al. A comprehensive review on novel liposomal methodologies, commercial formulations, clinical trials and patents. BioNanoSci. 2022;12:274–91. https://doi.org/10.1007/s12668-022-00941-x.
63. Abu Lila AS, Ishida T. Liposomal delivery systems: design optimization and current applications. Biol Pharm Bull. 2017;40:1–10. https://doi.org/10.1248/bpb.b16-00624.
64. Bulbake U, Doppalapudi S, Kommineni, et al. Liposomal formulations in clinical use: an updated review. Pharmaceutics. 2017;9:12. https://doi.org/10.3390/pharmaceutics9020012.
65. Alba Soto CD, Mirkin GA, Solana, et al. Trypanosoma cruzi infection modulates in vivo expression of major histocompatibility complex class II molecules on antigen-presenting cells and T-cell stimulatory activity of dendritic cells in a strain-dependent manner. Infect Immun. 2003;71:1194–9. https://doi.org/10.1128/iai.71.3.1194-1199.2003.
66. Mehnert W, Mäder K. Solid lipid nanoparticles: production, characterization and applications. Adv Drug Deliv Rev. 2012;64(2–3):83–101. https://doi.org/10.1016/j.addr.2012.09.021.
67. Müller RH, MaÈder K, Gohla S. Solid lipid nanoparticles (SLN) for controlled drug delivery–a review of the state of the art. Eur J Pharm Biopharm. 2000;50(1):161–77.
68. Tomiotto-Pellissier F, Miranda-Sapla MM, Machado LF, et al. Nanotechnology as a potential therapeutic alternative for schistosomiasis. Acta Trop. 2017;2017(174):64–71. https://doi.org/10.1016/j.actatropica.
69. Muller RH. Solid lipid nanoparticles (SLN)-an alternative colloidal carrier system for controlled drug delivery. Eur J Pharm Biopharm. 1995;41(1):62–9.
70. Carneiro ZA, Maia PID, Sesti-Costa R, et al. In vitro and in vivo trypanocidal activity of H(2)bdtc-loaded solid lipid nanoparticles. PLoS Negl Trop Dis. 2014;8(5):e2847. https://doi.org/10.1371/journal.pntd.0002847.
71. Kresge C, Leonowicz M, Roth W. Dendrimers and dendrons. Concepts, syntheses, applications. Weinheim: VCH; 2001.
72. Basu S, Sandanaraj BS, Thayumanavan S. Molecular recognition in dendrimers. In: Mark HF, editor. Encyclopedia of polymer science and technology. 4th ed. John Wiley & Sons, Inc; 2004.
73. Stiriba SE, Frey H, Haag R. Dendritic polymers in biomedical applications: from potential to clinical use in diagnostics and therapy. Angew Chem Int Ed. 2002;41(8):1329–34.
74. Gonzalez-Martin G, Figueroa C, Merino I, Osuna A. Allopurinol encapsulated in polycyanoacrylate nanoparticles as potential lysosomatropic carrier: preparation and trypanocidal activity. Eur J Pharm Biopharm. 2000;49:137–42. https://doi.org/10.1016/s0939-6411(99)00076-4.
75. Morgen M, Bloom C, Beyerinck R, et al. Polymeric nanoparticles for increased oral bioavailability and rapid absorption using celecoxib as a model of a low-solubility, high-permeability drug. Pharm Res. 2012;29:427–40. https://doi.org/10.1007/s11095-011-0558-7.
76. Prabhu RH, Patravale VB, Joshi MD. Polymeric nanoparticles for targeted treatment in oncology: current insights. Int J Nanomedicine. 2015;10:1001–18. https://doi.org/10.2147/ijn.s56932.
77. Zhao K, Li D, Shi C, et al. Biodegradable polymeric nanoparticles as the delivery carrier for drug. Curr Drug Deliv. 2016;13:494–9.
78. Muller RH, Lherm C, Herbort J, Couvreur P. In vitro model for the degradation of alkylcyanoacrylate nanoparticles. Biomaterials. 1990;11:590–5. https://doi.org/10.1016/0142-9612(90)90084-4.
79. Sposito PA, Mazzeti AL, Faria DO, et al. Ravuconazole self-emulsifying delivery system: in vitro activity against Trypanosoma cruzi amastigotes and in vivo toxicity. Int J Nanomedicine. 2017;12:3785–99.

80. Kohli K, Chopra S, Dhar D, et al. Self-emulsifying drug delivery systems: an approach to enhance oral bioavailability. Drug Discov Today. 2010;15:958–65. https://doi.org/10.1016/j.drudis.2010.08.007.
81. Branquinho RT, Mosqueira VC, Oliveira-Silva D, et al. Sesquiterpene lactone in nanostructured parenteral dosage form is efficacious in experimental Chagas disease. Antimicrob Agents Chemother. 2014;58:2067–75. https://doi.org/10.1128/aac.00617-13.
82. Hardman R. A toxicologic review of quantum dots: toxicity depends on physicochemical and environmental factors. Environ Health Perspect. 2006;114:165–72. https://doi.org/10.1289/ehp.8284.
83. Vieira CS, Almeida DB, Thomaz D, et al. Studying nanotoxic effects of CdTe quantum dots in Trypanosoma cruzi. Mem Inst Oswaldo Cruz. 2011;106:158–65.
84. Lee LY, Ong SL, Hu JY, et al. Use of semiconductor quantum dots for photostable immunofluorescence labeling of *Cryptosporidium parvum*. Appl Environ Microbiol. 2004;70:5732–6.
85. Tokumasu F, Fairhurst RM, Ostera GR, et al. Band 3 modifications in *Plasmodium falciparum*—infected AA and CC erythrocytes assayed by autocorrelation analysis using quantum dots. J Cell Sci. 2005;118:1091–8.
86. Chowdhury IH, Lokugamage N, Garg NJ. Experimental nanovaccine offers protection against repeat exposures to *Trypanosoma cruzi* through activation of polyfunctional T cell response. Front Immunol. 2020;11:595039. https://doi.org/10.3389/fimmu.2020.595039.
87. Barry MA, Wang Q, Jones KM, et al. A therapeutic nanoparticle vaccine against Trypanosoma cruzi in a BALB/c mouse model of Chagas disease. Hum Vaccin Immunother. 2016;12(4):976–87. https://doi.org/10.1080/21645515.2015.1119346.
88. Tamborrini M, Hauser J, Schäfer A, et al. Vaccination with virosomally formulated recombinant CyRPA elicits protective antibodies against *Plasmodium falciparum* parasites in preclinical in vitro and in vivo models. NPJ Vaccines. 2020;5:9. https://doi.org/10.1038/s41541-020-0158-9.

# Intertwining of Retinoic Acid and Cholesterol Pathway and its Consequences in *Leishmania donovani*-Infected Macrophages

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**Abstract**

Visceral leishmaniasis (VL) is the most severe disease among other forms of leishmaniasis and results in a fatality in more than 90% of cases if left untreated. It is a zoonotic disease caused by *Leishmania donovani* and is prevalent in the Indian subcontinent, affecting the population of poor socioeconomic backgrounds. People residing in endemic regions lacking proteins, iron, zinc, and vitamin A in their diet are more prone to develop this opportunist infection into a full-blown disease. The deficiency of a prominent micronutrient vitamin A favors the parasites to develop an infection in the human host, and WHO recommends 200,000 IU doses of vitamin A to VL patients on admission. Additionally, *Leishmania* entry to the host is favored by cholesterol present in the plasma membrane, and survival inside the host is achieved by utilizing host cholesterol as *Leishmania* and other trypanosomatids lack de novo synthesis of sterol. However, in our study, we have already reported that a deficit of retinoic acid (RA), a metabolite of vitamin A, favors the parasite to increase their number in *L. donovani*-infected macrophages by downregulating immune response. Along with this finding, we have also observed the restoration of cellular cholesterol levels in *L. donovani*-infected macrophages by RA. In this chapter, we have explained the connecting link between cholesterol and RA in visceral leishmaniasis.

**Keywords**

Visceral leishmaniasis · Cholesterol · Retinoic acid

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## 1 Introduction

Leishmaniasis is one of the seven neglected tropical diseases caused by 21 of 30 species of parasites of the genus *Leishmania*. *Leishmania* is an obligate intracellular parasite belonging to the Trypanosomatidae family and Kinetoplastida order, with a single flagellum and kinetoplast as the key attributes [1, 2]. Their geographical distribution categorizes them into "Old World" and "New World" species. The "Old World" species (*Leishmania aethiopica*, *Leishmania donovani*, *Leishmania infantum*, *Leishmania major*, and *Leishmania tropica*) cause leishmaniasis in Asia, Eastern Hemisphere, Africa, and Southern Europe, while the "New World" parasite species (*Leishmania amazonensis*, *Leishmania braziliensis*, *Leishmania chagasi*, and *Leishmania mexicana*) affect Central America, South America (except Chile, El Salvador, and Uruguay), and Mexico [3, 4]. Leishmaniasis is classified into three categories: cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL), and visceral leishmaniasis (VL or kala-azar). Certain previously treated VL patients exhibit yet another form of leishmaniasis called post-kala-azar dermal leishmaniasis (PKDL). This is a severe complication of VL that primarily manifests in the cutaneous parts of the body after completion of the treatment. Around 5–10% of previously treated VL patients in South Asia and 50–60% in East Africa are recorded to develop PKDL [5].

*Leishmania* has specific established routes for entry into the host cells, one being through the receptors in cholesterol-rich regions of the host macrophages. These cholesterol-rich plasma membrane regions, particularly lipid rafts, are centers of high cell signaling activities and are therefore vital to a cell's proper functioning. Studies suggest that when cholesterol was intentionally lowered using methyl-beta-cyclodextrin (MβCD), the extent of *L. donovani* infection reduced accordingly in J774A.1 macrophage (mφ) [6] helps us mark this route as vital in an anti-leishmanial response strategy.

Malnutrition is a major deciding factor that marks an individual prone to contracting VL. As per the WHO report, a malnourished person lacking protein energy, vitamin A, zinc, and iron in their diet is at a high risk of developing VL. Vitamin A is multifunctional and exerts various effects through its metabolite retinoic acid (RA). The conversion of retinol to retinoic acid is a two-step process that occurs via the retinaldehyde dehydrogenase (RALDH) enzyme pathway. RA plays a vital role in growth and development and contributes to immune modulation and cholesterol homeostasis [7, 8]. Our current findings show that RA restores the cellular cholesterol level by increasing the *npc1* and *npc2* expression in *L. donovani*-infected macrophages [9] and cholesterol depletion leads to a decrease in the parasitic burden [10], which could be a promising factor to use RA as an anti-leishmaniasis agent to treat VL patients.

## 2 Visceral Leishmaniasis Caused by *Leishmania donovani*

Visceral leishmaniasis (VL) is caused by *L. donovani* in the Indian subcontinent and is fatal if left untreated in >90% of cases. These parasites disseminate in the reticuloendothelial cells of the liver, bone marrow, spleen, and lymph node upon successful entry into the host [11]. High fever, enlargement of the spleen and liver, anemia, and weight loss are characteristics found in VL patients. Around 50,000 to 90,000 new cases of VL annually occur globally, but only 25–45% of cases are submitted to WHO [12]. Above 90% of new cases appear in Brazil, Bangladesh, India, Ethiopia, Sudan, and South Sudan. In the Indian subcontinent, a maximum number of cases occur in India, particularly in Bihar and certain adjoining districts of Jharkhand, West Bengal, and Uttar Pradesh. The World Health Organization [12] and the Government of India had set a goal to eliminate VL from India by 2020. As per the recent information provided by the National Vector Borne Disease Control Programme (NVBDCP), the number of VL and PKDL cases has decreased to 1267 and 770, respectively, in 2021. Despite all efforts, their development and reappearance are found in various localities of India [13–15].

*Leishmania* is transmitted to humans by the bite of an infected female sandfly vector of the genus *Phlebotomus* and *Lutzomyia*. Including humans, approximately 70 animal species act as natural reservoir hosts for *Leishmania* [12], but most of the reservoirs remain asymptomatic [16, 17]. *Leishmania* survives in two morphological forms with a heteroxenous life cycle; flagellated promastigote forms reside and differentiate in the gut of sandflies to produce infective metacyclic forms. Metacyclic forms of promastigote, transmitted to the human host via sandfly, are transformed into the second morphological form, i.e., a non-flagellated amastigote form, to disseminate the infection (Fig. 1) [18–20].

According to the World Health Organization (WHO), leishmaniasis has been placed in group 1 diseases, and its control is based on the following three points: vector control, control of animal reservoir, and vaccine development [21–24]. Leishmaniasis has been placed in neglected tropical diseases by WHO. That has endured in tropical and subtropical conditions affecting more than 1 billion people in 149 countries [1]. WHO categorized neglected tropical diseases into two classes: (a) preventive chemotherapy and transmission control and (b) innovative and intensified disease management. Under preventive chemotherapy and transmission control, cost-effective, efficient, and safe administration of drugs is carried out to susceptible populations from time to time. At the same time, innovative and intensified disease management is more challenging and requires significant efforts to develop innovative tools to overcome these diseases. Therefore, the former class is called "tool ready," while the latter is known as the "tool deficient" class. Leishmaniasis comes under the class of "tool deficient," not only because of its place in 20 neglected tropical diseases by WHO but also because of being the plan of action by the Centers for Disease Control and Prevention (CDC) [12, 25].

In 2012, Britain had taken a challenge to eradicate ten neglected tropical diseases by 2020. It was also pointed out that there is a need to spread the elimination programs to control their dissemination, and support is needed in the field of research

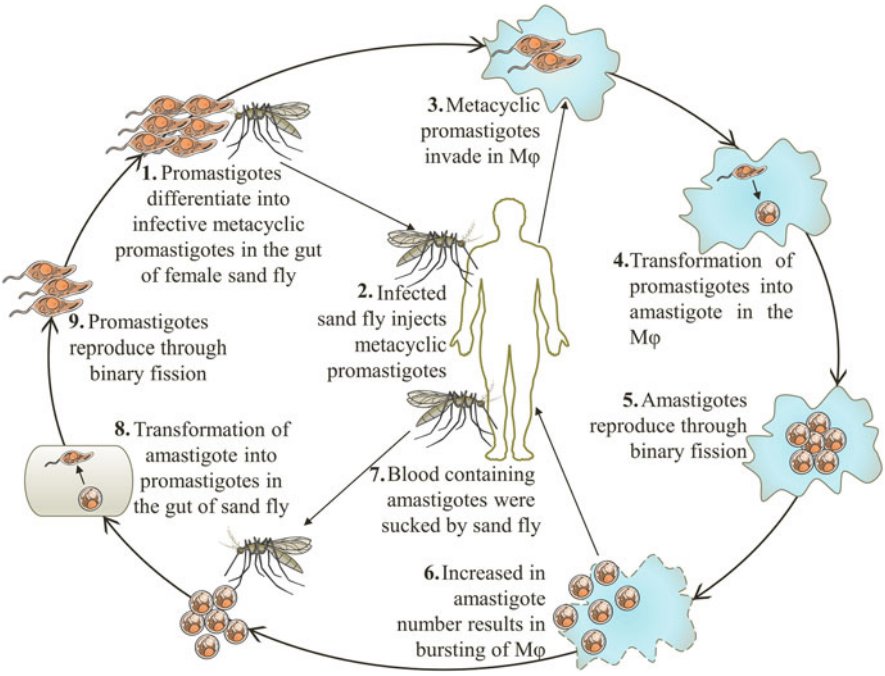


**Fig. 1** Heteroxenous life cycle of *Leishmania*: *Leishmania* undergoes heteroxenous life cycle due to existence in two morphological forms: (i) flagellated promastigotes, which reside and differentiate in the gut of sandfly, and (ii) non-flagellated amastigote form, responsible to cause disease in human host

and development to form innovative therapeutic tools to eradicate the parasites [26, 27]. Approximately 30,000 new cases of visceral leishmaniasis are reported annually by WHO, while over 20,000 deaths occur by visceral leishmaniasis annually [1]. However, some actions were taken to deal with the spread of parasites, including considerable efforts to control vector proliferation. Despite this, the discovery of novel antiprotozoal agents and vaccine development must be emphasized. Another primary concern is the lack of pediatric formulations [28]. Actually, in the case of visceral leishmaniasis, the death rate is high compared to other neglected tropical diseases due to a lack of proper drug formulations. That is why the following approaches have been undertaken either alone or in combination to contain the neglected tropical diseases: (i) intensive case management, (ii) preventive chemotherapeutic measures, (iii) vector control procedures, (iv) veterinary health, and (v) innocuous water, correct sanitation, and good hygiene habits [1].

## 3 Current Status of Therapy in VL

Visceral leishmaniasis is second in mortality and fourth in morbidity among neglected tropical diseases [29–32]. Its symptoms include weight loss, persistent fever, hepatosplenomegaly, hypergammaglobulinemia, and pancytopenia. Most of the drugs used to cure leishmaniasis are not ideal due to their long duration of treatment, high toxicity, and uncertain effectiveness and because they all require parenteral therapy. Instead, liposomal amphotericin B (AmB), miltefosine, and paromomycin for leishmaniasis have been increased [33]. Despite several clinical trials and research, the primary challenge is understanding the pharmacokinetic characteristics of drugs to release effective components primarily in inflicted sites such as the liver, spleen, bone marrow, and lymph nodes [34].

Several chemotherapeutics are in use or on trial for leishmaniasis, but most are new formulations or combinations of the available drugs [35]. Many leishmaniasis patients in India have become resistant to pentavalent antimony, the first successful drug to treat the disease, even though their effectiveness has been found in other areas. At the same time, there are limitations to antimony treatment, such as durability in the treatment; significant toxic effects on the liver, heart, and kidneys; and mandatory parenteral drug delivery [36–38].

Liposomal amphotericin B and amphotericin B deoxycholate are the two formulations of amphotericin B used to treat VL [39]. The primary use of amphotericin B is not recommended due to numerous adverse effects and should only be prescribed when patients show unresponsiveness to the pentavalent antimony during treatment. However, this drug has become relevant in India due to increased resistance against pentavalent antimony [40, 41]. Miltefosine, the oral drug to treat VL, has shown 94% activity in phase IV trials [37]. However, miltefosine is less toxic than others but has drawbacks like deformities in the embryo, gastrointestinal disturbances, management to avoid the emergence of drug resistance due to nonadherence, etc. [42]. Sitamaquine is another anti-leishmanial drug with the chemical name of 8-aminoquinoline, administrated orally to treat visceral leishmaniasis [43, 44].

Many of these drugs are costly, and no significant results have been observed when administrated orally. These drugs are given for a more extended period at low doses as parenteral therapy to minimize their side effects. Therefore, these could lead to significant non-compliances. From past experiences, it has been observed that the spread of disease and emergence of drug-resistant strains increase and pose a significant threat to the management of the disease [45, 46].

The combined formulation of anti-leishmanial drugs is another approach used to treat VL. These combinations are based on their properties, such as cost of treatment, therapy duration, adverse effects, recurrence of treatment failure, individual doses, and chances of drug resistance [47–49]. These combinations are planned in those areas where drug-resistant cases are rampantly increasing. For example, the use of miltefosine with amphotericin B or paromomycin against VL has increased in India due to the number of resistant patients to pentavalent antimonial [49, 50]. It will be early to conclude the outcome and consequences of treatment with combinational

drugs. So, long-term study is needed to understand their outcomes and possible chances to avoid resistance. Besides this, a combination of antiparasitic drugs and immunotherapy has been explored to treat leishmaniasis in the last few years with inconsistent outcomes [51–53]. An encouraging result was found when the combined formulation of sodium stibogluconate and paromomycin was used in Sudan against VL patients. A similar approach with intravenous sodium stibogluconate and oral allopurinol was tried in Kenya to treat VL patients. However, this method was initially effective but inconsistent and required redressal [54].

## 4 Immune Responses in Liver, Bone Marrow, and Spleen

Immediately after entering the host, the metacyclic promastigotes first evade the innate response employed by the host immune system. Specific promastigote kinases phosphorylate the complement proteins, disabling the complement pathways and thus inhibiting their clearance. Metacyclic promastigotes also express lipophosphoglycan (LPG) on their surface, which further plays a role in deactivating the complement-mediated parasite killing. Additionally, zinc-metalloprotease glycoprotein 63 (gp63) of *Leishmania* converts active C3b to inactive C3bi that assist the parasites in entering host cells through complement receptor 3 (CR3). Inactivation of complement components protects the parasites from phagocytes [55]. Studies further suggest lipid rafts, a cholesterol-rich region in the plasma membrane, as a potential entry point for the parasites into the host. Depleting the cholesterol reduced the *L. donovani* infection to J774A.1 mφ [6], confirming the role of lipid raft in the entry of parasites. Certain risk factors increase the spread of VL in different regions.

VL commonly affects lymphoid organs like the bone marrow, liver, lymph node, and spleen [56]. Infection and growth of parasites can be contained in the liver, but it persists in the spleen and bone marrow [57, 58]. In the first 4 weeks of infection, amastigote forms of *L. donovani* divide rapidly in the liver, but later infection gets retarded within 8 weeks. However, the parasitic burden increases slowly in the spleen and bone marrow, and the burden gradually increases even after 4 weeks [59]. Development of inflammatory granulomas curbs the parasitic infection in the liver [60]. Kupffer cells are the resident mφ in the liver and the primary target for the *L. donovani* amastigotes [61, 62]. Initially, monocytes and neutrophils are recruited at the site of infection in response to the cytokines and chemokines produced by resident mφ/DCs [63]. Early entry of *L. donovani* in neutrophils results in the visceralization, i.e., their dissemination to the spleen and bone marrow. Increased levels of IL-4 and IL-10 with the substantial rise in the ratio of *L. donovani*-specific serum IgG1/IgG2a levels skewed the mφ toward M2 type resulting in decreased inducible nitric oxide synthase (iNOS) and thus favoring the parasite growth [64].

Pattern recognition receptors (PRRs) sense a parasite's entry into the host body. Innate immune cells expressing PRRs like complement receptors, dectin receptors, mannose receptors, scavenger receptors, Toll-like receptors (TLR), etc. help to detect pathogen-associated molecular patterns (PAMPs). These receptors are found on the surface of dendritic cells, mφ, natural killer (NK) cells, and neutrophils

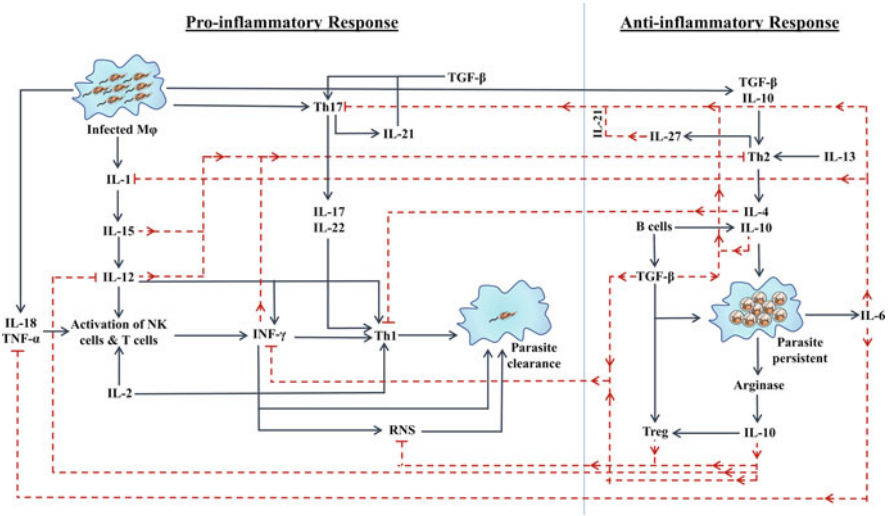


**Fig. 2** Pro-inflammatory cytokines' and anti-inflammatory cytokines' response in the parasite clearance and parasite dissemination, respectively. Th1 cytokines (like IFN-γ, IL-1, IL-2, IL-8, IL-12, IL-15, IL-17, IL-18, GM-CSF and TNF-α) are upregulated for stimulating inflammatory response to kill parasites by generating ROS and RNS, whereas upregulation of Th2 anti-inflammatory cytokines (such as IFN-α, IL-4, IL-6, IL-10, IL-13 and TGF-β) suppress the activity of pro-inflammatory cytokines to favor the parasites for the establishment of infection into the host

[65, 66]. Molecules like glycosyl inositol phospholipids, glycoprotein gp63, LPG, and proteophosphoglycan (PPG) are present on *Leishmania* parasites interrupting host cell signaling by manipulating the PRRs and altering the expression of numerous cytokines, antigen presentation, and microbicidal activities. This allows the parasites to escape from the innate immune response and increase their number to expand infection into full-blown disease [67]. Cytokines show a specific effect upon interaction with their receptors on target cells [68] and are categorized into pro-inflammatory cytokines and anti-inflammatory cytokines. Pro-inflammatory cytokines such as IFN-γ, IL-8, IL-12, IL-17, IL-18, TNF-α, etc. are responsible for stimulating inflammatory response upon parasite entrance, whereas anti-inflammatory cytokines such as IL-4, IL-6, IL-10, IL-13, TGF-β, etc. suppress the activity of pro-inflammatory cytokines (Fig. 2) [69, 70].

## 5 Cholesterol is Required for *Leishmania donovani* Infection: Implications in Leishmaniasis

Cholesterol ($C_{27}H_{46}O$) was first isolated from human gallstones in 1789; since then, it has been extensively studied. Its pathological and physiological importance is very well-elucidated. It is an essential component of the cell membrane in higher eukaryotes and helps make a semipermeable barrier between cellular compartments

because of its significant hydrophobicity like other sterol molecules. It interconnects with the lipids to regulate the bilayer's fluidity, rigidity, and permeability. Moreover, cholesterol helps maintain or change the conformations of several transmembrane proteins that ease its trafficking. It controls the subcellular distribution after associating with several sterol transport proteins. It also controls the transmembrane signaling processes through G protein-coupled receptor (GPCR) signaling and helps in membrane trafficking [71–73]. Although cholesterol is an indispensable component of mammalian cell membranes, the ratio of cholesterol and protein within cells varies [74]. Besides, it is heterogeneously dispersed between cellular membranes. The amount of cholesterol in the plasma membrane constitutes approximately 20–25% of the lipid molecules with various sphingomyelin, phospholipids, and glycolipids. Apart from these, cholesterol is also found in various parts of the Golgi complex and the endocytic recycling compartment [75, 76]. However, less than 1% of the total cell cholesterol is found in the endoplasmic reticulum (ER) [77]. Cholesterol concentration of the ER seems to act as a controller of numerous functions which are associated with the ER and ER-Golgi membrane transport, for example, the regulation of the sterol-homeostatic machinery [78] and the role of inhabitant proteins of ER, and leads to the release of freshly produced membrane proteins from the ER. It is interesting to know that a decrease of sterol from ER obstructs the transport of secretory marker protein from ER to Golgi [79–81] but increases sterol regulatory element-binding protein cleavage-activating protein (SCAP) [82]. However, it is not clear how this differential process is regulated. However, it seems to have sterol interference with the recruitment of COPII coat protein [81] and loading of cargo to COPII vesicles [83] and possibly COPII isoforms [84] that show differential sterol sensitivities.

Cholesterol can alter cellular processes after interacting with specific proteins and other membrane lipids. Packing and cohesion (ordering) of neighboring lipids with cholesterol are increased due to its unique four-ring structure, which provides special biophysical properties. Due to its rigid sterol backbone, cholesterol is positioned adjacent to saturated hydrocarbon chains of nearest lipids that are elongated and stiffer than those of unsaturated lipids. Cholesterol increases the lateral ordering of lipids and alters the membrane's biophysical properties, resulting in reduced fluidity and decreased permeability of polar molecules. This leads to the distribution of ions and solutes on either side of the membrane [85]. Few membrane-associated proteins are bound to cholesterol [86]. Besides, cellular cholesterol homeostasis and intracellular acquisition are performed by some essential regulatory proteins like Niemann-Pick C1 protein (NPC1), NPC2, sterol regulatory element-binding protein 2 (SREBP-2), hydroxymethylglutaryl-CoA (HMG-CoA) reductase, SCAP, etc. SCAP contains the conserved sterol-sensing domain (SSD), having a five-transmembrane helix domain [82]. However, the function of this domain is not clear yet. However, this domain binds to cholesterol, and this binding directs the SCAP recruitment to COPII vesicles [87].

It has been observed that *Leishmania donovani* parasites require host membrane cholesterol for their entry into the mφ. Reducing cholesterol levels in mφ either by cyclodextrins or by nystatin results in a decreased infection of *L. donovani*

promastigotes. It possibly perturbs the binding of parasites to the cell surface. However, the binding of *L. donovani* promastigotes to the cholesterol-depleted mφ cell surface can be restored by enrichment of membrane cholesterol. In the same study, the number of intracellular amastigotes decreases in infected cells upon enrichment of membrane cholesterol. However, a depleted level of cholesterol or sequestration does not affect the entrance of serum-opsonized *L. donovani* promastigotes in host mφ. This report suggests the importance of cholesterol for the entrance of *L. donovani* promastigotes in host mφ via a non-opsonic pathway [6, 88].

Rodríguez et al. [89] reported that cholesterol plays an indispensable role in the entry of *L. chagasi* into host bone marrow mφ through cholesterol-enriched caveolar domains [89]. However, a depleted level of cholesterol also affects the ingression of opsonized *L. chagasi* in bone marrow mφ, unlike *L. donovani*. Despite that, the development of primary infection in host cells requires entry of promastigote form of parasites, and the first entry may get inhibited if cholesterol gets depleted in mφ. Accordingly, it has been observed that the decrease in cholesterol level leads to a reduced load of intracellular amastigotes in host mφ [6, 88, 89]. However, in vivo studies are further required to confirm whether such a decline in intracellular amastigote levels could inhibit their re-infection with the neighboring cells.

Following the entry of parasites into host cells, it survives by perturbing the host's immunity [90]. Simultaneously, the competence of infected mφ to efficiently present the antigens to T cells decreases [91]. Infected mφ with inadequate antigen-presenting ability is associated with poor properties of cell membranes, and such impairment is intended to avert the host immune response by the parasites [92]. Since cholesterol plays several functions to affect the properties of the cell membrane [93], parasites could cause loss of physical properties of the cell membrane by the intake of host cholesterol. It has been shown that the physical properties can be restored by providing exogenous cholesterol to infected host cells, which helps restore antigen presentation [91]. Thus, these supporting results confirm that parasites inside the host cells require cholesterol. Although the exact mechanism is not fully understood, the functional aspects of membrane cholesterol and its role in the organization of integral membrane proteins have been studied extensively [94].

## 6 Malnutrition and Visceral Leishmaniasis

Amastigotes increase their numbers in host mφ through binary fission and spread in the reticuloendothelial system of the mammalian host. This leads to weakening of the immune system of the infected person and needs to be cured as soon as possible; otherwise, if it will persist with symptoms like high fever, cough, diarrhea, pancytopenia, hepatosplenomegaly, thrombocytopenia, etc. over a week or more, then the condition of the patient becomes worst and could result in death if left untreated [95–99]. Thus, the host's immune system plays a vital role in countering the growth of amastigotes. Primarily, the disease inflicts individuals of the socio-economically oppressed class. Due to improper intake of nutrients, persons become

immunocompromised and more vulnerable to the infection [47]. Optimum nutrition plays a countering role in the establishment and progression of the disease [100, 101]. From the same geographical regions, deficiency of micronutrients such as vitamin A is already reported indicating increased susceptibility to the infection and the disease [1].

## 7 Retinoic Acid (RA) and its Importance

Vitamin A is a lipid-soluble vitamin required for embryonic development and actively participates in several cellular functions like apoptosis, differentiation, and proliferation. Retinol, a precursor of RA, is not synthesized in our body, but we acquire it through our diet. β-Carotene from plants and retinyl esters from the animal are rich sources of vitamin A. β-Carotene and retinyl ester are converted into retinol with the help of an enzyme retinyl ester hydrolase (REH). Once absorbed in the intestine, this retinol is converted into retinyl esters using lecithin-retinol acyltransferase (LRAT). Retinyl ester is stored in the adipose tissue and liver (stellate cells) and requires retinol-binding proteins (RBP) and transthyretin to circulate in the blood (Fig. 3). Transthyretin-retinyl ester association averts the elimination of retinyl esters from the kidney. All-trans-retinoic acid (ATRA or

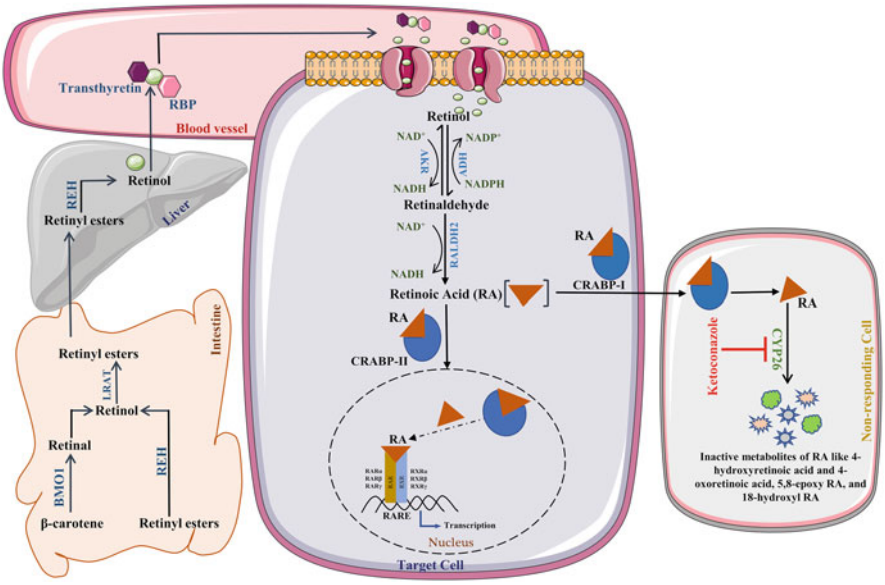


**Fig. 3** RA metabolism—β-carotene and retinyl ester are converted to retinol by different enzymes. Retinol in the plasma is carried to the target cells by transthyretin and retinol binding protein. Retinol in the target cell converted to retinoic acid (RA) by ADH and RALDH2 with intermediate retinaldehyde. RA is then transported to the nucleus by CRABPII, where it binds with the retinoic acid receptors and recognizes the RARE sequence. Thus, it helps to regulate gene expression

retinoic acid (RA)) and 11-cis-retinaldehyde (cis-RA) were two initial active metabolites found [102–107]. Along with ATRA and cis-RA, other natural and synthetic retinoids have been studied and listed in Table 1. Some are already in use to treat some diseases, while the remaining are under clinical trials.

## 8 RA Metabolism and Gene Regulation through Nuclear Receptors

Retinol is reversibly metabolized to retinaldehyde using cytosolic alcohol dehydrogenase (ADH, also called RALDH1) enzyme. Further, this retinaldehyde is oxidized to non-reversible retinoic acid (RA) using retinaldehyde dehydrogenase 2 (RALDH2) [107, 128–130]. This newly formed RA remains in the cytosol and has two fates after binding with cellular RA-binding proteins (CRABPs). If it binds with CRABPII, then RA is transported to the nucleus, where it interacts with the RAR-RXR heterodimer, while after binding with CRABPI, it is directed for oxidation into 4-hydroxyretinoic acid and 4-oxoretinoic acid metabolites by cytochrome P450 family 26 (CYP26) enzyme [131–134]. RA mediates the expression of genes at the transcriptional level after binding with nuclear receptors, retinoic acid receptor (RAR), and retinoid X receptor (RXR). These RAR and RXR, either alone or as a heterodimer, bind to the retinoic acid response element (RARE) on enhancer regions of RA target genes and regulate gene expression (Fig. 3). Three subtypes (α, β, and γ) of each RAR and RXR are found. Additionally, two or more isoforms are known [131, 135–138]. RARα generally acts as transcriptional activators but can also interact with RA outside the nucleus [135]. Standard RARE sequences are formed by di-hexameric motif (A/G)G(G/T)TCA separated with five bases known as direct repeat 5 (DR5). However, RAR-RXR heterodimer can also start the gene regulation after binding to any of the following: either with DR1 or DR2 [139–141]. ATRA and 9-cis-RA can activate RARs with the same efficiency, but ATRA is less effective than 9-cis-RA for activating RXRs [135].

## 9 Status of RA and its Pathway in Visceral Leishmaniasis

Retinoic acid (RA) is produced inside the cells from retinol with an intermediate retinaldehyde by the enzymes RALDH1 and RALDH2. Vitamin A, through its active metabolite, RA, acts in several biological conditions, essential for eyesight, embryonic development, and reproduction, and also, it plays a crucial role in the maintenance of the immune system. Immunoregulatory functions of RA are well explained in inflammatory bowel disease, neurological disorder, and other diseases. Our earlier findings show a compromised RA pathway in *L. donovani*-infected mφ due to a decrease in *RALDH1* and *RALDH2* levels. Moreover, inhibition of RALDH pathway by RALDH2 inhibitor favored the *L. donovani* parasites to establish the infection by increasing anti-inflammatory Th2 cytokines [142].

**Table 1** Natural and synthetic retinoids with their pharmacological role

| Derivatives | IUPAC name | Target | Half-life | Pharmacological role | References |
|---|---|---|---|---|---|
| Acitretin | (2E,4E,6E,8E)-9-(4-Methoxy-2,3,6-trimethylphenyl)-3,7-dimethylnona-2,4,6,8-tetraenoic acid | RAR and RXR | 33–96 h | Used for the treatment of psoriasis and also have anti-aging property | Kai-rong et al. [108], Saurat [109], Mukherjee et al. [110] |
| Adapalene | 6-[3-(Adamantan-1-yl)-4-methoxyphenyl]naphthalene-2-carboxylic acid | RARβ, RARγ, and RXR | Not available | Used for the treatment of acne | Pechère et al. [111], Thielitz et al. [112] |
| Alitretinoin (9-cis-retinoic acid) | (2E,4E,6Z,8E)-3,7-Dimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl) nona-2,4,6,8-tetraenoic acid | RAR and RXR | Not available | Used in treating cutaneous lesions of Kaposi's sarcoma and also found to prevent mammary and prostate cancer | Wu et al. [113], Christov et al. [114], Baumann et al. [115] |
| Amsilarotene (TAC101) | 4-[[3,5-bis(trimethylsilyl)benzoyl] amino]benzoic acid | RARα and RARβ | Not available | Used to inhibit the growth of hepatocellular carcinoma | Sano et al. [116], Sako et al. [117] |
| Bexarotene (Targretin) | 4-[1-(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)ethenyl] benzoic acid | RXR | 7 h | Used for the treatment of cutaneous T-cell lymphoma | Duvic et al. [118] |
| Etretinate | Ethyl 9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethylnona-2,4,6,8-tetraenoate | RAR and RXR | 120 days to 2.9 years | Used for the treatment of psoriasis and also have anti-aging property | Mukherjee et al. [110] |
| Fenretinide (4HPR) | (2E,4E,6E,8E)-N-(4-hydroxyphenyl)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl)nona-2,4,6,8-tetraenamide | RAR and RXR | Not available | Used as a chemopreventive against prostate cancer, growth inhibitory properties against human head-and-neck and lung cancer cells, also efficiently act as an antineoplastic agent | Sun et al. [119] |
| Isotretinoin (13-cis-retinoic acid) | (2Z,4E,6E,8E)-3,7-Dimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl) nona-2,4,6,8-tetraenoic acid | RXR | 7–39 h | Used for the treatment of acne and is under clinical trial for head-and-neck cancer, prostate cancer, lung cancer, and thyroid cancer | Pechère et al. [111], Baumann et al. [115], Thielitz et al. [112] |

| | | | | | |
|---|---|---|---|---|---|
| MDI 301 (9-cis-RA derivative) | (9cis)-O15-(3,3-Dimethyl-2-oxobutyl)retinoic acid | RARα | Not available | Used for the treatment of acne and efficacy in dermal repair | Appleyard et al. [120], Warner et al. [121] |
| Palovarotene (R667) | 4-[(E)-2-{5,5,8,8-Tetramethyl-3-[(1H-pyrazol-1-yl)methyl]-5,6,7,8-tetrahydronaphthalen-2-yl}ethenyl] benzoic acid | RARγ | Not available | Used to treat emphysema | Brennan et al. [122] |
| Seletinoid G | (5-Hydroxy-4-oxopyran-2-yl)methyl (E)-3-(1,3-benzodioxol-5-yl)prop-2-enoate | RARγ | Not available | Anti-aging property | Mukherjee et al. [110] |
| Tamibarotene (AM80) | 4-[(5,5,8,8-Tetramethyl-6,7-dihydronaphthalen-2-yl)carbamoyl] benzoic acid | RARα | Not available | Anti-diabetic activity and also used for Alzheimer's disease (AD) | Miwako and Kagechika [123], Kawahara et al. [124], Kwak et al. [125] |
| Tazarotene | Ethyl 6-[2-(4,4-dimethyl-3,4-dihydro-2H-1-benzothiopyran-6-yl)ethynyl] pyridine-3-carboxylate | Mainly with RAR | Approx. 18 h | Used for the treatment of acne | Pechère et al. [111], Thielitz et al. [112] |
| Tretinoin (all-trans-retinoic acid) | (2E,4E,6E,8E)-3,7-Dimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl) nona-2,4,6,8-tetraenoic acid | RAR | 0.5–2 h | Used to treat acute promyelocytic leukemia (APL), acne, and photodamaged skin | Wu et al. [113], Baumann et al. [115] |
| Vitamin A (11-cis-retinal) | (2E,4E,6E,8E)-3,7-Dimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl) nona-2,4,6,8-tetraen-1-ol | RAR and RXR | 1.9 h | Required for normal vision | Khorana [126], Cvekl and Wang [127] |

In the continuation of our previous study, we observed the loss in cellular cholesterol level in *L. donovani*-infected mφ after inhibiting the RALDH pathway. Additionally, during the infection, *Leishmania* uses the host cholesterol, as they lack de novo synthesis of sterol, and causes a decrease in the expression of Niemann-Pick C type 1 (*npc1*) and Niemann-Pick C type 2 (*npc2*) lysosomal genes involved in cholesterol uptake [9].

## 10 Crosstalk of RA and Cholesterol Pathways: Impact of RA on Cellular Cholesterol

Macrophages (mφ) promptly obtain lipoproteins from dying cells and develop mechanisms for eradicating cholesterol from the cell. In case too much cholesterol is accumulated in mφ, this could lead to the formation of foam cells [143]. So, two active and two passive pathways are held by mφ to transfer free cholesterol to HDLs [144]. On the other hand, free cholesterols are transported to the plasma membrane. The number of transcription factors such as the retinoid X receptor (RXR), liver X receptors α and β (LXRα, LXRβ), and members of the peroxisome proliferator-activated receptor (PPAR) family, including PPARα and PPARγ, is activated by these accumulated cellular cholesterols [145, 146]. Transcription factors like RXR and LXR form heterodimer and enhance the expressions of ATP-binding cassette subfamily A member 1 (ABCA1) and ATP-binding cassette subfamily G member 1 (ABCG1). ABCA1 and ABCG1 transporters direct free cholesterol efflux to lipid-deficient ApoA-I and other lipid-deficient apolipoproteins to ultimately form mature HDLs. These are immensely distributed on mφ and play an essential role in lipid and cholesterol metabolism through LXR [147–152]. Instead, free cholesterol can also be effluxed passively via an aqueous diffusion pathway or a scavenger receptor class B type I (SRBI)-mediated pathway [144]. RXR, other than RAR, also forms the heterodimer with LXR, pregnane X receptor (PXR), and farnesoid X receptor (FXR) that regulate systematic lipid metabolism. RA governs the lipid metabolism in the number of cells, including mφ, and is found to be efficiently involved in ABCA1 expression in mφ cell types [153, 154]. Zhou et al. [155] confirmed that protein expressions of ABCA1 and ABCG1 were enhanced upon 9-cis-RA treatment in J774A.1 mφ in a dose-dependent manner and increased protein expression of LXRα in the same condition [155]. In our current findings, we observed *L. donovani* infection in J774A.1 mφ resulted in depletion of cellular cholesterol by decreasing the expression of *npc1* and *npc2*. However, RA supplementation in the same condition restored the cellular cholesterol level by restoring the expression of lysosomal genes [9].

## 11 Conclusions

Retinoic acid (RA) shows pleiotropic effects such as regulating cellular development, their function(s), immune regulation, etc. RA shows dual immune modulatory effects in VL patients [156, 157]. It supports pro-inflammatory as well as anti-inflammatory response, which may further dictate the host's response toward the disease [158]. Another aspect which is affected by RA is cholesterol and lipid homeostasis [159–161].

Membrane cholesterol plays a prominent role in eliciting competent immune responses of the cells. However, dysfunction in the lipid rafts due to low cholesterol helps in the attachment and internalization of pathogens. On that account, to retain the normal physiological functions of the cells, it is imperative to maintain the intracellular cholesterol levels [9, 161–163]. How cellular cholesterol level is maintained is discussed earlier in the review. It is confirmed that cellular cholesterol depletion results in the weakening of immune response in terms of antigen presentation by the mφ cells and flawed recognition by the T cells.

For this reason, maintaining the cellular cholesterol in cells is crucial to fixing the immune response's essential functions [164]. Hence, to support this possibility by the experimental approach, cholesterol-rich liposomes were used to overcome the condition of low cellular cholesterol in infected mφ, which restored its functions as well [165]. Our previous findings showed that RA treatment in *L. donovani*-infected mφ enhanced the M1 phenotypes (IL-12 ↑, iNOS ↑, arginase-1 ↓, and IL-10 ↓) [142].

Further, we identified retinoic acid response element (RARE) sequences upstream of the npc1 and npc2 lysosomal genes. We observed that compromised cellular cholesterol level in *L. donovani*-infected mφ was restored by upregulating the expression of npc1 and npc2 genes following the treatment with RA and the parasitic load was also decreased eminently in the same condition [9].

Alternatively, it has also been observed that overall loss of cellular cholesterol in infected mφ was due to impaired expressions of other cholesterol-regulating genes (HMGCR ↓, ABCA1 ↓, SREBP-2 ↓, LDLR ↓, and PCSK9 ↓), and this loss of cellular cholesterol was restored upon RA supplementation in *L. donovani*-infected mφ [166]. The interesting thing to note here was that RA-mediated increase in cellular cholesterol level was not mediated through the mTOR pathway; however, RA does increase the Raptor expression, i.e., an essential constituent of mTORC1 assembly [161].

As most VL drug shows side effects, they become resistant over the period and require a more extended therapy period, making treatment very costly. So, providing RA would be a safe, cost-effective, and better adjunct option to cure VL patients along with standard therapy, as it will increase the level of cellular cholesterol and help reduce the burden of parasites by restoring the immune response. However, further in vivo studies are required to confirm the overall consequences of RA on VL patients.

## References


1. Ayi B. Leishmaniasis. xPharm Compr Pharmacol Ref: 1–10. 2007 https://doi.org/10.1016/B978-008055232-3.60933-X.
2. Hughes AL, Piontkivska H. Molecular phylogenetics of Trypanosomatidae: contrasting results from 18S rRNA and protein phylogenies. Kinetoplastid Biol Dis. 2003;2(1):1–10. https://doi.org/10.1186/1475-9292-2-15.
3. Kevric I, Cappel MA, Keeling JH. New world and old world leishmania infections: a practical review. Dermatol Clin. 2015;33(3):579–93. https://doi.org/10.1016/j.det.2015.03.018.
4. Reithinger R, Dujardin J-C, Louzir H, Pirmez C, Alexander B, Brooker S. Cutaneous leishmaniasis. Lancet Infect Dis. 2007;7(9):581–96. https://doi.org/10.1016/S1473-3099(07)70209-8.
5. DNDi. Symptoms, transmission, and current treatments for visceral leishmaniasis | DNDi. 2022. https://dndi.org/diseases/visceral-leishmaniasis/facts/. Accessed 5 Jan 2022.
6. Pucadyil TJ, Tewary P, Madhubala R, Chattopadhyay A. Cholesterol is required for leishmania donovani infection: implications in leishmaniasis. Mol Biochem Parasitol. 2004;133(2):145–52. https://doi.org/10.1016/j.molbiopara.2003.10.002.
7. Gerber LE, Erdman JW. Changes in lipid metabolism during retinoid administration. J Am Acad Dermatol. 1982;6(4):664–72. https://doi.org/10.1016/S0190-9622(82)80047-9.
8. Jie Z, Liang Y, Yi P, Tang H, Soong L, Cong Y, Zhang K, Sun J. Retinoic acid regulates immune responses by promoting IL-22 and modulating S100 proteins in viral hepatitis. J Immunol. 2017;198(9):3448–60. https://doi.org/10.4049/jimmunol.1601891.
9. Prakash S, Saini S, Kumari S, Singh B, Kureel AK, Rai AK. Retinoic acid restores the levels of cellular cholesterol in leishmania donovani infected macrophages by increasing npc1 and npc2 expressions. Biochimie. 2022;198:23–32. https://doi.org/10.1016/J.BIOCHI.2022.03.002.
10. Bansal D, Bhatti HS, Sehgal R. Role of cholesterol in parasitic infections. Lipids Health Dis. 2005;4:1–7. https://doi.org/10.1186/1476-511X-4-10.
11. Melchionda F, Varani S, Carfagnini F, Belotti T, Di Muccio T, Tigani R, Bergamaschi R, Pession A. Spleen nodules: a potential hallmark of visceral leishmaniasis in young children. BMC Infect Dis. 2014;14(1):1–5. https://doi.org/10.1186/s12879-014-0620-2.
12. WHO (2022) Leishmaniasis: World Health Organization. https://www.who.int/health-topics/leishmaniasis#tab=tab_1. Accessed 5 Jan 2022.
13. Kumar V, Mandal R, Das S, Kesari S, Dinesh DS, Pandey K, Das VR, Topno RK, Sharma MP, Dasgupta RK, Das P. Kala-azar elimination in a highly-endemic district of Bihar, India: a success story. PLoS Negl Trop Dis. 2020;14(5):e0008254. https://doi.org/10.1371/JOURNAL.PNTD.0008254.
14. NVBDCP (2022) Kala-azar: National Vector Borne Disease Control Programme (NVBDCP). In: NVBDCP. https://nvbdcp.gov.in/index1.php?lang=1&level=1&sublinkid=5774&lid=3692. Accessed 5 Jan 2022.
15. Saini P, Kumar NP, Ajithlal PM, Joji A, Rajesh KR, Reena KJ, Kumar A. Visceral leishmaniasis caused by leishmania donovani zymodeme MON-37, Western Ghats. India Emerg Infect Dis. 2020;26(8):1956. https://doi.org/10.3201/EID2608.200557.
16. Andrade-Narvaez FJ, Canto Lara SB, Van Wynsberghe NR, Rebollar-Tellez EA, Vargas A, Albertos-Alpuche NE. Seasonal transmission of Leishmania (Leishmania) mexicana in the state of Campeche, Yucatan Peninsula, Mexico. Mem Inst Oswaldo Cruz. 2003;98(8):995–8. https://doi.org/10.1590/S0074-02762003000800002.
17. Van Wynsberghe NR, Canto-Lara SB, Sosa-Bibiano EI, Rivero-Cárdenas NA, Andrade-Narváez FJ. Comparison of small mammal prevalence of Leishmania (Leishmania) mexicana in five foci of cutaneous leishmaniasis in the State of Campeche, Mexico. Rev Inst Med Trop Sao Paulo. 2009;51(2):87–94. https://doi.org/10.1590/S0036-46652009000200006.
18. Dostálová A, Volf P. Leishmania development in sand flies: parasite-vector interactions overview. Parasites Vectors. 2012;5(1):1–12. https://doi.org/10.1186/1756-3305-5-276.

19. Sacks DL. Metacyclogenesis in Leishmania promastigotes. Exp Parasitol. 1989;69(1):100–3. https://doi.org/10.1016/0014-4894(89)90176-8.
20. Serafim TD, Coutinho-Abreu IV, Oliveira F, Meneses C, Kamhawi S, Valenzuela JG. Sequential blood meals promote leishmania replication and reverse metacyclogenesis augmenting vector infectivity. Nat Microbiol. 2018;3(5):548–55. https://doi.org/10.1038/s41564-018-0125-7.
21. Abdian N, Gholami E, Zahedifard F, Safaee N, Rafati S. Evaluation of DNA/DNA and prime-boost vaccination using LPG3 against leishmania major infection in susceptible BALB/c mice and its antigenic properties in human leishmaniasis. Exp Parasitol. 2011;127(3):627–36. https://doi.org/10.1016/J.EXPPARA.2010.12.007.
22. John TJ, Dandona L, Sharma VP, Kakkar M. Continuing challenge of infectious diseases in India. Lancet. 2011;377(9761):252–69. https://doi.org/10.1016/S0140-6736(10)61265-2.
23. Matlashewski G, Arana B, Kroeger A, Battacharya S, Sundar S, Das P, Sinha PK, Rijal S, Mondal D, Zilberstein D, Alvar J. Visceral leishmaniasis: elimination with existing interventions. Lancet Infect Dis. 2011;11(4):322–5. https://doi.org/10.1016/S1473-3099(10)70320-0.
24. Stockdale L, Newton R. A review of preventative methods against human leishmaniasis infection. PLoS Negl Trop Dis. 2013;7(6):e2278. https://doi.org/10.1371/JOURNAL.PNTD.0002278.
25. CDC. Leishmaniasis: Centers for Disease Control and Prevention. 2022. https://www.cdc.gov/parasites/leishmaniasis/. Accessed 5 Jan 2022.
26. Mitra AK, Mawson AR. Neglected tropical diseases: epidemiology and global burden. Trop med. Infect Dis. 2017;2:3. https://doi.org/10.3390/tropicalmed2030036.
27. Rosenberg M, Utzinger J, Addiss DG. Preventive chemotherapy versus innovative and intensified disease management in neglected tropical diseases: a distinction whose shelf life has expired. PLoS Negl Trop Dis. 2016;10(4):e0004521. https://doi.org/10.1371/JOURNAL.PNTD.0004521.
28. Mackey TK, Liang BA, Cuomo R, Hafen R, Brouwer KC, Lee DE. Emerging and reemerging neglected tropical diseases: a review of key characteristics, risk factors, and the policy and innovation environment. Clin Microbiol Rev. 2014;27(4):949–79. https://doi.org/10.1128/CMR.00045-14.
29. Alvar J, Vélez ID, Bern C, Herrero M, Desjeux P, Cano J, Jannin J, den Boer M, Team the WLC. Leishmaniasis worldwide and global estimates of its incidence. PLoS One. 2012;7(5):e35671. https://doi.org/10.1371/JOURNAL.PONE.0035671.
30. Bern C, Maguire JH, Alvar J. Complexities of assessing the disease burden attributable to leishmaniasis. PLoS Negl Trop Dis. 2008;2(10):e313. https://doi.org/10.1371/JOURNAL.PNTD.0000313.
31. Burza S, Croft SL, Boelaert M. Leishmaniasis Lancet. 2018;392(10151):951–70. https://doi.org/10.1016/S0140-6736(18)31204-2.
32. Murray HW, Berman JD, Davies CR, Saravia NG. Advances in leishmaniasis. Lancet. 2005;366(9496):1561–77. https://doi.org/10.1016/S0140-6736(05)67629-5.
33. Silva-Jardim I, Thiemann OH, Anibal FF. Leishmaniasis and chagas disease chemotherapy: a critical review. J Braz Chem Soc. 2014;25(10):1810–23. https://doi.org/10.5935/0103-5053.20140229.
34. Kip AE, Schellens JHM, Beijnen JH, Dorlo TPC. Clinical pharmacokinetics of systemically administered antileishmanial drugs. Clin Pharmacokinet. 2018;57(2):151–76. https://doi.org/10.1007/s40262-017-0570-0.
35. Ponte-Sucre A, Gamarro F, Dujardin JC, Barrett MP, López-Vélez R, García-Hernández R, Pountain AW, Mwenechanya R, Papadopoulou B. Drug resistance and treatment failure in leishmaniasis: a 21st century challenge. PLoS Negl Trop Dis. 2017;11(12):1–24. https://doi.org/10.1371/journal.pntd.0006052.
36. Croft SL, Sundar S, Fairlamb AH. Drug resistance in leishmaniasis. Clin Microbiol Rev. 2006;19(1):111–26. https://doi.org/10.1128/CMR.19.1.111-126.2006.

37. Freitas-Junior LH, Chatelain E, Kim HA, Siqueira-Neto JL. Visceral leishmaniasis treatment: what do we have, what do we need and how to deliver it? Int J Parasitol Drugs Drug Resist. 2012;2:11–9. https://doi.org/10.1016/j.ijpddr.2012.01.003.
38. Monge-Maillo B, López-Vélez R. Therapeutic options for visceral leishmaniasis. Drugs. 2013;73(17):1863–88. https://doi.org/10.1007/s40265-013-0133-0.
39. Roberts CW, McLeod R, Rice DW, Ginger M, Chance ML, Goad LJ. Fatty acid and sterol metabolism: potential antimicrobial targets in apicomplexan and trypanosomatid parasitic protozoa. Mol Biochem Parasitol. 2003;126(2):129–42. https://doi.org/10.1016/S0166-6851(02)00280-3.
40. Sundar S, Chakravarty J. Liposomal amphotericin B and leishmaniasis: dose and response. J Glob Infect Dis. 2010;2(2):159–66. https://doi.org/10.4103/0974-777X.62886.
41. Sundar S, More DK, Singh MK, Singh VP, Sharma S, Makharia A, Kumar PCK, Murray HW. Failure of pentavalent antimony in visceral leishmaniasis in India: report from the Center of the Indian Epidemic. Clin Infect Dis. 2000;31(4):1104–7. https://doi.org/10.1086/318121.
42. Dorlo TPC, Balasegaram M, Beijnen JH, de Vries PJ. Miltefosine: a review of its pharmacology and therapeutic efficacy in the treatment of leishmaniasis. J Antimicrob Chemother. 2012;67(11):2576–97. https://doi.org/10.1093/jac/dks275.
43. Loiseau PM, Cojean S, Schrével J. Sitamaquine as a putative antileishmanial drug candidate: from the mechanism of action to the risk of drug resistance. Parasite. 2011;18(2):115–9. https://doi.org/10.1051/parasite/2011182115.
44. Pérez-Victoria JM, Bavchvarov BI, Torrecillas IR, Martínez-García M, López-Martín C, Campillo M, Castanys S, Gamarro F. Sitamaquine overcomes ABC-mediated resistance to miltefosine and antimony in Leishmania. Antimicrob Agents Chemother. 2011;55(8):3838–44. https://doi.org/10.1128/AAC.00065-11.
45. Neves LO, Talhari AC, Gadelha EPN, da Silva Júnior RM, Guerra JADO, Ferreira LCDL, Talhari S. A randomized clinical trial comparing meglumine antimoniate, pentamidine and amphotericin B for the treatment of cutaneous leishmaniasis by Leishmania guyanensis. An Bras Dermatol. 2011;86(6):1092–101. https://doi.org/10.1590/S0365-05962011000600005.
46. Oliveira LF, Schubach AO, Martins MM, Passos SL, Oliveira RV, Marzochi MC, Andrade CA. Systematic review of the adverse effects of cutaneous leishmaniasis treatment in the New World. Acta Trop. 2011;118(2):87–96. https://doi.org/10.1016/J.ACTATROPICA.2011.02.007.
47. Alvar J, Croft S, Olliaro P. Chemotherapy in the treatment and control of leishmaniasis. Adv Parasitol. 2006;61:223–74. https://doi.org/10.1016/S0065-308X(05)61006-8.
48. Meheus F, Balasegaram M, Olliaro P, Sundar S, Rijal S, Faiz MA, Boelaert M. Cost-effectiveness analysis of combination therapies for visceral leishmaniasis in the Indian subcontinent. PLoS Negl Trop Dis. 2010;4(9):e818. https://doi.org/10.1371/JOURNAL.PNTD.0000818.
49. Sundar S, Sinha PK, Rai M, Verma DK, Nawin K, Alam S, Chakravarty J, Vaillant M, Verma N, Pandey K, Kumari P, Lal CS, Arora R, Sharma B, Ellis S, Strub-Wourgaft N, Balasegaram M, Olliaro P, Das P, Modabber F. Comparison of short-course multidrug treatment with standard therapy for visceral leishmaniasis in India: an open-label, non-inferiority, randomised controlled trial. Lancet. 2011;377(9764):477–86. https://doi.org/10.1016/S0140-6736(10)62050-8.
50. Seifert K, Croft SL. In vitro and in vivo interactions between miltefosine and other antileishmanial drugs. Antimicrob Agents Chemother. 2006;50(1):73–9. https://doi.org/10.1128/AAC.50.1.73-79.2006.
51. Bimal S, Sinha S, Singh SK, Narayan S, Kumar V, Verma N, Ranjan A, Sinha PK, Das VNR, Pandey K, Kar SK, Das P. Leishmania donovani: CD2 biased immune response skews the SAG mediated therapy for a predominant Th1 response in experimental infection. Exp Parasitol. 2012;131(3):274–82. https://doi.org/10.1016/J.EXPPARA.2012.04.007.
52. Shakya N, Sane SA, Vishwakarma P, Bajpai P, Gupta S. Improved treatment of visceral leishmaniasis (kala-azar) by using combination of ketoconazole, miltefosine with an

immunomodulator-Picroliv. Acta Trop. 2011;119(2–3):188–93. https://doi.org/10.1016/j.actatropica.2011.05.017.
53. van Griensven J, Balasegaram M, Meheus F, Alvar J, Lynen L, Boelaert M. Combination therapy for visceral leishmaniasis. Lancet Infect Dis. 2010;10(3):184–94. https://doi.org/10.1016/S1473-3099(10)70011-6.
54. Atia AM, Mumina A, Tayler-Smith K, Boulle P, Alcoba G, Elhag MS, Alnour M, Shah S, Chappuis F, van Griensven J, Zachariah R. Sodium stibogluconate and paromomycin for treating visceral leishmaniasis under routine conditions in eastern Sudan. Trop Med Int Health. 2015;20(12):1674–84. https://doi.org/10.1111/TMI.12603.
55. Walker DM, Oghumu S, Gupta G, McGwire BS, Drew ME, Satoskar AR. Mechanisms of cellular invasion by intracellular parasites. Cell Mol Life Sci. 2013;71(7):1245–63. https://doi.org/10.1007/S00018-013-1491-1.
56. Kumar R, Nylén S. Immunobiology of visceral leishmaniasis. Front Immunol. 2012;3 (AUG):1–10. https://doi.org/10.3389/fimmu.2012.00251.
57. Smelt SC, Engwerda CR, McCrossen M, Kaye PM. Destruction of follicular dendritic cells during chronic visceral leishmaniasis. J Immunol. 1997;158:8.
58. Wilson ME, Streit JA. Visceral leishmaniasis. Gastroenterol Clin N Am. 1996;25(3):535–51. https://doi.org/10.1016/S0889-8553(05)70262-4.
59. Wilson ME, Jeronimo SMB, Pearson RD. Immunopathogenesis of infection with the visceralizing Leishmania species. Microb Pathog. 2005;38(4):147–60. https://doi.org/10.1016/j.micpath.2004.11.002.
60. Engwerda CR, Ato M, Kaye PM. Macrophages, pathology and parasite persistence in experimental visceral leishmaniasis. Trends Parasitol. 2004;20(11):524–30. https://doi.org/10.1016/j.pt.2004.08.009.
61. Beattie L, Peltan A, Maroof A, Kirby A, Brown N, Coles M, Smith DF, Kaye PM. Dynamic imaging of experimental leishmania donovani-induced hepatic granulomas detects Kupffer cell-restricted antigen presentation to antigen-specific CD8+ T cells. PLoS Pathog. 2010;6(3): e1000805. https://doi.org/10.1371/JOURNAL.PPAT.1000805.
62. Dixon LJ, Barnes M, Tang H, Pritchard MT, Nagy LE. Kupffer cells in the liver. Compr Physiol. 2013;3(2):785–97. https://doi.org/10.1002/CPHY.C120026.
63. McGovern K, Wilson E. Role of chemokines and trafficking of immune cells in parasitic infections. Curr Immunol Rev. 2014;9(3):157–68. https://doi.org/10.2174/1573395509666131217000000.
64. McFarlane E, Perez C, Charmoy M, Allenbach C, Carter KC, Alexander J, Tacchini-Cottier F. Neutrophils contribute to development of a protective immune response during onset of infection with Leishmania donovani. Infect Immun. 2008;76(2):532–41. https://doi.org/10.1128/IAI.01388-07.
65. Merida-De-Barros DA, Chaves SP, Belmiro CLR, Wanderley JLM. Leishmaniasis and glycosaminoglycans: a future therapeutic strategy? 11 medical and health sciences 1108 medical microbiology 06 biological sciences 0601 biochemistry and cell biology. Parasit Vectors. 2018;11(1):1–12. https://doi.org/10.1186/s13071-018-2953-y.
66. Mogensen TH. Pathogen recognition and inflammatory signaling in innate immune defenses. Clin Microbiol Rev. 2009;22(2):240–73. https://doi.org/10.1128/CMR.00046-08.
67. Gregory DJ, Sladek R, Olivier M, Matlashewski G. Comparison of the effects of Leishmania major or Leishmania donovani infection on macrophage gene expression. Infect Immun. 2008;76(3):1186–92. https://doi.org/10.1128/IAI.01320-07.
68. Zhang JM, An J. Cytokines, inflammation, and pain. Int Anesthesiol Clin. 2007;45(2):27–37. https://doi.org/10.1097/AIA.0B013E318034194E.
69. Andargie TE, Diro Ejara E. Pro- and anti-inflammatory cytokines in visceral leishmaniasis. J Cell Sci Ther. 2016;06:02. https://doi.org/10.4172/2157-7013.1000206.
70. Cavaillon J. Pro- versus anti-inflammatory cytokines: myth or reality. Cell Mol Biol (Noisy-le-Grand). 2001;47(4):695–702.

71. Cherezov V, Rosenbaum DM, Hanson MA, Rasmussen SGF, Foon ST, Kobilka TS, Choi HJ, Kuhn P, Weis WI, Kobilka BK, Stevens RC. High-resolution crystal structure of an engineered human β2-adrenergic G protein-coupled receptor. Science (80- ). 2007;318(5854):1258–65. https://doi.org/10.1126/SCIENCE.1150577.
72. Luo J, Yang H, Song B-L. Mechanisms and regulation of cholesterol homeostasis. Nat Rev Mol Cell Biol. 2019;21(4):225–45. https://doi.org/10.1038/s41580-019-0190-7.
73. Wong LH, Gatta AT, Levine TP. Lipid transfer proteins: the lipid commute via shuttles, bridges and tubes. Nat Rev Mol Cell Biol. 2018;20(2):85–101. https://doi.org/10.1038/s41580-018-0071-5.
74. Takahashi M, Murate M, Fukuda M, Sato SB, Ohta A, Kobayashi T. Cholesterol controls lipid endocytosis through Rab11. 2007;18(7):2667–77. https://doi.org/10.1091/MBC.E06-10-0924.
75. Coxey R, Pentchev P, Campbell G, Blanchette-Mackie E. Differential accumulation of cholesterol in Golgi compartments of normal and Niemann-pick type C fibroblasts incubated with LDL: a cytochemical freeze-fracture study. J Lipid Res. 1993;34(7):1165–76. https://doi.org/10.1016/S0022-2275(20)37704-X.
76. Mukherjee S, Zha X, Tabas I, Maxfield FR. Cholesterol distribution in living cells: fluorescence imaging using dehydroergosterol as a fluorescent cholesterol analog. Biophys J. 1998;75(4):1915–25. https://doi.org/10.1016/S0006-3495(98)77632-5.
77. Lange Y. Disposition of intracellular cholesterol in human fibroblasts. J Lipid Res. 1991;32(2):329–39. https://doi.org/10.1016/S0022-2275(20)42093-0.
78. Goldstein JL, DeBose-Boyd RA, Brown MS. Protein sensors for membrane sterols. Cell. 2006;124(1):35–46. https://doi.org/10.1016/J.CELL.2005.12.022.
79. Feng B, Yao PM, Li Y, Devlin CM, Zhang D, Harding HP, Sweeney M, Rong JX, Kuriakose G, Fisher EA, Marks AR, Ron D, Tabas I. The endoplasmic reticulum is the site of cholesterol-induced cytotoxicity in macrophages. Nat Cell Biol. 2003;5(9):781–92. https://doi.org/10.1038/ncb1035.
80. Ridsdale A, Denis M, Gougeon P-Y, Ngsee JK, Presley JF, Zha X. Cholesterol is required for efficient endoplasmic reticulum-to-golgi transport of secretory membrane. Proteins. 2006;17(4):1593–605. https://doi.org/10.1091/MBC.E05-02-0100.
81. Runz H, Miura K, Weiss M, Pepperkok R. Sterols regulate ER-export dynamics of secretory cargo protein ts-O45-G. EMBO J. 2006;25(13):2953–65. https://doi.org/10.1038/SJ.EMBOJ.7601205.
82. Nohturfft A, Brown MS, Goldstein JL. Sterols regulate processing of carbohydrate chains of wild-type SREBP cleavage-activating protein (SCAP), but not sterol-resistant mutants Y298C or D443N. Proc Natl Acad Sci. 1998;95(22):12848–53. https://doi.org/10.1073/PNAS.95.22.12848.
83. Sun L-P, Li L, Goldstein JL, Brown MS. Insig required for sterol-mediated inhibition of Scap/SREBP binding to COPII proteins in vitro*◆. J Biol Chem. 2005;280(28):26483–90. https://doi.org/10.1074/JBC.M504041200.
84. Miller E, Antonny B, Hamamoto S, Schekman R. Cargo selection into COPII vesicles is driven by the Sec24p subunit. EMBO J. 2002;21(22):6105–13. https://doi.org/10.1093/EMBOJ/CDF605.
85. Simons K, Vaz WLC. Model systems, lipid rafts, and cell membranes 1. Annu Rev Biophys Biomol Struct. 2004;33:269–95. https://doi.org/10.1146/ANNUREV.BIOPHYS.32.110601.141803.
86. Murata M, Peränen J, Schreiner R, Wieland F, Kurzchalia TV, Simons K. VIP21/caveolin is a cholesterol-binding protein. Proc Natl Acad Sci. 1995;92(22):10339–43. https://doi.org/10.1073/PNAS.92.22.10339.
87. Radhakrishnan A, Sun L-P, Kwon HJ, Brown MS, Goldstein JL. Direct binding of cholesterol to the purified membrane region of SCAP: mechanism for a sterol-sensing domain. Mol Cell. 2004;15(2):259–68. https://doi.org/10.1016/J.MOLCEL.2004.06.019.

88. Tewary P, Veena K, Pucadyil TJ, Chattopadhyay A, Madhubala R. The sterol-binding antibiotic nystatin inhibits entry of non-opsonized Leishmania donovani into macrophages. Biochem Biophys Res Commun. 2006;339(2):661–6. https://doi.org/10.1016/J.BBRC.2005.11.062.
89. Rodríguez NE, Gaur U, Wilson ME. Role of caveolae in Leishmania chagasi phagocytosis and intracellular survival in macrophages. Cell Microbiol. 2006;8(7):1106–20. https://doi.org/10.1111/J.1462-5822.2006.00695.X.
90. Olivier M, Gregory DJ, Forget G. Subversion mechanisms by which Leishmania parasites can escape the host immune response: a signaling point of view. Clin Microbiol Rev. 2005;18(2):293–305. https://doi.org/10.1128/CMR.18.2.293-305.2005.
91. Chakraborty D, Banerjee S, Sen A, Banerjee KK, Das P, Roy S. Leishmania donovani affects antigen presentation of macrophage by disrupting lipid rafts. J Immunol. 2005; https://doi.org/10.4049/jimmunol.175.5.3214.
92. Sen E, Chattopadhyay S, Bandopadhyay S, De T, Roy S. Macrophage heterogeneity, antigen presentation, and membrane fluidity: implications in visceral leishmaniasis. Scand J Immunol. 2001;53(2):111–20. https://doi.org/10.1046/J.1365-3083.2001.00856.X.
93. Mouritsen OG, Zuckermann MJ. What's so special about cholesterol? Lipids. 2004;39(11):1101–13. https://doi.org/10.1007/S11745-004-1336-X.
94. Pucadyil TJ, Chattopadhyay A. Cholesterol: a potential therapeutic target in leishmania infection? Trends Parasitol. 2007;23(2):49–53. https://doi.org/10.1016/j.pt.2006.12.003.
95. Alemayehu B, Alemayehu M. Leishmaniasis: a review on parasite, vector and reservoir host. Health Sci J. 2017;11(4):1–6. https://doi.org/10.21767/1791-809x.1000519.
96. Bailey MS, Lockwood DNJ. Cutaneous leishmaniasis. Clin Dermatol. 2007;25(2):203–11. https://doi.org/10.1016/J.CLINDERMATOL.2006.05.008.
97. Killick-Kendrick R. The biology and control of Phlebotomine sand flies. Clin Dermatol. 1999;17(3):279–89. https://doi.org/10.1016/S0738-081X(99)00046-2.
98. Nylén S, Eidsmo L. Tissue damage and immunity in cutaneous leishmaniasis. Parasite Immunol. 2012;34(12):551–61. https://doi.org/10.1111/PIM.12007.
99. Vannier-Santos M, Martiny A, Souza W. Cell biology of leishmania spp.: invading and evading. Curr Pharm Des. 2005;8(4):297–318. https://doi.org/10.2174/1381612023396230.
100. Gomes CMC, Giannella-Neto D, Gama MEA, Pereira JCR, Campos MB, Corbett CEP. Correlation between the components of the insulin-like growth factor I system, nutritional status and visceral leishmaniasis. Trans R Soc Trop Med Hyg. 2007;101(7):660–7. https://doi.org/10.1016/J.TRSTMH.2007.02.017.
101. Pearson RD, Cox G, Jeronimo SMB, Castracane J, Drew JS, Evans T, Alencar JED. Visceral leishmaniasis: a model for infection-induced cachexia. Am J Trop Med Hyg. 1992;47(1_Suppl):8–15. https://doi.org/10.4269/AJTMH.1992.47.8.
102. Cunningham TJ, Duester G. Mechanisms of retinoic acid signalling and its roles in organ and limb development. Nat Rev Mol Cell Biol. 2015;
103. Das BC, Thapa P, Karki R, Das S, Mahapatra S, Liu TC, Torregroza I, Wallace DP, Kambhampati S, Van Veldhuizen P, Verma A, Ray SK, Evans T. Retinoic acid signaling pathways in development and diseases. Bioorganic Med Chem. 2014;
104. Gilbert C. What is vitamin a and why do we need it? Community Eye Health. 2013;26(84):65.
105. MacDonald PN, Ong DE. A lecithin: retinol acyltransferase activity in human and rat liver. Biochem Biophys Res Commun. 1988;156(1):157–63. https://doi.org/10.1016/S0006-291X(88)80818-0.
106. Pawlikowski B, Wragge J, Siegenthaler JA. Retinoic acid signaling in vascular development. Genesis. 2019;57(7–8):e23287. https://doi.org/10.1002/DVG.23287.
107. Rhinn M, Dollé P. Retinoic acid signalling during development. Development. 2012;139(5):843–58. https://doi.org/10.1242/dev.065938.
108. Kai-rong T, Andrew WN, Chan-Lan Sun L, Ellen L. The isolation and characterization of purified heterocomplexes of recombinant retinoic acid receptor and retinoid X receptor ligand binding domains†. Biochemistry. 1997;36(19):5669–76. https://doi.org/10.1021/BI9627020.

