



Leishmania Vaccines: the Current Situation with Its Promising Aspect for the Future

Rasit Dinc*

INVAMED RD Global, Mutlukent Mah, 1961 Cd. No.27 Cankaya, Ankara 06810, Turkey

Abstract: Leishmaniasis is a serious parasitic disease caused by *Leishmania* spp. transmitted through sandfly bites. This disease is a major public health concern worldwide. It can occur in 3 different clinical forms: cutaneous, mucocutaneous, and visceral leishmaniasis (CL, MCL, and VL, respectively), caused by different *Leishmania* spp. Currently, licensed vaccines are unavailable for the treatment of human leishmaniasis. The treatment and prevention of this disease rely mainly on chemotherapeutics, which are highly toxic and have an increasing resistance problem. The development of a safe, effective, and affordable vaccine for all forms of vector-borne disease is urgently needed to block transmission of the parasite between the host and vector. Immunological mechanisms in the pathogenesis of leishmaniasis are complex. IL-12-driven Th1-type immune response plays a crucial role in host protection. The essential purpose of vaccination is to establish a protective immune response. To date, numerous vaccine studies have been conducted using live/attenuated/killed parasites, fractionated parasites, subunits, recombinant or DNA technology, delivery systems, and chimeric peptides. Most of these studies were limited to animals. In addition, standardization has not been achieved in these studies due to the differences in the virulence dynamics of the *Leishmania* spp. and the feasibility of the adjuvants. More studies are needed to develop a safe and effective vaccine, which is the most promising approach against *Leishmania* infection.

Key words: Leishmaniasis, prevention, new modality, vaccine, recombinant DNA

INTRODUCTION

Leishmaniasis is a serious parasitic disease caused by *Leishmania* species transmitted by sand fly bites [1]. The clinical manifestations can be classified as cutaneous (CL), mucocutaneous (MCL), and visceral leishmaniasis (VL). The clinical form depends on the complex interactions between the virulence characteristics of the infecting *Leishmania* spp. and the immune responses of the host [2]. This disease is a major public health concern. The CL form tends to heal spontaneously and often causes disfiguring scars on the skin [3]. Although a history of CL is not reported in all new MCL patients, MCL results from the spread of parasites to the oral, nasal, pharyngeal, and laryngeal mucosa following or simultaneously with CL. Spontaneous recovery from MCL is rare and can progress to severe deformities such as nasal septal destruction, airway obstruction, and eventually death [4]. The VL is the most se-

vere form and potentially fatal if left untreated [5]. The disease has been reported as endemic in 98 countries and territories in 2020. It primarily affects poor people in Africa, Asia, and Latin America [6]. Despite being the second most common parasitic disease after malaria and its devastating nature, it has remained one of the most neglected diseases worldwide until recent years, perhaps due to its rarity in developed countries [7,8].

Currently, licensed vaccines are unavailable for the treatment of human leishmaniasis [9]. The treatment of the disease and prevention of its spread in the community is mainly dependent on chemotherapy [10-12]. Of the more than 25 compounds, pentavalent antimonials (Sb5+) are the first-line chemotherapy drugs. Amphotericin B, pentamidine isethionate, miltefosine, and paromomycin are the second-line chemotherapeutics [1,13]. However, the existing drugs are not ideal because of their high toxicity and resistance [2,12]. Although the CL form can be treated with chemotherapy, it is not feasible for the other forms of the disease [14].

According to the World Health Organization (WHO), vaccination is most likely the best method to control the disease and avoid the unwanted effects of chemotherapies [14]. The development of a safe, effective, and affordable vaccine for all

•Received 9 September 2022, revised 9 November 2022, accepted 25 November 2022.

*Corresponding author (rasitdinc@hotmail.com)

© 2022, Korean Society for Parasitology and Tropical Medicine

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<https://creativecommons.org/licenses/by-nc/4.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

forms of the disease is one of the most promising approaches and the main priority of global public health [15]. Hence, this article aims to focus on the current status and promising results of the available vaccines evaluated in different modalities.

THE ROLE OF IMMUNOLOGICAL MECHANISMS IN LEISHMANIASIS

Unlike most parasitic infections, protective immunity occurs against reinfection in patients who recover from leishmaniasis or following leishmanization (LZ) [16,17]. This result reveals that immunological mechanisms play a major role in shaping the disease, and this globally important disease can potentially be prevented using vaccines [8,18,19]. It also provides a roadmap for the development of successful vaccines that can generate protective immunity against infection [20].

The immunological protective mechanism is complicated in leishmaniasis [21]. Cell-mediated immunity plays a crucial role in host immunity. While the primary immune response to infection is initiated through the innate branch, which develops protective innate and adaptive immunity, infection control is mainly mediated by IL-12-driven Th1-type immune response. T lymphocytes shape the host immune response to provide direct protective or non-protective immunity [22]. The production of IFN- γ by CD4+ T cells activates macrophages to kill parasites under nitric oxide (NO)-mediated conditions [23]. Disease progression is largely driven by the production of non-protective IL-4-driven Th2-associated cytokines IL-4, IL-10, IL-13, and TGF- β [24-26]. Some species, such as *L. mexicana* and *L. amazonensis* in the New World, unlike *L. major*, can survive in conditions of limited Th1 immune responses in the host [27]. Unlike Th1, the Th2-type immune response is unable to neutralize intracellular parasites, causing the parasite to spread into VL or, for New World species, disseminated cutaneous leishmaniasis (DCL) [28].

VACCINATION STRATEGIES IN LEISHMANIASIS

The availability of vaccines against one or more forms of leishmaniasis can reduce mortality and morbidity associated with this disease. However, *Leishmania* parasites have a complex life cycle, which consists of stages in animal/human and sand flies and is the most important barrier for vaccine development. Moreover, this neglected tropical disease substantially affects low- and lower-middle-income countries, which dis-

courages commercial developers from invest in the studies of vaccine [8]. In addition, differences in the virulence dynamics of *Leishmania* species and the immune responses induced by them, as well as the suitability of adjuvants negatively affect vaccine development and standardization efforts [7]. A vaccine against CL caused by *L. major* may not necessarily have effectiveness against the New World forms of diseases, including MCL and DCL [29]. Nonetheless, various vaccination strategies, such as recombinant antigens, DNA vaccines, salivary gland proteins, killed parasites, and live attenuated parasites exist to treat leishmaniasis [30-34].

An ideal antileishmanial vaccine may solve the current problems and must be safe, stable, reproducible, less risky, easily administered, stored, and delivered, not reversible to an infectious state, and able to induce long-term immunological memory [8,35]. However, no vaccine meets these ideal specifications [36]. Compared with other approaches, the strategy of using live vaccines is more attractive because of the induction of a response similar to the immunological response in the natural course of infection (Fig. 1). In the live vaccine strategy, the entire spectrum of antigens is presented to the host immune system without an adjuvant [21].

Potential interventions to induce an immune response against *Leishmania* can be analyzed using different strategies, such as LZ and saliva vaccines, as well as first-, second-, and third-generation vaccines [28]. Different novel approaches have been investigated for this purpose, such as delivery systems and chimeric peptides. These approaches can be designed as single or cocktail antigens [36-38].

Leishmanization (LZ) vaccine

LZ is an ancient practice of vaccination [28]. LZ is an intradermal low-dose inoculation of live and virulent *L. major* leading to a single lesion [39]. LZ provided greater than 90% protection against reinfection [16]. LZ has been used in several countries in the Middle East and the former Soviet Union. Except for Uzbekistan, which is an endemic country, it is no longer practiced due to safety concerns such as HIV spread, use of immunosuppressive drugs, ethical reasons, uncontrollable persistent skin lesions, and persistence of parasites [16,21]. The overall strategy should be to develop a safer vaccine by providing protective immunity without causing skin lesions, including in immunosuppressed individuals. However, vaccination with live parasites showed a stronger Th1 type of immune response than vaccination with killed parasites, which exhibited

