Chapter 21

Novel Therapeutic Approaches to Leishmania Infection

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Additional information is available at the end of the chapter

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1. Introduction

1.1. Leishmaniasis

Leishmaniasis is a parasitic disease transmitted by phlebotomine sandflies. Approximately 1.2 million cases of cutaneous leishmaniasis (CL) and 500,000 cases of visceral leishmaniasis (VL), which is lethal if untreated, occur annually across the globe as per world health organization (WHO) estimates [1-3]. Current statistics and information relevant to leishmaniasis are summarized in Table 1. Leishmaniasis currently affects about 12 million people and it is estimated that approximately 350 million people live in risk of infection [1-3].The number of cases of leishmaniasis is probably underestimated because only 40 of the 88 countries where diseases frequently occur report them on a regular basis [4]. Leishmaniasis, is caused by several *leishmania* spp., that are obligate intracellular and unicellular kinetoplastid protozoan flagellate that establish themselves within the phagolysosome of host immune competent cells, especially macrophages and dendritic cells (DCs). In 1903, W.B. Leishman and C. Donovan reported this new parasite at the turn of the century [5,6]. Ronald Ross christened the new genus leishmania and the new species donovani in year 1903 [7]. *L. major* infection (leishmaniasis) in mice is a widely used model of human infection that has yielded critical insights into the immunobiology of leishmaniasis [8-10]. Leishmaniasis as a parasitic disease manifests itself mainly in 3 clinical forms; visceral leishmaniasis (VL), cutaneous leishmaniasis (CL) and mucocutaneous leishmaniasis (MCL), of which VL is the most severe form of the disease. VL is lethal if untreated and spontaneous cure is extremely rare. Cutaneous leishmaniasis usually has milder course and often results into a self-healing of ulcers. Resolution of leishmanial infection is dependent on the coordinated interactions between components of cell mediated immune response, specifically the activation of targeted T-cell populations for appropriate cytokine production and activation of macrophages. *L. major* infection of B6 and BALB/c mouse strains drives predominant-

ly T_H1 and T_H2 responses, respectively [11-14]. In murine model, the development of Th1 response is associated with control of infection, and Th2 response is associated with disease progression. However, Th1 and Th2 dichotomy in the human system is not as distinct as in mice and the murine model does not strictly apply to human leishmaniasis.

Parameter	Statistic or Information
Geographical location	Worldwide tropical and subtropical regions
Population at risk in 2013	~350 million
Number of people affected	~12 million
Number of deaths in 2013	~20,000 – 30,000
Number of new cases in 2013	~1.3 million
Global disease burden in 2013 (DALYs)	~1.7 million
Multidrug-resistance in 2013	Resistance to antimonials only
Visceral Leishmaniasis (VL)	~200 000 to 400 000 new cases of VL occur worldwide each year. Over 90% of new cases occur in six countries: Bangladesh, Brazil, Ethiopia, India, South Sudan, and Sudan.
Cutaneous Leishmaniasis (CL)	~One-third of CL cases occur in the Americas, the Mediterranean basin, and the Middle East and Central Asia. An estimated 0.7 million to 1.3 million new cases occur worldwide annually
Mucocutaneous Leishmaniasis	Reported in Bolivia, Brazil and Peru.
Major risk factors	Socioeconomic conditions, Malnutrition, Population mobility, Environmental changes, Climate change
Prevention and control	Early diagnosis and effective case management, Vector control, Effective disease surveillance, Control of reservoir hosts, Social mobilization and strengthening partnerships

Abbreviations: CL, cutaneous leishmaniasis; DALYs, disability-adjusted life years; NK, not known; VL, visceral leishmaniasis, WHO, World Health Organization.

Table 1. Factfile: WHO leishmaniasis statistics for 2013 (Adapted from http://www.who.int/mediacentre/factsheets/fs375/en/)

2. Conventional treatment strategies and limitations

Chemotherapy is the primary method used to control leishmaniasis. Despite the existence of several drugs for chemotherapy of human leishmaniasis, many of them are new formulations of ancient drugs repurposed in the last decade [15,16]. The treatment options for leishmaniasis are limited and include penta-valent antimonials, pentamidine, amphotericin B (AmB) and its lipoidal formulations and miltefosine, which have been introduced recently in the group of antileishmanial drugs (Table 2). Among all of these drugs, pentavalent antimonials are the first

choice drugs in most of the developing countries as in these countries treatment strategy is governed by economic factors. But a large number of incidences of resistance have been observed for antimonials, particularly in India where the failure rate has been reported up to 65% [17,18]. AmB is very effective against leishmania parasite but frequent and severe adverse effects associated with it limit its application.

Drugs	Admin Route	Dosage	Known Toxicities	Mechanism of Action	Resistance	Comment	References
Antimonial drugs (sodium stibogluconate and meglumine antimoniate (Pentostam)	IM, IV	28 mg/kg/day (28-30 days)	Cardioto-xicity, nephroto-xicity, hepatotoxicity, pancreati-tis (frequent and severe)	Activated within the amastigote, but not in the promastigote, by conversion to a lethal trivalent form. Activation. Mechanism not known. Antileishmanial activity might be due to action on host macrophage.	Failure rates up to 65% (in India)	First line drugs but high incidences of resistance has been emerged	[16,19-22]
Amphotericin B (AmB) or Polyene antibiotic	IV	0.75-1 mg/kg/day (15-20 days, daily or alternately)	Severe nephrotoxicity, infusion-related reactions (frequent and severe)	Complexes with 24-substituted sterols, such as ergosterol in cell membrane, thus causing pores which alter ion balance and result in cell death	-	Severe toxicity	[16,22,23]
Lipoidal formulations of AmB (Amphotec or Amphocil; AmBisome; Abelcet and dimyristoyl phosphatidyl glycerol with AMB)	IV	10-30 mg/kg total dose (single dose 3-5 mg/kg/ dose)	Mild nephrotoxicity (infrequent and mild)	Am**B** formulation, act by binding to the sterol component cell membranes, leading to alterations in cell permeability and cell death. They bind to the	-	High market price	[16,22,24,25]

