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Chapter 1

Introductory Chapter: Leishmaniasis: An Emerging Clinical Syndrome

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Additional information is available at the end of the chapter

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1. Introduction

Leishmaniasis comprises a broad-spectrum of neglected vector-borne diseases ranging in severity from the self-healing but disfiguring and stigmatizing cutaneous lesions to mucocutaneous and fatal visceral manifestations, depending on the species and host characteristics. This syndrome primarily afflicts the impoverished population of low-income countries falling in the tropics and subtropics. Globally, 0.7–1.2 million new cases of cutaneous leishmaniasis (CL) occur every year while for visceral leishmaniasis (VL), 200,000–400,000 new cases and 20,000–40,000 deaths are reported each year, with 95% of fatal cases occurring in only six countries, namely, India, Bangladesh, Sudan, South Sudan, Ethiopia and Brazil [1]. The disease is transmitted by the bite of female *Phlebotomus* sandflies that transmit the promastigotes, which are then transformed into amastigotes within the mammalian macrophages. The goal of World Health Organization is to eliminate this public health problem in the South-east Asia Region by 2020 [2]. Despite intensive research, live vaccines are the only effective vaccines till date against CL while none exists for the visceral form that is the most severe of the various clinical forms of leishmaniasis. Moreover, there is an upward trend in development of resistance to most of the currently available drugs [3]. The chemotherapeutic arsenal is associated with need for hospitalization and prolonged periods of treatment, coupled with high toxicity, which limits the application and patient compliance. Combinations of drugs have also been explored. Absence of vaccines, progressive emergence of HIV-*Leishmania* co-infection and relapse after treatment delineate the gravity of leishmaniasis affliction [4]. A recent report indicated relapse of post kala-azar dermal leishmaniasis (PKDL) 1 year after successful treatment of VL with miltefosine and paromomycin [5]. Antimony therapy is also not advised in elderly patients with CL due to severe adverse side effects [6]. The potential of

the visceralizing species, *Leishmania donovani* to cause localized cutaneous lesions is also not fully understood [7].

This chapter gives a brief glimpse of the recent advances in immunopathogenesis and immune evasion strategies employed by the *Leishmania* parasite, vaccination and immunotherapeutic approaches, natural product-based drugs, nanomedicines, therapeutic targets and diagnosis of leishmaniasis. We have included citations of the latest research articles presenting the most recent results.

2. Immunopathogenesis and immune evasion strategies

Invasion of host macrophages by *Leishmania* triggers a multitude of signaling circuits to eliminate the pathogen. However, the parasite tries to subvert these defense mechanisms to create a safe haven for their survival. *Leishmania* secretes effector molecules to modulate the host immune transcriptome resulting in alterations in the host epigenome to alter cytokine and chemokine levels, their cross talks and downstream signaling hubs. This adversely affects the recruitment and activation of immune cells, respiratory burst and antigen presentation, leading to immune evasion. *Leishmania amazonensis* has been reported to induce histone deacetylase in infected macrophages, which contributes to down regulation of inducible nitric oxide synthase and subsequent parasite survival [8]. *L. donovani* infection causes hypoxic environment within the macrophages by activating hypoxia inducible factor-1α, that in turn up regulates micro RNA-210, while down regulating NF-κB mediated pro-inflammatory immune responses, to establish a safe niche for their survival [9].

Leishmania have evolved stratagems to neutralize macrophage defensive arsenals, the very heart of the immune system's defensive machinery, resulting in replication of the parasites within the phagolysosomal vacuoles of the infected macrophages. Unfolding of these host-pathogen interactions will help in development of effective drug targets that would enable to modulate the host immune system to ameliorate the pathogenesis of infection. Besides the host immune profile and the intrinsic parasite factors that may influence the clinical manifestations of the disease, *Leishmania* virus RNA 1 (LRV1) infecting *Leishmania guyanensis* has been implicated to contribute to immunopathogenesis of American tegumentary leishmaniasis [10]. Studies have also indicated that gut microbiota egested during infected sandfly bites is an important determinant of *Leishmania* dissemination via triggering of inflammasomes, leading to IL-1β production that sustains the neutrophilic infiltrate harboring the parasites [11].