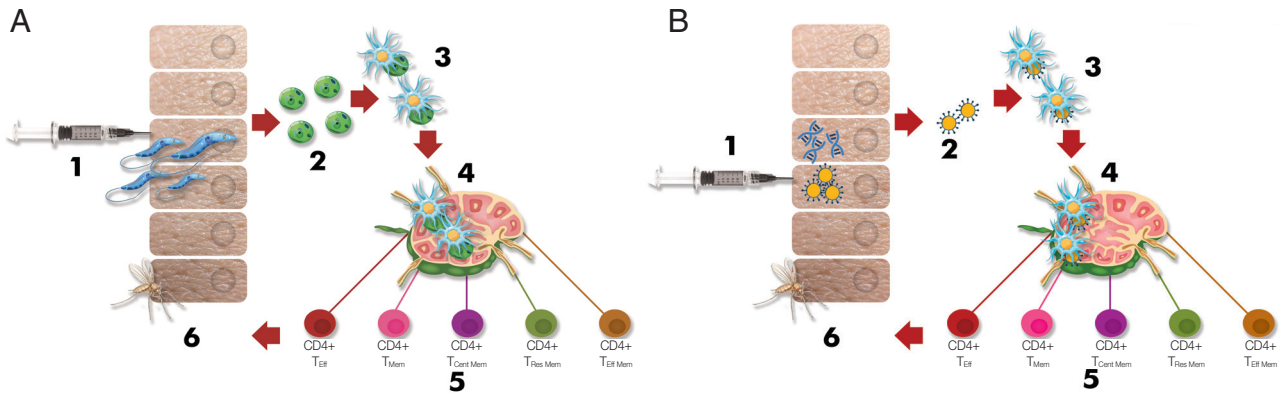


Fig. 1. Schematic depiction briefing the immunological response against *Leishmania* infection (Modified from Pacheco-Fernandez et al. [36], with permission). (A) Immunological response in live/attenuated *Leishmania* vaccination. 1) Injection of live/attenuated parasites, 2) Transforming promastigotes into amastigotes, 3) Internalization of the amastigotes by dendritic cells, 4) Presentation to T cells in the draining lymph nodes by dendritic cells, 5) Differentiation of T cells into effector and memory T cells, 6) Prevention of transmission to the sand fly because of long-term protective immunity. (B) Immunological response in *Leishmania* vaccination using DNA, recombinant antigen (Ag), or subunit Ag. 1) Injection of DNA, recombinant Ag, or subunit Ag, 2) Encountering of antigens and dendritic cells, 3) Internalization of the antigens by dendritic cells, 4) Presentation to T cells in the draining lymph nodes by dendritic cells, 5) Differentiation T cells into the effector and memory T cells, 6) Prevention of transmission to the sand fly because of long-term protective immunity. CD4+ T_{Eff}: effector T helper cell, CD4+ T_{Mem}: memory T helper cell, CD4+ T_{Cent Mem}: central memory T helper cell, Tissue T_{Res Mem}: tissue residence memory T cell, effector T helper cell, and CD8+ T_{Eff}: effector cytotoxic T cell.

limited protection. However, the reason for these differences is not well known [17].

Another study from Iran showed that leishmanization was found to reduce the incidence of the disease between 1/6 and 1/8 of its original level in a hyper-endemic region of Iran and thus was recommended for people at high risk of contracting the disease [39].

Saliva vaccine

Sand fly vectors (*Phlebotomus* and *Lutzomyia* spp.) are of particular interest and induce immune responses as adjuvants through certain immunogenic proteins such as LJM19 and LJL143 from *L. longipalpis* and PdSP15 from *P. duboscqui* [16,40,41]. Normally, sand fly saliva is known to enhance the infection caused by *Leishmania* spp. [41]. However, pre-exposure to saliva protected mice against parasitic infections. In a study on mice, Carregaro et al. [18] reported that the injection of sand fly salivary gland extract resulted in increase in IFN- γ and IL12 production in the site of inflammation and pre-exposure to saliva protects mice against parasitic infections. They concluded that the generation of new saliva vaccine strategies may help prevent *Leishmania* establishment in the host.

Immunization with a vehicle expressing the vector salivary protein expressing the PpSP15 protein, such as *Lactococcus lactis* and *Leishmania tarentolae*, has been shown to protect against

CL or VL [42,43].

First generation (whole-killed or fractioned parasite) vaccines

First-generation vaccines are composed of whole-killed parasites or partially purified fraction(s) or excreted from the parasite. These have been replaced with LZ [28]. The parasites were killed using different methods, such as long-term in vitro culture, temperature, pressure, γ -radiation, and chemicals [44-49]. The killed parasite vaccines present a huge repertoire of parasite antigens and can promote significant protection against infection by mimicking natural infections. However, these studies show that they induce a weaker Th1 type immune response than live parasites, and the results are associated with an inconsistent effect. For these reasons, adjuvants have been used in many studies (e.g., Bacillus Calmette-Guérin (BCG)), and the parasites have been administered through alternative routes such as the mucosal route [19,39, 49].

Whole-killed vaccines

The Leishvaccine, which comprised whole-killed promastigotes of *L. amazonensis* strain (IFLA/BR/1967/PH8) and BCG, plays an important role against canine leishmaniasis. The vaccine significantly increases cytokine expression, innate immu-

Table 1. Several example for given properties of different types of *Leishmania* vaccines [21,51,84-86]

Type of vaccines	Essential component	Clinical form	Benefits	Concerns
Leishmanization	Live and virulent <i>L. major</i> or <i>L. tropica</i>	CL	Single dose Long-term and strong immunity	Not safe
Killed vaccines	Killed <i>Leishmania</i> species	CL	Safer than live vaccines	Less powerful than live vaccines Require multiple doses
Live genetically modified vaccines (avirulent)	<i>BT1</i> -/ <i>Ldp27</i> -/ <i>HSP70-II</i> nul <i>KHARON1</i> <i>Cen</i> -/	VL VL; cross-protection for CL and MCL CL, VL VL CL, VL	Safer than general live vaccines Stimulate immune system as in natural infection Don't require multiple doses	Risk of reversion to virulent state Need cold chain for transportation
Recombinant and subunit vaccines	a. Recombinant LEISH-F1, LIESH-F2 and LEISH-F3 b. LdA2, Ldp27, eIF-2, NH, CPA, CPB, SMT, H1, HSP, LACK c. Sand fly saliva antigens: LJM19, LJL143, PdSP15	CL, VL CL, MCL, VL CL, MCL, VL	No risk Induce strong immune response	Need cold chain for transportation Need adjuvant
DNA vaccines	a. A2, LACK, TSA + LmSTI1, gp63, KMP-11, CPB, NH36, LelF, gp63+HSP70, MIDGE-Th1 vectors encoding conserved T-cell epitopes from KMP11, TSA, CPA and CPB b. Semian Adenovirus expressing NH and SMT	CL, VL PKDL	Safe No need adjuvant Elicit antigen-specific immune responses	Low potency in humans
Live nonpathogenic vaccines (avirulent)	<i>L. tarentolae</i>	VL	Life-long immunity No reversion to virulent state Cross-protection between species	Unknown memory formation and duration Need cold chain for transportation

CL, Cutaneous leishmaniasis; VL, visceral leishmaniasis; MCL, Cutaneous leishmaniasis; PKDL, post-kala-azar dermal leishmaniasis; BCG, Bacillus Calmette Guerin; BT1, Bioprotein transporter 1; Ldp27, *L. donovani* amastigote specific protein p27; Cen, centrin; HSP, Heat-shock protein; A2, Amastigote specific protein 2; LACK, *Leishmania* homolog of receptors for activated c-kinase; CPA or B, Cysteine peptidase A or B; NH, Nucleoside hydrolase; SMT, Sterol 24-c-methyltransferase; H1, Histone-1; LJM19, *A. L. longipalpis* salivary protein; LJL143, *A. L. longipalpis* salivary protein; PdSP15, *P. duboscqi* salivary protein-15; KMH-11, Kinetoplastid membrane protein 11; TSA, Thiol-specific antioxidant; gp63, Glikoprotein63; LmSTI1, *L. major* stress-inducible protein-1; LelF, *L. braziliensis* elongation and initiation factor; MIDGE, minimalistic immunogenically defined gene expression.

ity, and adaptive immune response [50]. The leishmania vaccine was successful in clinical trials of human Phases I and II for its safety and immunogenicity; however, it failed in Phase III. The application of autoclaved-killed *L. mexicana* adjuvanted with BCG resulted in low levels of leishmanin skin test (LST) conversion, which is a marker for cellular immune response [51]. Nevertheless, the incidence of leishmaniasis has significantly decreased in LST-converted participants [49]. Similarly, autoclaved-killed *L. major* (ALM), the old-world species associated with BCG, caused LST conversion in approximately one-third of healthy participants. However, there was a significant reduction in the incidence in individuals who LST-converted [52].

In addition to their preventive aims, the use of this type of vaccine for immunotherapy offers a safe option for severe forms of CL that do not respond to conventional chemotherapy. In a multicenter randomized controlled clinical trial (RCT)

that evaluated the effects of immunotherapy with a vaccine comprising heat-killed *L. mexicana*+*L. amazonensis* adjuvanted with BCG over 10 years, 95.7% of patients with CL were treated with mild adverse events and low cost [49]. The immunotherapeutic approach has been successful in cases of mucocutaneous and diffuse forms of CL [52]. Furthermore, a combination of alum-precipitated ALM (alum/ALM)+BCG and sodium stibogluconate (Stb) was shown to be more effective than Stb alone (87% vs. 53%) [53].