109. Saurat J-H. Retinoids and psoriasis: novel issues in retinoid pharmacology and implications for psoriasis treatment. J Am Acad Dermatol. 1999;41(3):S2–6. https://doi.org/10.1016/S0190-9622(99)70358-0.
110. Mukherjee S, Date A, Patravale V, Korting HC, Roeder A, Weindl G. Retinoids in the treatment of skin aging: an overview of clinical efficacy and safety. Clin Interv Aging. 2006;1(4):327–48. https://doi.org/10.2147/ciia.2006.1.4.327.
111. Pechère M, Pechère JC, Siegenthaler G, Germanier L, Saurat JH. Antibacterial activity of retinaldehyde against propionibacterium acnes. Dermatology. 1999;199(SUPPL. 1):29–31. https://doi.org/10.1159/000051375.
112. Thielitz A, Abdel-Naser MB, Fluhr JW, Zouboulis CC, Gollnick H. Topical retinoids in acne—an evidence-based overview. JDDG J der Dtsch Dermatologischen Gesellschaft. 2008;6(12):1023–31. https://doi.org/10.1111/J.1610-0387.2008.06741.X.
113. Wu K, Kim H-T, Rodriquez JL, Munoz-Medellin D, Mohsin SK, Hilsenbeck SG, Lamph WW, Gottardis MM, Shirley MA, Kuhn JG, Green JE, Brown PH. 9-cis-retinoic acid suppresses mammary tumorigenesis in C3(1)-simian virus 40 T antigen-transgenic mice. Clin Cancer Res. 2000;6:9.
114. Christov KT, Moon RC, Lantvit DD, Boone CW, Steele VE, Lubet RA, Kelloff GJ, Pezzuto JM. 9-cis-retinoic acid but not 4-(hydroxyphenyl)retinamide inhibits prostate intraepithelial neoplasia in noble rats. Cancer Res. 2002;62:18.
115. Baumann L, Vujevich J, Halem M, Martin LK, Kerdel F, Lazarus M, Pacheco H, Black L, Bryde J. Open-label pilot study of alitretinoin gel 0.1% in the treatment of photoaging. Cutis. 2005;76(1):69–73.
116. Sano K, Takayama T, Murakami K, Saiki I, Makuuchi M. Overexpression of retinoic acid receptor α in hepatocellular carcinoma. Clin Cancer Res. 2003;9(10):3679–83.
117. Sako T, Nakayama Y, Minagawa N, Inoue Y, Onitsuka K, Katsuki T, Tsurudome Y, Shibao K, Hirata K, Nagata N, Ohie S, Kohno K, Itoh H. 4-[3,5-bis(trimethylsilyl)benzamido] benzoic acid (TAC-101) induces apoptosis in colon cancer partially through the induction of fas expression. In Vivo (Brooklyn). 2005;19(1):125–32.
118. Duvic M, Hymes K, Heald P, Breneman D, Martin AG, Myskowski P, Crowley C, Yocum RC, Group for M of the BWS. Bexarotene is effective and safe for treatment of refractory advanced-stage cutaneous T-cell lymphoma: multinational phase II-III trial results. J Clin Oncol. 2001;19(9):2456–71. https://doi.org/10.1200/JCO.2001.19.9.2456.
119. Sun S-Y, Yue P, Kelloff GJ, Steele VE, Lippman SM, Hong WK, Lotan R. Identification of retinamides that are more potent than N-(4-hydroxyphenyl)retinamide in inhibiting growth and inducing apoptosis of human head and neck and lung cancer cells. Cancer Epidemiol Prev Biomarkers. 2001;10:6.
120. Appleyard VCL, O'Neill MA, Murray KE, Bray SE, Thomson G, Kernohan NM, Varani J, Zhang J, Thompson AM. Activity of MDI-301, a novel synthetic retinoid, in xenografts. Anti-Cancer Drugs. 2004;15(10):991–6. https://doi.org/10.1097/00001813-200411000-00009.
121. Warner RL, Bhagavathula N, Nerusu K, Hanosh A, McClintock SD, Naik MK, Johnson KJ, Ginsburg I, Varani J. MDI 301, a nonirritating retinoid, improves abrasion wound healing in damaged/atrophic skin. Wound Repair Regen. 2008;16(1):117–24. https://doi.org/10.1111/J.1524-475X.2007.00338.X.
122. Brennan BJ, Brown AB, Kolis SJ, Rutman O, Gooden C, Davies BE. Effect of R667, a novel emphysema agent, on the pharmacokinetics of midazolam in healthy men. J Clin Pharmacol. 2006;46(2):222–8. https://doi.org/10.1177/0091270005283836.
123. Miwako I, Kagechika H. Tamibarotene. Drugs Today. 2007;43(8):563–8. https://doi.org/10.1358/DOT.2007.43.8.1072615.
124. Kawahara K, Nishi K, Suenobu M, Ohtsuka H, Maeda A, Nagatomo K, Kuniyasu A, Staufenbiel M, Nakagomi M, Shudo K, Nakayama H. Oral administration of synthetic retinoid Am80 (tamibarotene) decreases brain β-amyloid peptides in APP23 mice. Biol Pharm Bull. 2009;32(7):1307–9. https://doi.org/10.1248/BPB.32.1307.

125. Kwak SH, Nam G-S, Bae SH, Jung J. Effect of specific retinoic acid receptor agonists on noise-induced hearing loss. Int J Environ Res Public Health. 2019;16:3428. https://doi.org/10.3390/IJERPH16183428.
126. Khorana HG. Rhodopsin, photoreceptor of the rod cell. An emerging pattern for structure and function. J Biol Chem. 1992;267(1):1–4. https://doi.org/10.1016/S0021-9258(18)48444-X.
127. Cvekl A, Wang WL. Retinoic acid signaling in mammalian eye development. Exp Eye Res. 2009;89(3):280–91. https://doi.org/10.1016/j.exer.2009.04.012.
128. Kedishvili NY. Enzymology of retinoic acid biosynthesis and degradation: thematic review series: fat-soluble vitamins: vitamin A. J Lipid Res. 2013;54(7):1744–60. https://doi.org/10.1194/JLR.R037028.
129. Parker RO, Crouch RK. Retinol dehydrogenases (RDHs) in the visual cycle. Exp Eye Res. 2010;91(6):788–92. https://doi.org/10.1016/J.EXER.2010.08.013.
130. Petkovich PM. Retinoic acid metabolism. J Am Acad Dermatol. 2001;45(5):S136–42. https://doi.org/10.1067/MJD.2001.113715.
131. Blomhoff R, Blomhoff HK. Overview of retinoid metabolism and function. J Neurobiol. 2006;66(7):606–30. https://doi.org/10.1002/NEU.20242.
132. Dong D, Ruuska SE, Levinthal DJ, Noy N. Distinct roles for cellular retinoic acid-binding proteins I and II in regulating Signaling by retinoic acid *. J Biol Chem. 1999;274(34):23695–8. https://doi.org/10.1074/JBC.274.34.23695.
133. Napoli JL. Biochemical pathways of retinoid transport, metabolism, and signal transduction. Clin Immunol Immunopathol. 1996;80(3):S52–62. https://doi.org/10.1006/CLIN.1996.0142.
134. Theodosiou M, Laudet V, Schubert M. From carrot to clinic: an overview of the retinoic acid signaling pathway. Cell Mol Life Sci. 2010;67(9):1423–45. https://doi.org/10.1007/S00018-010-0268-Z.
135. Heyman RA, Mangelsdorf DJ, Dyck JA, Stein RB, Eichele G, Evans RM, Thaller C. 9-cis retinoic acid is a high affinity ligand for the retinoid X receptor. Cell. 1992;68(2):397–406. https://doi.org/10.1016/0092-8674(92)90479-V.
136. Mangelsdorf DJ, Evanst RM. The RXR heterodimers and orphan receptors. Cell. 1995;83:841–50.
137. Rochette-Egly C, Germain P. Dynamic and combinatorial control of gene expression by nuclear retinoic acid receptors (RARs). Nucl Recept Signal. 2009;7:e005. https://doi.org/10.1621/NRS.07005.
138. Shulman AI, Mangelsdorf DJ. Retinoid x receptor heterodimers in the metabolic syndrome. N Engl J Med. 2005;353(6):604–15. https://doi.org/10.1056/NEJMRA043590.
139. Al Tanoury Z, Piskunov A, Rochette-Egly C. Vitamin a and retinoid signaling: genomic and nongenomic effects. J Lipid Res. 2013;54(7):1761–75. https://doi.org/10.1194/jlr.R030833.
140. Bastien J, Rochette-Egly C. Nuclear retinoid receptors and the transcription of retinoid-target genes. Gene. 2004;328(1–2):1–16. https://doi.org/10.1016/J.GENE.2003.12.005.
141. Cotnoir-White D, Laperrière D, Mader S. Evolution of the repertoire of nuclear receptor binding sites in genomes. Mol Cell Endocrinol. 2011;334(1–2):76–82. https://doi.org/10.1016/J.MCE.2010.10.021.
142. Verma P, Kureel AK, Saini S, Prakash S, Kumari S, Kottarath SK, Srivastava SK, Bhat M, Dinda AK, Thakur CP, Sharma S, Rai AK. Leishmania donovani reduces the levels of retinoic acid–synthesizing enzymes in infected macrophages and favoring its own survival. Parasitol Res. 2018;118(1):63–71. https://doi.org/10.1007/S00436-018-6115-0.
143. Moore KJ, Sheedy FJ, Fisher EA. Macrophages in atherosclerosis: a dynamic balance. Nat Rev Immunol. 2013;13(10):709–21. https://doi.org/10.1038/nri3520.
144. Phillips MC. Molecular mechanisms of cellular cholesterol efflux. J Biol Chem. 2014;289(35):24020–9. https://doi.org/10.1074/jbc.R114.583658.
145. Duffy D, Rader DJ. Update on strategies to increase HDL quantity and function. Nat Rev Cardiol. 2009;6(7):455–63. https://doi.org/10.1038/nrcardio.2009.94.

146. Klappacher GW, Glass CK. Roles of peroxisome proliferator-activated receptor γ in lipid homeostasis and inflammatory responses of macrophages. Curr Opin Lipidol. 2002;13(3):305–12.
147. Gelissen IC, Harris M, Rye K-A, Quinn C, Brown AJ, Kockx M, Cartland S, Packianathan M, Kritharides L, Jessup W. ABCA1 and ABCG1 synergize to mediate cholesterol export to ApoA-I. Arterioscler Thromb Vasc Biol. 2006;26(3):534–40. https://doi.org/10.1161/01.ATV.0000200082.58536.E1.
148. Hara H, Yokoyama S. Interaction of free apolipoproteins with macrophages. Formation of high density lipoprotein-like lipoproteins and reduction of cellular cholesterol. J Biol Chem. 1991;266(5):3080–6. https://doi.org/10.1016/S0021-9258(18)49957-7.
149. Kennedy MA, Barrera GC, Nakamura K, Baldán Á, Tarr P, Fishbein MC, Frank J, Francone OL, Edwards PA. ABCG1 has a critical role in mediating cholesterol efflux to HDL and preventing cellular lipid accumulation. Cell Metab. 2005;1(2):121–31. https://doi.org/10.1016/J.CMET.2005.01.002.
150. Nakamura K, Kennedy MA, Baldán A, Bojanic DD, Lyons K, Edwards PA. Expression and regulation of multiple murine ATP-binding cassette transporter G1 mRNAs/isoforms that stimulate cellular cholesterol efflux to high density lipoprotein *. J Biol Chem. 2004;279(44):45980–9. https://doi.org/10.1074/JBC.M408652200.
151. Tarling EJ, Edwards PA. ATP binding cassette transporter G1 (ABCG1) is an intracellular sterol transporter. Proc Natl Acad Sci. 2011;108(49):19719–24. https://doi.org/10.1073/PNAS.1113021108.
152. Wang N, Lan D, Chen W, Matsuura F, Tall AR. ATP-binding cassette transporters G1 and G4 mediate cellular cholesterol efflux to high-density lipoproteins. Proc Natl Acad Sci. 2004;101(26):9774–9. https://doi.org/10.1073/PNAS.0403506101.
153. Ayaori M, Yakushiji E, Ogura M, Nakaya K, Hisada T, Uto-Kondo H, Takiguchi S, Terao Y, Sasaki M, Komatsu T, Iizuka M, Yogo M, Uehara Y, Kagechika H, Nakanishi T, Ikewaki K. Retinoic acid receptor agonists regulate expression of ATP-binding cassette transporter G1 in macrophages. Biochim Biophys Acta Mol Cell Biol Lipids. 2012;1821(4):561–72. https://doi.org/10.1016/J.BBALIP.2012.02.004.
154. Zhang Y, Beyer TP, Bramlett KS, Yao S, Burris TP, Schmidt RJ, Eacho PI, Cao G. Liver X receptor and retinoic X receptor mediated ABCA1 regulation and cholesterol efflux in macrophage cells—messenger RNA measured by branched DNA technology. Mol Genet Metab. 2002;77(1–2):150–8. https://doi.org/10.1016/S1096-7192(02)00111-7.
155. Zhou W, Lin J, Chen H, Wang J, Liu Y, Xia M. Retinoic acid induces macrophage cholesterol efflux and inhibits atherosclerotic plaque formation in apoE-deficient mice. Br J Nutr. 2015;114(4):509–18. https://doi.org/10.1017/S0007114515002159.
156. Maciel BLL, Valverde JG, Rodrigues-Neto JF, Freire-Neto F, Keesen TSL, Jeronimo SMB. Dual immune modulatory effect of vitamin a in human visceral leishmaniasis. PLoS One. 2014;9:9. https://doi.org/10.1371/journal.pone.0107564.
157. Vellozo NS, Pereira-Marques ST, Cabral-Piccin MP, Filardy AA, Ribeiro-Gomes FL, Rigoni TS, DosReis GA, Lopes MF. All-trans retinoic acid promotes an M1-to M2-phenotype shift and inhibits macrophage-mediated immunity to Leishmania major. Front Immunol. 2017;8(NOV) https://doi.org/10.3389/fimmu.2017.01560.
158. Pino-Lagos K, Guo Y, Noelle RJ. Retinoic acid: a key player in immunity. Biofactors. 2010;36(6):430–6. https://doi.org/10.1002/BIOF.117.
159. Chen J, Costa LG, Guizzetti M. Retinoic acid isomers up-regulate ATP binding cassette A1 and G1 and cholesterol efflux in rat astrocytes: implications for their therapeutic and teratogenic effects. J Pharmacol Exp Ther. 2011;338(3):870–8. https://doi.org/10.1124/jpet.111.182196.
160. He Y, Gong L, Fang Y, Zhan Q, Liu HX, Lu Y, Guo GL, Lehman-McKeeman L, Fang J, Wan YJY. The role of retinoic acid in hepatic lipid homeostasis defined by genomic binding and transcriptome profiling. BMC Genomics. 2013;14(1):1. https://doi.org/10.1186/1471-2164-14-575.

161. Prakash S, Kumar Rai A. Retinoic acid increases the cellular cholesterol predominantly in a mTOR-independent manner. Immunol Res. 2022; https://doi.org/10.1007/S12026-022-09292-X.
162. Simons K, Toomre D. Lipid rafts and signal transduction. Nat Rev Mol Cell Biol. 2000;1(1):31–9. https://doi.org/10.1038/35036052.
163. Yang ST, Kreutzberger AJB, Lee J, Kiessling V, Tamm LK. The role of cholesterol in membrane fusion. Chem Phys Lipids. 2016;199:136–43. https://doi.org/10.1016/J.CHEMPHYSLIP.2016.05.003.
164. Roy K, Mandloi S, Chakrabarti S, Roy S. Cholesterol corrects altered conformation of MHC-II protein in leishmania donovani infected macrophages: implication in therapy. PLoS Negl Trop Dis. 2016;10(5):1–23. https://doi.org/10.1371/journal.pntd.0004710.
165. Ghosh J, Guha R, Das S, Roy S. Liposomal cholesterol delivery activates the macrophage innate immune arm to facilitate intracellular leishmania donovani killing. Infect Immun. 2014;82(2):607–17. https://doi.org/10.1128/IAI.00583-13.
166. Prakash S, Rai AK. Retinoic acid increases cellular cholesterol in Leishmania donovani-infected macrophages in an mTOR-independent manner. Microbiol Spectr. 2022:e0269922. https://doi.org/10.1128/spectrum.02699-22.

# Role and Pathophysiology of Protozoan Parasites Causing Liver Diseases

Amresh Kumar Singh, Suraiya Khanam Ansari, Alok Raghav, and Vivek Gaur

**Abstract**

Protozoans are responsible for numerous chronic and severe human diseases worldwide. These are transmitted through food and water along with blood transfusion and physical organ transplantation. Apart from tropical parasites, other protozoans including *Entamoeba histolytica*, *Cryptosporidium*, and *Giardia* cause debilitating and fatal human diseases. Countries with low socioeconomic status like India, Mexico, Africa, and Central and South America showed higher prevalence of protozoan infections. Protozoan species invading into the intestine can easily get access to the human liver through the bloodstream and cause chronic liver diseases. Impairment due to such protozoan in the liver causes abnormal liver enzyme activities. These infectious protozoan parasites cause liver abscesses and colitis and are responsible for epidemic in developing and tropical nations. Several drugs including metronidazole/tinidazole, iodoquinol, paromomycin, and diloxanide furoate are currently available for the treatment

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of hepatic abscess along with few proteolytic agents such as ritonavir, saquinavir, and indinavir that effectively and efficiently inhibit the growth of protozoans. Moreover, newer treatment and therapeutic approaches are needed to control protozoan-associated infection without any side effects that are associated with the drugs. Such newer therapies and research will help in the reduction of protozoan-related disease burden especially in developing countries.

**Keywords**

Pathophysiology of protozoan · Liver diseases · Protozoan parasites · Treatment for liver diseases by protozoans

## 1 Introduction

Protozoa belongs to the subkingdom of the kingdom Protista, although classical system designates them in the Animalia kingdom. Protozoans are present in each and every ecosystem and habitat. Historical evidences proved that Anton van Leeuwenhoek has firstly seen the protozoan using simple microscopes. It is believed that every human being is the habitat of protozoan, known as commensals. Protozoans invading humans are unicellular eukaryotes that demonstrate size of less than 50 μm. The smallest known protozoan ranges from 1 to 10 μm in size, while the longest species reported showed 150 μm in size [1]. Protozoan presents vesicular diffuse nucleus with scattered chromatin. Protozoan vesicular nucleus consists of a central body, which is referred to as endosome or karyosome. *Amebas* and *trypanosomes* are species that are devoid of such endosome having nucleic material. However, Apicomplexa contains more than one nucleolus having DNA, while ciliates have micro- and macronucleus both with homogeneous organization. Protozoan organelles perform the same function as the organs of higher animals, and their plasma membrane (PM) helps to perform locomotion functions through pseudopodia, flagella, and cilia. In some protozoan including *trypanosomes* and *Giardia*, pellicle plays an important role in providing rigidity and shape.

The structure of the cytoplasm in most protozoans can be easily seen in species such as amoebas in which there is distinct ectoplasm and endoplasm. Cytosome of the protozoans performed the function of engulfing fluids and solids, while the contractile vacuoles performed the function of osmoregulation in species like *Naegleria* and *Balantidium* [1]. Aplicomplexa have subpellicular microtubule and are devoid of external locomotory organelles. In some of the protozoans including trichomonads and trypanosomes, an undulating membrane flanked by flagellum and the body wall is present [1]. Protozoan also contains other vital organelles including food vacuoles, mitochondria, lysosomes, and Golgi complex [1].

However, some of the protozoans are considered to be non-harmful, while some are the causes of the various life-threatening diseases. Asymptomatic host

individuals and immunosuppressed patients are always the carriers of the protozoan disease, and they pass it to other individuals. In one of the standard instances, several individuals harbor *Pneumocystis carinii* in their lungs, sometimes causing pneumonia in immunosuppressed patients especially in those patients who are suffering from acquired immunodeficiency syndrome (AIDS) [2]. *Toxoplasma gondii* is also among the common protozoan that is the cause of toxoplasmic encephalitis in AIDS patients [3]. *Cryptosporidium* is also a common protozoan that is responsible for life-threatening infection in patients with AIDS [4]. Amebic meningoencephalitis is another fatal disease caused by the soil and water habitant protozoan including *Acanthamoeba* and *Naegleria.*

Intestine-invading protozoan species easily get access to the human liver through the bloodstream and cause chronic liver diseases. *Entamoeba histolytica*, a causative agent of amebiasis, which is an infection of the liver cells through hepatic portal blood supply, invade intra-hepatic portal vessels and initiate lytic necrosis. Involvement of the liver is a key presenting feature in amebiasis; however, *Cryptosporidium* and *Giardia* contribute to hepatobiliary changes especially in immunocompromised individuals. Amebic hepatic abscess is caused by the protozoan *Entamoeba histolytica* possibly due to consumption of feces-contaminated water and food. *E. histolytica* after invading the human body reaches the mesenteric vessels and finally targets the liver.

The World Health Organization (WHO) showed concern for protozoan disease including malaria, leishmaniasis, and trypanosomiasis and promoted several research and training programs. However, paucity of effective and efficient drugs and vaccines against protozoans increased the attention of scientist and researchers to explore newer therapeutic approaches and management skills. The present chapter focused on the protozoan epidemiology, diagnostic methods for the protozoans, and their role in liver diseases.

## 2 Epidemiology

### 2.1 *Entamoeba histolytica*

*E. histolytica* is a leading protozoan of several global diseases, and it remains the third leading cause of death due to parasitic infections [5]. However, *E. histolytica* contribute 90% to the asymptomatic infections with 50 million symptomatic individuals around the globe, contributing 1 lakh annual deaths [6]. It is evident from the previously published literature that prevalent infection was made by *E. histolytica* or *E. dispar* but the common cause of amoebic colitis and extra-intestinal amoebiasis is due to *E. histolytica* in comparison with other species. However, *E. dispar* is a non-pathogenic form of the protozoans [7].

Countries with low socioeconomic status like India, Mexico, Africa, and Central and South America showed higher prevalence of protozoan infections. In one of the studies conducted in Bangladesh, *E. histolytica* showed a prevalence of 2.2% in preschool children [8]. In another Mexican seroprevalence study conducted in rural

areas, 42% prevalence was observed caused by *E. histolytica* [9]. These low-income countries with high prevalence of infection demonstrated fecal-oral transmission as the major risk factor for the spread of protozoan infection due to poor hand hygiene and defecation in the drinking water bodies especially in the areas residing on the river banks. It is evident that developed nations like the United States showed a negligible death of five per year due to the amebiasis infection, and also in such nations, only travelers from endemic countries lead to these infection-related mortalities [9].

Authors from previously published study showed that amoebic colitis infection affects population of all ages and sexes. It has been also reported that gay and bisexual population are at higher risk of developing such infection due to risk of oral and anal sex transmitted through fecal-oral contamination [7]. Other factors that contributed in increasing the risk of mortality associated with the protozoan infection include corticosteroid interventions, pregnancy, alcoholism, malnutrition, and malignancies. Previously published study showed that amoebic liver abscess affects people with age range of 18 to 50 years three times than people with deranged age groups [7].

## 2.2 *Cryptosporidium* spp.

*Cryptosporidium* spp. is the major causal agent of cryptosporidiosis around the globe and infects the humans, thereby causing acute gastroenteritis, diarrhea, and abdominal pain [10]. Like *E. histolytica*, the mode of transmission in cryptosporidiosis is fecal contaminated water and food ingestion [10]. According to a report, there has been an upsurge in cases, with 3 cases per 100,000 population [10]. It has been seen that the incidence and prevalence of cryptosporidiosis are lowest in developed and industrialized countries compared to the developing countries, due to lack of proper sanitation and potable water in latter nations [11, 12]. Previously published studies reported that in immunocompetent individuals, cryptosporidiosis is rarely reported [11, 12]. Moreover, malnourished children below the age group of 5 years presented clinical symptoms of severe diarrhea with a rate of 10–15% [11, 12]. In several countries, the route of *Cryptosporidium* spp. transmission occurs due to the use of contaminated swimming pool and unhygienic source of drinking water [13, 14]. Studies showed that there are 30 known species of *Cryptosporidium* reported worldwide; however, few species including *C. hominis*, *C. parvum*, *C. meleagridis*, *C. felis*, and *C. canis* have been reported commonly in infected individuals [15, 16]. Among these species, *C. hominis* and *C. parvum* are the most prevalent intestinal species found in humans.

## 2.3 *Giardia lamblia*

Giardiasis is an intestinal infection caused by the species *G. lamblia* presenting diarrheal symptoms worldwide. Its mode of transmission includes fecal-oral,

physical contact, and sexual activity that originated as waterborne disease. Tropical countries showed trend of outbreak and endemic compared to the developed and developing nations. Infected persons showed clinical presentation of diarrhea, abdominal pain, and cramping. It has been known that till now ten *Giardia* cysts are responsible for giardiasis. Its prevalence and incidences are high among children below the age of 5 years especially in tropical and developing nations. Nevertheless, it has also been seen that many parts of the developed countries have outbreak of this disease especially in summer season, when swimming-related activities are at peak [17]. Previously published report showed there were 242 outbreaks of giardiasis that affected 41,000 individuals, out of them waterborne were 74.8%, food borne contributed 15.7%, and physical contact contributed 2.5%, while animal-mediated transmission contributed 1.2% transmission [18]. A surveillance-based study showed that giardiasis infected people with a trend of bimodal age distribution between 0–9 and 45–49 years without gender dependency but with a seasonal dependency [19]. Other risk factors associated with giardiasis include use of tap water located at endemic area and shallow well water [20, 21].

## 2.4 Malaria Parasite

Clinically tropical and subtropical regions of Africa and South America and Asia are affected due to malaria infection. Mortality due to malaria infections (93%) reported in sub-Saharan Africa is relatively higher [22]. However, even the risk varies widely, followed by 2% each in the South East Asian and Eastern Mediterranean regions, and rest contributed by the American and Western Pacific regions [23, 24]. Due to different geographical distribution of these parasites, all malaria species are not responsible for the transmission of infection in all regions. Despite the fact that urban areas have typically been less at risk, rapid unplanned population growth has contributed significantly and plays an important role to the growing issue of urban or peri-urban transmission [25]. Globally, about 3.2 billion populations are at risk of *P. vivax* infection and more than 200 million clinical cases registered annually [23].

In 2021, approximately 627,000 deaths and 241 million infective cases of malaria are reported in *World Malaria Report*. While approximately 67% of these deaths were due to disruptions during the SARS-CoV-2 pandemic, the remaining one third of deaths irrespective of COVID-19 disruptions due to a recent advancement in WHO's methodology for calculating malaria deaths [26]. More than 50% of all malaria deaths worldwide occurred in African nations, with Nigeria (31.9%), the Democratic Republic of the Congo (13.2%), United Republic of Tanzania (4.1%), and Mozambique (3.8%) accounting for the majority of these deaths. India's contribution to the global malaria burden is only 3%, according to the *WMR 2019*. India had a drop in reported malaria cases of 49% and deaths of 50.5% compared to 2017 despite having the largest malaria load in South East Asia [27].

## 2.5 *Toxoplasma gondii*

In developed countries, the distribution of toxoplasmosis is even common. Due to the differences in culture, eating systems, and the types of management of livestock, different regions of the world have different frequencies of infection [28]. *Toxoplasma gondii* is present in every country, and seropositivity rates range from under 10% to more than 90% [29]. In North America, South East Asia, Northern Europe, and the Sahelian countries of Africa, low seroprevalences (10 to 30%) have been reported. In Central and Southern European nations, moderate prevalences (30 to 50%) have been seen. Latin America and tropical African countries are the high prevalences' regions as shown in Table 1 [30]. However, it was shown that in regions with low seroprevalence, there was a low risk of perinatal transmission with untreated *T. gondii* infection [31]. In some studies, the overall estimated prevalence is 22.4% in India, but South India (37.3%) has the highest prevalence, followed by East India (21.2%) and North India (19.7%). The lowest seroprevalence (8.8%) was seen in West India. This variation was quite significant [32].

**Table 1** Possible co-relation with human diseases and geographical distribution of *Toxoplasma gondii* genotypes (courtesy: Robert et al.)

| Geographical area | Genotypes | Specific features of immunocompetent individuals with congenital toxoplasmosis |
|---|---|---|
| Europe | Type II (haplogroup 2), highly predominant; type III, more present in Southern Europe; other genotypes sporadically observed | Immunocompetent people with type II or type III are more likely to have an asymptomatic or benign condition; they also have a lower prevalence of retino-choroiditis than people of South America who have congenital toxoplasmosis |
| North America | Type II (haplogroup 2), haplogroup 12, type III (haplogroup 3), other genotypes | Asymptomatic or benign disease in immunocompetent individuals associated with type II or III; insufficient data for other haplogroups |
| South and Central America | High genotypic diversity; some haplogroups shared with Africa (haplogroup 6); type II sporadically present; type I rarely encountered; highly atypical genotypes in the Amazonian forest | Congenital toxoplasmosis patients and immunocompetent people have higher rates and severity of retino-choroiditis; disseminated, potentially fatal cases have been seen with the most unusual genotypes |
| Africa | African 1, 2, 3 (haplogroup 6); type III (haplogroup 3); type II | Higher rate of retino-choroiditis than in Europe |
| Asia | Less genotypic diversity than in South America; type III (haplogroup 3); a common haplogroup widespread across the continent | No significant data available |

### 2.6 *Leishmania donovani*

In 2012, the majority of cutaneous and visceral disorders are found in 89 different countries and account for 1.5 to 2 million new cases each year, according to the World Health Organization (WHO). Asia, Northern Africa, the Middle East, the Mediterranean region, South and Central America, and the Caribbean are all endemic to leishmaniasis. Globally, every year, approximately 70,000 deaths occur due to visceral disease [33]. Over the past 10 years, there has been a significant decline in the incidence of visceral leishmaniasis worldwide: from 200,000 to 400,000 new cases in 2012 to 50,000 to 90,000 new cases in 2017 [34].

## 3 Pathogenesis in Liver Disease

### 3.1 *Entamoeba histolytica*

*E. histolytica* is a protozoan with pseudopodia that initiate the proteolysis and lysis of tissues in humans. This infection is transmitted through ingestion of cysts that are present in fecally contaminated water and food. Trophozoites are released into the small intestine through excystation of mature cysts; later these trophozoites traveled to the large intestine. Moreover, trophozoites were doubled using the process of binary fission and produce cysts in large amount. The cysts produced by these protozoans are stable and survive from days to weeks even in the external environment. Chronic intestinal amoebiasis (CIA) is the acute phase of amoebiasis that needs to be treated pharmacologically as it is a chronic illness. CIA showed presentation of clinical symptoms including bloodless diarrhea, constipation, chronic ulcerative colitis, and colon irritability, enlargement of the liver, wasting, anemia, and low-grade fever [35].

Another protozoan-related disease includes amoebic hepatitis due to blood trophozoite transmission from the intestine to the liver. Amoebic hepatitis demonstrates enlarged liver soreness and increased body temperature with chills and perspirations. A study revealed an increase in ALT and AST enzyme activities in amoebic hepatitis [36]. Another protozoan-related disease includes amoebic liver abscess (ALA). It is a chronic complication of intestinal amoebiasis. The transmission is through the process of trofozoity and translocation to the liver from the intestine causing inflammatory changes along with fibrosis and necrosis of the liver lobules that initiates abscess with pus. Amoebic abscess of the liver presented with pains in the right upper quadrant with hepatomegaly, rise in body temperature, appetite loss, loss of weight, and positive Chelmonski sign along with elevated ESR [37].

Amoebic liver abscess (ALA) patients complains of diarrhea, dysentery in 38% of patients [38]. In one of the studies, it was found that 50% of the patients presented colonic ulcerations possibly due to portal entry. Moreover, some uncommon presentations include jaundice or icterus in 24% of the infected patients suggestive of multiple abscesses [38]. In adverse situation, complications associated with ALA include eruption of the abscess that further spread into the peritoneum with overall

presentation in 40% of the patients and peritoneal rupture in 7% of the patients [38]. In ALA cases, there are ongoing tissue damage leads to formation of abscess to the chest cavity contributes to 6 patients in study population [38]. Referring to the uncommon presentations, obstructive jaundice and inferior vena cava have also been presented in some patients.

## 3.2 *Cryptosporidium parvum*

*Cryptosporidium* is an intestinal coccidian parasite recognized worldwide, which is a known cause of diarrhea in immunocompromised and immunocompetent individuals [39]. In developing and tropical countries, *Cryptosporidium* is among the most prevalent infection in young children under the age of 5 years and AIDS patients [40]. *C. hominis* and *C. parvum* are among the most prevalent species causing infection in humans. *C. hominis* are found mostly in human, whereas *C. parvum* colonies are found mostly in domestic livestock and wild animals along with humans. On the basis of such distribution, it is divided into two genotypes termed as genotype I and genotype II, respectively, depending on the source of origin [28, 29]. Human-to-human transmission is seen in *Cryptosporidium*, which is a known zoonotic pathogen that can spread through fecal-oral mode through ingestion of the oocysts of *Cryptosporidium* [41, 42].

In one of the studies, it was seen that patients with AIDS and mutation in the gene CD154 (causing congenital X-linked immunodeficiency with hyper-IgM [XHIM]) demonstrated susceptibility to the *Cryptosporidium parvum* (CP)-associated chronic infections of the biliary tract that ultimately lead to biliary sclerosis and cholangiocarcinoma [43]. Previously published study showed an association between severe hepatic injury and hepatic failure along with decrease in cellular immunity [44]. *Cryptosporidium parvum*-related infection was presented in 32% infected patients having characteristics of hepatocellular carcinoma and severe diarrhea as compared to the 22% patients with liver cirrhosis without ascites, with 36% having liver cirrhosis with ascites [45]. In this study, *Cryptosporidium* oocytes were reported in 20% of people with hepatic illness [45].

Biliary system is the reservoir for *Cryptosporidium* protozoan, and its infection is well presented in immunocompromised patients having T-cell immunodeficiency in the form of cryptosporidiosis of the pancreato-biliary system along with sclerosing cholangitis [46]. In patients with organ transplant, significant increase in the levels of tacrolimus was observed in *Cryptosporidium* enteritis [46]. In one of the previously published studies, *Cryptosporidium parvum* has been found to initiate sclerosing cholangitis in patients with renal transplants [46]. In patients with nephrotic syndrome, *Cryptosporidium hominis* is related with the application of tacrolimus [47].

### 3.3 *Giardia lamblia*

*Giardia lamblia* is a protozoan pathogen causing diarrhea that is known to transmit through water, food, and fecal-oral transmission from infected person. In one of the previously published studies conducted on the liver of 20 rats previously infested with *Giardia muris* along with 25 patients with giardiasis, significant high levels of alanine aminotransferase were observed [48]. In similar study, it was observed in liver biopsy that 94% patients suffered from hepatic damage with confirmation of protozoan infestation into the tissue [48]. Additionally, researchers observed that the effects of giardiasis on the levels of serum retinol and the storage of vitamin A in the liver in school-age children and found that giardiasis impairs the liver retinol storage along with malabsorption of vitamin A in the intestine [49].

### 3.4 Invasion and Transition of Malaria Parasite

The way the disease develops, as well as the symptoms and signs of all kinds of malaria are similar, including fever as shown in Fig. 1a, and it can be treated easily during each symptomatic episode with specific antimalarials, and most of the patients can be easily cured from the infection when treated with proper compliance, but few of them may progress into severe malaria [50, 51].

Sporozoites directly interact with the hepatocyte surface, and several hepatocyte receptors are required for the invasion of sporozoites as shown in Fig. 1b. However, each cycle lasting between 24 and 72 hours relies on the parasite species infecting the red cells once the schizogony development is complete, lysis of infected RBCs, and release of newly developed merozoites and pigment hemozoin and other toxic substances such as glycosylphosphatidylinositol (GPI) that are also released into the blood along with them [52]. Anorexia, thrombocytopenia, immunosuppression, coagulopathy, fever with rigors, diarrhea, exhaustion, nausea, vomiting, and joint and muscle pain are some of the systemic clinical signs of malaria, and these products, specifically the GPI, activate macrophages and endothelial cells which helps to secrete cytokines and inflammatory mediators like tumor necrosis factor, IL-6, IL-8, interferon-γ, interleukin-1, macrophage colony-stimulating factor, lymphotoxin, and superoxide and nitric oxide (NO). As seen in Fig. 1b, numerous investigations have suggested that the merozoite surface proteins MSP-1, MSP-2, and MSP-4 are significant parasite toxins that cause severe sickness [51].

### 3.5 *Toxoplasma gondii*

Tachyzoite intracellular proliferation causes putrefaction, cellular inflammation, and direct cytopathic consequences. The primary goal of type I cell-mediated immunity (CMI) is to prevent both acute and chronic *T. gondii* infection. Therefore, any deficiencies in cell-mediated immunity cause the patient to get the deadly toxoplasmosis disease. The human gut's epithelial cells, damaged by tachyzoite invasion,

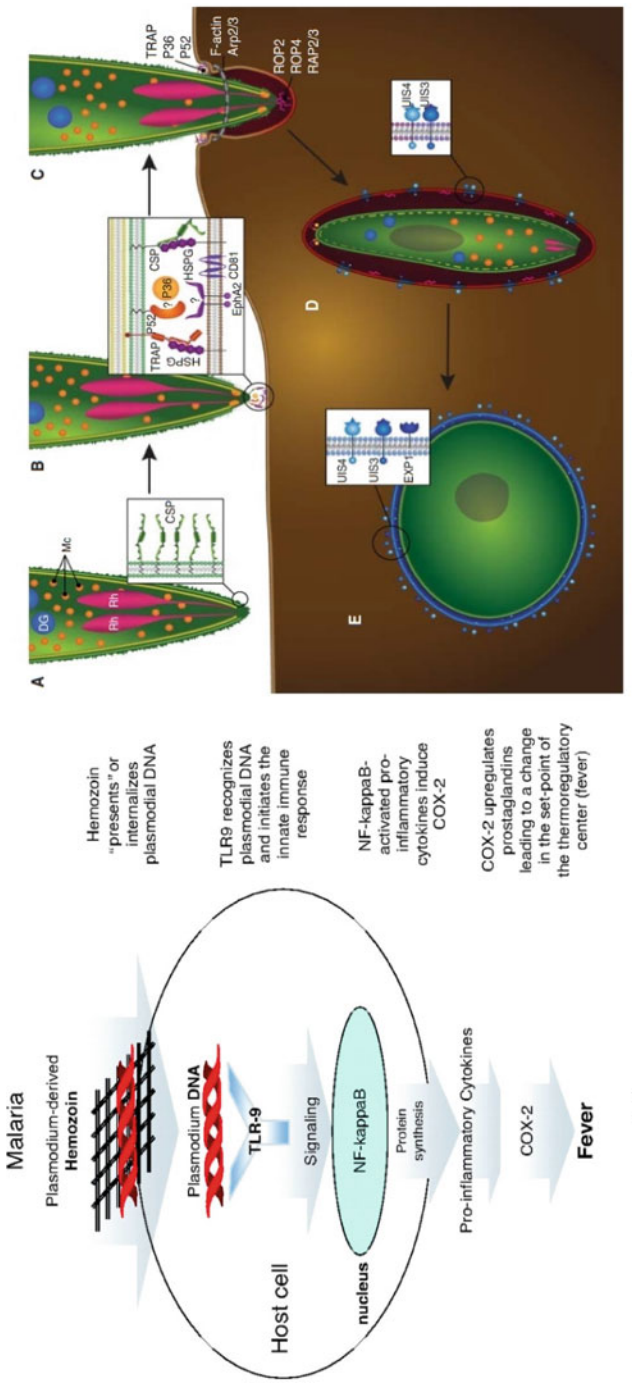


**Fig. 1** (**a**) Induction of fever by malaria parasites (courtesy: www.malariasite.com). (**b**) Sporozoite invasion of host hepatocytes (courtesy: Vaughan et al.)

release chemokines, which operate as chemical messengers and draw dendritic cells (DC), macrophages, and neutrophils to the site of the damage. Tachyzoites entering these inflammatory cells encourage and increase interleukin-12 production (IL-12). NK cells and T lymphocytes produce interferon-gamma (IFN-gamma) when IL-12 is present [53].

IFN-gamma synthesis helps to control both acute and chronic infections. Patients with acquired immunodeficiency syndrome (AIDS) and lower-than-normal CD4 counts had reduced IFN-gamma levels, which led to ongoing tachyzoite growth. An infection of cerebral and extracerebral toxoplasmosis developed in cases of acute infection and reactivation of bradyzoites during latent infection. However, humoral immunity also contributes to the control of the *T. gondii* infection by producing antibodies, regulating CD4 and CD8 T-cell responses, and increasing IFN-gamma production that is involved in CMI [54].

## 3.6 *Leishmania donovani*

The sandfly's foregut is through which the infected promastigote enters and begins to multiply. The disease spreads to the new host when the fly feeds on dogs, rodents, marsupials, or people [33]. Visceral leishmaniasis and cutaneous leishmaniasis are the two primary clinical subtypes. The most severe clinical form of this illness, known as visceral leishmaniasis, is marked by a long-lasting fever, splenomegaly, and pancytopenia [34]. Morphological changes occur in the hepatocytes, Kupffer cells, Ito cells, portal tracts, sinusoids, and hepatic veins within the liver in visceral leishmaniasis as shown in Fig. 2 [55]. Hypertrophy and hyperplasia of the Kupffer cells occur, and 44% of symptomatic cases show the presence of these cells. The pathogenesis of this change is unknown because the presentation as fulminant hepatitis is an even more unusual manifestation [55].

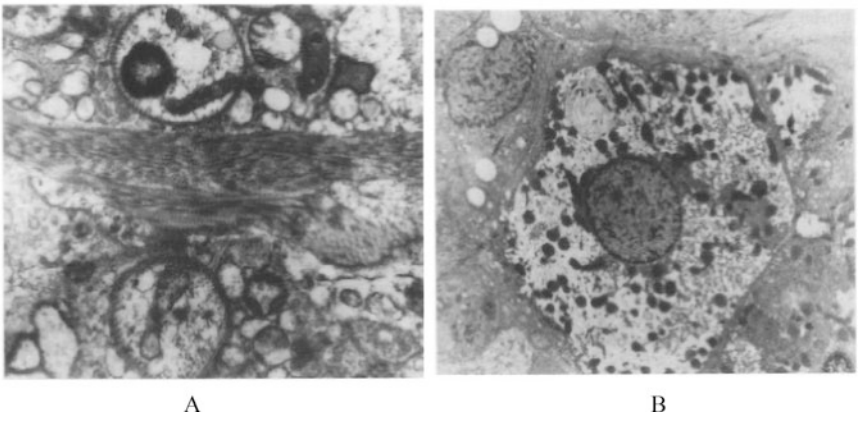


**Fig. 2** *Leishmania* parasite inside two adjacent Kupffer cells. There is collagen between the two cells (**a**). Hepatocytes showing pronounced edema of the cytoplasm (**b**) (courtesy: el Hag et al.)

## 4 Pharmacological Targeting of Drugs Against Protozoans Causing Liver Disease

*Entamoeba histolytica* is an infection-causing agent of amoebiasis that is transmitted by food and water. Several drugs are available that target *Entamoeba*, and these include oral antiparasitic medication. For treating asymptomatic infections, iodoquinol/paromomycin is commonly used [56, 57]. Metronidazole/tinidazole is the commonly available drug of choice to treat mild to moderate or severe intestinal disease along with extra-intestinal diseases including hepatic abscess [58, 59] followed by iodoquinol, paromomycin, and diloxanide furoate [56, 57]. In one of the previously published studies, the authors proposed a novel drug substitute including 2-methyl-5-nitroimidazole-1-ethanol derivatives for the treatment against *Entamoeba histolytica* [60]. In another study, metronidazole (25 mg $kg^{-1}$) and ornidazole (10 mg $kg^{-1}$) showed protective effect against *amoebic dysentery* [60]. Sharma and their coworkers synthesize new molecule 5-nitrofuran 2-carboxaldehyde thiosemicarbazones and its related bidentate $Cu^{II}$ complexes that showed positive therapeutic effect against HK-9 strain of *Entamoeba histolytica*. The above compounds showed better IC50 value for metronidazole having copper components [61, 62].

Giardiasis is also a food- and waterborne disease that is caused by the ingestion of cyst through oral route and initiates inflammation, nausea, and diarrhea-like symptoms. Recently, WHO included giardiasis in the list of neglected diseases. Currently available treatments against giardiasis include metronidazole, tinidazole, and nitazoxanide [63]. Among these available drugs, nitroimidazoles are considered to be the most effective drugs against these protozoans with mild, moderate, and transient side effects in humans [63]. In another study by Suk and their coworkers, anti-giardial agents were developed [64]. In another study, phosphonoxin was developed that exhibited anti-giardial activity by targeting giardia cyst [64].

Similarly, paromomycin drug showed therapeutic effects against *Cryptosporidium* through targeting bacterial ribosomes that hamper the synthesis of protein [65]. In another study, it is recommended that long term and low dose of azithromycin antibiotic provide protective role in chronic cryptosporidiosis in patients with AIDS [65]. Another antibiotic roxithromycin showed inhibitory role in cryptosporidiosis [66]. Nitazoxanide (NTZ) showed promising effect against the growth of protozoan and helminths. Studies showed that two metabolites of NTZ, namely, tizoxanide and tizoxanide glucuronide, showed inhibitory effect against *C. parvum* at concentration of 10 mg/L [67, 68]. Few studies used protease inhibitors including ritonavir, saquinavir, and indinavir to inhibit the growth of *C. parvum* infections [69, 70].

Leupeptin, E-64, and chymostatin used to inhibit different *Plasmodium* proteases, which include aspartate, serine, cysteine, metallo, threonine, and glutamate, are regulatory and catalytic enzymes essential to the parasite's survival. Some other protein inhibitors like azithromycin, clindamycin, doxycycline, and azithromycin + piperaquine are used as a combination drug in antenatal cases [71]. The combination of pyrimethamine and sulfadiazine/clindamycin with leucovorin added to prevent

hematologic toxicity produced by *Toxoplasma gondii* infection. Atovaquone or azithromycin might be the choice of drug used as alternate therapy in combination with pyrimethamine or sulfadiazine for the treatment and prophylaxis of toxoplasmosis [72]. Available drugs cannot be considered ideal due to their high toxicity, long duration of treatment, and severe adverse reactions for the treatment of leishmaniasis. Few alternative drugs like pentavalent antimonials, liposomal amphotericin B, amphotericin B, paromomycin, pentamidine, and miltefosine have emerged because the most commonly used drugs do not eliminate the parasites completely from all infected individuals [73].

## 5 Conclusion

The liver performs the vital function in the human body, and due to its intensive function, there is always a risk of exposure for various infectious diseases and other toxicants. These protozoans directly or indirectly affect the liver essential function depending upon the severity of the infectious phase. Several drugs including metronidazole/tinidazole, iodoquinol, paromomycin, and diloxanide furoate are currently available for the treatment of hepatic abscess along with few proteolytic agents such as ritonavir, saquinavir, and indinavir that effectively and efficiently inhibit the growth of protozoans. Moreover, newer treatment and therapeutic approaches are needed to control protozoan-associated infection without any side effects that are associated with the drugs. Such newer therapies and research will help in the reduction of protozoan-related disease burden especially in developing countries.

## References

1. Seed JR. Protozoa: pathogenesis and defenses. In: Baron S, editor. Medical Microbiology. 4th ed. University of Texas Medical Branch at Galveston; 1996.
2. White PL, Price JS, Backx M. *Pneumocystis jirovecii* pneumonia: epidemiology, clinical manifestation and diagnosis. Curr Fungal Infect Rep. 2019;13:260–73.
3. Basavaraju A. Toxoplasmosis in HIV infection: an overview. Trop Parasitol. 2016;6(2):129–35.
4. Utami WS, Murhandarwati EH, Artama WT, et al. *Cryptosporidium* infection increases the risk for chronic diarrhea among people living with HIV in Southeast Asia: a systematic review and meta-analysis. Asia Pac J Public Health. 2020;32(1):8–18.
5. Ghosh S, Padalia J, Moonah S. Tissue destruction caused by *Entamoeba histolytica* parasite: cell death, inflammation, invasion, and the Gut microbiome. Curr Clin Microbiol Rep. 2019;6(1):51–7.
6. Stauffer W, Ravdin JI. *Entamoeba histolytica*: an update. Curr Opin Infect Dis. 2003;16(5):479–85.
7. Stanley SL. Amoebiasis. Lancet. 2003;361(9362):1025–34.
8. Haque R, Huston CD, Hughes M, et al. Amebiasis. N Engl J Med. 2003;348(16):1565–73.
9. Shirley DT, Farr L, Watanabe K, et al. A review of the global burden, new diagnostics, and current therapeutics for Amebiasis. Open forum Infect Dis. 2018;5(7):ofy161.
10. Gerace E, Lo Presti VDM, Biondo C. *Cryptosporidium* infection: epidemiology, pathogenesis, and differential diagnosis. Eur J Microbiol Immunol (Bp). 2019;9(4):119–23.

11. Bouzid M, Kintz E, Hunter PR. Risk factors for cryptosporidium infection in low and middle income countries: A systematic review and meta-analysis. PLoS Neglected Trop Dis. 2018;12 (6):e0006553.
12. Shoultz DA, de Hostos EL, Choy RK. Addressing cryptosporidium infection among young children in low-income settings: the crucial role of new and existing drugs for reducing morbidity and mortality. PLoS Neglected Trop Dis. 2016;10(1):e0004242.
13. Fayer R, Farley CA, Lewis EJ, et al. T.K. potential role of the eastern oyster, Crassostrea virginica, in the epidemiology of *Cryptosporidium parvum*. Appl Environ Microbiol. 1997;63 (5):2086–8.
14. Fayer R, Morgan U, Upton SJ. Epidemiology of cryptosporidium: transmission, detection and identification. Int J Parasitol. 2000;30(12–13):1305–22.
15. Ayinmode AB, Oliveira BCM, Obebe OO, et al. Genotypic characterization of *cryptosporidium* species in humans and Peri-domestic animals in Ekiti and Oyo states, Nigeria. J Parasitol. 2018;104(6):639–44.
16. Slapeta J. Cryptosporidiosis and *cryptosporidium* species in animals and humans: a thirty colour rainbow. Int J Parasitol. 2013;43(12–13):957–70.
17. Pijnacker R, Mughini-Gras L, Heusinkveld M, et al. Different risk factors for infection with *Giardia lamblia* assemblages A and B in children attending day-care centres. Eur J Clin Microbiol Infect Dis. 2016;35:2005–13.
18. Adam EA, Yoder JS, Gould LH, et al. Giardiasis outbreaks in the United States, 1971-2011. Epidemiol Infect. 2016;144:2790–801.
19. Painter JE, Gargano JW, Collier SA, et al. Centers for disease C, prevention. Giardiasis surveillance—United States, 2011-2012. MMWR. 2015;64(3):15–25.
20. Gagnon F, Duchesne JF, Levesque B, et al. Risk of giardiasis associated with water supply in an endemic context. Int J Environ Health Res. 2006;16:349–59.
21. Odoi A, Martin SW, Michel P, et al. Determinants of the geographical distribution of endemic giardiasis in Ontario, Canada: A spatial modelling approach. Epidemiol Infect. 2004;132:967–76.
22. Malaria. World health organization. 2021. https://www.who.int/news-room/fact-sheets/detail/malaria#:~: text=In%202020%2C%20there%20were%20an,and%2096%25%20of%20malaria%20deaths.
23. Dayananda KK, Achur RN, Gowda DC. Epidemiology, drug resistance, and pathophysiology of *plasmodium vivax* malaria. J Vector Borne Dis. 2018;55(1):1–8.
24. Lal S, Dhillonm GP, Aggarwalm CS. Epidemiology and control of malaria. Indian J Pediatr. 1999;66(4):547–54.
25. Bloland PB, Williams HA. National Research Council (US) committee on population; program on forced migration and health at the mailman School of Public Health, Columbia University. Washington (DC): National Academies Press (US); 2002.
26. World malaria report. World health organization. 2021. World malaria report 2021 (who.int)
27. Malaria. World health organization. 2017. https://www.who.int/india/health-topics/malaria#:~: text= According%20to%20the%20WMR%202019,of%2050.5%25%20compared%20with%202017.
28. Abid A, Talha O, Asad U, et al. Epidemiological survey of *toxoplasma gondii* and associated risk factors in ruminant species of the Khyber Pakhtunkhwa Province of Pakistan. J Parasitol Res. 2021;1-8 https://doi.org/10.1155/2021/6653239.
29. Torgerson PR, Mastroiacovo P. The global burden of congenital toxoplasmosis: a systematic review. Bull World Health Organ. 2013;91(7):501–8.
30. Robert-Gangneux F, Dardé ML. Epidemiology of and diagnostic strategies for toxoplasmosis. Clin Microbiol Rev. 2012;25(2):264–96. https://doi.org/10.1128/CMR.05013-11. Erratum in: Clin Microbiol Rev 2012 Jul;25(3):583
31. Bigna JJ, Tochie JN, Tounouga DN, et al. Global, regional, and country seroprevalence of *toxoplasma gondii* in pregnant women: a systematic review, modelling and meta-analysis. Sci Rep. 2020;10:12102.

32. Singh S, Munawwar A, Rao S, Mehta S, Hazarika NK. Serologic prevalence of *toxoplasma gondii* in Indian women of child bearing age and effects of social and environmental factors. PLoS Negl Trop Dis. 2014;8(3):e2737.
33. Maxfield L, Crane JS. Leishmaniasis. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2022. https://www.ncbi.nlm.nih.gov/books/NBK531456/
34. Iratxe N, Alba D, Octavi B, Adriá C, Carla F, María V, et al. Acute liver failure due to visceral leishmaniasis in Barcelona: a case report. BMC Infect Dis. 2019;874:874. https://doi.org/10.1186/s12879-019-4553-7.
35. Boczo K, Deryo A, Drewa G, et al. Parasitology and medical acaroentomology. PWN Warszawa; 2002. p. 104–17.
36. Dziubek Z, Janeczko J, Juszczyk J, et al. Parasites and parasitic diseases. Infectious and parasitic diseases. Dziubek Z. PZWL Warsaw; 2003. p. 438–44.
37. Khan U, Mirdha BR, Samantaray JC, Sharma MP. Detection of *Entamoeba histolytica* using polymerase chain reaction in pus samples from amebic liver abscess. Indian J Gastroenterol. 2006;25:55–7.
38. Wuerz T, Kane JB, Boggild AK, et al. A review of amoebic liver abscess for clinicians in a nonendemic setting. Can J Gastroenterol. 2012;26(10):729–33.
39. Iqbal J, Hira PR, Al-Ali F, Khalid N. *Cyclospora cayetanensis*: first report of imported and autochthonous infections in Kuwait. J Infect Dev Ctries. 2011;5:383–90.
40. Wang KX, Li CP, Wang J, Pan BR. Epidemiological survey of cryptosporidiosis in Anhui Province China. World J Gastroenterol. 2002;8:371–4.
41. Strong WB, Gut J, Nelson RG. Cloning and sequence analysis of a highly polymorphic *Cryptosporidium parvum* gene encoding a 60-kilo-Dalton glycoprotein and characterization of its 15- and 45-kilodalton zoite surface antigen products. Infect Immun. 2000;68:4117–34.
42. Xiao L. Molecular epidemiology of cryptosporidiosis: an update. Exp Parasitol. 2010;124:80–9.
43. Stephens J, Cosyns M, Jones M, Hayward A. Liver and bile duct pathology following *Cryptosporidium parvum* infection of immunodeficient mice. Hepatology. 1999;30(1):27–35.
44. Da'as, H.A. Prevalence of cryptosporidium species among children ≤5 years old in northwest-Bank, Palestine/cross sectional study. Thesis for master's degree in public health, Faculty of Graduate studies. An-Najah National University, Nablus-Palestine; 2010.
45. Mor SM, Tumwine JK, Ndeezi G, et al. Respiratory cryptosporidiosis in HIV seronegative children in Uganda: potential for respiratory transmission. Clin Infect Dis. 2010;50(10):13661–72.
46. Chalmers RM, Davies AP. Minireview: clinical cryptosporidiosis. Exp Parasitol. 2010;124:138–46.
47. Cello JP. Acquired immunodeficiency syndrome cholangiopathy: spectrum of disease. Am J Med. 1989;86:539–46.
48. Sotto A, Alvarez JL, García B, et al. Lesión hepática aguda por *Giardia lamblia* [Acute hepatic lesion caused by *Giardia lamblia*]. Rev Esp Enferm Dig. 1990;77(1):24–8.
49. Astiazaran-Garcia H, Lopez-Teros V, Valencia ME, et al. *Giardia lamblia* infection and its implications for vitamin A liver stores in school children. Ann Nutr Metab. 2010;57(3–4):228–33.
50. Milner DA. Malaria pathogenesis. Cold Spring Harb Perspect Med. 2018;8(1):a025569.
51. https://www.malariasite.com/pathophysiology/
52. Vaughan AM, Kappe SHI. Malaria parasite liver infection and exoerythrocytic biology. Cold Spring Harb Perspect Med. 2017;7(6):a025486.
53. Frenkel JK. Pathophysiology of toxoplasmosis. Parasitol Today. 1988;4(10):273–8.
54. Madireddy S, Madireddy S, Rivas Chacon ED, Mangat R. Toxoplasmosis. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2022. https://www.ncbi.nlm.nih.gov/books/NBK563286/
55. el Hag IA, Hashim FA, el Toum IA, et al. Liver morphology and function in visceral leishmaniasis (kala-azar). J Clin Pathol. 1994;47(6):547–51.

56. Amazonas JN, Cosentino-Gomes D, Werneck-Lacerda A, et al. Exp Parasitol. 2009;121:15.
57. Hill DR, Gardner TB. Clin Microbiol Rev. 2001;14:114.
58. Huang TL, Eynde JJV, Mayence A, et al. WO Patent. 2008.
59. Bray PG, Barrett MP, Ward SA, et al. Trends Parasitol. 2003;19:232.
60. Cen J, Lv A. WO Patent. 2007:07079653.
61. Dorsch MR, Veal DA. WO Patent. 2000:0078781.
62. Raether W, Hänel H. Parasitol Res. 2003;90:S19.
63. Houghton RL, Reed SG, Raychaudhuri S. WO Patent. 2007:07056114.
64. Suk DH, Rejman D, Dykstra CC, Pohl R, et al. Bioorg. Med Chem Lett. 2007;17:2811.
65. Gargala G. Drug treatment and novel drug target against cryptosporidium. Parasite. 2008;15(3): 275–81.
66. Uip DE, Lima ALL, Amato VS, et al. Roxithromycin treatment for diarrhoea caused by cryptosporidium spp. in patients AIDS. J Antimicrob Chemother. 1998;41(Suppl. B):93–7.
67. Gargala G, Delaunay A, Li X, et al. Efficacy of nitazoxanide, tizoxanide and tizoxanide glucuronide against *Cryptosporidium parvum* development in sporozoite-infected HCT-8 enterocytic cells. J Antimicrob Chemother. 2000;46(1):57–60.
68. Cai X, Woods KM, Upton SJ, et al. Application of quantitative real-time reverse transcription-PCR in assessing drug efficacy against the intracellular pathogen *Cryptosporidium parvum* in vitro. Antimicrob Agents Chemother. 2005;49(11):4437–42.
69. Mele R, Gomez Morales MA, Tosini F, et al. Indinavir reduces *C. parvum* infection in both in vitro and in vivo models. Int J Parasitol. 2003;33(7):757–64.
70. Hommer V, Eichholz J, Petry F. Effect of antiretroviral protease inhibitors alone, and in combination with paromomycin, on the excystation, invasion and in vitro development of *C. parvum*. J Antimicrob Chemother. 2003;52(3):359–64.
71. Shibeshi MA, Kifle ZD, Atnafie SA. Antimalarial drug resistance and novel targets for antimalarial drug discovery. Infection and drug resistance. 2020;13:4047–60.
72. Alday PH, Doggett JS. Drugs in development for toxoplasmosis: advances, challenges, and current status. Drug Des Devel Ther. 2017;11:273–93.
73. de Menezes JP, Guedes CE, Petersen AL, et al. Advances in development of new treatment for leishmaniasis. Biomed Res Int. 2015;2015:1–11.

# Cognitive Impairment in Parasitic Protozoan Infection

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**Abstract**

The association between chronic and acute infections with cognitive decline has been established by considerable amount of evidences comprising laboratory-level and cohort studies. Infections caused by protozoan parasites have a systemic impact on host, often linked to altered psychosocial behaviours. Neuro-parasitology research gradually accumulated mechanistic understanding of the cognitive interface of parasite-host interaction. As revealed by clinical findings, cytokine-chemokine levels and direct neuroimaging of infections caused by neurotropic and non-neurotropic parasite factors like the shared molecular pathways, immunoinflammation affecting the central nervous system (CNS) and direct damage of CNS by parasitic invasion determine the association of the host cognition and parasitic infections. In this narrative, cognitive and neurological aspects of six important parasitic protozoan diseases, namely, toxoplasmosis, malaria, Chagas disease, human African trypanosomiasis, leishmaniasis and primary amoebic meningoencephalitis, have been discussed. The broader aim of the article is to emphasize significance of cognitive care in developing therapeutic strategies against the diseases.

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**Keywords**

Neuroinflammation · Cognitive impairment · Blood-brain barrier · Protozoan parasite · Infection

During the evolution of host-parasite interaction through a period of over millions of years, parasitic infection attained the capability to modulate several organ systems of the host directly or indirectly. Neuromodulation and the resultant behavioural aberrations including impairment of cognitive functions have started to gain attention in recent years. Thus, "neuro-parasitology" is emerging as a crucial stream of infection biology to unveil influence of parasitic infection on the nervous system [1]. Unravelling such mechanisms might offer fundamental insights into the study of cognition. For insect hosts of several parasitic protozoa and fungi, significant alteration has been highlighted in behaviours like [1] navigation of the host that leads to a suicidal behaviour, [2] bodyguard behaviour, [3] anti-social behaviour and [4] motivation to move [2]. In the past few years, both whole organism models for neurotropic parasite infections by *Naegleria fowleri*, *Toxoplasma gondii*, *Trypanosoma brucei* and *Plasmodium falciparum* pinpointed acute neuroinflammation as the cornerstone of cognitive dysfunction [3]. Also postinfection neuronal dystrophy and irreversible damages have been ascribed to contact dependent processes during brain or CNS invasion by the pathogen. Behavioural manifestation and cognitive impairment have been reported in typical non-neurotropic parasitic infections like Chagas disease (CD) and leishmaniasis. Cognitive challenge in CD, for example, has been strongly associated with brain atrophy [4], neuroinflammation and BBB damage [5], which conclusively place the brain among the organs affected by these parasites. For both kinds of infection, the general mechanistic aspect identified so far is neuroinflammation triggered by disruption of the delicate balance between pro-inflammatory and anti-inflammatory mediators [5, 6]. Here, an attempt is made to provide a comprehensive purview of cognitive impairment triggered by major parasitic protozoan infections. The narration begins with a brief outline of fundamental concepts of cognitive impairment and neuroinflammation, which is followed by elaborated discussion on case studies and mechanistic understanding of cognitive impairment for toxoplasmosis, malaria, CD, human African trypanosomiasis (HAT), leishmaniasis and primary amoebic meningoencephalitis (PAM).

## 1 Cognition and Cognitive Impairment

Responsiveness is the ability to gather, process, classify, analyse, store and retrieve information and act accordingly. Psychological processes that are measured in terms of our ability to perceive conditions, recall information and assign adequate attention and ability to think in defined and abstract way to connect diverse domains of sensitivity are referred to as cognition [7]. Sensory skills like perception, attention,

memory, balance and proprioception, motor skills like executive functioning and verbal and language skills are considered as key elements of cognition [8]. Critical elements like learning and memory need attention which involve emotional component in the process [9]. Most of such cognitive domains are compromised under various ailments like hormonal imbalance, genetic predisposition, infection and ageing [10–12]. Growing body of evidences underpin the strong association between neural systems of cognition and inflammatory processes. Investigation involving whole organism in vivo models has established close association between some aspects of the immune system and processes at the level of the neurons in the brain and CNS [13]. Cognitive impairment is also the state where a person has difficulties in remembering, learning new things, concentrating or making decisions that affect their normal life. It ranges from mild to severe form if not treated at proper time. It is a severe and global healthcare problem with 3–19% of elderly population suffering from mild cognitive impairment (MCI) of which >50% develop dementia, a severe form of cognitive decline [14]. Chronic neurodegenerative disorders like Alzheimer's disease (AD), manifested by the accumulation of amyloid-β (Aβ) and tau proteins in the brain, are the most prevalent cause of dementia with high prevalence co-occurrence. Mechanistically, infection-associated cognitive impairment can be an outcome of direct invasion of the central nervous system by a pathogen [15] or an indirect effect of systemic infections like triggering of pro-inflammatory cytokines and neuroinfammation [16], as depicted in Fig. 1. When such inflammation is restricted at the fluid and membrane of the brain, the symptoms are categorized as "meningitis", while inflammations of the brain parenchyma leading to neurologic dysfunctions are called "encephalitis" [17]. Both the conditions induct altered mental status and consciousness, personality change, lethargy and dementia for a sustained period. Animal models of several acute systemic infections revealed neuroinflammatory reaction in CNS [18]. With progressive evidences of intense molecular, biochemical, histological and anatomical association, infection-neurodegeneration-cognitive impairment triad is being unravelled with progressive development of efficacious therapeutics [19].

## 2 Neuroinflammation in Cognitive Impairment

An inflammatory response within the brain or CNS is delineated as neuroinflammation. Similar to other tissue systems, inflammation in CNS is spurred by cytokines, chemokines, burst of associated second messengers and signalling cascades in specific immune cells like microglia, astrocytes and peripheral lymphocytes [20]. Such immune activation elicits physiological and psychological consequences depending on the degree of inflammatory response [20]. Microglial cells, the macrophage equivalent of CNS, are involved in immune surveillance and production of cytokines and chemokines [21]. Microglia constitute 10% of cell population of the CNS and brain while residing in grey and white matter [22]. As evidenced by two photon imaging studies, microglial cells actively survey neural microenvironment [23]. While identifying infection/invasion or damage, it

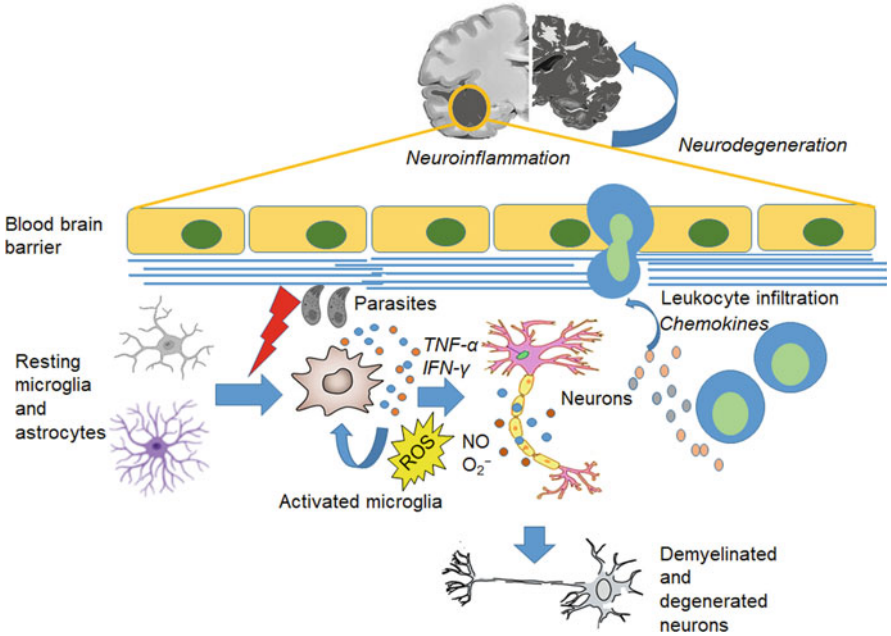


**Fig. 1** *Infection, neuroinflammation and neurodegeneration.* The resting microglial cell can be activated by different factors including detection of infection in the CNS environment. This triggers constant release of pro-inflammatory cytokines and generation of ROS that promote demyelination and other neuronal damage culminating into neurodegenerative processes. Inflammation is further exaggerated by pro-inflammatory factors serving as chemo-attractants for leucocytes from peripheral lymphoid organs, which, upon entry into the loci of infection, release chemokines to attract immune cells. Such sustained inflammation leads to meningitis or encephalitis or even atrophy of the brain

propagates inflammatory milieu for the peripheral region and facilitates infiltration of leucocytes to the brain [24]. Chronic or exaggerated activation of microglia can lead to behavioural disruptions including depression and cognitive deficits [25]. Key pro-inflammatory mediators for neuroinflammation are secondary messengers (NO and prostaglandins) and reactive oxygen species (ROS), cytokines (IL-1β, IL-6 and TNFα) and chemokines (CCL2, CCL5, CXCL1), mostly produced by microglia and astrocytes. IL-1β act on microglial progenitors to repopulate microglial cells [20]. The markers for neuroinflammation are summarized in Table 1. During severe infection or insults like vascular occlusion and ischaemia, profound neuroinflammation is triggered with cytokine-chemokine burst, enhanced permeability of blood-brain barrier (BBB) and recruitment of immune cells resulting in oedema. Often such responses are transient and do not involve profound infiltration of immune cells or breakage of BBB [20]. Conversely, for conditions like Alzheimer's, the inflammatory response can even become chronic and cause demyelination and fragmentation of axons. Such damages cause severe impairment of neurotransmittance [40]. The link between neuroinflammation and cognitive

**Table 1** Markers of neuroinflammation (table prepared)

| Marker | Category | Role in cognitive impairment | References |
|---|---|---|---|
| TNF-α | Pro-inflammatory | Polymorphism associated with risk of AD<br>Elevated in AD<br>Elevated levels linked to cognitive decline | [26]<br>[27]<br>[28] |
| IFN-γ | Pro-inflammatory | Deregulated in delirium | [29] |
| IL-1 | Pro-inflammatory | Enhances neuronal tau phosphorylation<br>Activates astrocytes<br>Elevated in AD | [30]<br>[31]<br>[32] |
| IL-6 | Pro-inflammatory | Elevated in delirium<br>Polymorphism linked to increased risk of AD<br>Linked to grey matter volume and memory function<br>Also elevated in non-AD dementia | [33]<br>[26]<br>[34] |
| IL-18 | Pro-inflammatory | Stimulate production of IL-1β and IFN-γ<br>Inhibit induction of long-term-potentiation (LTP)<br>Enhance tau phosphorylation<br>Dysregulation linked to cognitive deficit | [35]<br>[36]<br>[37]<br>[38] |
| IL-8 | Pro-inflammatory | Level elevated in delirium | [33] |
| IL-1RA | Anti-inflammatory | Level decreased in delirium | [29] |
| CRP | Pro-inflammatory | Elevation linked to risk of cognitive decline | [39] |

impairment is further reinforced by the reports of clinical improvement by implementing immunosuppressive therapy in a spectrum of cognitive compromised states including AD [41]. A double-blind clinical trial has already been conducted by Brod et al. (2021) using type 1 interferon to mitigate IL-1β and IL-6 secretion by activating lymphocytes of lamina propria in the gut-associated lymphoid tissue (GALT). The activated lymphocytes apparently elicited anti-inflammatory response in the brain following infiltration [42]. In accordance, earlier, a number of clinical studies evidenced that prolonged use of non-steroidal anti-inflammatory drugs (NSAIDs) may reduce AD pathology [43, 44]. NSAIDs may even prevent age-associated cognitive decline [45]. Cyclooxygenase 1 and 2 (COX-1 and COX-2) inhibition are possibly the major mechanistic aspect for NSAID-mediated neuroprotection, thereby attenuating cognitive impairment processes like deregulation of vasomotor activity and platelet activity, free radical generation and alteration of peroxisome proliferator-activated receptors [24].

## 3 Neural, Psychological and Cognitive Impairments in Parasitic Protozoan Diseases

Research in this avenue has been more restricted owing to the priority given to understand the pathophysiologies of the parasitic protozoan diseases for finding effective therapies. However, concerns are growing for systematic investigations of the neurological manifestations and psychological and cognitive impairments

associated with these diseases considering their potential to cause havoc on the mental structure of the communities. In this section, we have emphasized the important findings on the four prevalent protozoan diseases with an attempt to comprehend the mechanistic connections between neurological manifestations and behavioural and cognitive abnormalities during the pathological progressions of these diseases or even after their recoveries.

## 3.1 Toxoplasmosis

Toxoplasmosis, caused by *Toxoplasma gondii*, is a major protozoan parasitic disease that often features neurological, behavioural and cognitive abnormalities as understood from the investigations on human and animal models [46]. Among the various groups of parasites, Toxoplasma has a greater ability to infect and replicate within the mammalian and avian cells [47]. Also it can reside and directly cause tissue damage to the CNS and create a long-lasting severity to the brain, impairing the neurons and glial cells [46]. *The infectivity generally revolves between two kinds of hosts: one in their intermediate host, where they carry out their asexual stages, and another in their definitive host, where the sexual cycle is being carried out. There are mainly two distinct morphotypic asexual phases of Toxoplasma, namely, tachyzoites and bradyzoites* [48], that are detected in the host tissues depending upon the severity of infection, whether it is acute or chronic [48]. The first asexual phase of *Toxoplasma* is the tachyzoite [19]. *Within the infected organism, this tachyzoite differentiates into the slow-growing bradyzoite stage, where the encystment takes place within 7 to 10 days* [48]. Generally, the differentiation from bradyzoites to tachyzoites follows a slower kinetics, and our immune response efficiently prevents the dissemination of tachyzoites, but in case of immunocompromised patients, such differentiation takes place more frequently, which may lead to a massive and potentially fatal recrudescence [49]. *Bradyzoite, which is the cystic stage, develops in the brain areas and tissues* [50]. *As this cystic form is highly resistant to the drugs and also impervious to the host's immune response, it can cause benign chronic infection in the brain* [50] and the CNS [48], where the parasite can actually sustain itself for a longer period of time.

The transmission of the parasite from the blood to the brain is more obstructive than the transmission to other tissues due to the impermeable BBB [51]. It has been suggested that the parasite infects the dendritic cells and monocytes and spreads haematogenously via a "Trojan horse" mechanism [52]. Utilizing the "Trojan horse" mechanism, the infected monocytes and myeloid-derived cells can extravasate from the blood capillaries to the brain, thereby crossing the BBB [53, 54]. Another mechanism that is associated with the transmission of *Toxoplasma* to the CNS is the "gliding motility"—the movement that provides the effectiveness to cross the first line of barrier, that is, the epithelium of the intestinal barrier [55]. Study of the impacts of physiological shear force to live cells in microfluidic chambers has revealed that tachyzoites have the capabilities to adhere and to move to the vascular endothelium in this physiological condition [56]. It is suggested that through this

gliding motility along with the Trojan horse mechanism, the parasite can successfully invade the impregnable barriers like polarized cell monolayers and tight and paracellular junctions, including the BBB [46]. The infection elicits a combinatorial effect in increasing excitatory and decreasing the inhibitory neurotransmission, which often are associated with brain seizures [50]. As revealed in several histopathological and in vivo imaging data, T. gondii has a greater preference of residing mainly in the brain areas like the amygdala, hippocampus, frontal cortex, etc. [57]. *Thereby,* T. gondii *hampers the neuronal connectivity and also alters the protein composition of the synapse mainly in the neocortex and hippocampus areas* [58]. The neuromodulatory impacts of infection of the central nervous system (CNS) by this pathogen, as studied primarily in the murine models of toxoplasmosis, are chronic neuroinflammation; disruptions of functioning of the three prime neurotransmitters of the brain, viz. glutamate, GABA and dopamine; functional alterations of several areas of the CNS; and formations of Aβ plaques and tauopathy [46]. *T. gondii* infects the dendritic cells and microglia and brings about their hypermigration by hijacking the GABAergic machinery, which results in the cellular migration and dissemination of the pathogen in the brain parenchyma ("Trojan horse" mechanism) [54, 59, 60].

The CNS invasion by *Toxoplasma* initially elicits a profound pro-inflammatory response. The pathogen stimulates immune responses by activating nuclear factor kappa B signalling (in astrocytes) and the T cells in the brain, which results in influxes of the immune effector cells and subsequent actions of the pro-inflammatory cytokines, leading to CNS pathology, including neuronal apoptosis [61–63]. The affected cells produce different chemokines (GRO1, GRO2, LIF and MCP1) followed by various cytokines (IL-1β and IL-6) and other pro-inflammatory components by activating the series of transcription factors (REL-B, NF-κBp105 and I-κBα). Such triggering assures a strong IFN-γ-dependent immune response against this infection [64]. As revealed by two-photon image analysis, the infection and the subsequent lymphocyte infiltration culminate into tissue remodelling in the CNS [65]. The invasion of T cells in the infection milieu is essential for the prevention of the parasitic infection through a cell-mediated manner as T cells produce IFN-γ [66]. Followed by the production of IFN-γ, other pro- and anti-inflammatory cytokines and chemokines are found to surmount an effective immune response against this parasitic infection [67]. Although this cumulative protective response effectively kills tachyzoites, some can escape the assault and transform into cyst-forming bradyzoites within microglia, astrocyte and neurons [68] with majority of the cysts residing within the neurons [69], which are nonresponsive to stimulation with interferon-gamma (IFN-γ) or tumour necrosis factor α (TNF-α) and therefore are suitable niches for the cysts. Toxoplasmic encephalitis (TE) occurs as a result of neuronal cell death and inflammation following the reactivation of cysts [50]. Besides avoiding the activation of host's immune system, the parasite also manages an effective and delicate way to keep their metabolic and proliferative activity low upon entry into the CNS. Host-parasite interplay often is determined by the genotypic variations in the parasite [70]. Systemic study of *T. gondii* infection in

murine model of experimental TE has yielded profound level of understanding regarding the cellular immunoregulation of the *T. gondii* infection [71].

In addition, alterations in the composition of synaptic proteins in chronic toxoplasmosis have revealed the disruption in the key components of glutamatergic neurotransmission—downregulation of the NMDA and AMPA receptors and impairments of the astrocytic EAAT2 transporter [72]. The dysfunctioning of NMDA receptors in turn can decline the GABAergic signalling which together with the interference from the tachyzoite and bradyzoite stages of the pathogen on the GABAergic neurotransmission possibly disturb the neuronal inhibitory control in the infected animals and thus alter the behaviour [73, 74]. Furthermore, increased levels of dopamine in the brain because of *T. gondii* infection are also considered as one of the causal factors that leads to the damage of dendritic spines and subsequent cognitive impairment. Hyperfunction of dopamine neurotransmission has been reported to impair the cortical functions that include executive, motor, memory, motivational and emotional regulation with the possibility to induce psychiatric symptoms [61, 75–77]. In fact, several recent studies have identified toxoplasmosis as one of the risk factors in the pathophysiologies of neuropsychiatric disorders, including schizophrenia, bipolar disorder, migraine and obsessive-compulsive disorder [78]. Additionally, increased lesions in the somatosensory cortex with immune inflammation and abnormalities in the connectivity and neuronal structures in the somatosensory cortex and hippocampus, along with reduced synaptic density and efficacy in the neocortex, hippocampus and subcortical areas, comprehend the memory impairment and neuropsychiatric symptoms in this disease [57, 58, 79]. Altogether, the effects of molecular, cellular and immune pathologies in the CNS networks lead to behavioural and cognitive abnormalities in the *T. gondii*-infected individuals that manifest in the forms of impaired spatial, olfactory and associative learning and memories in the murine models and compromised processing, working and verbal memory and executive performance in the seropositive human subjects [80, 81]. Escalating our anxiety, this neurotropic parasite may also have the capacity to nucleate amyloid and tau pathologies in the hippocampus and prefrontal cortex [72]. Systemic analysis intricately unravelled the connection between toxoplasmosis and Alzheimer's disease [82]. Indeed, the intimidating similarities in the cellular and molecular abnormalities between Alzheimer's disease (AD) and *T. gondii* infection warrant future concentration of research efforts to untangle the nature of trigger that may lead to AD pathology in *T. gondii* infection [46, 82–84].

