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Review Article

An update on recombinant vaccines against leishmaniasis

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Leishmaniasis is a parasitic disease caused by various species of the *Leishmania* parasite, manifesting in visceral (VL), cutaneous (CL), and mucocutaneous (MCL) forms. To combat this debilitating disease, various vaccines candidates including proteins, DNA, vectors, adjuvants, and recombinant whole parasites have been developed and tested experimentally and preclinically against several *Leishmania* species. Some vaccines have already entered human clinical trials. These vaccines aim to induce protective immunity using specific antigens. This review examines all efforts to develop recombinant vaccines against the parasite, analyzing successes including commercially available canine vaccines and the overall challenges faced in the quest to eradicate the disease. Additionally, recent advances in vaccine delivery systems, such as viral vectors and non-pathogenic bacteria, offer promising avenues to enhance immunogenicity and improve the targeted delivery of antigens, potentially leading to more effective and long-lasting immune responses. By understanding past and current efforts, future strategies can be refined to create more effective vaccines and ultimately control or eradicate this parasitic disease.

Key words DNA vaccine - *Leishmania* - live attenuated vaccine - protein vaccine - recombinant vaccine - vaccine candidate

Recombinant vaccines against leishmaniasis represent a promising approach to combat this parasitic disease. Leishmaniasis, caused by various species of the *Leishmania* parasite, it manifests in different forms, including visceral (VL), cutaneous (CL), and mucocutaneous (MCL) leishmaniases[1]. These vaccines aim to induce protective immunity by using specific antigens derived from the parasite, produced through genetic engineering techniques, and by developing whole live attenuated parasite vaccine candidates[2]. Various antigens, singly or in combination, are tested with different adjuvants and delivered using diverse vectors to achieve optimal protection and efficacy. This review examines the efforts to develop recombinant vaccines for leishmaniasis, analyzing the successes and challenges in eradicating this debilitating disease. Understanding past and current efforts can refine future strategies to create more effective vaccines and ultimately control or eliminate leishmaniasis. The article reviews current vaccine candidates, including live attenuated, killed, and subunit vaccines, along with their mechanisms of action and efficacy in preclinical and clinical trials[2]. It also addresses challenges and limitations in vaccine development, such as antigen variability and immune response heterogeneity. Finally, it explores future directions in leishmaniasis vaccine research, emphasizing the role of novel technologies, such as CRISPR-Cas9, in developing more effective vaccine candidates. Figure illustrates the various recombinant antigens developed as vaccines, which

