Chapter

Modern Aspects of Leishmaniasis: Basis of Development New Approaches against Infection

Asli Pinar Zorba Yildiz, Gulnaz Yildirim Koken, Burcak Yavuz, Emrah Sefik Abamor, Ozlem Ayse Tosyali, Serap Yesilkir Baydar, Ikram Ullah, Ahmet Berkay Civan, Melahat Bagirova and Adil M. Allahverdiyev

Abstract

In this review, the basic principles of developing new approaches to leishmaniasis have been stated contrary to the available literature on *Leishmania*. In general, the morphology of parasites, life cycle, clinical forms, correspondence to epidemiology, and distribution according to species in the world were described. It has been expressed in various aspects of the interaction between host and parasite cell receptors. This plays an extremely important role in vaccine and drug development against leishmaniasis. Immunologically, natural immunocytes emphasize the importance of macrophages and dendritic cells in the *Leishmania* parasites' immunopathology. This review will also discourse on the possibilities and necessity for the generation of new treatment technologies for leishmaniasis.

Keywords: Leishmaniasis, vaccine, cytokines, parasite, infection

1. Introduction

Human infection is caused by approximately 21 of 30 species of *Leishmania* parasites that are pathogenic for humans. The species are found in two morphologic forms during their life cycle. In hosts (both human and mammalian), parasites exist within macrophages and monocytes as round to oval non-flagellate morphology. Macrophages can play a crucially important role in *Leishmania* infection for professional phagocytes. The *Leishmania parasites* can be viewed as carefully selecting the optimal phagocyte receptors to promote their survival in the host. Neutrophils are thought to participate in the containment of *Leishmania* parasites within an hour of infection and they are important to be the first cells recruited to the site of infection, and are also dendritic cells (DCs). The main obligation of DCs is the diagnosis and processing of foreign antigens and presentation to T cells. The parasitic surface molecules (LPG, GPI, GP63, *etc.*) give detailed information that plays an important role in infectivity in

the pathogenesis of leishmaniasis. The *Leishmania* parasites have a dense covering of glycocalyx, which as a significant role in host-parasite interactions. The glycocalyx is attached to the plasma membrane via GPI (glycophosphoinositol). The GPI anchor molecules (LPG, GP63) have different roles, such as promoting infectivity, helping parasite survival, and important virulence role. The *Leishmania* infection initiation mechanism can be explained by its interaction between the parasite-expressed lipophosphoglycan (LPG) and the host cell-expressed TLR-2. The third complement receptor (CR3), first complement receptor (CR1), mannose receptor (MR), Fc gamma receptors (FcgRs, in particular, FcgRII-B2), and fibronectin receptors (FnRs) have been reported to take place for the facilitator to include *Leishmania* internalization. On the other hand, in this review, we have included the cytokines and signal molecules that play an important role in the immunology of leishmaniasis, and these molecules, especially the interaction between natural and acquired immunity as well.

In the last part, it was stated that the basic principles of vaccine development against leishmaniasis, information about the diagnosis of leishmaniasis and different generations of vaccine types, and the important role of parasite-host interaction in the development of a vaccine. Treatment outcome is a complex phenomenon with the potential of multi-factors such as genetics, immunological response, characteristics, and clinical treatment of the patient. Also, drug quality, duration of therapy, compliance, and parasite characteristics, such as variable intrinsic susceptibility (species) and drug resistance should be considered.

As a result, this literature review titled "Modern Aspects of Leishmaniasis: Basis of Development New Approaches against Infection" provides current information on various aspects of the leishmaniasis problem and suggests new approaches to struggling with the disease.

2. About *Leishmania spp*

Leishmaniasis is a zoonotic disease caused by *Leishmania* protozoa. The genus of *Leishmania* has more than 20 species and they are all obligate intracellular parasites. Leishmaniasis is transmitted to humans by the bites of infected female *phlebotominae* sandflies (genus Lutzomyia) [1], which survive in natural areas such as sylvans, caves, or coves [2, 3]. World Health Organization (WHO) declares leishmaniasis threatens about 350 million adults and children in approximately 90 countries around the world. It is believed that 12 million people are currently infected. Every year about 2 million new cases consist of this illness [3, 4]. Endemic regions for the illness are expressed as the west side of Asia and Africa, India, China, and the Mediterranean region [5].

2.1 Epidemiological and clinical form of old and new world *Leishmania*

Kala-Azar discovered in India by Leishman and Donovan, is endemic in 88 countries. In addition to India, the majority of new cases are seen in Brazil, Somalia, South Sudan, Ethiopia, Kenya, and Sudan, according to the data announced by WHO (2018) [6]. The epidemiological distributions are detailed in **Table 1**.

Leishmaniasis has four main types and each type differs in specific properties [4].

Cutaneous leishmaniasis (CL): Skin ulcers usually form on exposed areas, such as the face, arms, and legs. Healing of the wounds takes a few months but generally leaves scars [4]. The incidence of the cases is seen in Afghanistan, Brazil, Iran, Peru, Saudi Arabia, and Syria with a ratio of 90% [8].