## 3.2 Chagas Disease

Though the strongest account on neurological, behavioural and cognitive dysfunction exists for *T. gondii* [46], reports are accumulating on the anxiety- and depression-like behaviour in American trypanosomiasis, or popularly known as Chagas disease (CD), caused by the protozoan parasite, *Trypanosoma cruzi* [85]. In the chronic symptomatic form of CD, parasite load, however, has been

reported to decline in the patients, but Chagas cardiomyopathy and neurological disturbances were observed [86, 87]. Further, several preclinical and clinical investigations have confirmed behavioural changes and psychiatric symptoms, especially depression, anxiety and mood disturbances, which were independent of the sickness symptoms, acute inflammation and the psychological status of the patients [88–90]. Neurocognitive and psychological disturbances, such as poor orientation, attention, learning and memory, non-verbal reasoning, problem solving and speed of information processing and increased confusion and delusion, and mood disorders were reported to associate with the chronic symptomatic Chagasic patients having different degree of depression and anxiety symptoms [85, 89, 91, 92]. Thus, the chronic nature of CD is considered as a risk factor for the development of emotional disturbances (stress), which eventually transform into psychological and physical symptoms of anxiety and depression, and lower levels of resilience in the symptomatic cases [93]. In fact, in the developing countries, the symptomatic Chagasic patients were reported to experience social and economic discrimination and social exclusion, which often culminated into stigma in these cases. This psychological burden together with the compromised health and quality of life (daily functioning, higher suicidal risk and substance abuse) contributes to the development of hopelessness and depressive episodes among these patients [3, 94, 95]. In addition, the chronic patients also exhibited various forms of neuropathies, like encephalopathy, speech disturbance, dyspraxia, abnormal gaits, myoclonic seizures, bradykinesia, paresis, dizziness and weakness of muscle-tendon reflexes [93]. Interestingly, the patients with different forms of CD have reported to experience different life standards, as administrations of the Beck Depression Inventory (BDI) and the questionnaire on *Quality of Life*, on a Brazilian study population, demonstrated significant differences in the life quality and depression symptoms among the different forms of CD (cardiac, digestive, indeterminate and mixed), with the highest BDI and psychological domain scores found, respectively, for the digestive and indeterminate forms of the disease [91]. We do not know why these differences have emerged among the different forms of CD, but it is imperative for the future systematic studies to comprehend if these disease forms are associated with differential susceptibilities to depression, anxiety and other forms of psychological debilitations. This is particularly important for the implementations of therapeutic strategies in the Chagasic patients at their early phases of infection for effective interventions.

To develop effective therapeutic approaches against the acute and chronic forms of CD, one needs to understand the mechanistic connections between cognitive, behavioural and neural impairments. The establishments of murine models of CD have been proving to be productive in this regard [96]. Human symptoms, such as compromised motor coordination, depression (as assessed through the tail suspension and forced swim tests) and increased anxiety (elevated plus maze and open field tests), were reproduced in the C57BL/6 mice after 150 days of chronic infection with the Columbian type 1 strain of *T. cruzi*, without causing any neuromuscular pathology [97]. In addition, impairments in the object recognition and associative memories were also found in the mouse model along with sleep disturbances in

rats, although these symptoms can be the consequences of depression per se [98]. Investigations to unravel mechanistic connections between depression and Chagas disease implicated several functional abrogations in the brains of *T. cruzi*-infected animals in pro-inflammatory environments. Prolonged depletion of tryptophan in the brain due to excess breakdown of this amino acid, mediated by the overt action of the enzyme, indoleamine 2,3-dioxygenase (IDO), in the acute and chronic infections leads to the decreased production of serotonin which paralleled the exhibition of depressive-like behaviour in mice [99]. In association with the excess activation of IDO, the other proposed mechanism points to the onset of depression owing to the neurotoxic effects of the metabolites of the TRYCAT pathway of tryptophan metabolism (3-hydroxykynurenine, quinolinic acid) and the associated IFN-α-, IL-6- and IL-8-mediated inflammation [85]. Prolonged activations of the immune-inflammatory signalling pathways probably play key roles in inducing depression in the animal models of CD. Characteristic activation of the cell-mediated immunity (Th-1 and Th-17 cells, CD8+ T lymphocytes, pro-inflammatory cytokines, such as TNF-α, IL-6, IL-17, IFN-γ) and elevated levels of TNF-α and pro-inflammatory cytokines (PICs) in the *T. cruzi*-infected mice were suggested to cause depression [99–101]. TNF-α and IFN-γ were shown to promote the astrocytic invasion and colonization by *T. cruzi* in vitro, especially astrocytic priming by TNF-α that led to the shifting of the mouse and human astrocytes into pro-inflammatory profiles. These infected, altered astrocytes secrete further PICs, TNF-α and IL-6 and express TNF receptor 1. Increased expression of TNFR1 sustains the operation of a TNF-activated self-sustaining inflammatory loop, which favours chronic *T. cruzi* infection in the central nervous system and subsequent neuroinflammation and impediment of neurotransmission [85]. These pathological alternations in the cellular and molecular mechanisms, particularly the prolonged activations of PICs and neuroinflammation, can explain the observed depression, mood dysfunction and other forms of behavioural alterations in CD.

### 3.3 Leishmaniasis

Another identified burden on the brain and mind is the tropical disease leishmaniasis, caused by several species of the parasitic protozoa, *Leishmania*. The different subtypes and manifestations of this disease, viz. visceral leishmaniasis (VL), cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL) and post-kala-azar dermal leishmaniasis (PKDL), are reported to exert overlapping as well as different neurological and psychological manifestations [102]; however, there exist massive gaps in literature on some of the subtypes. Hepatosplenomegaly or inflammation of the liver and spleen is the most documented symptom of VL, albeit clinical reports are accumulating on the inflammation of the nervous system in VL, despite the fact that leishmaniasis is typically not associated with neurological symptoms. Central, peripheral and cranial nerve malfunctioning and other symptoms were reported in the VL patients [102].

During the end of the twentieth century, investigations by some of the British pathologist in India on "dum-dum fever" found *Leishmania* amastigotes in the brain meninges of the patients, which was corroborated well with the findings of the parasite in the cerebrospinal fluid and meningeal vessel of the African patients, and their paralytic symptoms [103]. Previously, another report on a group of 13 Sudanese patients in 1965 also documented neurological findings, viz. pain and burning sensation of the feet, foot drop, impaired senses and mental disturbances [104]. Another notable study in 1995 in the Soba University Hospital in Khartoum, Sudan, reported 46% of 111 patients of VL with neurological symptoms; the 2 most prominent symptoms were burning feet sensation and foot drop, along with deafness, cranial nerve palsies, impairments in nerve conduction and other sensory-motor deficits. Importantly, these neurological signs largely disappeared after 2 weeks of particular anti-leishmanial therapy [105]. A case study of a 16-month old boy, diagnosed with VL, at the Hospital Infantil João Paulo II, Brazil, also showed several neurological symptoms, including a diminished tone all over the body, tremors of the extremities, facial myoclonus and a reduction of the brain volume in the area of the right frontal lobe, yet all of these neurological symptoms improved after the onset of treatment [106]. Another important case report of a 10-year-old boy, who was unresponsive to the known anti-leishmanial therapy and developed meningitis, found leishmanial amastigotes in his cerebrospinal fluid (CSF), and his meningitis symptoms were resolved after the treatment with amphotericin B, as a consequence of the dissemination of the drug through the BBB [107].

A report on the neurological symptoms in the patients of CL described diminished sensation among some of the patients with the demolition of nerves, visible in the cutaneous lesion biopsy specimens. This was due to macro-scale perineural inflammation because of cellular infiltrations, comprising of lymphocytes, macrophages, plasma cells, etc., with the active presence of amastigotes in the perineural sheath [108].

The account on the neurological manifestations in the canine model of VL includes neuroinflammation and functional abrogation in the central nervous system [103]. Presence of amastigotes in the choroid plexus and meninges, high titres of anti-leishmanial antibodies in the CSF, perivascular accumulation of parasitic DNA, severe infiltrations of inflammatory cells (primarily CD3+ T cells and other mononuclear cells) and deposition of IgG at the choroid plexus, as well as the upregulation of the chemokines, viz. CCL-3, CCL-4 and CCL-5, constitute the pro-inflammatory milieu in the brain of the infected dogs (the prevalent finding), as confirmed by the in situ hybridization, gene expression and immunohistological studies [109–113]. Furthermore, ocular pathologies, altered state of behaviour (aggression, photophobia, hind limb paralysis, seizures and olfactory impairment) and meningoencephalitis with neuronal death both in the grey and white matter were also described in the infected dogs [103, 114]. Importantly, some of these neurological and behavioural manifestations also mimic the symptoms of human patients [114]. The presence of antibodies in the aqueous humour and CSF of the infected animals and human patients and the entry of the anti-leishmanial drugs into the brain indicate

the disruptions of the BBB and blood-cerebrospinal fluid barrier that bring about the CNS pathologies in leishmaniasis. A potential mechanism that may contribute to the destructions of these impermeable barriers is the apparent elevated activities of the two matrix metalloproteinases (MMP-2 and MMP-9) found in the infected dogs [115] that can disrupt the BBB, although the functional connection between these metalloproteinases and neuroinflammation is unclear.

Investigations, demonstrating the effects of leishmaniasis on the mental health and wellbeing, showed association between the various forms of the disease with stigma, social and family rejection, anxiety and depression [102]. A systematic review on the perceived social stigma in neglected tropical diseases (NTDs) clearly pointed out the preponderant existence of stigma in the patients of NTDs with differences in the reason of stigma [94]. An exploratory study on Tunisian women with scars from zoonotic cutaneous leishmaniasis (ZCL) reported low self-esteem, feelings of inferiority, anticipation of social rejection, strong correlations between the perceived life stress and perception of the illness as well as significant negative association between the presence of ZCL scar and perceived quality of life [94]. A small cohort of patients in Belo Horizonte, Brazil, also disclosed the negative impact of CL on their quality of life, especially at their school and workplace [116]. Another case-controlled study, performed in Sanliurfa, Turkey, reported widespread psychological deterioration among the active CL patients. The patients had more anxiety and depression, less body satisfaction and lower perceived quality of life than the control group [117]. In fact, a related study, investigating on the predominant types of mental disorders (MDs) and their predictors among the women in Sanliurfa, Turkey, also identified CL as one of the significant predictors of one or the other MDs, in which the list of MD includes major depressive disorder [118]. Further, a cross-sectional study in Kerman, Iran, found lower self-reported quality of life among the patients with ulcerated lesions of CL [119]. Confirming to these results, an assessment of psychiatric morbidity in the children and adolescents, suffering from CL, and their parents also showed higher frequency of depression symptoms in the patient group than the healthy individuals as well as lower quality of life found in the CL cases and their parents [120]. Since the nodular lesions in CL appear on the exposed parts of the body (face, arms and legs), they are known to have strong associations with stigma, anxiety/depression and anticipation avoidance of stress in the patients (suggestive of both social stigma and self-stigma) [117, 120, 121]. Thus, with the current status of knowledge, it is conceivable that CL can cause self-stigma and social stigma which can culminate into psychiatric morbidity, mental illness and compromised quality of life in the patients, with the possibility that these effects can be particularly pronounced in the young people and women [102].

Similarly, the study of Pal and colleagues in 2017 on post-kala-azar dermal leishmaniasis (PKDL) patients in Bihar, India, also found significantly lower score in the Dermatology Life Quality Index, in the domain of personal relationship, especially for those of below 20 years [122]. The prominent dermal lesions that are common manifestations of both CL and PKDL, irrespective of their different pathological attributes, are visible cosmetic disfigurement that adversely affect the psychological wellbeing, social life quality and day-to-day activities of the patients,

and it is increasingly clear that stigma plays crucial roles in developing these mental health problems associated with CL and PKDL [102, 123].

The number of investigations on VL and mental health is rather low yet unfortunately represented by several studies that investigated the matter indirectly [124]. Even though it has been shown that VL could directly deteriorate the quality of life in the patients [125, 126], no evidence is there that can connect the neuroinflammation in VL with mental disorders. However, it is important to mention that the cross-sectional and longitudinal Ethiopian studies, mentioned before, used HIV patients with or without VL to assess the possible differences in the quality of life and depression between these groups [125, 126]. Although the results showed differences in quality of life and depression symptoms between the HIV-VL co-infected and the HIV-only patients, it is impossible to conclude anything based on these results as effects of VL-only on the mental health because of the presence of two diseases and the potential unknown factors that may contribute to mental dysfunctioning. The solitary evidence that connects VL with mental health problems was conducted in Bihar, India, which showed, like in the cases of CL and PKDL, social stigma among the patients along with compromised quality of life, psychiatric morbidity and mental illness, albeit these manifestations were also associated with economic loss [126]. Thus, a clear association of mental health outcomes with VL is still lagging, which most likely is due to social stigma and fear among the patients to document their disease, speculating financial loss that it may bring about, apart from the lack of efforts from the researchers to conduct cross-sectional or longitudinal studies. Unfortunately, no studies were conducted to follow up the work of Carswell (1953), which associated VL patients with prominent signs of depression [127].

At the end, it is important to point out here that the literature is surprisingly skewed when we look at the effects of the individual subtypes of leishmaniasis on the mental health outcomes, owing to the much less conducted investigations on VL and PKDL compared to CL. Therefore, we need more number of systematic investigations with conserved measurement tools as the prerequisite to assess and conclude on the parameters, related to life satisfaction and psychological morbidity in the various types of leishmaniasis patients.

## 3.4 Human African Trypanosomiasis (HAT) or Sleeping Sickness

Human African trypanosomiasis (HAT), popularly known as sleeping sickness, is a neglected, vector-borne, parasitic, tropical disease that is still endemic in the rural regions of at least 36 sub-Saharan African countries [128]. The bites of tsetse fly (genus: *Glossina*) transmit the disease to a healthy individual if this insect previously acquires the pathogen from another infected human or cattle harbouring the human pathogen. HAT is caused by the extracellular protozoan parasite, *Trypanosoma brucei* (*T. b.*), which has two subspecies—*T. b. gambiense* (found in West and Central Africa and causing a chronic form of the disease) and *T. b. rhodesiense* (found in East and South Africa and causing an acute form of the disease). Between the two variants, the chronic disease caused by *T. b. gambiense* currently accounts

for more than 95% of the reported cases of HAT [129]. The disease has a dual mode of pathogenesis. Earlier after the infection, trypanosomes develop inflammatory reactions in the skin and then reach the circulation through lymphatic drainage and eventually arrive at different visceral tissues and damage them by inflammatory reactions (lymph nodes, liver, spleen and heart) in the course of the successive waves of parasitaemia—the haemolymphatic stage I of the disease that manifests with non-specific symptoms, like episodes of fever, headache, joint pains and itching. During stage II, the pathogen crosses the blood-brain barrier (BBB) to infect the CNS and severely affects the subcortical regions and damages the sleep-wake cycle with a typical daytime somnolence and nocturnal insomnia commonly found in both types of HAT [130]. The disease, if remain untreated, then progresses from the haemolymphatic stage I to the CNS invasion or meningoencephalitic stage II, which is fatal owing to the development of unconsciousness, dementia, double incontinence, epileptic seizures, coma and co-infections at the terminal stage of the disease [130, 131].

During HAT, the circadian rhythmicity of sleep and wake and endocrine secretions are reversibly disturbed in the patients with the scope of complete recovery after therapeutic intervention [132]. According to the "behavioural manipulation" hypothesis, the parasite can change the behaviour of the host (sensory disturbance, confusion and poor coordination) to enhance its transmission and reproductive fitness [133, 134]. It has been found from the analysis of polysomnographic traces of several patients in Brazzaville that HAT is not a hypersomnia with no change found in the total sleeping time for a 24-h recording session. However, patients showed disrupted sleep-wake distribution, and the degree of this symptom was related to the disease severity [132]. Furthermore, this circadian dysrhythmia was also associated with ultradian alterations with REM sleep happening throughout a period of 24 consecutive hours in the form of frequent sleep onset REM episodes or SOREM [132].

Experimentally induced chronic sleeping sickness in rats, caused by *T. brucei brucei*, has been used, owing to the reliability of this disease model, for investigating the neural causes underlying the disturbance in circadian rhythm. In a study, neural recordings were taken from the master pacemaker of the brain for circadian rhythms, the suprachiasmatic nuclei (SCN) of hypothalamus, from the trypanosome-infected rats. In vitro recording of the slide preparation showed specific impairment in the excitatory (glutamatergic) but not inhibitory synaptic transmission. Interestingly, the results also indicated possible influences of the pro-inflammatory cytokines in reducing the SCN synaptic activity [135]. Although these findings have emerged for a subspecies (*T. brucei brucei*) that is non-pathogenic to humans, the study by Tesoriero and colleagues has shown, for the first time using the pathogenic *T. brucei gambiense*, that rat's SCN suffers from substantial (about 30%) neuronal death after the disease has reached its encephalitic stage albeit no neurodegeneration was visible in the hippocampal region [136]. This focal death of neurons in SCN strongly indicates that SCN neurons are particularly susceptible to the molecular products of infection caused by the pathogenic human African trypanosomes, which leads to reversible or irreversible damage to the circadian centre of the brain. In addition,

rodent disease model with *T. brucei brucei* also exhibited significant decline in behaviour and cognitive capacity (decreased exploratory activity and motor function, increased anxiety and altered social recognition), which were associated with increased generation of astrocytes and damage to the Purkinje cells in the cerebellum [137]. Thus, apart from the SCN, extended structural and functional impairments in other areas of the brain also contribute to the clinical symptoms in sleeping sickness. It is known that astrocytes are vital immune regulators of the injured CNS and their activity may exacerbate tissue-damaging inflammatory reactions or tissue-repairing immunosuppression in response to CNS insults [137]. Masocha and colleagues also showed that invasion of *T. brucei* into the rodent brain parenchyma is paralleled by robust inflammatory responses in the brain and the course of the disease [138]. These evidences emphasize a clear understanding of the interactions between the neuroimmune system and human African trypanosomes to comprehend the molecular pathways underlying the disease progression.

Although the precise mechanism employed by trypanosomes to cross the blood-brain barrier (BBB) is still unclear, several investigations have suggested that interactions between the enzymes of the host and parasite along with the calcium signalling pathways at the endothelial cell layer are the key events to make the parasite eligible to evade the BBB and enter into the CNS [139, 140]. Currently, the evidences on the road to CNS invasion are in equal favour of both the routes via the CSF and blood. On the one hand, confocal imaging, by Laperchia et al. (2016), has revealed direct infiltrations of *T. b. brucei* and T cells, crossing the blood vessel endothelium into the brain [134], and on the other hand, studies have suggested that the fenestrated vessels of the choroid plexus and circumventricular organs, which are populated by trypanosomes early after infection, are utilized by the parasite to gain entry into the brain parenchyma through various passages [141–144]. Altogether, these evidences are inconclusive on the precise route and mechanism that trypanosomes employ to invade the brain parenchyma. However, one aspect of the CNS invasion by this parasite is becoming more accepted nowadays that even before the onset of the late stage of the disease, deterioration in the BBB is evident (as understood from the rodent and murine disease models), which only deteriorates further as the disease progresses [6, 145, 146]. The early-onset and progressive damage in the integrity of the BBB also has been evident in human cases infected by *T. b. rhodesiense* [147]. Several other studies, using the powerful confocal microscopic recording of the fluorescently labelled parasites, also confirmed African trypanosome invasion of the CNS early after the infection [134, 148].

The mechanisms that trypanosomes use to insult the CNS are unclear, though it is known that tumour necrosis factor-α plays a vital role in this regard. In fact, TNF-α was discovered in rabbits infected with African trypanosomes [149]. In a chronically infected mouse model, infected with *T. b. brucei* for months, immunostaining marked TNF-α in the microvessels of brain parenchyma, the tissues within choroid plexus and the infiltrating inflammatory cells in meninges and perivascular spaces [150]. In human patients, the increased serum levels of TNF-α also showed correlations with the severity of neurological dysfunctioning and neuroendocrinal abnormalities [151, 152]. Nitric oxide or NO has been assigned as one of the other

prime candidates that can elicit inflammatory reactions in the CNS during HAT and damage neurons. A massive increase of NO was found in the brains of animals infected with *T. b. brucei*, and thus, its signalling pathways were implicated in the exacerbation of clinical symptoms, keeping in mind the cytotoxic potentials of NO and its derivative peroxynitrites [132]. Moreover, substantial NOS activity was also found in the neurons, choroid plexus and mononuclear inflammatory cells (present in the perivascular and parenchymal infiltrates) of animals chronically infected with African trypanosomiasis [132]. In addition to TNF-α, both IFN-α and IFN-β are also implicated in the neuroinflammatory reactions associated with trypanosome-CNS invasion. In several of the transgenic mice strains for TLR and associated signalling molecules (Toll-like receptor, an innate immune system player which stimulates the synthesis of pro-inflammatory molecules like TNF-α and IFN-α/IFN-β), such as TLR-$2^{-/-}$, TLR-$9^{-/-}$ and MyD$88^{-/-}$, reductions in migrations of the parasite, *T. brucei*, and inflammatory T cells into the CNS were found that paralleled the reduced transcription of TNF-α and IFN-α/IFN-β [153]. Interferon-gamma (IFN-γ) and CXCL10 have also been reported to play key roles in the co-invasion of the CNS by the parasite and T lymphocyte. The IFN-γ-dependent accumulation of CXCL10 in the brains of *T. b. brucei*-infected mice was found to be critical for the accumulation of the T cells and trypanosomes in the brain parenchyma as CXCL$10^{-/-}$ mice showed reduced accumulations of both [154]. In an IFN-$\gamma^{-/-}$ rodent strain, transmigrations of the infiltrating pathogenic *T. brucei* and both the CD4+ and CD8+ T cells from the cerebral blood vessels into the brain parenchyma were severely hindered with the parasite and the T cells found to confine between the endothelial and parenchymal basement membranes. Interestingly, this accumulation also showed dependence on the laminin subtypes that are present in the endothelial basement membrane [155]. Thus, it has become clearer that several molecules are playing important roles in the vital co-migration process of the lymphocyte and trypanosomes into the CNS by disrupting the BBB (that decides the successful CNS invasion by African trypanosomes) and subsequent inflammatory reactions. In the list of inflammatory mediators, IL-6 and IL-10 were also reported from the investigations done on animal models. However, in contrary to detrimental roles played by TNF-α and IFN-α/IFN-β/IFN-γ, a protective role of IL-10 against neuroinflammation has been deciphered, using the *Trypanosoma brucei brucei* GVR35 mice model, as systemic administration of IL-10 was found to reduce the plasma levels of both IFN-γ and TNF-α and parasite load in the CNS and ameliorate neuroinflammatory reactions and clinical symptoms [156]. With the current status of our knowledge, the only ray of hope for a possibly effective therapeutic agent is IL-10 against the series of robust pro-inflammatory candidates. This situation clearly demands systematic research in the direction to understand the fine details of the neuroinflammatory pathways, which are switched on by the crucial event of co-migration of the T cells and African trypanosomes into the CNS, for identifying more number of drug targets.

Interestingly, Laperchia and co-workers (2016), in their study, have also found that the timings of infiltrations of *T. b. brucei* and T cells into the rat brain parenchyma are unrelated to the manifestations of functional brain disturbances.

These disturbances appeared during the early phase of peripheral infection as well as they may not be the direct consequence of the entry of the parasite and T cells into the brain neuropile. Although the disease severity only went on increasing during the encephalitic stage, whether the earliest presence of trypanosomes in the CSF is causing the impairments in brain function or not is still elusive and thus demands comprehensive investigations [134]. This issue is directly associated with the identification of reliable biomarkers to stage the disease. In another study by Laperchia and colleagues (2017), aiming to fish out reliable biomarkers of the early encephalitic stage, the authors have found concomitant rise in the serum and CSF concentrations of CXCL10 (and upregulations of IFN-γ and several other IFN-inducible chemokine genes, such as CXCL9 and CXCL11 in the brain) and fragmentation of sleep-wake pattern at 14-day post infection [157]. These humoural markers along with the appearance of the disruption in sleep-wake circadian rhythm can be used to specify the onset of the fatal encephalitic stage of the disease; however, more precise molecular biomarkers that are matched with the cognitive and behavioural abnormalities are mandatory to accurately stage the infection for correct therapeutic interventions. It is indeed an immediate exigency that African trypanosomiasis is still threatening an estimated population of 65 million in the sub-Saharan Africa [128].

## 3.5 Malaria

Malaria, among the protozoan diseases, is one of the biggest global liabilities in terms of mortality and morbidity, with an estimated 241 million cases and 627,000 deaths globally in 2020. Following their histories, nations of the sub-Saharan Africa carried the inordinate burden of 95% of the global cases and 96% of the global deaths in 2020 associated with huge losses in finance and disability-adjusted life years ([http://www.rollbackmalaria.org/keyfacts.html], World Malaria Report. 2020 https://www.who.int/news-room/fact-sheets/detail/malaria). However, apart from the disease-related symptoms, the invisible burden of neurological, cognitive and behavioural impairments after the recovery from malaria can also be overwhelming. Studies published in the past two decades have largely contributed to our knowledge on the neural, cognitive and behavioural deficits after recovery from cerebral malaria (CM) or even uncomplicated malaria. These studies were predominantly done on the children (rarely on adult groups) and within Africa, and among them, here we have discussed some of the key ones.

*Plasmodium falciparum* can cause a range of acute neurological disturbances, including the most debilitating CM. The clinical spectrum of CM includes encephalopathy; impairments in consciousness (ranging from drowsiness to coma); multiple, prolonged, generalized or focal seizures; further development of epilepsy; and other neuronal impairments [158–161]. In addition, CM can also cause retinopathy with characteristic features of capillary whitening, macular whitening, retinal haemorrhages and papilloedema. The specificity of malarial retinopathy has been found to be high enough (~90%) to diagnose CM as was confirmed by many studies

[162]. This broad-spectrum neurological damage due to infection of the central nervous system in CM can have detrimental consequences on the developments of children that include their behavioural and neurocognitive functions.

Julie Carter and colleagues (2005) followed a cohort of children (6–9 years) from the coastal Kenya who were admitted to a district hospital and diagnosed either with CM or malaria with seizures (M/S). After 20–112 months of their sickness, their cognitive abilities were compared with a group of children, who had no history of severe malaria. Cognitive assessments were focusing on functions, viz. motor skills, memory, speech, language, audition, vision and behaviour. Children with a history of CM manifested significant impairments in speech and higher-level language functions (vocabulary, pragmatics, phonology) and non-verbal functioning (cognition). The M/S group, as speculated, also showed impaired functioning in speech and language tasks (syntax, pragmatics, phonology), but their other cognitive capacities were largely unaffected (attention and non-verbal functioning). Further, episodes of active epilepsy after severe malaria were found to impair speech, language, cognition and behaviour and have been identified as a risk factor for compromised cognition and behaviour in children after recovery from malaria [163]. The crucial component of the analysis was that even after controlling for the confounding factors (age, sex, nutrition, schooling, socioeconomic status), the results showed significant differences between the CM, M/S and healthy control groups. This was important in understanding whether CM acts as an independent causative factor for cognitive impairment or not because cognitive deficits, manifested in behavioural abnormalities and poor academic performance, can be the results of several confounding factors (malnutrition, poor parental care, irregular school going, low socioeconomic status, history of other microbial or non-microbial diseases, etc.). In another study, conducted in Kenya, the same research group has also pointed out that CM has a causal relationship with acquired childhood language disorder [164].

In a Ugandan study, the nature of functional impairments, behavioural alterations and pattern of brain injury were described among the children (12–79 months) who suffered from severe neurological sequelae after cerebral malaria (CM). Abnormal tightness in muscle, behavioural alterations, severe speech impairments, hearing problems, epileptic seizures, blindness and severe cognitive deficits were commonly found. Surprisingly, behavioural alterations included the symptoms of ADHD (hyperactivity, impulsiveness and inattention), and conduct disorders (CDs) with aggression and self-destructive behaviour, as well as pervasive developmental disorder. Furthermore, some of the neurological manifestations were observed to resolve within 6 months after the discharge from the hospital, and some persisted even after 6 months. The authors proposed that potentially different pathological mechanisms that affected different brain areas and focal or global injury have resulted in the different patterns of neurological sequelae. The results also indicated that CM can be a risk factor for ADHD and CDs. Importantly, no alternative cause other than CM was found to explain these manifestations because all clinical symptoms appeared only after the children recovered from their malarial episodes [165].

Comparisons between the children (5–12 years) who visited a hospital in Uganda with CM, and uncomplicated malaria, and healthy asymptomatic community children by John and colleagues (2008) found that CM is associated with long-term deficits in cognitive abilities among the survivors. Children, tested after 2-years of their malarial episodes, showed that even after adjusting for the confounding factors (age, gender, nutrition, environment at home and school level), those with CM had a 3.67-fold increased risk for a cognitive impairment than their healthy counterparts, together with possible neurological deficits, surfacing 3 months after their discharge [166].

In a prospective cohort study, Boivin and colleagues (2007) tested (6 months after the discharge of the cases) and compared some of the cognitive functions (working memory, attention and learning ability) between three groups of Ugandan children (5–12 years), two groups with respective admission histories with CM and uncomplicated malaria and the other group that was comprised of healthy children. Not all but 21.4% of the CM children manifested persisted cognitive deficits, after 6 months, in the areas of working memory and attention compared to the healthy controls. Importantly, among the children with CM, those who manifested cognitive deficits also had histories of multiple episodes of seizures before admission to the hospital and prolonged coma compared to those without any cognitive deficits. The authors also reported that children with CM had a 3.7-fold increased risk of a cognitive impairment compared with healthy community children—a result that John and colleagues also found [167].

Boivin also reported that in Senegalese children (5–12 years), CM is a developmental risk factor that along with the poor socioeconomic condition severely impedes the complete intellectual development of the children by impairing their vital cognitive functions (perception, attention, information processing capacity and memory ability). The author suggested that these manifestations were due to impaired development of neural networks in the brains of these cases, during a critical period of development [168].

Everyday memory was also tested and compared between three groups of Kenyan children (mean age of 7 years), two groups with respective admission histories with CM and CM with complex seizures and the other group that is composed of healthy community children with no history of either conditions. Children with exposure to CM showed significantly compromised everyday memory (weak recall and recognition) compared to the controls; however, no impairment was found in the M/S group. The health-related predictors (in multivariate analysis) of poor memory performance among the CM-exposed children were diagnosis of CM, schooling and nutrition but not seizures [169]. This analysis, however, has kept out seizures, commonly observed in severe CM, as a causal factor of cognitive deficits though other studies found opposite [163, 167], but the univariate analysis by the same authors has identified both the number and duration of seizures to be associated with impaired memory function.

Abnormalities were even detected in the electrical responses (P3a amplitudes), while evaluating the event-related potentials of cerebral activities, towards the novel auditory and visual stimuli among the children with histories of severe malaria (CM,

malaria with seizures and malaria with prostration) compared to the unexposed age-matched healthy counterparts [170]. Two important results that emerged from this investigation were, first, that the percentage of children, with severe malaria, who showed impaired ERP performance was within the range as specified by other studies that measured neuropsychological dysfunctioning and, second, that the impairments in processing of the novel stimuli indicated that severe malaria can adversely affect the electrical properties of the neurons present in the prefrontal and temporal cortices [170].

Investigations, assessing the impact of post-malarial neurocognitive impairments outside of Africa, also showed that malaria can exert adverse consequences on the brain development in children. Deepika Fernando and colleagues have carried out several investigations in the malaria-endemic districts of Sri Lanka between 1997 and 1999 to determine the detrimental impacts of malarial attacks on the cognitive performance of school-going children. In one of the studies, the results showed that children (aged 5–6 years) who experienced more number of malarial infections performed poorly in the tests, assessing writing (poor letter identification ability), language and mathematical skills, compared to those children who experienced less number of infections [171]. This clearly indicates that repeated attacks of malarial infections can have devastating consequences on the brain development in children and hence on their neurocognitive capacities. In the other study, the short-lasting effects of an acute attack of malaria were assessed on the schoolchildren (aged 6–11 years). Cognitive performances in language and mathematics both during the time of presentation and the 2-week follow-up were significantly lower in the children with malaria compared to the ones with non-malarial fever and healthy controls. This was an important finding that even an acute episode of uncomplicated malaria can cause substantial short-term cognitive impairment which may be more persistent in the cases suffering from repeated malarial attacks [172]. These two studies along with another one [173] confirmed that malarial infections are major predictors of performance in language and mathematics in the schoolchildren, after controlling for confounding variables.

In fact, it is not only the forms of symptomatic malaria that are burdens to the developing world but also it has been shown by Al Serouri and colleagues in 2000 that asymptomatic malaria is also able to dampen cognitive functions in the schoolchildren. In a case control study in the Yemen Republic, these authors have compared between two groups of, respectively, asymptomatic parasitaemic boys and non-parasitaemic boys and found that the first group performed poorly than the second one in fine motor function tests [174].

These evidences, discussed above, have several incompatibilities with respect to their experimental designs and evaluations of the outcome parameters (malaria cases were followed up with different symptoms and severity, age groups of the studied children were different, protocols used for enrolling participants from the community were different, measured cognitive deficits were different, the administered neuropsychological test batteries had inapplicability in the very young children and in sub-Saharan rural African societies, etc.); however, studies have largely controlled for confounding factors, and some of them even applied culturally

adopted neuropsychological tests. The results, altogether, demonstrate that severe forms of malaria, including the ones that are associated with repeated seizures, can cause irreversible damage to the brain during early childhood, when brains are more susceptible to pathological insults. Such damages may mature into prominent impairments in cognitive functions, such as perception, information processing, learning, memory and language processing during the childhood as well as defects can be visible in the later part of life in forms of incompatibilities in executive functions and social skills. Investigations conducted within the African continent and outside also made it visible that the burden is not just limited for severe forms of malaria but also associated with uncomplicated forms. Uncomplicated malaria may be overlooked for their shorter infection times and the availability of effective therapies, but repeated episodes of attacks are reported in the children in malaria-endemic areas [162]. Therefore, this form demands great concern as they are significant predictors of children's cognitive performance and thus have the potential to affect their school performance and contribute to the loss of school time and an overall ill health.

The cellular pathophysiological mechanism in CM is still obscure, but animal studies implicated the roles of CD8+ T cells in mediating damage to the blood-brain barrier, activation of microglia, increased apoptosis of astrocytes and neurons, reduced supply of oxygen (hypoxia) in the brain due to vascular obstruction, involvements of interferon-γ-regulated processes and a decrease in the ratio of neuroprotectant (kynurenic acid) to neuroexcitotoxic species (quinolinic acid) in the pathogenesis of CM [175, 176]. These mechanisms implicating neuroinflammation receive some support from a human study in which John and colleagues (2008) investigated the association between cognitive impairment and the levels of cytokines in the serum and cerebrospinal fluid (CSF) of the CM cases. The results showed that children with CM, compared to the controls, had elevated levels of the cytokines, IL-6, IL-8, granulocyte colony-stimulating factor and TNF-α, with the increased CSF levels (but not the serum levels) of TNF-α during the time of admission that were associated with an increased risk of neurological problems after a 3-month follow-up. The CSF levels of TNF-α were also negatively correlated with the scores in attention and working memory after 6 months. Although the findings of this study indicate the involvement of the overproduced TNF-α in the central nervous system in nucleating signalling cascades that leads to neurological and cognitive disturbances in children with CM, the uneven sizes of the CM [90] and control [8] groups have kept the conclusions uncertain [177].

To understand how the cognitive functions like perception, attention, information processing, executive function, speech, language and memory are impaired in CM, computed tomography scans were also performed by Potchen and colleagues (2010) on the CM children. The findings were inconsistent among the cases, albeit abnormalities were visible in the forms of diffuse cerebral oedema, oedema at the thalamic grey matter and localized cerebral atrophy [178]. This study, however, did not check for the possible roles of extensive inflammatory responses and cellular infiltrations in the central nervous system for causing cerebral oedema in the CM children, but a mouse model that mimics human CM showed migrations and

extravasations of immune cells in the areas of the thalamus, midbrain and cerebellum; expanded microglial activation and inflammation in different areas of the cerebral cortex, hippocampus and parahippocampal region; as well as persistent cognitive impairment even after the drug-mediated elimination of parasitaemia [179]. Considering our poor status of understanding of the cellular and molecular pathological mechanisms underlying the neurological consequences and cognitive and behavioural impairments in CM, more number of investigation is warranted to decipher the causal links. This is mandatory for the development of effective neuroprotective pharmacological interventions to mitigate neuropathologies both during the acute phase of the disease and after recovery.

## 3.6 Primary Amoebic Meningoencephalitis

*Naegleria fowleri* is a free-living amoeba, thermophilic and ubiquitous in nature, that is, prevalent in soil and warm waters [180, 181], and can cause fatal infections in humans named primary amoebic meningoencephalitis (PAM). PAM is a waterborne infection commonly observed in children and young adults [182]. The disease has low morbidity but high mortality rate (98%), and there is dearth of data and comprehensive understanding on pathogenesis of the infection. In its natural habitats, the amoeba can exist in at least three distinguishable morphotypical forms, transformation within which is condition triggered [183]. Under favourable condition, the only reproductive form of the parasite, trophozoites, develops. Trophozoites graze on bacteria, algae and yeast and display features of typical eukaryotic cells that divide by binary fission at a temperature between 35° and 45 °C. Under nutrient-depleted condition, the amoeba develops into transitory flagellate with two flagella attached to a peer-shaped cell with significant amount of vacuolar structures within [183]. In a more adverse and stringent condition, the parasite transforms in cyst with spherical shape contained by a thick endocyst, a thin ectocyst and some mucoid-plugged pores [183]. PAM was first described by Fowler and Carter in 1965 after four people died in Adelaide Children's Hospital due to amoeba invading their meninges resulting in severe inflammation and damage in the brain [184]. The clinical manifestations include headaches, fever, nausea, fatigue and vomiting [185] that typically emerge within 7 days of initial exposure. At later stages, confusion, anorexia, Kernig's sign, Brudzinski's signs, lethargy, photophobia, seizures and possible coma are recorded as clinical manifestation [186]. Autopsies have revealed focal demyelination, oedema, haemorrhage and accumulation of inflammatory exudates. At the base of the brain, hypothalamus, midbrain, subarachnoid and perivascular spaces, trophozoites could be identified [186]. The mechanism of infection includes attachment of the parasite to nasal mucosa, penetration and subsequent migration along olfactory nerves till olfactory bulb, following which it passes through olfactory nerve bundles to enter the brain and trigger inflammatory response [187, 188]. It has been demonstrated in several in vivo and experimental models that axenically grown *N. fowleri* are less virulent; however, exposure to the brain and CNS environment triggers specific signalling and

cytological reorganization to make it more pathogenic. Among the already identified factors, a Rho guanine nucleotide exchange protein plays a significant part in triggering invasion [189] in response to exposure to neural extracellular matrix (ECM). The trophozoites induce production of leucocyte adhesion molecules, like vascular cell adhesion molecule 1 (VCAM-1) and intracellular adhesion molecule 1 (ICAM-1), and simultaneously disrupt BBB by secreting cysteine proteases that degrade tight junction proteins like claudin-1 and occludins [190]. Cumulatively, this augments accumulation of immune cells in the brain.

The amoeba can also cause neural tissue damage by contact-dependent phagocytosis using their amoebastomes [186]. Trophozoite's pathogen-associated molecular patterns (PAMPs) can trigger TLR-mediated production of pro-inflammatory cytokines like IL-1β, IL-6, TNF-α and nitric oxide in microglial cells [188]. In rat astrocytes, the PAMPs can trigger MAPKs like the extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK) pathways to activate AP1 transcription factor for IL-6 and IL-1β [191]. Spike of such pro-inflammatory cytokines in response to *N. fowleri* contributes to brain tissue damage and induces hyper-inflammation of the brain escalated by immune cells from other tissue sites. The combined outcome of such neuroinflammatory and contact-dependent damage is a fulminant and fatal infection of CNS.

## 4 Concluding Remarks

To conclude, it is worth to re-emphasize the point that all of the six parasitic protozoan diseases cause prolonged inflammatory responses in the brain. Such harmful reactions in response to the pathogens and their antigens can engender changes in the profiles of the brain cells (microglia, astrocytes), from being harmless to pro-inflammatory types, as well as cause their death that includes the neurons. Damage to the blood-brain barrier (often mediated by T lymphocyte), migrations of the various inflammatory cell types and further actions of the pro-inflammatory cytokines through their respective downstream signalling cascades are the common mechanisms that these pathogens use to cause brain pathology (Fig. 2). These diseases show diverse neurocognitive and behavioural abnormalities; however, two of them are particularly intimidating from the standpoint of the future of the nations, which include the endemic areas for some of these diseases. The first one is the psychiatric symptom of depression that manifests among the patients of CD and leishmaniasis, and the second one is the general disability in learning and memory among the children, infected with different forms of malaria. The burden of neuroinflammation, especially the chronic, low-grade inflammatory responses, has been documented for depression and developmental damage to the brain that can cause life-long impairments in learning and intellectual ability [192, 193]. Thus, the demand of the current hour is to conduct systematic research to develop effective, low-cost and accessible therapeutic means to mitigate neuroinflammation in these protozoan patients for the sake of saving the generations of several nations from

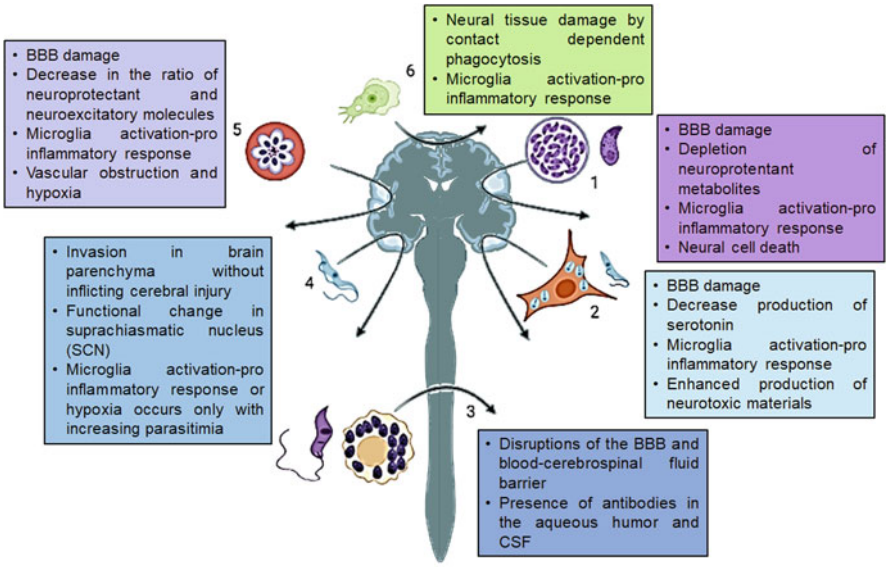


**Fig. 2** *Pathophysiological changes inflicted by parasitic infection in the central nervous system.* (1) Toxoplasmic encephalitis (TE) (*T. gondii*), (2) Chagas disease (*T. cruzi*), (3) leishmaniasis (*L. donovani/L. infantum*), (4) human African trypanosomiasis, (5) cerebral malaria (*P. falciparum*) and (6) primary amoebic meningoencephalitis (*N. fowleri*)

becoming psychologically and intellectually challenged. Taken together, acute systemic inflammation can cause havoc in the brain and thereby can mediate neurodegeneration, delirium and cognitive deterioration at the long term. Yet, there is a lack of longitudinal studies on cognitive outcomes of older people following an acute infection that correlate long-term patterns of cognitive decline with clinical variables and infection-related data, which could offer a deeper understanding on the relationship between these events. Moreover, deciphering the crosstalk between the peripheral and the local brain immune system might pave the avenue of developing novel therapeutic approaches for cognitive impairment.

**Conflicts of Interest** The authors declare no conflict of interest.

## References

1. Hughes DP, Libersat F. Neuroparasitology of parasite-insect associations. Annu Rev Entomol. 2018;63:471–87.
2. Libersat F, Kaiser M, Emanuel S. Mind control: how parasites manipulate cognitive functions in their insect hosts. Front Psychol. 2018;9:572.
3. Dare LO, Bruand PE, Gerard D, Marin B, Lameyre V, Boumediene F, et al. Associations of mental disorders and neurotropic parasitic diseases: a meta-analysis in developing and emerging countries. BMC Public Health. 2019;19(1):1645.

4. Oliveira-Filho J, Vieira-de-Melo RM, Reis PS, Lacerda AM, Neville IS, Cincura C, et al. Chagas disease is independently associated with brain atrophy. J Neurol. 2009;256(8):1363–5.
5. Melo GD, Goyard S, Fiette L, Boissonnas A, Combadiere C, Machado GF, et al. Unveiling cerebral Leishmaniasis: parasites and brain inflammation in Leishmania donovani infected mice. Sci Rep. 2017;7(1):8454.
6. Rodgers J, Bradley B, Kennedy PGE. Delineating neuroinflammation, parasite CNS invasion, and blood-brain barrier dysfunction in an experimental murine model of human African trypanosomiasis. Methods. 2017;127:79–87.
7. Khera T, Rangasamy V. Cognition and pain: A review. Front Psychol. 2021;12:673962.
8. Moriarty O, McGuire BE, Finn DP. The effect of pain on cognitive function: a review of clinical and preclinical research. Prog Neurobiol. 2011;93(3):385–404.
9. Tyng CM, Amin HU, Saad MNM, Malik AS. The influences of emotion on learning and memory. Front Psychol. 2017;8:1454.
10. Singh-Manoux A, Kivimaki M, Glymour MM, Elbaz A, Berr C, Ebmeier KP, et al. Timing of onset of cognitive decline: results from Whitehall II prospective cohort study. BMJ. 2012;344:d7622.
11. Magnuson A, Mohile S, Janelsins M. Cognition and cognitive impairment in older adults with cancer. Curr Geriatr Rep. 2016;5(3):213–9.
12. Katan M, Moon YP, Paik MC, Sacco RL, Wright CB, Elkind MS. Infectious burden and cognitive function: the northern Manhattan study. Neurology. 2013;80(13):1209–15.
13. Tangestani Fard M, Stough C. A review and hypothesized model of the mechanisms that underpin the relationship between inflammation and cognition in the elderly. Front Aging Neurosci. 2019;11:56.
14. Knopman DS, Petersen RC. Mild cognitive impairment and mild dementia: a clinical perspective. Mayo Clin Proc. 2014;89(10):1452–9.
15. Dando SJ, Mackay-Sim A, Norton R, Currie BJ, St John JA, Ekberg JA, et al. Pathogens penetrating the central nervous system: infection pathways and the cellular and molecular mechanisms of invasion. Clin Microbiol Rev. 2014;27(4):691–726.
16. Janicki-Deverts D, Cohen S, Doyle WJ, Turner RB, Treanor JJ. Infection-induced proinflammatory cytokines are associated with decreases in positive affect, but not increases in negative affect. Brain Behav Immun. 2007;21(3):301–7.
17. Damiano RF, Guedes BF, de Rocca CC, de Padua SA, Castro LHM, Munhoz CD, et al. Cognitive decline following acute viral infections: literature review and projections for post-COVID-19. Eur Arch Psychiatry Clin Neurosci. 2022;272(1):139–54.
18. Hamasaki MY, Machado MCC, Pinheiro da Silva F. Animal models of neuroinflammation secondary to acute insults originated outside the brain. J Neurosci Res. 2018;96(3):371–8.
19. Smith JE. A ubiquitous intracellular parasite: the cellular biology of toxoplasma gondii. Int J Parasitol. 1995;25(11):1301–9.
20. DiSabato DJ, Quan N, Godbout JP. Neuroinflammation: the devil is in the details. J Neurochem. 2016;139(Suppl 2):136–53.
21. Lee J, Hamanaka G, Lo EH, Arai K. Heterogeneity of microglia and their differential roles in white matter pathology. CNS Neurosci Ther. 2019;25(12):1290–8.
22. Bachiller S, Jimenez-Ferrer I, Paulus A, Yang Y, Swanberg M, Deierborg T, et al. Microglia in neurological diseases: A road map to brain-disease dependent-inflammatory response. Front Cell Neurosci. 2018;12:488.
23. Rotterman TM, Alvarez FJ. Microglia dynamics and interactions with Motoneurons Axotomized after nerve injuries revealed by two-photon imaging. Sci Rep. 2020;10(1):8648.
24. Gorelick PB. Role of inflammation in cognitive impairment: results of observational epidemiological studies and clinical trials. Ann N Y Acad Sci. 2010;1207:155–62.
25. Norden DM, Godbout JP. Review: microglia of the aged brain: primed to be activated and resistant to regulation. Neuropathol Appl Neurobiol. 2013;39(1):19–34.
26. McGeer PL, McGeer EG. Polymorphisms in inflammatory genes and the risk of Alzheimer disease. Arch Neurol. 2001;58(11):1790–2.

27. Bruunsgaard H, Andersen-Ranberg K, Jeune B, Pedersen AN, Skinhoj P, Pedersen BK. A high plasma concentration of TNF-alpha is associated with dementia in centenarians. J Gerontol A Biol Sci Med Sci. 1999;54(7):M357–64.
28. Holmes C, Cunningham C, Zotova E, Woolford J, Dean C, Kerr S, et al. Systemic inflammation and disease progression in Alzheimer disease. Neurology. 2009;73(10):768–74.
29. Adamis D, Lunn M, Martin FC, Treloar A, Gregson N, Hamilton G, et al. Cytokines and IGF-I in delirious and non-delirious acutely ill older medical inpatients. Age Ageing. 2009;38(3): 326–32. discussion 251
30. Li Y, Liu L, Barger SW, Griffin WS. Interleukin-1 mediates pathological effects of microglia on tau phosphorylation and on synaptophysin synthesis in cortical neurons through a p38-MAPK pathway. J Neurosci. 2003;23(5):1605–11.
31. Griffin WS. Inflammation and neurodegenerative diseases. Am J Clin Nutr. 2006;83(2):470S–4S.
32. Licastro F, Pedrini S, Caputo L, Annoni G, Davis LJ, Ferri C, et al. Increased plasma levels of interleukin-1, interleukin-6 and alpha-1-antichymotrypsin in patients with Alzheimer's disease: peripheral inflammation or signals from the brain? J Neuroimmunol. 2000;103(1): 97–102.
33. de Rooij SE, van Munster BC, Korevaar JC, Levi M. Cytokines and acute phase response in delirium. J Psychosom Res. 2007;62(5):521–5.
34. Sundelof J, Kilander L, Helmersson J, Larsson A, Ronnemaa E, Degerman-Gunnarsson M, et al. Systemic inflammation and the risk of Alzheimer's disease and dementia: a prospective population-based study. J Alzheimers Dis. 2009;18(1):79–87.
35. Sutinen EM, Pirttila T, Anderson G, Salminen A, Ojala JO. Pro-inflammatory interleukin-18 increases Alzheimer's disease-associated amyloid-beta production in human neuron-like cells. J Neuroinflammation. 2012;9:199.
36. Curran B, O'Connor JJ. The pro-inflammatory cytokine interleukin-18 impairs long-term potentiation and NMDA receptor-mediated transmission in the rat hippocampus in vitro. Neuroscience. 2001;108(1):83–90.
37. Ojala JO, Sutinen EM, Salminen A, Pirttila T. Interleukin-18 increases expression of kinases involved in tau phosphorylation in SH-SY5Y neuroblastoma cells. J Neuroimmunol. 2008;205 (1–2):86–93.
38. Wu JQ, Chen DC, Tan YL, Tan SP, Xiu MH, Wang ZR, et al. Altered interleukin-18 levels are associated with cognitive impairment in chronic schizophrenia. J Psychiatr Res. 2016;76:9–15.
39. Yaffe K, Lindquist K, Penninx BW, Simonsick EM, Pahor M, Kritchevsky S, et al. Inflammatory markers and cognition in well-functioning African-American and white elders. Neurology. 2003;61(1):76–80.
40. Kaur N, Chugh H, Sakharkar MK, Dhawan U, Chidambaram SB, Chandra R. Neuroinflammation mechanisms and Phytotherapeutic intervention: A systematic review. ACS Chem Neurosci. 2020;11(22):3707–31.
41. Sanchez-Sarasua S, Fernandez-Perez I, Espinosa-Fernandez V, Sanchez-Perez AM, Ledesma JC. Can we treat Neuroinflammation in Alzheimer's disease? Int J Mol Sci. 2020;21(22)
42. Brod SA. Anti-inflammatory agents: an approach to prevent cognitive decline in Alzheimer's disease. J Alzheimers Dis. 2022;85(2):457–72.
43. Group AR, Martin BK, Szekely C, Brandt J, Piantadosi S, Breitner JC, et al. Cognitive function over time in the Alzheimer's disease anti-inflammatory prevention trial (ADAPT): results of a randomized, controlled trial of naproxen and celecoxib. Arch Neurol. 2008;65(7): 896–905.
44. Szekely CA, Thorne JE, Zandi PP, Ek M, Messias E, Breitner JC, et al. Nonsteroidal anti-inflammatory drugs for the prevention of Alzheimer's disease: a systematic review. Neuroepidemiology. 2004;23(4):159–69.

45. Calvo-Rodriguez M, Nunez L, Villalobos C. Non-steroidal anti-inflammatory drugs (NSAIDs) and neuroprotection in the elderly: a view from the mitochondria. Neural Regen Res. 2015;10 (9):1371–2.
46. Ortiz-Guerrero G, Gonzalez-Reyes RE, de-la-Torre A, Medina-Rincon G, Nava-Mesa MO. Pathophysiological mechanisms of cognitive impairment and neurodegeneration by toxoplasma gondii infection. Brain Sci. 2020;10(6)
47. Dubey JP. Advances in the life cycle of toxoplasma gondii. Int J Parasitol. 1998;28(7): 1019–24.
48. Black MW, Boothroyd JC. Lytic cycle of toxoplasma gondii. Microbiol Mol Biol Rev. 2000;64(3):607–23.
49. Gazzinelli RT, Eltoum I, Wynn TA, Sher A. Acute cerebral toxoplasmosis is induced by in vivo neutralization of TNF-alpha and correlates with the down-regulated expression of inducible nitric oxide synthase and other markers of macrophage activation. J Immunol. 1993;151(7):3672–81.
50. Wohlfert EA, Blader IJ, Wilson EH. Brains and brawn: toxoplasma infections of the central nervous system and skeletal muscle. Trends Parasitol. 2017;33(7):519–31.
51. Chow BW, Gu C. The molecular constituents of the blood-brain barrier. Trends Neurosci. 2015;38(10):598–608.
52. Da Gama LM, Ribeiro-Gomes FL, Guimaraes U Jr, Arnholdt AC. Reduction in adhesiveness to extracellular matrix components, modulation of adhesion molecules and in vivo migration of murine macrophages infected with toxoplasma gondii. Microbes Infect. 2004;6(14): 1287–96.
53. Ueno N, Harker KS, Clarke EV, McWhorter FY, Liu WF, Tenner AJ, et al. Real-time imaging of toxoplasma-infected human monocytes under fluidic shear stress reveals rapid translocation of intracellular parasites across endothelial barriers. Cell Microbiol. 2014;16(4):580–95.
54. Bhandage AK, Kanatani S, Barragan A. Toxoplasma-induced Hypermigration of primary cortical microglia implicates GABAergic signaling. Front Cell Infect Microbiol. 2019;9:73.
55. Tardieux I, Menard R. Migration of Apicomplexa across biological barriers: the toxoplasma and plasmodium rides. Traffic. 2008;9(5):627–35.
56. Harker KS, Jivan E, McWhorter FY, Liu WF, Lodoen MB. Shear forces enhance toxoplasma gondii tachyzoite motility on vascular endothelium. MBio. 2014;5(2):e01111–3.
57. Berenreiterova M, Flegr J, Kubena AA, Nemec P. The distribution of toxoplasma gondii cysts in the brain of a mouse with latent toxoplasmosis: implications for the behavioral manipulation hypothesis. PLoS One. 2011;6(12):e28925.
58. Lang D, Schott BH, van Ham M, Morton L, Kulikovskaja L, Herrera-Molina R, et al. Chronic toxoplasma infection is associated with distinct alterations in the synaptic protein composition. J Neuroinflammation. 2018;15(1):216.
59. Fuks JM, Arrighi RB, Weidner JM, Kumar Mendu S, Jin Z, Wallin RP, et al. GABAergic signaling is linked to a hypermigratory phenotype in dendritic cells infected by toxoplasma gondii. PLoS Pathog. 2012;8(12):e1003051.
60. Kanatani S, Fuks JM, Olafsson EB, Westermark L, Chambers B, Varas-Godoy M, et al. Voltage-dependent calcium channel signaling mediates GABAA receptor-induced migratory activation of dendritic cells infected by toxoplasma gondii. PLoS Pathog. 2017;13(12): e1006739.
61. Wang T, Sun X, Qin W, Zhang X, Wu L, Li Y, et al. From inflammatory reactions to neurotransmitter changes: implications for understanding the neurobehavioral changes in mice chronically infected with toxoplasma gondii. Behav Brain Res. 2019;359:737–48.
62. Gonzalez-Reyes RE, Nava-Mesa MO, Vargas-Sanchez K, Ariza-Salamanca D, Mora-Munoz L. Involvement of astrocytes in Alzheimer's disease from a Neuroinflammatory and oxidative stress perspective. Front Mol Neurosci. 2017;10:427.
63. Liesenfeld O, Parvanova I, Zerrahn J, Han SJ, Heinrich F, Munoz M, et al. The IFN-gamma-inducible GTPase, Irga6, protects mice against toxoplasma gondii but not against plasmodium berghei and some other intracellular pathogens. PLoS One. 2011;6(6):e20568.

64. Carruthers VB, Suzuki Y. Effects of toxoplasma gondii infection on the brain. Schizophr Bull. 2007;33(3):745–51.
65. Wilson EH, Harris TH, Mrass P, John B, Tait ED, Wu GF, et al. Behavior of parasite-specific effector CD8+ T cells in the brain and visualization of a kinesis-associated system of reticular fibers. Immunity. 2009;30(2):300–11.
66. Dunay IR, Gajurel K, Dhakal R, Liesenfeld O, Montoya JG. Treatment of toxoplasmosis: historical perspective, animal models, and current clinical practice. Clin Microbiol Rev. 2018;31(4)
67. Sana M, Rashid M, Rashid I, Akbar H, Gomez-Marin JE, Dimier-Poisson I. Immune response against toxoplasmosis-some recent updates RH: Toxoplasma gondii immune response. Int J Immunopathol Pharmacol. 2022;36:3946320221078436.
68. Fischer HG, Nitzgen B, Reichmann G, Gross U, Hadding U. Host cells of toxoplasma gondii encystation in infected primary culture from mouse brain. Parasitol Res. 1997;83(7):637–41.
69. Ferguson DJ, Hutchison WM. An ultrastructural study of the early development and tissue cyst formation of toxoplasma gondii in the brains of mice. Parasitol Res. 1987;73(6):483–91.
70. Mukhopadhyay D, Arranz-Solis D, Saeij JPJ. Influence of the host and parasite strain on the immune response during toxoplasma infection. Front Cell Infect Microbiol. 2020;10:580425.
71. Hwang YS, Shin JH, Yang JP, Jung BK, Lee SH, Shin EH. Characteristics of infection immunity regulated by toxoplasma gondii to maintain chronic infection in the brain. Front Immunol. 2018;9:158.
72. Torres L, Robinson SA, Kim DG, Yan A, Cleland TA, Bynoe MS. Toxoplasma gondii alters NMDAR signaling and induces signs of Alzheimer's disease in wild-type, C57BL/6 mice. J Neuroinflammation. 2018;15(1):57.
73. Brooks JM, Carrillo GL, Su J, Lindsay DS, Fox MA, Blader IJ. Toxoplasma gondii infections Alter GABAergic synapses and signaling in the central nervous system. MBio. 2015;6(6): e01428–15.
74. Lucchese G. From toxoplasmosis to schizophrenia via NMDA dysfunction: peptide overlap between toxoplasma gondii and N-methyl-d-aspartate receptors as a potential mechanistic link. Front Psych. 2017;8:37.
75. McFarland R, Wang ZT, Jouroukhin Y, Li Y, Mychko O, Coppens I, et al. AAH2 gene is not required for dopamine-dependent neurochemical and behavioral abnormalities produced by toxoplasma infection in mouse. Behav Brain Res. 2018;347:193–200.
76. Martin HL, Alsaady I, Howell G, Prandovszky E, Peers C, Robinson P, et al. Effect of parasitic infection on dopamine biosynthesis in dopaminergic cells. Neuroscience. 2015;306:50–62.
77. Stibbs HH. Changes in brain concentrations of catecholamines and indoleamines in toxoplasma gondii infected mice. Ann Trop Med Parasitol. 1985;79(2):153–7.
78. Flegr J, Horacek J. Negative effects of latent toxoplasmosis on mental health. Front Psych. 2019;10:1012.
79. McConkey GA, Martin HL, Bristow GC, Webster JP. Toxoplasma gondii infection and behavior–location, location, location? J Exp Biol. 2013;216(Pt 1):113–9.
80. de Haan L, Sutterland AL, Schotborgh JV, Schirmbeck F, de Haan L. Association of Toxoplasma gondii Seropositivity with cognitive function in healthy people: A systematic review and meta-analysis. JAMA Psychiat. 2021;78(10):1103–12.
81. Kannan G, Pletnikov MV. Toxoplasma gondii and cognitive deficits in schizophrenia: an animal model perspective. Schizophr Bull. 2012;38(6):1155–61.
82. Bayani M, Riahi SM, Bazrafshan N, Ray Gamble H, Rostami A. Toxoplasma gondii infection and risk of Parkinson and Alzheimer diseases: A systematic review and meta-analysis on observational studies. Acta Trop. 2019;196:165–71.
83. Kusbeci OY, Miman O, Yaman M, Aktepe OC, Yazar S. Could toxoplasma gondii have any role in Alzheimer disease? Alzheimer Dis Assoc Disord. 2011;25(1):1–3.
84. Nayeri Chegeni T, Sarvi S, Moosazadeh M, Sharif M, Aghayan SA, Amouei A, et al. Is toxoplasma gondii a potential risk factor for Alzheimer's disease? A systematic review and meta-analysis. Microb Pathog. 2019;137:103751.

85. Duarte-Silva E, Maes M, Macedo D, Savino W, Peixoto CA. Shared neuroimmune and oxidative pathways underpinning Chagas disease and major depressive disorder. Transl Psychiatry. 2020;10(1):419.
86. Almeria S, Murata FHA, Cerqueira-Cezar CK, Kwok OCH, Shipley A, Dubey JP. Epidemiological and public health significance of toxoplasma gondii infection in wild rabbits and hares: 2010-2020. Microorganisms. 2021;9(3)
87. Clayton J. Chagas disease 101. Nature. 2010;465(7301):S4–5.
88. Silva WT, Avila MR, Oliveira LFF, Figueiredo PHS, Lima VP, Bastone AC, et al. Prevalence and determinants of depressive symptoms in patients with Chagas cardiomyopathy and predominantly preserved cardiac function. Rev Soc Bras Med Trop. 2020;53:e20200123.
89. Suman AC, Costa E, Bazan SGZ, Hueb JC, Carvalho FC, Martin LC, et al. Evaluating respiratory musculature, quality of life, anxiety, and depression among patients with indeterminate chronic Chagas disease and symptoms of pulmonary hypertension. Rev Soc Bras Med Trop. 2017;50(2):194–8.
90. Vilar-Pereira G, Ruivo LA, Lannes-Vieira J. Behavioral alterations are independent of sickness behavior in chronic experimental Chagas disease. Mem Inst Oswaldo Cruz. 2015;110(8):1042–50.
91. Ozaki Y, Guariento ME, de Almeida EA. Quality of life and depressive symptoms in Chagas disease patients. Qual Life Res. 2011;20(1):133–8.
92. Jackson Y, Castillo S, Hammond P, Besson M, Brawand-Bron A, Urzola D, et al. Metabolic, mental health, behavioral and socioeconomic characteristics of migrants with Chagas disease in a non-endemic country. Tropical Med Int Health. 2012;17(5):595–603.
93. Jorg ME, Zalazar RI. Encephalopathic forms of chronic Chagas disease seen in Argentina. Mem Inst Oswaldo Cruz. 1981;76(4):353–60.
94. Hofstraat K, van Brakel WH. Social stigma towards neglected tropical diseases: a systematic review. Int Health. 2016;8(Suppl 1):i53–70.
95. Guimaraes PM, Passos SR, Calvet GA, Hokerberg YH, Lessa JL, Andrade CA. Suicide risk and alcohol and drug abuse in outpatients with HIV infection and Chagas disease. Braz J Psychiatry. 2014;36(2):131–7.
96. Novaes RD, Santos EC, Fialho M, Goncalves WG, Sequetto PL, Talvani A, et al. Nonsteroidal anti-inflammatory is more effective than anti-oxidant therapy in counteracting oxidative/nitrosative stress and heart disease in T. cruzi-infected mice. Parasitology. 2017;144(7):904–16.
97. Lima-Costa MF, Castro-Costa E, Uchoa E, Firmo J, Ribeiro AL, Ferri CP, et al. A population-based study of the association between Trypanosoma cruzi infection and cognitive impairment in old age (the Bambui study). Neuroepidemiology. 2009;32(2):122–8.
98. Vilar-Pereira G, Castano Barrios L, Silva AAD, Martins Batista A, Resende Pereira I, Cruz Moreira O, et al. Memory impairment in chronic experimental Chagas disease: Benznidazole therapy reversed cognitive deficit in association with reduction of parasite load and oxidative stress in the nervous tissue. PLoS One. 2021;16(1):e0244710.
99. Vilar-Pereira G, Silva AA, Pereira IR, Silva RR, Moreira OC, de Almeida LR, et al. Trypanosoma cruzi-induced depressive-like behavior is independent of meningoencephalitis but responsive to parasiticide and TNF-targeted therapeutic interventions. Brain Behav Immun. 2012;26(7):1136–49.
100. da Matta Guedes PM, Gutierrez FR, Maia FL, Milanezi CM, Silva GK, Pavanelli WR, et al. IL-17 produced during Trypanosoma cruzi infection plays a central role in regulating parasite-induced myocarditis. PLoS Negl Trop Dis. 2010;4(2):e604.
101. Almeida MS, Lorena VMB, Medeiros CA, Junior WO, Cavalcanti M, Martins SM, et al. Alternative Th17 and CD4(+) CD25(+) FoxP3(+) cell frequencies increase and correlate with worse cardiac function in Chagas cardiomyopathy. Scand J Immunol. 2018;87(4):e12650.
102. Pires M, Wright B, Kaye PM, da Conceicao V, Churchill RC. The impact of leishmaniasis on mental health and psychosocial Well-being: A systematic review. PLoS One. 2019;14(10):e0223313.

103. Petersen CA, Greenlee MH. Neurologic manifestations of Leishmania spp. Infection. J Neuroparasitol. 2011:2.
104. Mustafa D. Neurological disturbances in visceral leishmaniasis. J Trop Med Hyg. 1965;68 (10):248–50.
105. Hashim FA, Ahmed AE, el Hassan M, el Mubarak MH, Yagi H, Ibrahim EN, et al. Neurologic changes in visceral leishmaniasis. Am J Trop Med Hyg. 1995;52(2):149–54.
106. Diniz LM, Duani H, Freitas CR, Figueiredo RM, Xavier CC. Neurological involvement in visceral leishmaniasis: case report. Rev Soc Bras Med Trop. 2010;43(6):743–5.
107. Prasad LS, Sen S. Migration of Leishmania donovani amastigotes in the cerebrospinal fluid. Am J Trop Med Hyg. 1996;55(6):652–4.
108. Kubba R, el Hassan AM, Al Gindan Y, Omer AH, Bushra M, Kutty MK. Peripheral nerve involvement in cutaneous leishmaniasis (Old World). Int J Dermatol. 1987;26(8):527–31.
109. Melo GD, Marcondes M, Vasconcelos RO, Machado GF. Leukocyte entry into the CNS of Leishmania chagasi naturally infected dogs. Vet Parasitol. 2009;162(3–4):248–56.
110. Lima VM, Goncalves ME, Ikeda FA, Luvizotto MC, Feitosa MM. Anti-leishmania antibodies in cerebrospinal fluid from dogs with visceral leishmaniasis. Braz J Med Biol Res. 2003;36(4): 485–9.
111. Melo GD, Silva JE, Grano FG, Souza MS, Machado GF. Leishmania infection and neuroinflammation: specific chemokine profile and absence of parasites in the brain of naturally-infected dogs. J Neuroimmunol. 2015;289:21–9.
112. Tafuri WL, Santos RL, Arantes RM, Goncalves R, de Melo MN, Michalick MS, et al. An alternative immunohistochemical method for detecting Leishmania amastigotes in paraffin-embedded canine tissues. J Immunol Methods. 2004;292(1–2):17–23.
113. Vinuelas J, Garcia-Alonso M, Ferrando L, Navarrete I, Molano I, Miron C, et al. Meningeal leishmaniosis induced by Leishmania infantum in naturally infected dogs. Vet Parasitol. 2001;101(1):23–7.
114. Maia CS, Monteiro MC, Gavioli EC, Oliveira FR, Oliveira GB, Romao PR. Neurological disease in human and canine leishmaniasis–clinical features and immunopathogenesis. Parasite Immunol. 2015;37(8):385–93.
115. Marangoni NR, Melo GD, Moraes OC, Souza MS, Perri SH, Machado GF. Levels of matrix metalloproteinase-2 and metalloproteinase-9 in the cerebrospinal fluid of dogs with visceral leishmaniasis. Parasite Immunol. 2011;33(6):330–4.
116. Toledo AC Jr, da Silva RE, Carmo RF, Amaral TA, Luz ZM, Rabello A. Assessment of the quality of life of patients with cutaneous leishmaniasis in Belo Horizonte, Brazil, 2009-2010. A pilot study. Trans R Soc Trop Med Hyg. 2013;107(5):335–6.
117. Yanik M, Gurel MS, Simsek Z, Kati M. The psychological impact of cutaneous leishmaniasis. Clin Exp Dermatol. 2004;29(5):464–7.
118. Simsek Z, Ak D, Altindag A, Gunes M. Prevalence and predictors of mental disorders among women in Sanliurfa, Southeastern Turkey. J Public Health (Oxf). 2008;30(4):487–93.
119. Vares B, Mohseni M, Heshmatkhah A, Farjzadeh S, Safizadeh H, Shamsi-Meymandi S, et al. Quality of life in patients with cutaneous leishmaniasis. Arch Iran Med. 2013;16(8):474–7.
120. Turan E, Kandemir H, Yesilova Y, Ekinci S, Tanrikulu O, Kandemir SB, et al. Assessment of psychiatric morbidity and quality of life in children and adolescents with cutaneous leishmaniasis and their parents. Postepy Dermatol Alergol. 2015;32(5):344–8.
121. Chahed MK, Bellali H, Ben Jemaa S, Bellaj T. Psychological and psychosocial consequences of zoonotic cutaneous Leishmaniasis among women in Tunisia: preliminary findings from an exploratory study. PLoS Negl Trop Dis. 2016;10(10):e0005090.
122. Mohammad NS, Nazli R, Zafar H, Fatima S. Effects of lipid based multiple micronutrients supplement on the birth outcome of underweight pre-eclamptic women: A randomized clinical trial. Pak J Med Sci. 2022;38(1):219–26.
123. Ganguly S, Saha P, Chatterjee M, Roy S, Ghosh TK, Guha SK, et al. PKDL–A Silent Parasite Pool for Transmission of Leishmaniasis in Kala-azar Endemic Areas of Malda District, West Bengal, India. PLoS Negl Trop Dis. 2015;9(10):e0004138.

124. Govil D, Sahoo H, Pedgaonkar SP, Chandra Das K, Lhungdim H. Assessing knowledge, attitudes, and preventive practices related to kala-A: A study of rural Madhepura, Bihar. India Am J Trop Med Hyg. 2018;98(3):857–63.
125. Alemayehu M, Wubshet M, Mesfin N, Tamiru A, Gebayehu A. Health-related quality of life of HIV infected adults with and without visceral Leishmaniasis in Northwest Ethiopia. Health Qual Life Outcomes. 2017;15(1):65.
126. Alemayehu M, Wubshet M, Mesfin N, Gebayehu A. Effect of health care on quality of life among human immunodeficiency virus infected adults with and without visceral Leishmaniasis in Northwest Ethiopia: a longitudinal follow-up study. Am J Trop Med Hyg. 2018;98(3): 747–52.
127. Carswell J. Kala-azar at Kitui. East Afr Med J. 1953;30(7):287–93.
128. Gao JM, Qian ZY, Hide G, Lai DH, Lun ZR, Wu ZD. Human African trypanosomiasis: the current situation in endemic regions and the risks for non-endemic regions from imported cases. Parasitology. 2020;147(9):922–31.
129. Aksoy S, Buscher P, Lehane M, Solano P, Van Den Abbeele J. Human African trypanosomiasis control: achievements and challenges. PLoS Negl Trop Dis. 2017;11(4):e0005454.
130. Blum J, Schmid C, Burri C. Clinical aspects of 2541 patients with second stage human African trypanosomiasis. Acta Trop. 2006;97(1):55–64.
131. Ponte-Sucre A. An overview of Trypanosoma brucei infections: an intense host-parasite interaction. Front Microbiol. 2016;7:2126.
132. Buguet A, Bourdon L, Bouteille B, Cespuglio R, Vincendeau P, Radomski MW, et al. The duality of sleeping sickness: focusing on sleep. Sleep Med Rev. 2001;5(2):139–53.
133. Masocha W, Rottenberg ME, Kristensson K. Migration of African trypanosomes across the blood-brain barrier. Physiol Behav. 2007;92(1–2):110–4.
134. Laperchia C, Palomba M, Seke Etet PF, Rodgers J, Bradley B, Montague P, et al. Trypanosoma brucei invasion and T-cell infiltration of the brain parenchyma in experimental sleeping sickness: timing and correlation with functional changes. PLoS Negl Trop Dis. 2016;10(12):e0005242.
135. Lundkvist GB, Hill RH, Kristensson K. Disruption of circadian rhythms in synaptic activity of the suprachiasmatic nuclei by African trypanosomes and cytokines. Neurobiol Dis. 2002;11 (1):20–7.
136. Tesoriero C, Xu YZ, Mumba Ngoyi D, Bentivoglio M. Neural damage in experimental Trypanosoma brucei gambiense infection: the Suprachiasmatic nucleus. Front Neuroanat. 2018;12:6.
137. Adebiyi OE, Omobowale TO, Abatan MO. Neurocognitive domains and neuropathological changes in experimental infection with Trypanosoma brucei brucei in Wistar rats. Heliyon. 2021;7(11):e08260.
138. Masocha W, Amin DN, Kristensson K, Rottenberg ME. Differential invasion of Trypanosoma brucei brucei and lymphocytes into the brain of C57BL/6 and 129Sv/Ev mice. Scand J Immunol. 2008;68(5):484–91.
139. Nikolskaia OV, Ana Paula CA de Lima de ALAP, Kim YV, Lonsdale-Eccles JD, Fukuma T, Scharfstein J, et al. Blood-brain barrier traversal by African trypanosomes requires calcium signaling induced by parasite cysteine protease. J Clin Invest 2006;116(10):2739–2747.
140. Grab DJ, Garcia-Garcia JC, Nikolskaia OV, Kim YV, Brown A, Pardo CA, et al. Protease activated receptor signaling is required for African trypanosome traversal of human brain microvascular endothelial cells. PLoS Negl Trop Dis. 2009;3(7):e479.
141. Mogk S, Bosselmann CM, Mudogo CN, Stein J, Wolburg H, Duszenko M. African trypanosomes and brain infection–the unsolved question. Biol Rev Camb Philos Soc. 2017;92(3):1675–87.
142. Mogk S, Meiwes A, Bosselmann CM, Wolburg H, Duszenko M. The lane to the brain: how African trypanosomes invade the CNS. Trends Parasitol. 2014;30(10):470–7.

143. Kristensson K, Nygard M, Bertini G, Bentivoglio M. African trypanosome infections of the nervous system: parasite entry and effects on sleep and synaptic functions. Prog Neurobiol. 2010;91(2):152–71.
144. Schultzberg M, Ambatsis M, Samuelsson EB, Kristensson K, van Meirvenne N. Spread of Trypanosoma brucei to the nervous system: early attack on circumventricular organs and sensory ganglia. J Neurosci Res. 1988;21(1):56–61.
145. Philip KA, Dascombe MJ, Fraser PA, Pentreath VW. Blood-brain barrier damage in experimental African trypanosomiasis. Ann Trop Med Parasitol. 1994;88(6):607–16.
146. Rodgers J, McCabe C, Gettinby G, Bradley B, Condon B, Kennedy PG. Magnetic resonance imaging to assess blood-brain barrier damage in murine trypanosomiasis. Am J Trop Med Hyg. 2011;84(2):344–50.
147. MacLean L, Reiber H, Kennedy PG, Sternberg JM. Stage progression and neurological symptoms in Trypanosoma brucei rhodesiense sleeping sickness: role of the CNS inflammatory response. PLoS Negl Trop Dis. 2012;6(10):e1857.
148. Frevert U, Movila A, Nikolskaia OV, Raper J, Mackey ZB, Abdulla M, et al. Early invasion of brain parenchyma by African trypanosomes. PLoS One. 2012;7(8):e43913.
149. Rouzer CA, Cerami A. Hypertriglyceridemia associated with Trypanosoma brucei brucei infection in rabbits: role of defective triglyceride removal. Mol Biochem Parasitol. 1980;2(1):31–8.
150. Keita M, Bouteille B, Enanga B, Vallat JM, Dumas M. Trypanosoma brucei brucei: a long-term model of human African trypanosomiasis in mice, meningo-encephalitis, astrocytosis, and neurological disorders. Exp Parasitol. 1997;85(2):183–92.
151. Reincke M, Arlt W, Heppner C, Petzke F, Chrousos GP, Allolio B. Neuroendocrine dysfunction in African trypanosomiasis. The role of cytokines. Ann N Y Acad Sci. 1998;840:809–21.
152. Rijo-Ferreira F, Takahashi JS. Sleeping sickness: A tale of two clocks. Front Cell Infect Microbiol. 2020;10:525097.
153. Amin DN, Vodnala SK, Masocha W, Sun B, Kristensson K, Rottenberg ME. Distinct toll-like receptor signals regulate cerebral parasite load and interferon alpha/beta and tumor necrosis factor alpha-dependent T-cell infiltration in the brains of Trypanosoma brucei-infected mice. J Infect Dis. 2012;205(2):320–32.
154. Amin DN, Rottenberg ME, Thomsen AR, Mumba D, Fenger C, Kristensson K, et al. Expression and role of CXCL10 during the encephalitic stage of experimental and clinical African trypanosomiasis. J Infect Dis. 2009;200(10):1556–65.
155. Masocha W, Robertson B, Rottenberg ME, Mhlanga J, Sorokin L, Kristensson K. Cerebral vessel laminins and IFN-gamma define Trypanosoma brucei brucei penetration of the blood-brain barrier. J Clin Invest. 2004;114(5):689–94.
156. Rodgers J, Bradley B, Kennedy PG, Sternberg JM. Central nervous system Parasitosis and Neuroinflammation ameliorated by systemic IL-10 Administration in Trypanosoma brucei-infected mice. PLoS Negl Trop Dis. 2015;9(10):e0004201.
157. Laperchia C, Tesoriero C, Seke-Etet PF, La Verde V, Colavito V, Grassi-Zucconi G, et al. Expression of interferon-inducible chemokines and sleep/wake changes during early encephalitis in experimental African trypanosomiasis. PLoS Negl Trop Dis. 2017;11(8):e0005854.
158. Molyneux ME, Taylor TE, Wirima JJ, Borgstein A. Clinical features and prognostic indicators in paediatric cerebral malaria: a study of 131 comatose Malawian children. Q J Med. 1989;71(265):441–59.
159. Oluwayemi IO, Brown BJ, Oyedeji OA, Oluwayemi MA. Neurological sequelae in survivors of cerebral malaria. Pan Afr Med J. 2013;15:88.
160. Annegers JF, Hauser WA, Shirts SB, Kurland LT. Factors prognostic of unprovoked seizures after febrile convulsions. N Engl J Med. 1987;316(9):493–8.
161. Vargha-Khadem F, Gadian DG, Watkins KE, Connelly A, Van Paesschen W, Mishkin M. Differential effects of early hippocampal pathology on episodic and semantic memory. Science. 1997;277(5324):376–80.