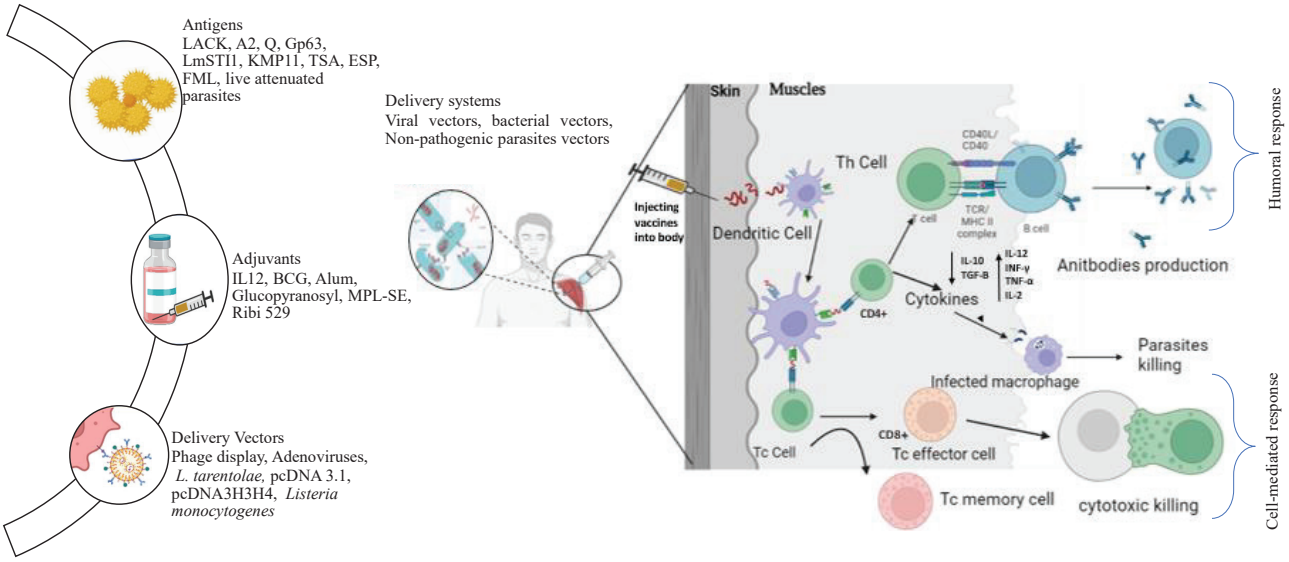

Figure. A schematic diagram depicting the types of recombinant antigens currently being investigated for the development of commercial vaccines against leishmaniasis, along with the potential immunity they may induce in the host. The figure was generated using the programmes 'Biorender' and 'PowerPoint'. LACK, *Leishmania* activated C kinase; Gp63, Leishmanolysin; LmSTI1, *L. major* stress-inducible protein; KMP-11, kinetoplastid membrane protein-11; TSA, thiol-Specific Antioxidant; ESP, excreted-secreted proteins; FML, fucose mannose ligand; BCG, Bacillus Calmette-Guerin; IL, interleukin.

aim to induce long-term immunity in the host. Table[3-41] lists these recombinant antigens as reported in the literature.

Recombinant antigens

Recombinant vaccines exploit various antigens critical to the survival or pathogenicity of the *Leishmania* parasite. These antigens can include surface-expressed molecules and secretory proteins, which are recognized by the body's immune cells, thereby stimulating a protective immune response[42]. By targeting these specific antigens, recombinant vaccines aim to induce immunity that can effectively prevent infection or reduce its severity.

Stress-inducible protein (STI): The eukaryotic homolog of STI, is one of the most commonly used antigens in vaccine studies. This conserved protein is expressed in both forms of the parasite and has been shown to protect against *Leishmania major* infection effectively[43]. The recombinant LmSTI1 vaccine, formulated with Ag-M720 and Ag-M50 adjuvants, was administered to BALB/c mice at 3-week intervals, and the immune response was monitored. A lower parasitic burden and a smaller lesion size were observed in the Ag-M720 group, indicating better protection[18]. Results showed a higher level of IFN-γ and a lower induction of IL-4, IL-10, and IL-17 cytokines (and/or a higher IL-10/IL-17 ratio) in the immunized/protected animals compared to the control group. Another approach involved using STI1 from *L. major* in fusion with SP15 from *Phlebotomus papatasi* expressed as self-amplifying mRNA (SAM) through alphavirus. These SAM constructs show transient expression and do not integrate into the host genome. Such constructs mimic viral infection and enhance the immune response against the fused antigens[44].

Kinetoplastid membrane protein-11 (KMP-11): A conserved protein found on the surface of *Leishmania* parasites, KMP-11is differentially expressed in both amastigote, and promastigote forms and has been shown to elicit strong immune responses, protecting experimental models[20] Vaccinated hamsters exhibited a reversal of T cell inactivity, leading to IL-2 production and a strong specific response from cytotoxic T lymphocytes (CTLs). In a separate study, elevated levels of IFN-γ, IL-12, and TNF-α, along with increased splenic $CD3^+$, $CD4^+$ and $CD8^+$ T cells, hepatic granulomas, and an 86 per cent reduction in splenic load were observed in immunized mice post-infection, suggesting the immune-protective nature of the vaccine[45].