Fractionated *Leishmania* antigens

Four fractionated vaccines, Leishmune (Zoetis Industria de Produtos Veterinarios LTDA, São Paulo, Brazil), Leish-Tec (Hertape Calier Saúde Animal S/A, Juatuba, Brazil), CaniLeish (Virbac, Carros, France), and LetiFend (3P Biopharmaceuticals SL, Navarra, Spain), have been licensed and have achieved impressive success in preventing canine leishmaniasis. Of these,

Table 2. Main mechanisms of action and status of *Leishmania* vaccines at the clinical trial stage [51,69,82,83,86,87]

Name of vaccine	Essential component/antigen	Adjuvant	Mechanism of action	Clinical phase
Leishvaccine	Whole-killed promastigotes of <i>L. amazonensis</i>	BCG	CD4+, CD8+, B cell activation	III
ALM ^a	Autoclave-killed <i>L. major</i>	BCG	T cell activation	II
Leishmune	FML	Saponin	T cell activation	III
CaniLeish	LiESP	Saponin	Induction of Th1 cell	III
GALM ^a	Gentamicin-attenuated <i>L. major</i>		T and B cell activation	II
LEISH-F1	TSA+LmSTI1+LeIF	MPL-SE	T cell activation	I
LEISH-F2	LEISH-F2, designed from LEISH-F1	MPL-SE	T cell activation	II
LEISH-F3	NH+SMT	GLA-SE	T cell activation	I
Leish-Tec	<i>L. donovani</i> A2 protein	Saponin	T cell activation	III
SMT ^a + NH ^a	NH+SMT	GLA-SE	T cell activation	I
ChAd63-KH	KMP-11+HASPB		Broad CD8+ T cell activation	II

ALM, Autoclaved-killed *L. major*; GALM, Gentamycin-attenuated *L. major*; NH, Nucleoside hydrolase; SMT, Sterol 24-c-methyltransferase; FML, fucose-mannose ligand; LiESP, *L. infantum* excreted–secreted protein; KMH-11, Kinetoplastid membrane protein 11; HASPB, Hydrophilic acylated surface protein B; BCG, Bacillus Calmette Guerin; MPL-SE, Monophosphoryl lipid A; GLA-SE, Glucopyranosyl lipid A-stable oil-in-water nano emulsion.

Leishmune and Leish-Tec in Brazil, and CaniLeish and LetiFend in Europe have been commercialized [54].

Leishmune is composed of the fucose-mannose ligand (FML) of *L. donovani* and saponin adjuvant [55]. In phase III studies conducted in dogs, 92% protection against disease was observed. Because no clinical signs or *Leishmania* DNA were detected in these animals, Leishmune was evaluated as a transmission-blocking vaccine. However, the lack of sample randomization or blinded evaluation of trial individuals did not allow for full validation of the results. In 2014, the production and marketing license of Leishmune was withdrawn [54]. The CaniLeish vaccine is composed of purified excreted–secreted proteins of *L. infantum* (LiESP) and adjuvanted with saponin (named QA-21) [56]. The vaccine elicited predominantly Th1-type cellular immune responses, and the infection protection rate was 99.4% in a field study [57].

Leish-Tec and LetiFend are recombinant vaccines. Leish-Tec was formulated with recombinant protein A2 from *L. donovani* amastigotes and saponin as a vaccine adjuvant. In studies that assessed Leish-Tec, it was observed that it was effective not only as preventive, but also in immunotherapeutic approaches [54]. LetiFend contains a chimeric protein (protein Q) formed by 5 antigenic fragments from 4 different *L. infantum* proteins (ribosomal proteins Lip2a, Lip2b, and Lip0, and histone H2A), to which no adjuvant has been added. It was shown it would be the potential of protein Q in the immunization against *L. infantum* in preliminary studies in mice [58].

Fractionated *Leishmania* vaccines seem to be efficiently used in areas that are crucial to the control of *Leishmania* infection [56].

Second generation (subunit or genetically modified parasite) vaccines

Although first-generation vaccines are still being evaluated, several studies have focused on second-generation vaccines. Second-generation vaccines include different recombinant proteins, which are produced through genetically engineered cells such as viruses and bacteria, purified native protein fractions of parasite antigens, synthetic peptides, and even genetically modified parasites [28]. Second-generation vaccines are more feasible for mass vaccination, and their recombinant nature facilitates accessibility to large-scale and cost-effective production [54].

Subunit vaccines

The several subunits or recombinant vaccine candidates such as LeIF, gp63, p36/LACK, A-2, PSA-2/gp46/M-2, FML, LCR1, ORFE, KMP11, LmSTI1, TSA, HASPB1, protein Q, cysteine protease B (CPB), and A (CPA) have been extensively studied [40,55,59–67]. Many subunit vaccine candidates stimulate an effective protective immune response in the prevention of *Leishmania* infection. One of the advantages of subunit vaccines is that they pose no risk of infection, which ensures their suitability for immunocompromised individuals [59].

Application of a surface-expressed glycoprotein (gp63), another subunit protein, in a cationic liposome increased the number of IFN- γ -producing effector T cells. Furthermore, vaccination based on gp63 DNA elicited immune responses and conferred protection [60]. In a couple of experiments assessing protective immunological effects, viruses expressing the LACK (*Leishmania* homologue for receptors of activated C kinase)

antigen, with or without adjuvant, showed good protective effects against *Leishmania* infection [61,62]. Similarly, the virus expressing the promastigote protein surface of G46/M-2/PSA-2 protected against *L. amazonensis* [63].

Notably, recombinant antigen vaccines such as LEISH-F1, LIESH-F2, and LEISH-F3 have reached phase II clinical trials, demonstrating their potential as vaccine candidates against leishmaniasis [68-70]. LEISH-F1 is an artificial protein encoded by 3 genes: *L. major* homologue of eukaryotic thiol-specific antioxidant (TSA), *L. major* stress-inducible protein-1 (LmSTI1), and *L. braziliensis* elongation and initiation factor (LeIF). LEISH-F1 protein applications were evaluated following emulsification of monophosphoryl lipid A in a structure-stimulating toll-like receptor (MPL-SE). LEISH-F1 + MPL-SE (IDRI, Seattle, Washington, USA) efficiently treated patients with CL or ML and induced protective immunity in healthy volunteers [68-70]. This vaccine is also safe and tolerated [71]. Among the other adjuvanted artificial proteins, LEISH-F2 + MPL-SE and LEISH-F3 + GL-SE (glucopyranosyl lipid A-stable oil-in-water nanoemulsion) showed promising results against infection [68-70]. A NS recombinant vaccine, consisting of enzyme nucleoside hydrolase (NH) and sterol 24-c-methyltransferase (SMT) and adjuvanted with "glucopyranosyl lipid A-stable oil-in-water nanoemulsion" (GLA-SE), is also in clinical trial phase [51].

Genetically modified parasite vaccines

In *Leishmania* vaccine research, live attenuated vaccines made by genetic modifications are another research topic [16]. In this strategy, the parasite genes responsible for its survival and/or virulence are modified or deleted. Unlike live virulent parasites, they do not pose any danger associated with infection. However, they ensure that the induction of immune responses is consistent with protection, since they closely mimic natural infection [72].

Various live attenuated vaccine strains of *L. major*, *L. mexicana*, *L. amazonensis*, and *L. donovani* have been produced by deleting the targeted genes. They were observed to provide significant protection against CL and VL in susceptible mice. Among them, Biopterin transporter 1 (BT1)-deleted *L. donovani* parasites and A2-rel gene cluster in *L. donovani* as well as SIR2, Hsp70-II, and KH1 *L. infantum* null mutants, protected the mice against virulent strains [73-76]. The p27 gene, encoding an amastigote-specific cytochrome C oxidase component, knockout (gene Ldp27-/-) *L. donovani* reduced parasitic

loads and provided long-lasting protection against the development of CL and VL associated with virulent strains [77,78].

Leishmania centrin gene-1 is necessary for parasite growth and differentiation. The generation of a potential mutant vaccine candidate appears to be an interesting target. Volpedo et al. [28] reported that immunization with LdCen-/- led to a significant influx of MHC-II-expressing macrophages, resulting in higher levels of IFN- γ -secreting CD4+ Th1 cells and lower levels of IL-10- and IL-4-secreting CD4+ Th2 cells. This response ensures protection against the virulent parasites.

In general, the most promising strategic alternative against VL can be claimed to be the use of live, non-pathogenic/genetically engineered strains of these species [26].

Third generation (naked DNA) vaccines

Third-generation vaccines utilize the DNA [51]. In this relatively new approach, naked plasmid DNA or DNA encapsulated in a viral vector is injected intradermally or intramuscularly [28,79]. DNA vaccines are safe because they do not contain any pathogenic organisms that may revert virulence [79]. They also efficiently induce interferon-gamma production and dendritic cell activation, which protects against *Leishmania* infection [80].