162. Fernando SD, Rodrigo C, Rajapakse S. The 'hidden' burden of malaria: cognitive impairment following infection. Malar J. 2010;9:366.
163. Carter JA, Ross AJ, Neville BG, Obiero E, Katana K, Mung'ala-Odera V, et al. Developmental impairments following severe falciparum malaria in children. Tropical Med Int Health. 2005;10(1):3–10.
164. Carter JA, Lees JA, Gona JK, Murira G, Rimba K, Neville BG, et al. Severe falciparum malaria and acquired childhood language disorder. Dev Med Child Neurol. 2006;48(1):51–7.
165. Idro R, Kakooza-Mwesige A, Balyejjussa S, Mirembe G, Mugasha C, Tugumisirize J, et al. Severe neurological sequelae and behavior problems after cerebral malaria in Ugandan children. BMC Res Notes. 2010;3:104.
166. John CC, Bangirana P, Byarugaba J, Opoka RO, Idro R, Jurek AM, et al. Cerebral malaria in children is associated with long-term cognitive impairment. Pediatrics. 2008;122(1):e92–9.
167. Boivin MJ, Bangirana P, Byarugaba J, Opoka RO, Idro R, Jurek AM, et al. Cognitive impairment after cerebral malaria in children: a prospective study. Pediatrics. 2007;119(2): e360–6.
168. Boivin MJ. Effects of early cerebral malaria on cognitive ability in Senegalese children. J Dev Behav Pediatr. 2002;23(5):353–64.
169. Kihara M, Carter JA, Holding PA, Vargha-Khadem F, Scott RC, Idro R, et al. Impaired everyday memory associated with encephalopathy of severe malaria: the role of seizures and hippocampal damage. Malar J. 2009;8:273.
170. Kihara M, de Haan M, Garrashi HH, Neville BG, Newton CR. Atypical brain response to novelty in rural African children with a history of severe falciparum malaria. J Neurol Sci. 2010;296(1–2):88–95.
171. Fernando D, Wickremasinghe R, Mendis KN, Wickremasinghe AR. Cognitive performance at school entry of children living in malaria-endemic areas of Sri Lanka. Trans R Soc Trop Med Hyg. 2003;97(2):161–5.
172. Fernando D, de Silva D, Wickremasinghe R. Short-term impact of an acute attack of malaria on the cognitive performance of schoolchildren living in a malaria-endemic area of Sri Lanka. Trans R Soc Trop Med Hyg. 2003;97(6):633–9.
173. Fernando SD, Gunawardena DM, Bandara MR, De Silva D, Carter R, Mendis KN, et al. The impact of repeated malaria attacks on the school performance of children. Am J Trop Med Hyg. 2003;69(6):582–8.
174. Al Serouri AW, Grantham-McGregor SM, Greenwood B, Costello A. Impact of asymptomatic malaria parasitaemia on cognitive function and school achievement of schoolchildren in the Yemen Republic. Parasitology. 2000;121(Pt 4):337–45.
175. Hunt NH, Golenser J, Chan-Ling T, Parekh S, Rae C, Potter S, et al. Immunopathogenesis of cerebral malaria. Int J Parasitol. 2006;36(5):569–82.
176. Lovegrove FE, Gharib SA, Patel SN, Hawkes CA, Kain KC, Liles WC. Expression microarray analysis implicates apoptosis and interferon-responsive mechanisms in susceptibility to experimental cerebral malaria. Am J Pathol. 2007;171(6):1894–903.
177. John CC, Panoskaltsis-Mortari A, Opoka RO, Park GS, Orchard PJ, Jurek AM, et al. Cerebrospinal fluid cytokine levels and cognitive impairment in cerebral malaria. Am J Trop Med Hyg. 2008;78(2):198–205.
178. Potchen MJ, Birbeck GL, Demarco JK, Kampondeni SD, Beare N, Molyneux ME, et al. Neuroimaging findings in children with retinopathy-confirmed cerebral malaria. Eur J Radiol. 2010;74(1):262–8.
179. Dai M, Reznik SE, Spray DC, Weiss LM, Tanowitz HB, Gulinello M, et al. Persistent cognitive and motor deficits after successful antimalarial treatment in murine cerebral malaria. Microbes Infect. 2010;12(14–15):1198–207.
180. Guemez A, Garcia E. Primary amoebic meningoencephalitis by Naegleria fowleri: pathogenesis and treatments. Biomol Ther. 2021;11(9)
181. Maciver SK, Pinero JE, Lorenzo-Morales J. Is Naegleria fowleri an emerging parasite? Trends Parasitol. 2020;36(1):19–28.

182. Dzikowiec M, Goralska K, Blaszkowska J. Neuroinvasions caused by parasites. Ann Parasitol. 2017;63(4):243–53.
183. Siddiqui R, Ali IKM, Cope JR, Khan NA. Biology and pathogenesis of Naegleria fowleri. Acta Trop. 2016;164:375–94.
184. Cope JR, Ali IK. Primary amebic meningoencephalitis: what have we learned in the last 5 years? Curr Infect Dis Rep. 2016;18(10):31.
185. Gompf SG, Garcia C. Lethal encounters: the evolving spectrum of amoebic meningoencephalitis. IDCases. 2019;15:e00524.
186. Grace E, Asbill S, Virga K. Naegleria fowleri: pathogenesis, diagnosis, and treatment options. Antimicrob Agents Chemother. 2015;59(11):6677–81.
187. Pugh JJ, Levy RA. Naegleria fowleri: diagnosis, pathophysiology of brain inflammation, and antimicrobial treatments. ACS Chem Neurosci. 2016;7(9):1178–9.
188. Moseman EA. Battling brain-eating amoeba: enigmas surrounding immunity to Naegleria fowleri. PLoS Pathog. 2020;16(4):e1008406.
189. Jamerson M, Schmoyer JA, Park J, Marciano-Cabral F, Cabral GA. Identification of Naegleria fowleri proteins linked to primary amoebic meningoencephalitis. Microbiology (Reading). 2017;163(3):322–32.
190. Coronado-Velazquez D, Betanzos A, Serrano-Luna J, Shibayama M. An in vitro model of the blood-brain barrier: Naegleria fowleri affects the tight junction proteins and activates the microvascular endothelial cells. J Eukaryot Microbiol. 2018;65(6):804–19.
191. Kim JH, Song AR, Sohn HJ, Lee J, Yoo JK, Kwon D, et al. IL-1beta and IL-6 activate inflammatory responses of astrocytes against Naegleria fowleri infection via the modulation of MAPKs and AP-1. Parasite Immunol. 2013;35(3–4):120–8.
192. Vogelzangs N, de Jonge P, Smit JH, Bahn S, Penninx BW. Cytokine production capacity in depression and anxiety. Transl Psychiatry. 2016;6(5):e825.
193. Di Marco B, Bonaccorso CM, Aloisi E, D'Antoni S, Catania MV. Neuro-inflammatory mechanisms in developmental disorders associated with intellectual disability and autism Spectrum disorder: A neuro- immune perspective. CNS Neurol Disord Drug Targets. 2016;15(4):448–63.

# Cycling Within a Cell: Cell Cycle of Intracellular Kinetoplastid Parasites

Anindita Bhattacharjee and Arunima Biswas

**Abstract**

Kinetoplastida forms a group of flagellated protozoan organisms with a unique organelle kinetoplastid associated with the mitochondria. They are generally well-known parasites, while a few are also known to be free-living. The disease-causing unique groups of organisms are *Leishmania* causing leishmaniasis and *Trypanosoma* causing trypanosomiasis. These parasites generally infect humans in a particular life cycle stage residing in the blood or tissues of human bodies. Kinetoplastida are generally known to have long and slender morphological forms in some stages of their life cycles, while they are also known to have aflagellate forms associated with their infectivity in humans. The cell cycles of kinetoplastid intracellular parasites have various morphological forms, and the cell division in these parasites is associated with the disease manifestation. This chapter will deal with the process and molecular regulation of cell cycles of the intracellular kinetoplastid parasites.

**Keywords**

Kinetoplastida · Cell cycle · Paraflagellar rod · *Leishmania* · *Trypanosoma*

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## 1 Introduction

Cell cycle can be generally classified as differentiation cell cycle and proliferative cell cycle. Proliferative cell cycles are those in which both progenies have similar features as the parent, whereas differentiation cell cycles are those in which one or both daughters have a distinct cell shape or function compared to the parent. Animal stem cells are frequently described as having the ability to divide in both proliferative symmetric divisions and asymmetric self-renewing divisions, in which one cell differentiates, while the other remains proliferative [1, 2]. Proliferative cell division is a coordinated process in kinetoplastid parasites belonging to the genus *Leishmania* and *Trypanosoma* which seizes the attention of the scientist for exploring the much complicated process because of the presence of flagella, numerous single-copy organisms and a kinetoplast in both [3]. Because of their variety of intriguing cellular processes and zoonotic significance, kinetoplastids among the other protists have received much interest from the scientific world. The Trypanosomatidae family (order: Kinetoplastida) is entirely made up of parasitic protozoa and comprises the digenetic genera *Leishmania* and *Trypanosoma*, which are responsible for a variety of human and animal diseases. These parasites spread by hematophagous insects and have evolved to survive in several hostile environments: the macrophages in mammalian host and the digestive system of insects [3]. Parasitic protozoa belonging to the genus *Leishmania* are the causative agents of a spectrum of clinical manifestations including cutaneous, visceral and mucocutaneous diseases collectively known as leishmaniasis [4]. Those manifestations range in severity from spontaneously healing cutaneous ulcers by *L. major* infection to potentially fatal visceral disease by *L. donovani* infection. These parasites show a digenetic life cycle which includes a sand fly host housing the extracellular promastigote form developing in the digestive tract and a human host with the intracellular amastigote form. Following infection of humans, the highly motile promastigotes are phagocytosed by the mammalian macrophages, and under the influence of the acidic phagolysosome conditions, the promastigotes are transformed into non-motile aflagellar amastigote forms inside the macrophage.

During the progression of cell cycle in eukaryotes, the multiplication and division of organelles that produce two identical daughter cells must be meticulously regulated. This comprises the duplication of a single mitochondrion harbouring a DNA network, known as the kinetoplast, and a flagellum emerges from a cytoplasmic basal body that outgrows from the cell in kinetoplastid protozoa. The sequence of the morphological processes that take place during the *Trypanosoma cruzi* epimastigote cell cycle has been studied, and it was found that a new flagellum emerged from the flagellar pocket at the mid-point of G2 stage which extends outwards from the cell body. The kinetoplast starts to segregate, and the onset of mitosis occurs even before the proper elongation of the flagellum is completed. The new flagellum obtains its ultimate size when the new cell is formed during cytokinesis [3]. The essential component in understanding the basic biology of both *Trypanosoma* and *Leishmania* is their cell cycle and how they are regulated and play important role in the infectivity of these protozoan parasites. There are two key

morphologies involved with the life cycle of *Leishmania*: the amastigote form that infects the mammalian host and the promastigote forms infecting the sand fly vector. Like the *Trypanosoma cruzi* cell cycle, *Leishmania mexicana* promastigotes were analysed for the same. A wide range of morphological variants were found in the culture medium which depicts that different morphologies of *Leishmania* are associated with different phases of the cell cycle of the parasites. The growth of the flagellum in the promastigotes occurs over multiple events of cell division. Regulation of flagellar length, differentiation of various stages of the life cycle and division of trypanosomatids in general all have evident consequences. As a result, this data collection serves as a foundation for postgenomic studies of *Leishmania* and *Trypanosoma* cell cycle in connection to differentiation and infectivity [5].

## 2 Cell Division in Kinetoplastids

Duplication, which is one of the major prerequisites for all living cells, is meticulously controlled by a series of multifaceted mechanisms that is found in a vast array of cells including single-celled eukaryotes like protista and the complex multi-celled metazoans. The eukaryote cell division has two stages: karyokinesis followed by cytokinesis. The nuclear division is further subdivided into five phases. The G1 phase prepares the cell for entering the S phase where the nuclear DNA synthesis takes place. G2 phase separates the S phase from the M-phase, i.e. the mitotic phase which is followed by the C-phase, i.e. cytokinesis. However, due to the presence of numerous copies of organelles like mitochondria, chloroplasts and kinetoplasts, the classical cell cycle phenomenon does not contain the steps required for the duplication of the extra-nuclear genetic material these organelles contain. There is only one mitochondrion in certain primitive eukaryotes like the Kinetoplastida [6, 7]. Both human and animals host pathogens like *Trypanosoma brucei*, *Trypanosoma cruzi*, *Trypanosoma evansi* and *Leishmania* that belong to this category. Mitochondrial DNA is packaged into a compact disc-shaped structure called kinetoplast (K) in these species, making K duplication events and nuclear duplication phases easier to detect and identify. Thus, apart from the nuclear division, kinetoplast division should also be taken into consideration in the case of the kinetoplastid parasites. Two organelles containing DNA in a single organism raise critical issues about how DNA replication is coordinated during the cell cycle. It has been observed in *Trypanosoma brucei* that kinetoplast DNA replication occurs prior to the nuclear division [3, 8].

### 2.1 Role of kDNA in the Regulation of Cell Cycle

The unique feature of Kinetoplastida is described as the presence of an exclusive DNA structure that they contain inside their single mitochondrion in the form of a two-dimensional mesh-like network packaged into a disc-shaped structure inside the cell. This network is composed of two different types of duplex DNA circles,

consisting of 5000–10,000 minicircles and 25–50 maxicircles architecturally interlocked into an enormous DNA catenane. Other types of kDNA networks, made up of unlinked DNA minicircles, have recently been discovered [9]. Kinetoplast biogenesis has been found to be synchronised with cell cycle. The presence of a single kinetoplast and nucleus (1K1N) is observed in trypanosomes in G1 phase. Synthesis of kinetoplast occurs in S phase resulting in the occurrence of 2K1N cells in trypanosomes in G2 phase [10]. 2K1N nuclei of the trypanosomes divide to produce 2K2N cells which further generate 1K1N cells following cytokinesis [11]. Limitation of kinetoplast replication to G2 phase specifies that a kinetoplast is approved for scission or segregation at this particular stage of the cell cycle [12].

### 2.2 Role of Paraflagellar Rod (PFR) in Cell Division of Kinetoplastid Parasites

The central structure of kinetoplastid flagellum is embellished with an array of extra-axonemal structures known as paraflagellar rod (PFR), a huge lattice-like structure extending along the side of the axoneme starting from the flagellar pocket and ending to the flagellar tip. With the exception of *Trypanosoma cruzi* and *Leishmania* spp. amastigote phases, where the flagellum is almost obliterated, the PFR is found in all stages of life cycle in kinetoplastids. The role of flagellar component is different in different forms of the parasites. For example, in *Trypanosoma brucei*, the bloodstream form is highly sensitive to the removal of flagellar components and seems to be dependent on the flagellar function for the completion of cytokinesis. Flagellar proteins, when ablated in the bloodstream forms, produce cell population with multiple nuclei, kinetoplast and flagella. Cells are gigantic, indicating an unsuccessful cytokinesis. Such catastrophic cytokinesis failure in bloodstream form is found to be caused by the loss of function of flagellar protein, PFR2. However, no such change in growth rate is observed in the procyclic forms under similar condition [13–17]. Moreover, evidence against impaired cell proliferation and morphogenesis is found to be existing in procyclic forms with unusual structure and functioning of the flagellar components [18].

## 3 Cell Cycle in *Trypanosoma*

The eukaryotic cell cycle is defined by a series of interconnected activities that allow identical genetic material to be distributed into progeny [19]. With regard to the role of cellular metabolism, DNA synthesis and mitosis, the processes that ensure accurate segregation have been widely explored, including the generation of networks of macromolecular chains produced at different periods of the cell cycle [20]. The connection between organelle duplication and segregation and advancement of cell cycle, on the other hand, is far less well understood. This is due to the fact that most of the eukaryotes have several copies of organelles in each cell. Some protists are tremendously desirable systems in this regard owing to the fact that they

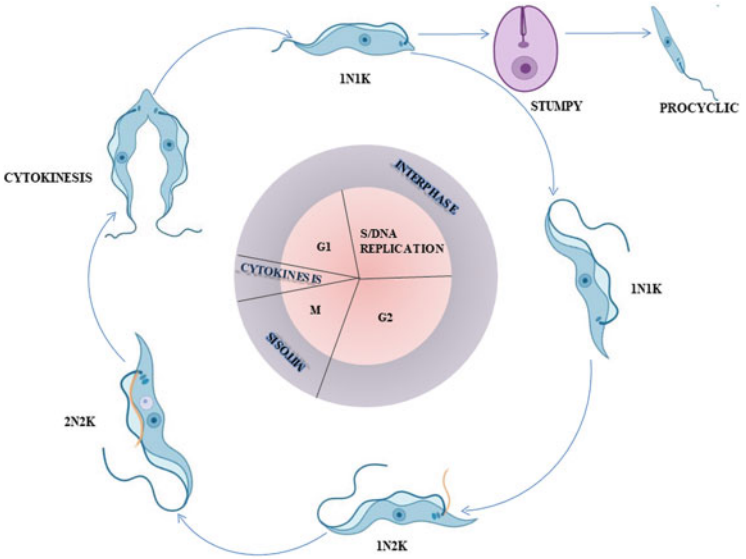


**Fig. 1** Schematic representation of the cell cycle events and morphological alterations of the phases of the *Trypanosoma* cell cycle. Cell cycle comprises the following stages: G1 (gap 1), S (DNA synthesis period), G2 (gap 2), M (mitosis) and C (cytokinesis) were indicated. Two flagella of equal length were observed at M phase, but the time point for initial assembly of the new flagellum might occur in either S or G2 phase, according to related studies in *T. brucei* or *T. cruzi*

have single-copy as well as specialised organelles. Trypanosomes are unicellular, flagellated protists with a single Golgi complex and a huge mitochondrion containing a single kinetoplast made up of an interconnected network of DNA minicircles and maxicircles [21]. The single flagellum emerging from a flagellar pocket is attached to the kinetoplast with its basal body. A single flagellum with its basal body attached to the kinetoplast develops from a flagellar pocket, a plasma membrane invagination [22, 23]. The morphological events that occur during the cell cycle of *Trypanosoma brucei*, the causative agent of African trypanosomiasis, are explored in detail [24]. Regardless of the fact that *T. brucei* is recognised as a stereotypical organism among trypanosomes, especially when structural organisation is described, morphological processes that happen during the cell cycle of other trypanosome species must be different owing to their diversified cell shapes, position of kinetoplasts and pattern of flagellar insertion. *T. brucei*, for example, does not replicate its nucleus and kinetoplast DNA in the same sequence as *Crithidia* and *Leishmania* species [25, 26]. The basal body does not migrate in most situations, and kinetoplast division occurs in close association with nuclear mitosis. Furthermore, despite the lack of a comprehensive explanation, attachment of new flagellum to the previous one does not occur.

The procyclic cell cycle of *Trypanosoma brucei*, among the kinetoplastid parasites that include *Leishmania* species and *Trypanosoma cruzi* also, is the best studied of any trypanosomatid, as depicted in Fig. 1. Its examination necessitates an

understanding of the finely defined cellular organisation containing single copies of several organelles. The procyclic trypanosomes in the G1 phase are termed as 1K1N owing to the presence of one kinetoplast and one nucleus in the cell. As a result, when studying how a G1 (1K1N) trypanosome divides, three subcycles come into consideration: the replication and segregation of (a) the nucleus, (b) the kinetoplast and (c) a series of sophisticated cytoskeletal structures in an orderly and regulated way [27]. *T. brucei* may reach a G0 stage, which is evidenced by the generation of the stumpy form inside the mammalian host, formally establishing that *T. brucei* has control over whether or not the cell cycle is initiated. The signals originating from the oligopeptide accumulation produced by the released peptidases induce the slender bloodstream forms of the parasite to differentiate into G0 stumpy forms [28]. This signalling pathway which is responsible for the release from G0 must be a trigger for the cell to enter nuclear S phase – the primary event of the three subcycles – followed by kinetoplast and cytoskeletal divisions. In mitosis, trypanosomes have the same issues as other eukaryotes: detecting DNA damage before entering S phase, assuring single-firing origin of replication, managing spindle construction and forming a bipolar spindle that precisely separates multiple chromosomes. Like other eukaryotes, trypanosomes possess various cyclins, cyclin-related kinases and MAP kinases, some of which are characterised and are found to have significant roles in regulating nuclear processes associated with cell cycle. The cyclins CYC2, CYC4, CYC5 and CYC7, as well as Cdc2-related kinases, are associated with the onset of nuclear S phase [29]. Chromosomal replication of both *Trypanosoma* and *Leishmania* starts from one or more replication origins [30–33]. CYC6 and CYC8 (cyclin B-like cyclins) and CDK1-like kinases such as CRK3 and CRK9 are involved with the progress of the cells from G2 to the start of mitotic phase [29, 34]. Mitosis is dependent on nuclear S phase, but it seems to be unrelated to kinetoplast S phase and cytoskeletal functions. It occurs in *Trypanosoma brucei* and *Trypanosoma cruzi* epimastigotes [3], during division of kinetoplast in *Leishmania* [4, 5] and at different times during kinetoplast division in *Trypanosoma abeli* [35]. In spite of the fact that trypanosomatids possess putative condensins [36], their chromosomes do not visibly condense, and the nucleolus is intact. With the exception of MAD2, which, possibly instructively, is confined to the basal body (BB), trypanosomes lack apparent orthologs for a spindle assembly complex [37, 38]. The Aurora B homologue AUK1 interacts with trypanosome-specific components (CPC1 and CPC2) [39], a Tousled-like kinase TLK1 and spindle kinesins in the chromosomal passenger complex. The nuclear envelope and connected endomembrane system must separate once the nucleus has advanced through mitosis. The cytoskeleton's architecture and mutant phenotypes show that cytokinesis is unaffected by nuclei position; hence, daughter nuclei must be positioned correctly for inheritance after cytokinesis.

A tripartite attachment complex (TAC) is required not only for kinetoplast division but also for the segregation of mitochondrial genome [40]. The latter stage of replication of kinetoplastid DNA marks the beginning of the process of segregation [41]. One of the most important parts of the eukaryotic cell division is the cytoskeletal remodelling. However, it is not surprising for an organism like

*Trypanosoma* to possess an extreme level of cellular regulation owing to the presence of highly defined cytoskeletal structures and multiple single-copy cell organelles. Along with the nuclear and kinetoplast subcycles, duplication and segregation of cytoskeletal elements are performed in a precise manner.

## 4 Cell Cycle in *Leishmania*

*Leishmania* is a genus including various species of protozoan parasites that are responsible for leishmaniases, a group of vector-borne infections that are endemic in tropical and subtropical areas. The procyclic promastigotes and amastigotes which are found in the sand fly insect vector and the phagolysosome of mammalian macrophages, respectively, are the two key replicative developmental stages in the life cycle of *Leishmania*. Procyclic promastigotes of *Leishmania* are highly asymmetric cells with numerous single-copy organelles in specific subcellular sites. The nucleus, Golgi apparatus, basal body, mitochondrion (which includes the kinetoplast) and flagellum, which protrudes from the cell body via the flagellar pocket, are among these structures. The replication and segregation of these organelles must be precisely controlled in order to produce viable progeny [5, 42–44]. The cell cycle of *Trypanosoma brucei* procyclic form has been widely studied, and it serves as a foundation for comparative studies with other trypanosomatids, such as *Leishmania*. In *T. brucei*, nuclear and kinetoplast DNA replication (S phase) begins apparently simultaneously, while the nucleus (M and C phases, respectively) and kinetoplast (D and A phases, respectively) divide and segregate at different times. Kinetoplast division is finished before the commencement of nuclear mitosis in procyclic *T. brucei* and *L. tarentolae* [45], but in *L. mexicana* and *L. donovani*, these processes seem to occur in the opposite order [5, 42, 43]. Although comprehensive changes in *L. mexicana* promastigote cell shape are observed from entry of the cells into mitosis to early cytokinesis, no trypanosomatid has yet been described that undergoes the extensive remodelling of the microtubular cytoskeleton that occurs during mammalian cell cytokinesis. Polymerisation or depolymerisation of microtubule has been demonstrated to alter trypanosome basal body duplication, segregation of kinetoplast, flagellar axoneme development, cell division and proliferation and finally cytokinesis [23, 24, 27, 46]. Microtubule-facilitated processes are also important in the advancement of the cell cycle of *Leishmania* [47–49], and antimicrotubule drugs inhibited not only nuclear mitosis and cytokinesis but also promoted inexact positioning of kinetoplast inside the cell [42].

In *Leishmania* spp., like in most trypanosomatids, the G1 phase accounts for the majority of the cell cycle (shown in Fig. 2), whereas the remaining phases differ in length significantly [8, 50]. There have been no investigations on the metabolic alterations and key events in *Leishmania* spp. during the G1 phase. Nevertheless, when compared to other trypanosomatids, it was deduced that during the G1 phase, there is an increase in transcription rate and intensive protein synthesis of DNA replication-related proteins [8, 51]. Furthermore, during the G1 phase, a distinct prereplication protein complex forms at certain sites on the chromosomes known as

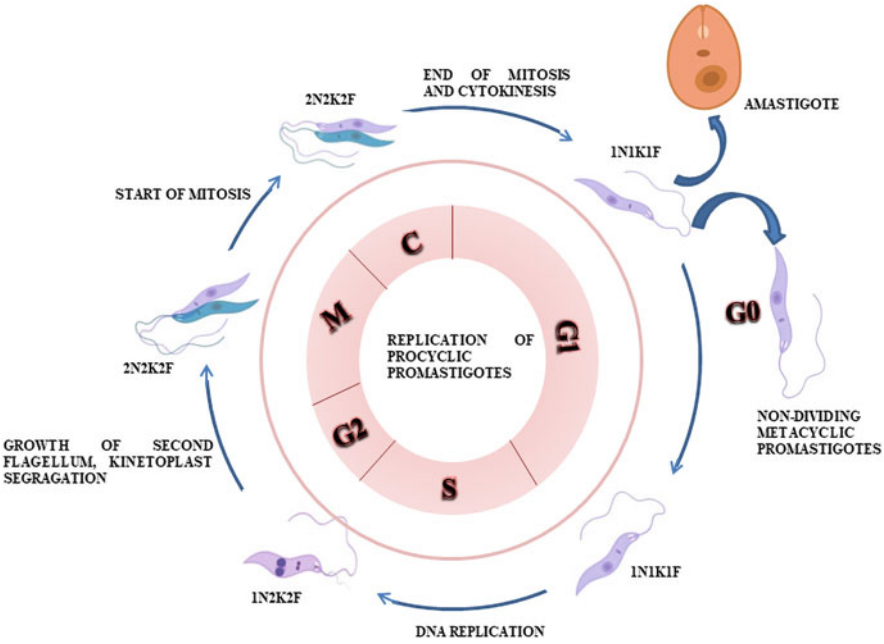


**Fig. 2** Schematic representation of *Leishmania* promastigote cell cycle. G1, S, G2, M, C and G0 indicate the different phases of the cell cycle. 1N1K1F, 1N2K2F and 2N2K2F are the number of nuclei, kinetoplast and flagella corresponding to the growth phase, DNA replication or S phase and mitotic phase, respectively. Although the pattern of kinetoplast segregation varies among different species, it can occur either or before the nuclear division. *L. donovani* exhibits both the patterns distributed in their population

the origin of replication, which might result in the formation of a replication bubble [8]. The activation of replication origins initiates the S phase, which is characterised by efficient replication of DNA. Eukaryotes often have multiple origins of replication on each chromosome. However, the number of origin of replications per chromosome in *Leishmania* spp. is a subject of controversy. In a research work of Marquez et al., in 2015, it was demonstrated that during the S phase, *L. major* uses a single replication origin to duplicate each of its chromosomes. Nonetheless, more sensitive tests are needed to determine the actual number of replication origins utilised per chromosome in *Leishmania* spp. during the S phase. The duplication of cell organelles and centrioles characterises the G2 phase in model eukaryotes [52]. Based on research with other species, we may deduce that during the G2 phase, *Leishmania* spp. accelerate the rate of transcription and resume intensive protein synthesis, both of which are required for cell division completion. The volume and size of the cells expand as a result of this process [5, 53]. *Leishmania* spp. and other trypanosomatids do not dismantle their nuclear envelope during the M phase; instead, they conduct a closed mitotic process regulated by spindle pole body-like structures [54]. Furthermore, *Leishmania* spp. and other trypanosomatids are

incapable of condensing their chromosomes into 30 nm fibres due to the lack of the N-terminal region and globular domain of histone H1 as well as phosphorylation on serine 10 of histone H3 (H3S10) [55, 56].

The multiplication and segregation of the kinetoplast are two phenomena that ought to be noted during the *Leishmania* spp. cell cycles. The chronology of these processes in the *Leishmania* cell cycle differs from that in model eukaryotes, where mitochondrial DNA replication takes place at any stage of the cell cycle [9, 57]. Although S phases of the nucleus and kinetoplast occur nearly together, the successful division of these organelles might take place at various times depending on the species studied. The nucleus and kinetoplast division trends in *L. donovani* and *L. amazonensis* are not fixed. When comparing the cell cycle of various *Leishmania* species, the order and timing of kinetoplast and nuclear division are inconsistent and difficult to generalise [5, 45]. For example, kinetoplast and nucleus segregation patterns are consistent in *L. mexicana*, *L. major* and *L. tarentolae*. *L. mexicana*, on the other hand, segregates its kinetoplast mostly after the nucleus [5], whereas *L. major* and *L. tarentolae* do the reverse [43, 45]. The fact that these parasites belong to the same genus yet having significant evolutionary distance [58] might be one of the explanations for their disparate behaviour. In other words, this evolutionary divergence might represent species-specific changes in kinetoplast segregation, implying that some *Leishmania* species have less tight control over the manner in which their organelles containing the DNA are divided (nucleus and kinetoplast). More research is needed to identify the possible factors involved in regulating cell division and organelle segregation, as some of them might be investigated for precise parasite cell cycle therapies.

## 5 Conclusion

The pursuit for new therapeutic targets necessitates a thorough understanding of the organism's cellular biology, and substantial research is underway in this area. Many of these investigations involve the examination of cells at various stages of the cell cycle. However, synchronising *Leishmania* promastigotes is incredibly tricky; most compounds that synchronise higher eukaryotic cells struggle to do the same in *Leishmania* promastigotes. The appearance of the kinetoplast and its partitioning pattern have been utilised to identify different stages of cell cycle in an asynchronous population of *Trypanosoma* species. There is a decent understanding of the trypanosome cell cycle process, more than a decade and a thorough description of molecular and cellular processes inside each of the three separate subcycles within the overall cell cycle has been described, and much knowledge is gained about the sequence and timing of events. The genomes of the kinetoplastid parasites including various species of *Trypanosoma* and *Leishmania* have been sequenced, and comparative identification of key molecules involved in cell cycle control should be useful in better understanding the various regulatory pathways in these organisms, as well as providing a foundation for understanding organelle segregation in eukaryotes.

## References


1. Ruijtenberg S, van den Heuvel S. Coordinating cell proliferation and differentiation: antagonism between cell cycle regulators and cell type-specific gene expression. Cell Cycle. 2016;15:196–212. https://doi.org/10.1080/15384101.2015.1120925.
2. Santoro A, Vlachou T, Carminati M, Pelicci PG, Mapelli M. Molecular mechanisms of asymmetric divisions in mammary stem cells. EMBO Rep. 2016;17:1700–20. https://doi.org/10.15252/embr.201643021.
3. Elias MC, da Cunha JPC, de Faria FP, Mortara RA, Freymüller E, Schenkman S. Morphological events during the Trypanosoma cruzi cell cycle. Protist. 2007;158:147–57. https://doi.org/10.1016/j.protis.2006.10.002.
4. Ambit A, Woods KL, Cull B, Coombs GH, Mottram JC. Morphological events during the cell cycle of Leishmania major. Eukaryot Cell. 2011;10:1429–38. https://doi.org/10.1128/EC.05118-11.
5. Wheeler RJ, Gluenz E, Gull K. The cell cycle of Leishmania: morphogenetic events and their implications for parasite biology. Mol Microbiol. 2011;79:647–62. https://doi.org/10.1111/j.1365-2958.2010.07479.x.
6. Lukeš J, Butenko A, Hashimi H, Maslov DA, Votýpka J, Yurchenko V. Trypanosomatids are much more than just trypanosomes: clues from the expanded family tree. Trends Parasitol. 2018;34:466–80. https://doi.org/10.1016/j.pt.2018.03.002.
7. Maslov DA, Opperdoes FR, Kostygov AY, Hashimi H, Lukeš J, Yurchenko V. Recent advances in trypanosomatid research: genome organization, expression, metabolism, taxonomy and evolution. Parasitology. 2019;146:1–27. https://doi.org/10.1017/S0031182018000951.
8. da Silva MS, Pavani RS, Damasceno JD, Marques CA, McCulloch R, Tosi LRO, Elias MC. Nuclear DNA replication in Trypanosomatids: there are no easy methods for solving difficult problems. Trends Parasitol. 2017b;33:858–74. https://doi.org/10.1016/j.pt.2017.08.002.
9. Shlomai J. The structure and replication of kinetoplast DNA. Curr Mol Med. 2004;4:623–47. https://doi.org/10.2174/1566524043360096.
10. Siegel TN, Hekstra DR, Cross GAM. Analysis of the Trypanosoma brucei cell cycle by quantitative DAPI imaging. Mol Biochem Parasitol. 2008;160:171–4. https://doi.org/10.1016/j.molbiopara.2008.04.004.
11. Sherwin T, Gull K. The cell division cycle of Trypanosoma brucei brucei: timing of event markers and cytoskeletal modulations. Philos Trans R Soc Lond Ser B Biol Sci. 1989;323:573–88. https://doi.org/10.1098/rstb.1989.0037.
12. Mensa-Wilmot K, Hoffman B, Wiedeman J, Sullenberger C, Sharma A. Kinetoplast division factors in a trypanosome. Trends Parasitol. 2019;35:119–28. https://doi.org/10.1016/j.pt.2018.11.002.
13. Branche C, Kohl L, Toutirais G, Buisson J, Cosson J, Bastin P. Conserved and specific functions of axoneme components in trypanosome motility. J Cell Sci. 2006;119:3443–55. https://doi.org/10.1242/jcs.03078.
14. Broadhead R, Dawe HR, Farr H, Griffiths S, Hart SR, Portman N, Shaw MK, Ginger ML, Gaskell SJ, McKean PG, Gull K. Flagellar motility is required for the viability of the bloodstream trypanosome. Nature. 2006;440:224–7. https://doi.org/10.1038/nature04541.
15. Farr H, Gull K. Functional studies of an evolutionarily conserved, cytochrome b5 domain protein reveal a specific role in axonemal organisation and the general phenomenon of post-division axonemal growth in trypanosomes. Cell Motil Cytoskeleton. 2009;66:24–35. https://doi.org/10.1002/cm.20322.
16. Ralston KS, Hill KL. Trypanin, a component of the flagellar dynein regulatory complex, is essential in bloodstream form African trypanosomes. PLoS Pathog. 2006;2:e101. https://doi.org/10.1371/journal.ppat.0020101.

17. Ralston KS, Lerner AG, Diener DR, Hill KL. Flagellar motility contributes to cytokinesis in Trypanosoma brucei and is modulated by an evolutionarily conserved dynein regulatory system. Eukaryot Cell. 2006;5:696–711. https://doi.org/10.1128/EC.5.4.696-711.2006.
18. Portman N, Gull K. The paraflagellar rod of kinetoplastid parasites: from structure to components and function. Int J Parasitol. 2010;40:135–48. https://doi.org/10.1016/j.ijpara.2009.10.005.
19. Murray AW. Recycling the cell cycle: cyclins revisited. Cell. 2004;116:221–34. https://doi.org/10.1016/s0092-8674(03)01080-8.
20. Tyers M. Cell cycle goes global. Curr Opin Cell Biol. 2004;16:602–13. https://doi.org/10.1016/j.ceb.2004.09.013.
21. He CY, Ho HH, Malsam J, Chalouni C, West CM, Ullu E, Toomre D, Warren G. Golgi duplication in Trypanosoma brucei. J Cell Biol. 2004;165:313–21. https://doi.org/10.1083/jcb.200311076.
22. Gull K. Host-parasite interactions and trypanosome morphogenesis: a flagellar pocketful of goodies. Curr Opin Microbiol. 2003;6:365–70. https://doi.org/10.1016/s1369-5274(03)00092-4.
23. Robinson DR, Gull K. Basal body movements as a mechanism for mitochondrial genome segregation in the trypanosome cell cycle. Nature. 1991;352:731–3. https://doi.org/10.1038/352731a0.
24. Woodward R, Gull K. Timing of nuclear and kinetoplast DNA replication and early morphological events in the cell cycle of Trypanosoma brucei. J Cell Sci. 1990;95(Pt 1):49–57. https://doi.org/10.1242/jcs.95.1.49.
25. Bhattacharya A, Ghosh M. Cell cycle of Leishmania donovani. Indian J Exp Biol. 1985;23:629–34.
26. Cosgrove WB, Skeen MJ. The cell cycle in Crithidia fasciculata. Temporal relationships between synthesis of deoxyribonucleic acid in the nucleus and in the kinetoplast. J Protozool. 1970;17:172–7. https://doi.org/10.1111/j.1550-7408.1970.tb02350.x.
27. McKean PG. Coordination of cell cycle and cytokinesis in Trypanosoma brucei. Curr Opin Microbiol. 2003;6:600–7. https://doi.org/10.1016/j.mib.2003.10.010.
28. Silvester E, McWilliam KR, Matthews KR. The cytological events and molecular control of life cycle development of Trypanosoma brucei in the mammalian bloodstream. Pathogens. 2017;6:E29. https://doi.org/10.3390/pathogens6030029.
29. Zhou Q, Hu H, Li Z. New insights into the molecular mechanisms of mitosis and cytokinesis in trypanosomes. Int Rev Cell Mol Biol. 2014;308:127–66. https://doi.org/10.1016/B978-0-12-800097-7.00004-X.
30. Calderano SG, Drosopoulos WC, Quaresma MM, Marques CA, Kosiyatrakul S, McCulloch R, Schildkraut CL, Elias MC. Single molecule analysis of Trypanosoma brucei DNA replication dynamics. Nucleic Acids Res. 2015;43:2655–65. https://doi.org/10.1093/nar/gku1389.
31. Marques CA, McCulloch R. Conservation and variation in strategies for DNA replication of Kinetoplastid nuclear genomes. Curr Genomics. 2018;19:98–109. https://doi.org/10.2174/1389202918666170815144627.
32. Stanojcic S, Sollelis L, Kuk N, Crobu L, Balard Y, Schwob E, Bastien P, Pagès M, Sterkers Y. Single-molecule analysis of DNA replication reveals novel features in the divergent eukaryotes Leishmania and Trypanosoma brucei versus mammalian cells. Sci Rep. 2016;6:23142. https://doi.org/10.1038/srep23142.
33. Tiengwe C, Marques CA, McCulloch R. Nuclear DNA replication initiation in kinetoplastid parasites: new insights into an ancient process. Trends Parasitol. 2014;30:27–36. https://doi.org/10.1016/j.pt.2013.10.009.
34. Duncan SM, Myburgh E, Philipon C, Brown E, Meissner M, Brewer J, Mottram JC. Conditional gene deletion with DiCre demonstrates an essential role for CRK3 in Leishmania mexicana cell cycle regulation. Mol Microbiol. 2016;100:931–44. https://doi.org/10.1111/mmi.13375.

35. Borges AR, Toledo DA, Fermino BR, de Oliveira JC, Silber AM, Elias MC, D'Avila H, Scopel KKG. In vitro cellular division of Trypanosoma abeli reveals two pathways for organelle replication. J Eukaryot Microbiol. 2019;66:385–92. https://doi.org/10.1111/jeu.12678.
36. Hirano T. Condensins: universal organizers of chromosomes with diverse functions. Genes Dev. 2012;26:1659–78. https://doi.org/10.1101/gad.194746.112.
37. Fort C, Bonnefoy S, Kohl L, Bastin P. Intraflagellar transport is required for the maintenance of the trypanosome flagellum composition but not its length. J Cell Sci. 2016;129:3026–41. https://doi.org/10.1242/jcs.188227.
38. Han X, Li Z. Comparative analysis of chromosome segregation in human, yeasts and trypanosome. Front Biol (Beijing). 2014;9:472–80. https://doi.org/10.1007/s11515-014-1334-y.
39. Li Z, Lee JH, Chu F, Burlingame AL, Günzl A, Wang CC. Identification of a novel chromosomal passenger complex and its unique localization during cytokinesis in Trypanosoma brucei. PLoS One. 2008;3:e 2354. https://doi.org/10.1371/journal.pone.0002354.
40. Schneider A, Ochsenreiter T. Failure is not an option–mitochondrial genome segregation in trypanosomes. J Cell Sci. 2018;131:jcs 221820. https://doi.org/10.1242/jcs.221820.
41. Gluenz E, Povelones ML, Englund PT, Gull K. The kinetoplast duplication cycle in Trypanosoma brucei is orchestrated by cytoskeleton-mediated cell morphogenesis. Mol Cell Biol. 2011;31:1012–21. https://doi.org/10.1128/MCB.01176-10.
42. Havens CG, Bryant N, Asher L, Lamoreaux L, Perfetto S, Brendle JJ, Werbovetz KA. Cellular effects of leishmanial tubulin inhibitors on L. donovani. Mol Biochem Parasitol. 2000;110:223–36. https://doi.org/10.1016/s0166-6851(00)00272-3.
43. Minocha N, Kumar D, Rajanala K, Saha S. Kinetoplast morphology and segregation pattern as a marker for cell cycle progression in Leishmania donovani. J Eukaryot Microbiol. 2011;58:249–53. https://doi.org/10.1111/j.1550-7408.2011.00539.x.
44. Tammana TVS, Sahasrabuddhe AA, Bajpai VK, Gupta CM. ADF/cofilin-driven actin dynamics in early events of Leishmania cell division. J Cell Sci. 2010;123:1894–901. https://doi.org/10.1242/jcs.068494.
45. Simpson L. Effect of acriflavin on the kinetoplast of Leishmania tarentolae. Mode of action and physiological correlates of the loss of kinetoplast DNA. J Cell Biol. 1968;37:660–82. https://doi.org/10.1083/jcb.37.3.660.
46. Ploubidou A, Robinson DR, Docherty RC, Ogbadoyi EO, Gull K. Evidence for novel cell cycle checkpoints in trypanosomes: kinetoplast segregation and cytokinesis in the absence of mitosis. J Cell Sci. 1999;112(Pt 24):4641–50. https://doi.org/10.1242/jcs.112.24.4641.
47. Borges VM, Lopes UG, De Souza W, Vannier-Santos MA. Cell structure and cytokinesis alterations in multidrug-resistant Leishmania (Leishmania) amazonensis. Parasitol Res. 2005;95:90–6. https://doi.org/10.1007/s00436-004-1248-8.
48. Jayanarayan KG, Dey CS. Altered tubulin dynamics, localization and post-translational modifications in sodium arsenite resistant Leishmania donovani in response to paclitaxel, trifluralin and a combination of both and induction of apoptosis-like cell death. Parasitology. 2005;131:215–30. https://doi.org/10.1017/s0031182005007687.
49. Jayanarayan KG, Dey CS. Microtubules: dynamics, drug interaction and drug resistance in Leishmania. J Clin Pharm Ther. 2002;27:313–20. https://doi.org/10.1046/j.1365-2710.2002.00431.x.
50. Archer SK, Inchaustegui D, Queiroz R, Clayton C. The cell cycle regulated transcriptome of Trypanosoma brucei. PLoS One. 2011;6:e18425. https://doi.org/10.1371/journal.pone.0018425.
51. da Silva MS, Cayres-Silva GR, Vitarelli MO, Marin PA, Hiraiwa PM, Araújo CB, Scholl BB, Ávila AR, McCulloch R, Reis MS, Elias MC. Transcription activity contributes to the firing of non-constitutive origins in African trypanosomes helping to maintain robustness in S-phase duration. Sci Rep. 2019;9:18512. https://doi.org/10.1038/s41598-019-54366-w.
52. Harashima H, Dissmeyer N, Schnittger A. Cell cycle control across the eukaryotic kingdom. Trends Cell Biol. 2013;23:345–56. https://doi.org/10.1016/j.tcb.2013.03.002.

53. da Silva MS, Monteiro JP, Nunes VS, Vasconcelos EJ, Perez AM, Freitas-Júnior L, Elias MC, Cano MIN. Leishmania amazonensis promastigotes present two distinct modes of nucleus and kinetoplast segregation during cell cycle. PLoS One. 2013;8:e81397. https://doi.org/10.1371/journal.pone.0081397.
54. Campbell PC, de Graffenried CL. Alternate histories of cytokinesis: lessons from the trypanosomatids. Mol Biol Cell. 2020;31:2631–9. https://doi.org/10.1091/mbc.E19-12-0696.
55. Hecker H, Betschart B, Bender K, Burri M, Schlimme W. The chromatin of trypanosomes. Int J Parasitol. 1994;24:809–19. https://doi.org/10.1016/0020-7519(94)90007-8.
56. Hecker H, Gander ES. The compaction pattern of the chromatin of trypanosomes. Biol Cell. 1985;53:199–208. https://doi.org/10.1111/j.1768-322x.1985.tb00368.x.
57. Liu B, Liu Y, Motyka SA, Agbo EEC, Englund PT. Fellowship of the rings: the replication of kinetoplast DNA. Trends Parasitol. 2005;21:363–9. https://doi.org/10.1016/j.pt.2005.06.008.
58. Valdivia HO, Reis-Cunha JL, Rodrigues-Luiz GF, Baptista RP, Baldeviano GC, Gerbasi RV, Dobson DE, Pratlong F, Bastien P, Lescano AG, Beverley SM, Bartholomeu DC. Comparative genomic analysis of Leishmania (Viannia) peruviana and Leishmania (Viannia) braziliensis. BMC Genomics. 2015;16:715. https://doi.org/10.1186/s12864-015-1928-z.

# Elaborating the Role of Aspartyl Protease in Host Modulation and Invasion in Apicomplexan Parasites *Plasmodium* and *Toxoplasma*

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**Abstract**

The phylum Apicomplexa comprising the widest group of protozoan pathogens causes lethal diseases to humans and commercially crucial animals. Two facts have triggered the grim need for research in this field, the absence of chemotherapeutics including vaccines and the swift spread of resistance against existing drugs. So, it's essential that we identify new targets for drug therapy. Recent elucidations have recognized the critical role of apicomplexan aspartyl proteases involved in infection and transmission of these pathogens, proposing them as potential drug targets. Aspartyl proteases are enzymes present in all eukaryotes carrying out a variety of functions including signal transduction, protein maturation and secretion of virulence factors. In this chapter we have focused on two classes of aspartyl proteases in apicomplexan parasites playing a broad role in host modulation along with host invasion and transmission. This book chapter imparts a vital analysis of the ongoing knowledge as well as recent developments in the biological aspects and biochemical properties of this prime parasitic enzyme involved in pathogenesis of the apicomplexan parasites. These advanced understanding into the structure-function property of this crucial class of parasitic enzyme should be of help in developing discrete inhibitors and also unfold the future line of research.

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**Keywords**

Apicomplexan · Aspartyl protease · *Toxoplasma* · *Plasmodium* · Host modulation · Invasion

## 1 Introduction

The phylum Apicomplexa comprises obligate intracellular parasites, which consist of the largest group of protozoan pathogens responsible for causing life-threatening human and animal diseases across the world. The two most common parasites in this group are *Plasmodium* and *Toxoplasma*, the infectious agents which cause malaria and toxoplasmosis, respectively.

Both *Plasmodium* and *Toxoplasma*, like any other apicomplexan parasites, use an array of several proteases to carry out the essential processes of host infection, which is indispensable for their survival. In general, proteases can be defined as enzymes that catalyse the hydrolysis of peptide bonds between amino acids. Generally, proteases are divided into two main groups according to their cleavage sites which are exopeptidases and endopeptidases. Exopeptidases remove the terminal amino acids from proteins, while endopeptidases are responsible for cleaving internal peptide bonds. Exopeptidases can also be classified as aminopeptidases and carboxypeptidases based on whether cleavage takes place from the amino-terminal or carboxy-terminal. Moreover, proteases are also classified as aspartyl proteases, threonine proteases, cysteine proteases, metalloproteases, glutamic proteases and serine proteases based on their catalytic site.

Among these, aspartyl proteases play a crucial role in apicomplexan parasites, which include several processes like enzymatic maturation, signal transduction, virulence factors and many more. This chapter primarily elaborates on the role of aspartyl proteases which are used by *Plasmodium* and *Toxoplasma* for performing successful infection, egress and modulating their host for successful intracellular survival.

### 1.1 Aspartyl Proteases in Pathogens and Their Mode of Action

The class of enzymes called aspartyl proteases (ASPs) are found in all eukaryotes performing a variety of activities, as represented in Table 1.

After analysis of kinetic and X-ray crystallographic data from various sources, it can be inferred that two catalytically proficient carboxyl groups of aspartate residues, one of which acts as a general acid while the other is in an ionized state and acts as a general base that can react with a water molecule and remove a proton from it, are present. The activated water molecule then acts as the nucleophile that attacks the carbonyl carbon of the scissile peptide bond. Concurrently, the aspartyl residue acting as a general acid provides carbonyl oxygen atom of the substrate's scissile bond with a proton, forming in the process a tetrahedral intermediate. Finally, a

**Table 1** Classification of aspartyl proteases and their functions

| | ASPs | Gene ID | Localization | Function |
|---|---|---|---|---|
| GROUP A | PfPM I | PF3D7_1407900 | Food vacuole | Heamogobin degradation |
| | PfPM II | PF3D7_1408000 | | |
| | PfPM IV | PF3D7_1407800 | | |
| | HAP | PF3D7_1408100 | | |
| | TgASP1 | TGME49_201840 | IMC | |
| GROUP B | PfPM VI | PF3D7_0311700 | n.d. | n.d. |
| | PfPM VIII | PF3D7_1465700 | | |
| | TgASP 2 | TGME49_262940 | | |
| | TgASP 4 | TGME49_209620 | | |
| GROUP C | PfPM IX | PF3D7_1430200 | Rhoptry | Invasion/Egress |
| | PfPM X | PF3D7_0808200 | Exoneme | |
| | TgASP 3 | TGME49_246550 | Trans-Golgi Network | |
| GROUP D | PfPMV | PF3D7_1323500 | Endoplasmic reticulum | Protein export |
| | TgASP 5 | TGME49_242720 | Golgi | |
| | TgASP 7 | TGME49_261530 | | |
| GROUP E | PfPM VII | PF3D7_1033800 | n.d. | n.d. |
| | TgASP 6 | TGME49_272510 | | |

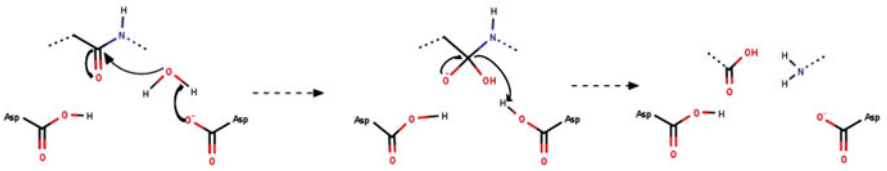

**Fig. 1** Mechanism of catalytic action of aspartyl protease

switch of the configuration surrounding the nitrogen atom of the substrate's scissile bond takes place, with the shift of a hydrogen atom from the aspartic residue acting as a base to the nitrogen atom. In the meantime, the transfer of a proton takes place from the oxygen atom of the aspartic acid residue acting as an acid for the carbonyl oxygen present on the peptide bond, which is being cleaved, breaking the C-N bond and discharging the two peptide products (Fig. 1).

## 1.2 ASPs in Apicomplexan *Plasmodium* and *Toxoplasma*

The apicomplexan ASPs are distributed between five groups of different evolutionary lineages (Table 1). Ten putative ASPs were identified in *P. falciparum*, known as

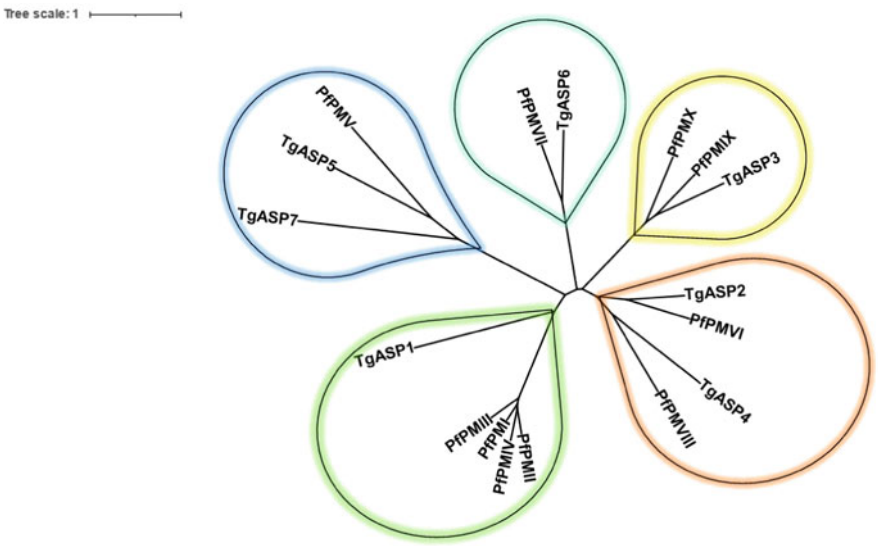


**Fig. 2** Aspartyl protease phylogeny cluster

plasmepsins (PfPMI, II and IV–X and histo-aspartic protease (HAP)). In contrast, *T. gondii* encodes only seven ASPs (TgASP1–TgASP7). These apicomplexan ASPs are clustered on the basis of their phylogenetic relationship, the function they perform and the stage at which they are expressed, as represented in Table 1 and Fig. 2.

PfPMI, II and IV and HAP share 50–70% sequence homology and are all grouped into one clade, which is consistent with their specific role in haemoglobin digestion, a process that is absent from other apicomplexan parasites. These food vacuole plasmepsins are also related to TgASP1 even though *T. gondii* lacks food vacuole and is not involved in haemoglobin digestion keeping its function obscure. PfPMV, while being the most divergent (19–23% sequence homology), groups with TgASP5, again sharing similar roles in protein export. PfPMVI and PfPMVIII with orthologues TgASP2 and TgASP4, respectively, are 36% similar to each other and are characterized by the presence of glycophosphatidylinositol (GPI) anchor signal, indicating an association with membrane, with their expression, limited to only transmission stage parasites. PfPMIX and PfPMX are 37% similar to each other, with TgASP3 forming another important clade involved in the maturation steps of rhoptry and micronemal proteins. PMVII is distantly related to PMVI and PfPMVIII with TgASP6 as its uncharacterized orthologue [1]. In this book chapter, we will particularly focus on the emerging role of aspartyl proteases involved in the export mechanism of *Plasmodium* and *Toxoplasma* (PMV/TgASP5) and recently characterized maturases (PMIX, PMX and TgASP3) that play a pivotal role in the process on invasion and egress of these two parasites.

## 2 Aspartyl Protease in Export

Based on phylogeny, PfPMV and TgASP5 come into one clad, which is related to the transport of proteins of parasite origin to the infected host. While sharing 75% similarity, both play pivotal roles in effector export. PfPMV is localized in the endoplasmic reticulum (ER), while TgASP5 is a Golgi resident protein [2, 3]. As the malarial parasite infects the RBC, which lacks a nucleus, secretory pathway, protein synthesis and other trafficking processes, the parasite modifies the RBC in order to get essential nutrients to carry out its replication process and evade the host immune system. *P. falciparum* achieves this by exporting a huge number of effector proteins in the RBC that takes part in changing the host cell's rigour, absorption of nutrient and endothelial attachment features. PfPMV takes part in the processing of exported proteins near the PEXEL motif in the ER. PEXEL motif marks the proteins for transport to the host, and cleavage at this site by PMV is vital for the export [4, 5]. Similarly, *Toxoplasma gondii* also, after successfully invading the host cell, secretes several effector proteins from the specialized secretory organs. In particular, dense granule proteins (GRAs) that are secreted after the PV is formed act as effector proteins and are involved in a wide repertoire of processes necessary for the initiation of infection, along with the establishment of the membranous nanotubular network (MNN), modulating cell cycle and remodelling host cell signalling pathways to block immune response [6, 7].

### 2.1 PMV

#### Structural Insights and Substrate Specificity

The activity of PMV as other aspartyl proteases is dependent on its catalytic dyad, which is composed of two aspartate residues, Asp118N and Asp365N (one from each active site region). It has been found that mutation of Asp365 had more prominent consequences on the catalytic efficiency than that of Asp118, which suggests that the D365 may act as a catalytic nucleophile to stimulate the water molecule [8]. PMV structure is similar to vacuole plasmepsins except for a few differences, which include the presence of a longer flap region as compared to the equivalent region of vacuole plasmepsins that perhaps contributes to the PMV's high conformational flexibility and unique substrate specificity. An unpaired cysteine residue, a well-conserved helix-turn-helix (HTH) motif close to the C-terminus and also in the N-terminal subdomain, a surface loop (Y116–G131) and "nepenthesin 1-type" aspartyl protease (NAP1) fold correlate with the opening/closing movement of the flap [9, 10]. The presence of one disulphide bond (C5–C7) within the NAP1 region directly links this loop to the N-terminal β-strand of the flap region. PvPMV structure suggests a protein-protein interaction role involving the NAP1 insert as a molecular gate confining entry to the PEXEL proteins [11]. A total of seven disulphide bonds are present in PfPMV, with two disulphide bonds in the N-terminal domain, the second pair of disulphide bridges in the Insert-1 domain, and the remaining three located in the C-terminal domain. The presence of these

sulphide bridges may have a function in conserving the structural integrity of PMV. Moreover, molecular dynamics simulation of the PfPMV-PEXEL complex suggested that distinct locations of Glu179 and Gln222 are accountable for lending PEXEL substrate specificity with arginine at the P3 position and also explained the presence of Ile94, Ala98, Phe370 and Tyr472 in the S4 binding pocket makes the catalytic site inaccessible to pepstatin, a powerful inhibitor of most pepsin-like aspartic proteases [12].

Among the malarial proteins which are most likely speculated to be exported into the infected erythrocyte, nearly half of them are membrane adhesins, i.e. ∼120 RIFINs (repetitive interspersed family), 50 *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1s) and 30 subtelomeric variant open reading frames (STEVORs). It has been shown that some of the RIFINs and STEVORs have a role in resetting of RBCs [13]. At the same time, it has been observed that certain STEVORs and surface-associated interspersed gene families are involved in the infiltration of the merozoites. The expression of various RIFINs and STEVORs has further been found in gametocytes [14, 15]. PMV also has a role in the maturing process for dense granule proteins. Along with the canonical PEXEL, PMV cleaves the "relaxed PEXEL" (RXLXXE) of the dense granule protein ring-infected erythrocyte surface antigen (RESA) [16]. The daughter merozoites store RESA in their dense granules after cleavage, and when they re-enter fresh RBCs, RESA is secreted into the lumen of the vacuole and rapidly transported into the host cell via PTEX [17]. Following infection, *Plasmodium* increases the rigidity of the erythrocyte by modulating its cytoskeleton via exporting some proteins like RESA, knob-associated histidine-rich protein (KAHRP) and the *Plasmodium* helical interspersed subtelomeric-domain proteins (PHIST) into the cytoplasm or at the cell membrane [5, 18]. Moreover, recent work has shown that modules that contain KAHRP are trafficked to the membrane of the red blood cell, where it assembles into ring-shaped complexes at the bottom of the knob, suggesting its role in knob formation [19]. PHIST proteins also participate in PfEMP1 trafficking and infected RBC (iRBC) cytoadherence. Several PHIST members are known to be present in the extracellular vesicles that have vital effects on malaria pathogenesis, with one of these taking part in the generation of these extracellular vesicles [20]. Investigations have shown that PMV also primes gametophyte exported proteins (GEXPs) such as GEX 07 and GEX 10, found on parasite-infected RBC plasma membranes. GEX 10 is involved in the cytoadherence of debilitated erythrocyte to chemokine CX3CL1, whose expression showed to control the deployment of monocytes [21–23]. Further recent reports on the basis of homology-directed repair and techniques like CRISPR-Cas9 found that the ΔGEXP07 parasite line expressed remarkable ultrastructural modifications, along with swelling and cleft fragmentation. Knockout of GEXP07 further exhibited the development of enlarged anomalous knobs and knob clusters, suggesting that GEXP07 is somehow involved in PfEMP1 uploading [24]. A complete list of identified substrates of PMV with their localization is presented in Table 2.

**Table 2** PMV and ASP5 substrates and their localization

| Substrates | | | Localization | | | |
|---|---|---|---|---|---|---|
| | | | Nucleus | Cytoplasm | PVM | PV |
| Toxoplasma effector proteins | MYR dependent | GRA16 | ✓ | | | |
| | | GRA17 | ✓ | | | |
| | | GRA18 | ✓ | | | |
| | | GRA19 | ✓ | | | |
| | | GRA20 | ✓ | ✓ | | |
| | | GRA21 | ✓ | | | |
| | | GRA22 | ✓ | | | |
| | MYR Independent | GRA15 | | | ✓ | |
| | | GRA17 | | | ✓ | |
| | | GRA23 | | | ✓ | |
| | | MAF1b | | | ✓ | |
| | | GRA7 | | | ✓ | |
| | | GRA60 | | | ✓ | |
| | | GRA 45 | | | ✓ | |
| | | GRA2 | | | | ✓ |
| | | GRA6 | | | | ✓ |
| | | GRA12 | | | | ✓ |
| | Presence of PEXEL | | Localization | | | |
| Plasmodium effector proteins | × | PfEMP 1 | RBC membrane | | | |
| | ✓ | RESA | RBC membrane | | | |
| | | | Dense granules | | | |
| | ✓ | STEVOR | RBC membrane | | | |
| | × | Clag 3 | RBC membrane/Cytosol | | | |
| | ✓ | RIFINS | RBC membrane | | | |
| | ✓ | GEX 07 | MC/ RBC membrane | | | |
| | ✓ | GEX 10 | | | | |
| | ✓ | KAHRP | RBC membrane | | | |
| | ✓ | PHIST | iRBCM/ MC/ Extracellular vesicle | | | |
| | ✓ | HSP70x | Cytosol | | | |
| | ✓ | HSP 40s | | | | |
| | × | SBP1 | MC | | | |

## Mechanism of Action

Upon invasion, *P. falciparum* remodels the physiological properties of host RBC by trafficking an extensive repertoire of proteins into and across the parasitophorous vacuole (PV), where the parasite inhabits and multiplies inside the host without getting fused with the host endolysosomal system. The exported *P. falciparum* effector proteins are involved in reconstituting a critical trafficking system in the RBC cytosol and changing the rigour of the host cell, absorption of nutrients and endothelial binding features, which is thus crucial for its survival and virulence inside the host [4, 5]. Cytoadherence-linked asexual gene 3 (CLAG3), for example, that is transported to the host cytosol and membrane, along with rhoptry proteins RhopH2 and RhopH3, forms a complex, referred to as RhopH complex, and acts as plasmodial surface anion channel (PSAC), contributing to the establishment of the new permeation pathways (NPP), which are essential for the import of small solutes including the amino acid isoleucine and water-soluble vitamin pantothenic acid across the red blood cell membrane. Also, RhopH2 and RhopH3 have been known to be vital for parasite's survival inside the intracellular niche. Their specific role is still obscure, but current research developments have indicated their role in escorting soluble CLAG to the RBC membrane [25–27]. In this regard, another protein PfEMP that is trafficked to knob-like structures on the erythrocyte surface possesses adhesive properties allowing parasite-infected erythrocytes to interact with cell surface receptors on uninfected erythrocytes or to vascular endothelium, a process known as cytoadherence that helps in escaping from splenic clearance [28]. PfEMP1 is involved in the interaction and binding of parasite-infected RBCs with endothelial cells via adhesive ligands like CSA, ICAM-1 and CD36, which also help to escape splenic clearance [13, 29].

The total parasite proteins that are exported (also regarded as exportome) can be classified into two groups on the basis of the presence of pentameric amino acid motif (RxLxE/Q/D), also known as *Plasmodium* export element (PEXEL) or host-targeting sequence. The PEXEL motif is located ~30 amino acids downstream of a signal sequence, often indented from the N-terminus, and acts as the site for cleavage by the ER-resident plasmepsin [30–32]. Recent advancements have elucidated that after translation, some PEXEL proteins utilize a novel pathway of ER translocation involving PfSec61-PfSec62-PfSPC25 interactome with PMV binding to signal peptidase complex 25 (PfSPC25), a non-catalytic component of the signal peptidase complex (SPC)15 instead of canonical PfSec61-SPC complex which is used for classical secretion. Following PMV cleavage at the C-terminal axis of conserved leucine residue of the motif, proteins are acetylated at the newly formed N-terminal [33–35]. The processed PEXEL proteins are then released into the parasitophorous vacuole and transported through PVM into the host cell cytoplasm or Maurer's clefts (MC) via *Plasmodium* Translocon of Exported protein (PTEX) complex. PTEX is a large PVM-associated compound composed of three core proteins: HSP101, a chaperon that unfolds proteins, PTEX150 which acts as the adapter between HSP101 and EXP2 and EXP2 that forms a channel for protein transport across PVM. Two accessory proteins, thioredoxin-2 (TRX2) and PTEX88, are also

required for the successful effector export [36–39]. Along with being an integral part of PTEX, EXP2 also forms a channel that serves as a molecular sieve which allows the absorption of nutrients and waste export. Another parasite protein, EXP1, has also been suggested to support this channel formation by EXP2 [40–42]. PEXEL-negative exported proteins (PNEPs) like PfEMP1, SBP1 and REX2 don't contain PEXEL motif and thus are not cleaved by PMV; however, most of them have an internal transmembrane domain. In contrast, some have been associated with a regular signal sequence towards the N-terminal [5, 30]. As PNEPs don't have a PEXEL motif, the N-terminus in conjunction with its transmembrane domain is sufficient for its export [43]. The exact number of PNEPs is still unrecognized due to the lack of any signature; however, more than 40 PNEPs have been identified till date. A recent report involving large-scale genetic screens and targeted gene-disruption studies has estimated that among the total exportome of *P. falciparum*, about 71 protein genes, including both PEXEL/PNEP exportome, equating to ~13% of the total exportome are critical for the parasite's viability inside the host RBC [25]. The entire process of PMV action along with its substrate is represented in Fig. 3a.

## 2.2 TgASP5

### Structural Insights and Substrate Specificity

Similar to the *Plasmodium falciparum* aspartyl protease plasmepsin V (PfPMV) that cleaves and licenses the effector proteins for their export through PVM, its orthologue, TgASP5, which is Golgi resident, can cleave its substrate at either N-terminal or C-terminal contrary to PMV that cleaved specifically at N-terminally located PEXEL motif. ASP5 cleaves the substrates at a fixed motif, known as the TEXEL (Toxoplasma Export Element), and a motif, which is conserved, comprising of RRLxx, which may be found at the C- or N-terminus of the substrate [2, 44, 45]. Proteolytic cleavage by TgASP5 at PEXEL like N-terminally located RRLAE motif is essential for the nuclear localization of GRA16 and GRA24 despite the fact that GRA24 is devoid of the motif. It also cleaves the parasitophorous vacuole membrane located MYC regulation 1 (MYR1) at its C-terminally located PEXEL; however, this cleavage has not been found to be essential for its translocation activity [46]. Further, while in *Plasmodium*-infected RBC, only proteins transiting the PVM required proteolytic cleavage by PMV, TgASP5 cleaves substrates of various fates. As an example, GRA15 localizes in the PVM towards the host cytosolic side, where it initiates the host NF-kB pathway to evoke a protective immune response to negate the action of the parasite by activating NF-kB-mediated transcription of cytokines like IL 12 and additional related molecules [47]. GRA17 and GRA23 that span the PMV help in the formation of a "molecular sieve" in the PVM that aids the parasite in accessing host cell nutrients, whereas the PV transmembrane protein mitochondrial association factor 1b (MAF1b) recruits host mitochondria towards the PVM [48, 49]. PV lumen resident GRA2 and GRA6 have been implicated in the MNN formation inside the

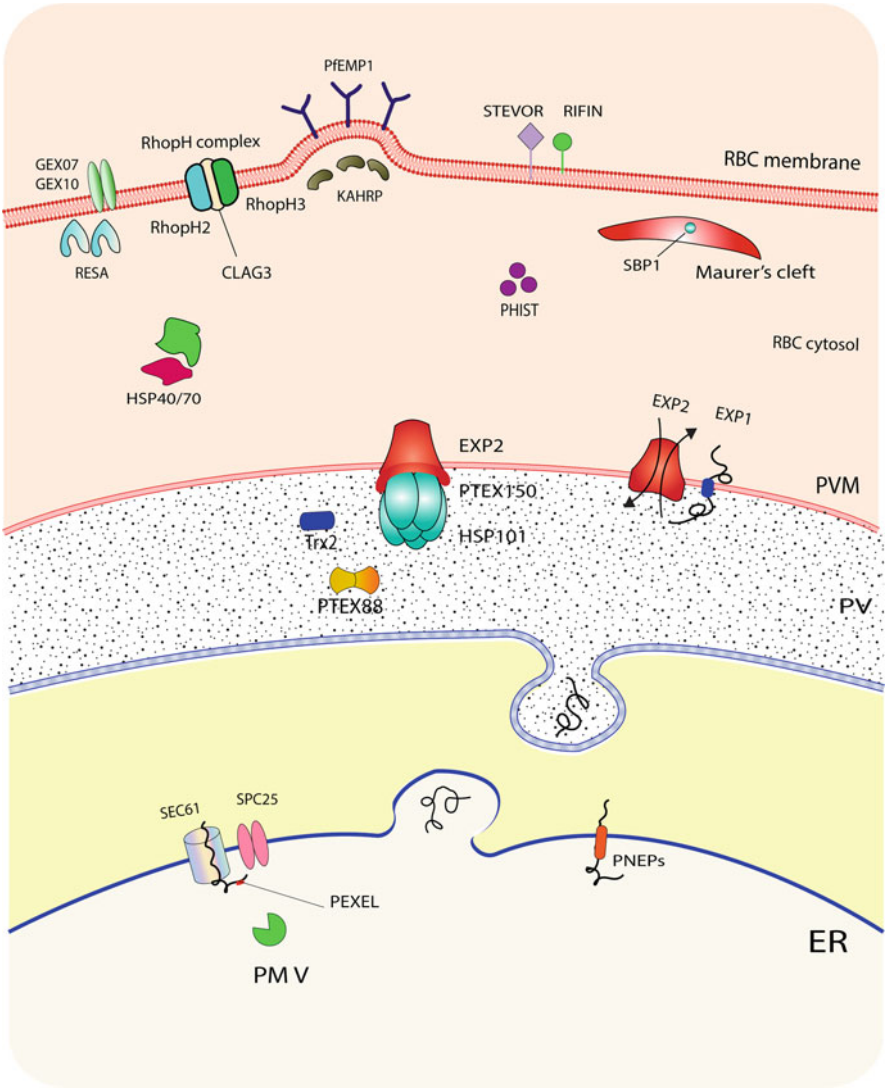


**Fig. 3** (**a**) Schematic representation of *Plasmodium*-infected erythrocyte, showing processing of exported proteins by PfPMV in ER, export pathway of processed proteins and their localization in the cell. (**b**) Schematic representation of cell infected with *Toxoplasma* parasite, showing the aspartyl protease ASP5 and various parasite effector proteins and their localization in parasitophorous vacuole (PV), parasitophorous vacuole membrane (PVM), host cell cytosol and nucleus

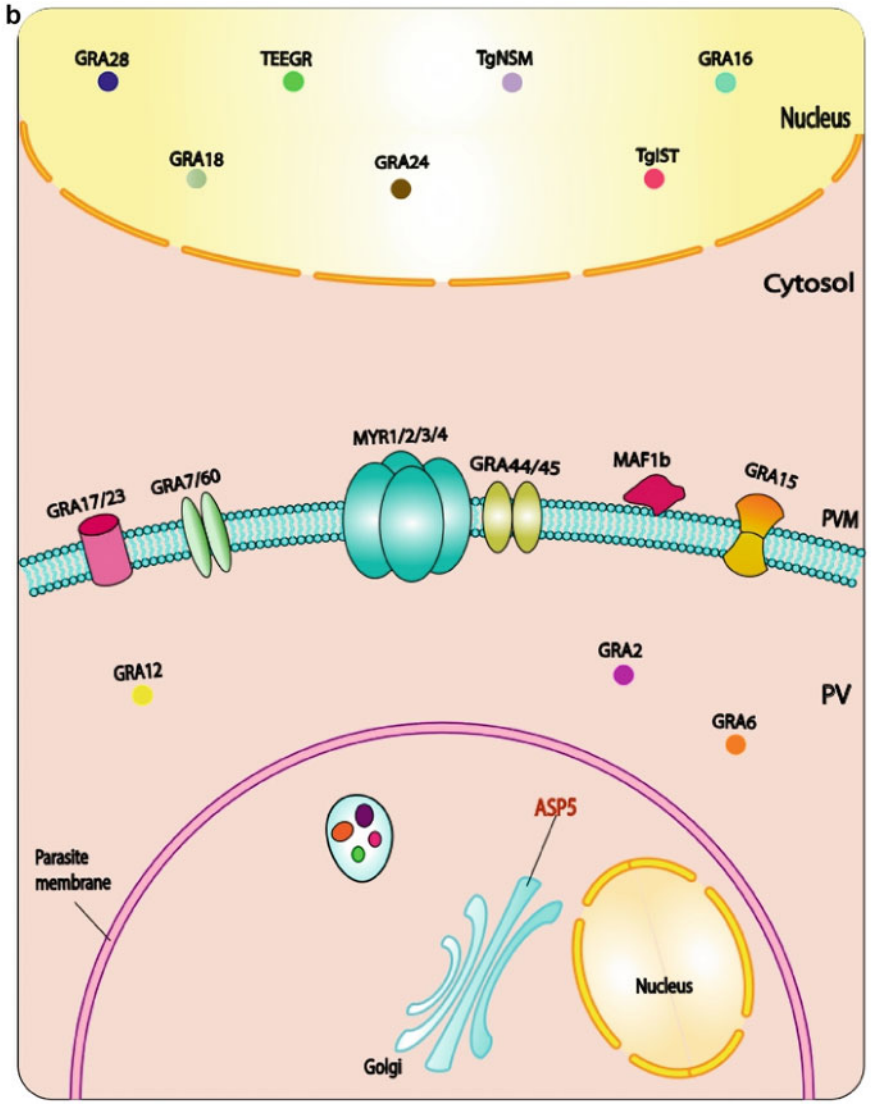


**Fig. 3** (continued)

PV space; GRA12 also remains in association with GRA2. However, recently it has been demonstrated that polymorphic dense granule protein GRA6 activates the host signalling cascade for activation of a host transcription factor nuclear factor of activated T cells 4 (NFAT4) via calcium modulating ligand (CAMLG) binding. Interferon-gamma (IFN-γ) is a very crucial cytokine in the elicitation of both innate and adaptive immune responses. Cells infected with *Toxoplasma* show inhibition of IFN-γ-induced expression of necroptotic genes, including protein kinase R (PKR)

that further activates mixed lineage kinase domain-like pseudokinase (MLKL), a mediator of necrosis signalling and thus preventing necroptotic death, ensuring parasite survival and transmission. This is achieved with the help of combinatory action of nuclear localized TgASP5 processed dense granule proteins IST (inhibitor of STAT1-dependent transcription) and TgNSM (Toxoplasma NCoR/SMRT modulator). TgIST also binds to activated STAT1 dimers and nucleosome remodelling and deacetylase Mi2/NURD complex, recruiting a chromatin remodelling complex to block the transcription of STAT1-dependent promoters and IFN-gamma-dependent gene expression and thus developing the protective immunity [6, 50, 51]. HCE1/TEEGR is another TgASP5-dependent dense granule protein which is involved in modulating the cell cycle by interacting with E2F3 and E2F4 and upregulating CDK2 and host cyclin E, which are master regulators of transition from G1 to S phase [52, 53]. GRA18 and GRA28 help the parasite in eliciting a protective immune response. GRA18 suppresses the Th1 response by activating the Th2 response via upregulation of chemokines of the Th2 branch, whereas GRA28 upregulates CCL22 in the cytoplasm, and GRA18 binds to β-catenin degrading complex, preventing the degradation of β-catenin and activating β-catenin-dependent WNT pathway [54, 55]. Comparative analysis of the N-terminal between Δ*asp5* tachyzoites and wild type (WT) with two different proteomic approaches (hydrophobic tagging-assisted N-terminal enrichment (HYTANE) and terminal amine isotopic labelling of substrates (TAILS)) identified additional TgASP5-dependent effector proteins GRA44, GRA45, GRA46, WNG1 and WNG2, although their role in parasite survival is yet to be characterized [3]. A complete list of substrates for ASP5 with their localization is presented in Table 2.