Clinical Forms	Species	Country or Territory
Cutaneous Leishmaniasis	*L. tropica*	Afghanistan, Azerbaijan, Egypt, Ethiopia, Turkey
	L. major	Afghanistan, Azerbaijan, Egypt, Ethiopia India, Iran, Iraq, Kenya, Morocco, Nigeria, Pakistan, Tunisia, and Yemen
	L. infantum	Albania, Bulgaria, China Ethiopia, Kenya
	L. aethiopica	Cyprus, Israel Belize, Colombia, Mexico
	L. donovani	Argentina, Brazil, Bolivia, Colombia, Mexico, Panama, Paraguay, Peru
	L. mexicana	Argentina, Brazil, Guyana, Peru, and Venezuela
	L. braziliensis	Colombia, Costa Rica, Ecuador, Honduras, Panama
	L. guyanensis	Peru
	L. panamensis	
	L. peruviana	
Mucocutaneous Leishmaniasis	*L. braziliensis*	Argentina, Bolivia, Brazil, Colombia, Mexico, Paraguay, Peru
	L. major	America, Mexico
	L. panamensis	Colombia, Costa Rica, Honduras, Panama
	L. braziliensis guyanensis	Peru
Diffuse cutaneously Leishmaniasis	*L. aethiopica*	Ethiopia, Kenya
	L. Mexicana	America, Costa Rica, Ecuador, Mexico Guatemala
	L. amazonensis	Bolivia, Colombia, Ecuador, Venezuela
Visceral Leishmaniasis	*L. donovani*	Africa, Bangladesh, India, Sudan
	L. infantum	Afghanistan, African Republic, Albania, Brazil, Central Asia, Central Iraq, France, the Mediterranean basin, Tunisia, Turkey
	L. chagasi	New World
Post-kala-azar dermal	*Leishmaniasis*	Bangladesh, Ethiopia, India, Nepal, Sudan
	L. donovani	

Table 1.
Leishmaniasis clinical forms, Leishmania *species and territory [7].*

Diffuse cutaneous leishmaniasis (DCL): The heel expands on the skin. It is a difficult type to treat because of chronic lesions [4].

Mucocutaneous leishmaniasis (MCL): It takes place on mucous membranes of the mouth, nose, and throat damaging or destroying the tissues [4]. The incidence of the cases is seen in Bolivia, Brazil, and Peru with a ratio of 90% [8].

Visceral leishmaniasis (Kala-azar, VL): Interior organs are affected by this type and have no treatment. Symptoms of VL include high said to be high fever, significant loss in weight, increase in sizes of the spleen and liver, and anemia. Sometimes death occurs within 2 years [4]. It is observed in humans but not in other mammals [9]. The incidence of the cases is seen in Bangladesh, Brazil, India, Nepal, and Sudan with a ratio of 90% [8]. Detailed leishmaniasis territory and its' vectors are shown in a table with Clinical Forms (**Table 1**).

2.2 *Leishmania spp.* lifespan

It has two stages: sand fly stage and human stage. At different stages of their life cycle, which includes vertebrates and invertebrates as hosts, parasites exist in several morphologies according to flagellum location and nucleus-kinetoplast location.

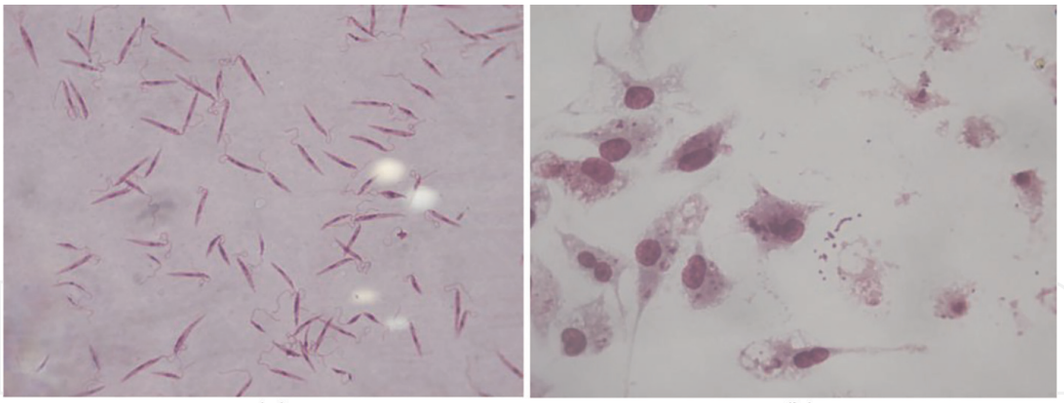

Figure 1.
a. L.infantum *(MONI/EP126) promastigotes (Giemsa staining, x100), b. amastigote form - phagocytosed by macrophage (Giemsa staining, x100) [10].*

Basically, *Leishmania* parasites are divided into two classes: promastigote form and amastigote form.

Promastigote form: Extracellular form of the parasite is called a promastigote. This form of parasite exists in the invertebrate region of the host's (sand fly) body at 270°C. They have a flagellum, and so they are motile. Their cell body length is 15–30 μm with a 5 μm width. The proliferation of the parasites follows longitudinal binary fission (**Figure 1a**).

Amastigote form: The parasite's intracellular form is called an amastigotes. This non-motile form exists in the vertebrate host's body at 37°C. They do not have a flagellum and their cell body length is 3–6 μm with a 1.5–3.0 μm width. The proliferation of the parasites follows longitudinal binary fission (**Figure 1b**) [10].

2.3 Vectors and reservoirs

Human infection is caused by approximately 21 of 30 species of *Leishmania* parasites that are pathogenic for humans [11, 12]. *Leishmania* reservoirs are expanded worldwide [13]. Expansion of the infection is commonly caused by mice, foxes, cats, and especially dogs [14]. Reservoir hosts show changes according to geographical location [15] in American Bradypus, opossums, rodents, and domestic dogs are the main hosts [13], and in India foxes, opossums, black rats, and domestic cats [14], and also humans are known as reservoirs [13]. Several parameters affect reservoir hosts. The factors that affect the system are the ecological properties of the homeland (habitat, geologic structure, and biotope), structure population (age, movement, dispersion, and reproduction), and sociology (feeding and customs) important to understand the behavior and type of the infection.