3. Current vaccination and immunotherapeutic approach

A major challenge to mitigation of this endemic disease is to achieve safe, efficacious and low-cost prophylactic or therapeutic vaccines with long-lasting protection. These vaccines should be effective against both stages of the parasite curbing its progression and accompanying pathology that stems from an imbalance between the pathogen and the host immune

system. The plethora of candidate vaccines range from the live non-pathogenic vectors to the recombinant subunit vaccines, alone or together with adjuvants and/or delivery systems for induction of cell-mediated immunity. Some of these include *Leishmania*-activated C-kinase antigen (LACK) [12], *Leishmania* cysteine peptidase A, B in poly-lactic-co-glycolic acid (PLGA) nanoparticles [13], soluble *Leishmania* antigens in nanoliposomes co-delivered with saponin and imiquimod [14], DNA vaccine encoding ornithine decarboxylase [15]. Inclusion of salivary proteins in antileishmanial vaccines has been reported to result in a synergistic protective effect [16]. A live recombinant amastigote 2 antigen vaccine vector using *Trypanosoma cruzi* non-virulent strain, and live attenuated centrin gene–deleted *Leishmania donovani* [17] have been reported to induce strong T cell-mediated protective immune responses against VL and hence could represent promising alternatives for translation to human clinical trials [18]. Recombinant small myristoylated protein-3, a virulence factor has been found to be immunogenic in both mice and humans, with induction of protective immunity against murine VL [19]. In case of CL, intranasal immunization has been found to reduce numbers of $CD4^{+}Foxp3^{+}$ regulatory T cells with increased Th1 response and associated protection [20].

Immunotherapy on the other hand has been found to promote sterilizing cure. However, immunotherapeutic intervention with *L. amazonensis* antigens plus saponin was not found to maintain long-lasting low parasitism in dogs naturally infected with *Leishmania infantum* [21]. Therapy with anti-PDL-1 antibody has been found to promote parasite clearance with concomitant induction of protective immunity against VL by inhibiting autophagy, that is hijacked by *Leishmania* [22]. Immunotherapeutic approach with Th1 stimulating antigens (aldolase, enolase, p45 and triose phosphate isomerase) has also been attempted [23].

An emerging therapeutic modality for CL is photodynamic therapy of zinc porphyrin that results in loss of plasma membrane integrity and hyperpolarization of the mitochondrial membrane potential [24].

4. Therapeutic targets and inhibitors

Identification of new drug targets can contribute towards designing inhibitors and strengthen the pipeline for disease elimination. DNA topoisomerases that control the over- or under-winding of DNA have been reported as deadly targets for topoisomerase inhibitors that may act as potential antileishmanial drugs [25]. Computational tools using *in silico* approaches targeting key enzymes in metabolic pathways of *Leishmania* have led to identification of several potential druggable targets such as cytochrome P450 sterol 14α-demethylase [26], dihydrofolate reductase-thymidylate synthase [27], methylglyoxal degradation superpathway [28], trypanothione reductase [29]. Trypanothione reductase is absent in humans and neutralizes the reactive oxygen species generated inside the infected macrophages. Inhibitors such as chalcones that block the activity of these trypanosomatid enzymes may be effective in treatment of leishmaniasis [29]. β-carbonic anhydrase [30], acid phosphatases [31], uracil DNA glycosylase [32] and Type 2 NADH dehydrogenase [33] are other potential therapeutic targets that are being explored. NLR (NOD-Like Receptor) family member NOD2 has also been implicated as an essential therapeutic target [34].

5. Natural products as source of antileishmanial drugs

In view of looming chemotherapeutic drug resistance, natural products and scaffolds from medicinal plants are being emphasized as leads for drug discovery. Plant-based bioactive compounds have merit over synthetic compounds, considering their unique structural variety, providing an unlimited source of molecules and biological activities [35]. A host of plant extracts or oils and their phytoconstituents (alkaloids, terpenoids, quinones, flavonoids, saponins, phenylpropanoids, flavonoids, lignoids, naphthoquinones, iridoids, and more) have shown promise *in vitro* and/or *in vivo* [36–41]. In some cases, the leishmanicidal effect is potentiated by immunomodulation [3, 42]. Besides plants, secondary metabolites from microorganisms such as fungi [43] and marine organisms have also been reported. Plant defensins have been found to eliminate *Leishmania* parasites via plasma membrane perturbation, mitochondrial membrane collapse, and reactive oxygen species induction [44].