## Mechanism of Action

*Toxoplasma gondii*, after successfully invading the host cell, secretes several effector proteins from the specialized secretory organs. Till date, all known effectors for *Toxoplasma* are either secreted from rhoptry or dense granules, which are destined to the parasitophorous vacuole (PV) lumen, parasitophorous vacuole membrane (PVM), host cell cytoplasm or nucleus. In particular, dense granule proteins (GRAs) that are secreted after the establishment of PV act as effector proteins and have been involved in a wide repertoire of processes necessary for the infection initiation, along with the establishment of the membranous nanotubular network (MNN), modulating cell cycle and remodelling host cell signalling pathways to block the immune response. The total number of GRAs and their role are still not clear, but recent studies are helping in uncovering many mechanisms.

The indication that translocation of GRA protein across PV membrane is also modulated by some complex came from a series of experiments. A study in 2013 showed that in *Toxoplasma*-infected cells, there was upregulation of host cell transcription regulator, C-MYC, which may have been mediated by parasite effector protein. Later whole-genome sequencing of mutant tachyzoites that were unable in upregulation of host C-MYC revealed MYR1 located at PVM, which is responsible for not only C-MYC upregulation but also altered pathways of other dense granule proteins, indicating its role as exporter protein across PVM. It was further reported

that MYR2 and MYR3 are also membrane-bound and crucial for PVM transiting GRA translocation [56–58]. Immunoprecipitation followed by affinity purification of MYR1 has revealed new MYR1-interacting proteins, like GRA44, GRA45 and MYR4, that along with MYR1, MYR2, MYR3, ROP17 and ASP5 are essential for GRA translocation. GRA45 was shown to have a chaperone-like domain vital for the proper localization of GRAs into the PV membrane and secretion of GRA effectors into the cytoplasm of the host [59]. Recent data has identified GRA24 as a key role player in inducing protective immunity, which works autonomously, without the TLR/MyD88 cascade, through prolonged p38 mitogen-activated protein kinase (MAPK) activation and regulation of IL-12 and other cytokines [60]. After cleavage, GRA16 is trafficked to the nucleus, where it interacts with herpesvirus-associated ubiquitin-specific protease (HAUSP) with N-terminal and PP2A (protein phosphatase 2A) via C-terminal and regulates genes which take part in the progression of cell cycle and the p53 tumour suppression mechanism. It also leads to C-MYC prolonged activation by halting the proteolytic degsradation of phosphorylated C-MYC [61, 62]. Another comparative genetic analysis of ΔGRA mutants generated by CRISPR-Cas9 genome editing system found the non-essentiality of 17 GRA genes, including GRA11, GRA12, GRA13, GRA14, GRA20, GRA21, GRA28–31, GRA33–38 and GRA40, for parasite virulency suggesting their role in some other parasite-mediated pathway [63]. A schematic representation of TgASP5 processing and mechanism of action has been represented in Fig. 3b.

## 3 Aspartyl Protease in Invasion and Egress

Numerous proteins that the parasite secrete while invasion are proteolytically cleaved either while passing via the secretory pathway or on the parasite cell surface while it is still inside the cell, aiding in the entry to the host body. In *Toxoplasma* parasites, aspartyl protease 3 (TgASP3) is the primary enzyme that acts as a maturase for several virulence factors, enabling these parasites to invade and exit from the host. In the context of phylogeny, TgASP3 conglomerates with PfPMIX (plasmepsin IX) and PfPMX (plasmepsin X) (Fig. 2) and functions as maturase for rhoptry and microneme proteins which helps in the process of invasion and egress of these parasites. For understanding the intracellular localization of these proteins, the 3′ end of the endogenous genes was tagged with epitopes by Nasamu et al. and visualized via immunoelectron microscopy. It has been observed by them that PMIX largely localizes in the rhoptry secretory organelle bulbs, which primarily take part in the invasion. On the other hand, they concluded that PMX localizes in exonemes that are released during egress into the PV surrounding the parasite [64–66]. A different point of view has been brought out by Pino et al.; they have altered the PMIX locus so as to add the loxP sites and also a Ty epitope tag in the C-terminal in the DiCre recombinase-expressing parasites [67, 68]. The recombinant PMIX partly colocalized with the secreted protein RhopH3 (high molecular weight rhoptry protein 3) but not with PfAMA1 (*Plasmodium falciparum* apical membrane antigen-1). With the help of immune electron microscopy, they confirmed the localization to

be in the vicinity of the rhoptries and is in accordance with transcriptomics, specifying that it is manifested before secretory organelle proteins [67, 69]. Due to the absence of any predicted transmembrane domains, PMIX and PMX are grouped as soluble proteins. Separate studies have already underlined the critical roles of PMIX and PMX in the invasion and egress of RBCs, two extremely vital processes controlled by the discharge of the effector molecules from the micronemes/exonemes and rhoptries, which are the secretory organelles [64, 67]. Plasmepsin IX acts on the rhoptry secretory organelle biogenesis, which is important for the invasion of the erythrocytes. On the contrary, plasmepsin X is vital for egress as well as invasion, directing the maturation of the subtilisin-like serine protease SUB1 in exoneme secretory vesicles. Micronemes/exonemes store the secretory proteins, which engage in the invasion and egress; meanwhile, the rhoptry-resident proteins are involved specifically in the invasion. Proteases of serine and cysteine play key roles in the PV and RBC membrane breakdown during egress and also take part in the invasion via the activation of ligands or removing those of them involved in the host-parasite interactions. Effector proteins like perforins, adhesins and even proteases, which are involved in egress as well as invasion, are processed pre- and post-exocytosis [70]. Studies have also revealed that conditional knockdown of plasmepsin IX caused a defect in rhoptries biogenesis which is associated with a vital invasion phenotype. On the other hand, depletion of plasmepsin X resulted in a drastic blockage in both invasion and egress [64, 67]. PfPMIX was shown to specifically process two rhoptry-resident proteins, apical sushi protein (PfASP) and the rhoptry-associated protein 1 (PfRAP1), whereas PfPMX process micronemal adhesin PfAMA1 and exoneme subtilisin-like serine protease PfSUB1 which in turn process another group of cysteine proteases (SERAs) and a cluster of merozoite surface proteins (MSPs) during egress [67]. It's important to mention here that the question of whether drugs directed towards molecules that take part in invasion will succeed is already elucidated by further research [64, 67]. Certain protease inhibitors are known to inhibit invasion and egress, which hints that there is ample amount of time for an efficient drug-target interaction.

## 3.1 TgASP3, PfPMIX and PfPMX

### Structural Insights and Substrate Specificity

As already mentioned in previous sections, PfPMIX and PfPMX phylogenetically cluster with TgASP3, which has an identical part in the invasion and egress of *Toxoplasma gondii* [71]. Although the crystal structure for PfPMI, II, IV and V has been determined, the crystal structure for TgASP3, PfPMIX and PfPMX is not available yet. Recent research has elucidated a comparative homology model for the catalytic domain of TgASP3 (Ile273–Val603), PfPMIX (Ile 221–Val608) and PfPMX (Ile229–Lys564), which addresses the selectivity of substrates and inhibitors for these enzymes [72]. Three basic parts of this structure can be distinguished as follows: the N-terminal domain, the central domain containing five antiparallel β-sheets, forming the active site's backbone, and the two catalytic aspartic acids

(D299 and D490 in TgASP3), which are conserved, and the C-terminal lobe. In the N-terminal of TgASP3, the β-hairpin structure, known as a flap (F344–G347), forms a perpendicular structure to the active site, facing the C-terminal flexible loop. PPMIX presents a very peculiar and unusual loop (V428–N490 not modelled in the PfPMIX structure) close to the flexible region, which can be regarded as a gatekeeper regulating the ligand access or in the protein-protein interaction, similar to the NAP1 insert on PfPMV. This model clearly explains the substrate specificity for each of these aspartyl proteases by docking their natural specific substrate peptides, which resulted in a conserved productive binding for a shared substrate TgROP1, which is efficiently cleaved by all three proteases. On the other hand, for PfPMX and PfPMIX, specific substrates PfSUB1 and PfRAP1 manifest a greater amount of useful binding modes, respectively. The model speculates that a single residue change, from threonine to serine, in the flap contributes to these substrate specificities. The model also recognizes a conserved motif like SFVE, which precedes the glutamic acid cleavage site, specifying a conserved motif that is recognized by these proteases. Interestingly, TgMIC6 (*Toxoplasma gondii* microneme protein 6), which is cleaved exclusively by ASP3, is not known to possess this usual motif and the specific point mutations. Furthermore, analysis of the N-terminal by TAILS (terminal amine isotopic labelling of substrates) revealed that several MICs, RONs and ROPs proteins could be substrates of TgASP3 (Table 3). Among the substrates identified for ASP3, another predicted kinase is RON13, which localizes to the rhoptry neck [71, 73, 74]. Parasites who are exhausted of ASP3 cannot invade the host cells, primarily due to an acute deficiency in the rhoptry discharge.

In the context of inhibitors, PfPMIX and PfPMX are an attractive chemotherapeutic target to develop antimalarial drugs since it obstructs the merozoite's potential to invade the host, reducing the risk of tolerance and any chance of resistance. It has been reported that an hydroxylethylamine aspartic protease inhibitor, 49c, inhibits the activity of TgASP3 and PfPMIX and PfPMX, without affecting other food vacuole plasmepsins, that emphasizes on a uniform structural characteristic between the members present in this cluster, which would directly become important for the targeted drug design [67, 71]. Docking of 49c into the 3D model of these proteases showed a notable depth of resemblance at the level of the active site, specifically in the flap area, that is critical for the accommodation of the substrate and ligand [72]. Studies on the binding mode of 49c at the active site divulged the need for a stretched conformation for accommodating the compound for proper inhibition efficacy. Further, a recent pharmacophore model which compares the free energy related to PfPMIX and PfPMX 49c complex underlined two conserved phenylalanine residues, one of which is in the flap (F304 in PfPMX) and another one in the hydrophobic cavity (F386 in PfPMX), vital for the binding of 49c [75, 76]. This phenylalanine region in the flap area is preserved only in PfPMIX, PfPMX and TgASP3. In aspartic proteases of the rest of the apicomplexans, this region is substituted by the tyrosine (Y) residue. Furthermore, F-Y mutations in the flap have given rise to decreased sensitivity of compound 49c against all of these aspartyl proteases in vitro. Various efforts to recapitulate this mutation in vivo by generating

**Table 3** PMIX, PMX and ASP3 substrates and their localization

| ASP | Substrate | Localization | | Function |
|---|---|---|---|---|
| | | Rhoptry | Microneme/ Exoneme | |
| PM IX | PfRAP1 | ✓ | ✗ | Invasion |
| | PfASP | ✓ | ✗ | |
| | R0N3 | ✓ | ✗ | |
| PM X | PfAMA1 | ✗ | ✓ | Invasion/ Egress |
| | PfSUB1 | ✗ | ✓ | |
| | PfSUB2 | ✗ | ✓ | |
| | EBL | ✗ | ✓ | |
| ASP 3 | M2AP | ✗ | ✓ | Invasion/ egress |
| | MIC3 | ✗ | ✓ | |
| | MIC6 | ✗ | ✓ | |
| | TLN4 | ✗ | ✓ | |
| | TLN1 | ✓ | ✗ | |
| | ROPI | ✓ | ✗ | |
| | ROP2 | ✓ | ✗ | |
| | ROP3 | ✓ | ✗ | |
| | ROP4 | ✓ | ✗ | |
| | ROP7 | ✓ | ✗ | |
| | ROP13 | ✓ | ✗ | |
| | ROP18 | ✓ | ✗ | |
| | RON2 | ✓ | ✗ | |
| | RON4 | ✓ | ✗ | |
| | RON9 | ✓ | ✗ | |

49c resistant cell lines of *P. falciparum* have failed, probably strengthening the idea of dual targets of 49c in *Plasmodium* parasites [67]. A complete list of substrates for TgASP3, PMIX and PMX and their localization is presented in Table 3.

## Mechanism of Action

Phylogenetically, since TgASP3 clusters together with PfPMIX and PfPMX, their mechanism of action is also coherent. A recent study revealed that TgASP3 localized in the endosome-like compartment (ELC); they also showed it to be critical for

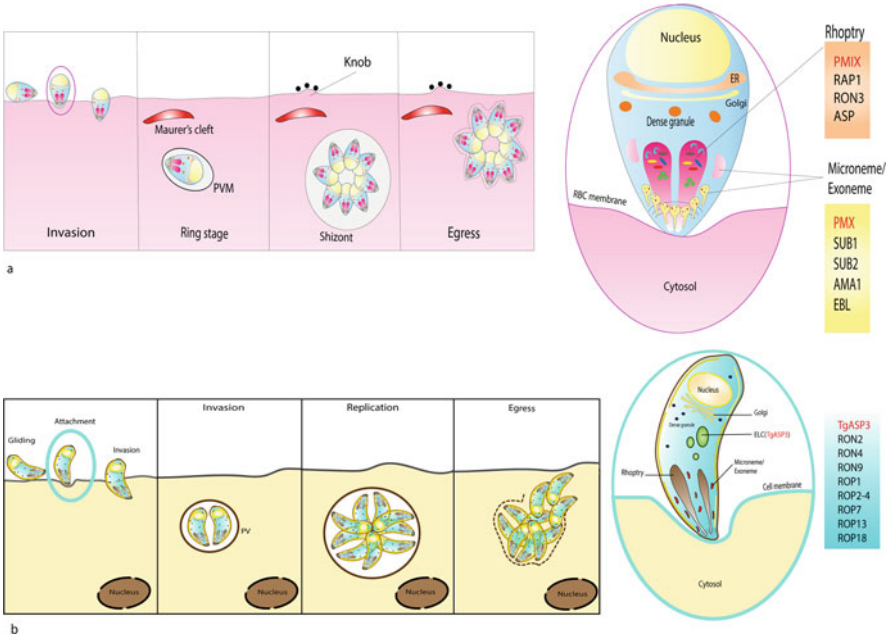


**Fig. 4** (**a**) Schematic representation showing various steps involved in the establishment of infection in erythrocyte by *Plasmodium* and the localization of the maturases, i.e. PMIX and PMX, and various rhoptry and microneme resident proteins processed by them. (**b**) Schematic representation showing various stages of *Toxoplasma* infection cycle and the localization of TgASP3, the only characterized maturates and its substrates

invasion and egress without impacting on intracellular growth [71]. Now just like in *Plasmodium*, *T. gondii* micronemes and rhoptry proteins also undergo proteolytic cleavage in the ELC while being trafficked from the Golgi to the secretory organelles. Processing of microneme proteins takes place once during the pre-exocytosis by unknown proteases and further during the post-exocytosis by subtilisin and rhomboid-like proteases, similar to PfSUB1. Owing to the comparable phenotypes of TgASP3 and PfPMIX/X, and, at the same time, with TgASP3 being the only *T. gondii* proteases in this particular cluster, it is plausible that the parasites process rhoptries and micronemes proteins in a common compartment before their trafficking to their respective organelles (Fig. 4a, b). It has also been observed that TgASP3 depletion leads to aggregation of unprocessed forms of M2AP, MIC3 and MIC6 protein without impacting their localization; as compared to that, MIC2, MIC4, CPL and MIC8 proteins were not affected. In the absence of TgASP3, the processing of toxolysin 4 (TLN4), a microneme metalloproteinase involved in invasion and egress, was also altered [77]. Similarly, in the case of *Plasmodium*, owing to its inactivation, SUB1 processing was affected. Moreover, several rhoptry proteins showed altered processing patterns (ROP1, ROP2–4, ROP7, ROP13, ROP18, RON2, RON2, RON4 and RON9) without impacting their localization

and mode of action [71]. A schematic representation of TgASP3, PfPMIX and PfPMX processing and mechanism of action has been represented in Fig. 4a and b.

## 4 Concluding Remarks

The development of resistance to all medications currently in use against the malaria parasite is rising at an alarming rate and thus presents one of the most prominent threats to malaria treatment and control; hence novel antimalarial drugs are urgently needed. The indispensable role of PMV in the development of malaria transmission stages as well as asexual stages, regardless of the fact that there are major differences in the asexual parasite and gametocyte, makes protein export a promising target for drug development. Also, treatment with peptidomimetic inhibitor WEHI-842 has been shown to obstruct the transmission of gametocytes. Furthermore, the interplay of the extensive repertoire of GRA and ROP effectors orchestrated by the parasite can elucidate the renovation mechanism of host cells by the parasite. The presence of conserved components in protein export and host modulation pathways among apicomplexan parasites can also help in understanding the microevolutionary process that may have happened. Plasmepsin IX and X are crucial drug targets for treating malaria owing to their specific nature towards *Plasmodium* and their important function as moderators in the development of the disease [78]. The current scenario calls for focus on the distinct targets in the parasite where no human homologue is present, such that it will decrease the chances of on-target drug toxicity.

## References

1. Nasamu AS, Polino AJ, Istvan ES, Goldberg DE. Malaria parasite plasmepsins: more than just plain old degradative pepsins. J Biol Chem. 2020;295(25):8425–41. https://doi.org/10.1074/jbc.REV120.009309.
2. Russo I, Babbitt S, Muralidharan V, Butler T, Oksman A, Goldberg DE. Plasmepsin V licenses plasmodium proteins for export into the host erythrocyte. Nature. 2010;463(7281):632–6. https://doi.org/10.1038/nature08726.
3. Coffey MJ, Dagley LF, Seizova S, Kapp EA, Infusini G, Roos DS, Boddey JA, Webb AI, Tonkin CJ. Aspartyl protease 5 matures dense granule proteins that reside at the host-parasite Interface in toxoplasma gondii. mBio. 2018;9(5) https://doi.org/10.1128/mBio.01796-18.
4. Matthews KM, Pitman EL, de Koning-Ward TF. Illuminating how malaria parasites export proteins into host erythrocytes. Cell Microbiol. 2019;21(4):e13009. https://doi.org/10.1111/cmi.13009.
5. Spillman NJ, Beck JR, Goldberg DE. Protein export into malaria parasite-infected erythrocytes: mechanisms and functional consequences. Annu Rev Biochem. 2015;84:813–41. https://doi.org/10.1146/annurev-biochem-060614-034157.
6. Panas MW, Boothroyd JC. Seizing control: how dense granule effector proteins enable toxoplasma to take charge. Mol Microbiol. 2021;115(3):466–77. https://doi.org/10.1111/mmi.14679.
7. Hammoudi P-M, Jacot D, Mueller C, Di Cristina M, Dogga SK, Marq J-B, Romano J, Tosetti N, Dubrot J, Emre Y, Lunghi M, Coppens I, Yamamoto M, Sojka D, Pino P, Soldati-

Favre D. Fundamental roles of the Golgi-associated toxoplasma aspartyl protease, ASP5, at the host-parasite Interface. PLoS Pathog. 2015;11(10):e1005211. https://doi.org/10.1371/journal.ppat.1005211.
8. Loymunkong C, Sittikul P, Songtawee N, Wongpanya R, Boonyalai N. Yield improvement and enzymatic dissection of plasmodium falciparum plasmepsin V. Mol Biochem Parasitol. 2019;231:111188. https://doi.org/10.1016/j.molbiopara.2019.111188.
9. Sittikul P, Songtawee N, Kongkathip N, Boonyalai N. In vitro and in silico studies of naphthoquinones and peptidomimetics toward plasmodium falciparum plasmepsin V. Biochimie. 2018;152:159–73. https://doi.org/10.1016/j.biochi.2018.07.002.
10. Xiao H, Bryksa BC, Bhaumik P, Gustchina A, Kiso Y, Yao SQ, Wlodawer A, Yada RY. The zymogen of plasmepsin V from plasmodium falciparum is enzymatically active. Mol Biochem Parasitol. 2014;197(1–2):56–63. https://doi.org/10.1016/j.molbiopara.2014.10.004.
11. Hodder AN, Sleebs BE, Czabotar PE, Gazdik M, Xu Y, O'Neill MT, Lopaticki S, Nebl T, Triglia T, Smith BJ, Lowes K, Boddey JA, Cowman AF. Structural basis for plasmepsin V inhibition that blocks export of malaria proteins to human erythrocytes. Nat Struct Mol Biol. 2015;22(8):590–6. https://doi.org/10.1038/nsmb.3061.
12. Bedi RK, Patel C, Mishra V, Xiao H, Yada RY, Bhaumik P. Understanding the structural basis of substrate recognition by plasmodium falciparum plasmepsin V to aid in the design of potent inhibitors. Sci Rep. 2016;6:31420. https://doi.org/10.1038/srep31420.
13. Wahlgren M, Goel S, Akhouri RR. Variant surface antigens of plasmodium falciparum and their roles in severe malaria. Nat Rev Microbiol. 2017;15(8):479–91. https://doi.org/10.1038/nrmicro.2017.47.
14. Sargeant TJ, Marti M, Caler E, Carlton JM, Simpson K, Speed TP, Cowman AF. Lineage-specific expansion of proteins exported to erythrocytes in malaria parasites. Genome Biol. 2006;7(2):R12. https://doi.org/10.1186/gb-2006-7-2-r12.
15. Yam XY, Niang M, Madnani KG, Preiser PR. Three is a crowd - new insights into Rosetting in plasmodium falciparum. Trends Parasitol. 2017;33(4):309–20. https://doi.org/10.1016/j.pt.2016.12.012.
16. Boddey JA, Carvalho TG, Hodder AN, Sargeant TJ, Sleebs BE, Marapana D, Lopaticki S, Nebl T, Cowman AF. Role of plasmepsin V in export of diverse protein families from the plasmodium falciparum exportome. Traffic (Copenhagen, Denmark). 2013;14(5):532–50. https://doi.org/10.1111/tra.12053.
17. Polino AJ, Nasamu AS, Niles JC, Goldberg DE. Assessment of biological role and insight into Druggability of the plasmodium falciparum protease Plasmepsin V. ACS Infectious Dis. 2020;6(4):738–46. https://doi.org/10.1021/acsinfecdis.9b00460.
18. de Koning-Ward TF, Dixon MWA, Tilley L, Gilson PR. Plasmodium species: master renovators of their host cells. Nat Rev Microbiol. 2016;14(8):494–507. https://doi.org/10.1038/nrmicro.2016.79.
19. Looker O, Blanch AJ, Liu B, Nunez-Iglesias J, McMillan PJ, Tilley L, Dixon MWA. The knob protein KAHRP assembles into a ring-shaped structure that underpins virulence complex assembly. PLoS Pathog. 2019;15(5):e1007761. https://doi.org/10.1371/journal.ppat.1007761.
20. Kumar V, Behl A, Sharma R, Sharma A, Hora R. Plasmodium helical interspersed subtelomeric family-an enigmatic piece of the plasmodium biology puzzle. Parasitol Res. 2019;118(10):2753–66. https://doi.org/10.1007/s00436-019-06420-9.
21. Hermand P, Cicéron L, Pionneau C, Vaquero C, Combadière C, Deterre P. Plasmodium falciparum proteins involved in cytoadherence of infected erythrocytes to chemokine CX3CL1. Sci Rep. 2016;6:33786. https://doi.org/10.1038/srep33786.
22. Hamon P, Loyher P-L, Baudesson de Chanville C, Licata F, Combadière C, Boissonnas A. CX3CR1-dependent endothelial margination modulates Ly6C(high) monocyte systemic deployment upon inflammation in mice. Blood. 2017;129(10):1296–307. https://doi.org/10.1182/blood-2016-08-732164.
23. Jennison C, Lucantoni L, O'Neill MT, McConville R, Erickson SM, Cowman AF, Sleebs BE, Avery VM, Boddey JA. Inhibition of Plasmepsin V activity blocks plasmodium falciparum

Gametocytogenesis and transmission to mosquitoes. Cell Rep. 2019;29(12):3796–3806.e4. https://doi.org/10.1016/j.celrep.2019.11.073.
24. McHugh E, Carmo OMS, Blanch A, Looker O, Liu B, Tiash S, Andrew D, Batinovic S, Low AJY, Cho H-J, McMillan P, Tilley L, Dixon MWA. Role of plasmodium falciparum protein GEXP07 in Maurer's cleft morphology, knob architecture, and P. falciparum EMP1 trafficking. *mBio*, *11*(2). 2020;11 https://doi.org/10.1128/mBio.03320-19.
25. Jonsdottir TK, Gabriela M, Crabb BS, de Koning-Ward F, T., & Gilson, P. R. Defining the essential Exportome of the malaria parasite. Trends Parasitol. 2021;37(7):664–75. https://doi.org/10.1016/j.pt.2021.04.009.
26. Schureck MA, Darling JE, Merk A, Shao J, Daggupati G, Srinivasan P, Olinares PDB, Rout MP, Chait BT, Wollenberg K, Subramaniam S, Desai SA. Malaria parasites use a soluble RhopH complex for erythrocyte invasion and an integral form for nutrient uptake. eLife. 2021;10 https://doi.org/10.7554/eLife.65282.
27. Ahmad M, Manzella-Lapeira J, Saggu G, Ito D, Brzostowski JA, Desai SA. Live-cell FRET reveals that malaria Nutrient Channel proteins CLAG3 and RhopH2 remain associated throughout their tortuous trafficking. mBio. 2020;11(5) https://doi.org/10.1128/mBio.01354-20.
28. Maier AG, Rug M, O'Neill MT, Brown M, Chakravorty S, Szestak T, Chesson J, Wu Y, Hughes K, Coppel RL, Newbold C, Beeson JG, Craig A, Crabb BS, Cowman AF. Exported proteins required for virulence and rigidity of plasmodium falciparum-infected human erythrocytes. Cell. 2008;134(1):48–61. https://doi.org/10.1016/j.cell.2008.04.051.
29. Smith JD, Rowe JA, Higgins MK, Lavstsen T. Malaria's deadly grip: cytoadhesion of plasmodium falciparum-infected erythrocytes. Cell Microbiol. 2013;15(12):1976–83. https://doi.org/10.1111/cmi.12183.
30. Boddey JA, Hodder AN, Günther S, Gilson PR, Patsiouras H, Kapp EA, Pearce JA, de Koning-Ward TF, Simpson RJ, Crabb BS, Cowman AF. An aspartyl protease directs malaria effector proteins to the host cell. Nature. 2010;463(7281):627–31. https://doi.org/10.1038/nature08728.
31. Hiller NL, Bhattacharjee S, van Ooij C, Liolios K, Harrison T, Lopez-Estraño C, Haldar K. A host-targeting signal in virulence proteins reveals a secretome in malarial infection. Science (New York, N.Y.). 2004;306(5703):1934–7. https://doi.org/10.1126/science.1102737.
32. Marti, M., Good, R. T., Rug, M., Knuepfer, E., & Cowman, A. F. (2004). Targeting malaria virulence and remodeling proteins to the host erythrocyte. Science (New York, N.Y.), 306(5703), 1930–1933. https://doi.org/10.1126/science.1102452.
33. Marapana DS, Dagley LF, Sandow JJ, Nebl T, Triglia T, Pasternak M, Dickerman BK, Crabb BS, Gilson PR, Webb AI, Boddey JA, Cowman AF. Plasmepsin V cleaves malaria effector proteins in a distinct endoplasmic reticulum translocation interactome for export to the erythrocyte. Nat Microbiol. 2018;3(9):1010–22. https://doi.org/10.1038/s41564-018-0219-2.
34. Boddey JA, Moritz RL, Simpson RJ, Cowman AF. Role of the plasmodium export element in trafficking parasite proteins to the infected erythrocyte. Traffic (Copenhagen, Denmark). 2009;10(3):285–99. https://doi.org/10.1111/j.1600-0854.2008.00864.x.
35. Chang HH, Falick AM, Carlton PM, Sedat JW, DeRisi JL, Marletta MA. N-terminal processing of proteins exported by malaria parasites. Mol Biochem Parasitol. 2008;160(2):107–15. https://doi.org/10.1016/j.molbiopara.2008.04.011.
36. Ho C-M, Beck JR, Lai M, Cui Y, Goldberg DE, Egea PF, Zhou ZH. Malaria parasite translocon structure and mechanism of effector export. Nature. 2018;561(7721):70–5. https://doi.org/10.1038/s41586-018-0469-4.
37. Bullen HE, Charnaud SC, Kalanon M, Riglar DT, Dekiwadia C, Kangwanrangsan N, Torii M, Tsuboi T, Baum J, Ralph SA, Cowman AF, de Koning-Ward TF, Crabb BS, Gilson PR. Biosynthesis, localization, and macromolecular arrangement of the plasmodium falciparum translocon of exported proteins (PTEX). J Biol Chem. 2012;287(11):7871–84. https://doi.org/10.1074/jbc.M111.328591.
38. de Koning-Ward TF, Gilson PR, Boddey JA, Rug M, Smith BJ, Papenfuss AT, Sanders PR, Lundie RJ, Maier AG, Cowman AF, Crabb BS. A newly discovered protein export machine in malaria parasites. Nature. 2009;459(7249):945–9. https://doi.org/10.1038/nature08104.

39. Marti M, Spielmann T. Protein export in malaria parasites: many membranes to cross. Curr Opin Microbiol. 2013;16(4):445–51. https://doi.org/10.1016/j.mib.2013.04.010.
40. Garten M, Nasamu AS, Niles JC, Zimmerberg J, Goldberg DE, Beck JR. EXP2 is a nutrient-permeable channel in the vacuolar membrane of plasmodium and is essential for protein export via PTEX. Nat Microbiol. 2018;3(10):1090–8. https://doi.org/10.1038/s41564-018-0222-7.
41. Mesén-Ramírez P, Bergmann B, Tran TT, Garten M, Stäcker J, Naranjo-Prado I, Höhn K, Zimmerberg J, Spielmann T. EXP1 is critical for nutrient uptake across the parasitophorous vacuole membrane of malaria parasites. PLoS Biol. 2019;17(9):e3000473. https://doi.org/10.1371/journal.pbio.3000473.
42. Nessel T, Beck JM, Rayatpisheh S, Jami-Alahmadi Y, Wohlschlegel JA, Goldberg DE, Beck JR. EXP1 is required for organisation of EXP2 in the intraerythrocytic malaria parasite vacuole. Cell Microbiol. 2020;22(5):e13168. https://doi.org/10.1111/cmi.13168.
43. Grüring C, Heiber A, Kruse F, Flemming S, Franci G, Colombo SF, Fasana E, Schoeler H, Borgese N, Stunnenberg HG, Przyborski JM, Gilberger T-W, Spielmann T. Uncovering common principles in protein export of malaria parasites. Cell Host Microbe. 2012;12(5):717–29. https://doi.org/10.1016/j.chom.2012.09.010.
44. Coffey MJ, Sleebs BE, Uboldi AD, Garnham A, Franco M, Marino ND, Panas MW, Ferguson DJ, Enciso M, O'Neill MT, Lopaticki S, Stewart RJ, Dewson G, Smyth GK, Smith BJ, Masters SL, Boothroyd JC, Boddey JA, Tonkin CJ. An aspartyl protease defines a novel pathway for export of toxoplasma proteins into the host cell. eLife. 2015;4 https://doi.org/10.7554/eLife.10809.
45. Yaeno T, Shirasu K. The RXLR motif of oomycete effectors is not a sufficient element for binding to phosphatidylinositol monophosphates. Plant Signal Behav. 2013;8(4):e23865. https://doi.org/10.4161/psb.23865.
46. Curt-Varesano A, Braun L, Ranquet C, Hakimi M-A, Bougdour A. The aspartyl protease TgASP5 mediates the export of the toxoplasma GRA16 and GRA24 effectors into host cells. Cell Microbiol. 2016;18(2):151–67. https://doi.org/10.1111/cmi.12498.
47. Rosowski EE, Lu D, Julien L, Rodda L, Gaiser RA, Jensen KDC, Saeij JPJ. Strain-specific activation of the NF-kappaB pathway by GRA15, a novel toxoplasma gondii dense granule protein. J Exp Med. 2011;208(1):195–212. https://doi.org/10.1084/jem.20100717.
48. Gold DA, Kaplan AD, Lis A, Bett GCL, Rosowski EE, Cirelli KM, Bougdour A, Sidik SM, Beck JR, Lourido S, Egea PF, Bradley PJ, Hakimi M-A, Rasmusson RL, Saeij JPJ. The toxoplasma dense granule proteins GRA17 and GRA23 mediate the movement of small molecules between the host and the Parasitophorous vacuole. Cell Host Microbe. 2015;17(5):642–52. https://doi.org/10.1016/j.chom.2015.04.003.
49. Pernas L, Adomako-Ankomah Y, Shastri AJ, Ewald SE, Treeck M, Boyle JP, Boothroyd JC. Toxoplasma effector MAF1 mediates recruitment of host mitochondria and impacts the host response. PLoS Biol. 2014;12(4):e1001845. https://doi.org/10.1371/journal.pbio.1001845.
50. Gay G, Braun L, Brenier-Pinchart M-P, Vollaire J, Josserand V, Bertini R-L, Varesano A, Touquet B, De Bock P-J, Coute Y, Tardieux I, Bougdour A, Hakimi M-A. Toxoplasma gondii TgIST co-opts host chromatin repressors dampening STAT1-dependent gene regulation and IFN-γ-mediated host defenses. J Exp Med. 2016;213(9):1779–98. https://doi.org/10.1084/jem.20160340.
51. Olias P, Etheridge RD, Zhang Y, Holtzman MJ, Sibley LD. Toxoplasma effector recruits the Mi-2/NuRD complex to repress STAT1 transcription and block IFN-γ-dependent gene expression. Cell Host Microbe. 2016;20(1):72–82. https://doi.org/10.1016/j.chom.2016.06.006.
52. Braun L, Brenier-Pinchart M-P, Hammoudi P-M, Cannella D, Kieffer-Jaquinod S, Vollaire J, Josserand V, Touquet B, Couté Y, Tardieux I, Bougdour A, Hakimi M-A. The toxoplasma effector TEEGR promotes parasite persistence by modulating NF-κB signalling via EZH2. Nat Microbiol. 2019;4(7):1208–20. https://doi.org/10.1038/s41564-019-0431-8.
53. Panas MW, Naor A, Cygan AM, Boothroyd JC. Toxoplasma controls host cyclin E expression through the use of a novel MYR1-dependent effector protein, HCE1. mBio. 2019;10(2) https://doi.org/10.1128/mBio.00674-19.

54. He H, Brenier-Pinchart M-P, Braun L, Kraut A, Touquet B, Couté Y, Tardieux I, Hakimi M-A, Bougdour A. Characterization of a toxoplasma effector uncovers an alternative GSK3/β-catenin-regulatory pathway of inflammation. eLife. 2018;7 https://doi.org/10.7554/eLife.39887.
55. Heiseke AF, Faul AC, Lehr H-A, Förster I, Schmid RM, Krug AB, Reindl W. CCL17 promotes intestinal inflammation in mice and counteracts regulatory T cell-mediated protection from colitis. Gastroenterology. 2012;142(2):335–45. https://doi.org/10.1053/j.gastro.2011.10.027.
56. Marino ND, Panas MW, Franco M, Theisen TC, Naor A, Rastogi S, Buchholz KR, Lorenzi HA, Boothroyd JC. Identification of a novel protein complex essential for effector translocation across the parasitophorous vacuole membrane of toxoplasma gondii. PLoS Pathog. 2018;14(1):e1006828. https://doi.org/10.1371/journal.ppat.1006828.
57. Franco M, Panas MW, Marino ND, Lee M-CW, Buchholz KR, Kelly FD, Bednarski JJ, Sleckman BP, Pourmand N, Boothroyd JC. A novel secreted protein, MYR1, is central to Toxoplasma's manipulation of host cells. mBio. 2016;7(1):e02231–15. https://doi.org/10.1128/mBio.02231-15.
58. Franco M, Shastri AJ, Boothroyd JC. Infection by toxoplasma gondii specifically induces host c-Myc and the genes this pivotal transcription factor regulates. Eukaryot Cell. 2014;13(4):483–93. https://doi.org/10.1128/EC.00316-13.
59. Cygan AM, Theisen TC, Mendoza AG, Marino ND, Panas MW, Boothroyd JC. Coimmunoprecipitation with MYR1 identifies three additional proteins within the toxoplasma gondii Parasitophorous vacuole required for translocation of dense granule effectors into host cells. mSphere. 2020;5(1) https://doi.org/10.1128/mSphere.00858-19.
60. Mercer HL, Snyder LM, Doherty CM, Fox BA, Bzik DJ, Denkers EY. Toxoplasma gondii dense granule protein GRA24 drives MyD88-independent p38 MAPK activation, IL-12 production and induction of protective immunity. PLoS Pathog. 2020;16(5):e1008572. https://doi.org/10.1371/journal.ppat.1008572.
61. Panas MW, Boothroyd JC. Toxoplasma uses GRA16 to upregulate host c-Myc. mSphere. 2020;5(3) https://doi.org/10.1128/mSphere.00402-20.
62. Bougdour A, Durandau E, Brenier-Pinchart M-P, Ortet P, Barakat M, Kieffer S, Curt-Varesano-A, Curt-Bertini R-L, Bastien O, Coute Y, Pelloux H, Hakimi M-A. Host cell subversion by toxoplasma GRA16, an exported dense granule protein that targets the host cell nucleus and alters gene expression. Cell Host Microbe. 2013;13(4):489–500. https://doi.org/10.1016/j.chom.2013.03.002.
63. Bai M-J, Wang J-L, Elsheikha HM, Liang Q-L, Chen K, Nie L-B, Zhu X-Q. Functional characterization of dense granule proteins in toxoplasma gondii RH strain using CRISPR-Cas9 system. Front Cell Infect Microbiol. 2018;8:300. https://doi.org/10.3389/fcimb.2018.00300.
64. Nasamu AS, Glushakova S, Russo I, Vaupel B, Oksman A, Kim AS, Fremont DH, Tolia N, Beck JR, Meyers MJ, Niles JC, Zimmerberg J, Goldberg DE. Plasmepsins IX and X are essential and druggable mediators of malaria parasite egress and invasion. Science (New York, N.Y.). 2017;358(6362):518–22. https://doi.org/10.1126/science.aan1478.
65. Sharma P, Chitnis CE. Key molecular events during host cell invasion by apicomplexan pathogens. Curr Opin Microbiol. 2013;16(4):432–7. https://doi.org/10.1016/j.mib.2013.07.004.
66. Yeoh S, O'Donnell RA, Koussis K, Dluzewski AR, Ansell KH, Osborne SA, Hackett F, Withers-Martinez C, Mitchell GH, Bannister LH, Bryans JS, Kettleborough CA, Blackman MJ. Subcellular discharge of a serine protease mediates release of invasive malaria parasites from host erythrocytes. Cell. 2007;131(6):1072–83. https://doi.org/10.1016/j.cell.2007.10.049.
67. Pino P, Caldelari R, Mukherjee B, Vahokoski J, Klages N, Maco B, Collins CR, Blackman MJ, Kursula I, Heussler V, Brochet M, Soldati-Favre D. A multistage antimalarial targets the plasmepsins IX and X essential for invasion and egress. Science (New York, N.Y.). 2017;358(6362):522–8. https://doi.org/10.1126/science.aaf8675.

68. Collins CR, Das S, Wong EH, Andenmatten N, Stallmach R, Hackett F, Herman J-P, Müller S, Meissner M, Blackman MJ. Robust inducible Cre recombinase activity in the human malaria parasite plasmodium falciparum enables efficient gene deletion within a single asexual erythrocytic growth cycle. Mol Microbiol. 2013;88(4):687–701. https://doi.org/10.1111/mmi.12206.
69. Bozdech Z, Llinás M, Pulliam BL, Wong ED, Zhu J, DeRisi JL. The transcriptome of the intraerythrocytic developmental cycle of plasmodium falciparum. PLoS Biol. 2003;1(1):E5. https://doi.org/10.1371/journal.pbio.0000005.
70. Dowse TJ, Koussis K, Blackman MJ, Soldati-Favre D. Roles of proteases during invasion and egress by plasmodium and toxoplasma. Subcell Biochem. 2008;47:121–39. https://doi.org/10.1007/978-0-387-78267-6_10.
71. Dogga SK, Mukherjee B, Jacot D, Kockmann T, Molino L, Hammoudi P-M, Hartkoorn RC, Hehl AB, Soldati-Favre D. A druggable secretory protein maturase of toxoplasma essential for invasion and egress. eLife. 2017;6 https://doi.org/10.7554/eLife.27480.
72. Mukherjee B, Tessaro F, Vahokoski J, Kursula I, Marq J-B, Scapozza L, Soldati-Favre D. Modeling and resistant alleles explain the selectivity of antimalarial compound 49c towards apicomplexan aspartyl proteases. EMBO J. 2018;37(7):10.15252/embj.201798047.
73. Sidik SM, Huet D, Ganesan SM, Huynh M-H, Wang T, Nasamu AS, Thiru P, Saeij JPJ, Carruthers VB, Niles JC, Lourido S. A genome-wide CRISPR screen in toxoplasma identifies essential apicomplexan genes. Cell. 2016;166(6):1423–1435.e12. https://doi.org/10.1016/j.cell.2016.08.019.
74. Lentini G, Ben Chaabene R, Vadas O, Ramakrishnan C, Mukherjee B, Mehta V, Lunghi M, Grossmann J, Maco B, Visentin R, Hehl AB, Korkhov VM, Soldati-Favre D. Structural insights into an atypical secretory pathway kinase crucial for toxoplasma gondii invasion. Nat Commun. 2021;12(1):3788. https://doi.org/10.1038/s41467-021-24083-y.
75. Munsamy G, Agoni C, Soliman MES. A dual target of Plasmepsin IX and X: unveiling the atomistic superiority of a core chemical scaffold in malaria therapy. J Cell Biochem. 2018; https://doi.org/10.1002/jcb.28062.
76. Munsamy G, Soliman MES. Unveiling a new era in malaria therapeutics: a tailored molecular approach towards the Design of Plasmepsin IX inhibitors. Protein J. 2019;38(6):616–27. https://doi.org/10.1007/s10930-019-09871-2.
77. Huynh M-H, Roiko MS, Gomes AO, Schinke EN, Schultz AJ, Agrawal S, Oellig CA, Sexton TR, Beauchamp JM, Laliberté J, Sivaraman KK, Hersh LB, McGowan S, Carruthers VB. Toxoplasma gondii Toxolysin 4 contributes to efficient parasite egress from host cells. MSphere. 2021;6(3):e0044421. https://doi.org/10.1128/mSphere.00444-21.
78. Barber J, Sikakana P, Sadler C, Baud D, Valentin J-P, Roberts R. A target safety assessment of the potential toxicological risks of targeting plasmepsin IX/X for the treatment of malaria. Toxicol Res. 2021;10(2):203–13. https://doi.org/10.1093/toxres/tfaa106.

# Leishmaniasis: Tissue Tropism in Relation to the Species Diversity

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**Abstract**

*Leishmania* is a vector-borne, obligatory, and anaerobic protozoan parasite that causes a spectrum of clinical conditions in its hosts. The disease has several outcomes and targets different parts of the host body ranging from infection in the dermal to the visceral organs. The fate of this disease depends highly on the availability of specific drugs and their penetration into the precise location of pathogen residence. Unavailability of specific medicines can and has caused death worldwide. This decision of sustenance vs. remission depends on various factors such as initial encounters of the host immune system with the pathogen during entry and the level of dissemination allowed after that. This in turn highly depends on nutritional availability and safe residence for the parasite inside the host system and, hence, to be precise its tropism. Therefore, to understand disease pathogenesis, it is important to explore pathogen-host interactions in light of their tropism. This chapter discusses several manifestations following *Leishmania* invasion into their mammalian host and the factors responsible for them. We also summarize various exceptions and their possible reasons including both parasitic and host-related factors influencing different disease outcomes.

**Keywords**

Leishmaniasis · Tissue tropism · Parasite diversity · Vector competence · Cytokine

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## 1 Introduction

Leishmaniases are a group of vector-borne diseases caused by *Leishmania*, an obligate anaerobic parasite, belonging to the genus *Leishmania* (Kinetoplastida: Trypanosomatidae). *Leishmania* is transmitted through the bite of infected female *phlebotomine* sand flies during a blood meal from their vertebrate hosts. About 53 *Leishmania* species have been reported (classified into 5 subgenera and complexes: *Leishmania, Viannia, Sauroleishmania, L. enriettii complex, and Paraleishmania*) [1, 2] (Table I). Among these, around 31 species can infect mammals and 20 species are reported as human pathogens. Currently, this disease threatens over 1 billion people globally, and about 1 million new incidences are reported annually, with a death toll of approximately 26,000 to 65,000 every year [3]. Although all species of *Leishmania* share a similar digenic life cycle and are inoculated through the skin, different species show a heterogeneous affinity for their shelter in specific organs of the hosts. Dermotropic (DT) parasites confine themselves in the skin and cause a localized infection with self-healing cutaneous sores as in cutaneous leishmaniasis (CL), while the viscerotropic parasites spread out causing a life-threatening parasitemia in the visceral organs accompanied by severe fever and hepatosplenomegaly known as visceral leishmaniasis (VL). Several factors have been reported over the years that contribute to this differential tropism in leishmaniasis. In this chapter, we first discuss several aspects of tropism and the evolution of such heterogeneity and then the possible mechanisms conferring the differential tropism in *Leishmania*.

## 2 What Is Tropism?

Tropism can be defined as an integral property to orient oneself toward the direction of an external stimulus. From multicellular organisms to unicellular bacteria, pathogenic or nonpathogenic, every organism practices tropism. In pathogens, tropism refers to the site-specific migration and residence of the disease-causing agent (fungal, viral, bacterial, or parasitic) inside host tissue and/or organs, for its survival and proliferation [4]. Studying this host cell interaction for pathogens, specifically, the ones with variable infectivity and different clinical forms, may not only lead to a better understanding of the disease severity but may also aid in the development of novel therapeutics for infectious diseases. Some pathogens can infect many or most of the host organs and are, hence, broadly tropic, whereas some are confined to specific tissues or even to certain tissue niches [5]. Based on this heterogeneity of site preference, tropism can be divided into the following:

**Pleiotropic:** Some pathogens localize in different tissues and organs depending on the disease stage.

**Organ and tissue tropism:** Some pathogens, intracellular and/or extracellular, may prefer to establish in parts or a whole tissue or organ in the host.

**Cellular tropism:** Certain pathogens prefer to colonize intracellularly into specific cell types. Subsequent infection is determined by the host's immune status and capability to resist infection internally.

**Subcellular tropism:** In some cases, pathogens localize into specific subcellular niches which may be only transient stages during pathogen uptake, while some take shelter inside various subcellular compartments throughout their life cycle.

## 3 Tropism in Leishmaniasis

Tropism in leishmaniasis gives rise to diverse clinical forms ranging from self-healing sores to severe disfigurations and even death. Based on their niche preferences and their clinical counterparts, tropism in leishmaniasis can be broadly classified into two main groups: dermotropic and viscerotropic.

### 3.1 Dermotropic

Dermotropic parasites as *L. major*, *L. tropica*, and *L. aethiopica* in the Old World and the *L. mexicana* complex (*L. mexicana*, *L. amazonensis*, *L. pifanoi*, *L. garnhami*, and *L. venezuelensis*) or the subgenus *Viannia* (*L. braziliensis*, *L. guyanensis*, *L. panamensis*, *L. naiffi*, *L. shawi*, *L. lainsoni*, and *L. peruviana*) in the New World (Table I) cause a localized form of infection in their vertebrate hosts called cutaneous leishmaniasis (CL) [1, 6]. The parasites enter during the bite of a sand fly and localize in the skin tissues at the site of the bite. CL is characterized by erythematous papules on the skin in the area around the bite that turn into ulcerated nodules over time and eventually crust over with swollen lesions having distinct borders. More than one lesion can occur, and the infection can spread into the lymphatic system causing lymph node enlargement and sporotrichoid lesions [7, 8]. CL lesions are generally self-healing and restitutes within a month leaving a scar behind, but in some cases they can also become chronic and recurrent calling for serious medical assistance for a cure. In some cases, in hosts with immune-suppressed conditions, specifically, in patients co-infected by HIV, a more diffused form of the CL (DCL) is reported [7–9]. DCL is caused by *L.* (*L.*) *amazonensis*, *L.* (*L.*) *mexicana*, and *L.* (*L.*) *aethiopica*. The parasites disseminate to the subcutaneous tissue and form non-ulcerated papules, with a high parasite burden, involving most of the skin resulting in a leonine-like appearance similar to lepromatous leprosy [7–9]. On the other end of the spectrum, an uncontrolled level of T-cell-mediated immune response can cause a more life-threatening form of this disease called mucocutaneous leishmaniasis (MCL) following infection with *L.* (*Viannia*) *braziliensis* and *L.* (*V.*) *guyanensis*. Parasites spread through the bloodstream or the lymphatic system with the nasopharyngeal mucosa developing severely ulcerated lesions that can spread further to the oropharynx and larynx and affect the cartilage and the vocal cords causing severe facial disfiguration. Treatment is essential or the situation might get fatal [7–9].

### 3.2 Viscerotropic

*L. donovani* and *L. infantum* in the Old World and *L. chagasi* (synonym with *L. infantum*) in the New World (Table I) are causative agents for visceral leishmaniasis (VL). *L. infantum* has a zoonotic transmission pattern (from animal to vector to human), while *L. donovani* has an anthroponotic transmission, i.e., from human to vector to human [8, 10]. Several animals mostly dogs are considered to be the reservoirs for the transmission of VL. This is the most fatal of all leishmaniasis and is fatal without treatment. Parasites visceralize into the internal organs and take residence in the cells of the reticuloendothelial system infecting the macrophages of the bone marrow, spleen and liver causing hepatosplenomegaly and lymph node enlargement [11]. Parasites take over the macrophage immune response and rechannel its metabolic pathways into producing pro-parasitic prolines and polyamines as nutrition [11]. They establish themselves in long-term HSCs in the bone marrow and utilize them as their safe haven evading treatments by chemotherapeutics [12]. Spleen is the active site of infection and acute stages include splenomegaly followed by self-tissue damage.

### 3.3 Exceptions

Although CL and VL are very distinct forms of leishmaniasis, the causative agents and their tropism are not always so strictly bona fide. Species of *L. donovani* complex are generally known to cause VL, and *L. tropica* is commonly considered to be CL causative agents; however, exceptions have been documented many times. Seven soldiers who served during operation desert storm during 1990–1991 returned with VL symptoms but were found to be infected with a viscerotropic variant of *L. tropica* instead of *L. donovani* or *L. infantum* [13]. On the other hand, some strains of *L. donovani* can cause a dermal form of the disease, called post-kala-azar dermal leishmaniasis (PKDL), in recovering VL patients that occurs due to treatment failure [14], stating the loss of visceralization that can be caused due to multiple factors. This could be further analyzed in the case of HIV/*Leishmania* co-infection patients who develop VL irrespective of the parasite infecting them [15–18], emphasizing the contribution of several factors from host immune status alongside the parasite's internal attributes. Additionally, several CL cases were reported from VL endemic regions and were caused by *L. infantum* instead of the traditional CL-causing parasite agents in the Old World [19]. In Morocco, sporadic CL was caused due to a DT variant of *L. infantum* and, though limited to the northern parts of the country, spread to new geographical areas, such as central and even southern regions where *L. major* was endemic [20–22]. This invasion of DT variants of *L. infantum* parasites into the CL endemic regions is raising concerns in terms of medications and diagnosis [21], and hence, the dual tropism of *L. infantum* is of growing interest [10].

## 4 Evolution of *Leishmania*

The dissimilitude in disease outcome resulting from the differential tropism in different strains of *Leishmania* can be attributed to the continuous adaptations forced on the protozoa throughout the course of evolution. Protists arrived as free-living forms billions of years ago [23]. Several free-living protozoa are still widespread in nature. Some of them chose an endosymbiotic lifestyle, while others chose parasitism to avoid entirely the potentially hazardous phase of existence outside the host that included ever-changing climates and lack of nutritional sources [23]. Thus, it could be concluded that the forces that drove diversification of the ancestral forms of these protozoa are the biggest reasons for its tissue tropism as well. According to Sleigh (1991), the eukaryotes evolved as "cells with a lifestyle that apparently involved engulfing another organism for food" [24]. This was probably when some initially free-living protozoa adapted to a parasitic mode of survival. In 1994, Baker mentions "Development of endoparasitism from an initially ectoparasitic stage is a real possibility in the case of Kinetoplastids and, perhaps, some ciliate parasites" [23]. Hence, protozoan parasites arose from such an internalization that started their monogenetic lifestyle.

The appearance of the member of the supergroup Excavata containing two sister phyla Euglenids and Kinetoplastida took place in the Ordovician (nearly 450 million years ago) [1]. Once internalized, some protozoa, as the Kinetoplastids, ended up in the alimentary canals of some lower invertebrates where they could adapt to several life forms and developmental stages to become better suited in the higher host's tissues. It was in the insects, however, that these parasites evolved extensively. Some members of the bodonids are parasitic on the fish *(Trypanoplasma* sp.) and snail (*Cryptobia* sp.). The relic of the connection between free-living euglenoids and parasitic trypanosomatids is represented by monoxenous *Paratrypanosoma confusum* which infects insects [25, 26]. Though the evolutionary kins of *Leishmania* appeared early in the geological timescale, only the heteroxenous life forms appeared after the evolution of hematophagous winged insects in the Cretaceous period (approximately 140 million years ago). Before the insects developed the blood-sucking organs, these parasites were transmitted from insects to insects and ingested with subsequent hosts. After the emergence of the hematophagous insects, these protozoa entered the blood of the vertebrates. The blood was an enriched source of nutrition for the flagellates, and two new groups emerged from this transition: *Leishmania* and *Trypanosoma*. So, the evolution toward a parasitic lifestyle took millions of years to make a shift from the free-living ones. The 100 million-year-old Burmese amber fossil record of extinct sand fly *Palaeomyia burmitis* which evidenced promastigotes and amastigotes of *Paleoleishmania proterus* was probably the first fossil evidence of a digenetic mode [1]. The studies of blood cells from the insect gut confirmed later that the blood was of reptilian origin. A second amber fossil, aged 20–30 million years, was also found which was of the extinct sand fly *Lutzomyia adiketis.* This one presented another fossil genus of the parasite, *Paleoleishmania neotropicum*, and its various life forms in the fossilized insect specimen. However, vertebrate blood cells were missing in this

fossil [1, 27]. So, it was clear that the co-evolution of *Leishmania* and its insect vectors helped to manage the parasite to sustain and complete its life cycle within the vertebrate system first.

In the context of tissue tropism, the spread of *Leishmania* and invasion into the host tissues have been influenced by various major shifts during various evolutionary time points. Three theories were put forward about the origin of the parasite and the disease spreading, i.e., Palaearctic origin hypothesis by Lysenko (1971) which was supported later by Kerr in 2000, the Neotropical origin hypothesis by Lainson and Shaw (1987) which was elaborated by Noyes in 1998 and the supercontinent hypothesis by Momen and Cupollili (2000) [1, 27]. Each of them has its supportive shreds of evidence and some flaws also. The recent supercontinent origin theory of leishmaniasis pointed out that the diversity of *Leishmania* tissue tropism originated from the appearance of different subgenera – *Leishmania* and *Sauroleishmnia* in the Old World and *Viannia* in the New World – after the separation of the Gondwana land in the Mesozoic era [1, 27]. This theory also supports the African origin of *Leishmania* which favors infection of the mammals and is subsequently transmitted via human migration.

The impact of parasite-insect co-evolution in disease tropism can be better understood with the molecular mechanisms that influence the restrictive and permissive nature of the insects and the parasites. It has been recognized for a long time that the activity of digestive enzymes affects *Leishmania* development in sand flies. Several studies showed reduced parasite numbers and even dead or destroyed parasites in the midguts of 'noncompatible' sand fly species in the early phase of infections, that is, the time of the onslaught of the proteolytic activity [28–30]. Based on a pioneer study by Adler (1938), enhanced survival of *L. donovani* was reported in *P. papatasi* following meals devoid of serum and showed that this was correlated with delayed timing and decreased levels of peak protease activities [30, 31]. Moreover, other studies revealed that even in 'compatible' parasite-vector combinations, up to 50% of the initial amastigote parasite inoculum is killed within the first day after blood feeding [32, 33]. The abundance of respective hosts also can determine exposure to the pathogens that could later determine the extent of visceralization in the vertebrate hosts.

## 5 What Determines VL vs. CL?

This differential dissemination and diverse clinical manifestation of different *Leishmania* species cannot be attributed to a single factor but should be viewed as coordination and balance within several intrinsic and extrinsic factors, deciding the fate of infection. Intrinsic factors include the genetic and metabolic differences among the parasites, and the extrinsic factors include the host immune system and contributions made by sand fly salivary components and their gut microbiota. All of these factors individually contribute to deciding the outcome of *Leishmania* infection. However, they coincide with each other during the course of infection, thereby aiding or hindering the process of visceralization [10]. Here we discuss the detailed

roles of these factors: The journey of *Leishmania* parasites from the sand fly gut to the visceral targets can be divided into two major stages – pre-dissemination and post-dissemination.

## 5.1 Pre-dissemination

### Size of the Inoculum

In 2011, Maia et al. reported that the number of parasites, i.e., the size of the inoculum introduced into the skin of the host during a female sand fly blood meal, had a significant role in deciding whether or not the parasites would visceralize [34, 35]. Female *L. longipalpis* one of the major vectors for *L. infantum* in the New World transmitted more DT *L. infantum* strains than VT strains at the site of their bites. Also, the feeding behavior had a critical impact on the rate of transmission as DT-carrying insects needed more time to complete their blood meal as compared to VT-carrying flies which take comparatively longer time than the non-infected flies. Also, the pre-bite load inside the insects carrying DT parasites was higher as compared to the VT-carrying flies. Parasites causing CL such as *L. mexicana* undergo rapid metacyclogenesis inside their vector host unlike their VL counterparts as *L. donovani* and *L. infantum* resulting in a higher parasite load in the gut of the sand fly. Also, rapid divisions inside the vectors lead to a higher expression of the filamentous proteophosphoglycan (fPPG) coating the parasites, thereby blocking the digestive tract and the option for a complete blood meal in one attempt. Hence, insects transmitting DT parasites are compelled to take repetitive blood meals as compared to the VT parasite-infected flies. This causes the regurgitation of more DT parasites into the vertebrate skin than the VT strains [10, 36]. The importance of the initial inoculum and its size was also shown with experimental *L. major* infection in mice with a high and low inoculum size. When injected in a low dose, these parasites could visceralize easier and spread to internal organs, whereas at a higher dose, these parasites formed a localized infection [37]. A contrasting result was found by Rostamian et al., in 2018, where *L. tropica* visceralized at a lower inoculum size but a higher dose caused CL-like symptoms [38]. This shows that the initial inoculum only plays a partial role in tropism; however, other factors ultimately decide the path and its target. Another cause for this variability may be because of the mode of infection, i.e., the use of needles instead of the natural bite which would include salivary components and the gut microbiota of the sand flies.

### Sand Fly Components

Sand flies do not feed directly from the blood vessels of the host but rely on the blood pool that follows the skin injury due to their bite [39]. This process allows them to deposit several salivary and gut contents into the host along with the parasites at the site of the bite. Saliva from DT vectors of *L. infantum* has been reported to show a lower vasodilator effect in vertebrate hosts as compared to the VT vectors of the same. These DT vectors also lack maxadilan, a 61 amino acid vasodilator peptide found in the salivary gland of sand flies [40–42]. Maxadilan acts through the PAC1

receptor in the host cells and delays the hypersensitive DTH reactions in the skin helping in the establishment of infection. Only a pictogram of this vasodilator injected into the mammalian skin can prolong erythema for more than 48 h [43]. One of the biggest examples of the significance of this difference would be the *L. longipalpis*, a vector for the DT *L amazonensis*, whose saliva is rich in maxadilan and was isolated from VL dogs in the VL endemic regions of southeast Brazil. According to Warburg, high salivary vasodilators attract circulatory monocytes that transport parasites to the visceral regions. Thus, lack or low vasodilator effects restrict the infection to the dermal (resident) monocytes causing CL [10, 40].

### Host Immune Response

Circulatory monocytes and neutrophils are recruited to the sites of infection by a local inflammatory response mediated partly via the host keratinocytes, the most abundant cells in the skin which act differentially in presence of DT and VT parasites [44]. Keratinocytes upon exposure to VT strains of *L. infantum* can induce the expression of the pro-inflammatory cytokines that can attract monocytes and neutrophils from the blood [44]. Neutrophils welcome *Leishmania* momentarily inside them and then pass them to macrophages without raising any concern for the cells as a Trojan horse and, hence, provide a safe passage inside the parasite's target cell for proliferation [11]. Pre-exposure of keratinocytes to VT *L. infantum* shows induction of TNF-α and IL-1β in monocytes that can activate the macrophages and can, hence, account for asymptomatism, whereas expression of IL8 and CXCL5 can attract neutrophils. Keratinocytes either act through direct cell-mediated interactions with LPG, GIPLs, and GP63 or through parasite internalization that exposes enough parasitic components internally that consort the tipping of the balance [44]. Exposure to CL-causing *L. major* increased slight expression of IL-4 in keratinocytes, an anti-inflammatory cytokine after 24 h, followed by the minimal effect of the pro-inflammatory response as compared to the VL-causing *L. infantum*, whereas *L. braziliensis* (causing MCL) induced higher pro-inflammation to the DT parasites but lower to the VT strains [44]. It is possible that keratinocytes are more instrumental in establishing a visceral infection, perhaps only aiding in the recruitment of the vehicles by creating a microenvironment required for visceralization but having minimal effect on dermotropism. As with the induction of pro-inflammation in VL to attract monocytes into the site of the bite for visceralization, it is also important to note that it is the same pro-inflammatory response that is responsible for the elimination of the pathogens as well. Hence, it would be wise for the parasites to overcome this pro-inflammatory state; otherwise an active Th1 response would follow and hinder parasite sustenance in the visceral organs.

### Pro-inflammation vs. Anti-inflammation

There are certain contrasting results where IFN-γ neutralization visceralized *L. major* infections whereas high IFN-γ and concomitant lower expression of IL-10 was imperative in DT *L. donovani* strain from Sri Lanka [45, 46]. This was

corroborated by Cardoso et al. (2020) who suggested that polymorphism in LPG of *Leishmania* has a greater role in deciding their tropism. They showed that the LPG of DT *L. infantum* was more pro-inflammatory than the VT strains and, hence, a profound pro-inflammation truly contains the parasites in the dermal regions [47]. This also supports the earlier notion of parasite inoculum during bite where a high parasite number induced a pro-inflammation that prohibited parasite visceralization. Also, LPG from *L. infantum* strains induces the upregulation of prostaglandin 2 or PGE2 which is known to break the lymph node barrier and help in *L. donovani* visceralization [10, 48]. There are reports of neutrophil activation and recruitment of monocytes-derived dendritic cells in the skin to be responsible for the elimination of CL infections. However, several extrinsic factors act to lower these pro-inflammatory responses to a basal non-harmful level and recruit neutrophils to deliver the parasites to the resident macrophages to establish infection instead [7, 9, 49]. It is possible that initially pro-inflammatory cytokines and chemokines are used by the VT parasites as mere transportation and while inside, the activation of these cells is slowed down. In this context, components of gut microbiota have been reported to exert similar effects as were seen with the role of maxadilan, mentioned earlier. Gut microbiota act to recruit circulatory neutrophils and monocytes aiding in the transportation of the *L. donovani* parasites from the site of the bite to the visceral organs [50]. This suggests that the tipping of balance between several parasitic and vector-derived factors shapes the ultimate environment that decides viscerotropism over dermotropism in VT parasites.

### Matrix Metalloproteinase

Irrespective of their tropism, parasites take shelter in the macrophages, and hence, macrophage mobility also plays a crucial role in the visceralization of *Leishmania*. Different *Leishmania* species regulate the secretion of matrix metalloproteinase (MMP) to restrict macrophage mobility producing several forms of the disease. *L. infantum*-mediated activation of phosphoinositide 3-kinase (PI3K) gamma pathway [51] has considerable effects on macrophage mobility. Simultaneously collagen degradation due to an increase in the secretion of MMPs induces macrophage mobility by promoting visceralization. In contrast, overexpression of metalloproteinase inhibitors in the tissue during PKDL caused by DT *L. donovani* impairs extracellular matrix (ECM) degradation, by inhibiting MMP secretion [52]. Similarly, macrophages infected with *L. braziliensis* isolated from ML patients show increased secretion of MMP-9, as compared to the macrophages obtained from the LCL [53].

### Apoptotic Mimicry

In addition to ECM degradation, dermotropism is enhanced by apoptosis mimicry by these parasites. *L. amazonensis* mimics mammalian apoptotic cells with the expression of high phosphatidylserine (PSP) than the VT parasites. This enables them to get internalized by the macrophages [54] that trigger the expression of anti-inflammatory molecules such as TGF-β and IL-10 [55], thereby decreasing the production of IL-17 as is evident in the CL patients. Downregulation of IL-17 in

CL cases can cause a reduction in the recruitment of neutrophils, thereby limiting parasite dissemination [56].

## 5.2 Post-dissemination

The skin forms the first line of physical barrier against pathogenic invasion. After its first interaction with the skin, now that the primary decision of DT vs. VT has been established, the VT parasite must now set out into the visceral organs and overcome the harsh and hostile environment of the viscera. VT *L. donovani* migrates to the bone marrow, spleen, and liver and takes residence in the monocyte-derived cells as macrophages [57]. They manipulate their host cells and eventually suppress their inflammatory response to ensure sustenance [57]. In the bone marrow, parasites take shelter in the long-term hematopoietic cells and induce emergency myelopoiesis of monocyte and macrophages that are already primed to be permissive to the parasites [12]. This allows a continuous source of residence for the *Leishmania*, and the host suffers from a prolonged state of immune suppression with high IL-10 in the system and very low Th1-mediated pro-inflammation. However, to establish an infection, parasites have to first overcome the temperature difference between the skin and the viscera. Viscera have a temperature of 37° C which is higher than the skin microenvironment [58] parasites were first adapted to, and hence, new strategies are now devised for further survival. Several genes and enzymes of *L. donovani* have been shown to function better at higher temperatures than *L. major* parasites. In this context, several genes and enzymes are upregulated in VT parasites than the DT parasites [10].

### A2 Genes and Oxidative Stress

A2 family of genes were found to be expressed in the amastigotes of *L. donovani* and found as pseudogenes in the DT species. Also, the DT *L. donovani* had very few copies of this gene. These genes encode a stress response protein in *Leishmania* that protects against high temperatures and ROS-mediated stress in the host cells by decreasing the levels of intracellular ROS. However, A2 did not confer any protection against ER stress response even though they co-localized in the ER with the ER chaperons like BiP [59–63].

### Nucleotide Sugar Transporter (NST)

Another gene Ld1590 NST specific to *L. donovani* also helped in the survival of the parasites during visceralization. Transfecting this gene into *L. major* aided in better survival inside the visceral organs of BALB/c mice. Although this load was comparatively lower as compared to the classical VT parasites, it does bring out the critical role of this gene during the visceralization of the VT strains. Also, this presents the probability of the involvement of other genes in this process. Ld1590 NST is a nucleotide sugar transporter (NST) encoding gene whose main function is to translocate nucleotide sugars (UDP-sugar, GDP-sugar, and CMP-Sia) from the cytoplasm to the Golgi lumen for glycosylation of the protein and, hence, allow

synthesis of several leishmanial surfaces and soluble glycoproteins as LPG and GP63. This gene might also account for the differential presence of glycoconjugates in several *Leishmania* spp. which also differentially activates the keratinocytes as mentioned earlier. Also, the high distribution of sialic acid on leishmanial glycoconjugates has been known to help parasites resist NO-mediated killing by the host [10, 64, 65]. This proves the importance of this gene in the establishment of infection in the VT species [66].

#### Lipid Composition

Varied lipid composition between leishmanial species can also add stress response and differential tropism. For example, phosphatidylethanolamine plasmalogen (PEP) is found in abundance in *L. infantum* as compared to *L. amazonensis* and helps to create tolerance to host-induced stress [10].

### 5.3 Visceralization in HIV/*Leishmania*

A unique scenario presents itself during HIV/*Leishmania* co-infection. HIV induces a state of immune suppression in the host and infects similar cells as DCs, macrophages, and lymphocytes, as do *Leishmania*. Hence synergistic parasitism is displayed in these patients. HIV is known to promote visceralization of DT *Leishmania* and helps in the progression of VT infection to a more severe state [15, 16]. In 2014 Zijlstra postulated that in this HIV *Leishmania* co-infection state, the boundaries between the parasite's tropism and their respective clinical manifestation become blurred due to high levels of immune suppression. Also, the body is open to other invasions by trypanosomatids or commensal bacteria from self microbiota that might cause severe ramifications in the host body [67].

### 5.4 *Leishmania* Genomics

Considering that species specificity can account for the varying tropism in *Leishmania*, several attempts for studying the genomic differences between distantly related species were made. Comparative genomics of *L. infantum*, *L. major*, and *L. braziliensis* revealed the conservative nature of the *Leishmania* genome worldwide which could be due to their short subtelomeric regions as compared to the other trypanosomatids [68]. In their studies, they report these strains to have 99% synteny with only 200 genes distributed variably along the length of the chromosomes [68, 69]. With such restrictions in species-specific genes, genetic diversity is derived from the formation of pseudogenes and tandem gene amplification and copy number variation (CNV) in the disomic chromosomes [69]. Certain genes specific to *L. infantum* for oxidative response and required for sugar transport and glucose metabolism were identified to be important in viscerotropism.

### RagC

Whole genome analysis of both CL and VL strains of Sri Lankan *L. donovani* correlated to 9 CNV regions and 117 non-synonymous SNPs that had a role in DT *L. donovani* tropism. The homozygous pseudogene LdBPK_311390.1 formed by frameshifts and generation of stop codons resulting from SNPs and indels, along with some non-synonymous SNPs, was found specific for these DT strains. One of the RagC genes in these regions was compared between both the DT and VT strains, and it was found that transfection of the VT counterpart of this gene into the DT *L. donovani* led to infection in the spleen. In contrast, induced mutations in the RagC region of the VT strains led to a loss of visceralization [70–72] in these parasites. RagC is a member of the mTOR signaling pathway and is crucial to the parasite's survival and proliferation [72]. Thus, any variation in this region negatively impacts protein expression, thereby reducing parasite survival.

### Mini-exon Copy Number Variation

Posttranscriptional regulation of a gene mediated by mini-exon transcripts plays an important role in influencing the expression of species-specific genes in different species of *Leishmania*. Hence, it goes without saying that these mini-exons also act on the genes that modulate tropism in these parasites. The mini-exon genes were found in higher copy numbers in *L. donovani* than in *L. major*. Introducing these mini-exons or sequences related to it into *L. major* strains resulted in increased virulence of the *L. major* strains in both CL and VL endemic regions, thereby establishing the significance of the mini-exon CNV in *Leishmania* tropism [73].

### Loss of Visceralization due to Genome Hybridization

Genome alignment of DT *L. donovani* strains with other DT and VT strains revealed several possibilities of hybridization between *L. donovani* with *L. major* and *L. tropica* followed by several recombination events resulted in the formation of new *L. donovani* strains with gradually decreasing viscerotropism [10, 70, 71]. *L. donovani* causing PKDL show higher expression of several genes as PA2 and GP63 with reduced A2 gene expression. As expected, loss of A2 can affect visceralization, and hence, these strains are restricted to causing cutaneous lesions. Apart from gene hybridizations, the inadequacy of chemotherapeutics can also lead to the formation of new strains. VL patients treated with sodium antimony gluconate (SAG) have higher probabilities of developing PKDL than those treated with amphotericin B [74] due to the differential susceptibility of *L. donovani* to antileishmanial drugs [75].

## 6 Differential Tissue Tropism: Bliss or Curse for *Leishmania*

Pathogen localization can highly influence the disease severity and its remission. Both hosts and the parasite can benefit from such tissue heterogeneity. Dispersal of *Leishmania* into the visceral organs can lead to severe fatalities and, hence, reduce

the life span of the host including the parasite longevity. In contrast, dermal residence can provide long-term sustenance to the intracellular amastigotes allowing proliferation at a considerate rate. Also, dermal infections have higher rates of transmission because of frequent exposure to the vector bites. A higher load in the dermal lesion can introduce a higher level of parasite number into the vector for successful transmission into the next mammalian host. In the case of visceralized infections, chemotherapeutics can cause rapid elimination of the pathogens. However, inconspicuous residence in the bone marrow of the host can provide a prolonged escape from the chemotherapeutics while giving a chance for the parasites to develop into resistant strains that can now spread in the endemic regions causing reduced activity of the drugs [12]. Moreover, treatment failures due to inadequate dosage or failed follow-ups can continuously cause changes in the parasites adapting dermotropic characteristics as seen in PKDL patients [10, 70]. Certain drugs have variable effects on CL and VL infections. Antimony resistance has been seen higher in VL strains of *Leishmania* as compared to the CL strains, whereas amphotericin B acts comparably on both infections [76]. Variable host immune responses in terms of the generation of cytokines and chemokines are also other concerns [11, 44, 77]. However, toxicity and emerging resistant strains limit their use in remission. Hence, understanding tropism in *Leishmania* might present new targets for drugs and parasite control.