DNA vaccines can consist of genes encoding single antigens, such as gp63, LACK, or PSA-2. They can also include multiple genes encoding various antigens such as TSA, KMP11, A-2, NH36, LmSTI1, cysteine proteases, and histones [51]. To increase the immunogenicity of DNA vaccines, these vaccines were primed by boosting the associated protein expressed on a recombinant virus-like modified vaccinia virus Ankara [81]. This strategy selectively elicits a wide range of CD8+ T cells specific for *Leishmania* antigens [28,79].

Collectively, DNA vaccines are stable, do not require adjuvants, produce antigens over long periods, can easily be produced in large quantities, and are effective. Nonetheless, they bear some safety concerns, such as the integration of DNA into the mammalian genome, which can result in the induction of autoimmune diseases or cancer [28].

DNA vaccines, known as third-generation vaccines, are of particular interest because they can effectively induce both CD8+ and CD4+ T cells, produce long-lived antigens and properly folded polypeptides, etc. [82]. Recently, a phase II study is being conducted to evaluate the therapeutic effects of ChAd63-KH, which consists of 2 genes encoding the *L. donovani* KMP-11 and HASPB antigens, in patients with persistent post-kala-azar dermal leishmaniasis (PKDL) [83].

New strategy vaccines

Advances in the field of science, in addition to the above-mentioned, have also led to the investigation of new strategies in vaccine development, including certain bioengineering approaches, such as delivery systems and chimeric vaccines, and the use of nonpathogenic *Leishmania* spp. [19].

Vaccine antigen delivery systems

Drug delivery systems have been extensively studied for the treatment of cancer and infectious diseases [1,88,89]. Nanoparticles (NP) are considered ideal vaccine delivery systems. Owing to their structures, vaccine delivery systems present several advantages, such as controlled antigen release, protection of the vaccine antigens from degradation, and site-specific delivery. They can also have adjuvant effect [89]. All these characteristics of NP carriers lead to enhanced bioavailability of antigens, which results in the activation of the immune response. In a study using cationic solid lipid nanoparticles (cSLN) for carrier and delivery, the injection of a fusion gene, pCDNA-A2-CPA-CPB-CTE, enhanced protective cell mediated immunity [90].

Another study demonstrated that vaccination with multifunctionalized PLGA NPs encapsulating sLiAg and/or MPLA provided strong protection against infection with *L. infantum* [89]. In both studies, the increased production of IFN γ followed by suppression of IL-4 and IL-10 production and very few parasitic loads were considered protective markers. In conclusion, nanoparticle carriers of *Leishmania* antigen vaccine may be a highly effective strategy against leishmaniasis.

Chimeric vaccines

Lage et al. [91] designed an in silico synthetic recombinant vaccine, named ChimeraT. It contained specific T-cell epitopes from *Leishmania* prohibitin, eukaryotic initiation factor 5a, and hypothetical LiHyp1 and LiHyp2 proteins. After injecting ChimeraT with saponin as an adjuvant, a Th1-type immune response was induced and BALB/c mice were protected against *L. infantum* infection [91]. In another study, the F1F3 chimera protein (C-terminal domain of nucleoside hydrolase NH36) showed a strong reduction in ear lesion size induced by *L. braziliensis*. It also promoted the highest CD4+ and CD8+ cytokine-secreting T cell responses, with predominant frequencies of multifunctional CD4+ and CD8+IL-2+TNF- α +IFN- γ + T cells [92]. Thus, chimeric proteins could be considered potential vaccine candidates to protect against human diseases.

Nonpathogen *Leishmania* spp. in vaccination

L. tarentolae, isolated from a reptile animal, is a nonpathogenic *Leishmania* specie in humans [93,94]. In contrast, this parasite activates the dendritic cell maturation process, which results in the production of interferon gamma and induction of a Th1-type immune response. Furthermore, it mimics the natural development of immunity better than other strategies as an important advantage [89]. Breton et al. [95] also observed that intraperitoneally injected *L. tarentolae* elicited protective immunity against *L. donovani* in BALB/C mice. In this regard, they proposed that *L. tarentolae* is a promising live vaccine candidate against *Leishmania* infections, without causing any infection in humans.

Breton et al. [95] proposed the idea that it can be improved by generating recombinant *L. tarentolae* expressing selected *Leishmania* immunodominant epitopes or by combining the *L. tarentolae* recombinant parasite with a DNA vaccine as part of a prime-boost strategy to elicit more effective and long-lasting protection. In fact, in a parallel study with a cSLN carrier of the pCDNA-A2-CPA-CPB-CTE fusion gene by Taslime et al. [90], it was reported that recombinant *L. tarentolae*-A2-CPA-CPB-CTE vaccination was protective against *L. infantum* infection in BALB/c mice.

Viral-like particle vaccines

One of the latest approaches to developing a safe and cost-effective large-scale vaccine is the use of virus-like particles (VLPs). VLPs are molecules that are morphologically identical to the native virus but cannot replicate because they do not contain viral genetic material (VLPs) [96]. The VLP-based antigen formulation has the potential to generate not only cellular but also humoral immunity that is very similar to that elicited by a natural viral infection VLPs can be produced in different expression systems such as bacterial, yeast, plant, insect or mammalian cells. Panasiuk et al. [86] revealed that VLPs derived from *L. tarentolae* can induce the production of potent neutralizing antibodies. Maura et al. [97] tested a polyvalent α -Gal, carbohydrates essential for the virulence and viability of many parasites, conjugated to an immunogenic Q β virus-like particle in a C57BL/6 α -galactosyltransferase knockout mouse model. This vaccine protected knockout mice against *L. infantum* and *L. amazonensis*, the aetiological agents of visceral and cutaneous leishmaniasis, respectively.

mRNA vaccines

An effective vaccine both should boost the natural innate and adaptive immune response and induce a memory immune response that provides long-term protection against infection. Recent research reveals that mRNA vaccines significantly enhance these properties [98]. The mRNA platform also allows simultaneous expression of multiple proteins, eliciting immunity against different epitopes from different targets [99]. In a mice model, Duthie et al. [100] observed a significant reduction in the parasite burden in the liver by administering F2-RNA as a prime vaccination and then boosting with the recombinant LEISH-F2 protein. RNA vaccine technology has the potential to offer an effective and practical solution to vaccine development. Development of RNA vaccine requires only knowledge of the target gene sequence, eliminating the need for pathogen culture or scale-up recombinant protein production [101].

CONCLUDING REMARK

A safe and efficacious vaccine is urgently required to provide long-lasting protective immunity for the control of parasitic infections. Although cell-mediated immunity is known to play a crucial role in host protection, the immunological protective mechanism of leishmaniasis is complicated. While there is currently no licensed vaccine for human leishmaniasis, extensive efforts are underway to develop a variety of vaccine modalities with promising results worldwide. Some of these modalities are in clinical phase and successful results are obtained in terms of safety and immunogenicity. Meanwhile, it is considered that animal vaccines will play an important role in preventing the transmission of Leishmaniasis to humans. Furthermore, several vaccine candidates are being evaluated at different phases of clinical trials, including those in the first, second, and even third generation.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that may have influenced the work reported in this paper.