Table. List of important recombinant vaccines/vaccine candidates. It describes the names and type of important recombinant vaccine candidates developed and tested against leishmaniasis. Few canine vaccines that are commercialized have also been included

Vaccine candidate	Adjuvant used	Leishmaniasis against & the host details	Study remarks	Current status in vaccine development	References
Self-amplifying mRNA (SAM) vaccines					
Nano vaccine with C1 lipid nanoparticle (LNP)	Self-adjuvant	Localization of the nano vaccine occurred in the lymph nodes & lungs	Highest concentration of CD8+ T cells observed in the lymph nodes, Toll-like receptor 4 (TLR4) activation	Preclinical studies conducted	3
DNA antigens					
Leishmania activated C kinase (LACK)	None	This antigen is highly conserved among *Leishmania* strains but lacks in providing cross-protection	LACK is involved in the intracellular signaling pathways of the parasite & generates protective immunity in recombinant vaccines	Preclinical studies conducted	4,5
Leishmanolysin (GP63)	None	A major surface protein found in both forms of parasites It rapidly acts on whole range of host cell substrates involved in cell signaling pathways and their functional regulation	Vaccines targeting GP63 aim to neutralize its function, thereby impairing the parasite's ability to infect host cells	Preclinical studies conducted	6,7
Ankara vaccine (LACK)	TRYP	TRYP is tandemly repeated & highly conserved across *L. major* and highly expressed in promastigotes & amastigotes	It induces protective immunity against virulent challenge with *L. major* in susceptible BALB/c mice as shown by reduction in footpad lesion size following injection of promastigotes	Preclinical studies conducted	8
Oligomeric recombinant fusion protein vaccine (ORFF)	None	Presented in both promastigote & amastigote forms	Elicit an Ag-specific cell-mediated immune response	Preclinical studies conducted	9
Leishdnavax	None	Lipophosphoglycan 3 (LPG3) is essential for the synthesis of glycoconjugates as parasite virulence factors	Vaccination with LPG3 of *L. infantum* induces parasite-specific protective Th1 responses	Preclinical studies conducted	10
Leishmune	FML-saponin	FML inhibits the penetration of promastigotes as well as amastigotes. It provides protection from VL in canines	It reduces parasite infection incidences in humans	Commercialized as canine vaccine as 'Leishmune'	11
Coctail DNA antigens					
Pleish-dom	IL-12	It incorporates antigenic regions from four proteins (LACK, TSA, KMP11, and LmSTI1)	The immunized mice show lower parasite burden &, significant protection against infection	Preclinical studies conducted	12

Contd...

Vaccine candidate	Adjuvant used	Leishmaniasis against & the host details	Study remarks	Current status in vaccine development	References
Recombinant Canine Distemper Virus - *Leishmania*-activated C-kinase (rCDV-LACK)		A promising vaccine candidate against CL infection	Dogs immunized by the rCDV-LACK protected against *L. major* infection	Preclinical studies conducted	13
Cocktail antigens (HLA-DR and HLA-A2 from *L. major* GP63) HLA is human leukocyte antigen	Montanide	TSA antigen provides protection against *L. major* infection	Immunized animals demonstrated enhanced IgG levels, lymphoproliferative activity with no cytotoxicity observed in renal & liver tissues	Preclinical studies conducted	14
Fucose-Mannose Ligand (FML/ VR1012-NH36)	SAP	A vaccine against Nucleoside hydrolase gene (NH36) Immunoprotective against visceral (*L. chagasi*) and cutaneous *(Leishmania mexicana*) murine leishmaniasis	Significant reduction of parasitic load observed	Preclinical studies conducted	15
Multicomponent DNA vaccine (with 10 antigens)	IL-12 or GM-CSF	This induced a delayed type hypersensitivity (DTH) response to viable *L. donovani* promastigotes and led to a reduction of parasite burden in an *in vitro* intracellular infection model, & in the draining lymph node of dogs	This multicomponent DNA vaccine primed dogs for a parasite-specific type 1 cellular immune response which restricted parasite growth	Preclinical studies conducted	16
ChAd63KH (ChAd63 is adenovirus vector derived from chimpanzees)		It utilizes a chimpanzee adenovirus vector (ChAd63) to deliver a synthetic gene encoding two key *Leishmania* antigens: kinetoplastid membrane protein-11 (KMP-11) & hydrophilic acylated surface protein B (HASPB)	This vaccine has shown promise in preclinical and clinical studies in Sudan, Africa, leading to the induction of strong CD4+ and CD8+ T cell responses, especially for therapeutics against persistent Post Kala Dermal Leishmaniasis (PKDL)	Phase III clinical studies in humans conducted	17
Protein antigens					
L. major Stress-inducible protein (LmSTI1)	Ag-M720 & Ag-M50	The STI1 protein is expressed in both *L. major* promastigotes & amastigotes	Lower parasitic burden & lesion size observed in the Ag-M720 group, indicating better protection against *L. major* infection	Preclinical studies carried out	18
Leishmania homolog of eukaryotic ribosomal elongation & initiation Factor 4a (LeIF)	MPL	LeIF is an important protein for the parasite's protein synthesis machinery. *L. infantum* LeIF protein inhibits translation in yeast	LeIF induces intramacrophage parasite growth inhibition, microbicidal activity; thereby induces a strong immune response.	Preclinical studies conducted	19