Drugs	Admin Route	Dosage	Known Toxicities	Mechanism of Action	Resistance	Comment	References
				cholesterol component of the mammalian cell.			
Miltefosine (Hexadecylphosphocholine)	Oral	100 mg/day (28 days)	GIT problems, nephrotoxicity, hepatotoxicity, chances of teratogenicity (frequent, mild and transient)	Primary effect uncertain, possible inhibition of ether remodelling, phosphatidylcholine biosynthesis, signal transduction and calcium Homeostasis.	Common in laboratory isolates	Effective orally but its long half-life may encourage emergence of resistance on prolonged use	[16,22,26,27]
Paromomycin (Monomycin or Aminosidine	IM	15 mg/day (21 days)	Nephrotoxicit-ototoxicity and hepatotoxicity (infrequent)	In bacteria, paromomycin inhibits protein synthesis by binding to 30S subunit ribosomes, causing misreading and premature termination of mRNA translation. In Leishmania, paromomycin also affects mitochondrion.	Common in laboratory isolates	Low cost; being investigated by non-profit groups	[16,22,26]

Adapted with modifications from Jain and Jain, 2013 and van Griensven, J. and Diro, E. 2012)

Abbreviations: AmB, Amphotericin B; IM, Intramascular; IV, Intravenous;

Table 2. Standard treatment protocols for leishmaniasis, characteristics and mechanisms of action

The development of lipoidal formulations of AmB reduced the severity and frequency of adverse effects but resulted in high cost of formulation [22-25]. The conventional treatment schedule for visceral leishmaniasis suffers from a lot of problems like invasive route of administration (parenteral), long treatment course, severe toxicity (nephrotoxicity, cardiotoxicity, among others), high cost of treatment, few treatment regimens, emergence of resistance and variable patient response [17,28,29]. Thus there is continuous need for alternative new treatment strategies, vaccine candidates and new chemotherapeutic agents to provide

complete cure from leishmaniasis taking into account the fatality of disease, high toxicity, high cost and inefficiency of current treatment protocols.

2.1. Nano-based antileishmanial agents

Currently, the pharmaceutical industry has undergone a profound transformation with the advent of nano-science. With a rapid growth of nanotechnology, different nanoparticles have been presented for medical science applications. Nanomaterials have unique chemical and physical properties, and may be used in the treatment of different severe or chronic diseases in the future [30]. Hitherto, it has been shown that some metal and metal oxide nanoparticles have antimicrobial activities [31]. It has long been demonstrated that silver ions, silver nanoparticles (Ag NPs), and nanosilver-containing complexes have antimicrobial behavior with high ability to inactivate bacteria and viruses [32]. Other reports indicate that gold nanoparticles (Au NPs), titanium dioxide nanoparticles (TiO2 NPs), zinc oxide nanoparticles (ZnO NPs), magnesium oxide nanoparticles (MgO NPs), etc. have antibacterial properties [33-37]. Nanotechnology has enabled the creation of nano-particle formulations such as liposomes, microemulsions and microcapsules [34]. Liposomes are microscopic vesicles composed of one or more concentric lipid bilayers separated by aqueous media. They can encapsulate hydrophilic and lipophilic substances in the aqueous compartment of the membrane respectively. Since liposomes are biodegradable, biocompatible and non-immunogenic, they are highly versatile for research, therapeutic and analytical applications [38]. In spite of the reported antileishmanial properties of nanoparticles under UV, IR, and dark conditions, these nanoparticles have the some cytotoxicity on immune competent cells such as macrophages and other antigen presenting cells and this must be considered in future applications and studies.

2.2. Leishmaniasis vaccines

Despite the knowledge about various life stages of the parasite and the ongoing work, designing an effective vaccine against leishmaniasis is still a matter of research, there are hundreds of potent vaccine candidates but issues regarding the cost, antigenic complexity along with the variability of organisms and the mixed type of responses produced in the host are limiting the progress in the relevant direction. Thus the technical challenges and the complexity in the immunity against the parasites clearly contribute to the absence of vaccines. There are three vaccines known: two in Brazil and one in Europe out of which one is highly efficient in treating VL and CL, thus still enlightening the ray of hope for progress in this field [39]. A glimpse of various antigens that has been used as vaccine candidates in last two decades are summarized in (Figure 1).