2.3.1 Interaction with Leishmania *parasites and host cells*

Leishmania specifically selects macrophages of mammalian cells as infected host cells [16, 17]. The transition of the parasite to the extracellular and intracellular environments is affected by different factors such as nutrients, pH, temperature, and availability of oxygen. Nutritional requirements, growth rate and ability to divide, the regulated expression of their surface molecules, and morphology are the factors that

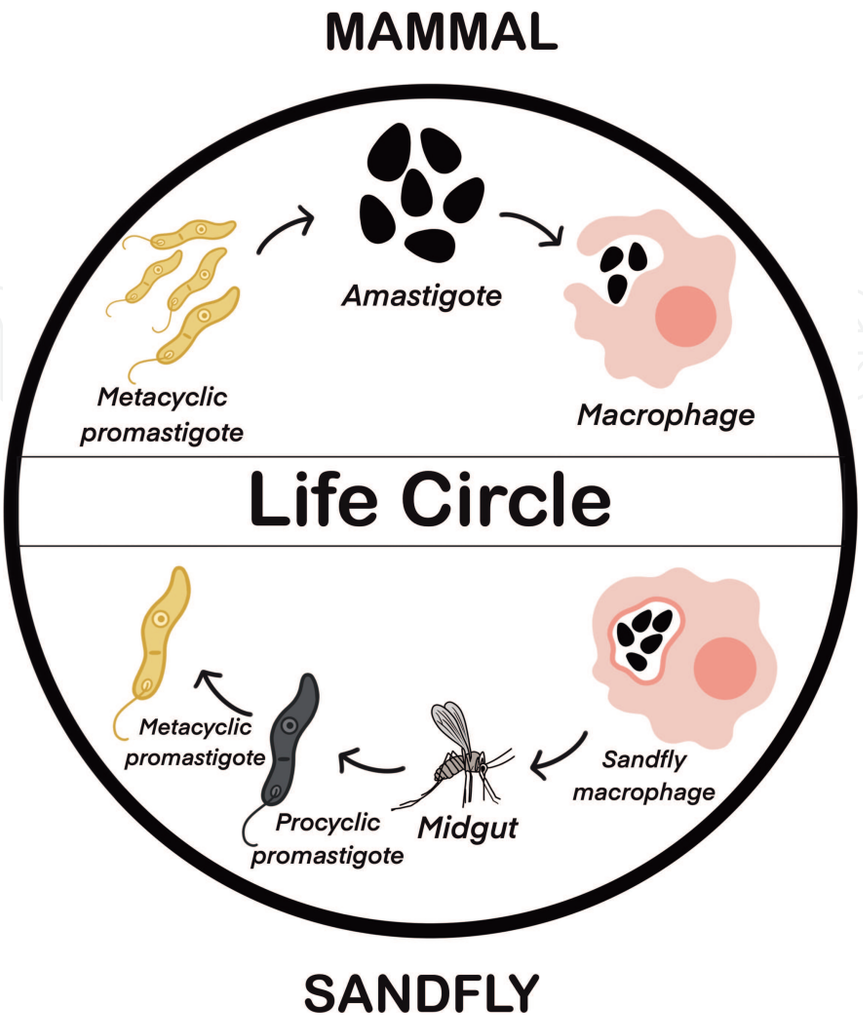

Figure 2.
Life circle of Leishmania *parasites.*

distinguish the developmental forms. The procyclic to metacyclic differentiation of promastigotes (also called metacyclogenesis) and the metacyclic promastigote are the two differentiation events studied with *Leishmania* to amastigote transformation [18] inside the host macrophage (**Figure 2**).

Procyclic phase promastigotes existing in the insect's midgut are not only non-disjunctional but, mammalian-infective, metacyclic phase promastigotes in the thoracic midgut and proboscis of the sandfly as well [19].

The metacyclic promastigotes differentiate into the intracellular flagellate amastigote form by inoculating into a mammalian host through a sandfly bite. This form of the parasite is named the parasitophorous vacuole by residing within a vacuole with lysosomal features [20].

Metacyclic promastigotes become resistant to complement by expressing stage-specific surface molecules. In this respect, it differs from the procyclic forms and is pre-adapted for survival in the mammalian host. Amastigotes are formed in macrophages by multiplying within the parasitophore vacuole. They are also intracellular, immobile forms, having a reduced size and a very reduced flagellum that does not protrude from the flagellum pocket. In addition, amastigotes are acidophiles with an energy metabolism adapted to the low pH of this compartment. They are characterized *in vitro* by low pH, lack of oxygen, and nutritional depletion of

tetrahydrobiopterin. The conditions such as low pH, 37°C temperature, and high CO_2 that mimic a phagolysosome-like environment can cause promastigote to amastigote differentiation [18]. Although environmental factors influencing *Leishmania* differentiation are known, little is known about the molecular processes that mediate cellular remodeling. Polycistronic RNAs transcripted *Leishmania* protein codes are not regulated at a transcriptional level, also they identify the stage-specific genes problematic [16]. There are many studies on different *Leishmania* species related to transcoelomic and proteomic approaches for identifying stage-regulated genes and proteins [17]. Some clear-cut stage-specific markers have functions that differ from nutrient acquisition to cellular reshaping and recycling and these markers involve peptidases. Moreover, they have a relationship with the mammalian virulence of *Leishmania* [21–23]. The peptidases are effective in the differentiation of the parasite. Host factors (species, concomitant infections/health, sex, age, behavioral patterns), parasite traits (generation time, dispersion strategies, molecular and biochemical characteristics of its sub-populations), exposure (inoculum size), and local environmental conditions (influenced, e.g., by stress and availability of natural resources) are the main factors that determine the patterns of *Leishmania* infection in any mammalian host species [23].

