Review Article

Perceptions on therapeutic modalities regarding the virulence and immunity of cutaneous leishmaniasis

Enas A El Saftawy[1,2], Ahmed A Hamed[2], Ahmed Sameh[2], Rania M Sarhan[2,3]

Departments of Medical Parasitology, Faculties of Medicine, Cairo[1] and Ain Shams[3] Universities, Armed Forces College of Medicine[2], Cairo, Egypt

ABSTRACT

Despite the wide variety of *Leishmania* spp. virulence, the present repertoire of drugs has limited effects, showing increased resistance. The effect depends on host immune factors which differ between immunocompetent and immunocompromised patients, and among various clinical forms of the disease. Recently, metallocomplexes have been increasingly shown to be potent delivery systems for conventional treatments. Additionally, lasers were suggested as an efficacious treatment tool due to their potentials in the clinical applications and resolution of the disease. This review suggests that the promising leishmanicidal activity of the metallocomplexes and laser treatment comprise a new hopeful alternative in the search for definitive cutaneous leishmaniasis (CL) cure.

Keywords: cutaneous leishmaniasis, leishmanial drug resistance; laser, metallocomplexes, virulence factors.

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Corresponding Author: Rania M Sarhan, **Tel.:** +20 1223493624, **E-mail:** raniasarhan99@gmail.com

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INTRODUCTION

The phlebotamine insect vector deposits metacyclic promastigotes during its blood meal that initiate the infection. The earliest sign of cutaneous leishmaniasis (CL) is a small erythema that proceeds into a papule and then into a nodule that gradually ulcerates in two weeks to six months, producing the distinctive lesion of localized CL (LCL)[1]. Once in the skin, the parasites interrelate with the extra-cellular matrix of connective tissue and basement membrane proteins, until the establishment of infection within phagolysosomes of the macrophages[2]. Currently available drugs for treatment of this dermal disease have restricted therapeutic effects, due to their frequent adverse reactions, drug resistance, or the parasite-host immune relationship. This drug resistance was found to be provoked by the virulence of the parasite and the immune aspects in the host[3,4]. Recently, substantial efforts have been made to modernize drug delivery systems to boost the bioavailability and pharmacokinetic profiles of conventional drugs[4,5]. In addition, phototherapy has been regarded as an updated therapeutic modality in leishmaniasis[5]. The current review discusses the therapeutic modalities as regards: I) the drug resistance; II) *Leishmania* virulence; III) The ability of *Leishmania* amastigotes to cause immunomodulation and immune-evasion and thus the severity of the disease; IV) New therapeutic modalities.

[I] Resistance to current medical therapeutics

1. Antimonial drugs

Since the 1950s, pentavalent antimony (SbV), sodium antimonate gluconate (SSG) or meglumine antimonate have been widely used to treat all clinical forms of leishmaniasis[6], as replacement for the Tartar emetics that were extremely effective in treating leishmaniasis but were abandoned due to their toxicity[7].

Dose and route of administration: Parenteral administration for at least three weeks (20-30 d, 20 mg SbV/kg/d)[5]. SbV drugs are rapidly absorbed into the blood, with a half-life of 2 h and an average terminal half-life of 76 h when administered intravenously[8]. In endemic areas intralesional treatment is recommended due to its standard systemic efficiency, fewer side effects and lower economical costs[9].

Mechanism of action: Despite the long use of antimony, its mode of action is still poorly understood. Several studies have shown that SbV drugs which are biologically inactive pro-drugs become reduced to a toxic and active form of trivalent antimonials (SbIII) against *Leishmania*[10]; either in the cell of the host and/or the parasite[11] (Figure 1). The molecular targets of the drug involve the tryptophane redox system[12] that maintains the cytosolic redox homeostasis and the zinc finger motifs[13]. The later molecules bind to the surface glycoprotein in the parasite and are directly convoluted in its DNA replication. Additionally, it suppresses the purine transporters in *Leishmania*[14].

However, in visceral leishmaniasis (VL), the efficiency of antimonials deteriorates in immunocompromised patients[15] accentuating the essential role of immune competency. In the same context, SbV was found to be dependent on the

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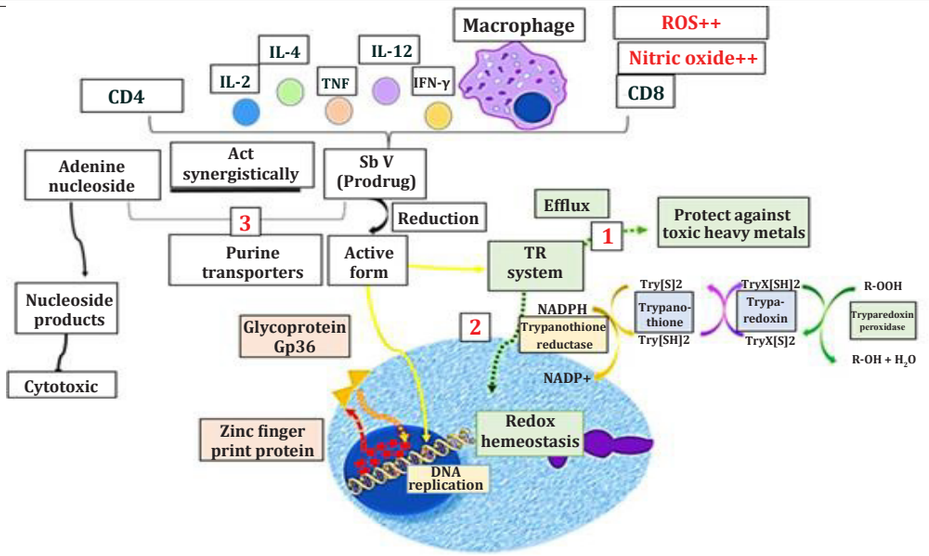