These candidates include major surface, intracellular, stress responsive molecules, as well as other biomolecules of various metabolic pathways that can be the targets for vaccine development. Vaccine design and development has focused on all the forms of leishmania because of the conserved nature of molecules in all the species of leishmania that have been selected as the targets. Many of these targets have been studied in mice models while others in humans during diseased state producing promising results (Table 3). The availability of complete

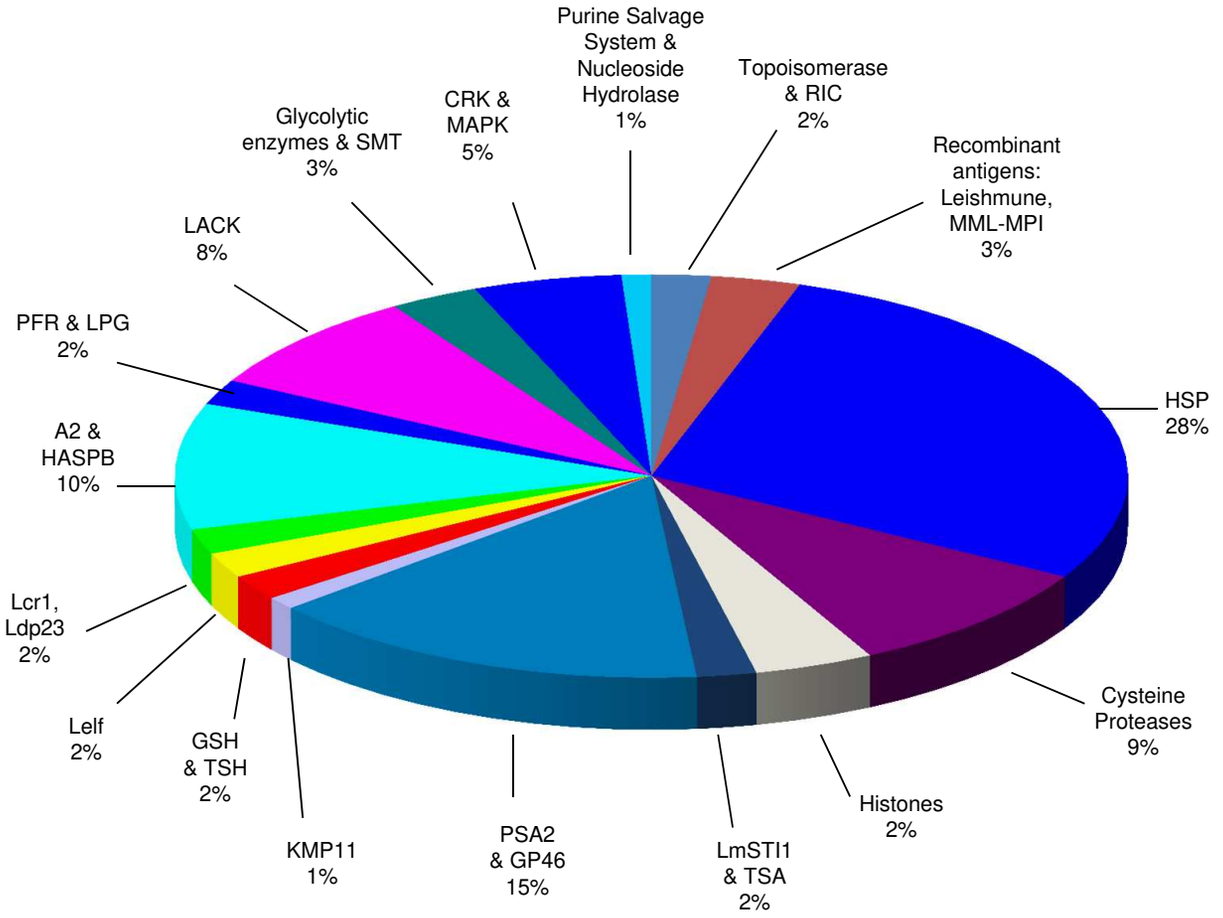

Figure 1. A glimpse of various antigens that have been used as vaccine candidates in past ten years (Adapted and modified with permission from Singh, and Sundar, 2012).

genome sequence of leishmania has provided hope for researchers to work with novel molecules as vaccine candidates [40]. The history of vaccination with the virulent forms of leishmania termed as "leishmanization" dates back to early 20th century but has since been banned for trials due safety concerns in human models [41]. First generation vaccines were limited in terms of the conferred immunity [42]. Second generation vaccines are currently in trial and are useful in providing protection of varying levels in different species along with the DNA and other subunit vaccines. The main hurdle in developing a potent vaccine stems from lack of multiple experimental study models necessary to provide all facets of immune responses in humans as well as safety issues [43].

2.3. Potential drug targets

Notwithstanding the significant progress of leishmanial research in the last few decades, identification and characterization of novel drugs and drug targets are far from satisfactory. The digenetic life cycle of leishmania consists of motile flagellated, extracellular promastigote forms which survive and multiply within the phagolysosomal compartments of macrophages and other antigen presenting cells. Therefore, the search for new

Antigenic Molecule	Vaccine type	Experimental model	References
LPG, gp 63, A2	Native antigen	Mouse	[44-47]
gp 63	Protein expressed in BCG	Mouse	[48]
gp63, gp 46, PSA-2, A2, KMP11	DNA Vaccine	Mouse	[49-53]
p36, LACK	DNA vaccine + protein expressed in vaccinia virus	Mouse	[54]
p36, LACK	Recombinant protein + IL12	Mouse	[55,56]
LmSTI1, TSA, HASPB1, CPB	Recombinant protein	Mouse	[57-59]
gp 63, KMP11	DC pulsed with native antigen	Mouse and Monkey	[60]
gp 63, LCR 1	Protein expressed in BCG	Mouse	[61,62]
Leish 111f	Recombinant polyprotein of TSA, LmSTI1 and LeIF + MPL-SE	Mouse	[63,64]
IDO 1	IDO Inhibitors / IDO specific vaccines	Mouse and Human	[65-68]

DC – dendritic cells CP – cysteine proteinase; BCG Mycobacterium bovis bacillus Calmette–Guerin; IDO, Indoleamine pyrrole 2,3-dioxygenase, IL, interleukin; MPL-SE, monophosphoryl lipid A soluble emulsion; TSA, thiol-specific-antioxidant antigen.