REFERENCES

1. Dinc R. New developments in the treatment of cutaneous

- leishmaniasis. *Asian Pac J Trop Med* 2022; 15: 196-205. <https://doi.org/10.4103/1995-7645.345944>
2. Yeşilova Y, Aksoy M, Sürücü HA, Uluat A, Ardic N, Yesilova A. Lip leishmaniasis: Clinical characteristics of 621 patients. *Int J Crit Illn Inj Sci*. 2015; 5: 265-266. <https://doi.org/10.4103/2229-5151.170849>
3. Alvar J, den Boer M, Dagne DA. Towards the elimination of visceral leishmaniasis as a public health problem in east Africa: reflections on an enhanced control strategy and a call for action. *Lancet Glob Health* 2021; 9: e1763-1769. [https://doi.org/10.1016/S2214-109X\(21\)00392-2](https://doi.org/10.1016/S2214-109X(21)00392-2)
4. Bezemer JM, Meesters K, Naveda CL, Machado PRL, Calvopiña M, Leeflang MMG, Schallig HDFH, de Vries HJC. Clinical criteria for Mucosal leishmaniasis diagnosis in rural South America: a systematic literature review. *PLoS Negl Trop Dis* 2022 16: e0010621. <https://doi.org/10.1371/journal.pntd.0010621>
5. Martins-Melo FR, Lima Mda S, Ramos AN Jr, Alencar CH, Heuelbach J. Mortality and case fatality due to visceral leishmaniasis in Brazil: a nationwide analysis of epidemiology, trends and spatial patterns. *PLoS One*. 2014; 9: e93770. <https://doi.org/10.1371/journal.pone.0093770>
6. World Health Organization. Leishmaniasis: Situation and trend [Internet]; [cited 2022 Aug 29]. Available from: <https://www.who.int/data/gho/data/themes/topics/topic-details/GHO/leishmaniasis#:~:text=As%20of%20September%202021%2C%205,Somalia%2C%20South%20Sudan%20and%20Sudan>
7. Srivastava S, Shankar P, Mishra J, Singh S. Possibilities and challenges for developing a successful vaccine for leishmaniasis. *Parasit Vectors* 2016; 9: 277. <https://doi.org/10.1186/s13071-016-1553-y>
8. Malvoti S, Malhame M, Mantel CE, Le Rutte EA, Kaye PM. Human leishmaniasis vaccines: Use cases, target population and potential global demand. *PLoS Negl Trop Dis* 2021; 15: e0009742. <https://doi.org/10.1371/journal.pntd.0009742>
9. Jha MK, Sarode AY, Bodhale N, Mukherjee D, Pandey SP, Srivastava N, Rub A, Silvestre R, Sarkar A, Saha B. Development and characterization of an avirulent *Leishmania* major strain. *J Immunol* 2020; 204: 2734-2753. <https://doi.org/10.4049/jimmunol.1901362>
10. Silva CFM, Pinto DCGA, Fernandes PA, Silva AMS. Evolution of acridines and xanthenes as a core structure for the development of antileishmanial agents. *Pharmaceuticals (Basel)* 2022; 15: 148. <https://doi.org/10.3390/ph15020148>
11. Santana W, de Oliveira SS, Ramos MH, Santos ALS, Dolabella SS, Souto EB, Severino P, Jain S. Exploring innovative leishmaniasis treatment: drug targets from pre-clinical to clinical findings. *Chem Biodivers* 2021; 18: e2100336. <https://doi.org/10.1002/cbdv.202100336>
12. Ardic N, Ardic AF, Gunel Z. Leishmaniasis during the increased Syrian refugee traffic. *Glob J Infect Dis Clin Res* 2018; 4: 013-016. <http://dx.doi.org/10.17352/2455-5363.000020>
13. de Moraes RC, de Melo MG, de Goes TC, e Silva RP, de Moraes

- RE, de Oliveira Guerra JA, de Brito ME, Brandão-Filho SP, de Paiva Cavalcanti M. Clinical-therapeutic follow-up of patients with American cutaneous leishmaniasis caused by different *Leishmania* spp. in Brazil. *Exp Parasitol* 2022; 240: 108338. <https://doi.org/10.1016/j.exppara.2022.108338>
14. World Health Organization. Leishmaniasis[Internet]; [cited 2022 Aug 29]. Available from: <https://www.who.int/news-room/fact-sheets/detail/leishmaniasis>
 15. Alves F, Bilbe G, Blesson S, Goyal V, Monnerat S, Mowbray C, Muthoni Ouattara G, Pécoul B, Rijal S, Rode J, Solomos A. Recent development of visceral leishmaniasis treatments: successes, pitfalls, and perspectives. *Clin Microbiol Rev* 2018; 31: e00048-18. <https://doi.org/10.1128/CMR.00048-18>
 16. Zhang WW, Karmakar S, Gannavaram S, Dey R, Lypaczewski P, Ismail N, Siddiqui A, Simonyan V, Oliveira F, Coutinho-Abreu IV, DeSouza-Vieira T, Meneses C, Oristian J, Serafim TD, Musa A, Nakamura R, Saljoughian N, Volpedo G, Satoskar M, Satoskar S, Dagur PK, McCoy JP, Kamhawi S, Valenzuela JG, Hamano S, Satoskar AR, Matlashewski G, Nakhasi HL. A second generation leishmanization vaccine with a markerless attenuated *Leishmania* major strain using CRISPR gene editing. *Nat Commun* 2020; 11: 3461. <https://doi.org/10.1038/s41467-020-17154-z>
 17. Miramin-Mohammadi A, Javadi A, Eskandari SE, Nateghi-Rostami M, Khamesipour A. Immune responses in cutaneous leishmaniasis: *In vitro* Thelper1/Thelper2 cytokine profiles using live versus killed *Leishmania major*. *J Arthropod Borne Dis* 2021; 15: 126-135. <https://doi.org/10.18502/jad.v15i1.6491>
 18. Carregaro V, Costa DL, Brodskyn C, Barral AM, Barral-Netto M, Cunha FQ, Silva JS. Dual effect of *Lutzomyia longipalpis* saliva on *Leishmania braziliensis* infection is mediated by distinct saliva-induced cellular recruitment into BALB/c mice ear. *BMC Microbiol* 2013; 13: 102. <https://doi.org/10.1186/1471-2180-13-102>
 19. Helou DG, Mauras A, Fasquelle F, Lanza JS, Loiseau PM, Betbeder D, Cojean S. Intranasal vaccine from whole *Leishmania donovani* antigens provides protection and induces specific immune response against visceral leishmaniasis. *PLoS Negl Trop Dis.* 2021; 15: e0009627. <https://doi.org/10.1371/journal.pntd.0009627>
 20. Karmakar S, Ismail N, Oliveira F, Oristian J, Zhang WW, Kaviraj S, Singh KP, Mondal A, Das S, Pandey K, Bhattacharya P, Volpedo G, Gannavaram S, Satoskar M, Satoskar S, Sastry RM, Oljuskin T, Sepahpour T, Meneses C, Hamano S, Das P, Matlashewski G, Singh S, Kamhawi S, Dey R, Valenzuela JG, Satoskar A, Nakhasi HL. Preclinical validation of a live attenuated dermatropic *Leishmania* vaccine against vector transmitted fatal visceral leishmaniasis. *Commun Biol* 2021; 4: 929. <https://doi.org/10.1038/s42003-021-02446-x>
 21. Saljoughian N, Taheri T, Rafati S. Live vaccination tactics: possible approaches for controlling visceral leishmaniasis. *Front Immunol* 2014; 5: 134. <https://doi.org/10.3389/fimmu.2014.00134>
 22. Nylén S, Maasho K, Söderstrom K, Ilg T, Akuffo H. Live *Leishmania* promastigotes can directly activate primary human natural killer cells to produce interferon-gamma. *Clin Exp Immunol* 2003; 131: 457-467. <https://doi.org/10.1046/j.1365-2249.2003.02096.x>
 23. Kopelyanskiy D, Desponds C, Prevel F, Rossi M, Migliorini R, Snäkä T, Eren RO, Claudinot S, Lye LE, Pasparakis M, Beverley SM, Fasel N. *Leishmania guyanensis* suppressed inducible nitric oxide synthase provoked by its viral endosymbiont. *Front Cell Infect Microbiol* 2022; 12: 944819. <https://doi.org/10.3389/fcimb.2022.944819>
 24. Cecílio P, Cordeiro-da-Silva A, Oliveira F. Sand flies: Basic information on the vectors of leishmaniasis and their interactions with *Leishmania* parasites. *Commun Biol* 2022; 5: 305. <https://doi.org/10.1038/s42003-022-03240-z>
 25. Cummings HE, Tuladhar R, Satoskar AR. Cytokines and their STATs in cutaneous and visceral leishmaniasis. *J Biomed Biotechnol* 2010; 2010: 294389. <https://doi.org/10.1155/2010/294389>
 26. Ikeogu NM, Akaluka GN, Edechi CA, Salako ES, Onyilgha C, Barazandeh AF, Uzonna JE. *Leishmania* immunity: advancing immunotherapy and vaccine development. *Microorganisms* 2020; 8: 1201. <https://doi.org/10.3390/microorganisms8081201>
 27. Natarajan G, Oghumu S, Varikuti S, Thomas A, Satoskar A. Mechanisms of immunopathology of leishmaniasis. In Satoskar AR, Durvasula R eds, *Pathogenesis of Leishmaniasis*. Springer. New York, USA. 2014, pp 1-13.
 28. Volpedo G, Pacheco-Fernandez T, Bhattacharya P, Oljuskin T, Dey R, Gannavaram S, Satoskar AR, Nakhasi HL. Determinants of innate immunity in visceral leishmaniasis and their implication in vaccine development. *Front Immunol* 2021; 12: 748325. <https://doi.org/10.3389/fimmu.2021.748325>
 29. Abdellahi L, Irají F, Mahmoudabadi A, Hejazi SH. Vaccination in leishmaniasis: a review article. *Iran Biomed J* 2022; 26: 1-35. <https://doi.org/10.52547/ibj.26.1.35>
 30. Hosseini Farash BR, Mohebbali M, Kazemi B, Fata A, Hajjaran H, Akhouni B, Raoofian R, Mastroeni P, Moghaddas E, Khaledi A, Salehi Sangani G. Validation of a mixture of rK26 and rK39 antigens from Iranian strain of *Leishmania infantum* to detect anti-*Leishmania* antibodies in human and reservoir hosts. *Sci Rep* 2022; 12: 10426. <https://doi.org/10.1038/s41598-022-14490-6>
 31. Gomes DC, Souza BL, Schwedersky RP, Covre LP, de Matos Guedes HL, Lopes UG, Ré MI, Rossi-Bergmann B. Intranasal immunization with chitosan microparticles enhances LACK-DNA vaccine protection and induces specific long-lasting immunity against visceral leishmaniasis. *Microbes Infect* 2022; 24: 104884. <https://doi.org/10.1016/j.micinf.2021.104884>
 32. Sumova P, Sanjoba C, Willen L, Polanska N, Matsumoto Y, Noiri E, Paul SK, Ozbel Y, Volf P. PpSP32-like protein as a marker of human exposure to *Phlebotomus argentipes* in *Leishmania donovani* foci in Bangladesh. *Int J Parasitol* 2021; 51: 1059-1068. <https://doi.org/10.1016/j.ijpara.2021.05.006>