Fig. 1. Paradigm showing the actions of the SbIII on parasite-derived molecular targets: **(1)** TR system. **(2)** Zinc finger print protein. **(3)** Synergistic action with adenine and deoxynucleoside complexes. **IFN-γ:** Interferon-γ; **TR:** Trypanotione reductase; **Gp63:** Glycoprotein 63; **ROS:** Reactive oxygen species. Illustrated by E. Elsaftawy.

subsets of $CD4^+$ and $CD8^+$ T cells, and their cytokines profiles[16] as well as the triggered production of reactive oxygen species (ROS) and nitric oxide (NO) in mouse macrophages[17]. Interestingly, co-treatment of infected macrophages with exogenous IFN-γ and TNF-α can considerably destroy the parasites and lead to SbV accumulation[16]. It is also organ-dependent, being more efficient in the liver than the spleen or bone marrow[18] due to the pharmacokinetic profile of the drug[19].

Side effects and drug resistance: Injection pain and systemic side effects have been recorded[20]. Sodium antimony gluconate despite being described with minor side effects at the therapeutic doses[6], has cumulative effects such as acute interstitial nephritis and cardiotoxicity during or after a long course of drug administration[20,21]. Dangerous cardiotoxicity features occur in 50% of the patients and include a concave ST segment, corrected QT interval prolongation followed by multiple ventricular ectopic foci, then ventricular tachycardia, torsade de pointes, ventricular fibrillation[15] and diminution in the height of T waves and T-wave inversion[22]. This was attributed to the high affinity for sulfhydryl groups that affect the calcium channels[23].

In accordance, it has been found to prolong the action potential of ventricular myocytes in guinea pigs at therapeutic doses with developed QT prolongation and life-threatening arrhythmias[24]. Higher doses of SbV were found to be associated with increased pancreatitis[25] especially in AIDS patients[26]. In New World CL, elevation of pancreatic and liver enzymes was also observed in a study at the dose of 20 mg/kg/d for 20 d[27]. In Brazil, a higher frequency of skin reactions was observed in some patients with CL treated with meglumine antimoniate, due to the greater concentrations of total and trivalent antimony, lead, cadmium, arsenic and lower values of osmolarity and pH[28]. These effects can lead to cessation of treatment before attaining curative levels[29].

Additionally, the emergence of parasite resistance against SbIII was recorded in some areas suffering from VL e.g., India[30-32]. Drug resistance was suggested to be related to parasite proteins involved in the drug efflux e.g., aquaglyceroporin-1[33] and *Leishmania* adenosine triphosphate (ATP)-binding cassette-G2 (LABCG2)[34]. *L. mexicana* is less sensitive to SbV than *L. braziliensis* while *L. major* amastigotes in mouse macrophages were found to be less sensitive to SSG than *L. donovani* amastigotes[35]. Nevertheless, it has been considered as the first line treatment due to deficiency of vaccines and limited therapies[36].

2. Pentamidine

Dose and route of administration: The intramuscular dose of pentamidine is 4 mg/kg, and the peak plasma concentration is about 0.5 mg/l, reached within 1 h; and continues to be identified in the plasma for 6-8 w after administration, due to wide tissue binding of the drug[37].

Mechanism of action: It causes inhibition of the active transport system and mitochondrial topoisomerase II, which ultimately destroys the parasite[38].

Side effects and drug resistance: Tubular nephrotoxicity due to renal accumulation of the drug[39]. In addition, it is believed that direct cytotoxic effect on pancreatic islet cells can cause hypoglycemia (through initial insulin release), followed by hyperglycemia (through cell lysis and insulin consumption)[40]. Other adverse reactions include hypotension, and abnormal

hypoglycemic or hyperglycemic reactions, leukopenia, abnormal liver function, hypocalcemia, and local irritation at the intramuscular injection site in up to 45% of recipients, which hinders continuity of the treatment[41]. However, among AIDS patients, the incidence of adverse reactions caused by pentamidine are lower than with the trimethoprim compound (45% and 65%, respectively).

Relapses[42] and opportunistic respiratory tract infections[43] were recorded in a small number of patients. However, the reduced efficacy of the drug has been recorded and attributed to possible drug resistance[44]. In the same context, in *in vitro* studies, parasites have been found to develop drug resistance by gradually increasing the drug concentrations[45]. In 2003, pentamidine resistance protein-1 (1807 amino acids) that belongs to P-glycoprotein/MRP ABC transporters was reported. However, the same study reported that verapamil can reverse its action[46]. In *L. mexicana*, resistance to pentamidine involved the efflux of the drug from the mitochondrion of the parasite[47].

3. Miltefosine

Dose and route of administration: The reported dose for miltefosine in post Kala Azar dermal leishmaniasis (PKDL) is 100–150 mg/d for 60 or 90 d orally. While in New World CL, it is administrated for 20–28 d orally with the same doses. In *L. panamensis* in Colombia, *L. braziliensis* in Brazil and Bolivia, and *L. guyanensis* in Brazil, miltefosine (2.5–3.3 mg/kg/d) is administrated orally for 28–42 d[48]. Miltefosine is the first oral drug with obvious curative effect on both types of diseases; visceral and cutaneous with curative rate > 90%[49]. In *L. amazonensis*, animals treated with miltefosine (20–50 mg/kg/d) revealed a substantial dose-dependent reduction in lesion size; moreover, in mice that received higher doses, the lesions disappeared after treatment[50].

Mechanism of action: Pinto-Martinez *et al.*[51] reported two mechanisms of action for miltefosine in *L. donovani*, both related to disruption of parasite $Ca2^{+}$ homeostasis by (1) stimulation of the plasma membrane $Ca2^{+}$ channels, and (2) rapid alkalization of acidocalcisomes (Figure 2). In addition, the treatment of macrophages with miltefosine increases the phagocytosis, NO production by infected and non-infected macrophages[52], and the expression of macrophages' IFN-γ and IL-12 by enhancing CD40 and inducing Th1 responses[53]. In addition, it is assumed that the drug disrupts lipid metabolism, causes mitochondrial dysfunction, and induces apoptosis of the parasite[54].