Table 3. Current Potent/effective vaccines against leishmaniasis

potential drug targets mainly focuses on biochemical and metabolic pathways essential for parasite survival [69-71]. The strategy to target more than one enzyme of a metabolic pathway simultaneously may prove more useful and effective. Important biochemical and enzymatic machineries that are utilized as putative drug targets for generations of true antileishmanial drugs are as follows: enzymes of polyamine synthesis [72,73], enzymes of the glycosomal machinery [74,75], enzymes of thiol-metabolic cyclin dependent kinases, enzymes of sterol biosynthesis [76,77], Pepsidases, Mitogen activated protein kinases (MAPK), dihydrofolate reductases (DHFR) [78], topoisomerases metacaspases [79,80]; and leishmanial antigens that modulate host immune functions [81].

Polyamines are not only involved in parasite growth and differentiation, but also down regulate lipid peroxidation generated by oxidant compounds to make the environment compatible for survival [82-84]. The leishmania genome has 154 peptidases namely serine, cysteine, aspartic, threonine and metallopeptidases. These enzymes play a role in reducing viability and induction of morphological changes [85,86]. Roles of other enzyme systems include but limited to roles in metabolic activities like glycolysis, oxidation of fatty acids, lipid biosynthesis and purine salvage pathways [87-103]. Other Functions of the above mentioned biochemical and metabolic pathways include: Cell division cycle, transcription, apoptosis, cell proliferation, cell differentiation and innate immunity to activation of adaptive immunity [104-113]. All these functions are essential for parasite survival, hence can be targeted to disrupt the unique targeting signal sequences.

3. Phytotherapy

Phytotherapy is the study of the use of extracts from natural origin as medicines or health-promoting agents. The main difference of phytotherapy medicines from the medicines containing the herbal elements is in the methods of plant processing. Methods of plant processing to receive medicines containing herbal elements are aimed on extraction of the chemical clean active substances, but methods of plants processing to obtain phytotherapy medicines are aimed to preserve all complex of active substances of plant in the most simple and close to the natural form. The biological activity of plant extracts has been attributed to compounds belonging to diverse chemical groups including alkaloids, flavonoids, phenylpropanoids, steroids, and terpenoids. [114-116]. Phytotherapy can be an important tool in the search for novel antileishmanial agents with fewer side effects and low cost. Firstly, the chemical diversity of plants makes them a valuable source of metabolites with pharmacological relevance [117]. Secondly, metabolites isolated from plants extracts or essential oils can be used in several different ways in the development of drugs. To obtain a herbal medicine or an isolated active compound, different research strategies can be employed, among them, investigation of the traditional use, the chemical composition, the toxicity of the plants, or the combination of several criteria [118]. In the extraction processes, different plant parts and different solvents have generally been used. In screening for biological activity, there is clearly substantial room for improvement in the extraction methodologies, since a variety of techniques can be used to prepare extracts [119-121]. Usually, solvents of different polarity are employed for the extractions. For purification and isolation, the active extracts of the plant are sequentially fractionated, and each fraction and/or pure compound can be evaluated for biological activity and toxicity. This strategy is called bioactivity-guided fractionation, which allows tests that are simple, reproducible, rapid, and low-cost [120,121]. In the last two decade, much attention has been given to the search of novel drug delivery systems for herbal drugs. The development of nanoparticles loaded with herbal drugs presents several advantages including: increase of drug solubility and bioavailability, protection against the toxicity, enhancement of pharmacological activity, increase of stability, and protection against degradation [122]. The tendency of nanoparticules, especially liposomes, to be captured by the mononuclear phagocyte system may be an additional advantage in the treatment of a variety of intracellular infectious diseases. Intraperitoneal and intravenous administration of liposomes proved to be a good bio-distribution system for drugs in the treatment of visceral leishmaniasis, since it allows increasing of drug accumulation in macrophage-rich tissues such as liver and spleen thus reducing the level of toxicity to other tissues and organs [123].

In vitro screenings are only the first steps to prove the efficacy and safety of medicinal plants for application in the treatment of leishmaniasis. In addition, variation in the efficacy of drugs in treating leishmaniasis may often result from differences in the drug sensitivity of leishmania species, the immune status of the patient, or the pharmacokinetic properties of the drug [4]. A review of the literature on the use of natural products, including plant crude extracts, fractions, isolated compounds, and essential oils, shows that there is a massive effort by scientists around the world to identify and characterize natural plant compounds with antileishmanial activity [124-126]. These efforts are now bearing fruit,

obtaining good results and validating natural products as genuine sources for drug discovery. A fitting example would be essential oils that are known to possess a wide variety of hydrophobic compounds with antimicrobial potential. The ability to diffuse across cell membranes certainly gives to those molecules some advantage in targeting cellular components, being a valuable research option for the search of bioactive compounds [124-126]. The *Ocimum gratissimum* essential oil and eugenol, its major component, was tested on the growth, viability, and ultrastructural alterations of the amastigote and promastigote forms of *L. amazonensis*, as well as on the interaction of these flagellates with mouse peritoneal macrophages, concomitant with nitric oxide production stimulation by the infected macrophages. Significant mitochondrial alterations occurred at the ultrastructural level of the parasite, such as remarkable swelling, disorganization of the inner membrane, and an increase in the number of cristae after treatment of parasites with *O. gratissimum* essential oil [125, 126]. Additionally, the linalool-rich essential oil extracted from the leaves of *Crotoncajucara*, also has effects on *L. amazonensis* parasites, on the interaction of these flagellates with mouse peritoneal macrophages and on nitric oxide production by the infected macrophages [125].