2.3.2 Receptors

The mechanism of initiation of *Leishmania* infection can be explained by the interaction between the parasite-expressed lipophosphoglycan (LPG) and the host cell-expressed TLR-2 [24, 25]. The third complement receptor (CR3), first complement receptor (CR1), mannose receptor (MR), Fc gamma receptors (FcgRs, in particular, FcgRII-B2), and fibronectin receptors (FnRs) [16] have been reported to be involved in the process. A definitive understanding of the roles of various receptors in parasite survival during natural infection has remained elusive [20]. Previous reports show that TLR-2 may not necessarily be host-protective, as proposed for all TLR anti-pathogen functions [20, 24–26]. However, nine types of TLRs are implicated differentially in infections with different *Leishmania* species [24, 27]. It is indicated that activation of TLRs leads to activation for adaptive immune responses; maturation of antigen-presenting cells and T cell activation can be observed first [24, 28, 29]. A significant role is taken in adaptive immune responses to *Leishmania* infection by CD40 [30, 31].

2.3.3 Host cells

Macrophages can play a crucially important role in *Leishmania* infection as professional phagocytes. The parasites make use of their phagocytic function as a strategy for learning and subsequent replication within the macrophage phagolysosomes as well. Thus, macrophages figure likewise both the host cells for effector cells that kill the parasites and parasite replication. Learning of *Leishmania* by macrophages triggers the production of reactive oxygen species [32] and leads to the generation of nitric oxide (NO) [33] and N-hydroxy-L-arginine (LOHA) [34] as mediators of parasite killing. The macrophage entry mechanism represents, in part, the dynamic nature of the parasite surface. The most abundant surface membrane components are the extracellular promastigote form, which can be found in insects, and the intracellular amastigote form, which is typically found in mammals [6]. Metacyclogenesis, the developmental process leading promastigotes to transform into a virulent form in the sand fly gut, is characterized by further modifications in surface proteins and other

glycoconjugates [10, 35]. Different sources of macrophages and different *Leishmania spp*. vary in their surface molecule composition, leading to unique parasite–host receptor interactions for each species-cell pair [2, 7].

Neutrophils are important in that they are the first cells to head to the site of infection with bite of a sand fly. Neutrophil uptake occurs by *Leishmania* parasites 1 hour after infection [36, 37]. Apoptotic neutrophils and neutrophils are more likely to play a role in promoting disease advance, instead of resisting. CR3 (CD18/CD11b) is an integrin expressed on the surface of neutrophils (PMNs) and they play a significant role in the immune defense system. iC3b and pathogen ligands are adhesion factors during phagocyte migration [16].

The main obligation of DCs is the diagnosis and processing of foreign antigens and presentation to T cells [38]. They are considered to be gatekeepers in the defense against invading pathogens. The body has the most efficient antigen-presenting cells (APCs) like skin DCs, Langerhans cells, and dermal DCs [39, 40]. Dermal DCs appear to present antigens directly to T cells during *Leishmania* infection [41]. Afterward, the infection, small numbers of parasites are involved directly by dermal DCs [42], although the majority of the DCs become infected through contact with parasitized neutrophils [43]. After weeks of post-infection, the number of DCs (CD11c (+) cells) in the lesion increases due to their recruitment [44], and those infected DCs can prime native CD4 (þ) and CD8 (þ) T cells [45].

2.3.4 Surface molecules

Among the species of *Leishmania,* the amounts of secreted proteophosphoglycans show differences. This means that different proteophosphoglycans have different functions, but the knowledge about this is still insufficient. In all *Leishmania* species, these GPI-anchored molecules include LPG, glycoinositolphospholipids (GIPLs), glycoproteins 63 (GP63), and the proteophosphoglycan (PPG) [46, 47].

Proteophosphoglycans: The family of these molecules includes several secreted or membrane-anchored proteins lustily modified by phosphoglycan chains [48]. A protein modification like this, called phosphoglycosylation, is common in Protozoa and includes the [6Galβ1,4Manα1PO4]n units, which are combined with serine-containing regions of proteophosphoglycan [47–49].

Lipophosphoglycan: In short, LPG is the main surface glycoconjugate of promastigotes. The most studied glycoconjugate is LPG, which forms a dense glycocalyx covering the entire surface of the parasite and the flagellum with approximately 5×10^6 copies/cell [20]. LPG is predominantly expressed in promastigotes, whereas it is absent in intracellular amastigotes. LPG has been chemically characterized and shown to vary in different species and during promastigote development in the sandfly. This LPG structure consists of four structures: a GPI anchor, a glycan core, a linear phosphoglycan chain (PG), and a terminator oligosaccharide cap (**Figure 3**). The structure and number of phosphoglycan repeat units vary and depend on the stage of differentiation and the species of *Leishmania*. The polymorphisms in its structure are important in allowing *Leishmania* parasites to colonize the host [46].

The GPI anchor includes an alkyl phosphatidylinositol containing either C24 or C26 as the aliphatic chain. The LPG glycan core is a consisting of the structure Gal (α1,6) Gal(α1,3) Galf(β1,3) [Glc(α1)-PO4] Man(α1,3) Man(α1,4)-GlcN(α1). There are 15–40 phosphodisaccharide (Galβ1,4Manα1-PO4) units between a linear PG and the glycan core. Eventually, an oligosaccharide cap structure is in the termination of LPG [46, 50].

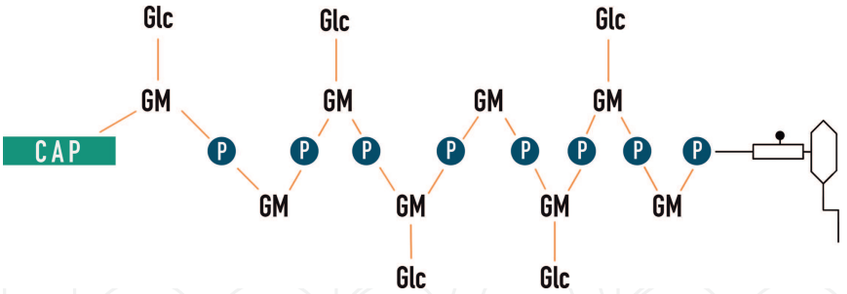

Figure 3.
Structure of LPG.