Side effects and drug resistance: Long-term side effects include gastric manifestations, dizziness, motion sickness, and headache. Despite the minimal side effects, it possesses genotype dependent drug sensitivity[55]. For example, *L. donovani* is the most vulnerable species to miltefosine rendering it the only oral drug used to treat VL[55,56]. The emergence of miltefosine resistance is speculated to be due to the inactivation of the aminophospholipid miltefosine transporter (MT) which is crucial for the drug action or the overexpression of ABC transporter; P-glycoprotein[57]. However, miltefosine resistance was reported only in one strain and the reduction in drug efficacy was described in India in 2012[58] and Nepal in 2016[59]; but investigations of drug susceptibility did not link relapse with increased resistance to the drug[60,61]. However, in 2017 Srivastava *et al.*[62] identified isolates with enhanced resistance to miltefosine indicating that if the drug use is not controlled its efficacy might be compromised.

Interestingly, Eberhardt *et al.*[63] proved that MT-deficient parasites have severely lost the ability to invade their host cells, reproduce, and to produce the typical pattern of VL infection in BALB/c mice. A condition that could not be restored even with immune suppression. Hendrickx *et al.*[64] reported that MT gene is harbored on chromosome 13.

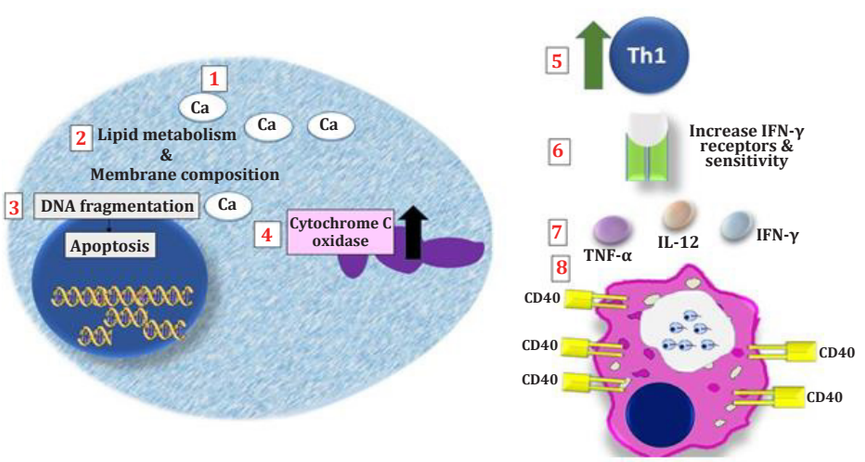

Fig. 2. Paradigm showing the actions of miltefosine. **(1-2)** Disruption of parasite calcium (Ca^{+}) homeostasis and lipid metabolism. **(3-4)** Apoptosis and mitochondrial dysfunction. **(5-8)** Enhanced immune response. Illustrated by E. Elsaftawy.

4. Amphotericin B (AMB)

Dose and route of administration: Although there are regional differences in drug susceptibility, a total dose of 20 mg/kg is effective for patients with normal immune function. Despite the high cost, toxicity, and undetermined dosing regimen, liposomal AMB is an accepted alternative for the management of cutaneous leishmaniasis[65]. However, the majority of clinical trials were directed to optimize treatment for the HIV-VL subgroups[66].

Mechanism of action: As with the binding of AmBisome (amphotecrine B liposomes) to fungal cells, also in *Leishmania* it is released from the liposome[67], traverses through the cell wall, and binds to ergosterol in the target cell membrane[68], forming pores that leak ions to induce metabolic shock, and cell death[69]. Release of AmB from the liposome occurs most efficiently at normal body temperature[70], with higher binding affinity to fungal and parasitic ergosterol compared to cholesterol[71].

Side effects and drug resistance: Johnson *et al*.[72] reported the good tolerance to intravenous amphotericin B. However, they recommended novel amphotericin B preparations for minor emergence of AmB intolerance. The major adverse effect is nephrotoxicity[73] and resistance to AmB was clinically identified in 2012. Drug resistance was related mainly to alterations in ABC transporter, membrane composition, ROS clearance, and upregulation of thiol metabolism pathways[74]. Mutation in the 14 α demethylase enzyme was reported as a marker of AmB resistance due to changes in sterol metabolism[75].

5. Paromomycin

Dose and route of administration: Paromomycin (PR) is a low-cost antibiotic with broad-spectrum activity against intestinal bacteria and parasites[76]. Its preparations are administered either topically or parenterally to treat CL at a dose of 15 mg/kg (11 mg base) for 21 d[77]. Topical PR preparations are the most commonly used for Old World and New World CL[78].

Mechanism of action: The main objective is to inhibit the production of proteins through disrupting the small subunit A decoding site of the cytoplasmic ribosome[79]. PR also affects the lipid bilayer of the parasite, the respiratory chain, the basic mitochondrial activity, and lipid metabolism[80] (Figure 3).

Side effects and resistance: Nephrotoxicity, vestibule, and cochlea malfunction are the most related side effects[74]. Induction of resistance to PR performed experimentally on *Leishmania* promastigote and amastigote forms[81] suggested its association with lipidomic and metabolomic strain-specific changes[82].

[II] Virulence factors of *Leishmania*

The molecules and cellular structures that lead several reproductive, nutritive, and locomotive vital processes to maintain the life of the parasite are called virulence factors (Figure 4). Notably, the association between these virulence factors and drug resistance have been suggested[83]. In addition, targeting of these virulence factors can aid in identification of new specific drugs with less side effects[7,84].