3.1. Alternative synthetic compounds

Screenings of synthetic compounds and their derivatives for antileishmanial activity has been carried out. In the last 10 years several compounds have been tested to identify potential new drugs, with the desirable characteristics. Most of the compounds exhibited antileishmanial activity against the promastigote form of L. major at non-cytotoxic concentrations and these compounds are also effective against intracellular L. major, and significantly decrease the infectivity index [127,128].

The stilbene trans-3,4 0,5-trimethoxy-3 0 -amino-stilbene (TTAS) has potent effect with low toxicity on *Leishmania infantum* (LD 50 value of 2.6 g/mL). The mechanism of action involves the disruption of the mitochondrial membrane potential and the ability to block leishmania parasites in the G2–M phase of the cell cycle [129,130]. N -Butyl-1-(4-dimethylamino) phenyl-1,2,3,4- tetrahydro- β -carboline-3-carboxamide is effective against *Leishmania amazonensis*.

BTB 06237 (2-[(2,4-dichloro-5-methylphenyl)sulfanyl]-1,3-dinitro-5-(trifluoromethyl) benzene) and its analogues, a compound previously identified through quantitative structure-activity relationship (QSAR) has also been shown, to possesses potent and selective activity against leishmania parasites. This compound and its analogues has the ability to reduce parasitemia levels in immune competent cells especially peritoneal macrophages, and additionally possess the ability to generate reactive oxygen species (ROS) in L. donovani promastigotes [131]. The in vitro antileishmanial activity of 44 derivatives of 1,3,4-thiadiazole and related compounds against promastigote forms of L. donovani have also been tested. Micromolar concentrations of these agents were used to study the inhibition of multiplication of promastigotes. Seven compounds were identified as potential anti-growth agents against the parasite [132]. Additionally, a series of 2,4,6-trisubstituted pyrimidines and 1,3,5-triazines following synthesis and screening display antileishmanial activity against L. donovani. Nitroimidazolyl-1,3,4-thiadiazole-based antileishmanial agents

against L. major also exhibits antileishmanial activity against the promastigote form of L. major at non-cytotoxic concentrations [128]. Other compounds with antileishmanial activity, both in vitro and in vivo include a series of 1- phenylsubstituted b-carbolines containing an N-butylcarboxamide group at C-3 of the b-carboline nucleus, tetrahydrobenzothieno-pyrimidines, R(+)-limonene derivatives, quinoline tripartite hybrids from chloroquine, ethambutol, and isoxyl drugs, and (4-butoxyphenyl)-N0-{2-[(7-chloroquinolin-4-yl)amino]ethyl}urea [133,136]. The urgent need to develop cost-effective new drugs and to discover novel molecules with potent antiparasitic activity and improved pharmacological characteristics cannot be overemphasized. Although many advances have been made in the treatment of leishmaniasis, much still remains to be understood.

3.2. A potential role of indoleamine 2,3-dioxygenase-specific T cells in leishmania vaccination

IDO is an immunoregulatory enzyme implicated in immunity under normal and pathological settings [137,138], and provides a potential mechanism for the development of dendritic cell (DC)–mediated T-cell tolerance [139]. IDO1 DCs inhibit T-cell proliferation due to tryptophan depletion and accumulation of toxic tryptophan metabolites [138]. 1-Methyl-D-tryptophan is an inhibitor of the enzymes IDO and INDOL1 (indolamine 2,3-dioxygenase 1 and 2) with selectivity for INDOL1 [140-142]. The enzymes perform similar transformations and are responsible for catalyzing the rate-limiting step of oxidative tryptophan catabolism in the kynurenine pathway. IDO activity is correlated with an induction of tolerance and immune-suppression through activation of regulatory T cells by metabolites generated from tryptophan catabolism. Inhibition of IDO by 1-Methyl-D-tryptophan blocks this induced immune suppression, which has shown utility in suppressing acquired immunities of tumors and indicates potential for chemical intervention of chronic inflammatory diseases [143-145]. In a recent report, Makala and colleagues [65, 66] elegantly showed that IDO is implicated in suppressing T-cell immunity to parasite antigens, and IDO inhibition reduced local inflammation and parasite burdens. The findings by Makala and colleagues support a counter-regulatory role for IDO that benefits the pathogen, not the host. In this regard, an interesting aspect of IDO is that systemic inactivation at the organism level, either pharmacologically or genetically, does not appear to cause autoimmunity [65-68,138]. A conceptual model of IDO-mediated activation and effector T cell suppression following *L. major* infection is summarized in Figure 2 [Makala, 2012].

The model depicts interactions between IDO+ DCs, Tregs and naïve T cells that drive suppressive and non-suppressive outcomes under IDO-sufficient (+) and IDO-deficient (-) conditions in response to *L. major* infection. Induced IDO activity in DCs triggers cell stress responses blocks IL6 production by pDCs themselves, and by other cells (e.g. macrophages) capable of producing IL6. Under conditions of IDO ablation the same stimuli do not create suppression, and instead DCs stimulate naïve T cells, and express IL6, which converts Tregs to TH17 T cells or promotes TH17 differentiation from naïve CD4+ T cells. The chemical structures of IDO and its inhibitors are shown in Figure 3.