**Table 1** Global distribution of *Leishmania* species, their potential vectors, and disease forms associated [1]

| Category | Subgenera | Found in (Old/New World) | Leishmania species involved | Vectors involved | Disease caused |
|---|---|---|---|---|---|
| Euleishmania | Subgenera: *Leishmania* | Old World | *L. tropica* (syn. of *L. killicki*)* proven vectors | *P. (La.) aculeatus, P. (Ad.) arabicus*, P. (Par.) chabaudi, P. (La.) guggisbergi*, P. (Syn.) rossi*, P. (Pa.) saevus*, P. (Par.) sergenti** | CL |
| | | | *L. aethiopica* | *P. (Lar.) longipes*, P. (Lar.) pedifer*, P. (Par.) sergenti** | CL, DCL |
| | | | *L. major* | *P. (Syn.) ansarii, P. (P.) bergeroti, P. (Par.) caucasicus*, P. (P.) duboscqi*, P. (P.) papatasi*, P. (P.) salehi** | CL, DCL |
| | | | *L. gerbilli* | *P. (P.) papatasi* | CL |
| | | | *L. turanica* | | |
| | | | *L. arabica* | | |
| | | | *L. donovani* (syn. of *L. archibaldi*) | *P. (Pa.) alexandri*, P. (Eu.) argentipes*, P. (Syn.) celiae*, P. (Ad.) chinensis, P. (Syn.) martini*, P. (La.)* | VL, PKDL |

(continued)

**Table 1** (continued)

| Category | Subgenera | Found in (Old/New World) | Leishmania species involved | Vectors involved | Disease caused |
|---|---|---|---|---|---|
| | | | | *orientalis*, P. (Ad.) longiductus, P. (Sy.) vansomerenae, P. (Ad.) sichuanensis* | |
| | | | *L. infantum* (syn. of *L. chagasi*)<br><br>**also found in New World | *P. (Pa.) alexandri, Lu. (Lu.) almerioi*, P. (Ad.) balcanicus*, P. (Ad.) brevis, L. (Lu.) atunesi, P. (Ad.) halepensis, P. (La.) longicuspis, P. (La.) kandelakii*, P. (La.) langeroni*, P. (Ad.) longiductus*, P. (La.) major*, P. (La.) perfiliewi s.i.*, P. (Ad.) chinensis*, P. (La.) perniciosus*, P. (Ad.) sichuanensis*, P. (La.) tobbi*, P. (La.) smimovi*, Lu. (Lu.) forattenii, Lu. (Lu.) cruzi*, Lu. (Pf.) evansi*, Lu. (Lu.) longipalpis*, Lu. (Lu.) pseudolongipalpis, Lu. (Lu.) migonei, Lu. (V.) ovallesi, Lu. (N.) olmecaolmeca, Lu. (Lu.) sallesi* | VL, CL |
| | | New World | *L. mexicana* | *Lu. (D.) anthophora, Lu. (Hel.) ayacuchenisis*, Lu. (C.) christophei, Lu. (V.) columbiana, Lu. (Lu.) cruciata, Lu. (Lu.) diabolica, Lu. (N.) flaviscutellata, Lu. (Lu.) gomezi, Lu. (Lu.) longipalpis, Lu. (Lu.) migonei, Lu. (N.)<br><br>olmecaolmeca*, Lu. (V.) ovallesi*, Lu. (Psy.) panamensis, Lu. (Ps.) shannoni, Lu. (N.) ylephiletor* | CL, DCL |
| | | | *L. amazonensis*<br><br>(syn of *L. garnhami*) | *Lu. (Lu.) diabolica, Lu. (N.) flaviscutellata*, Lu. (Lu.) longipalpis*, Lu. (Lu.) nuneztovarianglesi*, Lu. (N.) olmeca novica*, Lu. (N.) olmeca reducta*, Lu. (V.) townsendi, Lu. (N.) ylephiletor, Lu. (V.) youngi* | CL, DCL, MCL |

(continued)

**Table 1** (continued)

| Category | Subgenera | Found in (Old/New World) | Leishmania species involved | Vectors involved | Disease caused |
|---|---|---|---|---|---|
| | | | *L. venezuelensis* | *Lu. (Lu.) lichyi, Lu. (N.) olmecabicolor, Lu. (Ps.) panamensis, Lu. (V.) spinicrassa* | CL, DCL, MCL |
| | | | *L. aristidesi* | *Lu. (N.) olmecabicolor, Lu. (N.) trapidoi* | |
| | Subgenera: *Viannia* | New World | *L. braziliensis* | *Lu. (N.) anduzei, Lu. (Psy.) ayrozai, Lu. (Ps.) carrerai*, Lu. (V.) columbiana, Lu. (Ps.) complexa*, Lu. (Lu.) cruciata, Lu. (Lu.) edwardsi, Lu. (Pi.) fischeri*, Lu. (Lu.) gomezi*, Lu. (N.) intermedia, Lu. (Lu.) lichyi, Lu. (Ps.) llanosmartinsi*, Lu. (Lu.) longipalpis, Lu. (Lu.) migonei*, Lu. (N.)neivai*, Lu. (Lu.) nuneztovarianglesi*, Lu. (V.) ovallesi*, Lu. (Psy.) panamensis*, Lu. (Psy.) paraensis, Lu. (V.) pescei, Lu. (Lu.) pessoai, Lu. (V.) pia, Lu. (X.) shawi*, Lu. (V.) spinicrassa*, Lu. (Psy.) squamiventris, Lu. (Hel.) tejadai, Lu. (Lu.) townsendi, Lu. (Lu.) trinidadensis, Lu. (N.) trapidoi, Lu. (N.) umbralitis, Lu. (N.) whitmani*, Lu. (Ps.) wellcomei*, Lu. (N.) ylephiletor*, Lu. (Lu.) youngi, Lu.(Psy.) yucumensis** | CL, MCL |
| | | | *L. peruviana* | | |
| | | | *L. guyanensis* | *Lu. (N.) anduzei*, Lu. (Hel.) ayacuchensis*, Lu. (N.) flaviscutellata, Lu. (V.) longiflocosa, Lu. (Psy.)*<br><br>*llanosmartinsi, Lu. (Lu.) migonei, Lu. (V.) ovallesi, Lu. (N.) shawi*, Lu. (N.) umbratilis*, Lu. (N.) whitmani** | CL, MCL |
| | | | *L. panamensis* | *Lu. (T.) cruciata, Lu. (N.) flaviscutellata, Lu. (Lu.) gomezi*, Lu. (Hel.) hartmanni*, Lu. (Mig.) migonei, Lu. (V.) ovallesi,* | CL, MCL |

(continued)

**Table 1** (continued)

| Category | Subgenera | Found in (Old/New World) | Leishmania species involved | Vectors involved | Disease caused |
|---|---|---|---|---|---|
| | | | | *Lu. (Psy.) panamensis*, Lu. (Hel.) sanguinaria, Lu. (V.) spinicrassa, Lu. (N.) trapidoi*, Lu. (N.) umbratilis, Lu. (N.) ylephiletor, Lu. (N.) yuilli** | |
| | | | *L. shawi* | *Lu. (N.) whitmani** | CL, MCL |
| | | | *L. lainsoni* | *Lu. (V.) nuneztovarianglesi*, Lu. (N.) olmecabicolor, Lu. (T.) ubiquitalis*, Lu. (N.) whitmani* | CL, MCL |
| | | | *L. naiffi* | *Lu. (Psy.) amazonensis, Lu. (Ps.) ayrozai*, Lu. (Lu.) gomezi, Lu. (Psy.) paraensis, Lu. (Ps.) squamiventris*, Lu. (N.) trapidoi* | CL |
| | | | *L. lindenbergi* | *L. (Lu.) atunesi* | CL |
| | | | *L. utingensis* | *Lu. (Vi.) tuberculata* | CL |
| | Subgenera: *Sauroleishmania* | Old World | *L. adleri* | *S. (Si.) clydei, S. (S.) dentata* | |
| | | | *L. helioscopi* | | |
| | | | *L. senegalensis* | *S. (S.) dubia* | |
| | | | *L. agamae* | *P. (Pa.) caucasicus, P. (P.) papatasi, S. (S.) sintoni* | |
| | | | *L. hemidactyli* | | |
| | | | *L. sofieffi* | | |
| | | | *L. ceramodactyli* | *P. (Pa.) caucasicus, P. (P.) papatasi, S. (S.) sintoni* | |
| | | | *L. henrici* | | |
| | | | *L. tarentolae* | *S. (S.) antennata, S. (S.) minuta, P. (P.) papatasi* | |
| | | | *L. chameleonis* | | |
| | | | *L. hoogstraali* | *S. (Si.) clydei* | |
| | | | *L. zmeevi* | *S. (S.) arpaklensis, P. (P.) papatasi* | |
| | | | *L. davidi* | | |
| | | | *L. nicollei* | | |
| | | | *L. zuckermani* | | |
| | | | *L. gulikae* | | |
| | | | *L. phrynocephali* | | |
| | | | *L. gymnodactyli* | | |

(continued)

**Table 1** (continued)

| Category | Subgenera | Found in (Old/New World) | Leishmania species involved | Vectors involved | Disease caused |
|---|---|---|---|---|---|
| | | | | *P. (Pa.) caucasicus, S. (Si.) clydei, S. (S.) dentata, P. (P.) papatasi, S. (S.) sintoni* | |
| | | | *L. platycephala* | | |
| | Subgenera: *Mundinia* (*L. enriettii* complex) | New World | *L. enriettii* | *Lu. (Lu.) gasparviannai, Lu. (Lu.) gomezi, Lu. (Pf.) monticola* | |
| | | Old World | *L. australiensis* | *Midges* | |
| | | | *L. siamensis* | *S. (Ne.) gemmea* | CL, VL |
| | | | *L. martiniquensis* | | CL, VL |
| *Paraleishmania* | | New World | *L. herreri* | *Lu. (Ps.) shannoni, Lu. (N.) trapidoi, Lu. (N.) ylephiletor* | |
| | | | *L. hertigi* | *Lu. (Psy.) chagasi, Lu. (Psy.) claustrei, Lu. (Psy.) davisi, Lu. (Psy.) squamiventris* | |
| | | | *L. deanei* | *Lu. (Vi.) furcata* | |
| | | | *L. equatorensis* | *Lu. (Hel.) hartmanni* | |
| | | | *L. columbiensis* | *Lu. (Lu.) gomezi, Lu. (Hel.) hartmanni*, Lu. (Psy.) panamensis* | CL, VL |
| | | | *Endotrypanum* | | |
| | | | *E. schaudinni* | | |
| | | | *E. monterogeii* | | |

## References


1. Akhoundi M, Kuhls K, Cannet A, Votýpka J, Marty P, Delaunay P, Sereno D. A historical overview of the classification, evolution, and dispersion of Leishmania parasites and sandflies. PLoS Negl Trop Dis. 2016;10(3):e0004349.
2. Alvar J, Vélez ID, Bern C, Herrero M, Desjeux P, Cano J, Jannin J, Boer MD, WHO Leishmaniasis Control Team. Leishmaniasis worldwide and global estimates of its incidence. PLoS One. 2012;7(5):e35671.
3. WHO, 2021. Leishmaniasis [WWW document]. WHO. URL. https://www.who.int/health-topics/Leishmaniasis.
4. McCall LI, Siqueira-Neto JL, McKerrow JH. Location, location, location: five facts about tissue tropism and pathogenesis. PLoS Pathog. 2016;12(5):e1005519.
5. McCall LI. Quo vadis? Central rules of pathogen and disease tropism. Front Cell Infect Microbiol. 2021;25(11):640987.
6. MacMorris-Adix M. Leishmaniasis: a review of the disease and the debate over the origin and dispersal of the causaitive parasite Leishmania. Macalester Reviews in Biogeography. 2008;1(1):2.
7. David CV, Craft N. Cutaneous and mucocutaneous leishmaniasis. Dermatol Ther. 2009;22(6):491–502.
8. Abadías-Granado I, Diago A, Cerro PA, Palma-Ruiz AM, Gilaberte Y. Cutaneous and mucocutaneous leishmaniasis. Actas Dermo-Sifiliográficas (English Edition). 2021;112(7):601–18.

9. Reithinger R, Dujardin JC, Louzir H, Pirmez C, Alexander B, Brooker S. Cutaneous leishmaniasis. Lancet Infect Dis. 2007;7(9):581–96.
10. Maatallah IA, Akarid K, Lemrani M. Tissue tropism: is it an intrinsic characteristic of Leishmania species? Acta Trop. 2022;12:106512.
11. Awasthi A, Mathur RK, Saha B. Immune response to Leishmania infection. Indian J Med Res. 2004;1(119):238–58.
12. Dirkx L, Hendrickx S, Merlot M, Bulté D, Starick M, Elst J, Bafica A, Ebo DG, Maes L, Van Weyenbergh J, Caljon G. Long-term hematopoietic stem cells as a parasite niche during treatment failure in visceral leishmaniasis. Commun Biol. 2022;5(1):1–5.
13. Centers for Disease Control (CDC. Viscerotropic leishmaniasis in persons returning from Operation Desert Storm--1990-1991. MMWR Morb Mortal Wkly Rep. 1992;41(8):131–4.
14. Mcgwire BS, Satoskar AR. Leishmaniasis: clinical syndromes and treatment. QJM An Int J Med. 2014;107(1):7–14.
15. Alvar J, Aparicio P, Aseffa A, Den Boer M, Canavate C, Dedet JP, Gradoni L, Ter Horst R, López-Vélez R, Moreno J. The relationship between leishmaniasis and AIDS: the second 10 years. Clin Microbiol Rev. 2008 Apr;21(2):334–59.
16. Gramiccia M. The identification and variability of the parasites causing leishmaniasis in HIV-positive patients in Italy. Ann Trop Med Parasitol. 2003;97(sup1):65–73.
17. Jafari S, Hajiabdolbaghi M, Mohebali M, Hajjaran H, Hashemian H. Disseminated leishmaniasis caused by Leishmania tropica in HIV-positive patients in the Islamic Republic of Iran. EMHJ-Eastern Mediterranean Health J. 2010;16(3):340–3.
18. Liautaud B, Vignier N, Miossec C, Plumelle Y, Kone M, Delta D, Ravel C, Cabié A, Desbois N. First case of visceral leishmaniasis caused by Leishmania martiniquensis. Am J Trop Med Hygiene. 2015;92(2):317.
19. Rioux JA, Lanotte G, Maazoun R, Perello R, Pratlong F. Leishmania infantum Nicolle, 1908, the agent of the autochthonous oriental sore. Apropos of the biochemical identification of 2 strains isolated in the eastern Pyrenees. Comptes Rendus des Seances de L'academie des sciences. Serie D, Sciences Naturelles. 1980;291(8):701–3.
20. Kbaich MA, Mhaidi I, Ezzahidi A, Dersi N, El Hamouchi A, Riyad M, Akarid K, Lemrani M. New epidemiological pattern of cutaneous leishmaniasis in two pre-Saharan arid provinces, southern Morocco. Acta Trop. 2017;173:11–6.
21. Asmae H, Fatima A, Hajiba F, Mbarek K, Khadija B, Mohamed R, Faiza S. Coexistence of Leishmania tropica and Leishmania infantum in Sefrou province, Morocco. Acta tropica. 2014;130:94–9.
22. Hakkour M, Hmamouch A, El Alem MM, Rhalem A, Amarir F, Touzani M, Sadak A, Fellah H, Sebti F. New epidemiological aspects of visceral and cutaneous leishmaniasis in Taza Morocco. Parasit Vector. 2016;9(1):1–9.
23. Baker JR. The origins of parasitism in the protists. Int J Parasitol. 1994;24(8):1131–7.
24. Sleigh MA. The nature of protozoa. In: Kreier JP, Baker JR, editors. Parasitic Protozoa. San Diego: Academic Press; 1991. p. l–53.
25. Flegontov P, Votýpka J, Skalický T, Logacheva MD, Penin AA, Tanifuji G, Onodera NT, Kondrashov AS, Volf P, Archibald JM, Lukeš J. Paratrypanosoma is a novel early-branching trypanosomatid. Curr Biol. 2013;23(18):1787–93.
26. Stevens JR. Free-living bodonids and derived parasitic trypanosomatids: but what lies in between? Trends Parasitol. 2014;30(3):113–4.
27. Steverding D. The history of leishmaniasis. Parasit Vectors. 2017;10(1):1–0.
28. Shatova SM, Shul'ga MA, Saf'ianova VM, Avakian AA. Comparative electron microscopy study of Leishmania major and L. tropica in experimental infestation of the sandfly Phlebotomus papatasi. Parazitologiia. 1984;18(2):154–9.
29. Lawyer PG, Ngumbi PM, Anjili CO, Odongo SO, Mebrahtu YB, Githure JI, Koech DK, Roberts CR. Development of Leishmania major in Phlebotomus duboscqi and Sergentomyia schwetzi (Diptera: Psychodidae). Am J Trop Med Hygiene. 1990;43(1):31–43.

30. Schlein Y, Jacobson RL. Resistance of Phlebotomus papatasi to infection with Leishmania donovani is modulated by components of the infective bloodmeal. Parasitology. 1998;117(5): 467–73.
31. Adler S. Factors determining the behaviour of Leishmania sp. in sandflies. Harefuah. 1938;14: 1–2.
32. Rogers ME, Chance ML, Bates PA. The role of promastigote secretory gel in the origin and transmission of the infective stage of Leishmania mexicana by the sandfly Lutzomyia longipalpis. Parasitology. 2002;124(5):495–507.
33. Pimenta PF, Modi GB, Pereira ST, Shahabuddin M, Sacks DL. A novel role for the peritrophic matrix in protecting Leishmania from the hydrolytic activities of the sand fly midgut. Parasitology. 1997;115(4):359–69.
34. Maia C, Seblova V, Sadlova J, Votypka J, Volf P. Experimental transmission of Leishmania infantum by two major vectors: a comparison between a viscerotropic and a dermotropic strain. PLoS Negl Trop Dis. 2011;5(6):e1181.
35. Ribeiro-Romão RP, Moreira OC, Osorio EY, Cysne-Finkelstein L, Gomes-Silva A, Valverde JG, Pirmez C, Da-Cruz AM, Pinto EF. Comparative evaluation of lesion development, tissue damage, and cytokine expression in golden hamsters (Mesocricetus auratus) infected by inocula with different Leishmania (Viannia) braziliensis concentrations. Infect Immun. 2014;82(12): 5203–13.
36. Rogers ME, Bates PA. Leishmania manipulation of sand fly feeding behavior results in enhanced transmission. PLoS Pathog. 2007;3(6):e91.
37. Kimblin N, Peters N, Debrabant A, Secundino N, Egen J, Lawyer P, Fay MP, Kamhawi S, Sacks D. Quantification of the infectious dose of Leishmania major transmitted to the skin by single sand flies. Proc Natl Acad Sci. 2008;105(29):10125–30.
38. Rostamian M, Jafari D, Abolghazi M, Farahani H, Niknam HM. Leishmania tropica: suggestive evidences for the effect of infectious dose on pathogenicity and immunogenicity in an experimental model. Parasitol Res. 2018;117(9):2949–56.
39. Cecílio P, Cordeiro-da-Silva A, Oliveira F. Sand flies: basic information on the vectors of leishmaniasis and their interactions with Leishmania parasites. Commun Biol. 2022;5(1):1–2.
40. Warburg A, Saraiva E, Lanzaro GC, Titus RG, Neva F. Saliva of Lutzomyia longipalpis sibling species differs in its composition and capacity to enhance leishmaniasis. Philos Trans Royal Soc London Series B: Biol Sci. 1994;345(1312):223–30.
41. Valdivia HO, Almeida LV, Roatt BM, Reis-Cunha JL, Pereira AA, Gontijo C, Fujiwara RT, Reis AB, Sanders MJ, Cotton JA, Bartholomeu DC. Comparative genomics of canine-isolated Leishmania (Leishmania) amazonensis from an endemic focus of visceral leishmaniasis in Governador Valadares, southeastern Brazil. Sci Rep. 2017;7(1):1–1.
42. Cardoso MS, Bento GA, de Almeida LV, de Castro JC, Reis-Cunha JL, Barbosa VD, de Souza CF, Brazil RP, Valdivia HO, Bartholomeu DC. Detection of multiple circulating Leishmania species in Lutzomyia longipalpis in the city of Governador Valadares, southeastern Brazil. PLoS One. 2019;14(2):e0211831.
43. Lerner EA, Iuga AO, Reddy VB. Maxadilan, a PAC1 receptor agonist from sand flies. Peptides. 2007;28(9):1651–4.
44. Scorza BM, Wacker MA, Messingham K, Kim P, Klingelhutz A, Fairley J, Wilson ME. Differential activation of human keratinocytes by Leishmania species causing localized or disseminated disease. J Investig Dermatol. 2017;137(10):2149–56.
45. Diefenbach A, Schindler H, Donhauser N, Lorenz E, Laskay T, MacMicking J, Röllinghoff M, Gresser I, Bogdan C. Type 1 interferon (IFNα/β) and type 2 nitric oxide synthase regulate the innate immune response to a protozoan parasite. Immunity. 1998;8(1):77–87.
46. Kariyawasam KK, Selvapandiyan A, Siriwardana HV, Dube A, Karunanayake P, Senanayake SA, Dey R, Gannavaram S, Nakhasi HL, Karunaweera ND. Dermotropic Leishmania donovani in Sri Lanka: visceralizing potential in clinical and preclinical studies. Parasitology. 2018;145 (4):443–52.

47. Cardoso CA, Araujo GV, Sandoval CM, Nogueira PM, Zúniga C, Sosa-Ochoa WH, Laurenti MD, Soares RP. Lipophosphoglycans from dermotropic Leishmania infantum are more pro-inflammatory than those from viscerotropic strains. Mem Inst Oswaldo Cruz. 2020;21:115.
48. Gregory DJ, Sladek R, Olivier M, Matlashewski G. Comparison of the effects of Leishmania major or Leishmania donovani infection on macrophage gene expression. Infect Immun. 2008;76(3):1186–92.
49. Scott P, Novais FO. Cutaneous leishmaniasis: immune responses in protection and pathogenesis. Nat Rev Immunol. 2016;16(9):581–92.
50. Karmakar S, Nath S, Sarkar B, Chakraborty S, Paul S, Karan M, Pal C. Insect vectors' saliva and gut microbiota as a blessing in disguise: probability versus possibility. Future Microbiol. 2021;16(9):657–70.
51. Rocha MI, Dias F, Resende M, Sousa M, Duarte M, Tomas AM, Castro H. Leishmania infantum enhances migration of macrophages via a phosphoinositide 3-kinase γ-dependent pathway. ACS Infect Dis. 2020;6(7):1643–9.
52. Ansari NA, Katara GK, Ramesh V, Salotra P. Evidence for involvement of TNFR1 and TIMPs in pathogenesis of post-kala-azar dermal leishmaniasis. Clin Exp Immunol. 2008;154(3):391–8.
53. Maretti-Mira AC, de Pinho Rodrigues KM, de Oliveira-Neto MP, Pirmez C, Craft N. MMP-9 activity is induced by Leishmania braziliensis infection and correlates with mucosal leishmaniasis. Acta Trop. 2011;119(2–3):160–4.
54. Negrao F, Abanades DR, Jaeeger CF, Rocha DF, Belaz KR, Giorgio S, Eberlin MN, Angolini CF. Lipidomic alterations of in vitro macrophage infection by L. infantum and L. amazonensis. Mol BioSyst. 2017;13(11):2401–6.
55. de Freitas Balanco JM, Moreira ME, Bonomo A, Bozza PT, Amarante-Mendes G, Pirmez C, Barcinski MA. Apoptotic mimicry by an obligate intracellular parasite downregulates macrophage microbicidal activity. Curr Biol. 2001;11(23):1870–3.
56. Oliveira WN, Ribeiro LE, Schrieffer A, Machado P, Carvalho EM, Bacellar O. The role of inflammatory and anti-inflammatory cytokines in the pathogenesis of human tegumentary leishmaniasis. Cytokine. 2014;66(2):127–32.
57. Cotterell SE, Engwerda CR, Kaye PM. Leishmania donovani infection of bone marrow stromal macrophages selectively enhances myelopoiesis, by a mechanism involving GM-CSF and TNF-α. Blood J Am Soc Hematol. 2000;95(5):1642–51.
58. Quiñonez-Díaz L, Mancilla-Ramírez J, Avila-García M, Ortiz-Avalos J, Berron A, González S, Paredes Y, Galindo-Sevilla N. Effect of ambient temperature on the clinical manifestations of experimental diffuse cutaneous leishmaniasis in a rodent model. Vector-Borne Zoonotic Dis. 2012;12(10):851–60.
59. Lypaczewski P, Hoshizaki J, Zhang WW, McCall LI, Torcivia-Rodriguez J, Simonyan V, Kaur A, Dewar K, Matlashewski G. A complete Leishmania donovani reference genome identifies novel genetic variations associated with virulence. Sci Rep. 2018;8(1):1–4.
60. Zhang WW, Matlashewski G. Characterization of the A2–A2rel gene cluster in Leishmania donovani: involvement of A2 in visceralization during infection. Mol Microbiol. 2001;39(4):935–48.
61. Zhang WW, Mendez S, Ghosh A, Myler P, Ivens A, Clos J, Sacks DL, Matlashewski G. Comparison of the A2 gene locus in Leishmania donovani and Leishmania major and its control over cutaneous infection. J Biol Chem. 2003;278(37):35508–15.
62. Zhang WW, Matlashewski G. Loss of virulence in Leishmania donovani deficient in an amastigote-specific protein, A2. Proc Natl Acad Sci. 1997;94(16):8807–11.
63. Sharma P, Gurumurthy S, Duncan R, Nakhasi HL, Salotra P. Comparative in vivo expression of amastigote up regulated Leishmania genes in three different forms of Leishmaniasis. Parasitol Int. 2010;59(2):262–4.
64. Zhang WW, Matlashewski G. Screening Leishmania donovani-specific genes required for visceral infection. Mol Microbiol. 2010;77(2):505–17.

65. Zhang WW, Chan KF, Song Z, Matlashewski G. Expression of a Leishmania donovani nucleotide sugar transporter in Leishmania major enhances survival in visceral organs. Exp Parasitol. 2011;129(4):337–45.
66. Ghoshal A, Gerwig GJ, Kamerling JP, Mandal C. Sialic acids in different Leishmania sp., its correlation with nitric oxide resistance and host responses. Glycobiology. 2010;20(5):553–66.
67. Zijlstra EE. PKDL and other dermal lesions in HIV co-infected patients with leishmaniasis: review of clinical presentation in relation to immune responses. PLoS Negl Trop Dis. 2014;8 (11):e3258.
68. Peacock CS, Seeger K, Harris D, Murphy L, Ruiz JC, Quail MA, Peters N, Adlem E, Tivey A, Aslett M, Kerhornou A. Comparative genomic analysis of three Leishmania species that cause diverse human disease. Nat Genet. 2007;39(7):839–47.
69. Rogers MB, Hilley JD, Dickens NJ, Wilkes J, Bates PA, Depledge DP, Harris D, Her Y, Herzyk P, Imamura H, Otto TD. Chromosome and gene copy number variation allow major structural change between species and strains of Leishmania. Genome Res. 2011;21(12): 2129–42.
70. Lypaczewski P, Matlashewski G. Leishmania donovani hybridisation and introgression in nature: a comparative genomic investigation. Lancet Microbe. 2021;2(6):e250–8.
71. Lypaczewski P, Zhang WW, Matlashewski G. Evidence that a naturally occurring single nucleotide polymorphism in the RagC gene of Leishmania donovani contributes to reduced virulence. PLoS Negl Trop Dis. 2021;15(2):e0009079.
72. Zhang WW, Ramasamy G, McCall LI, Haydock A, Ranasinghe S, Abeygunasekara P, Sirimanna G, Wickremasinghe R, Myler P, Matlashewski G. Genetic analysis of Leishmania donovani tropism using a naturally attenuated cutaneous strain. PLoS Pathog. 2014;10(7): e1004244.
73. Zhang WW, Peacock CS, Matlashewski G. A genomic-based approach combining in vivo selection in mice to identify a novel virulence gene in Leishmania. PLoS Negl Trop Dis. 2008;2 (6):e248.
74. Mukhopadhyay D, Dalton JE, Kaye PM, Chatterjee M. Post kala-azar dermal leishmaniasis: an unresolved mystery. Trends Parasitol. 2014;30(2):65–74.
75. Baek KH, Piel L, Rosazza T, Prina E, Späth GF, No JH. Infectivity and drug susceptibility profiling of different Leishmania-host cell combinations. Pathogens. 2020;9(5):393.
76. Wijnant GJ, Dumetz F, Dirkx L, Bulte D, Cuypers B, Van Bocxlaer K, Hendrickx S. Tackling drug resistance and other causes of treatment failure in Leishmaniasis. Front Trop Dis. 2022;3: 837460. https://doi.org/10.3389/fitd.2022.837460.
77. Ghosh S, Roy K, Rajalingam R, Martin S, Pal C. Cytokines in the generation and function of regulatory T cell subsets in leishmaniasis. Cytokine. 2021;147:155266.

# Transcriptional Control in *Entamoeba*: Something Old, Something New

Shreyasee Hazra and Dipak Manna

**Abstract**

*Entamoeba histolytica* is an extracellular protozoan parasite and is a global health problem that kills approximately 100,000 people annually. The life cycle of *Entamoeba* consists of two stages – infective trophozoite form and dormant cyst form. *Entamoeba* infection begins with the entry of cysts into our body with contaminated hands touching to our mouth or intake of contaminated water and food. Trophozoites emerged from the resting cysts in the intestine and relocate to the colon where they multiply by binary fission and can cause invasive or noninvasive disease. The key aspects of their host cell-killing activities are engulfing small/dead host cells by the process called "phagocytosis," nibbling bigger/live host cells by the process called "trogocytosis," or inducing apoptotic death of the host cell. It is evident that the pathogenesis, virulence, and development of *Entamoeba* are controlled by the fine tuning of the process called transcription; however, not much is known about the transcriptional regulation and gene expression in this parasite. Transcription regulatory networks play a key role in global gene expression which control a vast range of biological processes and mostly are well characterized in model organisms like yeast, *Drosophila*, and mammals; however, these processes are not well understood in a non-model organism like *Entamoeba histolytica.* In *Entamoeba* only a few transcription factors (TFs) and DNA motifs have been characterized so far. In this chapter we give an overview of transcriptional regulation features in *Entamoeba*, summarizing all transcription factors identified up to date and their significant roles in *Entamoeba* biology.

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Entamoeba · Transcription factor · Development · Stress response · Virulence · Encystation

## 1 Introduction

Many unusual features in the transcriptional regulation were demonstrated in this early branching protozoan parasite *Entamoeba* including the following: (1) an atypical RNA polymerase present in *Entamoeba* that is resistant to alpha-amanitin [1]; (2) during mitosis chromatin doesn't condense; (3) histone H3 and H4 comprise a variable N-terminal tail, and the TATA box that is present upstream of the (Inr) initiator region is unusual [2–5]; (4) very short untranslated regions (UTRs) [3, 6–8]; (5) a GAAC element (AATGAACT) or GAAC-like element comprises different locations in the main promoter [6, 8–10]; and (6) an Inr element (AAAAATTCA) present adjacent the transcription start position [6, 10]. Furthermore, the putative TATA-binding protein in *E. histolytica* (EhTBP) shows notable sequence divergence from the TATA-binding protein of other higher eukaryotes [11]. Conjointly, it comes out that the transcriptional regulation in *Entamoeba* is controlled by unusual mechanisms. The core promoters of *Entamoeba* consist of three elements: (a) putative TATA element (GTATTTAAA) at approximately 30 nt upstream of the transcription start site, (b) GAAC element (AATGAACT) with different locations in the core promoter, and (c) an Inr element (AAAAATTCA) adjacent the transcription start site [6, 10, 12]. A GAAC-like motif (EiCPM-GL) (GAACTACAAA) that shows high similarity with GAAC element has also been identified in *E. invadens* which create similar diarrheal disease in reptiles.

The unavailability of genetic manipulations in *Entamoeba* was the main hurdle to identify and characterize the transcription factors in this parasite for a long time. Once Nickel and Tannich developed the transfection protocols to introduce plasmid DNA into *Entamoeba*; it gives a new dimension to study and characterize the transcription factors [13–15]. The development of several *Entamoeba* vectors with reporter genes was helpful for the characterization of several *Entamoeba* cis-regulatory elements and core promoter. Additionally, a putative TATA box was identified in this parasite which is 30 nt upstream from the transcription start site along with an Inr element (adjacent to the transcription initiation site) and a GAAC element [6]. In protozoan system, this GAAC element is unique and is capable of controlling the transcription initiation independent of either the TATA box or the Inr element [16]. Further, in silico analysis of gene promoter along with biochemical approaches identified few more TFs (e.g., EhCudA, HRM-BP, ERM-BP) in this parasite characterized through deletion and replacement analysis.

## 1.1 *Entamoeba* Genome Sequencing and Transcriptomic Data Improves the Field

*E. histolytica* genome sequencing was an important advancement in understanding the *Entamoeba* biology, and further refinement of *Entamoeba* genomic features was achieved by reassembly of the genome [17, 18]. The whole genome size of *Entamoeba* is predicted to be ~20 Mbp comprising the following characteristics relevant to gene structure and transcription: (1) ~ 8200 gene codes for protein along with a median gene length of 1260 bp, (2) small number of intron (~ 24% genes carry introns), (3) a unique RNA polymerase II having several especial features comprising a highly variant α-amanitin-binding region, which explains why this organism shows resistance to this drug, (4) TATA-binding proteins encoded by three genes in *Entamoeba* [19, 20], (5) Myb domain containing proteins in *Entamoeba* which are greatly expanded [21], (6) histone acetyl transferases and histone deacetylases (these two histone-modifying proteins are identified in this parasite) [22], (7) demethylase domains containing protein not identified in this parasite, and (8) presence of one DNA methyltransferase (cytosine-5) protein in *Entamoeba* [23].

*Entamoeba* genome sequencing and genome annotation provide an inauguration platform for many studies in this parasite. For an example 32 Myb domain-containing proteins were identified by comparative in silico analysis and are further classified into three families [21]. Family I consists of two Myb domains and structurally resembles the plant Myb domain proteins. Moreover, the individual domains of *Entamoeba* Myb share closest homology with human c-Myb. On the other hand, families II and III both comprise a single Myb domain.

Despite the effort from many groups, there are no methods developed yet to study the encystation in *E. histolytica*, and *E. invadens* which is a closely related parasite in reptiles has been developed as a model for the study of stage conversion in this parasite.

The genome sequence of both *E. histolytica* and *E. invadens* is extremely repetitive, and it appears that only 50% of the genome size is accounted for genic and intergenic sequence. The genome of *E. invadens* accounted for 11,549 predicted genes compared to 8306 in *E. histolytica.* The genome analysis in *Entamoeba* showed that the length of the genes of *E. histolytica* and *E. invadens* is very similar; however, the intergenic regions in *E. histolytica* tend to be shorter compared to *E. invadens*.

In *E. invadens* out of 11,549 predicted genes, 9865 showed a BLASTP (E-value $<10^{-5}$) hit to 7216 genes (out of 8306 predicted genes in *E. histolytica*), and among those 5227 are putative orthologs. Alignment of orthologs showed an average amino acid identity around 69%, indicating that these species are distantly related. Of the *E. invadens* genes which do not have orthologs in *E. histolytica*, 77% (4815/6218) have at least considerate RNA-seq support, compared to 98% (5206/5331) of genes that shared with *E. histolytica* [24]. This result indicates that a fraction of these genes may be false positive predictions; additionally this is also consistent that many of these genes are not constitutively expressed.

To point out the conserveness between the genes in these two *Entamoeba* species, all collinear gene pairs that were adjacent in both *E. histolytica* and *E. invadens* were analyzed. This analysis showed only 561 genes that preserved their neighboring gene in both species (out of 5227 total genes). Hence it is quite clear that there has been extensive rearrangement in the genome between these two species and most of the biological processes are also conserved.

### 1.2 Gene Expression Profiling and Transcriptional Regulation

Expression profiling with advanced technologies like microarray and RNA-seq has revolutionized the research field in understanding transcriptional regulation on a genome-wide scale [24]. These approaches have been significantly used in studying *Entamoeba* gene expression in different conditions as well as throughout the different stages of development [24]. Different types of microarray platforms in *Entamoeba* have been developed such as that generated from genomic DNA, short oligonucleotides and long oligonucleotides. These tools help in identifying transcript abundance in different developmental time points as well as during stress and host invasion. Moreover, many factors that are responsible for virulence and pathogenicity have been identified by comparative gene expression studies, genes that are upregulated in virulent strain but downregulated in non-virulent *Entamoeba*. All together, these findings have provided insights on molecular aspects of important amebic biology, e.g., stage conversion and pathogenic potential, and allow researcher with the first intuition to identify prospective novel drug targets against amebic disease.

## 2 Transcription Factors in Cellular Function

The fundamental step in gene expression is "transcription" process, where an mRNA is synthesized from a DNA template, followed by the second step "translation" that strings the amino acid together to make protein. Developmental studies have shown the upregulation and downregulation of sets of transcript level during the different stages of development as well as at different growth conditions. The transcription process is controlled by the orchestrated function of several proteins, e.g., a protein can bind DNA, and this DNA-binding proteins may involve in regulation in gene expression. Most of our knowledge on the basic elements of transcription regulation is achieved from early work on prokaryotic systems, where genes are arranged in sets of contiguous genes that comprise regulatory sequences and structural genes. A classic example is the lactose (*lac*) operon of *E. coli*. The transcription in eukaryotes is much more complex than in prokaryotes. First, the prokaryotes utilize only one RNA polymerase; however, in eukaryotes there are three different RNA polymerases: I, II, and III. Second, the eukaryotic RNA polymerases require additional proteins called general transcription factors (TFs) to position them at the correct start site. However, during transcription in prokaryotes, RNA polymerases

also require accessory polypeptides called sigma factors (σ), which are considered as a subunit of the RNA polymerase. On the other hand, a large, multi-subunit transcription initiation complex is formed in eukaryotic transcription initiation. For example, RNA polymerase II requires a multi-subunit complex of seven general transcription factors to constitute the initiation complex, and each of the subunits must be added in an orchestrated way.

Transcription factors normally have three structural features: a domain that binds to DNA, a transcription-activating domain, and a domain that binds to a ligand. The DNA-binding domain binds to a specific DNA sequence through the formation of hydrogen, ionic, and hydrophobic bonds, although the particular combination and spatial distribution of such interactions are distinctive for each sequence. In silico analysis of many DNA-binding proteins guided the identification of a number of highly conserved DNA-binding structural motifs; these are (1) HTH (helix-turn-helix) motif, (2) ZnF (zinc finger) motif, (3) HLH (helix-loop-helix) motif, (4) leucine zipper motif, and (5) basic zipper motif.

Cellular responses consist of a cascade of events both in prokaryotes and in eukaryotes which involves many intracellular signaling pathways (e.g., PKA, MAPks, JAKs, PKCs) that control the fine tuning of gene regulation by many transcription factors. Transcription factors in bacteria are generally classified by comparison of amino acid sequence with prototypic members of families of DNA-binding proteins, such as LysR-like and AraC-like protein families. TFs are often classified based on the structural motifs that constitute their binding domains, for example, TBP (TATA-binding protein), TBP-associated factors (TAF), and recently identified p300/CBP coactivator family. There are several families of TFs that exist, and each of which shows structural and functional features. The examples of such families are helix-turn-helix (e.g., Oct1), helix-loop-helix (e.g., E2A), zinc finger (e.g., GATA proteins, TFIIIA), leucine zipper motif (cAMP, CREB, AP-1), and beta-sheet motif (e.g., nuclear factor-kB) [25].

In eukaryotes, there is a class of transcription factors called GTFs (general transcription factors) involved in basal transcription regulation which includes TFIIA, TFIIB, TFIID, TFIIE, and THIIH. Jump-start of different transcription factors, for example p53, NF-κB (nuclear factor-κB), AP-1 (activated protein-1), Nrf2 (nuclear erythroid-derived 2-related factor 2), and CREB (cAMP-responsive element-binding) protein associate with various cellular function like p53 and NF-kB are involved in cellular damage response. NF-κB family play critical roles in immunity, inflammation, differentiation, cell proliferation, and survival [26]. AP-2 family transcription factors are evolutionarily conserved that bind to the DNA consensus sequence GCCNNNGGC and upregulate target gene expression. In mammals, four different isoforms of AP-2 have been identified, termed AP-2 $\alpha$, $\beta$, $\gamma$, and $\delta$. Studies have identified the role of AP-2 TF in *Plasmodium* ApiAP2 transcription factor (PfAP2-EXP2) – controlling the gene expression in the intraerythrocytic developmental cycle of plasmodium parasite. AP-1 on the other hand participates in control of proliferation, senescence, differentiation, and apoptosis [27]. Sp1 is a member of transcription factors which include Sp2, Sp3, and Sp4 playing a role in DNA repair. CREB is a phosphorylation-dependent nuclear

transcription factor that is involved in different important cellular functions including apoptosis and cell proliferation. The cAMP-CRP protein is considered as lying between the conventional transcription regulators and histone-like proteins, and it can bind specifically to a consensus DNA sequence. Another TF is FOXO3a protein, a fork-head transcription factor that is a member of FOXO subfamily and mediates a variety of cellular process including proliferation, cell cycle progression, DNA damage, and apoptosis [28]. The next important TF is E2F that is activated by E1A protein that is a viral oncoprotein and needed for adenovirus gene expression. E2F transcription factors are recognized as key players in controlling the cell cycle, transformation, and differentiation, and it has been found that the E2F/pRB pathway acts as a key regulator on cell cycle and development. Quite a few important TFs and DNA motifs have been characterized in protozoan parasites. For instance, a member of the HMGB was identified in *Entamoeba histolytica*, and some Myb family members were characterized from *Trichomonas vaginalis*, and a cell cycle-dependent ApiAP2 transcription factor, TgAP2IX-5, was found in *Toxoplasma gondii* [29]. The list of transcription factors identified so far in *Entamoeba* is shown in Table 1, and their functions are depicted in the schematic in Fig. 1. However, the biological role of many TFs in this parasite is still poorly understood, and further characterization is needed for better understanding.

## 2.1 TATA-Box-Binding Protein

In the past two decades, important improvements have been achieved in terms of molecular biology techniques that expanded our perception of transcriptional regulation *in E. histolytica.* Several groups have identified a number of TFs and the core promoter region in *Entamoeba*. However, very little is known about the transcription machinery and especially transcription regulation during the development of this parasite.

In the late 1990s or early 2000s, the approaches used by different groups to identify the transcription factors in this parasite were mainly based on comparative amino acid sequence analysis of known transcription factors, present in other systems [30], or yeast one-hybrid assay [31] or deletion or replacement analysis of consensus motifs in the promoter region [31, 32]. Among the earlier approaches, comparative analysis of amino acid sequence TATA-box-binding protein from *Acanthamoeba castellanii* identified the *Entamoeba* transcription factor as TATA-box-binding protein (EhTBP) [30]. The EhTBP is more unselective compared to higher eukaryotes and binds a wide variety of *E. histolytica* TATA-box sequence [11, 19]. Later on, genome sequencing, gene expression profiling, and proteomics approaches advanced to study the transcriptional networks and help identify novel transcription factors [12, 20, 21, 33–36]. Subsequently, the sequencing and annotation of *Entamoeba* genome provide identification of two more amoebic TATA-binding proteins (TBP) [20]. TBP and TRF1 transcription factors in *E. histolytica* are GAAC-box-binding proteins that represent distinctive expression of genes under

**Table 1** *Entamoeba* transcription factors, their representative DNA-binding motifs, and information gained

| Transcription factors | DNA-binding motif | Information gained | References |
|---|---|---|---|
| EhTBP<br>TATA-binding protein | TATTTAAA | Shows specific binding in vitro to TATA box<br>EhTBP is more unselective compared to TBP of human and yeast | Luna-Arias et al. [30]<br>de Dios-Bravo et al. [19] |
| EhTRF1<br>TBP-related factor 1 | GAAC-box-binding proteins | Two EhTRF identified. TRF1 binds to GAAC box and displays distinctive expression of genes under stress response and during host-*Entamoeba* interaction | Castañon-Sanchez et al. [20] |
| URE3-BP<br>Upstream regulatory element-binding protein | TATTCTATT<br>(URE3) | DNA motif identified. URE3-BP identified.<br>Identification of target genes | Purdy et al. [6]<br>Gilchrist et al. [56]<br>Gilchrist et al. [59]<br>Gilchrist et al. [58] |
| EhEBP1 and EhEBP2<br>Enhancer-binding proteins | AAAAATGAATGGAAAAATGAA<br>(URE4) | DNA motif identified. Identification of EhEBP1 and 2 | Schaenman et al. [32]<br>Schaenman et al. [31] |
| EhMyb10 | TAACGG | 32 Myb domain proteins identified | Meneses et al. [21] |
| EhMyb-dr | CCCCCC | EhMyb-dr identifiedEhMyb-dr DNA motif was identified | Ehrenkaufer et al. [50] |
| ECudA | AGAATTTTCT | CudA homolog identified in *Entamoeba* | Yamada et al. [34] |
| C/EBP | TGTTTGGTAGTTGAATTGGAAAGAA | C/EBP binding DNA motif was identified. Partial purifications of proteins | Marchat et al. [36] |
| Ehp53 | GGACATGCCCGGGCATGTCC | Shows specific binding in vitro | Mendoza et al. [39] |
| HMGB1<br>(high mobility group box protein 1) | DNA structure,<br>e.g., stem-loops | Proof of DNA bendingHMGB1 increase DNA binding of protein p53. Genes regulated by HMGB1 were identified | Gilchrist et al. [33]<br>Abhyankar et al. [40] |
| EhHSTF | 5′-GAA-3′ motif into heat shock elements of the EhPgp5 gene | Play an important function in multidrug resistance activity in *E. histolytica* | Bello et al. [62] |
| HRM-BP | AAACCTCAATGAAGA | Transcriptional control of genes involved in oxidative stress response | Pearson et al. [35] |

(continued)

**Table 1** (continued)

| Transcription factors | DNA-binding motif | Information gained | References |
|---|---|---|---|
| EhPC4 | – | Induce cell migration, multinucleation, and polykaryon formation promoted by EhPC4 | Cruz et al. [37, 38] |
| ERM-BP (encystation regulatory motif-binding protein) | CAACAAA | An $NAD^+$ regulated TF. Upregulated during encystation. Control cyst-specific gene expression | Manna et al., [51, 52] |
| NF-Y complex (nuclear factor-Y) | CCAAT | CCAAT motif specifically binds to *Entamoeba* NF-Y complex<br>Control encystation<br>NF-Y works downstream of ERM-BP transcriptional control | Manna et al. [53, 54] |
| EhGATA-TF | GATA | Modulates genes involved in phagocytosis | Huerta et al. [61] |

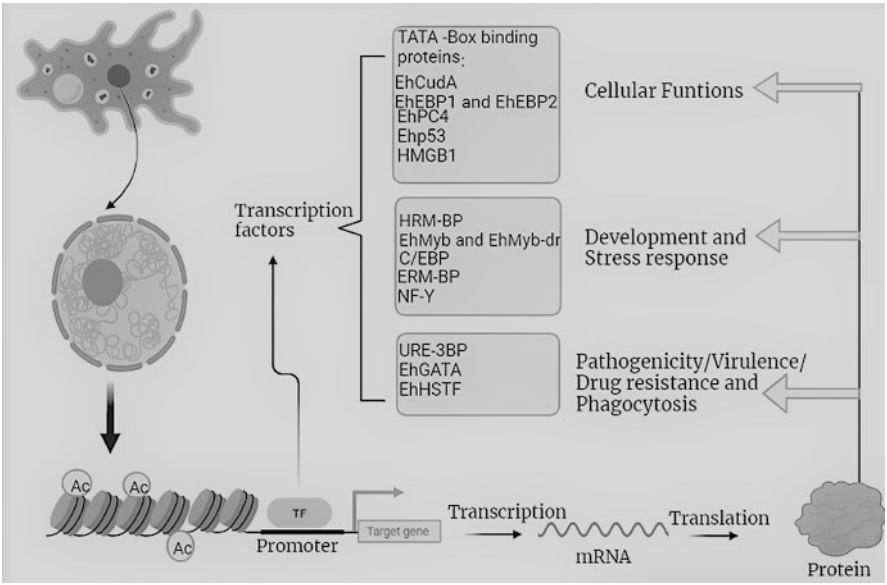


**Fig. 1** Transcription factors and their roles in *Entamoeba*

stress response and during the interaction of *Entamoeba* with mammalian cells. However, the biological role of these two new TBP is yet to be determined.

## 2.2 EhCudA

The transcription factor EhCudA was identified by a comparative in silico approach by utilizing *Dictyostelium* CudA as a query [34]. In *Dictyostelium* this protein is necessary for pre-spore-specific gene expression and has significant homology in *Entamoeba* protein [34]. Yamada et al. expressed CudA protein in bacteria and used recombinant protein and were able to identify the DNA-binding motif AGAATTTTCT which shows specific interaction with CudA in vitro; however the functional characterization of *Entamoeba* CudA is yet to be determined [34].

## 2.3 EhEBP1 and EhEBP2

Two enhancer-binding proteins (EhEBP1 and EhEBP2) which specifically bind to the URE4-binding domain were discovered by using nuclear extracts from amoeba and DNA affinity chromatography followed by mass spectrometry [31]. Some unique features were reported in these TFs; both EhEBP1 and EhEBP2 comprise

an RNA recognition motif RRM; however; no recognizable DNA-binding domain was identified.

## 2.4 EhPC4

Analysis of genome-wide microarray data from virulent trophozoites isolated from hamster liver abscesses identified a transcription factor, EhPC4 (*E. histolytica*-positive cofactor), which significantly upregulated during the infection [37]. The author has reported the potential role of EhPC4 in liver abscess formation by controlling the expression of vital genes involved in cytoskeleton dynamics, cell migration, and invasion [37]. The transcription factor EhPC4 also possesses important role in regulating DNA replication and genome stability [38].

## 2.5 Ehp53

A p53-like *E. histolytica* protein (Ehp53) was identified which binds to the human p53-binding consensus DNA sequence confirmed by human p53 antibodies [39]. It has been reported that monoclonal antibody against human p53 protein could recognize the recombinant *Entamoeba* Ehp53 suggesting that in *Entamoeba* this protein may be evolutionarily conserved [39]. In mammalian cells, p53 takes part in several cellular processes like cell cycle regulation, DNA repair, precluding uncontrolled cellular division, and apoptosis; however the functional characterization of this TF is yet to be determined.

## 2.6 HMGB1

The TF HMGB1 (high mobility group box protein 1) was identified by the analysis of genome-wide transcriptome data during *Entamoeba* colonization and invasion to the intestine [40]. HMGB proteins can bind a diverse sequence of DNA in a conformation-dependent way which includes stem-loops, palindromes, four-way junctions, B-Z junctions, and even single-stranded or cruciform DNA [41]. This protein contains one or more units of the HMG box DNA-binding motif, and it is observed that it can increase DNA binding in a sequence-specific manner. This protein is involved in many important cellular functions, e.g., transcription, recombination, and repair. In a recent study, it was shown that *Entamoeba* when in contact with macrophage induced the secretion of HMGB1 which functions as a pro-inflammatory cytokine and can also act as a chemoattractant during the *Entamoeba* infection [42].

## 3 TF in Development and Stress Response

Development needs the conscientious orchestration of many biological episodes in order to generate an entire multicellular organism, and in case of unicellular organisms, this orchestration is equally important throughout the different stages of development. Many transcription factors (TFs) involved in the development are conserved evolutionarily from yeast to humans. For example, there are four TF families that play a determining role and have been characterized immensely both during the development of embryo and in cancer. These are (1) GATA, (2) the high mobility group box (HMG), (3) paired box (PAX), and (4) basic helix-loop-helix (bHLH) [43–45]. Living organisms constantly face diverse types of physiological and environmental stress. To survive with the detrimental consequences of stress or to protect against further exposure to the same or other forms of stress, cells have evolved rapid molecular responses to repair the damage. TF plays an important role by upregulation or downregulation of set of genes which makes the organism more resistant in the adverse condition, and many stress-controlled transcription factors have been discovered and characterized in different systems [46, 47].

Transcription factor activation is a complicated process that may involve numerous signal transduction pathways, including several kinases, e.g., PKA, MAPKs, JAKs, and PKCs, which are activated by cell-surface receptors [48]. Major TF families, together with WRKY, MYB, NAC, and AP2/ERF, are important regulators of diverse genes associated with various stressors. WRKY, as one of the most well-studied plant TFs, regulates a wide range of developmental, physiological, and metabolic activities. The WRKY family has been recognized as a major group of transcription factors in many plant species. These function as activators, repressors, or corepressors of essential pathways, such as the generation of alkaloids, terpenes, and other specialized metabolites, and have been proven to be significant in the activation of diverse immune response pathways, making them important in biotic stress. WRKY transcription factors were found to be useful in relieving infection stress produced by biotic or abiotic agents via self-regulation or hormone-mediated signal transduction pathways. In *Entamoeba*, a few transcription factors were identified which control the expression of important genes pertinent to several important facets of *Entamoeba* biology which includes stage conversion and oxidative stress (Table 1 and Fig. 1) [35, 49].

### 3.1 HRM-BP

A novel $H_2O_2$ stress-responsive motif HRM was identified by in silico analysis of the promoter sequences of genes that are upregulated in $H_2O_2$ stress, and the transcription factor HRM-binding protein (HRM-BP) was identified by biochemical analysis and mass spectrometry [35]. The interaction of HRM-BP with the HRM motif is very specific, and alteration of HRM-BP expression either by silencing or by overexpression in *Entamoeba* showed changes in the basal expression of stress responsiveness or $H_2O_2$-responsive genes [35].

### 3.2 EhMyb and EhMyb-dr

A set of 32 Myb domain-containing proteins were identified in Eh by analyzing the c-Myb protein sequences as the query from human [21]. Electrophoretic mobility shift assays (EMSA) by using recombinant *Entamoeba* Myb10 (family I) showed that this Myb10 protein could bind the canonical Myb-binding motif (TAACGG) as reported in other eukaryotes and in *Entamoeba*, EhHSP70 gene promoter comprises a Myb DNA-binding motif suggesting its important role in heat shock gene expression in stress response.

Studies in recent years evidenced that transcriptional control has an important contribution in stage conversion in *Entamoeba*, and three transcription factors were identified. Myb transcription factor (EhMyb-dr) is a SHAQKY family Myb gene which binds to a hexa-nucleotide motif CCCCCC; upregulating this protein in *E. histolytica* trophozoites results a transcriptional profile that highly resembles with the transcriptome profile of amoebic encystation [50]. The interplay between the EhMyb-dr protein and the DNA sequence is eventually confirmed by EMSA as well as by chromatin immunoprecipitation (ChIP) analysis, and it is evident that EhMyb-dr regulates a set of cyst-specific genes [50].

### 3.3 ERM-BP

An encystation regulatory motif (ERM) that is a hepta-nucleotide sequence was (CAACAAA) identified in the promoter of 131 cyst-specific genes in *E. invadens* which is used as model system for developmental studies. Electrophoretic mobility shift assay showed specific binding of *Entamoeba* cyst protein only, not by the trophozoite protein suggesting that the protein bind to ERM may be specifically expressed in cyst only. ERM-binding protein (ERM-BP) was identified by electrophoretic mobility shift assay followed by mass spectrometry. Metabolic cofactor $NAD^+$ positively regulates the binding of recombinant ERM-BP with ERM, and downregulation of ERM-BP significantly decreased encystation efficiency, and ghost-like abnormal cysts with defective cyst wall are produced suggesting that ERM-BP plays an important role in encystation [51]. The ERM-BP is conserved among other *Entamoeba* species, and upregulating ERM-BP in *E. histolytica* (EHI_146360) produced quadri-nucleate cyst-like structures and makes the parasite more resistant due to heat stress, supporting the idea that heat stress response and encystation might have a potential overlap and some interconnection and share common signaling pathways [52, 53].

### 3.4 NF-Y (Nuclear Factor Complex)

Nuclear factor complex (NF-Y) is made up of three subunits, namely, NF-YA, NF-YB, and NF-YC, that very specifically bind to a pentanucleotide motif CCAAT and this TF complex conserved throughout evolution [54]. NF-Y plays

crucial roles in higher eukaryotes, controlling many cellular processes (e.g., cell cycle regulation, development, response to growth, stress, DNA damage, and apoptosis) by regulating the expression of genes that comprise CCAAT promoter motif [54].

In *E. invadens* the expression of NF-YA is constitutive; however NF-YB and NF-YC are expressed during encystation. Silencing of the NF-YC subunit in *Entamoeba* showed significant reduction in DNA-binding ability of the NF-Y complex and also reduced encystation efficiency [54].

## 4 Transcription Factors in Pathogenicity, Virulence, Drug Resistance, and Phagocytosis

Transcription factors (TFs) are central components which play a critical role in the gene expression. A little change in the TF expression and specificity can alter the entire gene expression. During the infection, pathogenic organisms upregulate or downregulate many genes those are downregulated by their TFs which helps in the adaptation of host or tissue specific environment and adaptation of various physiological changes and in the activation of virulence and pathogenicity. The main aim of the identification of TFs is to block the virulence factors in any pathogenic organism. For developing in-depth knowledge about host-pathogen interaction, it is necessary to identify the interplay of signal exchange mechanism which will be helpful to identify the virulence factor and outcome of the infection. Very little information was known regarding the transcriptional switch that helps cell to adjust in response to immune signals and infection. In 2016 Gray et al., identified Fcγ receptor that helps TFEB transcription factor to enhance lysosome-based degradation and killing bacteria [55]. So, it is uncovered thereafter that IgG immune complexes instruct macrophages to transform it as super killers by the upregulated activation of the lysosomes through a transcriptional circuit. It is evident that in *Entamoeba* pathogenesis, virulence and development are controlled transcriptionally.

### 4.1 URE-3BP

The upstream regulatory element DNA sequence motif TATTCTATT (URE3) was first discovered in the promoter region of the heavy chain subunit of the lectin gene *hgl5* in *E. histolytica* and later on also found in the promoter of ferredoxin (*fdx*) 1 gene [6, 40, 49, 56, 57]. Upstream regulatory element-binding protein (URE3-BP) was identified through a yeast one-hybrid screen by using URE3 as bait [30]. It was reported that the promoter activity increases due to the mutation in URE3 motif in the promoter of *hgl*5 lectin; on the contrary mutation in URE3 motif in the *fdx* 1 gene promoter decreases the promoter activity by twofold of the reporter gene activity, suggesting that URE3 can act as either a negative or positive regulator in gene expression [57]. This transcription factor comprises two calcium-binding motifs (EF hands), and URE3-BP detach from URE3 DNA in the presence of higher

level of calcium, suggesting that calcium acts as negative regulator [58, 59]. The transcription factor URE3-BP is regulated by calcium and controls the expression of two virulence genes in *Entamoeba*, the Gal/GalNac lectin and ferredoxin. It also has been reported that upregulation of URE3-BP leads to the changes in the morphology of trophozoite and boosts parasite invasion in different organs like the colon and liver, suggesting that transcription factor URE3-BP plays a salient role in *Entamoeba* virulence [60].

## 4.2 EhGATA

The GATA transcription factors are conserved and a part of the DNA-binding domain (ZFBD) family that contains zinc finger and recognizes the consensus DNA sequence (A/T)GATA(A/G). This ZFBD superfamily TF moderates a wide range of cellular functions.

In 2020, Huerta et al. reported the existence of a single *gata* gene in *E. histolytica* (*Ehgata*) by bioinformatic analysis and the GATA domain ensured in 80% similarity to the GATA protein of human [61]. *Ehgata* codes for a noncanonical EhGATA transcription factor that contains an AT-Hook motif and only one zinc finger DNA-binding domain. Bioinformatic prediction showed the presence of GATA-binding sequence over 1600 gene promoters in *Entamoeba* genome [61]. Electrophoretic mobility shift assay with the bacterially expressed and purified EhGATA protein, additionally with trophozoite nuclear extracts, showed binding to the consensus GATA-DNA sequence. Moreover, Huerta et al. showed that EhGATA especially binds to the promoters of *Ehadh* and *Ehvps32* genes in vivo and eventually controls EhADH and EhVps32 gene expression in the course of phagocytosis. Additionally, overexpressing of EhGATA in trophozoites showed significant changes in morphology, alteration in cell proliferation, change in adherence efficiency, and change in rate of phagocytosis. These findings suggest that EhGATA TF is capable to bind DNA and fine-tune the expression of several genes those involved in cell proliferation, adhesion to surface, and phagocytosis [61].

## 4.3 EhHSTF

When bacteria or any other organisms are exposed to a certain drug or antibiotic, they alter their cellular mechanism to survive. Continuous and excessive exposure of any drug can lead to the rise of a drug-resistant population of cells. For decades the main drug of choice against amebiasis is metronidazole, but due to emergence of drug resistance (DR) in most of the pathogens, it is really alarming that DR will cause a major public health problem worldwide. It has been reported that methionine γ-lyase (*EhMGL*) gene silencing resulted in resistance to trifluoromethionine, revealing a novel mechanism of drug resistance in *E. histolytica.*

In *Entamoeba* it has been observed that emetine stress induces the expression of the multidrug resistance *EhPgp5* gene [62]. Bello et al. showed that the transcription

factor EhHSTF7 recognizes the 5′-GAA-3′ motif into the heat shock element of EhPgp5 gene and is involved in the transcriptional activation of the EhPgp5 gene [62].

## 5 Summary and Conclusions

The most constructive way to understand the functions of different genes in an organism is by genetic manipulation, and most of the genetic analyses are achieved by alteration in the transcriptome level. In *Entamoeba*, gene expression and their fine tuning by transcriptional controls are still not well understood, and the majority of genes or proteins are hypothetical. Recent advancement in RNA-seq analysis, proteome analysis, and gene editing by CRISPR/Cas9 opens the avenue to analyze their expression pattern during different stages of development, stress condition, and also the differential gene expression between pathogenic and nonpathogenic strains. For example, during oxidative stress it is reported that 57 genes are upregulated in response to $H_2O_2$ exposure and the expression of these genes is controlled by transcription factor HRM-BP. A Myb domain protein EhMyb-dr binds to CCCCCC motif and upregulates a set of genes during encystation, and another transcription factor ERM-BP that binds to CAACAAA motif and 131 cyst-specific genes which were upregulated was identified having this motif. It has been seen that the TFs that bind to cis-regulatory sequence can either positively or negatively regulate the transcription regulation. In *Entamoeba* TF URE3-BP is reported to regulate the transcription in both ways. URE3-BP positively regulates the expression of lectin heavy chain and negatively regulates ferredoxin 1 gene. However, only a few TFs have been characterized in this parasite till now, and definitely there is an urgency to extend this line of research for better understanding of many unrevealed area of amoebic biology.

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## References

1. Lioutas C, Tannich E. Transcription of protein-coding genes in Entamoeba histolytica is insensitive to high concentrations of alpha-amanitin. Mol Biochem Parasitol. 1995;73:259–61. https://doi.org/10.1016/0166-6851(95)00101-6.
2. Torres-Guerrero H, Peattie DA, Meza I. Chromatin organization in Entamoeba histolytica. Mol Biochem Parasitol. 1991;45:121–30. https://doi.org/10.1016/0166-6851(91)90033-3.
3. Bruchhaus I, Leippe M, Lioutas C, Tannich E. Unusual gene organization in the protozoan parasite Entamoeba histolytica. DNA Cell Biol. 1993;12:925–33. https://doi.org/10.1089/dna.1993.12.925.
4. Födinger M, Ortner S, Plaimauer B, et al. Pathogenic Entamoeba histolytica: cDNA cloning of a histone H3 with a divergent primary structure. Mol Biochem Parasitol. 1993;59:315–22. https://doi.org/10.1016/0166-6851(93)90229-q.

5. Binder M, Ortner S, Plaimauer B, et al. Sequence and organization of an unusual histone H4 gene in the human parasite Entamoeba histolytica. Mol Biochem Parasitol. 1995;71:243–7. https://doi.org/10.1016/0166-6851(94)00044-n.
6. Purdy JE, Pho LT, Mann BJ, Petri WA. Upstream regulatory elements controlling expression of the Entamoeba histolytica lectin. Mol Biochem Parasitol. 1996;78:91–103. https://doi.org/10.1016/s0166-6851(96)02614-x.
7. Hon C-C, Weber C, Sismeiro O, et al. Quantification of stochastic noise of splicing and polyadenylation in Entamoeba histolytica. Nucleic Acids Res. 2013;41:1936–52. https://doi.org/10.1093/nar/gks1271.
8. Manna D, Ehrenkaufer GM, Singh U. Regulation of gene expression in the protozoan parasite Entamoeba invadens: identification of core promoter elements and promoters with stage-specific expression patterns. Int J Parasitol. 2014;44:837. https://doi.org/10.1016/j.ijpara.2014.06.008.
9. Singh U, Rogers JB, Mann BJ, Petri WA. Transcription initiation is controlled by three core promoter elements in the hgl5 gene of the protozoan parasite Entamoeba histolytica. Proc Natl Acad Sci U S A. 1997;94:8812–7. https://doi.org/10.1073/pnas.94.16.8812.
10. Singh U, Gilchrist CA, Schaenman JM, et al. Context-dependent roles of the Entamoeba histolytica core promoter element GAAC in transcriptional activation and protein complex assembly. Mol Biochem Parasitol. 2002;120:107–16. https://doi.org/10.1016/s0166-6851(01)00441-8.
11. McAndrew MB, Read M, Sims PF, Hyde JE. Characterisation of the gene encoding an unusually divergent TATA-binding protein (TBP) from the extremely a+T-rich human malaria parasite plasmodium falciparum. Gene. 1993;124:165–71. https://doi.org/10.1016/0378-1119(93)90390-o.
12. Hackney JA, Ehrenkaufer GM, Singh U. Identification of putative transcriptional regulatory networks in Entamoeba histolytica using Bayesian inference. Nucleic Acids Res. 2007;35:2141. https://doi.org/10.1093/nar/gkm028.
13. Nickel R, Tannich E. Transfection and transient expression of chloramphenicol acetyltransferase gene in the protozoan parasite Entamoeba histolytica. Proc Natl Acad Sci U S A. 1994;91:7095–8. https://doi.org/10.1073/pnas.91.15.7095.
14. Purdy JE, Mann BJ, Pho LT, Petri WA. Transient transfection of the enteric parasite Entamoeba histolytica and expression of firefly luciferase. Proc Natl Acad Sci U S A. 1994;91:7099–103. https://doi.org/10.1073/pnas.91.15.7099.
15. Hamann L, Nickel R, Tannich E. Transfection and continuous expression of heterologous genes in the protozoan parasite Entamoeba histolytica. Proc Natl Acad Sci U S A. 1995;92:8975–9. https://doi.org/10.1073/pnas.92.19.8975.
16. Singh U, Rogers JB. The novel core promoter element GAAC in the hgl5 gene of Entamoeba histolytica is able to direct a transcription start site independent of TATA or initiator regions. J Biol Chem. 1998;273:21663–8. https://doi.org/10.1074/jbc.273.34.21663.
17. Loftus B, Anderson I, Davies R, et al. The genome of the protist parasite Entamoeba histolytica. Nature. 2005;433:865–8. https://doi.org/10.1038/nature03291.
18. Lorenzi HA, Puiu D, Miller JR, et al. New assembly, reannotation and analysis of the Entamoeba histolytica genome reveal new genomic features and protein content information. PLoS Negl Trop Dis. 2010;4:e716. https://doi.org/10.1371/journal.pntd.0000716.
19. de Dios-Bravo G, Luna-Arias JP, Riverón AM, et al. Entamoeba histolytica TATA-box binding protein binds to different TATA variants in vitro. FEBS J. 2005;272:1354–66. https://doi.org/10.1111/j.1742-4658.2005.04566.x.
20. Castañon-Sanchez CA, Luna-Arias JP, de Dios-Bravo MG, et al. Entamoeba histolytica: a unicellular organism containing two active genes encoding for members of the TBP family. Protein Expr Purif. 2010;70:48–59. https://doi.org/10.1016/j.pep.2009.12.007.
21. Meneses E, Cárdenas H, Zárate S, et al. The R2R3 Myb protein family in Entamoeba histolytica. Gene. 2010;455:32–42. https://doi.org/10.1016/j.gene.2010.02.004.

22. Ramakrishnan G, Gilchrist CA, Musa H, et al. Histone acetyltransferases and deacetylase in Entamoeba histolytica. Mol Biochem Parasitol. 2004;138:205–16. https://doi.org/10.1016/j.molbiopara.2004.09.002.
23. Bernes S, Siman-Tov R, Ankri S. Epigenetic and classical activation of Entamoeba histolytica heat shock protein 100 (EHsp100) expression. FEBS Lett. 2005;579:6395–402. https://doi.org/10.1016/j.febslet.2005.09.101.
24. Ehrenkaufer GM, Weedall GD, Williams D, et al. The genome and transcriptome of the enteric parasite Entamoeba invadens, a model for encystation. Genome Biol. 2013;14:R77. https://doi.org/10.1186/gb-2013-14-7-r77.
25. Hellweg CE, Spitta LF, Henschenmacher B, et al. Transcription factors in the cellular response to charged particle exposure. Front Oncol. 2016;6 https://doi.org/10.3389/fonc.2016.00061.
26. Oeckinghaus A, Ghosh S. The NF- B family of transcription factors and its regulation. Cold Spring Harb Perspect Biol. 2009;1:a000034. https://doi.org/10.1101/cshperspect.a000034.
27. Shang X, Wang C, Shen L, et al. PfAP2-EXP2, an essential transcription factor for the Intraerythrocytic development of plasmodium falciparum. Front Cell Dev Biol. 2021;9:782293. https://doi.org/10.3389/fcell.2021.782293.
28. Liu Y, Ao X, Ding W, et al. Critical role of FOXO3a in carcinogenesis. Mol Cancer. 2018;17:104. https://doi.org/10.1186/s12943-018-0856-3.
29. Khelifa AS, Guillen Sanchez C, Lesage KM, et al. TgAP2IX-5 is a key transcriptional regulator of the asexual cell cycle division in toxoplasma gondii. Nat Commun. 2021;12:116. https://doi.org/10.1038/s41467-020-20216-x.
30. Luna-Arias JP, Hernandez-Rivas R, de Dios-Bravo G, et al. The TATA-box binding protein of Entamoeba histolytica: cloning of the gene and location of the protein by immunofluorescence and confocal microscopy. Microbiology. 1999;145(Pt 1):33–40. https://doi.org/10.1099/13500872-145-1-33.
31. Schaenman JM, Gilchrist CA, Mann BJ, Petri WA. Identification of two Entamoeba histolytica sequence-specific URE4 enhancer-binding proteins with homology to the RNA-binding motif RRM. J Biol Chem. 2001;276:1602–9. https://doi.org/10.1074/jbc.M006866200.
32. Schaenman JM, Driscoll PC, Hockensmith JW, et al. An upstream regulatory element containing two nine basepair repeats regulates expression of the Entamoeba histolytica hgl5 lectin gene. Mol Biochem Parasitol. 1998;94:309–13. https://doi.org/10.1016/s0166-6851(98)00081-4.
33. Gilchrist CA, Houpt E, Trapaidze N, et al. Impact of intestinal colonization and invasion on the Entamoeba histolytica transcriptome. Mol Biochem Parasitol. 2006;147:163–76. https://doi.org/10.1016/j.molbiopara.2006.02.007.
34. Yamada Y, Wang HY, Fukuzawa M, et al. A new family of transcription factors. Development. 2008;135:3093–101. https://doi.org/10.1242/dev.026377.
35. Pearson RJ, Morf L, Singh U. Regulation of H2O2 stress-responsive genes through a novel transcription factor in the protozoan pathogen Entamoeba histolytica. J Biol Chem. 2013;288:4462. https://doi.org/10.1074/jbc.M112.423467.
36. Marchat LA, Gómez C, Pérez DG, et al. Two CCAAT/enhancer binding protein sites are cis-activator elements of the Entamoeba histolytica EhPgp1 (mdr-like) gene expression. Cell Microbiol. 2002;4:725–37. https://doi.org/10.1046/j.1462-5822.2002.00220.x.
37. de la Cruz OH, Muñiz-Lino M, Guillén N, et al. Proteomic profiling reveals that EhPC4 transcription factor induces cell migration through up-regulation of the 16-kDa actin-binding protein EhABP16 in Entamoeba histolytica. J Proteome. 2014;111:46–58. https://doi.org/10.1016/j.jprot.2014.03.041.
38. Cruz OH de la, Marchat LA, Guillén N, et al (2016) Multinucleation and Polykaryon formation is promoted by the EhPC4 transcription factor in Entamoeba histolytica. Sci Rep 6:19611. https://doi.org/10.1038/srep19611.
39. Mendoza L, Orozco E, Rodríguez MA, et al. Ehp53, an Entamoeba histolytica protein, ancestor of the mammalian tumour suppressor p53. Microbiology. 2003;149:885–93. https://doi.org/10.1099/mic.0.25892-0.

40. Abhyankar MM, Hochreiter AE, Hershey J, et al. Characterization of an Entamoeba histolytica high-mobility-group box protein induced during intestinal infection. Eukaryot Cell. 2008;7:1565–72. https://doi.org/10.1128/EC.00123-08.
41. Bustin M, Reeves R. High-mobility-group chromosomal proteins: architectural components that facilitate chromatin function. Prog Nucleic Acid Res Mol Biol. 1996;54:35–100. https://doi.org/10.1016/s0079-6603(08)60360-8.
42. Begum S, Moreau F, Leon Coria A, Chadee K. Entamoeba histolytica stimulates the alarmin molecule HMGB1 from macrophages to amplify innate host defenses. Mucosal Immunol. 2020;13:344–56. https://doi.org/10.1038/s41385-019-0233-6.
43. Roy N, Hebrok M. Regulation of cellular identity in cancer. Dev Cell. 2015;35:674–84. https://doi.org/10.1016/j.devcel.2015.12.001.
44. Knudson AG. Mutation and cancer: statistical study of retinoblastoma. Proc Natl Acad Sci U S A. 1971;68:820–3. https://doi.org/10.1073/pnas.68.4.820.
45. Huilgol D, Venkataramani P, Nandi S, Bhattacharjee S. Transcription factors that govern development and disease: an Achilles heel in cancer. Genes (Basel). 2019;10 https://doi.org/10.3390/genes10100794.
46. Ruis H, Schüller C. Stress signaling in yeast. BioEssays. 1995;17:959–65. https://doi.org/10.1002/bies.950171109.
47. Estruch F. Stress-controlled transcription factors, stress-induced genes and stress tolerance in budding yeast. FEMS Microbiol Rev. 2000;24:469–86. https://doi.org/10.1111/j.1574-6976.2000.tb00551.x.
48. Gilmore TD. Introduction to NF-kappaB: players, pathways, perspectives. Oncogene. 2006;25:6680–4. https://doi.org/10.1038/sj.onc.1209954.
49. Pearson RJ, Singh U. Approaches to characterizing Entamoeba histolytica transcriptional regulation. Cell Microbiol. 2010;12:1681–90. https://doi.org/10.1111/j.1462-5822.2010.01524.x.
50. Ehrenkaufer GM, Hackney JA, Singh U. A developmentally regulated Myb domain protein regulates expression of a subset of stage-specific genes in Entamoeba histolytica. Cell Microbiol. 2009;11:898. https://doi.org/10.1111/j.1462-5822.2009.01300.x.
51. Manna D, Lentz CS, Ehrenkaufer GM, et al. An NAD+-dependent novel transcription factor controls stage conversion in Entamoeba. eLife. 2018; https://doi.org/10.7554/eLife.37912.
52. Manna D, Lozano-Amado D, Ehrenkaufer G, Singh U. The NAD+ responsive transcription factor ERM-BP functions downstream of cellular aggregation and is an early regulator of development and heat shock response in Entamoeba. Front Cell Infect Microbiol. 2020;10:363. https://doi.org/10.3389/fcimb.2020.00363.
53. Manna D, Ehrenkaufer GM, Lozano-Amado D, Singh U. Entamoeba stage conversion: progress and new insights. Curr Opin Microbiol. 2020;58:62–8. https://doi.org/10.1016/j.mib.2020.09.005.
54. Manna D, Singh U. Nuclear factor Y (NF-Y) modulates encystation in Entamoeba via stage-specific expression of the NF-YB and NF-YC subunits. mBio. 2019;10 https://doi.org/10.1128/mBio.00737-19.
55. Gray MA, Choy CH, Dayam RM, et al. Phagocytosis enhances lysosomal and bactericidal properties by activating the transcription factor TFEB. Curr Biol. 2016;26:1955–64. https://doi.org/10.1016/j.cub.2016.05.070.
56. Gilchrist CA, Holm CF, Hughes MA, et al. Identification and characterization of an Entamoeba histolytica upstream regulatory element 3 sequence-specific DNA-binding protein containing EF-hand motifs. J Biol Chem. 2001;276:11838–43. https://doi.org/10.1074/jbc.M007375200.
57. Gilchrist CA, Mann BJ, Petri WA. Control of ferredoxin and gal/GalNAc lectin gene expression in Entamoeba histolytica by a cis-acting DNA sequence. Infect Immun. 1998;66:2383–6. https://doi.org/10.1128/IAI.66.5.2383-2386.1998.
58. Gilchrist CA, Baba DJ, Zhang Y, et al. Targets of the Entamoeba histolytica transcription factor URE3-BP. PLoS Negl Trop Dis. 2008;2:e282. https://doi.org/10.1371/journal.pntd.0000282.

59. Gilchrist CA, Leo M, Line CG, et al. Calcium modulates promoter occupancy by the Entamoeba histolytica Ca2+-binding transcription factor URE3-BP. J Biol Chem. 2003;278:4646–53. https://doi.org/10.1074/jbc.M211271200.
60. Gilchrist CA, Moore ES, Zhang Y, et al. Regulation of virulence of Entamoeba histolytica by the URE3-BP transcription factor. mBio. 2010;1 https://doi.org/10.1128/mBio.00057-10.
61. Huerta M, Reyes L, García-Rivera G, et al. A noncanonical GATA transcription factor of *Entamoeba histolytica* modulates genes involved in phagocytosis. Mol Microbiol. 2020;114:1019–37. https://doi.org/10.1111/mmi.14592.
62. Bello F, Orozco E, Benítez-Cardoza CG, et al. The novel EhHSTF7 transcription factor displays an oligomer state and recognizes a heat shock element in the Entamoeba histolytica parasite. Microb Pathog. 2022;162:105349. https://doi.org/10.1016/j.micpath.2021.105349.

# A Perspective on Mathematical Modeling and Machine Learning Models to Predict Visceral Leishmaniasis

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**Abstract**

In this chapter, we have demonstrated different aspects and development for the study of visceral leishmaniasis. People around the world assumed that the world is free from the disease, but some recently found cases indicate the existence of the disease in the world. We have also tried to circumvent different challenges and future scopes of the study on visceral leishmaniasis.

**Keywords**

Visceral leishmaniasis · Kala-azar · Mathematical modeling · Machine learning

## 1 Introduction

Leishmaniasis, popularly known as Kala-azar, is a human sickness achieved by the protozoan parasite named *Leishmania* parasite through sand fly chomps in tropical and subtropical regions. Researchers established that out of the 54 known kinds of this parasite, only 21 species in 98 countries can lead to the disease and around 350 million species are at chance of contamination [1]. In view of its clinical signs, the sickness is portrayed by self-recuperating skin leishmaniasis (SSL), skin mucosa

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that structures mucocutaneous leishmaniasis (ML), and harmful visceral leishmaniasis (VL), which can be trailed by a dermal disease called PKDL. Post-kala-azar dermal leishmaniasis (PKDL) is a dermal confusion of VL, which might happen later or during treatment [2]. It occurs half a year or longer after the previous VL, and in 15% of PKDL cases, no previous kala-azar disease has been observed [3]. Yet, a past clinic put together a review concerning Indian PKDL issues and viewed that in 20% of cases, VL has no point of reference [4]. PKDL can be analyzed by slit skin smear (SSS), culture, and/or polymerase chain response (PCR) [5]. There are wide contrasts between the clinical signs of VL and PKDL, with the previous comprising persistent fever, hepatosplenomegaly, iron deficiency, and weight reduction, trailed by macular, papular, or nodular injuries [6]. The topographical circulation of PKDL incorporates various intracellular leukocyte sores, remembering papular or nodular injuries in East Africa (Sudan) and broad polymorphic sores (co-event of macules and patches) in the South Asian locale (India, Bangladesh, and Nepal) [7].

In tropical and subtropical region, VL is found as a protozoan disease. WHO reported yearly around 200,000–400,000 VL cases and out of which 20,000–40,000 are death cases. In 2015 WHO data discovered that more than 90% of new cases occurred in seven countries: Brazil, Ethiopia, India, Kenya, Somalia, South Sudan, and Sudan. Regardless of the severity of VL, the number of cases has declined in some endemic countries, primarily in Bangladesh with a decline in new VL cases, with unequivocal recommendations to eliminate transmission by 2020. Of course, other regional countries have a degree of VL cases, such as in Brazil, which presents an annual average of 3800 new cases [8].

According to a 2016 WHO report, 36.7 million people worldwide are infected with HIV of these, 25.6 million live in Africa, 3.5 million in Southeast Asia, 3.3 million in the Americas, 2.4 million in Europe, and 1.4 million in the western Pacific Ocean. In countries where the leishmaniasis disease is more prevalent, the number of patients with HIV varies. A 2016 survey reported 710,000 (570,000–880,000) cases in Ethiopia, 2,100,000 (1,700,000–2,600,000) cases in India, 830,000 (610,000–1,100,000) cases in Brazil, 1,600,000 (1,400,000–1,800,000) cases in Kenya, 24,000 (16,000–33,000) cases in Somalia, 200,000 (130,000–290,000) cases in South Sudan, and 56,000 (34,000–87,000) cases in Sudan [8]. A move past note can be taken among VL and HIV disease regions. Leishmaniasis-HIV infection is represented in 35 local countries. VL-HIV co-infection occurs when people with HIV are exposed to the VL disease. A large study with a high co-existence rate found that VL accelerates HIV replication simultaneously.