1. **Lipophosphoglycan (LPG):** LPG is one of the most abundant heterogeneous cell surface glycoconjugate molecules, present mainly in the promastigote stage and is strongly down-regulated or absent in amastigotes[85]. These molecules are characteristic virulence factors in the variable species during the life cycle of the parasite[86]. It seems to be involved also in the selective competency of their sand fly vectors[87]. In addition, LPG activates toll like receptors (TLRs) 1 and 2 in the cells of the innate immunity[88]. The variations in the structure of surface LPG is mandatory for the tissue tropism of different *Leishmania* spp.[86].

Notably, LPG plays a key role in the resistance of the parasite. In *L. infantum*, they are agonists to the TLR2/TLR4 and trigger the assembly of prostaglandin E2 and heme-oxygenase[89,90]. Other effects of LPG include activation of complement classical pathway, phagocytosis of promastigotes, stimulation of modulatory immune cells, modulation of the macrophages and the impairment of nuclear factor kappa of activated B cells (NF-κB) translocation in the monocytes and thus reduce

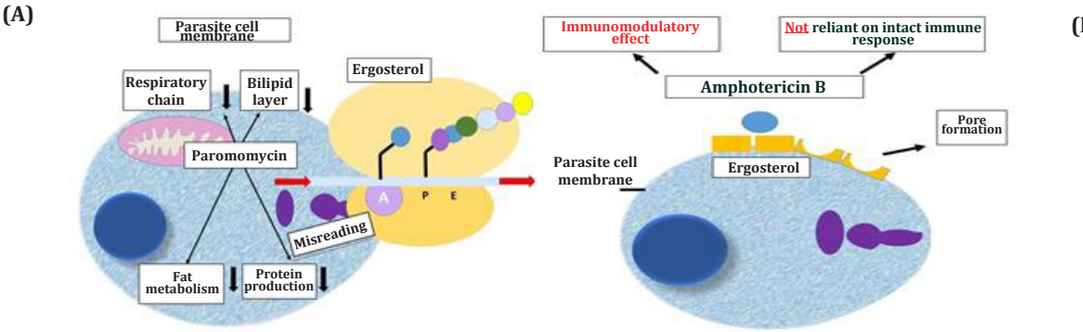

Fig. 3. Paradigm for the mode of drugs action. **(A)** Paromomycin disrupts protein production fat metabolism, and respiratory chain in the mitochondria. **(B)** Amphotericin binds to ergosterol in the target cell membrane forming pores, causes immune modulation, and is not related to the immune status of the host. Illustrated by E. Elsaftawy.

the production of IL-12[91-93]. Liu *et al.*[94] reported that LPG can modulate the dendritic cells and hence the inhibition of antigen presentation and earlier production of IL-4. Interestingly, studies that manipulated mutants of *L. major* deficient in LPG1 gene showed the vital role of LPG in the survival of the parasite in *Phlebotomus duboscqi* vectors but not in *P. argentipes* or *P. perniciosus*[87].

2. **Glycoinositolphospholipids (GIPLs):** These molecules play a significant inhibitory role in the survival of *L. major* in macrophages by inhibiting the inducible nitric oxide synthase (iNOS) and protein kinase C[95,96]. This factor seems to need various studies on the detailed spectrum of its action.

3. **Proteophosphoglycans (PPGs):** The function of membrane PPGs is not fully understood. However, they were found to trigger infection in an insulin like growth factor dependent pattern[97]. Interestingly, the parasite secretes mucin-like gel called promastigote secretory gel (PSG), composed mainly of PPGs localized in the mouth part and mid gut of sand fly vectors. These molecules aid the parasite to adapt to their vectors and enhance the regurgitation of metacyclic promastigotes during blood meal as a result of blocking the stomach valve, anterior mid gut, and mouth part of the vector[98]. More importantly, PSGs in association with the saliva of the sand fly influence the action of macrophages, recruitment of the neutrophils at the site of infection, increase the arginase enzyme activity, immune suppression and the survival of the parasite in the hostile environment with establishment of the infection[99].

4. **11 kDa Kinetoplastid Membrane Protein (KMP-11):** This hydrophobic protein is involved in the motility of the parasite and its attachment to the mammalian host cells[86]. Additionally, it stimulates the expression of IL-10 in cases of CL and MCL and inhibits the fusion of phagosomes and lysosomes in the macrophages[100,101]. However, it has been speculated in the construction of vaccines[102].

5. **Acid Phosphatases (ACPs):** These enzymes participate by stimulating the humoral immunity responses and facilitating attainment of nutrients from host cells. They also encourage the adaptation of the parasite in acidic media through inhibition of respiratory burst restraining the assembly of the oxidative products in neutrophils[103].

6. **Proteinases:** In addition to their intracellular degeneration of proteins, these enzymes are also involved in creating favorable conditions for the survival and growth mechanisms of amastigotes in macrophages. Host proteases, such as threonine, aspartyl, cysteine, and matrix metalloproteinases, can disrupt the host's immune response[104]. However, the activities of cysteine proteinases (CP) on hosts differ according to the infecting species. In this regard, CP triggered the Th2 profile in BALB/c mice infected with *L. mexicana*, in addition to their role in the induction of lesions, the production of IL-4 and IL-5, and the inhibition of IL-12 and NO production by cleaving the STAT-1 and AP-1 transcription factors. Meanwhile, in *L. chagasi* and *L. major*, CP targeted the Th1 profile and enhanced the expression of its associated cytokines[105,106], and in