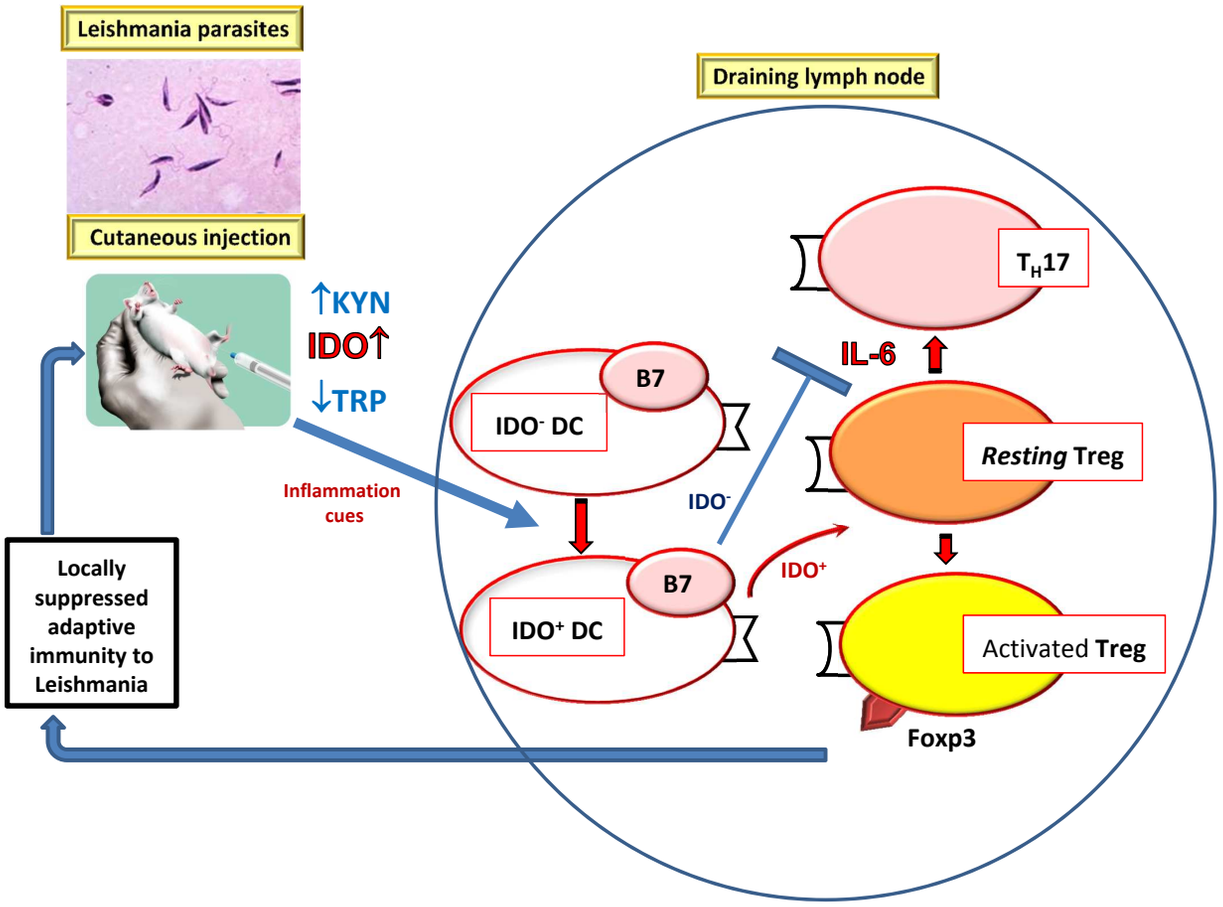

Figure 2. A conceptual model of IDO-mediated activation and effector T cell suppression following *L. major* infection. The model depicts interactions between IDO+ DCs, Tregs and naïve T cells that drive suppressive and non-suppressive outcomes under IDO-sufficient (+) and IDO-deficient (-) conditions in response to *L. major* infection. Induced IDO activity in DCs triggers cell stress responses blocks IL6 production by pDCs themselves, and by other cells (e.g. macrophages) capable of producing IL6. Under conditions of IDO ablation the same stimuli do not create suppression, and instead DCs stimulate naïve T cells, and express IL6, which converts Tregs to TH17 T cells or promotes TH17 differentiation from naïve CD4+ T cells (Adapted and modified from Makala, 2012).

To examine the possible effects (and/or side effects) of the induction of IDO-specific T cells, a phase I vaccination study is ongoing at the Center for Cancer Immune Therapy, Copenhagen University Hospital, in which patients with non-small-cell lung cancer are vaccinated with an IDO-derived peptide with Montanide adjuvant (www.clinicaltrials.gov; NCT01219348). Different species of leishmania are responsible for cutaneous, mucocutaneous, or visceral leishmaniasis infections in millions of people and animals. Adverse reactions caused by antileishmanial drugs, emergence of resistance, and lack of a vaccine have motivated the search for new therapeutic options to control this disease. There have been notable advances in molecular diagnostics, in the understanding of host immune responses to infection, and in vaccine development. The fact that IDO may be involved in tolerance to non-self-antigens, may have key attractive implications for IDO-based immune therapy as boosting immunity to neo-antigens, but not normal self-antigens, by the activation of IDO-specific T cells. Makala and colleagues [65-68, 138] demonstrated that IDO suppresses adaptive immunity, supporting