33. Goyal DK, Keshav P, Kaur S. Potential of TLR agonist as an adjuvant in *Leishmania* vaccine against visceral leishmaniasis in BALB/c mice. *Microb Pathog* 2021; 158: 105021. <https://doi.org/10.1016/j.micpath.2021.105021>
34. Bhaumik SK, Singh MK, Karmakar S, De T. UDP-Gal: N-acetylglucosamine beta 1-4 galactosyltransferase expressing live attenuated parasites as vaccine for visceral leishmaniasis. *Glycoconj J* 2009; 26: 663-673. <https://doi.org/10.1007/s10719-008-9212-y>
35. De Luca PM, Macedo AB. Cutaneous leishmaniasis vaccination: a matter of quality. *Front Immunol* 2016; 7: 151. <https://doi.org/10.3389/fimmu.2016.00151>
36. Pacheco-Fernandez T, Volpedo G, Gannavaram S, Bhattacharya P, Dey R, Satoskar A, Matlashewski G, Nakhasi HL. Revival of leishmanization and leishmanin. *Front Cell Infect Microbiol* 2021; 11: 639801. <https://doi.org/10.3389/fcimb.2021.639801>
37. Ostolin TL, Gusmão MR, Mathias FA, de Oliveira Cardoso JM, Roatt BM, de Oliveira Aguiar-Soares RD, Ruiz JC, de Melo Resende D, de Brito RC, Reis AB. A chimeric vaccine combined with adjuvant system induces immunogenicity and protection against visceral leishmaniasis in BALB/c mice. *Vaccine* 2021; 39: 2755-2763. <https://doi.org/10.1016/j.vaccine.2021.04.004>
38. Athanasiou E, Agallou M, Tastsoglou S, Kammona O, Hatzi-georgiou A, Kiparissides C, Karagouni E. A poly (lactic-co-glycolic) acid nanovaccine based on chimeric peptides from different *Leishmania infantum* proteins induces dendritic cells maturation and promotes peptide-specific IFN γ -producing CD8 $^+$ T cells essential for the protection against experimental visceral leishmaniasis. *Front Immunol* 2017; 8: 684. <https://doi.org/10.3389/fimmu.2017.00684>
39. Mohebbi M, Nadim A, Khamesipour A. An overview of leishmanization experience: A successful control measure and a tool to evaluate candidate vaccines. *Acta Trop* 2019; 200: 105173. <https://doi.org/10.1016/j.actatropica.2019.105173>
40. Alvar J, Croft SL, Kaye P, Khamesipour A, Sundar S, Reed SG. Case study for a vaccine against leishmaniasis. *Vaccine* 2013; 31: B244-B249. <https://doi.org/10.1016/j.vaccine.2012.11.080>
41. Oliveira F, Rowton E, Aslan H, Gomes R, Castrovinci PA, Alvarenga PH, Abdeladhim M, Teixeira C, Meneses C, Kleeman LT, Guimarães-Costa AB, Rowland TE, Gilmore D, Doumbia S, Reed SG, Lawyer PG, Andersen JF, Kamhawi S, Valenzuela JG. A sand fly salivary protein vaccine shows efficacy against vector-transmitted cutaneous leishmaniasis in nonhuman primates. *Sci Transl Med* 2015; 7: 290ra90. <https://doi.org/10.1126/scitranslmed.aaa3043>
42. Zahedifard F, Gholami E, Taheri T, Taslimi Y, Doustdari F, Seyed N, Torkashvand F, Meneses C, Papadopoulou B, Kamhawi S, Valenzuela JG, Rafati S. Enhanced protective efficacy of non-pathogenic recombinant *Leishmania tarentolae* expressing cysteine proteinases combined with a sand fly salivary antigen. *PLoS Negl Trop Dis* 2014; 8: e2751. <https://doi.org/10.1371/journal.pntd.0009123>
43. Davarpanah E, Seyed N, Bahrami F, Rafati S, Safaralizadeh R, Taheri T. *Lactococcus lactis* expressing sand fly PpSP15 salivary protein confers long-term protection against *Leishmania major* in BALB/c mice. *PLoS Negl Trop Dis* 2020; 14: e0007939. <https://doi.org/10.1371/journal.pntd.0007939>
44. Mitchell GF, Handman E, Spithill TW. Vaccination against cutaneous leishmaniasis in mice using nonpathogenic cloned promastigotes of *Leishmania major* and importance of route of injection. *Aust J Exp Biol Med Sci* 1984; 62: 145-153. <https://doi.org/10.1038/icb.1984.14>
45. Gorczynski RM. Immunization of susceptible BALB/c mice against *Leishmania braziliensis*: II. Use of temperature-sensitive avirulent clones of parasite for vaccination purposes. *Cellular Immunol* 1985; 94: 11-20. [https://doi.org/10.1016/0008-8749\(85\)90081-4](https://doi.org/10.1016/0008-8749(85)90081-4)
46. Rivier D, Shah R, Bovay P, Mauel J. Vaccine development against cutaneous leishmaniasis. Subcutaneous administration of radioattenuated parasites protects CBA mice against virulent *Leishmania major* challenge. *Parasite Immunol* 1993; 15: 75-84. <https://doi.org/10.1111/j.1365-3024.1993.tb00587.x>
47. Kimsey PB, Theodos CM, Mitchen TK, Turco SJ, Titus RG. An avirulent lipophosphoglycan-deficient *Leishmania major* clone induces CD4 $^+$ T cells which protect susceptible BALB/c mice against infection with virulent *L. major*. *Infect Immun* 1993; 61: 5205-5213. <https://doi.org/10.1128/iai.61.12.5205-5213.1993>
48. Daneshvar H, Coombs GH, Hagan P, Phillips RS. *Leishmania mexicana* and *Leishmania major*: attenuation of wild-type parasites and vaccination with the attenuated lines. *J Infect Dis* 2003; 187: 1662-1668. <https://doi.org/10.1086/374783>
49. Convit J, Ulrich M, Polegre MA, Avila A, Rodríguez N, Mazzedo MI, Blanco B. Therapy of Venezuelan patients with severe mucocutaneous or early lesions of diffuse cutaneous leishmaniasis with a vaccine containing pasteurized *Leishmania* promastigotes and bacillus Calmette-Guerin: preliminary report. *Mem Inst Oswaldo Cruz* 2004; 99: 57-62. <https://doi.org/10.1590/S0074-02762004000100010>
50. Araújo MS, de Andrade RA, Vianna LR, Mayrink W, Reis AB, Sathler-Avelar R, Teixeira-Carvalho A, Andrade MC, Mello MN, Martins-Filho OA. Despite Leishvaccine and Leishmune trigger distinct immune profiles, their ability to activate phagocytes and CD8 $^+$ T-cells support their high-quality immunogenic potential against canine visceral leishmaniasis. *Vaccine* 2008; 26: 2211-2224. <https://doi.org/10.1016/j.vaccine.2008.02.044>
51. Moafi M, Rezvan H, Sherkat R, Taleban R. *Leishmania* vaccines entered in clinical trials: A review of literature. *Int J Prev Med* 2019; 10: 95. https://doi.org/10.4103/ijpvm.IJPVM_116_18
52. Convit J, Ulrich M, Zerpa O, Borges R, Aranzazu N, Valera M, Villarroel H, Zapata Z, Tomedes I. Immunotherapy of american cutaneous leishmaniasis in Venezuela during the period 1990-99. *Trans R Soc Trop Med Hyg* 2003; 97: 469-472. [https://doi.org/10.1016/S0035-9203\(03\)90093-9](https://doi.org/10.1016/S0035-9203(03)90093-9)
53. Khamesipour A. Therapeutic vaccines for leishmaniasis. *Expert Opin Biol Ther* 2014; 14:1641-9. <https://doi.org/10.1517/14712>