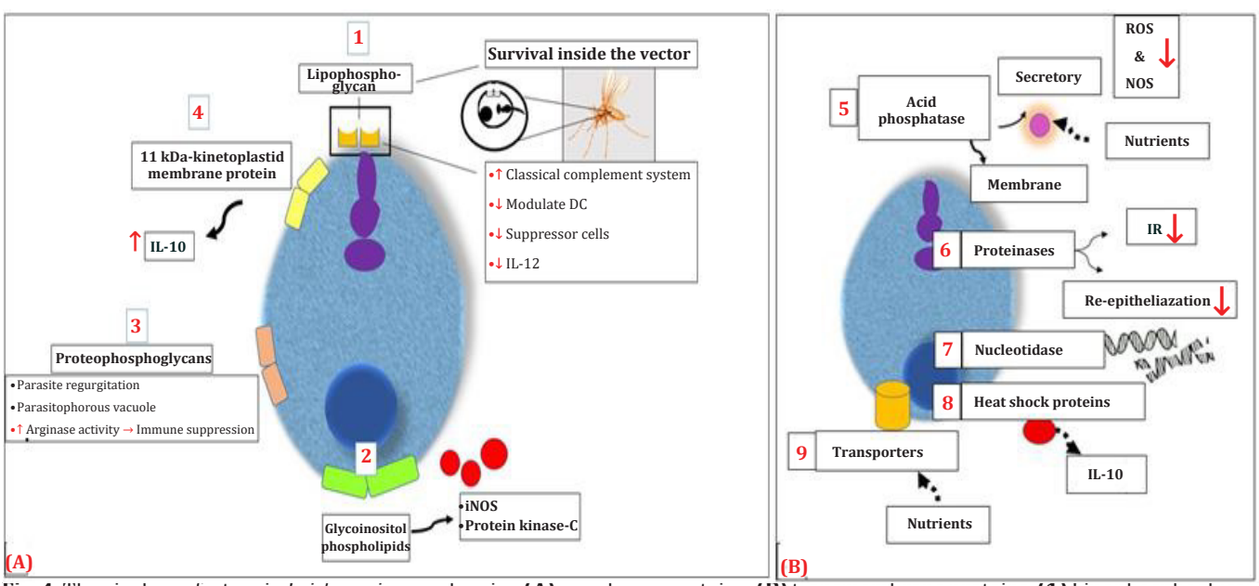

Fig. 4. The virulence factors in *Leishmania* spp. showing **(A)** membrane proteins; **(B)** transmembrane proteins. **(1)** Lipophosphoglycan; **(2)** Glycoinositolphospholipids; **(3)** Proteophosphoglycan; **(4)** 11-kDa kinetoplastid membrane protein; **(5)** Acid phosphatase; **(6)** Proteinases; **(7)** Nucleotidase; **(8)** Heat shock proteins; **(9)** Transporters. **DC:** Dentritic cell; **IL-10:** Interleukin-10; **IL-12:** Interleukin-12; **iNOS:** inducible nitric oxide synthase; **IR:** immune response; **NOS:** nitric oxide synthase; **ROS:** Reactive oxygen species; Illustrated by E. Elsaftawy.

L. amazonensis, the production of CP was associated with cleavage of MHC class II gene, the stimulation of Th1 or Th2-related cytokines; as well as the $CD8^+$ T lymphocyte[107].

Matrix metalloprotease-9 is another protease enzyme that delays the re-epithelization of chronic wounds through the stimulation of TNF-α and pro-inflammatory cytokines[108]. Furthermore, glycoprotein 63 (Gp63) is one of the main glycoproteins of the surface antigen protease that participates in parasite-host interaction and parasite virulence through binding to macrophages[109]. Additionally, a previous report documented the protective effect of Gp63 on liposome-encapsulated proteins during phagolysosome degradation in *L. mexicana* infections[110]. Another study revealed that Gp63 plays a key role in the activation and regulation of the major tyrosine phosphatase proteins involved in the JAK2/STAT1a pathway. Subsequently, this affects the IFN-γ mediated signal transduction and regulates the production of NO[111].

7. **Nucleotidases:** These are extracellular enzymes participating in the hydrolysis of tri- and/or diphosphate nucleotides into monophosphate products. Subsequently, they are hydrolyzed into adenosine and play an important role in the purinergic signal transduction. In addition, they can modulate the host's immune system, to maintain infection[112].

8. **Heat shock proteins (HSPs):** Are exosomes that play an important role in the folding, assembly, intracellular localization, secretion, regulation, stabilization, degradation of other proteins and survival at high temperatures[113]. In addition, it is related to drug tolerance[114]. HSPs contribute in immune-modulation of the innate and adaptive immunity by promoting the production of IL-10[115].

9. **Transporters:** *Leishmania* spp. express many membrane transporters for parasites' nourishment. These include pentose-phosphate pathway and purine salvage. These molecules are also mandatory for beta-oxidation of fatty acids, biosynthesis of pyrimidines, and ether-lipids[116]. Transporters are not only for nourishment, but also for several functions such as PgpA related to ABC transporters involved in drug resistance against arsenic, antimonite, and AmB[74,117].

[III] Parasitic burden and influence of immunity

After infection, *Leishmania* parasites are instantly engulfed by immune neutrophils, dendritic cells (DC), and monocytes recruited at the site of infection[118]. Neutrophils play diverse roles in the process of infection because they can destroy the parasites. However, they can also function as supplementary carriers for the parasites[119]. For example, the extracellular neutrophil trap (ENT) was shown to eradicate the promastigotes of *L. amazonensis*[120] (Figure 5A). On the other hand, the phagocytosis of apoptotic neutrophils infected by *L. major* hinders stimulation of macrophages and DCs, leading to the persistence of parasites[121] (Figure 5B). Although neutrophils are literally defined as the foremost cells enrolled after *Leishmania* infection, new evidence proposes that a cluster of inflammatory lymphocyte antigen 6 complex, locus C ($Ly6C^+$) monocytes are the first cells to migrate into the inflamed tissue. It was revealed that these monocytes can eradicate most of parasites through prompt release of ROS during phagocytosis (respiratory burst)[122]. However, other researchers have shown that $Ly6C^+$ monocytes contribute to the pathogenesis of disease by functioning as a reservoir for the propagation and cell-to-cell spread of the parasite[123]. In the later stages of infection, macrophages become the hosts for the *Leishmania* parasites[119].