The molecular functions of LPG are;

1. Inhibiting phagosome-endosome fusion

2. Disruption of macrophage signaling pathways

3. Protection of promastigotes against oxidative burst during phagocytosis

4. Regulation of NO synthesis expression in macrophages

5. Retarding maturation of macrophagic phagolysosomes

6. Prevention of IL12 secretion by macrophages [51, 52].

Glycosylphosphatidylinositols: GPIs are the most abundant molecules, which bind a lot of proteins and carbohydrates settled in the membranes of the *Leishmania* parasites.

Glycoinositolphospholipids: GIPLs, other glycoconjugates are the glycoinositol-phospholipids (GIPLs), which are expressed both in promastigotes and amastigotes (approximately 107 molecules per cell), the replicating form during the infection in the host. GIPLs are also polymorphic like LPG in both glycan and lipid structures, however, a conserved main core are shown in all *Leishmania* species studied to date. It contains the Manα1,4GlcNα1,6 myoinositol1phospholipid structure. In the other trypanosomatids, there is no unsaturated fatty acid component of the lipid moiety [53].

The metalloproteinase GP63 (leishmanolysin): GP63 is the main GPI-anchored zinc-dependent metalloprotease of the cell surface of *Leishmania* parasites and was first discovered in the 1980s. The molecular mass is 58–65 kDa and an average of 5×10^5 GP63 copies are expressed per cell, which makes up about 1% of the total cellular protein [47]. The GP63 has expressed promastigotes however is down-regulated in amastigotes [54]. The GP63 probably plays different roles in both forms of the parasite. The GP63 was found to cleave C3b into iC3b so helping *L. amazonensis* and L. major promastigotes to avoid complement-mediated lysis [55]. Also, the GP63 molecules can bind to macrophages and can easily survive and replicate in them [56, 57]. The role of structural differentiation of GP63 molecules in both amastigotes and promastigotes of *Leishmania* is still unknown [47].

2.4 Cytokines and signaling molecules

A variety of different cytokines are associated with Th1 (gamma interferon [IFN-gamma], interleukin-2 [IL-2]) and Th2 cells (IL-4 and IL-10), and they have

important roles in host-parasite infection in leishmaniasis. The cellular immune responses (Th1 type) play a role against the disease [58]. There are four main steps in *Leishmania* infection, as explained below:

In the first step, *Leishmania* promastigotes calmly enter macrophages. The macrophages cannot manage to induce innate immune responses and this result may cause a delayed induction of an adaptive immune response. The delayed adaptive immunity may provide the parasite to replicate inside macrophages. In the second step, the replication of a parasite is prevented by macrophages which finally generate the immune signals. In the third step, amastigote spreads by blasting the macrophages and they infect other macrophages. Amastigotes coat themselves in host IgG and the IgG binds to macrophage FcγR which results in the overproduction of IL-10 cytokine. This cytokine can prevent macrophage responses to IFN γ and also allow the parasites to survive [45].

It is known that the production of IFN-γ induces the macrophages which were infected before. Hence the induced macrophages provide the upregulation of nitric oxide (NO) synthesis. NO has an important role in killing *Leishmania* parasites [59].

The cellular response to leishmaniasis is elicited by the T cell response after infection. First, the amastigotes proliferate and turn into the phagolysosome in the macrophage. Then both the parasites and their antigens can be taken up by antigen-presenting cells (APCs). These APCs transfer them to $CD4^+$ and $CD8^+$ T cells. After that, these cells induce specific T cells for the production of different types of cytokines like TNFα, IL-6, IL-10, IL-12, and IFN-γ. This condition has the ability to activate macrophages to kill amastigotes [60]. Specific $CD8^+$ T cells, which are triggered during infection, have cytolytic antileishmanial activity. Possibly it can do this by inducing the production of IFN-γ and TGF-b. $CD4^+$ T cells are major cytokine-expressing cells that can produce both type 1 and type 2 cytokines [61]. IL-4 was shown to induce Th2 differentiation and lead to IL-4, IL-5, and IL-10 production.IL-10 has a major important role in *Leishmania* disease progression. In contrast, the cytokine IL-12 was shown to be linked to Th1 differentiation, cell proliferation, and secretion of IFN-γ. It supports protective immunity against the disease. A strong and specific cellular immune response against leishmaniasis is interested in long-term protection.

3. Importance of host-parasite interactions in vaccine technology

Type-1, polytypic, and peripheral membrane proteins are important plasma membrane components, reflecting the different microbicidal responses and nutritional environments encountered. Recently, single or multiple surface components missing in *Leishmania* mutants have been developed. Wild-type parasites are more deadly than some mutant parasites. Mutant parasites may have little or no loss of infectivity. Identification of other surface or intracellular components with these mutants would be helpful, as it is necessary for determining virulence in macrophages [62]. Although there are several leishmanial antigens tested for potential vaccine candidates, vaccine candidates whose efficacy and phase studies have been completed are limited [63]. There are some difficulties in the selection of these vaccine candidates. The cellular components of complex organisms, such as protozoa, are highly immunogenic and initiate complex immune processes. Immunogenicity and induction of mixed immune system on the immune system are also quite mixed. Some cellular components may cross-react with the host, causing pathological responses or aggravating infection-

related disease. The mouse model of leishmaniasis shows some similarities with human diseases. In addition, there is limited evidence for the protection of vaccine candidates in multiple animal models. Advances in technology in molecular biology and immunological techniques may provide some important insights that answer the challenge. In addition, potential vaccine candidates can be found with the availability of genome sequences with gene encoding [63].