In the 1990s, there is an increasing rate of VL-HIV infection in the Mediterranean. Between 1998 and 2001, a level of cases was observed after the 1997 peak. Highly Active antiretroviral therapy (HAART) is steadily reducing the number of VL HIV cases in the comparable region in 2001. Recent cases of VL-HIV in the Mediterranean Basin are comparatively less [9]. On the contrary, a development of VL-HIV infection has been observed in other districts, mainly in northwestern Ethiopia, where the recurrence rate of VL-HIV infection is exceptionally high [10]. A steady growth of VL-HIV co-infection is found in Latin America and precisely in Brazil. Recently a study [8] has shown around 9% of the patients with

HIV who are infected by VL. However, about 40% of VL patients must have no serology for HIV. In addition, this rate only indicates problems with the clinical indications of VL. Due to immune suppression, which occurs in HIV contamination and leishmaniasis contamination, recurrence may occur, and these patients may similarly act as reservoirs of leishmaniasis [11].

## 2 History of Kala-azar and PKDL

History of kala-azar is more established than the dated records. Back then, jungle fever was pervasive, and a few plagues of kala-azar were passed as poisonous intestinal sickness. Twining's writing in 1835 depicted a condition that he called "endemic cachexia of the tropical regions that are liable to paludal exhalations." The disease went unnoticed for a long time, but a closer look at the human mind's nature could make a final determination, however, many aspects of the disease continue to be ignored. The word kala-azar comprises "Kala" (in Hindi signifies "dark") and "Azar" (in Hindi signifies "fever").

Leishmaniasis is an ignored tropical illness that taints the world's least fortunate individuals in excess of 90 nations across Asia, Africa, the Middle East, and Central and South America. Albeit maybe less detailed, the ongoing appraisal shows that 700,000 to 1.2 million instances of cutaneous leishmaniasis (CL) happen every year, with around 95% happening in the Americas, the Mediterranean Basin, the Middle East, and Central Asia. Yearly instinctive leishmaniasis (VL) gauges are right now under 100,000, a huge drop from prior evaluations of 400,000, with over 95% of cases detailed from Brazil, China, Ethiopia, Kenya, Nepal, Somalia, Sudan, India to the World Health Organization (WHO). Risk factors for leishmaniasis include neediness, populace relocation, lack of healthy sustenance, uncleanliness, and an immunocompromised condition [available from: https://www.who.int/newsroom/realitysheets/detail/leishmaniasis].

VL is brought about by parasites from the *Leishmania donovani* complex in the Indian subcontinent [12, 13]. It is spread from one individual to another by female sand flies (*Phlebotomus argentipes*), and there are no known creature repositories [14]. Sand flies are generally dynamic and feed around evening time [15], with female movement topping preceding 12 PM. They normally look for cover in creature tunnels or other safeguarded spots [16] and flourish in unacceptable abodes [17, 18]. They are many times unfortunate flyers, flying in short leaps close to the ground [19]. All the while, there is proof that they are prepared to do longer and more drawn-out flight, as well as being more exophilic and exophagic than recently suspected [20]. Fifteen years subsequent to marking a memorandum of understanding by the governments of India, Nepal, and Bangladesh, focused on killing it as a general medical condition, the objective is being accomplished. Despite this, understanding the role of potentially highly infectious subgroups in preventing contagious leishmaniasis disease is becoming increasingly important as VL prevalence decreases. In humans, the parasite taints the reticuloendothelial framework, causing relentless fever and iron deficiency and influencing a few interior organs, generally

the spleen, liver, and bone marrow [17]. Since the side effects persevere, the people normally look for treatment, particularly in Bihar where treatment is accessible [21]. Regardless, the social disgrace connected to VL brings about an enormous level of people looking for treatment at private as opposed to general well-being offices [22], which brings about under-detailing genuine rate and pervasiveness of the illness [23]. After recuperation of intense disease, around 5–10% of patients foster an ongoing cutaneous structure called PKDL [14]. Besides, a couple of PKDL patients have had no set of experiences of VL [24]. Since PKDL is definitely not very fatal and the means of treatment are often be quite troubling and terrible, numerous PKDL cases remain untreated [25]. As a result, in the Indian subcontinent, regarding the transmission from one person to another of *L. donovani* [14], the PKDL patients are found to be the carrier of disease, but there is a possibility of co-infection of the disease [26]. However, the function of asymptomatic people in transmitting is not very clear [27]. Moreover, HIV-VL co-infection is a matter of major concern in the state of Bihar [28]. People infected with HIV-VL are often backslid and they take longer time for their treatment [29]. Generally, HIV decreases the manageability of an effective VL disposal program [23].

Post-kala-azar dermal leishmaniasis (PKDL) is a skin sequel that follows VP in approximately 5% to 10% of treatments in Asia, usually 1–3 years after the end of therapy [30, 31]. PKDL, however, is contagious to sand fly vectors and, if left untreated, can remain noticeable for years [27, 32]. After VL was almost eradicated from the Indian subcontinent in the 1970s, after the last VL case was reported in West Bengal, PKDL was suspected to be the inter-epidemic reservoir responsible for a new VL outbreak [26]. Such PKDL is considered a largely hidden but endless reservoir of infection, and it remains a significant threat to the sustainability of eradication initiatives (Fig. 1).

## 3 History of HIV

According to the WHO, out of 36.7 million people with HIV in 2016, Africa has 25.6 million, the Americas 3.3 million, Southeast Asia 3.5 million, Europe 2.4 million, and the Western Pacific 1.4 million cases—resulting in 1 million deaths caused by HIV/AIDS.

According to the as of late delivered India HIV Estimation 2019 report, overall, the assessed grown-up (15–49 years) HIV commonness pattern has been declining in India since the pandemic's top in the year 2000 and has been balancing out lately. The gauge for this marker was 0.22% (0.17–0.29%) in 2019. Around the same time, HIV commonness among grown-up guys (15–49 years) was assessed at 0.24% (0.18–0.32%) and among grown-up females at 0.20% (0.15–0.26%).

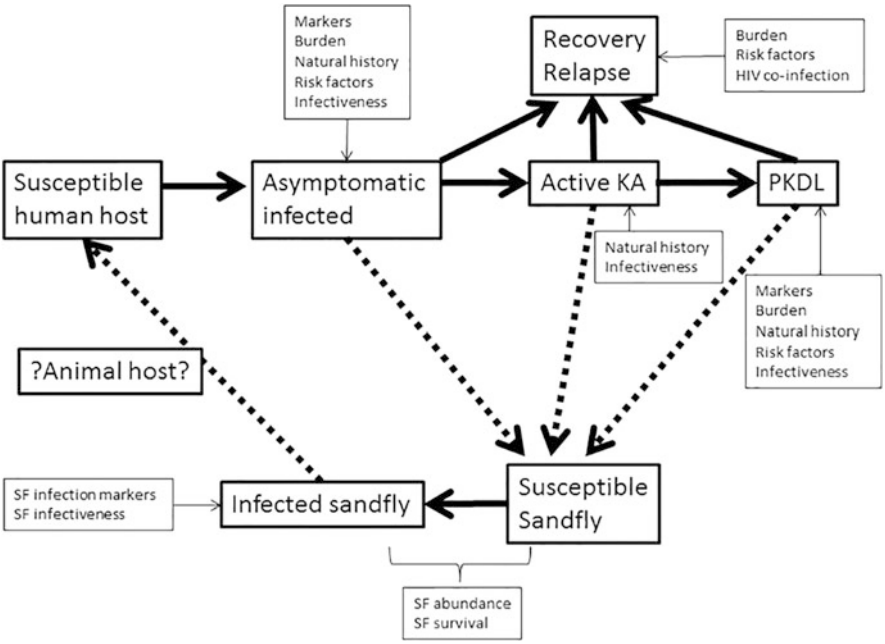


**Fig. 1** Transmission dynamics of VL in the Indian subcontinent

## 4 Overview of HIV and PKDL Co-infection

In Bihar, the most localized state for VL in India, approximately 2%–7% of VL cases are co-infected with HIV, although this is probably due to lack of data [23, 29, 33, 34]. Data from other settings in the Indian subcontinent are limited, partly due to the lack of regular testing for HIV in patients [23]. VL patients co-infected with HIV are highly contagious to sand flies [35]. HIV infection and leishmaniasis divide the path of an immune-pathological cell that enhances replication of both pathogens and accelerate the progression of both VL and HIV [36–38]. A concomitant HIV infection increases the risk of becoming active VL by 100 to 2320 times [39]. Diagnosing VL in HIV-infected patients is also a big challenge, as VL symptoms are less common and existing diagnostic tools are less accurate [40]. In addition, VL-HIV+ patients experience lower therapeutic success rates for VL and are more likely to experience drug-related toxicity and recurrence than non-HIV-infected patients [28, 36, 41]. As each new episode of VL becomes increasingly challenging to treat, these patients are more likely to have long-term infectious leishmaniasis [42]. However, their exact contribution to VL infection has not yet been determined.

## 5 Related Work and Methodologies

### 5.1 Mathematical Model on VL

There are numerous numerical models of VL components for ongoing monitoring [43–45]. A model of VL transmission at Bihar area was formulated in [46] to identify the effect of unreported cases causing the spread of the disease. Various vector control policies were applied for VL to eradicate it from the system in [47]. On VL disease, various types of optimization techniques like multistate Markov model were found in [48]; an individual-based stochastic model of the VL was developed where temperature-driven sand fly was taken into consideration in [49], which has similarly mimicked the effects of the use of drugs administered to cattle in the control of the vector. Chapman et al. [50] created techniques to investigate longitudinal spatial occurrence information on visceral leishmaniasis and post-kala-azar dermal leishmaniasis. A bunch of three age-structured model variations in view of [51], each with people from an alternate sickness stage being the primary sources of transmission, asymptomatic people, beforehand safe people in whom disease has reactivated, and people with PKDL, was made in [52]. The expense viability of different medication medicines was concentrated in [53, 54]. According to information from the community intervention trial, the greatest probability advancement technique was used to locate a significant portion of the problem at hand boundaries [51]. Models developed by them broaden the SIR (susceptible-infected-recovered) structure for the human populace by fragmenting it into five overlapping stages as per a singular's disease status not entirely settled by the consequences of three demonstrative classes:

(a) A polymerase chain response (PCR) test, the faster disease diagnosis ready to get the existence of antigens [55]
(b) DAT, an immediate agglutination test [56] which estimates the immunizer reaction
(c) LST, the leishmanin skin test, likewise called Montenegro test [57], which recognizes the cell insusceptibility [58]

The model integrates the functionality and behavior of asymptomatic people on VL transmission that remains questionable right up to the present day [59]. The model likewise incorporates two paths of treatment of suggestive VL patients, a potential treatment disappointment, backslide toward PKDL and treatment of PKDL. A large portion of the problems under consideration boundaries were tracked down by fitting to information available at KalaNet, primarily using greatest probability advancement technique.

### 5.2 Statistical Model on VL

VL has been drawn up considering critical epidemiological investigations; lots of measurable data were collected, and an account of the overall VL epidemic

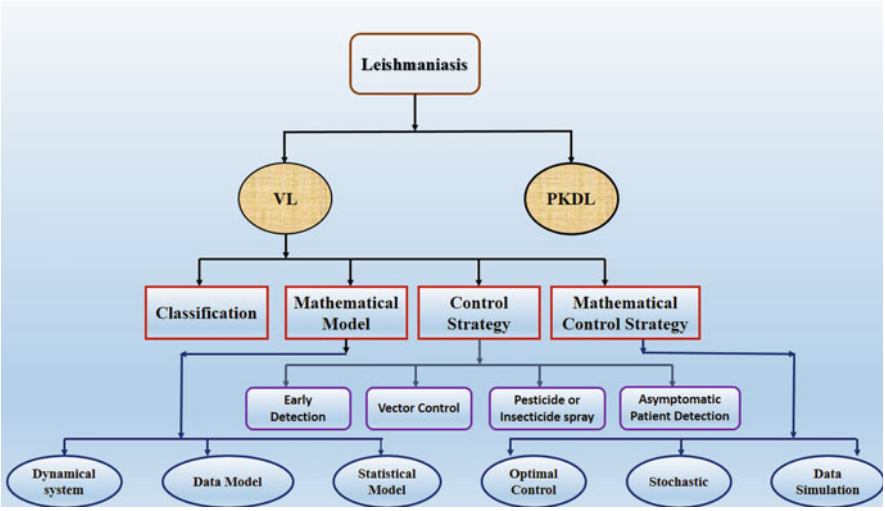


**Fig. 2** A flowchart of leishmaniasis

momentum was given by researchers and experts. Numerous analysts understood the significance of the use of data in the VL model turns of events. Illness data is largely used in three ways: the use of published data to create measurable models, the use of verifiable data for future dominance, and the use of existing data to align model boundaries in numerical epidemiological models.

The essential goal of creating a VL statistical model is to identify the actual parameters of VL transmission and to determine the connection between the extent of the parameters and the amount of the contaminated population. Miranda et al. [60] have considered statistical models with data and observed that spatial data are more reliable and accurate for VL epidemic review and testing. Using the geographic information system (GIS), the ENM model can predict risks at three levels (high, medium, and low). The predictive model has shown undeniable level and high accuracy (more than 90%) of intermediate-level data when approved with authentic data. Comparative methods were used to predict VL prevalence in North America and the Middle East [61, 62]. Oshaghi et al. [63] created a precise degree-day model for VL using a single triangle technique. Karagiannis-Voules et al. [64] have used Bayesian geo-statistical models to match event data from Brazil, and they have separated environmental and financial indicators using Bayesian factors.

Bi et al. [65] summed up age designs of VL contaminations in different areas. Biswas et al. [66] determined the previous distribution of various parameters and initial parameters in the light of perception information. The use of boundary assignments allows rearrangement tests to reflect multiple results with exceptionally conceivable results. Although most numerical models use estimates or evaluations with existing text as their system parameters [67–70], a growing number of studies

are using actual data to more accurately measure system parameters and approve their models (Fig. 2).

## 6 Future Research Directions

The major challenges in the diagnosis and treatment of VL are the following:

- Presence of asymptomatic patient in the system.
- PKDL patients are mostly unaware of their infectious stage.
- Proper awareness campaign in the VL-prone area.
- Since domestic animals like dogs or cats live in proximity to the human habitat, dogs act as amplifying hosts for VL.
- Like other disease outbreaks, it is almost impossible to predict when the disease can again reappear in the next phase. As a result, for the policymakers, it becomes tough to correctly predict the nature of the illness or the analysis of disease dynamics and thereby control the same with proper strategies.
- There is very less amount of reliable data available as open source.
- Due to the wide spread of the disease, fixing the statistical or mathematical modeling parameters is also a huge matter of concern.
- Since VL was almost abandoned for a long period, as a result, the virus must have changed its characteristics (mutated).
- In the poor economic zones where the VL is in the active state, people who live in slum areas and with poor hygienic zone are often found to have VL with HIV co-infection without showing any clinical symptoms. Most of the time, people are reluctant to undergo pathological tests, and there are many more.

The abovementioned challenges can be overcome, but rigorous efforts are needed, which leads to multidirectional research opportunities for the future, namely:

- Analysis of VL-PKDL with HIV co-infection and major focus on asymptotic patients.
- Theoretically mathematical model on VL-PKDL-HIV co-infection can be formulated, and optimal control strategies can be obtained to minimize the infectious population as well as a side effect of overdoses of the control measures.
- Rigorous and mass-scale awareness program to reduce the unsafe use of injection syringes.
- To reduce the amplification of the disease caused by the amplifying host like domestic dogs or domestic cats, proper hygiene and vaccination can be applied.
- Predictive model considering the multiple waves of the VL-PKDL can help find the possibilities of reoccurrence of the VL-PKDL.
- Some AI tools can also be deployed to handle a wide range of data and also the large deviation of the existing data.

- Proper mathematical modeling considering the genetic mutation of VL disease can analyze the current state and future of the disease.
- In poor economic zones where people are more exposed to both the disease and the co-infection, the proper awareness campaign, conducting a mass testing camp for both VL-PKDL and HIV, and ensuring the proper hygiene and nutrition to help malnutrition will reduce the impact of both diseases in the specific zones.

## 7 Conclusion

Additionally, future work in these four parts of VL numerical displaying should use present-day logical devices. The disservice of current displaying is the restricted variety of model kinds. A greater part of existing VL numerical models is ODE models, which are generally utilized but produce restricted anticipated results without subtleties. Accordingly, more factual, AI, and PDE models are expected to fabricate refined, thorough numerical models of VL. Measurable and AI models can all the more profitably use genuine information to guarantee model forecast precision, while utilization of a PDE model can improve anticipated results with age, orientation, financial status, morals, and spatial data. For the subsequent perspective, the consideration of true information, most test information as of now used to approve and check hidden numerical models is assessed or on the other hand accepted, thusly restricting the numerical model to reflect just information from past VL plague episodes. Future exploration endeavors ought to use late pandemic information with worldly and spatial information during the demonstrating stage, making the displaying system progressively powerful and reflecting ongoing information while foreseeing potential patterns of a continuous plague. The ongoing essential impediment of the third perspective, investigating conceivable control methodologies, is the control procedures' absence of pertinence in reality.

As a matter of fact, the best control methodologies recommended by the numerical models may not be operable, or they might be excessively cost restrictive to be executed. Operable control systems ought to be painstakingly quantized, like explicit thought of the ideal degree of canine winnowing in a specific time span or the degree of insect spray showering in every space impacted by VL. For the fourth perspective, current efforts to use mathematical simulations in games frequently yield insufficient information from simulation results. Most reproductions of VL models can foresee the pattern of VL contaminations. Future exploration ought to zero in on spatial reenactment and specialist-based reproduction as well as the investigation of the collaborations between numerous districts or conditions. Taking everything into account, the utilization of numerical models to study, break down, and foresee VL plagues and to investigate compelling furthermore, implementable control systems stays a functioning and study-commendable area of future examination. Anyway, research results from additional exhaustive examinations utilizing current logical devices will help general well-being associations comprehend and forestall the VL infection.

## References


1. Alvar J, Velez ID, Bern C, Herrero M, Desjeux P, Cano J, Jannin J, den Boer M, WHO Leishmaniasis Control Team. Leishmaniasis worldwide and global estimates of its incidence. PLoS One. 2012;7(5):e35671.
2. Trindade MAB, Luiza L, da Cruz Silva LMAB, Amato VS, Naafs B, Sotto MN. Post-kala-azar dermal leishmaniasis and leprosy: case report and literature review. BMC Infect Dis. 2015;15 (1):1–8.
3. Hossain M, Jamil KM. Pathology and mechanism of disease in kala-azar and post-kala-azar dermal leishmaniasis. In Kala Azar in South Asia. Springer, 2011. p. 11–4.
4. Ramesh V, Kaushal H, Mishra AK, Singh R, Salotra P. Clinico-epidemiological analysis of post kala-azar dermal leishmaniasis (PKDL) cases in India over last two decades: a hospital based retrospective study. BMC Public Health. 2015;15(1):1–8.
5. El Hassan AM, Khalil EAG, Elamin WM, El Hassan LAM, Ahmed ME, Musa AM. Misdiagnosis and mistreatment of post-kala-azar dermal leishmaniasis. Case Rep Med. 2013;2013
6. Mukhopadhyay D, Dalton JE, Kaye PM, Chatterjee M. Post kala-azar dermal leishmaniasis: an unresolved mystery. Trends Parasitol. 2014;30(2):65–74.
7. Quazi Tarikul Islam and Ariful Basher. Atypical presentation of post-kala-azar dermal leishmaniasis. Lancet Infect Dis. 2017;17(11):1218.
8. Lindoso JAL, Moreira CHV, Cunha MA, Queiroz IT. Visceral leishmaniasis and HIV coinfection: current perspectives, vol. 10. HIV/aids (Auckland, NZ); 2018. p. 193.
9. Rogelio Lopez-Velez GL, Mokni M. Manual on case management and surveillance of the leishmaniases in the WHO European region. 2017.
10. Diro E, Lynen L, Ritmeijer K, Boelaert M, Hailu A, van Griensven J. Visceral leishmaniasis and HIV coinfection in east Africa. PLoS Negl Trop Dis. 2014;8(6):e2869.
11. Ferreira GR, et al. Human competence to transmit Leishmania infantum to Lutzomyia longipalpis and the influence of human immunodeficiency virus infection. Am J Trop Med Hyg. 2018;98(1):126.
12. Sundar S, Singh OP, Chakravarty J. Visceral leishmaniasis elimination targets in India, strategies for preventing resurgence. Expert Rev Anti-Infect Ther. 2018;16(11):805–12.
13. Thakur L, Singh KK, Shanker V, Negi A, Jain A, Matlashewski G, Jain M. Atypical leishmaniasis: a global perspective with emphasis on the Indian subcontinent. PLoS Negl Trop Dis. 2018;12(9):e0006659.
14. Singh OP, Singh B, Chakravarty J, Sundar S. Current challenges in treatment options for visceral leishmaniasis in India: a public health perspective. Infect Dis Poverty. 2016;5(02): 1–15.
15. Dinesh DS, Ranjan A, Palit A, Kishore K, Kar SK. Seasonal and nocturnal landing/biting behaviour of Phlebotomus argentipes (Diptera: Psychodidae). Ann Trop Med Parasitol. 2001;95 (2):197–202.
16. Feliciangeli MD. Natural breeding places of phlebotomine sandflies. Med Vet Entomol. 2004;18(1):71–80.
17. Gawade S, Mangesh Nanaware RM, Gokhale, Adhav PS. Visceral leishmaniasis: a case report. Australas Med J. 2012;5(2):130.
18. Younis LG, Kroeger A, Joshi AB, Das ML, Omer M, Singh VK, Gurung CK, Banjara MR. Housing structure including the surrounding environment as a risk factor for visceral leishmaniasis transmission in Nepal. PLoS Negl Trop Dis. 2020;14(3):e0008132.
19. Goddard J. Physician's guide to arthropods of medical importance. CRC; 2016.
20. Richard M, Poche RG, Elnaiem D-EA, Perry D, Poche D. The role of Palmyra palm trees (Borassus flabellifer) and sand fly distribution in northeastern India. J Vector Ecol. 2012;37(1): 148–53.

21. Das A, Karthick M, Dwivedi S, Banerjee I, Ma T, Hapatra SS, Chaudhuri I. Epidemiologic correlates of mortality among symptomatic visceral leishmaniasis cases: findings from situation assessment in high endemic foci in India. PLoS Negl Trop Dis. 2016;10(11):e0005150.
22. Ranjan A, Sur D, Singh VP, Siddique NA, Manna B, Lal CS, Sinha PK, Kishore K, Bhattacharya SK. Risk factors for Indian kala-azar. Am J Trop Med Hyg. 2005;73(1):74–8.
23. Akuffo H, Costa C, van Griensven J, Burza S, Moreno J, Merce Herrero. New insights into leishmaniasis in the immunosuppressed. PLoS Negl Trop Dis. 2018;12(5):e0006375.
24. Rahman KM, Islam S, Rahman MW, Kenah E, Galive CM, Zahid MM, Maguire J, Rahman M, Haque R, Luby SP, et al. Increasing incidence of post-kala-azar dermal leishmaniasis in a population-based study in Bangladesh. Clin Infect Dis. 2010;50(1):73–6.
25. Islam S, Kenah E, Bhuiyan MAA, Rahman KM, Goodhew B, Ghalib CM, Zahid MM, Masayo Ozaki MW, Rahman RH, et al. Clinical and immunological aspects of post–kala-azar dermal leishmaniasis in Bangladesh. Am J Trop Med Hyg. 2013;89(2):345.
26. Addy M, Nandy A. Ten years of kala-azar in West Bengal, part i. did post-kala-azar dermal leishmaniasis initiate the outbreak in 24-Parganas? Bull World Health Organ. 1992;70(3):341.
27. Das VNR, Pandey RN, Siddiqui NA, Chapman LAC, Kumar V, Pandey K, Matlashewski G, Das P. Longitudinal study of transmission in households with visceral leishmaniasis, asymptomatic infections and PKDL in highly endemic villages in Bihar, India. PLoS Negl Trop Dis. 2016;10(12):e0005196.
28. Burza S, Mahajan R, Sanz MG, Sunyoto T, Kumar R, Mitra G, Mar´ıa Angeles Lima. HIV and visceral leishmaniasis coinfection in Bihar, India: an underrecognized and underdiagnosed threat against elimination. Clin Infect Dis. 2014;59(4):552–5.
29. Burza S, Mahajan R, Sinha PK, van Griensven J, Pandey K, et al. Visceral leishmaniasis and HIV co-infection in Bihar, India: long-term effectiveness and treatment outcomes with liposomal amphotericin b (AmBisome). PLoS Negl Trop Dis. 2014;8(8):e3053.
30. Zijlstra EE, Alves F, Rijal S, Arana B, Alvar J. Post-kala-azar dermal leishmaniasis in the Indian subcontinent: a threat to the south-east Asia region kala-azar elimination programme. PLoS Negl Trop Dis. 2017;11(11):e0005877.
31. Zijlstra EE, Musa AM, Khalil EAG, El Hassan IM, El-Hassan AM. Post-kala-azar dermal leishmaniasis. Lancet Infect Dis. 2003;3(2):87–98.
32. Garapati P, Pal B, Siddiqui NA, Bimal S, Das P, Murti K, Pandey K. Knowledge, stigma, health seeking behaviour and its determinants among patients with post kalaazar dermal leishmaniasis, Bihar, India. PLoS One. 2018;13(9):e0203407.
33. Purva Mathur JC, Samantaray MV, Samanta P. Visceral leishmaniasis/human immunodeficiency virus co-infection in India: the focus of two epidemics. J Med Microbiol. 2006;55(7):919–22.
34. Directorate of National Vector Borne Disease Control Programme et al. Accelerated plan for kala-azar elimination 2017. Ministry of Health and Family Welfare, government of India, New Delhi; 2017.
35. Molina R, Gradoni L, Alvar J. HIV and the transmission of Leishmania. Ann Trop Med Parasitol. 2003;97(Suppl 1):29–45.
36. Alvar J, Aparicio P, Aseffa A, Den Boer M, Cana- C, Vate J-PD, Gradoni L, Ter Horst R, L'opez-Velez R, Moreno J. The relationship between leishmaniasis and aids: the second 10 years. Clin Microbiol Rev. 2008;21(2):334–59.
37. Mock DJ, Hollenbaugh JA, Daddacha W, Overstreet MG, Lazarski CA, Fowell DJ, Kim B. Leishmania induces survival, proliferation and elevated cellular dNTP levels in human monocytes promoting acceleration of HIV co-infection. PLoS Pathog. 2012;8(4):e1002635.
38. Tremblay M, Olivier M, Bernier R. Leishmania and the pathogenesis of HIV infection. Parasitol Today. 1996;12(7):257–61.
39. Lopez-Velez R, Perez-Molina JA, Guerrero A, Baquero F, Villarrubia J, Escribano L, Bellas C, Perez-Corral F, Alvar J. Clinicoepidemiologic characteristics, prognostic factors, and survival analysis of patients coinfected with human immunodeficiency virus and Leishmania in an area of Madrid, Spain. Am J Trop Med Hyg. 1998;58(4):436–43.

40. Singh S. Changing trends in the epidemiology, clinical presentation, and diagnosis of Leishmania–HIV co-infection in India. Int J Infect Dis. 2014;29:103–12.
41. Cota GF, De Sousa MR, Rabello A. Predictors of visceral leishmaniasis relapse in HIV-infected patients: a systematic review. PLoS Negl Trop Dis. 2011;5(6):e1153.
42. Van Griensven J, Carrillo E, Lopez-Velez R, Lynen L, Moreno J. Leishmaniasis in immunosuppressed individuals. Clin Microbiol Infect. 2014;20(4):286–99.
43. DebRoy S, Prosper O, Mishoe A, Mubayi A. Challenges in modeling complexity of neglected tropical diseases: a review of dynamics of visceral leishmaniasis in resource limited settings. Emerg Themes Epidemiol. 2017;14(1):1–14.
44. Hirve S, Boelaert M, Matlashewski G, Mondal D, Arana B, Kroeger A, Olliaro P. Transmission dynamics of visceral leishmaniasis in the Indian subcontinent–a systematic literature review. PLoS Negl Trop Dis. 2016;10(8):e0004896.
45. Rock KS, le Rutte EA, de Vlas SJ, Adams ER, Medley GF, Deirdre T, Hollingsworth. Uniting mathematics and biology for control of visceral leishmaniasis. Trends Parasitol. 2015;31(6): 251–9.
46. Mubayi A, Castillo-Chavez C, Chowell G, Kribs-Zaleta C, Siddiqui NA, Kumar N, Das P. Transmission dynamics and underreporting of kala-azar in the Indian state of Bihar. J Theor Biol. 2010;262(1):177–85.
47. Stauch A, Duerr H-P, Picado A, Ostyn B, Sundar S, Rijal S, Boelaert M, Dujardin J-C, Eichner M. Model-based investigations of different vector-related intervention strategies to eliminate visceral leishmaniasis on the Indian subcontinent. PLoS Negl Trop Dis. 2014;8(4):e2810.
48. Chapman LAC, Dyson L, Courtenay O, Chowdhury R, Bern C, Medley GF, Deirdre T, Hollingsworth. Quantification of the natural history of visceral leishmaniasis and consequences for control. Parasit Vectors. 2015;8(1):1–13.
49. Poche DM, Grant WE, Wang H-H. Visceral leishmaniasis on the Indian subcontinent: modelling the dynamic relationship between vector control schemes and vector life cycles. PLoS Negl Trop Dis. 2016;10(8):e0004868.
50. Chapman LAC, Spencer SEF, Pollington TM, Jewell CP, Mondal D, Alvar J, Hollingsworth TD, Cameron MM, Bern C, Medley GF. Inferring transmission trees to guide targeting of interventions against visceral leishmaniasis and post–kala-azar dermal leishmaniasis. Proc Natl Acad Sci. 2020;117(41):25742–50.
51. Stauch A, Sarkar RR, Picado A, Ostyn B, Sundar S, Rijal S, Boelaert M, Dujardin J-C, Duerr H-P. Visceral leishmaniasis in the Indian subcontinent: modelling epidemiology and control. PLoS Negl Trop Dis. 2011;5(11):e1405.
52. Le Rutte EA, Coffeng LE, Bontje DM, Hasker EC, Ruiz Postigo JA, Argaw D, Boelaert MC, De Vlas SJ. Feasibility of eliminating visceral leishmaniasis from the Indian subcontinent: explorations with a set of deterministic age-structured transmission models. Parasit Vectors. 2016;9(1):1–14.
53. Meheus F, Balasegaram M, Olliaro P, Sundar S, Suman Rijal M, Faiz A, Boelaert M. Cost-effectiveness analysis of combination therapies for visceral leishmaniasis in the Indian subcontinent. PLoS Negl Trop Dis. 2010;4(9):e818.
54. Stauch A, Duerr H-P, Dujardin J-C, Vanaerschot M, Sundar S, Eichner M. Treatment of visceral leishmaniasis: model-based analyses on the spread of antimony-resistant l. Donovani in Bihar, India. PLoS Negl Trop Dis. 2012;6(12):e1973.
55. Das VNR, Bimal S, Siddiqui NA, Kumar A, et al. Conversion of asymptomatic infection to symptomatic visceral leishmaniasis: a study of possible immunological markers. PLoS Negl Trop Dis. 2020;14(6):e0008272.
56. Chappuis F, Sundar S, Hailu A, Ghalib H, Rijal S, Peeling RW, Alvar J, Boelaert M. Visceral leishmaniasis: what are the needs for diagnosis, treatment and control? Nat Rev Microbiol. 2007;5(11):873–82.
57. Manson-Bahr PEC, Heisch RB, Garnham PCC, et al. Studies in leishmaniasis in east Africa. iv. The Montenegro test in kala-azar in Kenya. Trans R Soc Trop Med Hyg. 1959;53(5):380–3.

58. Bern C, Amann J, Haque R, Chowdhury R, Ali M, Kurkjian KM, Vaz L, Wagatsuma Y, Breiman RF, Evan Secor W, et al. Loss of leishmanin skin test antigen sensitivity and potency in a longitudinal study of visceral leishmaniasis in Bangladesh. Am J Trop Med Hyg. 2006;75(4): 744–8.
59. Molina R, Jimenez M, Garcıa-Martınez J, Martın JVS, Carrillo E, Sanchez C, Moreno J, Alves F, Alvar J. Role of asymptomatic and symptomatic humans as reservoirs of visceral leishmaniasis in a Mediterranean context. PLoS Negl Trop Dis. 2020;14(4):e0008253.
60. Miranda VE, Araújo LCP, de Mattos Almeida MC, de Menezes FC, Morais MHF, Reis IA, Assuncao RM, Carneiro M. Relative risk of visceral leishmaniasis in Brazil: a spatial analysis in urban area. PLoS Negl Trop Dis. 2013;7(11):e2540.
61. Colacicco-Mayhugh MG, Masuoka PM, Grieco JP. Ecological niche model of Phlebotomus alexandri and P. papatasi (Diptera: Psychodidae) in the Middle East. Int J Health Geogr. 2010;9 (1):1–9.
62. Gonzalez C, Wang O, Strutz SE, Gonzalez-Salazar C, Sanchez-Cordero V, Sarkar S. Climate change and risk of leishmaniasis in north America: predictions from ecological niche models of vector and reservoir species. PLoS Negl Trop Dis. 2010;4(1):e585.
63. Oshaghi MA, Maleki Ravasan N, Javadian E, Rassi Y, Sadraei J, Enayati AA, Hassan Vatandoost Z, Zare SN, et al. Application of predictive degree day model for field development of sand-fly vectors of visceral leishmaniasis in northwest of Iran. J Vector Borne Dis. 2009;46 (4):247–55.
64. Karagiannis-Voules D-A, Scholte RGC, Guimaraes LH, Utzinger J, Vounatsou P. Bayesian geostatistical modeling of leishmaniasis incidence in Brazil. PLoS Negl Trop Dis. 2013;7(5): e2213.
65. Bi K, Chen Y, Zhao S, Kuang Y, Chih-Hang John W. Current visceral leishmaniasis research: a research review to inspire future study. Biomed Res Int. 2018;2018
66. Biswas S. Mathematical modeling of visceral leishmaniasis and control strategies. Chaos, Solitons Fractals. 2017;104:546–56.
67. Le Rutte EA, Chapman LAC, Coffeng LE, Jervis S, Hasker EC, Dwivedi S, Karthick M, Das A, Mahapatra T, et al. Elimination of visceral leishmaniasis in the Indian subcontinent: a comparison of predictions from three transmission models. Epidemics. 2017;18:67–80.
68. Shimozako HJ, Jianhong W, Massad E. Mathematical modelling for zoonotic visceral leishmaniasis dynamics: a new analysis considering up-dated parameters and notified human Brazilian data. Infect Dis Modell. 2017;2(2):143–60.
69. Wang Y, Wei H, Wang J, Liu J, Guo J, Zhang X, Weeks BL, Shen TD, Wei S, Guo Z. Electropolymerized polyaniline/manganese iron oxide hybrids with an enhanced color switching response and electrochemical energy storage. J Mater Chem A. 2015;3(41): 20778–90.
70. Zhao S, Kuang Y, Chih-Hang W, Ben-Arieh D, Ortigao MR, Bi K. Zoonotic visceral leishmaniasis transmission: modeling, backward bifurcation, and optimal control. J Math Biol. 2016;73 (6):1525–60.

# Elucidating the Role of miRNA in Inflammasome-Mediated Immune Response in Leishmaniasis

Ria Bhar, Kuntal Pal, Srijan Haldar, and Joydeep Paul

**Abstract**

The inflammasome is a cell cytoplasm-localized multimeric protein complex of host defense mechanism which gets activated due to infection in the innate immune system via pathogens or due to receiving cell damage signals or due to induction of dendritic, antigen-presenting cells, etc. Usage of various pattern recognition receptors or PRRs helps to identify various factors which are responsible for generating innate inflammatory responses. MicroRNAs are one of the major posttranscriptional regulators which even change immune regulatory mechanisms through inflammasome signaling modulation. Deregulation of these noncoding RNAs may lead to various diseases. Previous study reports suggested that *Leishmania* infection induced inflammasome activation especially in macrophages as a protective measure of the host immune system. According to current research, excessive productions of cytokines are inhibited by regulatory miRNAs, thus maintaining a homeostasis between pro- and anti-inflammation to optimize the inflammatory response in host cells. In this article, we have discussed different inflammasome activation and its regulation mechanism via miRNA in light of leishmaniasis.

**Keywords**

Inflammasome · miRNA · NLR · Leishmaniasis · Epigenetic regulation

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## 1 Introduction

Inflammation is a biological response of the host's body against harmful stimulation induced by pathogens in all metazoans. Any change in physiological homeostatic balance may generate an inflammatory response at micro level [1]. Innate immune response responds against signals like pathogen as well as sterile incursions like trauma, tumor, ischemia, metabolic perturbation, etc. Along with innate immune response, dendritic cell and APCs, also called antigen-presenting cells, transfer host immune response to B and T lymphocytes to induce second level of immune response, i.e., adaptive immune system. Lymphocytes have the advantage of antigen receptor gene rearrangement mechanism through which it can generate variety of antigen receptor to recognize diverse antigen [2]. However in case of innate immune system, pathogen recognition is instructed by a specific number of germ line-coded PRRs, also called pattern recognition receptors. The factors which are responsible for generating innate inflammatory response can be divided into three main categories: PAMPs (pathogen-associated molecular patterns), preserved components of different infectious agents, and DAMPs (damage-associated molecular patterns). PAMPs and DAMPs are being identified by different cells, belonging to both innate and adaptive immune system along with pattern recognition receptors (PRRs) [3, 4]. Based on the subcellular localization, there are two major categories of PRRs that exist. The first category includes Toll-like receptors/TLRs and C-type lectin receptors/CLRs which are transmembrane proteins mostly located on endosomal membrane and plasma membrane. TLRs and CLRs detect PAMPs and DAMPs located in the extracellular environment, whereas RIG-I-like receptor/RLR, the AIM2-like receptor/ALR, nucleotide-binding domain, leucine-rich repeat-containing proteins/NLR, and PRR-like proteins in the second class reside in intracellular compartments [5, 6]. Martinon et al. [6] first coined the term "inflammasome" in 2002 where he explained that these cytoplasmic supramolecular structures assemble in cytoplasm of activated immune cells. This inflammasome finally activates pro-inflammatory caspases 1 and 11 [6, 7] which leads to induced innate immune response along with inflammation. To restrict the collateral complications in host system, inflammasome signaling mechanism is highly regulated. The PRRs present in the cytoplasm, are assembled to form inflammasome complex, which can be categorized based on their domain structures like NLR, PYHIN, NBD, and NLR family proteins containing N-terminal effector domains which have four differently characterized domains, like acidic transactivation domain, pyrin domain, caspase recruitment domain/CARD, and BIR (baculoviral inhibitory repeat)-like domains [8]. PYHIN protein family members contain HIN200 and pyrin domains [9]. PRRs can be found in a variety of cell types including neutrophils, dendritic cells, epithelial cells, and macrophages [4]. Inflammation through the activation of caspases can be achieved through the assembly of some heterotrimeric scaffold protein complex like NLRP/NOD-like receptor-pyrin-containing proteins or proteins like AIM2. In some cases extra adapter and effector partner like apoptosis-associated speck-like protein containing CARD (ASC) [10] recruitment is necessary. Caspase activation due to rapid pro-caspase zymogen

conversion results in pro-inflammatory cytokine IL-1β (interleukin-1β) and/or IL-18 (interleukin-18) activation which in turn turns on a variety of inflammation regulations [11].

MicroRNAs are almost found in every eukaryotic cell that is conserved over the species. These miRNAs are small RNA molecules that play a significant function in translation repression through binding to target molecules and also in gene silencing. miRNA is small ncRNA of about 22 nucleotides long, which at the posttranscriptional level influences the expression of genes by barging in with the translation of the miRNA into the respective protein. Dysregulation of miRNA may lead to many diseases particularly cancer. Relevant studies have also shown that miRNA plays a crucial role in figuring out the adaptive and innate response against pathogen [12]. In various studies, miRNA has been shown playing a crucial role in immune response development and functions against leishmaniasis [13]. The miRNA expression shows differences that have been observed in the *Leishmania major* and *Leishmania donovani*, in vitro in infected human dendritic cells [14]. When the human phagocyte is infected in vitro with the *Leishmania donovani*, the parasite showed alteration or changes on the expression of miR-21, miR-155, and also miR-146b-5p [14]. Similarly, when the J774 murine macrophage is infected in vitro with *Leishmania infantum*, it increases the expression of miR-155. miRNAs are also found responsible in modulating cytokine response in host cells.

Post-transcriptionally in *Leishmania* infection to regulate both pro-inflammatory and anti-inflammatory response [15]. During infection, host macrophages activate the inflammatory response by producing pro-inflammatory cytokines and NO (nitric oxide) [16]. In uninfected cells, the production of these inflammatory cytokines is inhibited by the regulatory activity of miRNAs, which checks the uncontrolled cytokine synthesis. Hence, the role of miRNAs in mammalian cells is very important, as they maintain the balance between pro- and anti-inflammation to optimize the inflammatory response in host cells [17].

But only very few studies have been done to decipher the regulation of miRNAs in activating inflammation in leishmaniasis. In this current study, we discussed in detail different approaches of miRNA regulation in inflammasome formation and assembly in leishmaniasis. We have also discussed about the fundamentals of inflammasome and its types that are being reported in various pathogenic and nonpathogenic diseases.

## 2 Pathogen-Derived Activating Signals

Among various PAMPs bacteria-associated signals are the most well-studied example in the area of inflammasome activation. Gram-negative bacteria like *Pseudomonas*, *Salmonella*, *Shigella*, and *Legionella* spp. have rod-shaped secretion protein flagellin, or a type III (T3SS) or type IV (T4SS) which is recognized by NAIP the binding partner of NLRC inflammasome [18]. Potassium (K+) efflux caused due to pore formation by various bacteria like *Streptococcus pneumoniae*, *Staphylococcus aureus*, enterohemorrhagic *Escherichia coli*, etc. is responsible for NLRP3

inflammasome activation [19–21]. Lipoteichoic acid containing Gram-positive pathogens like *L. monocytogenes* [22] triggers NLRP6 inflammasome activation, whereas acetylated lipopeptides is recognized by NLRP7 of human macrophages [23]. Pyrin group of inflammasome activated by inactivated Rho GTPases resulted due to bacterial toxins like *Clostridium botulinum* ADP-ribosylating C3 toxin, *Clostridium* diffusible cytotoxin TcdB, etc. [24] (PMID: 24919149). Noncanonical pathway of inflammation was activated by caspase-4/caspase-5 (*Homo sapiens*) or caspase-11 (murine) in recognition of intracellular lipopolysaccharide (LPS) from Gram-negative bacteria [25]. It was also reported that specific protein factors present in various groups of pathogens induce inflammation (Table 1). Selective commensal bacteria from the huge mammalian microbiome able to activate NLRP6 inflammasome to induce IL-18 secretion from IECs [26] along with NLRP3 inflammasome signaling mediated IL-1β maturation in intestinal monocytes to promote intestinal inflammation [27].

## 3 Host-Derived Activating Signals

Endogenous DAMPs are another vital source of inflammasome activation, released due to tissue injury. Internal signals include mitochondrial dysfunction, ion efflux, and reactive oxygen species (ROS) inducing NLRP3 activation. NLRP3 signal initiates destruction of trans-Golgi network (TGN) where NLRP3 recruitment occurs followed by assembly [21]. Cytosolic K+ efflux plays important role in commencement of the pathways like noncanonical and canonical activation via NLRP3 and caspase-11, respectively [28]. NLRP3 oligomerization regulator *protein* NEK7 (a serine-threonine kinase) [29] and intracellular chloride efflux [30] also play important role in canonical pathway activation. However the role and mechanism of other ions like Ca++, Na+, and Zn++ efflux in NLRP3 activation remain unclear till now [31, 32].

Mitochondrial dysfunction is another key player of NLRP3 pathway activation. According to recent reports, TLR signaling-mediated oxidized mtDNA release in cytosol causes aberrant NLRP3 assembly [33]. Again cytosolic localization of mtDNA from mitochondria also requires NLRP3 which indicates the possible regulatory role of NLRP3 up- and downstream of mtDNA [34]. In macrophages along with mtDNA, activation of intrinsic apoptotic pathway, with caspase-3 and -7 activation, drives IL-1β secretion [35]. But generation and activation of NLRP3 inflammasome still remain highly controversial. ROS generation can also induce NLRP3 inflammasome activation either by increasing interaction between thioredoxin-interacting protein and NLRP3 [36] or NADPH oxidase [37], whereas other studies reported that NADPH oxidase activity and NLRP3 inflammasome activation are two independent events [38].

**Table 1** Involvement of inflammasomes in different diseases

| Types of inflammasome | miRNA | Regulation type | Types of diseases | References |
|---|---|---|---|---|
| NLRP3 | miR-233-3p | Negative | Inflammatory bowel diseases, hepatocellular carcinoma, acute lung injury/acute respiratory distress syndrome | [65, 66, 177] |
| NLRP3 | miR-133a-1 | Negative | Inflammatory diseases | [112] |
| NLRP3 | miR-22 | Negative | Gastric cancer | [113, 114] |
| NLRP3 | miR-30e | Negative | Parkinson's disease | [67] |
| NLRP3 | miR 7 | Negative | Parkinson's disease | [178] |
| NLRP3 | miR-146a-5p | | Autoimmune diseases, multiple sclerosis | [179] |
| NLRP3 | miR-20b-5p | | Multiple sclerosis | [180] |
| NLRP3 | miR-495-3p | | Cardiac injury, ALI | [181] |
| NLRP3 | miR-330-3p | | Renal inflammatory disease | [182] |
| NLRP3 | miR-21 | | Liver tissue, idiopathic pulmonary fibrosis | [183] |
| NLRP3 | mir-17-5p | | Obesity disease | [184] |
| NLRP3 | mir-141-3p | | Bladder cancer | [185] |
| NLRP4 | mir-141-3p | | Bladder cancer | [185] |
| NLRP1 | miR-199a-3p | Negative | | [118] |
| NLP7 | miR-18-b | Negative | Breast cancer | [119] |
| NLRP12 | miR-372 | | Ulcerative colitis | [186] |
| AIM2 | miR-143 | | Inflammatory diseases | [187] |

## 4 Canonical and Noncanonical Inflammasomes

Inflammasomes are made of three major units: a receptor molecule, an effector protein, and an adaptor protein. Receptor molecules like PRR (pattern recognition receptor) family are the proteins which first activate the assembly process by sensing the presence of foreign particles. Some examples of receptor molecule are NOD

(nucleotide-binding oligomerization domain), LRR (leucine-rich repeat), NLR (NOD-like receptor) family, ALR (absent in melanoma 2-like receptor) family, IFI-16 (interferon-inducible protein 16), and RIG-I (retinoic acid-inducible gene I). After activation via adaptor protein (ASC protein) which possesses a caspase activation and recruitment domain (CARD), sensor protein activates specific effector caspase protein [39, 40]. For activation, interaction between inflammasome sensor and inflammasome caspase is required. In the canonical inflammasome pathways, procaspase-1 catalytically is converted into caspase-1, whereas noncanonical inflammasome assembly promotes activation of procaspase-4, procaspase-5 in humans, or procaspase-11 in mice through an undefined mechanism. Aspartate-specific cysteine protease family proteins like caspase-1 and caspase-11 possess conserved pro-domains which interact with other proteins. These homotypic interacting domains can be classified into few subcategories like caspase activation and recruitment domain (CARD), pyrin domain (PYD), death domain (DD), or death effector domain (DED) [41].

## 5 Canonical Inflammasome Assembly

1. *NLRC4 Inflammasome Assembly*
   PAMP (e.g., type III secretion system and bacterial flagellin protein)-medicated activation is common in case of NLRC4 inflammasome, and the process requires involvement of another NLR family member, NAIP. NAIP has the ability to directly interact with bacterial ligands.

   NLRC4 inflammasome remains in a self-inhibited state due to regulation of its two domains, the NBD (nucleotide-binding domain) and WHD (winged-helix domain) [42]. Only one Pro J-bound NAIP2 molecule is required to activate NLRC4 oligomerization. After substantial structural reorganization, 1 activated NLRC4 starts a chain reaction to activate other dormant NLRC4 which finally leads to formation of 10 to 12 disk-like structure [43, 44]. In case of flagellin-mediated NLRC4 inflammasome activation, conserved regions of flagellin are recognized by NAIP5 which interacts with NLRC4 for its oligomerization [45]. The C-terminal side of different bacterial flagellin generates a species-specific structural epitope, which is further detected by NAIP5, although the binding efficiency varies across species. Thus NLRC4 induction potential also varies from species to species [46]. Phosphorylation at Ser533 by protein kinase Cδ (PKCδ) plays vital role in posttranslational regulation of NLRC4 inflammasome. The process can be induced by *Salmonella typhimurium* infection either via NAIP-mediated pathway or *Shigella* T3SS inner rod protein MxiI-mediated NAIP2 pathway [47, 48]. Direct interaction between NLRC4 S533A and NLRP3 recruits ASC and activates caspase [49]. NLRC4 is also regulated through ubiquitination via surg1, a 26S proteasome-associated protein. To understand the underlying molecular mechanism, studies are required on NLRC4 inflammasome [50].

2. *NLRP3 Inflammasome Assembly*
   As previously mentioned NE7 is required to activate NLRP3 inflammasome. During interphase stage to induce inflammasome assembly, NEK7 directly interacts with LRR of NALP3 resulting in its oligomerization [51]. NLRP3 activation was induced by fungal, viral, and bacterial pathogens, crystals, pore-generating toxins, and DAMPs such as hyaluronan and ATP [41].This inflammasome is activated by K+ efflux [21], translocation to mitochondria [52–54], mitochondrial ROS formation [54], cardiolipin and mtDNA release to cytosol, and cathepsin release from lysosome [55]. Basically NLRP3 inflammasome can be activated by two major signaling cascades. First one is NF-kB-activating stimulus to generate pro-IL-1β and required substantial increase of Nlrp3 expression [56], and this Nlrp3 activation further provides the second signal [41]. The TLR stimulation also occurs parallel to NLRP3 activation as TLR4 provides signals to release adaptors like MyD88, IRAK1, and IRAK4 [57–59], resulting in deubiquitination of Nlrp3 [58, 60, 61].

   This inflammasome is regulated by priming as well as multiple activation stages. Since the uncontrolled expression of inflammasome is responsible for autoimmune and metabolic disorders like cryopyrin-associated periodic syndromes (CAPS), type II diabetes, Alzheimer's disease (AD), etc., thus NLRP3-mediated inflammatory resonance is a closely regulated process. Cellular NLRP3 upregulation due to NF-κB activation, pro-IL-1β transcription, and translation initiation as pro-inflammatory stimuli are considered as priming step [56].
3. *NLRP3 Priming*
   Activation of nuclear protein NF-κB is mediated by FADD (Fas-associated death domain) and later by caspase-8 [62]. Transcription-independent regulation mechanism such as LPS-TLR4-MyD88 signaling through NF-κB activation can prime NLRP3 by stimulating its deubiquitination. dsRNA binding to TLR3 induces NLRP3 priming via TRIF/RIPK1/FADD-dependent pathways [63]. Moreover priming-independent NLRP3 assembly also occurred in case of TLR, IRAK-1, and IRAK-4-dependent activation [59].
4. *Posttranscriptional Regulation*
   Evidence showed that, due to *Mycobacterium tuberculosis* infection, epigenetic regulators (histone acetylation, DNA methylation) are able to upregulate NLRP3 mRNA expression [64]. A major posttranscriptional regulator, miR-223, downregulates NLRP3 expression by binding its UTR-binding sites in myeloid cells [65] and modulates innate immune response in case of intestinal inflammation [66]. Other miRNAs like miR-30e in Parkinson's disease [67], miR-22 in heart disease, and squamous cell in oral cancer [68] are reported to negatively regulate nlrp3 inflammasome. The role of another vital posttranscriptional regulator, long noncoding RNAs (lncRNAs), in NLRP3 expression still needs to be revealed. ANRIL, the antisense noncoding RNA in the INK4 locus, can positively regulate NLRP3 by sequestering miR-122-5p in uric acid nephropathy [69]. Some other lncRNAs also showed positive or negative regulation against inflammasome signaling.

5. *Posttranslational Modulation*
   Phosphorylation and deubiquitination are the major posttranslational modifications by which NLRP3 inflammasome is mostly regulated. JNK1-mediated Ser198 phosphorylation (in humans) and E3 ubiquitin ligase Pellino2-mediated ubiquitination lead to self-association and inflammasome assembly [70, 71]. However FBXL2 E3 ligase-mediated ubiquitination leads to negative regulation of NLRP3 via degradation [71]. The presence of bile acid PKA-induced phosphorylation of NLRP3 at mouse Ser 291 inactivates inflammasome [72]. Dephosphorylation at Tyr861 in NLRP-Golgi-mediated protein kinase-D by phosphatase 2A (PP2A) [73] and protein tyrosine phosphatase non-receptor 22 (PTPN22) turns on inflammasome activation [74].
6. *NLRP1 Inflammasome Assembly*
   The NLRP1 inflammasome is composed of five domains, NOD, LRRs, NLRP1 (amino terminal PYD domain-containing protein 1), CARD (carboxy-terminal domain), and FIIND (function-to-find domain). CARD domain of caspase-1 directly docks with NLRP1 inflammasome and initiates oligomerization which is increased due to pyrin domain of ASC binding [75].

   C-terminus CARD domain and function-to-find domain (FIIND) are located in human NLRP1 and murine NLRP1b. Autolytic proteolysis by FIIND results in auto-inhibited NLRP1. Inhibition of FIIND auto-processing activity causes blocking of NLRP1 activation [76, 77]. In response to anthrax lethal factor, both the rodent and human NLRP1 molecules are cleaved by proteolysis to activate inflammasome [77, 78]. NLRP1b N-terminus region degradation and instability caused by pathogen-derived proteases like lethal factor, IpaH7.8, or *Shigella* effector protein, etc. lead to the direct cleavage of NLRP1b. This resulted C-terminal fragment formation leading to caspase-1 activation [79]. As per genome-wide CRISPR/Cas9 knockout screening, N-terminus degradation of NLRP1 is catalyzed by UBR2 (the N-end rule ubiquitin ligase) and E2 ubiquitin-conjugating enzyme UBE2O [80, 81]. However C-terminal FIIND (UPA)-CARD segment of CASP1 protein is recruited, and NLRP1b inflammasome assembly is initiated [79]. NLRP1b acts as an inflammasome marker for [82] *Toxoplasma gondii* [83] and in case of cytosolic ATP depletion [84]. The crosstalk between NLRP1 with NLRP1 and murine NLRP1b and its role in ATP depletion still remain unclear. Also, the role of NBD and LRR sites in NLRP1 crosstalk is not clear, but according to few reports, PYD and LRR domains have an inhibitory regulation in NLRP1 self-organization. Disruption of this inhibition due to NLRP1 mutation leads to the inflammasome activation which may cause skin inflammation in humans [85]. However the role of these domains with respect to pathogen-encoded effectors needs further investigation. Moreover according to the current reports, cytosolic serine proteases Dpp8 and Dpp9 inhibition activates NLRP1b (in mice), which leads to pyroptosis induction in a proteasome-dependent activity [86]. In case of humans, Dpp9 binding with FIIND causes stabilization of NLRP1 in an auto-inhibited state [87]. However the molecular mechanism of Dpp8/Dpp9 pathways steel needs to be explored.

7. *NLRP6 Inflammasome Assembly*
   A previously less characterized inflammasome pathway, NLRP6, has already been well established regarding its involvement in colitis development and progression [88]. NLRP6 is a modulator of host-microbe interactions via both inflammasome-dependent and inflammasome-independent pathways. This inflammasome was reported to involve in innate immune defense regulation of the intestine against enteric viral or bacterial infections [89]. NLRP6 (previously known as PYPAF5) forms filamentous structures through the self-assembly mechanism of pyrin domain. The subsequent ASC binding through pyrin domain enhances the oligomerization of NLRP6. It was reported that this inflammasome regulates IL-18 [90], NF-$\kappa$B, and the mitogen-activated protein kinase (MAPK) signaling pathways [91] and also influences interferon type I and III production [92]. Taurine-like microbiota-associated metabolites regulate NLRP6 inflammasome signaling via direct or indirect interaction. NLRP6 is also activated by bacterial TLR ligands [93], lipoteichoic acid [22]. NLRP6 inflammasome is also inhibited by stress-induced corticotropin-releasing hormone [94]. However more studies will be needed to understand the mechanism of interaction between NLRP6 and its regulators' inflammasome-associated disease modulation.
8. *AIM2 and IFI16 Inflammasomes*
   ALRs such as AIM2 and IFI16 are cytosolic sensors which detect various kinds of endo- and exogenous ligands. Aim2 consists of PYD (N-terminal pyrin) domains and HIN200 domains and is expressed in various tissues like the spleen, peripheral blood, intestine, etc. Due to lack of NOD domain, the self oligomerization process was unable to take place for both AIM2 and IFI16. Both of these sensors detect cytosolic DNA with the help of their carboxy-terminal HIN or hematopoietic interferon-inducible nuclear domain. Negatively charged DNA backbone binds with the positively charged HIN domain [95], followed by helical assembly of amino terminal AIM2 pyrin domain to start polymerization process. This assembly also serves a platform to cluster the downstream $ASC_{CARD}$ domain through PYD-CARD interaction [96]. Although the DNA binding affinity of $IFI16_{HIN}$ domain is weak, $IFI16_{PYD}$ plays a vital role in inflammasome assembly on DNA filaments in a cooperative binding mode [97]. Aim2 is induced by type 1 IFN cytokine signaling downstream to NF-κB [98]. The HIN200 domain of AIM2 was able to detect at least 80 base-pair-long cytosolic dsDNA in a nonspecific way to activate this inflammasome [99]. Bacteriolysis is a prerequisite before detection of dsDNA by AIM2. Moreover viral DNA recognition is also done by AIM2 which leads to antiviral immune response. However some DNA virus has the capability to escape AIM2-like human herpes simplex virus (HSV) [79]. AIM2 activation leads to pyroptosis in caspase-1-mediated pathway or in caspase-8-dependent signaling, based on amount of dsDNA detected in cytosol [100]. Although AIM2 activation against RNA virus is reported, the mechanism is still unknown. AIM2 is mostly regulated by decoy proteins like PYD-only proteins (POPs), HIN-200 protein p202, etc. PYD-only proteins (POPs) interaction with the PYD of ASC

or PYD of PRRs leads to inhibition of PYD-PYD interactions, which is required for AIM2 inflammasome assembly [101, 102]. HIN-200 protein p202 lacks PYD domain but is able to interact with dsDNA which again causes inhibition to inflammasome activation [103].

9. *Recruitment of ASC and Caspase-1*
   The assembly of CARD domain-absent NLRs (like NLRP3, NLRP6, AIM2, NLRP7, IFI16, etc.) takes place through pyrin-pyrin domain interaction between NLRs and ASC adaptor protein. This interaction finally helps to bind effector protein caspase-1 through CARD-CARD interaction [92]. Clustering of multiple pro-caspase-1 at inflammasome initiates the self-cleavage and activation to generate active caspase-1 protein.
10. *Pyrin Inflammasome*
    Cells like neutrophils, monocytes, and DCs possess a 86KD pyrin inflammasome, which consists of four functional domains: a coiled coil (CC), a zinc finger domain (bBox), PYD domain, and a B30.2/SPRY domain [104]. Due to inactivating variation of the RhoA GTPase, pyrin-mediated caspase-1 activation occurred in an ASC-dependent manner [105]. Pyrin detects cytoskeletal remodeling of host proteins due to bacterial virulence [106]. Pyrin inflammasome contains two serine phosphorylation sites, which at the phosphorylated state binds with 14-3-3 proteins and remains at inactive state. Due to bacterial infection, dephosphorylation of pyrin occurs, which leads to an oligomeric pyrin-ASC inflammasome complex [106]. Inhibition due to dephosphorylation may cause immune escape strategy for pathogens against pyrin inflammasome [107]. Similarly aspirin associated with cytoskeleton inhibition in microtubule dynamics also inhibits pyrin inflammasome activation [108].

## 6 Noncanonical Inflammasome Activation

"Noncanonical inflammasome" functions in a NLR-ASC-caspase-1 paradigm-independent way via direct interaction between cytosolic LPS and CARD domains of caspase-4 and caspase-5 (in humans) and caspase-11 (in mice). In this system caspase-11 plays the role in LPS transfection in the cytosol [109]. Upon activation caspase-11 initiates IL-1β proteolytic maturation and dimerization. Activation of these noncanonical caspases leads to the cleavage of gasdermin D, which causes pyroptosis [110]. However, activation of noncanonical inflammasome is limited to penta- and hexa-acylated lipid A moieties of LPS. Tetra-acylated lipid A can evade this system as shown in *Francisella* spp. infection [111].

## 7 Epigenetic Mechanisms of Inflammasome Regulation

In humans, NLRP3 is regulated by miR-233-3p, which was first to be identified as a miRNA to regulate inflammasomes. NLRP3 mRNAs possess a conserved binding site (among mammals) for miR-233-3p in its 3′UTR region. Higher expression of

miR-233-3p was detected in myeloid cell lineage, especially in monocyte. However its expression is absent in B and T lymphocytes [65]. On the other hand, NLRP3 expression is low in neutrophils. During monocyte maturation and differentiation, NLRP3 expression increases and miR-233-3p expression levels decrease [66]. NLRP3 inflammasome activation is also indirectly regulated by miR-133a-1. This miRNA regulates IL-1β production and thus regulates the activation of effector protein caspase-1 [112]. In gastric cancer upregulated expression of NLRP3 has been reported, which leads to cancerous cell proliferation and tumor progression. Healthy gastric mucosa constitutively expresses miR-22 which attenuates NLRP3 expression by directly targeting its mRNA [113] and thus decreases the oncogenic effects of NLRP3. *Helicobacter pylori* infection causes NLRP3 inflammasome activation along with downregulation of miR-22 expression in gastric cell [114, 115].

miR-21 expression positively regulates NLRP3, ASC, and caspase-1-mediated inflammatory regulation [116]. NLRP3 is also a possible target of miR-30e which have conserved binding sites in NLRP3 3′UTR [67]. NLRP3 inflammasome is essential for neuro-inflammation highly expressed in active microglia. Excessive production of inflammatory cytokines leads to dopaminergic neuronal degeneration [117]. In Parkinson's disease (PD), higher expression of miR-30e via negative regulation of NLRP3 helps to improve the neuronal damage. NLRP3 regulation via miR-7 is also well established in PD [117].

NLRP1 inflammasome was reported to regulate via miR-199a-3p. In acute lung injury (ALI), NLRP1 inflammasome is significantly downregulated in ALI tissue sample having a high appearance of miR-199a-3p [118]. NLRP1 level increased when miR-199a-3p is downregulated and causes pro-IL-1β and pro-IL-18 activation in caspase-mediated manner. High expression of active IL-1β and IL-18 leads to immune response against disease [118].

NLRP7 was regulated by mir-18b which was overexpressed in breast cancer cell line. Downregulation of mir-18b causes NLRP3 upregulation which induces cellular migration and metastasis [119].

## 8 Inflammasome and Leishmaniasis

Intracellular pattern recognition receptors recognize pathogens or signals produced from damaged/cancerous cells to produce inflammasomes in innate immune cells. In this aspect, the role of the nucleotide-binding domain leucine-rich-repeat-containing proteins or NLRs in the detection of pathogens has been found to be of great importance [4]. Specifically, involvement of NLRP3 protein, a member of the NLR family, has been best studied [120]. It is activated by pathogens and chemicals that disrupt host cell membranes, ultimately leading to the activation of caspase-1. Interaction between pyrin-pyrin domains of NLRP3 oligomerizes and polymerizes ASC (apoptosis-associated speck-like protein containing a CARD domain), exposing numerous CARD domains in filaments, simultaneously activating multiple inflammatory genes, including Casp11 and IL-1, in response to the microbial

priming. A secondary signaling has also been initiated, when macrophage membranes open up and intracellular K+ decreases. Classical NLRP3 activation is related with an increased expression of ROS and lysosomal cathepsins. Caspase-11 stimulates noncanonical NLRP3 inflammasome pathway. Incorporating gasdermin-active D's N-terminal domain into macrophage membranes creates a channel for K+ exocytosis and noncanonical NLRP3 activation. No illness or dangerous substances are required to activate inflammasomes. Therefore these two signals prevent unnecessary activation of inflammation in host [121, 122]. *Leishmania* and other intracellular parasites negatively regulate these two signals to inhibit host response.

There are more than 1 million new cases of leishmanial infections each day, and an additional 300 million people are at more risk in 88 countries. Different species of *Leishmania* parasites are exhibiting clinical manifestations in human hosts, ranging from cutaneous skin lesions to the infections in visceral organs like the spleen and liver. *Leishmania* parasites having a digenetic life cycle survive in the sand fly in the form of flagellated promastigotes and in aflagellated amastigote form in the human host. In an immune response to the *Leishmania* infections, two types of signaling are found in host phagosomes: (i) Th-1 responses, which are critical to recovery against the infection, and Th-2 responses which are linked to high parasite burdens and illness transmission [123]. Reports from different research groups have been clearly established that infections with multiple *Leishmania* species trigger NLRP3 inflammasome as the outcome of activated host immune response to clear the parasite burden. Simultaneously, parasites adopt various strategies to evade this inflammatory signaling [124].

*L. amazonensis*, *L. major*, *L. braziliensis*, and *L. infantum* infections stimulated production of caspase-1 and IL-1 via Dectin-1 using mannose receptor, which subsequently stimulate p47phox protein and arachidonic acid-NADPH oxidase pathway to produce reactive oxygen species, leading to the restriction of parasite replication. Thus, inflammasome activation in the host macrophages was shown to be a key factor in limiting parasite reproduction [125, 126]. In *Leishmania*-infected BMDMs, caspase-11 activates the NLRP3 inflammasome. Also, reports are there to show that activation of caspase-11 in host macrophages is being stimulated by LPG (lipophosphoglycan) from different *Leishmania* species, establishing activation of noncanonical pathway of NLRP3 inflammasome [127, 128], although Gurung et al. (2015) showed that activation of NLRP3 inflammasome in *L. major*-infected mice skewed the host immunity toward Th-2-type responses by producing IL-18-mediated IL-4 cytokines [129]. Involvement of NLRP3 inflammasome in various types of leishmaniasis has been listed in Table 2.

## 9 miRNA and Leishmaniasis

Short, noncoding RNAs of 22–24 nucleotides long are regarded as miRNAs, synthesized by RNA polymerase II and III enzymes. Genes are being regulated by the miRNA-RISC complex after precursor miRNA undergoes cytoplasmic translocation from the nucleus, ultimately leading to the mRNA breakdown and

**Table 2** Role of NLRP3 inflammasome in various types of leishmaniasis

| Types of leishmaniasis | *Leishmania* sp. | Target protein | References |
|---|---|---|---|
| Localized cutaneous leishmaniasis | *Leishmania (Viannia) panamensis* | Th17 | [188] |
| Cutaneous leishmaniasis | Mixed infection | IL-1β, IL-6, and IL-17 | [176] [189] |
| Visceral/ cutaneous leishmaniasis | *Leishmania amazonensis* | CASP1, CASP-11, and IL-1β | [127, 190, 191] |
| Localized cutaneous leishmaniasis (LCL) | *Leishmania braziliensis* | IL-1β | [192, 193] |
| Cutaneous leishmaniasis | *Leishmania major* | Gbpb2/Gbp1 | [194] |
| Cutaneous leishmaniasis | *Leishmania major* | IL-4 | [129] |
| Visceral/ cutaneous leishmaniasis | *Leishmania amazonensis* | P2X7 receptor and LTB4 | [195] |
| Tegumentary/ mucosal leishmaniasis | *Leishmania (V.) braziliensis* | AIM2 inflammasome | [196] |
| Mucocutaneous leishmaniasis | *Leishmania* RNA virus *(LRV)* | TLR3 | [197, 198] |
| Cutaneous leishmaniasis | *Leishmania major* Seidman strain | IL-1β and neutrophil recruitment | [199] |
| Visceral leishmaniasis | *Leishmania donovani* | A20 and UCP2<br>IL-1β<br>CASP-1 | [200–203] |
| Cutaneous leishmaniasis | *Leishmania braziliensis* | IL-1β | [204] |
| Cutaneous leishmaniasis | *Leishmania guyanensis* | IL-1β | [205] |
| Visceral leishmaniasis | *Leishmania infantum* | Pyrin domain containing 3 and autophagosome-associated microtubule-associated protein 1 light chain 3 | [206] |
| Visceral leishmaniasis | *Leishmania amazonensis* | IL-1β | [207, 208] |
| Visceral leishmaniasis | *Leishmania infantum* | IL-1β, caspase-1, and IL-18 | [209, 210] |
| Visceral/ cutaneous leishmaniasis | *Leishmania amazonensis* | Dectin-1 | [211] |

(continued)

**Table 2** (continued)

| Types of leishmaniasis | *Leishmania* sp. | Target protein | References |
|---|---|---|---|
| Cutaneous leishmaniasis | *Leishmania mexicana* | IL-1β | [212] |
| Murine cutaneous leishmaniasis | *Leishmania major* | NLRP10 | [213] |
| American cutaneous leishmaniasis | *Leishmania amazonensis* | NLRP12/IL-1β/cNOS/NO pathway | [214] |

posttranscriptional alteration of the protein levels [130]. Because of their role in innate and adaptive immune responses, miRNA has been playing a vital role in immune response development and functions, having an impact on many disorders' symptomatology and pathogenesis [13, 131, 132].

MiRNAs play a significant function in regulating immune cell activity, targeting TLRs and other inflammation-related genes. Macrophage activation and the production of pro-inflammatory and microbicidal effector chemicals are controlled by TLRs in the fight against *Leishmania* infection [133]. *Leishmania* parasites may modify TLR signaling pathways and interfere with host immune responses by changing the expression levels of miRNAs in infected macrophages [134]. Additionally, miRNAs could induce TLR pathway by functioning as a physiological ligand for them to stimulate immune responses [135, 136]. Other protozoan parasites like *Toxoplasma*, *Plasmodium*, and *Trypanosoma* may also modify host cell signaling and biochemical pathways to enhance pathogenicity in infected host cells [137, 138]. miRNA-processing mechanism in *Toxoplasma* releases exosomes containing miRNAs [139]. Increased levels of miR-146a and/or miR-155 in the *Leishmania*, *Toxoplasma*, and *Plasmodium*-infected host cells interfere with the host immune response [137, 140, 141].

Several miRNA-like elements were reported through computational techniques in *Leishmania*, which are linked to MDR (multidrug-resistant) proteins like ribosomal protein, hydrolase, exonuclease, and ATP-binding cassette transporter, as well as RNA-binding proteins [142, 143], reported having an antiproliferative and apoptotic impact of DBA, also known as trans-dibenzalacetone (a synthetic analogue of curcumin), on *L. donovani* by downregulating several miRNAs, namely, hsa-miR-30c-1, hsa-miR-151a, and hsa-miR-15b [144]. Bcl-2 and caspases are targeted by miR-15b, which induces apoptosis in cell [145]. The cellular respiration and ATP synthesis in host cells are controlled by miR-151a that targets cytochrome b [144, 146]. DBA-treated *Leishmania* parasites are also reported to downregulate miR-30a-3p, resulting in the inhibition of *Leishmania* parasite replication and virulence [144]. ATG4 is the target of miR-30c, which is necessary for parasite survival. ATG4, which is necessary for the parasite survival, is regulated by the miR-30c [144]. MiRNAs are also involved in regulating autophagy and cell death induced by DBA, allowing parasites to survive and reproduce. Mukherjee et al.

(2016) [147] have shown that antimony-resistant *Leishmania* parasites could differentially regulate the expression of certain miRNA (miR-466i) to avert host inflammatory response.

Upon entering into the host body, *Leishmania* parasites, are immediately taken up by the highly acidic, oxidative, and antimicrobial components-filled professional macrophages, which include neutrophils [148, 149]. It has been revealed that *Leishmania* parasites modulate phagolysosomes through the formation of LPVs (*Leishmania* parasitophorous vacuoles), allowing them to be able to live successfully inside the macrophages [150–152]. The biogenesis of parasitophorous vacuole, which harbors the parasites, is regulated by Rab GTPases, making them potential targets for intracellular pathogens [153, 154]. Rab5a is upregulated by *L. donovani* through downregulating miR-494. This allows parasites to remain within early endosomes without merging with lysosomes by recruiting and keeping Rab5a on the PV. Rab5a inhibition by miR-494 is anticipated resulting in endosome-lysosome fusion, thereby reducing parasite survival and evasion [155]. For *Leishmania* parasites to thrive in human macrophages, both miR-494 and Rab5a must be present in the cells. *L. amazonensis*-infected macrophages exhibited distinct miRNA expression patterns when arginine was absent. WT macrophages had an increased expression level of the altered miRNAs (78 %), compared to only 32% in those infected with *L. amazonensis* in absence of arginine. When parasites were administered to macrophages in presence of arginine, the expression of two miRNAs involved in NOS2 regulation and NO production was decreased. These miRNAs increase NO production and target NOS2 to limit parasite infectivity [156].

miRNA targeting might lead to new treatments targeting TLRs. Few *L. guyanensis* strains are the host for an endosymbiotic virus: LRV1 (*Leishmania* RNA virus 1) [157]. TLR3 recognizes LRV1, leading to the increased *L. guyanensis* parasite burden and lesion edema [158]. *L. guyanensis* LRV1-infected macrophages upregulate miR-155 expression through TLR3-dependent TRIF (TLR3/TIR domain-containing adaptor-inducing IFN-β) pathway. This improves macrophage health and parasite survival, by inhibiting PI3K/AKT pathway simultaneously [158]. *L. donovani* elevates miR-210 and HIF-1 (hypoxia-inducible factor-1α) expression in macrophages. Increased HIF-1 boosts miR-210 in host, which further reduced pro-inflammatory cytokine release (TNF-α and IL-12) and improves parasite survival [159, 160]. HIF-1 siRNA or antagomir-210 treatment has shown reduced parasite load in macrophage cells [159].

*Leishmania* infection ensures the survival of the host macrophages through elevation of c-Myc expression by suppressing miR-34a [161]. Silencing c-Myc reduces *Leishmania* pathogenesis [162].

Melatonin is a critical modulator of macrophage stimulation and controlling inflammation in host cells [163, 164] during parasitic infection. Melatonin decreases *L. amazonensis* in BALB/c mouse macrophages. Melatonin suppressed IL-6, MCP-1, RANTES, and MIP-2 levels in infected macrophages by upregulating miR-294-3p, miR-30e-5p, and miR-302d-3p [165]. miR-294-3p also lowers NOS2, TNF, and Mcp-1/Ccl2 to increase parasite burden [156, 165]. miR-302d-3p/miR-30e-5p silencing reduced macrophage infectivity by increasing NOS2 and

NO levels [166]. Melatonin reduces arginase 1 and boosts NOS2 to reduce *Leishmania*-infected macrophage infectivity [165]. During leishmaniasis, miR-21 suppresses IL-12 mRNA, reducing expression of IL-12 cytokine in host dendritic cells by expressing miR-21. During severe infections, inhibiting MiR-21 boosts DC-induced IL-12 production. Lowering miR-21 levels in host cells may prove as an effective antileishmanial vaccine development strategy [167].

miRNAs in *Leishmania*-infected host cells regulate parasite replication, infectivity, and survival. Drug susceptibility may be reduced by modifying transporters, receptors, and ion channels. miRNAs may help in these aspects, helping in the advancement of drug resistance in *Leishmania* parasites. Drug-resistant *Leishmania* strains ($LD^R$) decrease miR-763, miR-1264, and miR-3473 expressions to increase the activity of efflux pumps, resulting in greater levels of TGF- and IL-10 [168]. IL-10 boosts antimony drug efflux from $LD^R$-infected cells. miRNAs also inhibit $LD^R$ parasites. miRNA-mediated control of HuR and PP2A (protein phosphatase 2A) balances pro- and anti-inflammatory cytokines in *L. donovani* [169]. Dephosphorylated Ago2 (Argonaute 2) is required for miRNA function [170]. PP2A and HuR regulate Ago2 phosphorylation. It prevents miRNAs from affecting mRNA expression. HuR prevents protozoan macrophage infection by regulating immunological responses. *Leishmania* infection boosts anti-inflammatory macrophage responses by downregulating HuR. Parasites may activate PP2A, which dephosphorylates $AgO_2$ and deactivates targeting miRNAs, reducing pro-inflammatory cytokines in infected macrophages. HuR's miRNA-modulating actions in macrophage immunology may reduce PP2A production. HuR and PP2A inhibition may promote pro-inflammatory macrophage responses to fight *L. donovani*. $LD^S$ and $LD^R$ increase PP2A and decrease HuR, causing pro-inflammatory cytokine production. It has also been shown that these miRNAs more specifically regulate IFN cytokine levels, as IFN transcripts have the highest predicted miRNA-binding sites in host, suggesting its role in $LD^R$ infection [17].

miRNAs play as a key modulator in the formation of inflammasome complex [171], but its mechanism in forming inflammasome in leishmaniasis is still not clear. Several reports suggested upregulation of different miRNAs like hsa-miR-346 [172], let-7a [173], miR9, miR132, miR-146a, miR-155, miR-187 [174], miR-193b, and miR-671 [175] inhibits host innate immune pathway, both in human visceral organs and also in the cutaneous lesions. But still there is no data showing involvement of these miRNAs in regulating assembly of inflammasomes in visceral/cutaneous leishmaniasis. Involvement of miRNAs in the development of different types of leishmaniasis has been listed in Table 3.