Despite the extreme significance of innate immunity, activation of cell-mediated adaptive immune responses is vital for prompt resolution of the disease and development of long-term immunity. Although mixed responses of Th1 and Th2 have been monitored during active infections, the strong immune properties of Th1 are chiefly responsible for clinical cure[124].

Notably, IL-12 promotes the Th1 response and provokes the production of IFN-γ; hence it is the key cytokine in the immune response[125]. Cases with acquired immunity to localized LCL were also shown to have increased levels of IFN-γ and TNF-α cytokines[126]. This in turn triggers a respiratory burst in the macrophage[127] composed mainly of ROS and NO[50] that eliminates the *Leishmania* parasites[128] in both mice and humans[129].

Nevertheless, the extensive stimulation of $CD8^+$ cytotoxic T lymphocytes is closely related to severity of the disease, and progression of mucosal leishmaniasis, in which parasites spread to the nasopharyngeal mucosa causing disfigurement[118]. In this context, previous studies reported that early in *L. braziliensis* infection there are low serum levels of IFN-γ and high levels of IL-10 that typically reverse as the infection progresses[125,130]. The early Th2 response allows the parasite infection to persist by producing its characteristic cytokines; IL-4, IL-10, and IL-13[118]. Studies have shown that both IL-10 and IL-4 are related to the proliferation of parasites and worsening of the disease. IL-4 hampers IFN-γ production and Th1 cell differentiation, while IL-10 inhibits IFN-γ induced macrophage activation[125]. This reaction is associated with diffuse CL and accompanied with high antibody titers[131]. These findings indicate that the keynote for complete resolution in LCL pathology is the balance between anti-inflammatory and pro-inflammatory

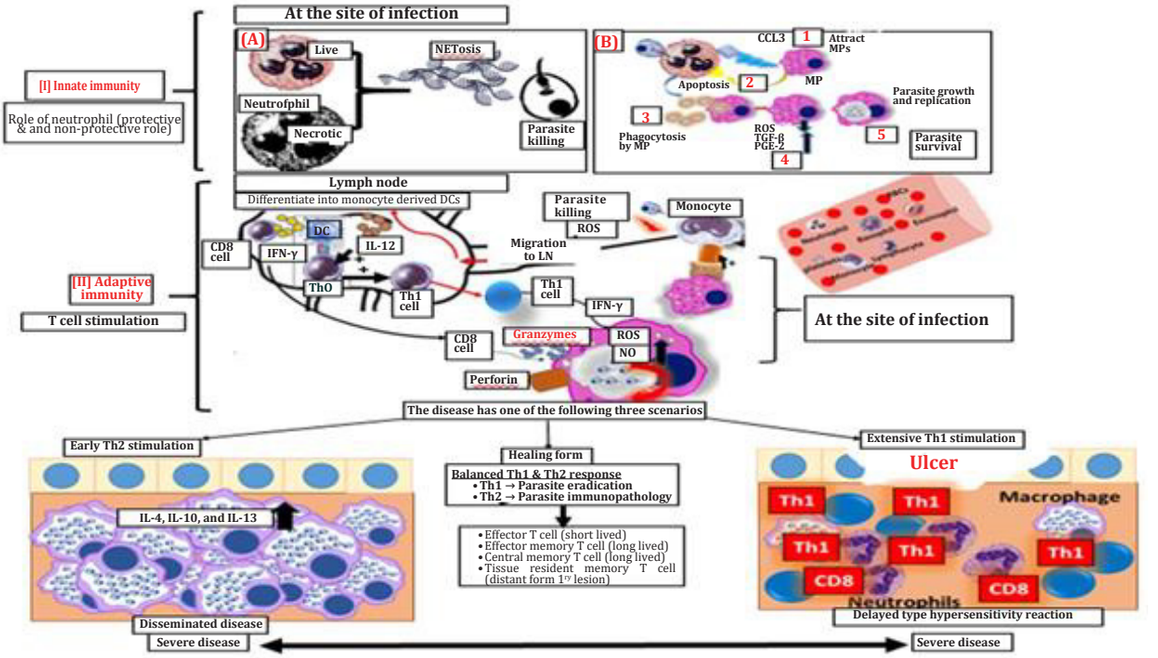

Fig. 5. Paradigm for immune response against CL. **[I] Innate immunity:** (A) Role of neutrophils in the killing of *Leishmania* parasites. (B) Phagocytosis of apoptotic neutrophils that inhibits activation of macrophages. **[II] Adaptive immunity** and the possible immune scenarios. **CCL-3:** CC chemokine ligand-3; **CCR-2:** CC-chemokine receptor-2; **DCs:** Dendritic cells; **Granzymes:** Serine protease enzymes; **IFN-γ:** Interferon-γ; **MP:** macrophage; **NETosis:** Neutrophil extracellular traps; **NO:** Nitric oxide; **PAF:** Platelet activating factor; **PGE-2:** Prostaglandin E2; **ROS:** Reactive oxygen species; **TGF-β:** Transforming growth factor–β; **Th0:** Naive T cell. Illustrated by E. Elsaftawy.

cytokines. For example, studies have shown an increase in the levels of both IL-10 and IFN-γ in the PBMC- LCL patients[125] (Figure 5).

[IV] New therapeutic modalities

(A) Drug delivery systems

A wide range of engineering technologies are concerned with formulations, manufacturing procedures, and storage systems to approach the target site efficiently and achieve the desired therapeutic effect[132]. In leishmaniasis, there are a number of controlled release delivery systems.