In the last century, vaccination strategies have been developed for those struggling with leishmaniasis [64]. However, no vaccine candidate whose efficacy against leishmaniasis has been completed has been found in phase studies. In general, there are three main categories of vaccines for leishmaniasis;

1. First-generation vaccines

2. Second-generation vaccines

    a. Live attenuated organisms as vaccine candidates

3. Subunit and recombinant vaccine candidates

4. Third-generation vaccines

Fractions of the parasite or whole-killed *Leishmania* with adjuvants or not can generate first-generation vaccines. The vaccines that are committed to treating leishmaniasis symbolize that first-generation vaccines are prepared with killed parasites or parasite fractions. Towards the end of the 1930s, Brazil carried out these vaccines. The big part of these vaccines that become phase III developed against CL [65] and VL [66]. Recombinant proteins, DNA vaccines, and their combinations form the second-generation vaccines against leishmaniasis [67]. Determining the exact ways of the cellular and molecular mechanism of pathogen-host interaction is the most important road to improving an ideal vaccine candidate. Also, researchers consisted of live-attenuated vaccines in search of a safe, stable, and efficient vaccine candidate. For this purpose, the attenuated parasite could be generated based on defined or undefined changing in the parasite. These methods applied to *in vitro* cultures such as gamma irradiation, chemical mutagenesis, and temperature sensitivity were preferred for the formation of attenuated strains [68, 69].

Wolff and colleagues established the concept of DNA vaccination that composes the third-generation vaccines. The colleagues determined that the protein expression was appointed with direct injection of recombinant plasmid DNA into mouse skeletal muscle [70, 71]. According to these findings, DNA vaccines that are produced for preventing infection with *Leishmania* parasites also represent vaccine candidates. In addition to that, these can be used as immunotherapeutic tools. The gene encoding of GP63 was used to produce the first DNA vaccine against *Leishmania* parasites. As a result, it is reported that the mice, immunized with plasmid DNA encoding GP63, developed a strong Th1 response [72].

There have been several studies associated with parasitic antigens and the advantages of vaccine technologies. Vaccination is the most important way to ensure the control of infectious diseases. The achievement of the development of vaccines depends on intellection the biology of between host and parasite interaction. Selection of suitable vaccine candidates, determining the correct adjuvants, and determination of appropriate carriers are also important. Nevertheless, the immunity that is

generated from vaccines must be long-standing. Immune regulatory pathways formed after infection and vaccination have been identified but our knowledge is limited to attenuating the advantages of available adjuvants and drugs. Vaccines must be effective against all of the agents that cause certain diseases. The vaccine against leishmaniasis development process is quite slow. However, information related to the construction and testing of new vaccines aimed at preventing and treating *Leishmania* has increased significantly in recent years. The control or treatment of such diseases may be possible with the accumulation of knowledge about the molecular mechanisms of parasite and host immune response. Different points in parasite metabolism are important strategies to prevent and treat the targeted area to increase efficiency and resistance. Immunomodulators are used to mansion and restore homeostasis to contribute to the treatment and may be appropriate to reduce tissue damage [67].

While the developments in exosome technology in recent years have discussed the functionality of cell-free vaccine candidates, the discovery of the exoproteome contents of *Leishmania* parasite reveals the vaccine potential [73].

As a result, even if agents such as pesticides developed against the parasite-vector-reservoir-host relationship produce temporary solutions by breaking the infection chain elements, the ecological cycle will eventually return to the system by gaining resistance. It should not be forgotten that the most important element in the prevention of the disease that will occur with this cycle is vaccination. Both patient survival, public health, psychology, and economic development approaches in health systems are made controllable by preventive medicine and vaccine applications.

4. Medical treatment

Treatment of leishmaniasis is dependent on especially chemical therapies used. Pentavalent antimonial, oral miltefosine, amphotericin B, liposomal amphotericin B, and paromomycin are the most commonly used drugs. There are several problems with these drugs including the cost of drugs, toxicity, length and duration of treatment, route of injection for treatment, and the development of parasite drug resistance [51]. The most used drug was pentavalent antimonial which was the first line of treatment for many years. According to this, increasing the ratio of parasite resistance in endemic regions has limited their usage for treatment [74]. Antileishmanial activity of the pentavalent antimony can be activated by reducing to its trivalent form inside macrophages. Although the mechanism of antimonial effects in the cells is not completely clarified, the inhibition of the glycolytic pathway, fatty acids, and trypanothione reductase is related to antimonial effects [75].

Second-line drugs for the treatment of leishmaniasis are pentamidine, amphotericin B, miltefosine, and paromomycin [76]. Although there is evidence that involves mitochondrial function intervention, the mechanism of pentamidine action is not well known [77]. Inhibition of protein synthesis of the parasite can be provided treatment with paromomycin. This drug is covalently bound to protein, which affects translation and vesicle-mediated trafficking [78]. Another drug for the treatment of leishmaniasis is miltefosine, which affect the cell signal transduction pathway by inhibiting protein kinase B. This pathway has an important role in the biosynthesis of sterols and phospholipids [79]. Ergosterol is a major component of the cellular membrane of *Leishmania*. Amphotericin B binds to ergosterol creating transmembrane channels that release monovalent ions (K^+, Na^+, H^+, and Cl^-) leading to cell death [80]. Various lipid formulations have been introduced leading to rapidly

concentrated in organs such as the liver and spleen to reduce the adverse effects of amphotericin B [81, 82].