- 598.2014.945415
54. Velez R, Gállego M. Commercially approved vaccines for canine leishmaniosis: a review of available data on their safety and efficacy. *Trop Med Int Health* 2020; 25: 540-557. <https://doi.org/10.1111/tmi.13382>
 55. Borja-Cabrera GP, Santos FN, Bauer FS, Parra LE, Menz I, Morgado AA, Soares IS, Batista LMM, Palatnik-de-Sousa CB. Immunogenicity assay of the Leishmune® vaccine against canine visceral leishmaniasis in Brazil. *Vaccine* 2008; 26: 4991-4997. <https://doi.org/10.1016/j.vaccine.2008.07.029>
 56. The European Public Assessment. CaniLeish: canine vaccine against *Leishmania infantum* adjuvanted [Internet]; The European Public Assessment; [cited 2022 Aug 29]. Available from: https://www.ema.europa.eu/en/documents/overview/canileish-epar-summary-public_en.pdf
 57. Moreno J, Vouldoukis I, Martin V, McGahie D, Cuisinier AM, Gueguen S. Use of a LIESP/QA-21 vaccine (CaniLeish) stimulates an appropriate Th1-dominated cell-mediated immune response in dogs. *PLoS Negl Trop Dis* 2012; 6: e1683. <https://doi.org/10.1371/journal.pntd.0001683>
 58. Parody N, Soto M, Requena JM, Alonso C. Adjuvant guided polarization of the immune humoral response against a protective multicomponent antigenic protein (Q) from *Leishmania infantum*. A CpG+Q mix protects Balb/c mice from infection. *Parasite Immunol* 2004; 26: 283-293. <https://doi.org/10.1111/j.0141-9838.2004.00711.x>
 59. Ahmed SB, Bahloul C, Robbana C, Askri S, Dellagi K. A comparative evaluation of different DNA vaccine candidates against experimental murine leishmaniasis due to *L. major*. *Vaccine* 2004; 22: 1631-1639. <https://doi.org/10.1016/j.vaccine.2003.10.046>
 60. Olobo JO, Anjili CO, Gicheru MM, Mbatia PA, Kariuki TM, Githure JJ, Koech DK, McMaster WR. Vaccination of vervet monkeys against cutaneous leishmaniosis using recombinant *Leishmania* 'major surface glycoprotein' (gp63). *Vet Parasitol* 1995; 60: 199-212. [https://doi.org/10.1016/0304-4017\(95\)00788-6](https://doi.org/10.1016/0304-4017(95)00788-6)
 61. Gurunathan S, Sacks DL, Brown DR, Reiner SL, Charest H, Glaichenhaus N, Seder RA. Vaccination with DNA encoding the immunodominant LACK parasite antigen confers protective immunity to mice infected with *Leishmania major*. *J Exp Med* 1997; 186: 1137-1147. <https://doi.org/10.1084/jem.186.7.1137>
 62. Yang DM, Fairweather N, Button LL, McMaster WR, Kahl LP, Liew FY. Oral Salmonella typhimurium (AroA-) vaccine expressing a major Leishmanial surface protein (gp63) preferentially induces T helper 1 cells and protective immunity against leishmaniasis. *J Immunol* 1990; 145: 2281-2285.
 63. McMahon-Pratt D, Rodriguez D, Rodriguez JR, Zhang Y, Manson K, Bergman C, Rivas L, Rodriguez JE, Lohman KL, Ruddle NH. Recombinant vaccinia viruses expressing GP46/M-2 protect against *Leishmania* infection. *Infect Immun* 1993; 61: 3351-3359. <https://doi.org/10.1128/iai.61.8.3351-3359.1993>
 64. Champs J, McMahon-Pratt D. Membrane glycoprotein M-2 protects against *Leishmania amazonensis* infection. *Infect Immun* 1988; 56: 3272-3279. <https://doi.org/10.1128/iai.56.12.3272-3279.1988>
 65. Handman E, Symons FM, Baldwin TM, Curtis JM, Scheerlinck JP. Protective vaccination with promastigote surface antigen 2 from *Leishmania major* is mediated by a TH1 type of immune response. *Infect Immun* 1995; 63: 4261-4267. <https://doi.org/10.1128/iai.63.11.4261-4267.1995>
 66. Santos WR, Aguiar IA, de Souza EP, de Lima VM, Palatnik M, Palatnik-de-Sousa CB. Immunotherapy against murine experimental visceral leishmaniasis with the FML-vaccine. *Vaccine* 2003; 21: 4668-4676. [https://doi.org/10.1016/S0264-410X\(03\)00527-9](https://doi.org/10.1016/S0264-410X(03)00527-9)
 67. Nagill R, Kaur S. Vaccine candidates for leishmaniasis: a review. *Int Immunopharmacol* 2011; 11: 1464-1488. <https://doi.org/10.1016/j.intimp.2011.05.008>
 68. Coler RN, Duthie MS, Hofmeyer KA, Guderian J, Jayashankar L, Vergara J, Rolf T, Misquith A, Laurance JD, Raman VS, Bailor HR, Cauwelaert ND, Reed SJ, Vallur A, Favila M, Orr MT, Ashman J, Ghosh P, Mondal D, Reed SG. From mouse to man: safety, immunogenicity and efficacy of a candidate leishmaniasis vaccine LEISH-F3+GLA-SE. *Clin Transl Immunology* 2015; 4: e35. <https://doi.org/10.1038/cti.2015.6>
 69. Gillespie PM, Beaumier CM, Strych U, Hayward T, Hotez PJ, Bottazzi ME. Status of vaccine research and development of vaccines for leishmaniasis. *Vaccine* 2016; 34: 2992-2995. <https://doi.org/10.1016/j.vaccine.2015.12.071>
 70. Chakravarty J, Kumar S, Trivedi S, Rai VK, Singh A, Ashman JA, Laughlin EM, Coler RN, Kahn SJ, Beckmann AM, Cowgill KD, Reed SG, Sundar S, Piazza FM. A clinical trial to evaluate the safety and immunogenicity of the LEISH-F1+MPL-SE vaccine for use in the prevention of visceral leishmaniasis. *Vaccine* 2011; 29: 3531-3537. <https://doi.org/10.1016/j.vaccine.2011.02.096>
 71. Duthie MS, Raman VS, Piazza FM, Reed SG. The development and clinical evaluation of second-generation leishmaniasis vaccines. *Vaccine* 2012; 30: 134-141. <https://doi.org/10.1016/j.vaccine.2011.11.005>
 72. Gannavaram S, Dey R, Avishek K, Selvapandiyani A, Salotra P, Nakhasi HL. Biomarkers of safety and immune protection for genetically modified live attenuated leishmania vaccines against visceral leishmaniasis-discovery and implications. *Front Immunol* 2014; 5: 241. <https://doi.org/10.3389/fimmu.2014.00241>
 73. Papadopoulou B, Roy G, Breton M, Kündig C, Dumas C, Fillion I, Singh AK, Olivier M, Ouellette M. Reduced infectivity of a *Leishmania donovani* bioprotein transporter genetic mutant and its use as an attenuated strain for vaccination. *Infect Immun* 2002; 70: 62-68. <https://doi.org/10.1128/IAI.70.1.62-68.2002>
 74. Silvestre R, Cordeiro-Da-Silva A, Santarém N, Vergnes B, Sereno D, Ouassii A. SIR2-deficient *Leishmania infantum* induces a defined IFN-gamma/IL-10 pattern that correlates with protection.