1. **Metallocomplexes system:** The discovery of a platinum compound (cisplatin) by Rosenberg in the 1960s was a milestone in the history of metal-based compounds used in the treatment of cancer[132]. In 2021, two studies[133,134] evaluated the effect of cobalt (Co) (II) complex on the promastigotes of *L. amazonensis*, and recorded several changes including the formation of autophagic vacuoles adjacent to the flagellar pocket. However, the lack of a clear distinction between therapeutic and toxic doses presents a challenge[135,136]. It was observed that AmB loaded on biogenic silver (Ag) nanoparticles (AgBIO) caused suppression in the parasitemia at 300-fold lower concentrations than the conventional treatment[135]. The ruthenium polypyridyl complex was speculated to reduce the numbers of infected cells *in vitro*, minimize the lesion size in the footpad of the hamster, and almost eradicated the parasites *in vivo*[136]. This complex was found to affect the parasite's biological activities, showing a high proportion of parasite fission forms, motility loss, and abundant vacuolization in the promastigotes of *L. mexicana*. In addition to growth inhibition, a leishmanistatic activity related to complexes-DNA parasite interactions was suggested[137,138]. Tetradentate Schiff base ligand combined with Co (II), Ni (II), and Zn (II), had higher leishmanicidal activity than the conventional treatments[139]. Similarly, the anti-leishmanial activity of [Au (dppz) 2] Cl3[140] and Cu (II) dimethoxy bipyridine[141] was reported. This signified the potential affinity between the metallo complexes and their targets on the molecular level.

2. **Liposomal system:** Sousa-Batista *et al.*[142] demonstrated the AmB-loaded on poly lactide-co-glycolide acid micro particles as a safe single-dosed remedy with low toxicity against *L. major* strain[142]. On the other hand, buparvaquone is a veterinary medication, whose formulation on the nanostructured lipid carriers showed high efficacy in an *in vitro* study[143].

3. **Nano-emulsions:** These proved to be an efficient delivery system for the lipophilic natural compounds. through the skin against *Leishmania* parasites[144]. Formulation of nano-emulsions comprising synthetic chalcone showed stable leishmanicidal activity in *in vitro* studies[145]. Propylene glycol is an emulsifier with lipophilic properties. Lanza *et*

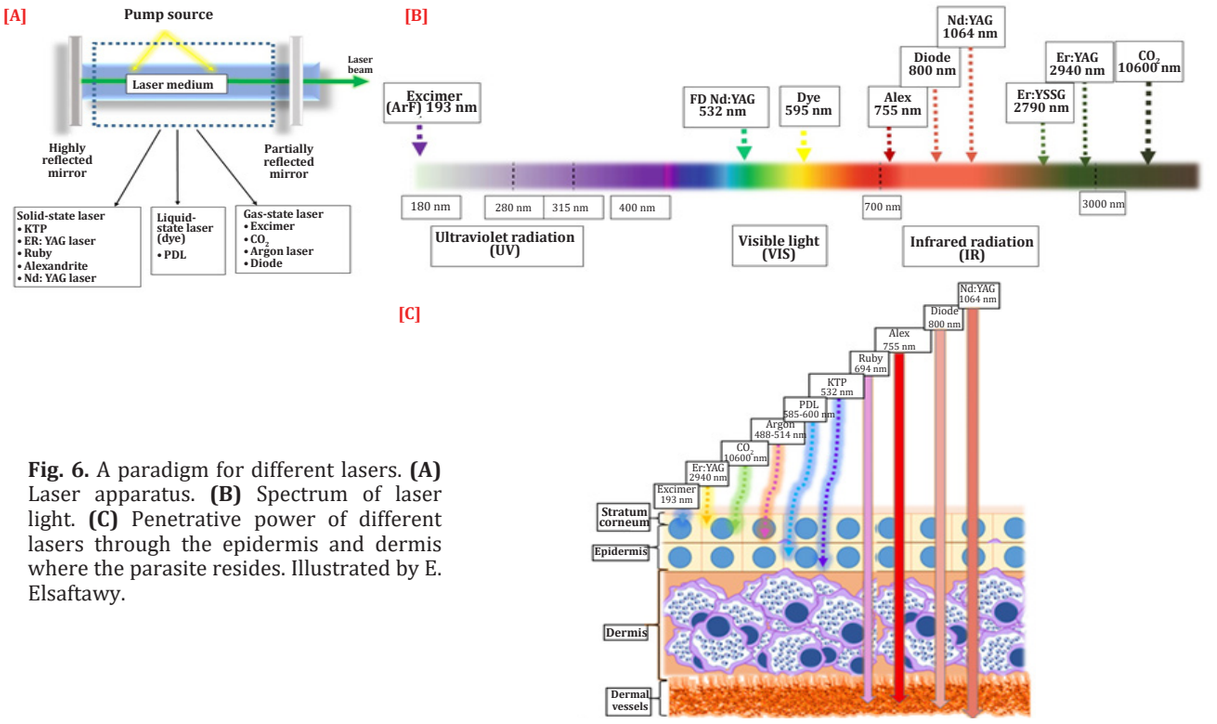

Fig. 6. A paradigm for different lasers. **(A)** Laser apparatus. **(B)** Spectrum of laser light. **(C)** Penetrative power of different lasers through the epidermis and dermis where the parasite resides. Illustrated by E. Elsaftawy.

al.[146] demonstrated a model for its use with the SbV complexes to form stabilized nano-assemblies in water against *L. amazonensis*.

4. **Chitosan scaffolds/dressings:** Chitosan based scaffolds have attracted attention, in the field of drug delivery for better tissue regeneration and wound healing. Chitosan matrix impregnated with nano-metallic components has wide potentiality due to its antimicrobial effects. Construction of wound dressing impregnated with chitosan-based nano-scaffolds showed positive impact on wound regeneration in *Leishmania* infections[147]. Studies revealed the immense effect of zinc oxide (ZnO) nanoparticles as incorporating metals in the chitosan dressings. Cu was found to increase the production of ROS. Also, acids like Zn^{++}, Ag^{+}, Au^{+}, Cu^{+} and Cu^{++} can form covalent bonds with thiols groups or proteins comprising a sulphur group[148].