Contd...

Vaccine candidate	Adjuvant used	Leishmaniasis against & the host details	Study remarks	Current status in vaccine development	References
KMP-11	None	The KMP-11 protein is differentially expressed both in amastigote and promastigote forms	KMP-11 elicits strong immune responses and provides protection in experimental models with Th1 protective response.	Preclinical studies conducted	20
Leish-Tec	Recombinant A2 protein & saponin	A2 antigen protects mice, monkeys, dogs. It efficiently protects the canine from VL	Involvement of A2 antigen enhanced humoral IG especially IG1 and IG2 levels in the vaccinated dogs	Commercialized as canine vaccine as 'LeishTec'	5,21-26
A2	IL-12, alum & saponin	A2 antigen leads to clearance of the parasites and provides cross-protection against *Leishmania* species	A2 formulated vaccines protect dogs, mice, and nonhuman- primates against VL	Preclinical & early clinical studies conducted	27
Q protein/LetiFend	Chimerical multi-component Q protein	Provide immunity against *L. infantum* in canines	High levels of anti-Q antibodies, globulin levels observed in vaccinated dogs A DTH response along with the production of NO observed	Commercialized as canine vaccine as 'LetiFend'	28,29
Phlebotomus duboscqi salivary protein (PdSP15)	Glucopyranosyl lipid	A salivary protein of *P. duboscqi* protects against vector-transmitted cutaneous leishmaniasis	The animal developed humoral mediated DTH response, clear signs of MNC recruitment and exhibited a positive IFN-γ response. Reduction in the parasitic burden and a lower no of lesions also found	Preclinical studies conducted in dogs	30
L. infantum excreted-secreted protein (LiESP/QA-21)	QA-21	Formulated from the excreted-secreted proteins (LiESP) of *L. infantum*	Dogs showed IG2 response against PSA Ag and IG1 response against ESP Ag along with cell mediated immunity	Commercialized as 'CaniLeish' as cainine vaccine	31
Polyprotein antigens					
Leishmania Glucopyranosyl Lipid A - Stable emulsion (LEISH-F3+/GLA-SE-)	GLA-SE	It provides protection against VL caused by *L. infantum & L. donovani*	The vaccine was safe and induced a strong antigen-specific immunity, as evidenced by the cytokine & immunoglobulin subclass	Undergone clinical trials (phase I)	32,33
LEISH-F3+/GLA-SE- CBP	GLA-SE		It protects against VL in mice & dogs. The addition of the truncated CBP shows a robust immune response	Undergone clinical trials (phase I)	33-36