Several studies have demonstrated that exosomes derived from MSCs have significantly improved the process of healing cutaneous wounds. Exosome formulations were developed to investigate their impact on the healing of cutaneous wounds caused by *Leishmania major* parasite on the skin. The study observed that the exosome formulations had an anti-leishmanial effect when compared to salicylic acid, which is known for its efficacy in wound healing and is commonly used in cosmetic products. Furthermore, *in vitro* wound model experiments showed that exosome formulations had a remarkable ability to inhibit *Leishmania* parasites while promoting wound healing, surpassing the effects of aloe-emodin, which is frequently used in cosmetic applications [83, 84].

5. Diagnostic

VL and KL diagnoses mostly are made with microscopic examinations from Giemsa painted smear preparations prepared from bone marrow and skin lesions. A smear is a simple sample, prepared easily and rapidly that is often the preferred diagnostic method, and finds out *Leishmania* amastigotes directly [85]. The microculture method and NNN medium (Novy-MacNeal-Nicolle) are used for the isolation of parasites [86]. The aspiration or biopsy material with a fine needle is mixed NNN medium for culture and microscopic examination [85]. The microculture method is the other culture method in which the development and identification of parasites are studied within microaerophilic niches developed by Allahverdiyev and his colleagues. These niches can supply optimal conditions for isolation, adaptation, and proliferation. The microculture method, which was compared with the NNN agar method, was found to be more sensitive in a short time [87]. The microculture method makes certain the microaerophilic environment (higher CO_2 concentrations, diminish O_2 concentrations, and pH) is essential for the growth of *Leishmania* parasites. In the following years, this method is used in asymptomatic leishmaniasis, the samples obtained from a blood donor, vaccine development, working mice without sacrificed in the studies, the isolation of stem cells, and the diagnosis of helicobacter [88, 89]. Various using molecular methods such as polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP), and DNA sequencing are performed for identifying *Leishmania* species in recent years although these methods require special equipment and are needed expertise [90]. It may not available infeasible to determine species easily in some clinical cases with molecular methods [91, 92]. Nowadays it is increased the works targeted different gene regions with DNA sequencing for *Leishmania* species and are also highly sensitive [91]. The other most important method is preferred isoenzyme gel electrophoresis except for PCR, RFLP, and real-time PCR (RT-PCR) used for the identification of *Leishmania* parasites. All these methods have disadvantages based on the number of parasites in the scar or blood samples. Moreover, they require costly technical equipment and specialist [93]. The sensitivity of CL and MCL are 20–30% and 55–70% respectively when using PCR-based methods in biopsy material or lesion aspires [94]. Serological methods are mainly based on a primer, or a secondary monoclonal antibody targeting parasite surface antigens and provide a great advantage for early diagnosis. For the diagnosis of leishmaniasis; serological methods such as enzyme-linked immunoassay (ELISA) test, western blot (WB), rk39 immunochromatographic diagnostic test (ICT), immunofluorescence

antibody test (IFAT) are used. The basic principle of these methods is that they should be non-invasive methods based on the detection of antibodies or antigens against the parasite in the patient's serum or urine [95–97]. IFAT, which is a method based on serological and microscopy, provide investigation taken in different concentration serum sample of consisting IgG by staining the detected parasites with fluorescence to determine the presence of parasites. In the study done by Ates et al., the blood bank serum samples were diluted to a 1/128 ratio and measured based on the immunofluorescence effect with the IFAT test for the detection of leishmanial parasites [98]. Affinity reagents, such as antibodies are mainly used as therapeutic, diagnostic, and biological [99] agents for infectious diseases [100]. Polyclonal and monoclonal antibodies are essential tools obtained from hybridomas actually [101].

The hybridomas method is discovered in 1975 by Kohler and Milstein and today, antibody engineering is a rising trend in modern life science technology [99, 100]. The polyclonal and monoclonal antibodies utilization are more proper to diagnose leishmaniasis in terms of specificity. Therefore technology of hybridoma is used typically [90, 100]. The rK39 Kalazar Detect rapid kit is commercially produced by using antibody engineering [98]. However, the kit is specific to the diagnosis of VL, and when compared to VL-KL serum sample, it is not inadequate in the diagnosis of CL. It was reported the sensitivity is less than 20% based on the ELISA method for diagnosis of KL [102]. Diagnostic systems for leishmaniasis based on antibody engineering are very insufficient. In vaccine studies developed against leishmaniasis is especially important the identification components of *Leishmania* parasites. In the production processes of polyclonal and monoclonal antibodies obtained by hybridoma technology, many factors such as the experimental animals used, adjuvant structure, antigen production technique, *etc*. affect the effectiveness of the diagnosis stage. In particular, the combination of antigen and adjuvant that affects the antigen-specific specificity of these antibodies during the immunization phase is very important. At this stage, there are various studies on the development of effective adjuvant systems that are not toxic, do not affect the antigen-specific specificity of hybridomas, do not create an immune response alone, and do not show the risk of cross-reactions [103].

6. Conclusions

For many years, researchers studied and tested potential vaccine candidates with leishmanial antigens. The spread of the disease of *Leishmania* has greatly increased incidence worldwide, therefore, today, we need *Leishmania* vaccines more than yesterday. Additionally, the conditions including lacking sensitive and specific diagnostics techniques, difficulties in obtaining cheap drugs and effective vaccines, emerging drug resistance, and toxicity of chemical drugs conduct us to produce new vaccines to treat leishmaniasis. Furthermore, new drugs, different from those used against human leishmaniasis, are needed. This slow translation of knowledge from the laboratories to the industry is probably the consequence of various factors. However, studies with leishmaniasis have gained in recent years. In particular, the activities of regenerative medicine products such as non-drug-based, cellular, and exosomes, or their synergistic effects with drugs, include new generation designs. In addition, diagnostic studies have gained momentum. The diagnosis of leishmaniasis has been approved by isolating, visualizing, and culturing the parasite from infected tissue, historically [104]. It explains that there is a serious need to develop more expensive, precise, sensitive, rapid, and specific diagnostic tests, especially for the detection of leishmaniasis [105].