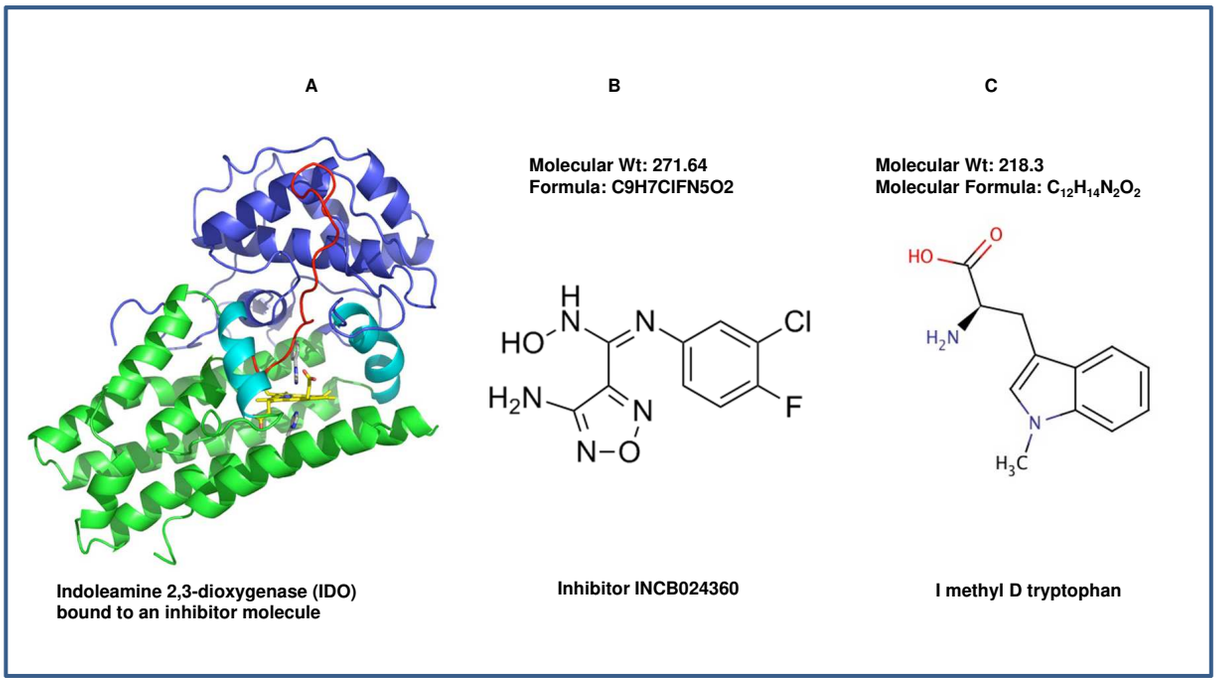

Figure 3. An illustration of the crystal structure for Indoleamine 2,3-dioxygenase (IDO) and chemical structures of its Inhibitors. A. bound to an inhibitor molecule.Indoleamine-2,3-dioxygenase inhibitors. Adapted from http://www.riken.go.jp/biometal/7_structures.files/index-e.htm. B. INCB024360 adapted from http://www.medchemexpress.com/product/INCB024360.html. C. I methyl D tryptophan (Ido inhibitor). Adapted from http://www.scbt.kr/datasheet-200313-1-methyl-d-tryptophan.html.

the notion that in clinical setting, the targeting of IDO could have synergistic effects in leishmania vaccine development. Thus, the induction of IDO-specific immune responses by therapeutic measures could function synergistically with additional immune therapy. Almost any successful immune therapy strategy aims at inducing immunological activation and inflammation. Since IDO-expressing cells might antagonize the desired effects of other immunotherapeutic approaches, targeting IDO-expressing cells by vaccination would be easily implementable and compatible with such therapeutic measures.

3.3. Multidrug treatment strategy

Combination therapy has increasingly been explored, particularly in highly endemic regions, aiming to identify a short, cheap, well-tolerated combination regimen that can preferably be given in an ambulatory way and requiring minimal clinical monitoring. To date combination therapy has shown promising results including improved treatment efficacy with reduced side effects, shorter duration of therapy, reduced cost as well as reduced incidence of resistance in phase 2 clinical trial, as has been used for diseases like malaria, tuberculosis, and HIV. [1,3,146]. A 17-day combination of antimonials with paromomycin was found effective in east Africa (93% efficacy). Owing to such fascinating results numerous phase 3 clinical trials are progressively being conducted in Asian and African continents to further investigate the clinical efficacy of combination therapy in treatment of leishmaniasis. Researchers have continued to study the effect of immunotherapy using combinations of two or more antileishmanial drugs

[146-149]. A list of completed or currently in progress clinical trials for treatment of leishmaniasis are shown in (Table 4). Sundar et al. [150] investigated the efficacy and safety of three combinations of three effective antileishmanial drugs (lipo-somal AmB, miltefosine, paromomycin) and compared their efficacy as duration of treatment with the standard monotherapy in India, that is, AmB infusion in an open-label, parallel-group, non-inferiority, randomized control trial conducted in two hospitals at Bihar, India. Combination regimens including liposomal amphotericin B (5 mg/kg single dose), paromomycin and/or miltefosine were also found highly effective (98%–99%) and safe, and are now included in WHO recommendations for the Indian subcontinent [3,146,147]. The multidrug treatment has been found equally effective as standard monotherapy even with fewer side effects and shorter course of administration [150]. Combination treatment approaches for leishmaniasis have been advocated by many scientists but they are also enforcing the simultaneous development of other measures in the control of this parasitic disease in the endemic regions of Asia and Africa including control of sandflies, clinical monitoring of treatment, advances in case detection and rapid methods of diagnosis as well as proper evaluation of various leishmania control programs [146,151,152]. The clinical efficacy of multidrug therapy has been confirmed and so far the results are convincing and give hope for the future in terms of treatment.