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Vaccine candidate	Adjuvant used	Leishmaniasis against & the host details	Study remarks	Current status in vaccine development	References
Leish-111f (TSA, LmSTI1 & LeIF) TSA is Thiol-specific antioxidant	MPL-SE and Ribi 529	Tried against CL & mucocutaneous leishmaniasis Promising to treat both canine & human populations	Immunized mice showed reduction in parasitic burden, an enhanced humoral response & cell-mediated response when administrated with Ribi whereas MPL generated humoral response Provides cross protection & stability Phase II clinical trial for CL in Brazil, where it was used as an adjunct therapy to enhance the standard treatment with sodium stibogluconate. The trial showed promise in boosting the immune response against *L. braziliensis* infection	Undergone clinical trials (phase II)	37
Live attenuated parasites as antigens					
L. donovani					
Laboratory & cGLP grade live attenuated *LdCen*$^{-/-}$parasites	None	Challenged against *L. donovani, L. major,* & *L. braziliensis.* Tested in BALB/c mice, hamsters, dogs & in human cells	Demonstrated safety and protection against homologous & heterologous challenges with Th1 protective immunity	Preclinical testing conducted	38
L. major					
Laboratory & cGLP grade live attenuated *LmCen*$^{-/-}$parasites	None	Challenged (sand fly mediated) against *L. major* & *L, donovani* in C57BL/6 and BALB/c mice & hamsters	Demonstrated safety & protection against homologous & heterologous challenges with Th1 protective immunity	Preclinical & animal toxicity testing with cGLP grade parasite conducted. Clinical trial in progress	39
L. mexicana					
Live attenuated *LmxCen*$^{-/-}$parasites	None	Tested against *L. mexicana* in BALB/c mice	Demonstrated strategy & protection with Th1 protective immunity	Preclinical testing conducted	40
L. infantum					
Live attenuated *Li Cen*$^{-/-}$parasites	None	Phase 1 study in limited number of dogs	Moderate protective efficacy with immunity was noticed	Preclinical testing conducted	41

Leishmania homologue of receptors for activated C kinase (LACK): This highly conserved antigen among *Leishmania* strains is involved in the parasite's intracellular signalling pathways and has been used in recombinant vaccines to generate protective immunity[4]. Crosslinked chitosan microparticles have also been exploited as a mucoadhesive delivery system for LACK DNA. When delivered intranasally to a mouse model, this vaccine reduced parasitic load upon challenge with *L. amazonensis*[46]. Mice that received this vaccine showed an enhanced Th1 immune response compared to those challenged with naked LACK DNA[46]. The use

of LACK antigens as a vaccine was further explored in a cocktail vaccine, which is discussed below.

Leishmania homolog of eukaryotic ribosomal elongation and initiation Factor 4A (LeIF): This important protein is integral to the parasite's protein synthesis machinery and is a target for recombinant vaccine development, known for inducing a strong immune response[19]. The *Leishmania infantum* LeIF protein is an ATP-dependent RNA helicase and an eIF4A-like factor that inhibits translation in yeast[47]. Additionally, LieIF inhibits the growth of intramacrophage parasites by promoting the production of TNF-α, which stimulates microbicidal activity through the generation of nitric oxide (NO) and reactive oxygen species (ROS)[19]. Its use in cocktail vaccines is detailed below.

GP63 (Leishmanolysin): GP63 is a major surface protease of *Leishmania* and is critical for the parasite's virulence and survival. Knockout studies of *L. major* for GP63 have shown reduced infection in mice[6]. It is a highly active protease that can rapidly act on a wide range of host cell substrates involved in cell signalling pathways and their functional regulation[7]. Vaccines targeting GP63 aim to neutralize its function, thereby impairing the parasite's ability to infect host cells. The catalytic epitope of GP63 combined with the B subunit of heat-labile enterotoxin (LTB) of *E. coli* as an adjuvant increased complement-mediated lysis of promastigotes *in vitro*, leading to elevated synthesis of antibodies against GP63[48].

Amastigote-specific stress response protein (A2): The A2 protein has been exploited as a target antigen in recombinant vaccines, and its association with various adjuvants, including IL-12, alum, and saponin, provides both humoral and Th1/Th2 mediated immunity[49,50]. The recombinant A2(rA2) antigen of *L. major* was able to cross-protect an animal model against infections from both *L. donovani* and *L. amazonensis*, as indicated by high levels of interferon-gamma (IFN-γ), in contrast to rLACK[18]. The primary role of the A2 antigen is known to be the clearance of the parasites rather than their dissemination. Such formulated vaccines have shown protective effects in dogs, mice, and nonhuman-primates against VL[27].