- J Immunol 2007; 179: 3161-3170. <https://doi.org/10.4049/jimmunol.179.5.3161>
75. Carrión J, Folgueira C, Soto M, Fresno M, Requena JM. *Leishmania infantum* HSP70-II null mutant as candidate vaccine against leishmaniasis: a preliminary evaluation. *Parasit Vectors* 2011; 4: 150. <https://doi.org/10.1186/1756-3305-4-150>
 76. Ghosh A, Labrecque S, Matlashewski G. Protection against *Leishmania donovani* infection by DNA vaccination: increased DNA vaccination efficiency through inhibiting the cellular p53 response. *Vaccine* 2001; 19: 3169-3178. [https://doi.org/10.1016/S0264-410X\(01\)00023-8](https://doi.org/10.1016/S0264-410X(01)00023-8)
 77. Dey R, Dagur PK, Selvapandian A, McCoy JP, Salotra P, Duncan R, Nakhasi HL. Live attenuated *Leishmania donovani* p27 gene knockout parasites are nonpathogenic and elicit long-term protective immunity in BALB/c mice. *J Immunol* 2013; 190: 2138-2149. <https://doi.org/10.4049/jimmunol.1202801>
 78. Walker PS, Scharton-Kersten T, Rowton ED, Hengge U, Bouloc A, Udey MC, Vogel JC. Genetic immunization with glycoprotein 63 cDNA results in a helper T cell type 1 immune response and protection in a murine model of leishmaniasis. *Hum Gene Ther* 1998; 9: 1899-1907. <https://doi.org/10.1089/hum.1998.9.13-1899>
 79. Mutiso JM, Macharia JC, Kiio MN, Ichagichu JM, Rikoi H, Gicheru MM. Development of *Leishmania* vaccines: predicting the future from past and present experience. *J Biomed Res* 2013; 27: 85-102. <https://doi.org/10.7555/JBR.27.20120064>
 80. Aunguldee T, Gerdrasert O, Tangteerawatana P, Jariyapongskul A, Leelayoova S, Wongsatayanon BT. Immunogenicity and potential protection of DNA vaccine of *Leishmania martiniquensis* against *Leishmania* infection in mice. *J Infect Dev Ctries* 2021; 15: 1328-1338. <https://doi.org/10.3855/jidc.14472>
 81. Rafati S, Zahedifard F, Azari MK, Taslimi Y, Taheri T. *Leishmania infantum*: prime boost vaccination with C-terminal extension of cysteine proteinase type I displays both type 1 and 2 immune signatures in BALB/c mice. *Exp Parasitol* 2008; 118: 393-401. <https://doi.org/10.1016/j.exppara.2007.10.004>
 82. Prasanna P, Kumar P, Kumar S, Rajana VK, Kant V, Prasad SR, Mohan U, Ravichandiran V, Mandal D. Current status of nanoscale drug delivery and the future of nano-vaccine development for leishmaniasis—a review. *Biomed Pharmacother* 2021; 141: 111920. <https://doi.org/10.1016/j.biopha.2021.111920>
 83. Kaye P, University of York. A study to assess the safety, efficacy and immunogenicity of *Leishmania* vaccine ChAd63-KH in PKDL [Internet]; [cited 2022 Dec 8]. Available from: <https://clinicaltrials.gov/ct2/show/NCT03969134>
 84. Taheri T, Rafati S. Leishmaniasis: recombinant DNA vaccination and different approaches for vaccine development. *Clin Invest* 2013; 3: 1023-1044. <https://doi.org/10.4155/CLI.13.99>
 85. Volpedo G, Huston RH, Holcomb EA, Pacheco-Fernandez T, Gannavaram S, Bhattacharya P, Nakhasi HL, Satoskar AR. From infection to vaccination: reviewing the global burden, history of vaccine development, and recurring challenges in global leishmaniasis protection. *Expert Rev Vaccines* 2021; 20: 1431-1446. <https://doi.org/10.1080/14760584.2021.1969231>
 86. Cecílio P, Pérez-Cabezas B, Fernández L, Moreno J, Carrillo E, Requena JM, Fichera E, Reed SG, Coler RN, Kamhawi S, Oliveira F, Valenzuela JG, Gradoni L, Glueck R, Gupta G, Cordeiro-da-Silva A. Pre-clinical antigenicity studies of an innovative multivalent vaccine for human visceral leishmaniasis. *PLoS Negl Trop Dis* 2017; 11: e0005951. <https://doi.org/10.1371/journal.pntd.0005951>
 87. Osman M, Mistry A, Keding A, Gabe R, Cook E, Forrester S, Wiggins R, Di Marco S, Colloca S, Siani L, Cortese R, Smith DF, Aebischer T, Kaye PM, Lacey CJ. A third generation vaccine for human visceral leishmaniasis and post kala azar dermal leishmaniasis: first-in-human trial of ChAd63-KH. *PLoS Negl Trop Dis* 2017; 11: e0005527. <https://doi.org/10.1371/journal.pntd.0005527>
 88. Pati R, Shevtsov M, Sonawane A. Nanoparticle vaccines against infectious diseases. *Front Immunol* 2018; 9: 2224. <https://doi.org/10.3389/fimmu.2018.02224>
 89. Margaroni M, Agallou M, Athanasiou E, Kammona O, Kiparisides C, Gaitanaki C, Karagouni E. Vaccination with poly(D,L-lactide-co-glycolide) nanoparticles loaded with soluble *Leishmania* antigens and modified with a TNF α -mimicking peptide or monophosphoryl lipid A confers protection against experimental visceral leishmaniasis. *Int J Nanomedicine* 2017; 12: 6169-6184. <https://doi.org/10.2147/IJN.S141069>
 90. Taslimi Y, Zahedifard F, Taheri T, Doroud D, Dizaji SL, Saljoughian N, Rafati S. Comparison of protective potency of DNA and live vaccines expressing A2-CPA-CPB^{CTE} antigens against visceral leishmaniasis in Syrian hamster as preliminary study. *Iran J Parasitol* 2020; 15: 383-392. <https://doi.org/10.18502/ijpa.v15i3.4203>
 91. Lage DP, Ribeiro PA, Dias DS, Mendonça DV, Ramos FE, Carvalho LM, de Oliveira D, Steiner BT, Martins VT, Perin L, Machado AS, Santos TTO, Tavares GSV, Oliveira-da-Silva JA, Oliveira JS, Roatt BM, Machado-de-Ávila RA, Teixeira AL, Humbert MV, Coelho EAF, Christodoulides M. A candidate vaccine for human visceral leishmaniasis based on a specific T cell epitope-containing chimeric protein protects mice Against *Leishmania infantum* infection. *NPJ Vaccines* 2020; 5: 75. <https://doi.org/10.1038/s41541-020-00224-0>
 92. Alves-Silva MV, Nico D, de Luca PM, Palatnik de-Sousa CB. The F1F3 recombinant chimera of *Leishmania donovani*-Nucleoside Hydrolase (NH36) and its epitopes induce cross-protection against *Leishmania (V.) braziliensis* infection in mice. *Front Immunol* 2019; 10: 724. <https://doi.org/10.3389/fimmu.2019.00724>
 93. Klatt S, Simpson L, Maslov DA, Konthur Z. *Leishmania tarentolae*: taxonomic classification and its application as a promising biotechnological expression host. *PLoS Negl Trop Dis* 2019; 13: e0007424. <https://doi.org/10.1371/journal.pntd.0007424>
 94. Mendoza-Roldan JA, Latrofa MS, Iatta R, RS Manoj R, Panarese R, Annoscia G, Pombi M, Zatelli A, Beugnet F, Otranto D. Detection of *Leishmania tarentolae* in lizards, sand flies and dogs in

- southern Italy, where *Leishmania infantum* is endemic: hindrances and opportunities. *Parasit Vectors* 2021; 14: 461. <https://doi.org/10.1186/s13071-021-04973-2>
95. Breton M, Tremblay MJ, Ouellette M, Papadopoulou B. Live nonpathogenic parasitic vector as a candidate vaccine against visceral leishmaniasis. *Infect Immun* 2005; 73: 6372-6382. <https://doi.org/10.1128/IAI.73.10.6372-6382.2005>
 96. Panasiuk M, Zimmer K, Czarnota A, Grzyb K, Narajczyk M, Peszyńska-Sularz G, Żołędowska S, Nidzworski D, Hovhannisyantsyan L, Gromadzka B. Immunization with *Leishmania tarentolae*-derived norovirus virus-like particles elicits high humoral response and stimulates the production of neutralizing antibodies. *Microbial Cell Factories* 2021; 20: 186. <https://doi.org/10.1186/s12934-021-01677-1>
 97. Moura AP, Santos LC, Brito CR, Valencia E, Junqueira C, Filho AA, Sant'Anna MR, Gontijo NF, Bartholomeu DC, Fujiwara RT, Gazzinelli RT, McKay CS, Sanhueza CA, Finn MG, Marques AF. Virus-like particle display of the α -Gal carbohydrate for vaccination against *Leishmania* infection. *ACS central science* 2017; 3: 1026-1031. <https://doi.org/10.1021/acscentsci.7b00311>
 98. De Beuckelaer A, Grooten J, De Koker S. Type I interferons modulate CD8+ T cell immunity to mRNA vaccines. *Trends Mol Med* 2017; 23: 216-226. <https://doi.org/10.1016/j.molmed.2017.01.006>
 99. Versteeg L, Almutairi MM, Hotez PJ, Pollet J. Enlisting the mRNA vaccine platform to combat parasitic infections. *Vaccines* 2019; 7: 122. <https://doi.org/10.3390/vaccines7040122>
 100. Duthie MS, Van Hoeven N, MacMillen Z, Picone A, Mohamath R, Erasmus J, Hsu FC, Stinchcomb DT, Reed SG. Heterologous immunization with defined RNA and subunit vaccines enhances T Cell responses that protect against *Leishmania donovani*. *Front Immunol* 2018; 9: 2420. <https://doi.org/10.3389/fimmu.2018.02420>
 101. Duthie MS, Machado BA, Badaró R, Kaye PM, Reed SG. Leishmaniasis vaccines: applications of RNA technology and targeted clinical trial designs. *Pathogens* 2022; 11: 1259. <https://doi.org/10.3390/pathogens11111259>