Besides these molecular methodology investigations, proteomic studies should go on to find out new diagnostic markers of *Leishmania*. Studies of these markers will also increase our understanding of disease progression on contribute to the search for new drug targets. Every *Leishmania spp*. infection may present different symptoms in the host. The genetic structure of the host also plays an active role in the determination of these symptoms. In this case, it affects the treatment process. The main antimonials, amphotericin B (AmpB), miltefosine, paromomycin, and pentamidine are used in the clinical treatment of VL [106]. High cost, drug resistance, and toxic side effects limit the use of traditional drugs. Therefore, the WHO strongly recommends the investigation and development of novel therapeutic alternatives against leishmaniasis. New synthetic drugs and antileishmanial plant extracts need to be rapidly evaluated both individually or together (combination therapy) in all forms of leishmaniasis by researchers. Recently, researchers, have special attention is given to innovative nanostructures based on unique physicochemical properties. Until today AmBisomes is the only nano-based antileishmanial drug, which has been using in clinically, and recently nano-liposomal AmB gel was approved for the treatment of CL, fungal, and tropical diseases. These nano-based drugs are unquestionably effective but their use remains restricted in some areas due to their high cost [107]. Also, polymeric nanoparticles [108], metallic nanoparticles [109], and plant-based solid lipid nanoparticles [110] used in recent studies have shown promising developments in treatments. Nanocarriers have not only been used for drug delivery but also for vaccine delivery. During the past decades, various types of *Leishmania* antigens (e.g., killed or attenuated whole parasites, synthetic or recombinant peptides, or recombinant live vaccine vectors) have been identified and tested with or without cytokines or other adjuvants for vaccine development to control parasite multiplication and dissemination, both in humans and dogs [111]. However, nowadays, new approaches are being researched for effective and safe vaccines thanks to nanotechnology. The nanocarriers provide to deliver antigens, antigen-adjuvant, or antigen cocktails to APCs that play a profound role in activating the host immune system. These small particles give a useful update on work on future vaccines. Likewise, exosomes produced from various cells among nanocargo carrier candidate structures, which have superior properties in terms of size and structure, can also carry active substances with antileishmanial activity. It has also been observed that combination treatments of cells and chemotherapeutic agents have more successful results in terms of antileishmanial effect compared to stem cell alone or chemical-based treatment options alone [112]. Immunotherapy, which is an emerging trend, has added a different perspective to parasitic diseases such as leishmaniasis as a different and personalized medicine. Especially in the treatment of VL, modulation provided by immunotherapy, and understanding the immunological dimension of the host-parasite relationship, may reduce the parasite burden and prevent drug resistance. Although there are studies showing that immunotherapeutic agents modulate leishmanial activities, the preclinical experiment remained in the size of animal studies [113, 114]. Immunotherapeutic agents, recombinant cytokines, or antibodies developed in this field, which are frequently used and patented, especially in diagnostics, have experienced a serious decline, especially with the Covid-19 pandemic, causing the neglect of two million new cases each year [115].

Science and technology develop day by day but the nature of the world has also changed. The climate change, wars, and immigrants are causing the disease to spread to areas where it has never been identified before. This means that leishmaniasis will not only affect developing countries but also others. Therefore, more improvement is

needed in leishmaniasis control. This can be achieved by considering parameters such as correct diagnosis and treatment, good analysis of public health effectiveness, and the natural course of the infection. In the future, researchers also must carefully design appropriate immunization protocols focusing on the parasite genotypes, powerful new adjuvants, development of new antigen-adjuvants in a different way (using advanced bioconjugation chemistries). These considerations can be providing a high level of protection and safety. In addition, designing new target drugs explored with shorter treatment duration, fewer adverse effects, and less risk of drug resistance provides an exciting future direction for rational treatment combinations at low cost.

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Conflict of interest

The authors declare no conflict of interest.

Author details

Asli Pinar Zorba Yildiz[1*], Gulnaz Yildirim Koken[2], Burcak Yavuz[3], Emrah Sefik Abamor[4], Ozlem Ayse Tosyali[5], Serap Yesilkir Baydar[6,7], Ikram Ullah[8], Ahmet Berkay Civan[9], Melahat Bagirova[10] and Adil M. Allahverdiyev[10]

1 Vocational of Health School, Department of Medical Services and Techniques, Istinye University, Topkapi Campus, Istanbul, Turkey

2 Sabancı University Nanotechnology Research and Application Center, Istanbul, Turkiye

3 Vocational School of Health Services, Altinbas University, Istanbul, Turkey

4 Department of Bioengineering, Davutpasa Campus Istanbul, Yildiz Technical University, Turkey

5 Faculty of Engineering and Architecture, Biomedical Engineering Department, Beykent University, Ayazaga Campus, Istanbul, Turkey

6 Faculty of Engineering and Architecture, Department of Biomedical Engineering, Istanbul Gelisim University, Istanbul, Turkey

7 Life Sciences and Biomedical Engineering Application and Research Center, Istanbul Gelisim University, Istanbul, Turkey

8 Department of Biotechnology and Genetic Engineering, Hazara University Mansehra, Pakistan

9 Stem Cell, and Tissue Engineering R&D Center, Istinye University, Istanbul, Turkey

10 V. Akhundov National Scientific Research Medical Prophylactic Institute, Baku, Azerbaijan Republic

*Address all correspondence to: aslipinarzorba@gmail.com

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