## 10 Conclusion

Activation of inflammasome is highly critical to clear parasites. It may be possible that few miRNAs could negatively regulate the formation of inflammasome in *Leishmania*-infected host, thus helping in the spread of the diseases, while the rest of the miRNAs help to regulate the activation of inflammasome complex formation

**Table 3** Involvement of miRNAs in different types of leishmaniasis

| Leishmaniasis | miRNA | References |
|---|---|---|
| *Leishmania donovani* | miRNA-21, miRNA-155, miRNA-146b-5p, miRNA-3620, miRNA-6385, miRNA-6973a, miRNA-6996, miRNA-328, miRNA-8113, miRNA-3473f, miRNA-763, miRNA-6540, miR-1264, miRNA-29a, miRNA-29b, miR-146a, miR-122, miR30A-3p | [14, 156, 174, 215–217] |
| *Leishmania infantum* | miRNA-21, miRNA-424, miRNA-194, miRNA-346, miRNA-192, miRNA-155, miRNA-503, miRNA-371 | [166, 172, 218] |
| *Leishmania braziliensis* | miRNA361-3P, miR-548d-3p, miR-193b, miR-671, miR-346 | [175, 219–221] |
| *Leishmania amazonensis* | miRNA-let-7e, miR-294, miR-721, miR-294, miR-30e, miR-302d, hsa-miR-346, miR-294, miR-410 | [156, 165, 172, 222, 223] |
| *Leishmania guyanensis* | miR-155, miR146-a | [158, 224] |
| *L. major* | miR-24-3p, miR-146a, miR-340, miR155, miR-15a, hsa-miR-346, let-7a, miR-210, miR-101c, miR-129, miR-210, miR-182, miR-10a | [172–174, 225–231] |
| *L. chagasi* (*Leishmania infantum chagasi*) | miR122 | [232] |

in a positive way. Recently, Mendonca et al. (2020) had performed a clinical study in 27 ACL (American cutaneous leishmaniasis) patients to decipher the correlation between inflammasome activation and miRNA regulation [176]. They have shown that levels of miR-7 and miR-223 go inversely proportional with the formation of inflammasomes in ACL patients, while miR-133a expression increases with the activation of inflammatory pathway, ultimately resulting in the upregulation of IL-1β, IL-6, and IL-17. But still the data is too few. To establish the role of miRNA in activation of inflammasomes in leishmaniasis, more studies are required. These data could also help us to understand the mechanism of various immune complex formations during leishmaniasis. Furthermore, clear understanding of miRNA's involvement in leishmaniasis could also help to use these miRNAs as probable biomarkers for the detection, diagnosis, and treatment against leishmaniasis.

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## References


1. Medzhitov R. Origin and physiological roles of inflammation. Nature. 2008;454(7203): 428–35.
2. Koch U, Radtke F. Mechanisms of T cell development and transformation. Annu Rev Cell Dev Biol. 2011;27:539–62.
3. Janeway CA Jr, Medzhitov R. Innate immune recognition. Annu Rev Immunol. 2002;20:197–216.
4. Schroder K, Tschopp J. The inflammasomes. Cell. 2010;140(6):821–32.
5. Takeuchi O, Akira S. Pattern recognition receptors and inflammation. Cell. 2010;140(6): 805–20.
6. Martinon F, Burns K, Tschopp J. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. Mol Cell. 2002;10(2):417–26.
7. Kayagaki N, et al. Non-canonical inflammasome activation targets caspase-11. Nature. 2011;479(7371):117–21.
8. Ting JP, et al. The NLR gene family: a standard nomenclature. Immunity. 2008;28(3):285–7.
9. Cridland JA, et al. The mammalian PYHIN gene family: phylogeny, evolution and expression. BMC Evol Biol. 2012;12:140.
10. Strowig T, et al. Inflammasomes in health and disease. Nature. 2012;481(7381):278–86.
11. Dinarello CA. A clinical perspective of IL-1beta as the gatekeeper of inflammation. Eur J Immunol. 2011;41(5):1203–17.
12. Tsitsiou E, Lindsay MA. microRNAs and the immune response. Curr Opin Pharmacol. 2009;9 (4):514–20.
13. Liu G, Abraham E. MicroRNAs in immune response and macrophage polarization. Arterioscler Thromb Vasc Biol. 2013;33(2):170–7.
14. Geraci NS, Tan JC, McDowell MA. Characterization of microRNA expression profiles in Leishmania-infected human phagocytes. Parasite Immunol. 2015;37(1):43–51.
15. Baltimore D, et al. MicroRNAs: new regulators of immune cell development and function. Nat Immunol. 2008;9(8):839–45.
16. Rossol M, et al. LPS-induced cytokine production in human monocytes and macrophages. Crit Rev Immunol. 2011;31(5):379–446.
17. Mukherjee B, et al. Probing the molecular mechanism of aggressive infection by antimony resistant Leishmania donovani. Cytokine. 2021;145:155245.
18. Zhao Y, et al. The NLRC4 inflammasome receptors for bacterial flagellin and type III secretion apparatus. Nature. 2011;477(7366):596–600.
19. Hayward JA, et al. Cytosolic recognition of microbes and pathogens: inflammasomes in action. Microbiol Mol Biol Rev. 2018;82(4).
20. Rathinam VA, et al. TRIF licenses caspase-11-dependent NLRP3 inflammasome activation by gram-negative bacteria. Cell. 2012;150(3):606–19.
21. Munoz-Planillo R, et al. K(+) efflux is the common trigger of NLRP3 inflammasome activation by bacterial toxins and particulate matter. Immunity. 2013;38(6):1142–53.
22. Hara H, et al. The NLRP6 inflammasome recognizes lipoteichoic acid and regulates gram-positive pathogen infection. Cell. 2018;175(6):1651–1664 e14.
23. Khare S, et al. An NLRP7-containing inflammasome mediates recognition of microbial lipopeptides in human macrophages. Immunity. 2012;36(3):464–76.
24. Xu H, et al. Innate immune sensing of bacterial modifications of rho GTPases by the pyrin inflammasome. Nature. 2014;513(7517):237–41.

25. Kayagaki N, et al. Noncanonical inflammasome activation by intracellular LPS independent of TLR4. Science. 2013;341(6151):1246–9.
26. Levy M, et al. Microbiota-modulated metabolites shape the intestinal microenvironment by regulating NLRP6 inflammasome signaling. Cell. 2015;163(6):1428–43.
27. Seo SU, et al. Distinct commensals induce interleukin-1beta via NLRP3 inflammasome in inflammatory monocytes to promote intestinal inflammation in response to injury. Immunity. 2015;42(4):744–55.
28. Ruhl S, Broz P. Caspase-11 activates a canonical NLRP3 inflammasome by promoting K(+) efflux. Eur J Immunol. 2015;45(10):2927–36.
29. He Y, et al. NEK7 is an essential mediator of NLRP3 activation downstream of potassium efflux. Nature. 2016;530(7590):354–7.
30. Tang T, et al. CLICs-dependent chloride efflux is an essential and proximal upstream event for NLRP3 inflammasome activation. Nat Commun. 2017;8(1):202.
31. Katsnelson MA, et al. K+ efflux agonists induce NLRP3 inflammasome activation independently of Ca2+ signaling. J Immunol. 2015;194(8):3937–52.
32. Gong T, et al. Orchestration of NLRP3 inflammasome activation by ion fluxes. Trends Immunol. 2018;39(5):393–406.
33. Luo H, et al. Mitochondrial stress-initiated aberrant activation of the NLRP3 inflammasome regulates the functional deterioration of hematopoietic stem cell aging. Cell Rep. 2019;26(4): 945–954 e4.
34. Nakahira K, et al. Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome. Nat Immunol. 2011;12 (3):222–30.
35. Vince JE, et al. The mitochondrial apoptotic effectors BAX/BAK activate caspase-3 and -7 to trigger NLRP3 inflammasome and caspase-8 driven IL-1beta activation. Cell Rep. 2018;25(9): 2339–2353 e4.
36. Zhou R, et al. Thioredoxin-interacting protein links oxidative stress to inflammasome activation. Nat Immunol. 2010;11(2):136–40.
37. Dostert C, et al. Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica. Science. 2008;320(5876):674–7.
38. Bauernfeind F, et al. Cutting edge: reactive oxygen species inhibitors block priming, but not activation, of the NLRP3 inflammasome. J Immunol. 2011;187(2):613–7.
39. Swanson KV, Deng M, Ting JP. The NLRP3 inflammasome: molecular activation and regulation to therapeutics. Nat Rev Immunol. 2019;19(8):477–89.
40. Martinon F, Tschopp J. Inflammatory caspases: linking an intracellular innate immune system to autoinflammatory diseases. Cell. 2004;117(5):561–74.
41. Lamkanfi M, Dixit VM. Inflammasomes and their roles in health and disease. Annu Rev Cell Dev Biol. 2012;28:137–61.
42. Hu Z, et al. Crystal structure of NLRC4 reveals its autoinhibition mechanism. Science. 2013;341(6142):172–5.
43. Hu Z, et al. Structural and biochemical basis for induced self-propagation of NLRC4. Science. 2015;350(6259):399–404.
44. Zhang L, et al. Cryo-EM structure of the activated NAIP2-NLRC4 inflammasome reveals nucleated polymerization. Science. 2015;350(6259):404–9.
45. Halff EF, et al. Formation and structure of a NAIP5-NLRC4 inflammasome induced by direct interactions with conserved N- and C-terminal regions of flagellin. J Biol Chem. 2012;287 (46):38460–72.
46. Yang X, et al. Structural basis for specific flagellin recognition by the NLR protein NAIP5. Cell Res. 2018;28(1):35–47.
47. Qu Y, et al. Phosphorylation of NLRC4 is critical for inflammasome activation. Nature. 2012;490(7421):539–42.
48. Suzuki S, et al. Shigella type III secretion protein MxiI is recognized by Naip2 to induce Nlrc4 inflammasome activation independently of Pkcdelta. PLoS Pathog. 2014;10(2):e1003926.

49. Qu Y, et al. NLRP3 recruitment by NLRC4 during salmonella infection. J Exp Med. 2016;213 (6):877–85.
50. Karki R, et al. IRF8 regulates transcription of Naips for NLRC4 inflammasome activation. Cell. 2018;173(4):920–933 e13.
51. Shi H, et al. NLRP3 activation and mitosis are mutually exclusive events coordinated by NEK7, a new inflammasome component. Nat Immunol. 2016;17(3):250–8.
52. Misawa T, et al. Microtubule-driven spatial arrangement of mitochondria promotes activation of the NLRP3 inflammasome. Nat Immunol. 2013;14(5):454–60.
53. Subramanian N, et al. The adaptor MAVS promotes NLRP3 mitochondrial localization and inflammasome activation. Cell. 2013;153(2):348–61.
54. Zhou R, et al. A role for mitochondria in NLRP3 inflammasome activation. Nature. 2011;469 (7329):221–5.
55. Hornung V, et al. Silica crystals and aluminum salts activate the NALP3 inflammasome through phagosomal destabilization. Nat Immunol. 2008;9(8):847–56.
56. Bauernfeind FG, et al. Cutting edge: NF-kappaB activating pattern recognition and cytokine receptors license NLRP3 inflammasome activation by regulating NLRP3 expression. J Immunol. 2009;183(2):787–91.
57. Fernandes-Alnemri T, et al. Cutting edge: TLR signaling licenses IRAK1 for rapid activation of the NLRP3 inflammasome. J Immunol. 2013;191(8):3995–9.
58. Juliana C, et al. Non-transcriptional priming and deubiquitination regulate NLRP3 inflammasome activation. J Biol Chem. 2012;287(43):36617–22.
59. Lin KM, et al. IRAK-1 bypasses priming and directly links TLRs to rapid NLRP3 inflammasome activation. Proc Natl Acad Sci U S A. 2014;111(2):775–80.
60. Lopez-Castejon G, et al. Deubiquitinases regulate the activity of caspase-1 and interleukin-1beta secretion via assembly of the inflammasome. J Biol Chem. 2013;288(4):2721–33.
61. Py BF, et al. Deubiquitination of NLRP3 by BRCC3 critically regulates inflammasome activity. Mol Cell. 2013;49(2):331–8.
62. Gurung P, et al. FADD and caspase-8 mediate priming and activation of the canonical and noncanonical Nlrp3 inflammasomes. J Immunol. 2014;192(4):1835–46.
63. Kang S, et al. Caspase-8 scaffolding function and MLKL regulate NLRP3 inflammasome activation downstream of TLR3. Nat Commun. 2015;6:7515.
64. Wei M, et al. NLRP3 activation was regulated by DNA methylation modification during mycobacterium tuberculosis infection. Biomed Res Int. 2016;2016:4323281.
65. Bauernfeind F, et al. NLRP3 inflammasome activity is negatively controlled by miR-223. J Immunol. 2012;189(8):4175–81.
66. Neudecker V, et al. Myeloid-derived miR-223 regulates intestinal inflammation via repression of the NLRP3 inflammasome. J Exp Med. 2017;214(6):1737–52.
67. Li D, et al. MicroRNA-30e regulates neuroinflammation in MPTP model of Parkinson's disease by targeting Nlrp3. Hum Cell. 2018;31(2):106–15.
68. Feng X, et al. MicroRNA-22 suppresses cell proliferation, migration and invasion in oral squamous cell carcinoma by targeting NLRP3. J Cell Physiol. 2018;233(9):6705–13.
69. Hu J, et al. LncRNA ANRIL promotes NLRP3 inflammasome activation in uric acid nephropathy through miR-122-5p/BRCC3 axis. Biochimie. 2019;157:102–10.
70. Song N, et al. NLRP3 phosphorylation is an essential priming event for inflammasome activation. Mol Cell. 2017;68(1):185–197 e6.
71. Humphries F, et al. The E3 ubiquitin ligase Pellino2 mediates priming of the NLRP3 inflammasome. Nat Commun. 2018;9(1):1560.
72. Guo C, et al. Bile acids control inflammation and metabolic disorder through inhibition of NLRP3 inflammasome. Immunity. 2016;45(4):802–16.
73. Stutz A, et al. NLRP3 inflammasome assembly is regulated by phosphorylation of the pyrin domain. J Exp Med. 2017;214(6):1725–36.
74. Spalinger MR, et al. NLRP3 tyrosine phosphorylation is controlled by protein tyrosine phosphatase PTPN22. J Clin Invest. 2016;126(5):1783–800.

75. Faustin B, et al. Reconstituted NALP1 inflammasome reveals two-step mechanism of caspase-1 activation. Mol Cell. 2007;25(5):713–24.
76. Levinsohn JL, et al. Anthrax lethal factor cleavage of Nlrp1 is required for activation of the inflammasome. PLoS Pathog. 2012;8(3):e1002638.
77. Finger JN, et al. Autolytic proteolysis within the function to find domain (FIIND) is required for NLRP1 inflammasome activity. J Biol Chem. 2012;287(30):25030–7.
78. Chavarria-Smith J, et al. Functional and evolutionary analyses identify proteolysis as a general mechanism for NLRP1 inflammasome activation. PLoS Pathog. 2016;12(12):e1006052.
79. Sandstrom A, et al. Functional degradation: a mechanism of NLRP1 inflammasome activation by diverse pathogen enzymes. Science. 2019;364(6435).
80. Chui AJ, et al. N-terminal degradation activates the NLRP1B inflammasome. Science. 2019;364(6435):82–5.
81. Xu H, et al. The N-end rule ubiquitin ligase UBR2 mediates NLRP1B inflammasome activation by anthrax lethal toxin. EMBO J. 2019;38(13):e101996.
82. Neiman-Zenevich J, et al. Listeria monocytogenes and Shigella flexneri activate the NLRP1B inflammasome. Infect Immun. 2017;85(11).
83. Ewald SE, Chavarria-Smith J, Boothroyd JC. NLRP1 is an inflammasome sensor for Toxoplasma gondii. Infect Immun. 2014;82(1):460–8.
84. Liao KC, Mogridge J. Activation of the Nlrp1b inflammasome by reduction of cytosolic ATP. Infect Immun. 2013;81(2):570–9.
85. Zhong FL, et al. Germline NLRP1 mutations cause skin inflammatory and cancer susceptibility syndromes via inflammasome activation. Cell. 2016;167(1):187–202 e17.
86. Okondo MC, et al. Inhibition of Dpp8/9 activates the Nlrp1b inflammasome. Cell. Chem Biol. 2018;25(3):262–267 e5.
87. Zhong FL, et al. Human DPP9 represses NLRP1 inflammasome and protects against autoinflammatory diseases via both peptidase activity and FIIND domain binding. J Biol Chem. 2018;293(49):18864–78.
88. Son MY, et al. A novel human model of the neurodegenerative disease GM1 gangliosidosis using induced pluripotent stem cells demonstrates inflammasome activation. J Pathol. 2015;237(1):98–110.
89. Anand PK, et al. NLRP6 negatively regulates innate immunity and host defence against bacterial pathogens. Nature. 2012;488(7411):389–93.
90. Souza AC, et al. TLR4 mutant mice are protected from renal fibrosis and chronic kidney disease progression. Physiol Rep. 2015;3(9).
91. Komada T, et al. Role of NLRP3 inflammasomes for rhabdomyolysis-induced acute kidney injury. Sci Rep. 2015;5:10901.
92. Zhuang Y, et al. Mitochondrial dysfunction confers albumin-induced NLRP3 inflammasome activation and renal tubular injury. Am J Physiol Renal Physiol. 2015;308(8):F857–66.
93. Birchenough GM, et al. A sentinel goblet cell guards the colonic crypt by triggering Nlrp6-dependent Muc2 secretion. Science. 2016;352(6293):1535–42.
94. Sun Y, et al. Stress-induced corticotropin-releasing hormone-mediated NLRP6 inflammasome inhibition and transmissible enteritis in mice. Gastroenterology. 2013;144(7):1478–87, 1487 e1-8.
95. Jin T, et al. Structure of the absent in melanoma 2 (AIM2) pyrin domain provides insights into the mechanisms of AIM2 autoinhibition and inflammasome assembly. J Biol Chem. 2013;288(19):13225–35.
96. Howard AD, et al. IL-1-converting enzyme requires aspartic acid residues for processing of the IL-1 beta precursor at two distinct sites and does not cleave 31-kDa IL-1 alpha. J Immunol. 1991;147(9):2964–9.
97. Morrone SR, et al. Cooperative assembly of IFI16 filaments on dsDNA provides insights into host defense strategy. Proc Natl Acad Sci U S A. 2014;111(1):E62–71.
98. Lugrin J, Martinon F. The AIM2 inflammasome: sensor of pathogens and cellular perturbations. Immunol Rev. 2018;281(1):99–114.

99. Jin T, et al. Structures of the HIN domain:DNA complexes reveal ligand binding and activation mechanisms of the AIM2 inflammasome and IFI16 receptor. Immunity. 2012;36(4):561–71.
100. Sagulenko V, et al. AIM2 and NLRP3 inflammasomes activate both apoptotic and pyroptotic death pathways via ASC. Cell Death Differ. 2013;20(9):1149–60.
101. Dorfleutner A, et al. Cellular pyrin domain-only protein 2 is a candidate regulator of inflammasome activation. Infect Immun. 2007;75(3):1484–92.
102. Stehlik C, et al. The PAAD/PYRIN-only protein POP1/ASC2 is a modulator of ASC-mediated nuclear-factor-kappa B and pro-caspase-1 regulation. Biochem J. 2003;373(Pt 1):101–13.
103. Roberts TL, et al. HIN-200 proteins regulate caspase activation in response to foreign cytoplasmic DNA. Science. 2009;323(5917):1057–60.
104. Heilig R, Broz P. Function and mechanism of the pyrin inflammasome. Eur J Immunol. 2018;48(2):230–8.
105. Chae JJ, et al. Targeted disruption of pyrin, the FMF protein, causes heightened sensitivity to endotoxin and a defect in macrophage apoptosis. Mol Cell. 2003;11(3):591–604.
106. Gao W, et al. Site-specific phosphorylation and microtubule dynamics control pyrin inflammasome activation. Proc Natl Acad Sci U S A. 2016;113(33):E4857–66.
107. Chung LK, et al. The Yersinia virulence factor YopM hijacks host kinases to inhibit type III effector-triggered activation of the pyrin inflammasome. Cell Host Microbe. 2016;20(3):296–306.
108. Van Gorp H, et al. Familial Mediterranean fever mutations lift the obligatory requirement for microtubules in pyrin inflammasome activation. Proc Natl Acad Sci U S A. 2016;113(50):14384–9.
109. Russo AJ, et al. Emerging insights into noncanonical inflammasome recognition of microbes. J Mol Biol. 2018;430(2):207–16.
110. Kayagaki N, et al. Caspase-11 cleaves gasdermin D for non-canonical inflammasome signalling. Nature. 2015;526(7575):666–71.
111. Hagar JA, et al. Cytoplasmic LPS activates caspase-11: implications in TLR4-independent endotoxic shock. Science. 2013;341(6151):1250–3.
112. Bandyopadhyay S, et al. MicroRNA-133a-1 regulates inflammasome activation through uncoupling protein-2. Biochem Biophys Res Commun. 2013;439(3):407–12.
113. Semper RP, et al. Helicobacter pylori-induced IL-1beta secretion in innate immune cells is regulated by the NLRP3 inflammasome and requires the cag pathogenicity island. J Immunol. 2014;193(7):3566–76.
114. Li S, et al. MiR-22 sustains NLRP3 expression and attenuates H. pylori-induced gastric carcinogenesis. Oncogene. 2018;37(7):884–96.
115. Castano-Rodriguez N, et al. The NOD-like receptor signalling pathway in Helicobacter pylori infection and related gastric cancer: a case-control study and gene expression analyses. PLoS One. 2014;9(6):e98899.
116. Xue Z, et al. miR-21 promotes NLRP3 inflammasome activation to mediate pyroptosis and endotoxic shock. Cell Death Dis. 2019;10(6):461.
117. Heneka MT, Kummer MP, Latz E. Innate immune activation in neurodegenerative disease. Nat Rev Immunol. 2014;14(7):463–77.
118. Chen Z, et al. Downregulation of miR-199a-3p mediated by the CtBP2-HDAC1-FOXP3 transcriptional complex contributes to acute lung injury by targeting NLRP1. Int J Biol Sci. 2019;15(12):2627–40.
119. Fonseca-Sanchez MA, et al. microRNA-18b is upregulated in breast cancer and modulates genes involved in cell migration. Oncol Rep. 2013;30(5):2399–410.
120. Yao M, et al. Berberine inhibits NLRP3 inflammasome pathway in human triple-negative breast cancer MDA-MB-231 cell. BMC Complement Altern Med. 2019;19(1):216.
121. Wen H, Miao EA, Ting JP. Mechanisms of NOD-like receptor-associated inflammasome activation. Immunity. 2013;39(3):432–41.

122. Horng T. Calcium signaling and mitochondrial destabilization in the triggering of the NLRP3 inflammasome. Trends Immunol. 2014;35(6):253–61.
123. Pradhan S, et al. Linking membrane fluidity with defective antigen presentation in leishmaniasis. Parasite Immunol. 2021;43(7):e12835.
124. Zamboni DS, Sacks DL. Inflammasomes and Leishmania: in good times or bad, in sickness or in health. Curr Opin Microbiol. 2019;52:70–6.
125. Lima-Junior DS, et al. Inflammasome-derived IL-1beta production induces nitric oxide-mediated resistance to Leishmania. Nat Med. 2013;19(7):909–15.
126. Lefevre L, et al. The C-type lectin receptors dectin-1, MR, and SIGNR3 contribute both positively and negatively to the macrophage response to Leishmania infantum. Immunity. 2013;38(5):1038–49.
127. de Carvalho RVH, et al. Leishmania lipophosphoglycan triggers caspase-11 and the non-canonical activation of the NLRP3 inflammasome. Cell Rep. 2019;26(2):429–437 e5.
128. Schauvliege R, et al. Caspase-11 gene expression in response to lipopolysaccharide and interferon-gamma requires nuclear factor-kappa B and signal transducer and activator of transcription (STAT) 1. J Biol Chem. 2002;277(44):41624–30.
129. Gurung P, et al. An NLRP3 inflammasome-triggered Th2-biased adaptive immune response promotes leishmaniasis. J Clin Invest. 2015;125(3):1329–38.
130. Thomson JM, et al. Extensive post-transcriptional regulation of microRNAs and its implications for cancer. Genes Dev. 2006;20(16):2202–7.
131. Raisch J, Darfeuille-Michaud A, Nguyen HT. Role of microRNAs in the immune system, inflammation and cancer. World J Gastroenterol. 2013;19(20):2985–96.
132. Cheng L, et al. Exosomes provide a protective and enriched source of miRNA for biomarker profiling compared to intracellular and cell-free blood. J Extracell Vesicles. 2014:3.
133. Galluzzi L, et al. Leishmania infantum induces mild unfolded protein response in infected macrophages. PLoS One. 2016;11(12):e0168339.
134. Muxel SM, et al. Arginine and polyamines fate in leishmania infection. Front Microbiol. 2017;8:2682.
135. He X, Jing Z, Cheng G. MicroRNAs: new regulators of toll-like receptor signalling pathways. Biomed Res Int. 2014;2014:945169.
136. Bayraktar R, Bertilaccio MTS, Calin GA. The interaction between two worlds: microRNAs and toll-like receptors. Front Immunol. 2019;10:1053.
137. Acuna SM, Floeter-Winter LM, Muxel SM. MicroRNAs: biological regulators in pathogen-host interactions. Cell. 2020;9(1).
138. Paul S, et al. Human microRNAs in host-parasite interaction: a review. 3 Biotech. 2020;10 (12):510.
139. Menard KL, Haskins BE, Denkers EY. Impact of Toxoplasma gondii infection on host non-coding RNA responses. Front Cell Infect Microbiol. 2019;9:132.
140. Guerfali FZ, et al. Simultaneous gene expression profiling in human macrophages infected with Leishmania major parasites using SAGE. BMC Genomics. 2008;9:238.
141. Hentzschel F, et al. AAV8-mediated in vivo overexpression of miR-155 enhances the protective capacity of genetically attenuated malarial parasites. Mol Ther. 2014;22(12):2130–41.
142. Sahoo GC, et al. Computational identification of microRNA-like elements in Leishmania major. Microrna. 2014;2(3):225–30.
143. Chauhan IS, et al. Chemoprevention of leishmaniasis: in-vitro antiparasitic activity of dibenzalacetone, a synthetic curcumin analog leads to apoptotic cell death in Leishmania donovani. Parasitol Int. 2018;67(5):627–36.
144. Singh N, Chauhan IS. MicroRNA expression profiling of dibenzalacetone (DBA) treated intracellular amastigotes of Leishmania donovani. Exp Parasitol. 2018;193:5–19.
145. Guo CJ, et al. miR-15b and miR-16 are implicated in activation of the rat hepatic stellate cell: an essential role for apoptosis. J Hepatol. 2009;50(4):766–78.
146. Zhou R, et al. Mitochondria-related miR-151a-5p reduces cellular ATP production by targeting CYTB in asthenozoospermia. Sci Rep. 2015;5:17743.

147. Mukherjee B, et al. Antimony-resistant Leishmania donovani exploits miR-466i to deactivate host MyD88 for regulating IL-10/IL-12 levels during early hours of infection. J Immunol. 2015;195(6):2731–42.
148. McMahon-Pratt D, Alexander J. Does the leishmania major paradigm of pathogenesis and protection hold for New World cutaneous leishmaniases or the visceral disease? Immunol Rev. 2004;201:206–24.
149. Uribe-Querol E, Rosales C. Control of phagocytosis by microbial pathogens. Front Immunol. 2017;8:1368.
150. Alexander J, Satoskar AR, Russell DG. Leishmania species: models of intracellular parasitism. J Cell Sci. 1999;112(Pt 18):2993–3002.
151. Rana T, et al. Mechanism of down-regulation of RNA polymerase III-transcribed non-coding RNA genes in macrophages by Leishmania. J Biol Chem. 2011;286(8):6614–26.
152. Levin R, Grinstein S, Canton J. The life cycle of phagosomes: formation, maturation, and resolution. Immunol Rev. 2016;273(1):156–79.
153. Seto S, Tsujimura K, Koide Y. Rab GTPases regulating phagosome maturation are differentially recruited to mycobacterial phagosomes. Traffic. 2011;12(4):407–20.
154. Spano S, Galan JE. Taking control: hijacking of Rab GTPases by intracellular bacterial pathogens. Small GTPases. 2018;9(1-2, 182):–191.
155. Verma JK, Rastogi R, Mukhopadhyay A. Leishmania donovani resides in modified early endosomes by upregulating Rab5a expression via the downregulation of miR-494. PLoS Pathog. 2017;13(6):e1006459.
156. Muxel SM, et al. Leishmania (Leishmania) amazonensis induces macrophage miR-294 and miR-721 expression and modulates infection by targeting NOS2 and L-arginine metabolism. Sci Rep. 2017;7:44141.
157. Ives A, et al. Leishmania RNA virus controls the severity of mucocutaneous leishmaniasis. Science. 2011;331(6018):775–8.
158. Eren RO, et al. Mammalian innate immune response to a leishmania-resident RNA virus increases macrophage survival to promote parasite persistence. Cell Host Microbe. 2016;20 (3):318–28.
159. Singh AK, et al. Intracellular pathogen leishmania donovani activates hypoxia inducible factor-1 by dual mechanism for survival advantage within macrophage. PLoS One. 2012;7 (6):e38489.
160. Kumar V, et al. Leishmania donovani activates hypoxia inducible factor-1alpha and miR-210 for survival in macrophages by downregulation of NF-kappaB mediated pro-inflammatory immune response. Front Microbiol. 2018;9:385.
161. Colineau L, et al. C-Myc is a novel Leishmania virulence factor by proxy that targets the host miRNA system and is essential for survival in human macrophages. J Biol Chem. 2018;293 (33):12805–19.
162. Whitfield JR, Beaulieu ME, Soucek L. Strategies to inhibit Myc and their clinical applicability. Front Cell Dev Biol. 2017;5:10.
163. Markus RP, et al. Immune-pineal axis - acute inflammatory responses coordinate melatonin synthesis by pinealocytes and phagocytes. Br J Pharmacol. 2018;175(16):3239–50.
164. Xia Y, et al. Melatonin in macrophage biology: current understanding and future perspectives. J Pineal Res. 2019;66(2):e12547.
165. Fernandes JCR, et al. Melatonin and Leishmania amazonensis infection altered miR-294, miR-30e, and miR-302d impacting on Tnf, Mcp-1, and Nos2 expression. Front Cell Infect Microbiol. 2019;9:60.
166. Rashidi S, et al. Potential therapeutic targets shared between leishmaniasis and cancer. Parasitology. 2021;148(6):655–71.
167. Gannavaram S, et al. miR-21 expression determines the early vaccine immunity induced by LdCen (-/-) immunization. Front Immunol. 2019;10:2273.

168. Guha R, et al. Antimony resistant leishmania donovani but not sensitive ones drives greater frequency of potent T-regulatory cells upon interaction with human PBMCs: role of IL-10 and TGF-beta in early immune response. PLoS Negl Trop Dis. 2014;8(7):e2995.
169. Goswami A, et al. MicroRNA exporter HuR clears the internalized pathogens by promoting pro-inflammatory response in infected macrophages. EMBO Mol Med. 2020;12(3):e11011.
170. Chakrabarty Y, Bhattacharyya SN. Leishmania donovani restricts mitochondrial dynamics to enhance miRNP stability and target RNA repression in host macrophages. Mol Biol Cell. 2017;28(15):2091–105.
171. Sutterwala FS, Haasken S, Cassel SL. Mechanism of NLRP3 inflammasome activation. Ann N Y Acad Sci. 2014;1319:82–95.
172. Diotallevi A, et al. Leishmania infection induces microRNA hsa-miR-346 in human cell line-derived macrophages. Front Microbiol. 2018;9:1019.
173. Hashemi N, et al. Locked nucleic acid -anti- let-7a induces apoptosis and necrosis in macrophages infected with Leishmania major. Microb Pathog. 2018;119:193–9.
174. Lemaire J, et al. MicroRNA expression profile in human macrophages in response to Leishmania major infection. PLoS Negl Trop Dis. 2013;7(10):e2478.
175. Nunes S, et al. Integrated analysis reveals that miR-193b, miR-671, and TREM-1 correlate with a good response to treatment of human localized cutaneous leishmaniasis caused by leishmania braziliensis. Front Immunol. 2018;9:640.
176. Mendonca LSO, et al. Characterization of serum cytokines and circulating microRNAs that are predicted to regulate inflammasome genes in cutaneous leishmaniasis patients. Exp Parasitol. 2020;210:107846.
177. Feng Z, et al. Ly6G+ neutrophil-derived miR-223 inhibits the NLRP3 inflammasome in mitochondrial DAMP-induced acute lung injury. Cell Death Dis. 2017;8(11):e3170.
178. Junn E, et al. Repression of alpha-synuclein expression and toxicity by microRNA-7. Proc Natl Acad Sci U S A. 2009;106(31):13052–7.
179. Boxberger N, Hecker M, Zettl UK. Dysregulation of inflammasome priming and activation by microRNAs in human immune-mediated diseases. J Immunol. 2019;202(8):2177–87.
180. Tezcan G, et al. MicroRNA post-transcriptional regulation of the NLRP3 inflammasome in immunopathologies. Front Pharmacol. 2019;10:451.
181. Yang Y, et al. miR-16 inhibits NLRP3 inflammasome activation by directly targeting TLR4 in acute lung injury. Biomed Pharmacother. 2019;112:108664.
182. Gu TT, et al. Fructose downregulates miR-330 to induce renal inflammatory response and insulin signaling impairment: attenuation by morin. Mol Nutr Food Res. 2017;61(8).
183. Ning ZW, et al. MicroRNA-21 mediates angiotensin II-induced liver fibrosis by activating NLRP3 inflammasome/IL-1beta Axis via targeting Smad7 and Spry1. Antioxid Redox Signal. 2017;27(1):1–20.
184. Coucha M, et al. High fat diet dysregulates microRNA-17-5p and triggers retinal inflammation: role of endoplasmic-reticulum-stress. World J Diabetes. 2017;8(2):56–65.
185. Mearini E, et al. Expression of urinary miRNAs targeting NLRs inflammasomes in bladder cancer. Onco Targets Ther. 2017;10:2665–73.
186. Shen M, Meng L. Peripheral blood miR-372 as a biomarker for ulcerative colitis via direct targeting of NLRP12. Exp Ther Med. 2019;18(2):1486–92.
187. Momeni M, et al. MiR-143 induces expression of AIM2 and ASC in Jurkat cell line. Iran J Immunol. 2013;10(2):103–9.
188. Gonzalez K, et al. Involvement of the inflammasome and Th17 cells in skin lesions of human cutaneous leishmaniasis caused by Leishmania (Viannia) panamensis. Mediat Inflamm. 2020;2020:9278931.
189. Santos D, et al. IL-1beta production by intermediate monocytes is associated with immunopathology in cutaneous leishmaniasis. J Invest Dermatol. 2018;138(5):1107–15.
190. de Carvalho RVH, et al. Macrophage priming is dispensable for NLRP3 inflammasome activation and restriction of Leishmania amazonensis replication. J Leukoc Biol. 2019;106(3):631–40.

191. Lecoeur H, et al. Leishmania amazonensis subverts the transcription factor landscape in dendritic cells to avoid inflammasome activation and stall maturation. Front Immunol. 2020;11:1098.
192. Gupta G, et al. Inflammasome gene expression is associated with immunopathology in human localized cutaneous leishmaniasis. Cell Immunol. 2019;341:103920.
193. Cardoso TM, et al. Inflammasome activation by CD8(+) T cells from patients with cutaneous leishmaniasis caused by Leishmania braziliensis in the immunopathogenesis of the disease. J Invest Dermatol. 2021;141(1):209–213 e2.
194. Sohrabi Y, et al. Genetic regulation of guanylate-binding proteins 2b and 5 during leishmaniasis in mice. Front Immunol. 2018;9:130.
195. Chaves MM, et al. Non-canonical NLRP3 inflammasome activation and IL-1beta signaling are necessary to L. amazonensis control mediated by P2X7 receptor and leukotriene B4. PLoS Pathog. 2019;15(6):e1007887.
196. Moreira RB, et al. AIM2 inflammasome is associated with disease severity in tegumentary leishmaniasis caused by Leishmania (V.) braziliensis. Parasite Immunol. 2017;39(7).
197. de Carvalho RVH, et al. Endosymbiotic RNA virus inhibits Leishmania-induced caspase-11 activation. iScience. 2021;24(1):102004.
198. de Carvalho RVH, et al. Publisher correction: Leishmania RNA virus exacerbates leishmaniasis by subverting innate immunity via TLR3-mediated NLRP3 inflammasome inhibition. Nat Commun. 2020;11(1):203.
199. Charmoy M, et al. The Nlrp3 inflammasome, IL-1beta, and neutrophil recruitment are required for susceptibility to a nonhealing strain of Leishmania major in C57BL/6 mice. Eur J Immunol. 2016;46(4):897–911.
200. Gupta AK, et al. Leishmania donovani inhibits inflammasome-dependent macrophage activation by exploiting the negative regulatory proteins A20 and UCP2. FASEB J. 2017;31(11):5087–101.
201. Dey R, et al. Gut microbes egested during bites of infected sand flies augment severity of leishmaniasis via inflammasome-derived IL-1beta. Cell Host Microbe. 2018;23(1):134–143 e6.
202. Saha G, et al. Leishmania donovani evades caspase 1 dependent host defense mechanism during infection. Int J Biol Macromol. 2019;126:392–401.
203. Saha G, et al. BLIMP-1 mediated downregulation of TAK1 and p53 molecules is crucial in the pathogenesis of kala-azar. Front Cell Infect Microbiol. 2020;10:594431.
204. Novais FO, et al. CD8+ T cell cytotoxicity mediates pathology in the skin by inflammasome activation and IL-1beta production. PLoS Pathog. 2017;13(2):e1006196.
205. Hartley MA, et al. Leishmania guyanensis parasites block the activation of the inflammasome by inhibiting maturation of IL-1beta. Microb Cell. 2018;5(3):137–49.
206. Esch KJ, et al. Activation of autophagy and nucleotide-binding domain leucine-rich repeat-containing-like receptor family, pyrin domain-containing 3 inflammasome during Leishmania infantum-associated glomerulonephritis. Am J Pathol. 2015;185(8):2105–17.
207. Giraud E, et al. Osteopontin in the host response to Leishmania amazonensis. BMC Microbiol. 2019;19(1):32.
208. Thorstenberg ML, et al. P2Y2 receptor induces *L. amazonensis* infection control in a mechanism dependent on caspase-1 activation and IL-1beta secretion. Mediat Inflamm. 2020;2020:2545682.
209. Saresella M, et al. Leishmania infantum infection reduces the amyloid beta42-stimulated NLRP3 inflammasome activation. Brain Behav Immun. 2020;88:597–605.
210. Gatto M, et al. Transcriptional analysis of THP-1 cells infected with Leishmania infantum indicates no activation of the inflammasome platform. PLoS Negl Trop Dis. 2020;14(1):e0007949.
211. Lima-Junior DS, et al. Dectin-1 activation during leishmania amazonensis phagocytosis prompts Syk-dependent reactive oxygen species production to trigger inflammasome assembly and restriction of parasite replication. J Immunol. 2017;199(6):2055–68.

212. Shio MT, et al. PKC/ROS-mediated NLRP3 inflammasome activation is attenuated by leishmania zinc-metalloprotease during infection. PLoS Negl Trop Dis. 2015;9(6):e0003868.
213. Clay GM, et al. An anti-inflammatory role for NLRP10 in murine cutaneous leishmaniasis. J Immunol. 2017;199(8):2823–33.
214. Miranda MM, et al. Kaurenoic acid possesses Leishmanicidal activity by triggering a NLRP12/IL-1beta/cNOS/NO pathway. Mediat Inflamm. 2015;2015:392918.
215. Tiwari N, et al. Identification and characterization of miRNAs in response to leishmania donovani infection: delineation of their roles in macrophage dysfunction. Front Microbiol. 2017;8:314.
216. Ganguly S, et al. Leishmania survives by exporting miR-146a from infected to resident cells to subjugate inflammation. Life Sci Alliance. 2022;5(6).
217. Singh AK, et al. MicroRNA expression profiling of leishmania donovani-infected host cells uncovers the regulatory role of MIR30A-3p in host autophagy. Autophagy. 2016;12(10): 1817–31.
218. Kumar A, et al. Differential regulation of miRNA profiles of human cells experimentally infected by leishmania donovani isolated from Indian visceral leishmaniasis and post-kala-azar dermal leishmaniasis. Front Microbiol. 2020;11:1716.
219. Lago TS, et al. The miRNA 361-3p, a regulator of GZMB and TNF is associated with therapeutic failure and longer time healing of cutaneous leishmaniasis caused by L. (viannia) braziliensis. Front Immunol. 2018;9:2621.
220. Souza MA, et al. miR-548d-3p alters parasite growth and inflammation in leishmania (Viannia) braziliensis infection. Front Cell Infect Microbiol. 2021;11:687647.
221. Buffi G, et al. The host micro-RNA cfa-miR-346 is induced in canine leishmaniasis. BMC Vet Res. 2022;18(1):247.
222. Muxel SM, et al. Toll-like receptor and miRNA-let-7e expression alter the inflammatory response in leishmania amazonensis-infected macrophages. Front Immunol. 2018;9:2792.
223. Acuna SM, et al. miR-294 and miR-410 negatively regulate Tnfa, arginine transporter Cat1/2, and Nos2 mRNAs in murine macrophages infected with leishmania amazonensis. Noncoding. RNA. 2022;8(1).
224. de Mesquita TGR, et al. Variants of MIRNA146A rs2910164 and MIRNA499 rs3746444 are associated with the development of cutaneous leishmaniasis caused by leishmania guyanensis and with plasma chemokine IL-8. PLoS Negl Trop Dis. 2021;15(9):e0009795.
225. Lasjerdi Z, et al. Comparative expression profile analysis of apoptosis-related miRNA and its target gene in leishmania major infected macrophages. Iran J Parasitol. 2020;15(3):332–40.
226. Nimsarkar P, Ingale P, Singh S. Systems studies uncover miR-146a as a target in leishmania major infection model. ACS Omega. 2020;5(21):12516–26.
227. Hamidi F, et al. Inhibition of anti-inflammatory cytokines, IL-10 and TGF-beta, in leishmania major infected macrophage by miRNAs: a new therapeutic modality against leishmaniasis. Microb Pathog. 2021;153:104777.
228. Varikuti S, et al. MicroRNA155 plays a critical role in the pathogenesis of cutaneous leishmania major infection by promoting a Th2 response and attenuating dendritic cell activity. Am J Pathol. 2021;191(5):809–16.
229. Gholamrezaei M, et al. MicroRNAs expression induces apoptosis of macrophages in response to leishmania major (MRHO/IR/75/ER): an in-vitro and in-vivo study. Iran J Parasitol. 2020;15(4):475–87.
230. Frank B, et al. Autophagic digestion of leishmania major by host macrophages is associated with differential expression of BNIP3, CTSE, and the miRNAs miR-101c, miR-129, and miR-210. Parasit Vectors. 2015;8:404.
231. Kelada S, et al. miR-182 and miR-10a are key regulators of treg specialisation and stability during schistosome and leishmania-associated inflammation. PLoS Pathog. 2013;9(6): e1003451.
232. Loria AD, et al. Expression of serum exosomal miRNA 122 and lipoprotein levels in dogs naturally infected by Leishmania infantum: a preliminary study. Animals (Basel). 2020;10(1).

# An Insight into Immunopathology of Leishmaniasis

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**Abstract**

Leishmaniasis is a disease complex with clinical manifestations ranging from systemic visceral leishmaniasis (VL) to cutaneous leishmaniasis (CL) with skin-restricted lesions to mucocutaneous leishmaniasis (MCL) that extends to mucous membranes. These classical disease outcomes are understood as an outcome of the infecting parasite species/subspecies along with the immune correlates that define host immune status. Further each of the visceral, cutaneous and/or muco-cutaneous disease forms exhibits heterogenous gradation of parasite load, extent of parasite dissemination and collateral host immunopathological damage that may result in asymptomatic, mild, moderate or severe disease phenotype. A complex network of crosstalk between immune cells, viz. neutrophils, macrophages and heterogenous T cells, with varied effector immune molecules defines the disease protective versus progressive response. Unlike a clear Th1 versus Th2 immune response in VL and CL murine models, the immune correlates in classical VL and CL human subjects exhibit a mixed response with considerable heterogeneity. A net balance of the inflammatory versus anti-inflammatory immune response induced by the complement of antigen pool presented by discrete parasite species along with the immune regulation mediated by T regulatory cells drives the immunopathological outcome. Such immune heterogeneity extends to a newer disease phenomenon of atypical leishmaniasis wherein the parasite species classically known to cause VL is reported to cause cutaneous disease and vice versa. The biology of such atypical leishmaniasis cases is beginning to be explored in terms of the host immune changes apart from the differences in the parasite determinants. The chapter seeks to highlight the

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host immune heterogeneity that is associated with different disease outcomes in a classical setting along with atypical clinical manifestations.

**Keywords**

Leishmaniasis · Immune heterogeneity · Classical leishmaniasis · Atypical leishmaniasis

## 1 Leishmaniasis

Leishmaniasis is an infectious disease complex with clinical manifestations including systemic visceral leishmaniasis (VL) caused by *Leishmania donovani* complex (*L. donovani*, *L. infantum/chagasi*) and cutaneous leishmaniasis (CL) with skin-restricted lesions caused by *L. major* complex (*L. major*, *L. tropica*, *L. aethiopica* and *L. mexicana*) and the subgenus *L. (Viannia)* complex (*L. (Viannia) braziliensis*, *L. (Viannia) amazonensis*, *L. (Viannia) guyanensis* and *L. (Viannia) panamensis*) causing CL and MCL as per the classical association of specific parasite species with distinct clinical outcomes. The varied disease outcome is understood as an outcome of the infecting parasite species/subspecies along with the immune correlates that define host immune status. A complex interaction between the species-specific parasite determinants and the host immune components is thought to play a role in determining disease progression and outcome. With this classical understanding of leishmaniasis as a visceral or cutaneous manifestation majorly driven by the VL- and CL-specific parasite species in different endemic regions of the world, a newer phenomenon with emergence of novel parasite variants is beginning to be registered in more recent years. In these unusual endemic sites, the parasite species classically known to cause VL is reported to cause cutaneous disease and vice versa. The biology of such atypical leishmaniasis cases is beginning to be explored in terms of the infecting species and the host immune changes. In this chapter, we provide a snapshot of the host immune changes induced by distinct parasite species/subspecies that determine parasite load, extent of parasite dissemination and host tissue damage that result in different disease outcome associated with heterogenous clinical manifestations.

## 2 Classical Leishmaniasis

Different clinical manifestations ranging from visceral to cutaneous to mucocutaneous leishmaniasis initiate as a skin infection involving host-parasite crosstalk resulting in varied disease outcomes. The initial host immune changes elicited by distinct parasite species/strains set the stage for differential parasite persistence, dissemination to different tissues and extent of host immunopathology that together determine the gradation of visceral and cutaneous disease manifestations. The different *Leishmania* species with discrete antigen compliment can induce

heterogenous immune modalities and can dictate the course of disease progression and outcome. Neutrophils are the first cells to arrive at the site of infection on the skin. Infected neutrophils or free parasites can be phagocytosed by macrophages that act as host cells for the parasitic cells [1, 2]. *Leishmania* survive as intracellular parasites within the macrophages and modulate the activity of host cells to its own survival and dissemination resulting in a gradation of immunopathological manifestations. As antigen-presenting cells, species-specific infections modulate macrophage activation status such that cell-mediated immune effector function is altered in a heterogenous manner (Fig. 1). Importance of such host immune changes in determining the disease outcome is well demonstrated in experimental *Leishmania major*-infected murine model wherein C57BL/6 mice exhibit a disease resistant outcome with T cell response dominated by a macrophage-activating $CD4^+$ T helper 1 (Th1) phenotype while BALB/c mice exhibit disease susceptibility with macrophage-deactivating $CD4^+$ T helper 2 (Th2) response [3, 4]. This dichotomy in protective versus progressive T cell response does not hold true in human patients with considerable heterogeneity. The disease outcome is a more complex phenomenon with involvement of heterogenous T cell subsets with inflammatory, anti-inflammatory and/or regulatory function that fine-tune the macrophage activity to clear/permit the parasite growth and thus shape the progressive versus protective disease outcomes with gradation of clinical outcome in both VL and CL [5–9]. Considerable studies on immune correlate that determine VL versus CL immunopathologies have been done. The gradation of clinical manifestation in VL as well as CL in relation to immune correlates further adds complexity to decipher the immunological basis of different disease outcomes.

## 2.1 Immunopathology of Visceral Leishmaniasis

Visceral infection with systemic parasite dissemination ranges from asymptomatic subclinical to active disease with varied degree of parasite load, dissemination and host tissue damage. Active and severe forms of VL are characterized with fever, hepatosplenomegaly, elevated liver enzymes, hepatocellular injury, anaemia, generalized adenopathy and leukopenia. VL patients have less number of immune cell counts as compared to healthy individuals and healed VL patients [10, 11]. Asymptomatic disease shows no clinical manifestations of disease, but exhibits positive Montenegro skin test (MST) and anti-*Leishmania* antibodies [12]. *Leishmania* species causing systemic disease infect Kupffer cells, spleen and bone marrow macrophages unlike cutaneous species such that parasite has better survival amidst the high visceral temperature and adverse oxidizing environment of host cells [13]. Classical human visceral infection is associated with elevated levels of macrophage-activating Th1 promoting pro-inflammatory cytokines, viz. IFN-γ, TNF-α and IL-12, that can potentiate nitric oxide-mediated parasite killing in principle. However, the counteracting high expression of immune-suppressive cytokines IL-10 and TGF-β conditions the infected macrophages towards a deactivated phenotype resulting in enhanced parasite survival and disease

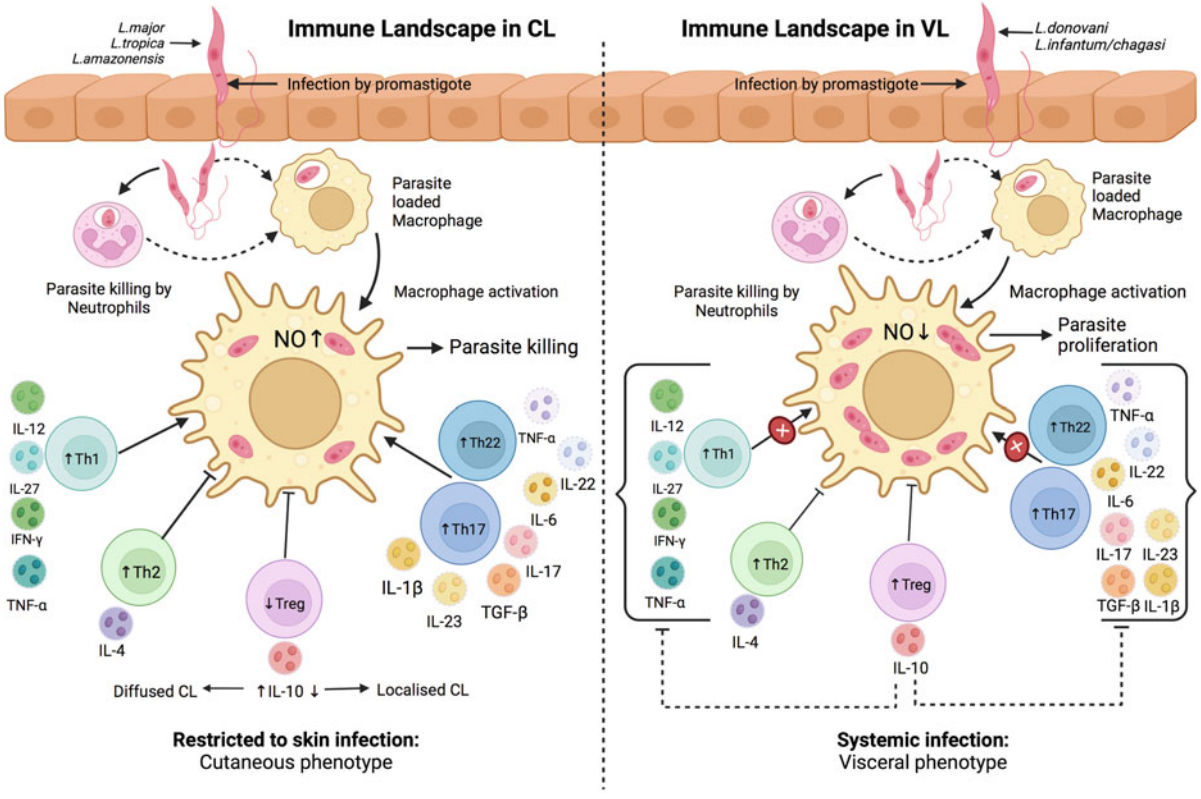


**Fig. 1** Immune landscape in CL vs. VL disease with different clinical outcomes. The figure depicts differential manifestations of Leishmaniasis initiated with infection by specific *Leishmania* species. The first cells to be recruited are the neutrophils with subsequent phagocytosis of infected, dying neutrophils and/or

free parasites by macrophages. Macrophages act as host cells and their activation status determines the degree of parasite multiplication and dissemination. Different T cell subset effector functions modulate the macrophage leishmanicidal activity and host tissue immunopathology. In the case of visceral disease, a high IL-10 activity counteracts protective response of Th1/Th17/Th22 inflammatory effector function. Relative IFN-γ to IL-10 levels act as an immune checkpoint in determining the parasite killing ability of infected macrophages. In CL, diffused versus localized cutaneous phenotype also correlate with different levels of IL-10 in relation to IFN-γ levels

progression with discrete immunopathological outcomes (Fig. 1 [5, 14–16]). The heterogeneous T cell subsets with their signature cytokines generate a gradation of immune correlates that define host ability to clear the parasite and get manifested as subclinical to mild to severe visceral disease outcome.

## T Cell Subset Cytokine Milieu with Effector Function in VL

With a major role of cell-mediated immunity in counteracting the parasitic infection, heterogenous T cell subsets with distinct effector cytokines direct the course of disease with varied infection load and collateral host immunopathology as shown in Fig. 1. Accordingly asymptomatic cases, post-kala-azar dermal leishmaniasis (PKDL) subjects, active VL patients with varied level of disease severity and those recovering after treatment exhibit a continuous gradation of T cell-associated immune correlates [7, 17–19]. The sum total of the heterogenous T cell effector responses, viz. Th1, Th2, Th17, Th22 and Treg cells, with differential expression of specific inflammatory and anti-inflammatory/regulatory cytokines acts as a useful readout of the nature of T cell response that determines the macrophage-driven parasite clearance [7, 19]. Systemic levels of different cytokines have been assessed in the plasma, serum and biopsy of VL patients compared to healthy persons [15, 19, 20]. Similar findings of increased level of IL-6, IL-10, IL-27, IFN-$\gamma$ and TNF-$\alpha$ in serum of VL patients compared to asymptomatic and control individuals have been found, with IL-6 positively correlated with severity of disease in VL patients [18, 21].

Th1 cell-specific pro-inflammatory cytokines such as IFN-$\gamma$, IL-12 and TNF-$\alpha$ lead to immunoprotection against the infecting parasite and correlate with the immunopathological damage to the host tissue [7, 8, 22]. In contrast, the Th2-specific IL-4 is a disease-promoting cytokine and counteracts IL-12-induced Th1 response. A clear opposing role of Th1-Th2 bias is however more heterogenous in human VL with the role of additional pro-inflammatory and regulatory cytokines in a crosstalk with the Th1-Th2 cytokines that modulate parasite survival and disease outcome [8]. The Th17-Th22 cytokines work in a cross-regulatory loop with antiparasitic role such that Th17 effector cytokines can lead to tissue inflammation if overproduced and Th22 effector response is understood more as tissue-protective [17]. Thus, differential expression of Th17 and Th22 cytokines together drives the degree of microbicidal activity, host tissue immunopathological damage and repair in VL with varying degree of severity. The higher Th17-Th22 type of response in VL patients can potentiate a protective phenotype by enhancing the Th1 response, neutrophil influx and production of antimicrobial peptides [23]. A landscape of pro-inflammatory antiparasitic pool of cytokines is reported in VL disease albeit with failure to control the parasite proliferation, dissemination and damage of visceral organs. This can be explained in terms of coexpression of immune-regulatory cytokines, viz. IL-10 and TGF-β, that normally help in restoration of immune homeostasis and prevent host immunopathological damage due to overt immune activation. IL-10 is the critical regulatory cytokine produced by activated macrophages, B lymphocytes, dendritic cells and T regulatory cells [24, 25]. Classical visceral disease is associated with elevated levels of IL-10 such that IL-10 promotes

systemic parasite dissemination and varied degree of tissue damage [12, 16, 19, 26–30]. The role of IL-10 as an immunosuppressive cytokine is evident with IL-10-deficient murine model exhibiting resistance to *L. donovani* infection irrespective of host genetic make-up (Balb/c and C57BL6 mice models) [26]. IL-10 modulates responsiveness of macrophages to activation signals and impacts the leishmanicidal potential of macrophages with reduced NO production and thus facilitates enhanced parasite proliferation and dissemination in VL [16].

An intact inflammatory Th1/Th17/Th22 response with IFN-γ as the key downstream macrophage-activating cytokine and an anti-inflammatory response with high IL-10 level dictates the read out in terms of IFN-γ/IL-10 ratio that determine the parasite killing and immunopathological outcome. In VL, an effective low IFN-γ/IL-10 ratio due to enhanced level of IL-10 dictates the downregulated leishmanicidal activity of infected macrophages albeit with elevated pro-inflammatory cytokines leading to visceral dissemination of the actively multiplying parasite as shown in Fig. 1.

#### Immunoglobulins and Visceral Leishmaniasis

A robust antibody response majorly comprising most abundant immunoglobulin, IgG specific to leishmanial antigens with a predominant increase in IgG1 and IgG3 subclasses and variable levels of IgG2 and IgG4 are reported in patients with VL [31–33]. Although the exact role of enhanced humoral response is not very clear in disease progression versus protection, antibody immune-complex formation and Fc-receptor engagement are reported to increase the parasite persistence via IL-10-mediated suppression of infected phagocytes such that parasite load is reported to correlate with increase in the plasma cells in systemic disease [18, 34–38].

### 2.2 Immunopathology of Cutaneous Leishmaniasis

Cutaneous leishmaniasis involves skin manifestation with subclinical to mild-to-moderate disease to extreme CL phenotypes. The cutaneous lesions may differ in number, nature, parasite load and persistence and localized versus diffuse lesions with varying timelines of healing [39]. The heterogeneous lesional and systemic host immune response associated with specific CL phenotype is modulated by the differences in local tissue interactions between the species-specific parasite determinants and the host immune components. Cutanotropic *Leishmania* species infect monocyte-derived macrophages and can downregulate the killing activity of macrophages at skin temperature ranging from 27 to 32 °C with cutaneous restricted growth and lesions and are not able to survive at higher visceral temperature [13]. Subclinical infection may show a positive delayed-type hypersensitivity (DTH) skin test with no cutaneous lesions [40]. Active CL cases may exhibit non-healing to healing lesions with heterogeneous characteristics depending on the infecting parasite species and host immune response with diverse pathological outcome. Overall, non-healing lesions correlate with a low IFN-γ and high IL-4 and IL-10 production, while healing lesions exhibit higher IFN-γ and low IL-4 and

IL-10 response. This is clearly exhibited in *L. major*-injected, resistant C57BL/6 mice model with self-resolving skin lesions associated with Th1-specific response (high levels of IFN-γ and low levels of IL-4) in comparison to susceptible BALB/c model with non-healing lesions dominated by Th2-specific response (high levels of IL-4 and low levels of IFN-γ) [9, 39].

### T Cell Subset Cytokine Milieu with Effector Function in CL

Similar to VL, differential activity of inflammatory/anti-inflammatory and regulatory T cells alters cutaneous as well as systemic immune environment in CL that culminates in varying lesional cutaneous phenotypes with varying phagocytic activity, parasite multiplication and persistence along with collateral tissue damage (Fig. 1 [9, 39, 41, 42]). Lesional cytokine environment corresponds with a mixed response comprising substantial expression of inflammatory Th1-type cytokines (IFN-γ, TNF-$\alpha$ and IL-12) with protective role along with Th2 cytokines (IL-4, IL-5 and IL-13) that counteract to facilitate parasite persistence [9, 41]. A mixed cytokine milieu may result in localized to diffused cutaneous manifestations with varying lesional outcomes, viz. wet, dry, nodular, papular or ulcerative lesions in different sizes, numbers and distribution [9, 39, 43]. Importantly, IFN-γ, TNF-$\alpha$, IL-12 and IL-4 have been found to be elevated in systemic circulation as well, although a high Th1-biased lesional protective response restricts systemic parasite circulation [41, 44]. Effector cytokines from other T cells other than Th1 and Th2 also regulate extent of parasite killing and concomitant host tissue damage. Th17-specific effector cytokine, IL-17, can add to the inflammatory cytokine milieu that can lead to enhanced neutrophil recruitment and disease progression and pathological damage [9, 39, 45, 46]. In opposition to Th17 effector response, Th22-specific cellular response is known to control parasite-induced immunopathology in CL and contributes to wound healing [47]. Thus the Th17-Th22 cytokine milieu along with Th1-Th2 cytokine balance drives the disease progression and extent of lesional pathology. Treg cell-specific cytokine IL-10 works to regulate the activated immune-inflammatory arm towards homeostasis. In the cutaneous disease with heterogeneous skin lesions, an effective Th1-Th17-Th22 parasite killing response with a gradation of low IL-10 production helps to restrict parasite multiplication albeit regulating the parasite persistence [9, 39, 48–50]. Interestingly, enhanced lesional pathology in CL is associated with absence or unresponsiveness to IL-10 as shown in IL-10-deficient and anti-IL-10-R mAb-treated *L. major* C57BL/6-resistant mouse model with large cutaneous lesions [45]. Low levels of IL-10 render effective activation of macrophage killing. In conclusion, a simplified model of cutaneous disease can be understood in terms of a mixed T cell response with effective IFN-$\gamma$-mediated parasite killing along with IL-4- and IL-10-driven disease exacerbating outcome. Overall IFN-γ/IL-4 ratio is higher in CL with effective parasite killing. Also a low IL-10 level facilitates macrophage-activating cascades such that a high IFN-γ/IL-10 ratio brings down the parasite survival. At the same time, basal parasite load persists in the lesions with varied degree of tissue damage.

### Immunoglobulins and Cutaneous Leishmaniasis

Cutaneous manifestations involve skin localized pathology, although systemic immune alterations almost always accompany the disease. With few reports of immunoglobulin assessment on CL patients, an increase in total antileishmanial IgG with variable changes in IgG subtypes has been reported. An increase in IgG1 and IgG3 levels and variable changes in IgG2 and IgG4 are documented as per the few studies available [51, 52]. The immunoglobulin isotypes are taken as a readout of differential Th1/Th2 response as seen in patients with gradation of CL manifestations.

## 2.3 Immunopathology of Atypical Leishmaniasis

Classical CL and VL with species-specific clinical outcomes, discussed in the previous section, are understood considerably well with respect to the parasite species-induced host immunopathological consequences. More recently, unusual disease cases designated as atypical leishmaniasis are emerging in known and newer endemic sites globally as well as in Indian subcontinent such that *L. donovani* complex that typically causes VL leads to cutaneous manifestation and *L. tropica* complex generally associated with CL is known to cause systemic visceral disease [53]. Specific research groups are trying to understand the basis of atypical disease aetiology in the respective atypical endemic sites. A combination of genetic variation in the causative parasite species and the accompanying host immune correlates is being deciphered to understand the biology of atypical disease outcome [54–57]. In this regard, significance of parasite determinants in directing disease outcome has been worked out with characterization of atypical parasite isolates from key endemic sites in Indian subcontinent, viz. Sri Lanka, Nepal and Kerala and Himachal Pradesh (HP) in India. The regions exhibit circulation of *L. donovani* genetic variants that unusually cause cutaneous disease [54–58].

Immunologically not much is known about the atypical cases with only few preliminary reports on lesional cytokine expression pattern in atypical CL patients from Sri Lanka [59–61]. The unexpected disease outcome as healing to non-healing skin lesions caused by variants of *L. donovani* exhibits Th1-dominating lesional cytokine environment with high IFN-γ and TNF-α expression along with marginal Th2-specific IL-4 such that the relative level of the protective IFN-γ correlates with the healing versus non-healing atypical CL [59–61]. With respect to the systemic immune profile of such atypical CL cases caused by *L. donovani*, a single study is available on such cases from the endemic state of Himachal Pradesh, India [19]. With a systemic immune-cytokine expression profile of atypical CL cases in comparison to classical VL cases, the inflammatory cytokine response that manifests parasite killing and host tissue damage was almost similar. The key IL-10 cytokine regulatory axis that differentially drives earlier discussed VL versus CL immunopathological outcomes acts as an immune checkpoint for *L. donovani*-mediated atypical CL as well [7, 9, 16, 17, 39]. Treg cells are known to secrete IL-10,

which plays a major role in deciding the fate of disease outcome such that an increase in its level leads to severity of the disease with visceral phenotype and decrease in expression is seen in cutaneous phenotype [7, 9, 16, 39, 48, 62]. With a suppressed Treg activity leading to decreased levels of IL-10, an effective Th1/Th17-Th22 parasite killing response is induced by the atypical *L. donovani* variant resulting in disease progression towards a cutaneous phenotype in the atypical CL cases over a visceral phenotype that classically correlates with high circulatory IL-10 levels. Thus differences in IL-10 levels seem to modulate a high IFN-γ/IL-10 ratio with restricted cutanotropic parasite load versus a low IFN-γ/IL-10 ratio that results in progressive visceral infection [17]. Interestingly a robust circulatory antibody response with high IgG titre is reported in atypical CL cases along with low residual circulating parasite load in some patients.

Thus genetic variations along with differences in the immunogenic antigens of the atypical CL causing *L. donovani* and the VL causing *L. donovani* strains seem to result in contrasting cutaneous versus visceral outcome [17]. With limited studies, the immunological correlates of atypical CL cases seem to fall somewhere in between VL and CL reflecting the role of immune heterogeneity in disease outcome.

## References

1. Thalhofer CJ, et al. Leukocytes infiltrate the skin and draining lymph nodes in response to the protozoan Leishmania infantum chagasi. Infect Immun. 2011;79(1):108–17.
2. Ribeiro-Gomes FL, et al. Efficient capture of infected neutrophils by dendritic cells in the skin inhibits the early anti-leishmania response. PLoS Pathog. 2012;8(2):e1002536.
3. Roberts M. Current understandings on the immunology of leishmaniasis and recent developments in prevention and treatment. Br Med Bull. 2006;75(1):115–30.
4. Sacks D, Noben-Trauth N. The immunology of susceptibility and resistance to Leishmania major in mice. Nat Rev Immunol. 2002;2(11):845–58.
5. Nylen S, Gautam S. Immunological perspectives of leishmaniasis. J Global Infect Dis. 2010;2 (2):135.
6. Samant M, et al. Role of cytokines in experimental and human visceral leishmaniasis. Front Cell Infect Microbiol. 2021;11:624009.
7. Dayakar A, et al. Cytokines: key determinants of resistance or disease progression in visceral leishmaniasis: opportunities for novel diagnostics and immunotherapy. Front Immunol. 2019;10:670.
8. Alexander J, Brombacher F. T helper1/t helper2 cells and resistance/susceptibility to leishmania infection: is this paradigm still relevant? Front Immunol. 2012;3:80.
9. Maspi N, Abdoli A, Ghaffarifar F. Pro-and anti-inflammatory cytokines in cutaneous leishmaniasis: a review. Pathogens Glob Health. 2016;110(6):247–60.
10. Kaushal H, et al. Role of CD8+ T cells in protection against *Leishmania donovani* infection in healed visceral leishmaniasis individuals. BMC Infect Dis. 2014;14(1):1–7.
11. Tanoli ZM, Rai ME, Gandapur ASK. Clinical presentation and management of visceral leishmaniasis. J Ayub Med Coll Abbottabad. 2005;17(4).
12. Costa ASA, et al. Cytokines and visceral leishmaniasis: a comparison of plasma cytokine profiles between the clinical forms of visceral leishmaniasis. Memorias do instituto oswaldo cruz. 2012;107:735–9.
13. McCall L-I, Zhang W-W, Matlashewski G. Determinants for the development of visceral leishmaniasis disease. PLoS Pathog. 2013;9(1):e1003053.

14. Caldas A, et al. Balance of IL-10 and interferon-γ plasma levels in human visceral leishmaniasis: implications in the pathogenesis. BMC Infect Dis. 2005;5(1):1–9.
15. Nylén S, Kumar R. Immunobiology of visceral leishmaniasis. Front Immunol. 2012;3:251.
16. Nylén S, Sacks D. Interleukin-10 and the pathogenesis of human visceral leishmaniasis. Trends Immunol. 2007;28(9):378–84.
17. Thakur L, et al. An insight into systemic immune response in *Leishmania donovani* mediated atypical cutaneous leishmaniasis in the new endemic state of Himachal Pradesh, India. Front Immunol. 2022;12:12.
18. Hailu A, et al. T cell subset and cytokine profiles in human visceral leishmaniasis during active and asymptomatic or sub-clinical infection with *Leishmania donovani*. Clin Immunol. 2005;117(2):182–91.
19. Ansari NA, Saluja S, Salotra P. Elevated levels of interferon-γ, interleukin-10, and interleukin-6 during active disease in Indian kala azar. Clin Immunol. 2006;119(3):339–45.
20. Peruhype-Magalhaes V, et al. Mixed inflammatory/regulatory cytokine profile marked by simultaneous raise of interferon-γ and interleukin-10 and low frequency of tumour necrosis factor-α+ monocytes are hallmarks of active human visceral leishmaniasis due to *Leishmania chagasi* infection. Clin Exp Immunol. 2006;146(1):124–32.
21. Dos Santos PL, et al. The severity of visceral leishmaniasis correlates with elevated levels of serum IL-6, IL-27 and sCD14. PLoS Negl Trop Dis. 2016;10(1):e0004375.
22. Osero BO, et al. Unravelling the unsolved paradoxes of cytokine families in host resistance and susceptibility to Leishmania infection. Cytokine: X. 2020;2(4):100043.
23. Pitta MG, et al. IL-17 and IL-22 are associated with protection against human kala azar caused by *Leishmania donovani*. J Clin Invest. 2009;119(8):2379–87.
24. Mantovani A, et al. The chemokine system in diverse forms of macrophage activation and polarization. Trends Immunol. 2004;25(12):677–86.
25. O'Garra A, et al. IL-10-producing and naturally occurring CD4+ Tregs: limiting collateral damage. J Clin Invest. 2004;114(10):1372–8.
26. Murphy ML, et al. IL-10 mediates susceptibility to *Leishmania donovani* infection. Eur J Immunol. 2001;31(10):2848–56.
27. Ghalib HW, et al. Interleukin 10 production correlates with pathology in human *Leishmania donovani* infections. J Clin Invest. 1993;92(1):324–9.
28. Bhattacharya P, et al. Induction of IL-10 and TGFβ from CD4+ CD25+ FoxP3+ T cells correlates with parasite load in Indian kala-azar patients infected with *Leishmania donovani*. PLoS Negl Trop Dis. 2016;10(2):e0004422.
29. Khoshdel A, et al. Increased levels of IL-10, IL-12, and IFN-in patients with visceral leishmaniasis. Braz J Infect Dis. 2009;13:44–6.
30. Mege JL, et al. The two faces of interleukin 10 in human infectious diseases. Lancet Infect Dis. 2006;6(9):557–69.
31. Ghosh AK, Dasgupta S, Ghose AC. Immunoglobulin G subclass-specific antileishmanial antibody responses in Indian kala-azar and post-kala-azar dermal leishmaniasis. Clin Diagn Lab Immunol. 1995;2(3):291–6.
32. Chatterjee M, et al. Distribution of IgG subclasses in antimonial unresponsive Indian kala-azar patients. Clin Exp Immunol. 1998;114(3):408–13.
33. Anam K, et al. Immunoglobulin subclass distribution and diagnostic value of *Leishmania donovani* antigen-specific immunoglobulin G3 in Indian kala-azar patients. Clin Diag Lab Immunol. 1999;6(2):231–5.
34. Kane MM, Mosser DM. The role of IL-10 in promoting disease progression in leishmaniasis. J Immunol. 2001;166(2):1141–7.
35. Galvão-Castro B, et al. Polyclonal B cell activation, circulating immune complexes and autoimmunity in human American visceral leishmaniasis. Clin Exp Immunol. 1984;56(1): 58–66.
36. Buxbaum LU, Scott P. Interleukin 10-and Fcγ receptor-deficient mice resolve *Leishmania mexicana* lesions. Infect Immun. 2005;73(4):2101–8.

37. Miles SA, et al. A role for IgG immune complexes during infection with the intracellular pathogen Leishmania. J Exp Med. 2005;201(5):747–54.
38. Elshafie AI, et al. Circulating immune complexes (IC) and IC-induced levels of GM-CSF are increased in Sudanese patients with acute visceral *Leishmania donovani* infection undergoing sodium stibogluconate treatment: implications for disease pathogenesis. J Immunol. 2007;178 (8):5383–9.
39. Scorza BM, Carvalho EM, Wilson ME. Cutaneous manifestations of human and murine leishmaniasis. Int J Mol Sci. 2017;18(6).
40. Lucas PC, et al. Epidemiologic and immunologic findings for the subclinical form of *Leishmania braziliensis* infection.
41. Castellano LR, et al. Th1/Th2 immune responses are associated with active cutaneous leishmaniasis and clinical cure is associated with strong interferon-gamma production. Hum Immunol. 2009;70(6):383–90.
42. Ajdary S, et al. Comparison of the immune profile of nonhealing cutaneous Leishmaniasis patients with those with active lesions and those who have recovered from infection. Infect Immun. 2000;68(4):1760–4.
43. Heinzel FP, et al. Production of interferon y, interleukin 2, interleukin 4, and interleukin 10 by CD4' lymphocytes in vivo during healing and progressive murine leishmaniasis. Proc Natl Acad Sci. 1991;88:7011–5.
44. Kumar R, Bumb RA, Salotra P. Evaluation of localized and systemic immune responses in cutaneous leishmaniasis caused by *Leishmania tropica*: interleukin-8, monocyte chemotactic protein-1 and nitric oxide are major regulatory factors. Immunology. 2010;130(2):193–201.
45. Gonzalez-Lombana C, et al. IL-17 mediates immunopathology in the absence of IL-10 following Leishmania major infection. PLoS Pathog. 2013;9(3):e1003243.
46. Lopez Kostka S, et al. IL-17 promotes progression of cutaneous leishmaniasis in susceptible mice. J Immunol. 2009;182(5):3039–46.
47. Gimblet C, et al. IL-22 protects against tissue damage during cutaneous leishmaniasis. PLoS One. 2015;10(8):e0134698.
48. Scott P, Novais FO. Cutaneous leishmaniasis: immune responses in protection and pathogenesis. Nat Rev Immunol. 2016;16(9):581–92.
49. Katara GK, et al. Analysis of localized immune responses reveals presence of Th17 and Treg cells in cutaneous leishmaniasis due to *Leishmania tropica*. BMC Immunol. 2013;14(1):1–9.
50. Belkaid Y, et al. CD4+ CD25+ regulatory T cells control Leishmania major persistence and immunity. Nature. 2002;420(6915):502–7.
51. Rodriguez V, Centeno M, Ulrich M. The IgG isotypes of specific antibodies in patients with American cutaneous leishmaniasis; relationship to the cell-mediated immune response. Parasite Immunol. 1996;18(7):341–5.
52. Ozbılge H, et al. IgG and IgG subclass antibodies in patients with active cutaneous leishmaniasis. J Med Microbiol. 2006;55(10):1329–31.
53. Thakur L, et al. Atypical leishmaniasis: a global perspective with emphasis on the Indian subcontinent. PLoS Negl Trop Dis. 2018;12(9):e0006659.
54. Kumar NP, et al. Cutaneous leishmaniasis caused by *Leishmania donovani* in the tribal population of the Agasthyamala Biosphere Reserve forest, Western Ghats, Kerala, India. J Med Microbiol. 2015;64(Pt 2):157–63.
55. Bastola A, et al. A case of high altitude cutaneous leishmaniasis in a non-endemic region in Nepal. Parasitol Int. 2020;74:101991.
56. Siriwardana Y, et al. *Leishmania donovani* induced cutaneous leishmaniasis: an insight into atypical clinical variants in Sri Lanka. J Trop Med. 2019;2019:4538597.
57. Thakur L, et al. *Leishmania donovani* infection with atypical cutaneous manifestations, Himachal Pradesh, India, 2014-2018. Emerg Infect Dis. 2020;26(8):1864–9.
58. Lypaczewski P, et al. An intraspecies *Leishmania donovani* hybrid from the Indian subcontinent is associated with an atypical phenotype of cutaneous disease. iScience. 2022;25(2): 103802.

59. Atapattu D, et al. The first documentation of the immune response to cutaneous leishmaniasis caused by *Leishmania donovani* in Sri Lanka. J Infect Dis. 2017;7(2):76.
60. Manamperi NH, et al. In situ immunopathological changes in cutaneous leishmaniasis due to *Leishmania donovani*. Parasite Immunol. 2017;39(3):e12413.
61. Galgamuwa LS, et al. Assessment of intralesional cytokine profile of cutaneous leishmaniasis caused by *Leishmania donovani* in Sri Lanka. BMC Microbiol. 2019;19(1):14.
62. Gautam S, et al. IL-10 neutralization promotes parasite clearance in splenic aspirate cells from patients with visceral leishmaniasis. J Infect Dis. 2011;204(7):1134–7.