Antimicrobial peptides have been reported to improve the therapeutic outcome of antileishmanial drugs [45]. Synergistic drug-natural product combinations have also been explored [46, 47].

6. Nanomedicines

In recent years, numerous advances in drug discovery have been made for treating leishmaniasis, exploiting nanotechnological approaches to target the immune cell phagolysosomes that harbors the *Leishmania* amastigotes. A plethora of nanoparticles have been reported to elicit protection with modulation of the immune response via reduction in anti-inflammatory cytokine IL-10, and increased nitric oxide production [48]. Recently, antileishmanial activity of sulphonamide nanoemulsions have been reported that target the leishmanial β-carbonic anhydrase [30]. Linalool-loaded gold nanoparticles have also been found to exhibit therapeutic effectiveness against *Leishmania* [49]. A short-course AmBisome regimen has been found to be safe and effective in the treatment of clinically diagnosed PKDL patients in Bangladesh, and may be considered as a viable option for routine programmatic use, contributing towards the VL elimination drive [50]. Biodegradable PLGA microparticles loaded with an antileishmanial nitrochalcone has proved therapeutic effectiveness when administered subcutaneously in BALB/c mice with cutaneous lesions [51].

Green nanoparticles, that is, plant-based synthesis of nanoparticles have an upper edge over the synthetic nanoparticles owing to their biosynthesis being rapid, eco-friendly, non-pathogenic and economical. An array of biogenic nanoparticles from plant extracts has been reported to have antileishmanial activity with boosting of anti-oxidant activity [52, 53].

Miltefosine- and ketoconazole-loaded nanoniosomes with improved antileishmanial activity have also been reported [54]. AmBisome-miltefosine combination therapy for VL-HIV co-infected patients has been reported in Ethiopia with 83.8% cure rate [55].

7. Diagnosis of leishmaniasis

A definitive diagnosis of leishmaniasis is crucial to guide timely and appropriate therapy. The disease is often confused with other co-endemic diseases and HIV co-infections may result in atypical clinical presentation [4]. Differential diagnosis of VL should be considered in patients of endemic areas after organ transplantation [56]. This underscores the need for highly sensitive and specific diagnostic modalities. In this regard, molecular techniques such as real-time polymerase chain reaction (qPCR)-based methods are gaining ground for detection and quantification of *Leishmania* as well as for species identification [57]. However, to rule out false negatives, combination of two PCR techniques is advisable in patients with cutaneous lesions [58]. For VL, serological diagnosis with recombinant antigen rK39-based immunochromatography and direct agglutination test based on the whole parasite antigens have been reported to have high sensitivity and specificity [59]. Nonetheless, amastigote detection in bone marrow aspirates and positive rK39 immunochromatographic test should be further validated by nested PCR [60]. Recently, a loop-mediated isothermal amplification (LAMP) assay based on 18S rDNA and the conserved region of minicircle kDNA has been implicated with high sensitivity for visceral as well as CL diagnostics [61]. Further, *Leishmania* urine antigen has been explored as a probable biomarker for predicting treatment failure and relapse in VL/HIV-coinfected patients [62].

8. Conclusions and future perspectives

To strengthen the leishmaniasis elimination drive, particular emphasis has to be laid on the diagnosis, chemotherapeutics and new targets identification and vaccination strategies for control of this endemic disease. This underscores renewed efforts to combat upcoming challenges in the quest for new drug targets in achieving definitive cure and/or safe, cost-effective prophylactic vaccines with long-lasting immunity against leishmaniasis. An effective therapeutic vaccine may further boost the immunosuppressed state and thus control the visceralizing form of leishmaniasis that is mainly harbored in the South Asian region.

Conflict of interest

The authors declare no conflict of interest.

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