Condition	Intervention	Study Phase	Study Objective
ML MCL	• Meglumine antimoniate (MA)	2 3	To compare efficacy of the standard recommended schedule with an alternative regimen of MA in the treatment of ML /MCL
CL	• Paramomycin • WR279, 396 (Paramomycin / Gentamycin)	3	To determine if WR279, 396 results in statistically superior final clinical cure rates compared to Paramomycin alone
VL	• Antimoniate of N-Methylglucamine (Fungizone) • Amphotericin B Deoxycholade (Anforicin) • Liposomal Amphotericin B (Ambisome)	4	To compare efficacy and safety of medications in Brazil
L	• Sodium Stibogluconate (SSG) (Pentosam)	2	To collect safety and efficacy data on the use of Pentosam
VL	• AmBisome + Miltefosine • AmBisome + Paromomycin sulfate • Miltefosine + Paromomycin sulfate • Amphotericin B Deoxycholate	3	To identify a safe and effective combination for short course treatment of visceral leishmaniasis with reduced risk of parasite resistance

Condition	Intervention	Study Phase	Study Objective
VL	• Sodium stibogluconate • Paromomycin sulfate • Sodium stibogluconate + Paromomycin sulfate	3	To assess the efficacy and safety of sodium stibogluconate 30 days alone, paromomycin sulfate 21 days alone and sodium stibogluconate and paromomycin sulfate as a combination course of 17 days in the treatment of patients with visceral leishmaniasis
VL	• Ambisome • AmBisome + Miltefosine • AmBisome + Paromomycin • Miltefosine + Paromomycin	3	To evaluate efficacy and safety of various combinations of the three drugs; AmBisome, paromomycin and miltefosine at reduced total dosage against the standard treatment with a total dose of 15 mg/kg of AmBisome
VL	• AmBisome + Sodium stibogluconate • AmBisome + Miltefosine • Miltefosine	2	To assess combinations of sodium stibogluconate plus single dose AmBisomeW, miltefosine plus single dose AmBisomeW and miltefosine alone in treatment of visceral leishmaniasis in Eastern Africa
VL	• Miltefosine + AmBisome	2	To sequential design to combine miltefosine and AmBisome in different doses
VL	• AmBisome + Miltefosine	2	To evaluate the final cure after six months on sequential administration of both drugs. AmBisome will be given on day 1, followed by miltefosine for 14 days
VL	• Sitamaquine	2	To evaluate the final cure after six months on sequential administration of both drugs. AmBisome will be given on day 1, followed by miltefosine for 14 days

Mucosal Leishmaniasis (LM), Mucocutaneous Leishmaniasis (MCL), Cutaneous Leishmaniasis (CL), Visceral Leishmaniasis (VL), Meglumine antimoniate (MA), Leishmaniasis (L).

Table 4. Clinical trial completed or currently recruiting for treatment of leishmaniasis (at: http://clinicaltrials.gov/ (accessed 10-10-2013))

4. Concluding remarks

Leishmaniasis is one of the major neglected infectious diseases. Progress has been achieved in terms of treatment, including the development of combination therapy as well as our understanding of the molecular nature of potential vaccine candidates following the completion of the genome sequence. The occurrence of drug resistance in disease-endemic countries is concerning and should be closely monitored. In spite of all these drawbacks, there is presently rapid progress in our understanding of the molecular nature of potential vaccine candidates. There is a need to develop more potent, cost effective drugs and vaccine candidates. Total eradication of leishmaniasis will depend on the combined efforts of governments, the scientific research community, the pharmaceutical industry and people with a view to reduce the

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transmission of disease, rapid diagnosis and appropriately targeted treatment of the various forms of leishmanisis. Understanding of the molecular nature of potential vaccine candidates could potentially lead to novel gene-based, plant-based and synthetic-based therapeutic approaches or a dependable cure for leishmaniasis.

Abbreviations

LM: Mucosal leishmaniasis

MCL: Mucocutaneous leishmaniasis

CL: Cutaneous leishmaniasis,

VL: Visceral leishmaniasis

MA: Meglumine antimoniate

AmB: Amphotericin B

IM: Intramascular

IV: Intravenous

DC: dendritic cells

CP: cysteine proteinase

BCG: Mycobacterium bovis bacillus Calmette–Guerin

IDO: Indoleamine-pyrrole 2,3-dioxygenase

IL: Interleukin

MPL-SE: monophosphoryl lipid A soluble emulsion

TSA: thiol-specific-antioxidant antigen

pDC: Plasmacytoid Dendritic Cell

HSP: Heat Shock Proteins

LmSTI1: L. major stress-inducible 1

LeIF: Leishmania elongation initiation factor

KMP-11: Kinetoplastid membrane protein-11

GSH: Glutathione complex

TSH: thyroid - stimulating hormone

LACK: Leishmania analogue of the receptor kinase C

Lcr1: T-cell antigens from an amastigote of L. chagasi containing homologous 67-amino-acid repeats

Ldp23: 23 kDa highly hydrophilic protein rich in lysine residues present on the surface of L. donovani and L. major

LPG: Leishmania major lipophosphoglycan

T(SH)2: trypanothione;

CRK: cdc-2 related kinase

RIC: RNA import complex

A2: amastigote stage-specific protein family in L. donovani

HASPB: hydrophilic acylated surface protein B

PFR-2paraflagellar rod protein

MAPK: Mitogen-activated Protein (MAP) kinases

SMT: sterol 24-cmethyltransferase

GPI/GP46: glycosylphosphatidylinositol

PSA: Promastigote surface antigen

MML: multi-subunit recombinant leishmanial vaccine

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