In the past decade, the selection of targets for vaccination has significantly expanded. Sand fly salivary proteins are immunogenic, making them promising candidates for vaccination. These proteins are advantageous because they exhibit minimal homology with human proteins. Efforts have been made to test these salivary proteins as target antigens, with notable examples including PdSP15 from *Phlebotomus duboscqi*[51], and LJL143 and LJM19 from *Lutzomyia longipalpis*[52]. The salivary protein PdSP15 of *P. duboscqi* provides protection against vector-transmitted CL. Animals exposed to bites from uninfected sand flies developed a humoral-mediated delayed-type hypersensitivity (DTH) response (63%), accompanied by clear signs of mononuclear cell (MNC) recruitment. The study also noted a reduction in parasitic burden and fewer lesions. Reverse antigen screening identified PdSP15 as the key protein responsible for significant protection. Non-primate animals immunized with DNA encoding PdSP15 and boosted with rPdPS15 plus glucopyranosyl lipid (as an adjuvant) tested positive for anti-rPdPS15 antibodies. Furthermore, these animals exhibited a positive IFN-γ response and demonstrated enhanced protection against vector-transmitted infections[30]. Similarly, the rLSA protein of *L. infantum*, one of the many antigens, efficiently generates a cellular immune response in dogs. The effectiveness of rLSA, in terms of antibody levels, is significantly higher compared to rKMP-11[53].

Cocktail antigens

A cocktail vaccine combining multiple antigens in a single formulation could potentially improve efficacy by targeting various aspects of the immune response and covering multiple pathways essential for the parasite's survival. For example, formulations containing HLA-DR and HLA-A2 peptides from *L. major* GP63, using Montanide as an adjuvant, were evaluated both separately and in combination for cytotoxicity and protection against infection. Animals immunized with this formulation demonstrated enhanced IgG levels and lymphoproliferative activity with no cytotoxicity observed in renal and liver tissues[14]. In another case, mice immunized with the Thiol-Specific Antioxidant (TSA) antigen DNA either alone or in combination with LmSTI1 DNA exhibited CD4 and CD8 T cell-mediated immunity against the infection[54]. Additionally, the DNA vaccine known as ‘pleish-dom’, which incorporates antigenic regions from four proteins (LACK, TSA, KMP11, and LmSTI1) was tested. These regions were cloned and administered to BALB/c mice alongside IL-12 as an adjuvant (pIL-12). The immunized mice showed a lower parasite burden and, consequently, significant protection against infection[12]. Similarly, recombinant canine distemper virus (CDV) was cloned with various *Leishmania* antigens like LACK (rCDV-

LACK), Thiol-Specific Antioxidant (rCDV-TSA) and LmSTI1 (rCDV - LmSTI1). These recombinant CDVs were evaluated for their protection against virulent *L. major* in dogs. Only the dogs immunized by the rCDV-LACK were able to protect dogs against *L. major* infection, whereas the other two rCDVs did not protect. The rCDV-LACK came out as a promising vaccine candidate for CDV and CL[13].

The cocktail of LACKp24, TSA, LmSTI1, and CoPa *Leishmania* antigens was evaluated for protection against CL. Individual antigens provided only a limited extent of Th1 cell-mediated protection. Different combinations of these antigens were tested for their protective efficacy, and the cocktail containing all four antigens proved to be a more effective approach for vaccination. Mice immunized with this combination demonstrated an enhanced Th1 cell-mediated immune response[55]. Whether the enhanced protection results from additive or synergistic effects, remains a topic of debate. Overall, the concept behind using multivalent antigens is to present multiple epitopes to immune cells, thereby generating a more robust immune response compared to using single antigens. It is anticipated that these multivalent formulations will exhibit additive, if not synergistic, effects.

Vaccine delivery systems

Various delivery systems have been used for the recombinant vaccines, both preclinically and clinically, as detailed below.

Viral vectors: Viral vectors are engineered viruses that deliver *Leishmania* antigens into host cells, eliciting a strong immune response. Adenoviruses (AdV) are widely used viral vectors for this purpose. Engineering these viruses to deliver antigens is complex; the gene of interest is integrated into the AdV DNA either by homologous recombination or ligation, followed by delivery into the host. Adenoviruses are also popular for delivering CRISPR-Cas9 machinery for genomic editing[56].

Bacteriophages are also commonly used as delivery vectors. The main principle behind this approach is the expression of *Leishmania* antigens on the phage surface. The mimotopes B10 and C01 from *L. infantum* were selected and cloned into the phage, either alone or in combination. This formulation has been shown to provide cross-protection in mice against *L. amazonensis*[57].