(B) Laser efficacy as recent medical therapeutic modality:

The word "LASER" is an abbre*via*tion for "Light Amplification by Stimulated Emission of Radiation." The intended indications for lasers to treat diseases vary with laser wavelength. Lasers produce light energy in the form of a beam of photons released from the laser medium, which usually gives the laser its name and defines its exact wavelength (Figure 6). For visible light lasers and some near-infrared lasers, the chief target chromophores are oxy- and deoxyhemoglobin and melanin. Current medical lasers produce wavelengths ranging from the ultraviolet to the mid-infrared portions of the light spectrum. The penetration depth increases with the increasing wavelength; however, the maximum penetration depth is 5378 µm. Recently, lasers were suggested as a successful treatment tool for viral and bacterial infections in soft tissues[149]. Interestingly, combination of medical treatment and laser therapy demonstrated the best results in bacterial infections. Since *Leishmania* amastigotes reside in the epidermal (85%) and dermal (100%) layers of skin biopsies, laser has been considered an important therapeutic issue[150].

1. **Neodymium-Doped Yttrium Aluminum Garnet (Nd:YAG) laser:** Its wavelength is 1064 nm, with the highest penetration depth among the different types of lasers, and hemoglobin is its main chromophore. ND: YAG lasers influence the *via*bility of *L. donovani* and *L. major* promastigotes in culture media[151]. Goldberg and Metzler[152] demonstrated the safety of Q-switched Nd:YAG laser treatment for cosmetic resurfacing of solar-damaged skin and improvement of skin texture, elasticity, and appearance. Additionally, Cannarozzo *et al*.[153] determined its efficacy in the removal of a cosmetic tattoo despite being in proximity to sensitive areas. The efficacy of Nd:YAG laser versus intralesional meglumine antimoniate showed a significant reduction in the formed scars[154]. Nd:YAG laser is also suggested to be more effective than CO_2 laser for the cure of leishmaniasis being of minor side effects. Activation of the immune system and inflammatory reaction in addition to the cytokine changes are the other passive mechanisms of Nd:YAG laser[155]. Al-Muslet

and Khaled[156] confirmed the excellent response with minimal side effects in CL patients treated with low-level laser therapy.

2. **CO_2 Laser:** CO_2 laser showed clinical healing nearly similar to the normal appearance[157], but differed according to the concentration of the CO_2-injected NaCl[158]. Artzi *et al*.[159] determined the potent effect of topical sodium stibo-gluconate following CO_2 laser. Continuous CO_2 laser for wounds improves the healing speed with no recurrence during follow-up[160]. A similar conclusion was deduced[161], and Nieva *et al*.[162] recommended this method as a promising new prospective treatment for CL. In 2006, Asilian *et al*.[163] showed the efficacy of CO_2 laser for lupoid leishmaniasis, the chronic form with papules and nodules at the borders of a previous leishmaniasis scar, which is more common with *L. tropica* infection. Basnett *et al*.[164] reported that fractional CO_2 laser plus topical paromomycin are useful for resistant cases of CL. CO_2 laser showed higher efficacy than glucantime alone or combined with topical trichloroacetic acid (50%)[165]. In case of *Leishmania* scars, fractional CO_2 laser is more potent than ablative CO_2 laser[166].

3. **Other new lasers and light sources**
   - **Argon laser:** Zhong *et al*.[167] approved its high efficacy in *L. tropica* lesion within 6 sessions at intervals of 4-5 d.
   - **Diode laser:** It has been introduced as an alternative treatment for CL with potent cure rates[168].
   - **Pulsed dye laser (PDL):** It is recommended for the treatment of erythematous papules and nodules of leishmaniasis, and the more superficial lesions respond better to the PDL therapy. However, the larger, deeper, and more indurated lesions require more treatment sessions[169].

CONCLUDING REMARKS

1. Current medical anti-leishmanial therapies include antimonials (SSG), pentamidine, miltefosine, amphotericin B (AmB), and paromomycin (PR). The existing medications face multiple challenges; 1) generation of several side effects, 2) emergence of drug-resistance, 3) targeting of a limited range of virulence factors; and 4) host immune status.
2. Side effects include interstitial nephritis and cardiotoxicity (SSG), nephrotoxicity and cytotoxicity of pancreatic islet cells (Pentamidine), nephrotoxicity (AmB) and nephrotoxicity and ototoxicity (PR). However, miltefosine was recorded with minimal side effects.
3. Regarding drug resistance, SSG shows drug resistance in a species-related manner. Pentamidine resistance was documented to ABC transporters or drug extrusion from the parasite's mitochondrion. Miltefosine resistance was selectively identified in some isolates thus compromising its efficacy. Resistance to PR is related to lipidomic and metabolomic alterations. However, several mechanisms were suggested for AmB resistance such as ABC transporters, membrane composition, ROS clearance, upregulation of thiol metabolism pathways, and mutation in the 14α-demethylase enzyme.
4. Although *Leishmania* spp. possess several virulence factors, the current medications target few of them. Besides, drug efficiency is deteriorating in the immunocompromised patients.
5. Raised disputes initiated the search for new therapeutic modalities including drug delivery systems and laser therapy. Metallocomplexes, liposomes, nano-emulsion and chitosan scaffolds/ dressings are the most common delivery systems reported in several studies with AmB.
6. Clinical studies showed the high efficiency of Nd:YAG laser in comparison to other lasers and medical therapies through the triggering of immune responses. Moreover, combination of topical sodium stibogluconate and continuous CO_2 lasers are reported with improved healing speed. The fractional CO_2 laser is more effective than ablative CO_2.

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