Bacterial vectors: Bacterial vectors involve recombinant bacteria expressing *Leishmania* antigens to stimulate an immune response. One example is *Listeria monocytogenes*, a Gram-positive organism used as a live vector. This model relies on both CD8 and CD4-mediated immune responses[58,59]. For instance, the LACK antigen of *L. major* was cloned into this bacterium, and when co-administered with IL-12 in BALB/c mice, it induced a robust immune response and showed promising resistance to infection[60].

The most common method of using bacteria for delivery is through the exploitation of their plasmids. Plasmids are small DNA carriers that can replicate independently within the host cell without integrating into the host genome for extended periods. Their small size and ease of replication make them an excellent choice for delivering constructs of interest[61]. For example, the pcDNA3H3H4 plasmid was used to deliver the H3 and H4 histone proteins *of L. major*[61], and the pcDNA 3.1 vector was utilized for the pLeishdom vaccine, among other vectors. While viral vectors are effective for delivering larger DNA constructs, they tend to be more complex to use compared to bacterial vectors. In contrast, bacterial vectors are easier to handle and are well-suited for delivery.

Non-pathogenic parasitic vectors: Recently, non-pathogenic parasites like *Leishmania tarentolae* have been exploited to deliver vaccines or DNA directly to dendritic cells and lymph nodes. Recombinant *L. tarentolae* expressing pathogenic parasite or viral proteins can induce protection in infected subjects. For instance, *L. tarentolae* expressing the A2 protein protects against *L. donovani* infection[21,62]. Studies have also demonstrated successful immunomodulation by dendritic cells using recombinant *L. tarentolae* expressing SARS-CoV-2 Spike protein[63]. Despite being non-pathogenic to mammals, *L. tarentolae* shares a high genetic similarity (90%) with pathogenic species[64], raising questions about its commercial viability.

In the race of the vaccine discovery, the *L. amazonensis* antigens linked with two nanoformulations was checked for the potential immunological response by taking hamsters as a model. The results have shown that the hamsters injected with LAPSmG and LAPSmP had identical immune responses for the anti-leishmania IgG test, demonstrating exceptional protection against the infection[65].

Recombinant vaccine candidates edging for human use and commercially available for dogs

Protein vaccines:

ChAd63KH: The ChAd63-KH vaccine is a recombinant vaccine designed to protect against leishmaniasis. It utilizes a chimpanzee adenovirus vector (ChAd63) to deliver a synthetic gene encoding two key *Leishmania* antigens: kinetoplastid membrane protein-11 (KMP-11) and hydrophilic acylated surface protein B (HASPB)[17]. The ChAd63-KH vaccine has shown promise in preclinical and clinical studies in Sudan, Africa, leading to the induction of strong CD4+ and CD8+ T cell responses, especially for therapeutics against persistent Post Kala Dermal Leishmaniasis (PKDL)[66-68]. These responses are crucial for controlling and eliminating *Leishmania* infections.

LEISH-F3+/GLA-SE: LEISH-F3+/GLA-SE is a di-fusion protein (nucleoside hydrolase and sterol 24-C-methyltransferase) vaccine candidate against Leishmania formulated with the adjuvant GLA-SE (glucopyranosyl lipid adjuvant-stable emulsion). The next-generation vaccine was developed by adding a third antigen, truncated CBP (*Leishmania* cysteine protease B). This protein is known to protect against VL in mice and dogs[34,35]. Adding truncated CBP elicited a robust immune response like earlier vaccines, with the truncation not affecting vaccine efficacy. The immune response in Leish-F3 with full-length and truncated CBP showed similar protection rates of 94.9 per cent and 95.2 per cent, respectively. LEISH-F3+/GLA-SE protects mice against VL caused by *L. infantum* and *L. donovani*[32]. Based on such experimental data, the researchers developed cGMP grade of it and conducted a phase 1 study in healthy, uninfected adults in the USA[33,36]. The vaccine was shown to be safe and induced a strong antigen-specific immunity, as evidenced by the cytokine and immunoglobulin subclass[32], indicating its commercial potential.

Leish-111f: Leish-111f is a recombinant polyprotein vaccine that combines three antigens: TSA, LmSTI1, and LeIF, with adjuvants such as MPL-SE and Ribi 529. Initially developed to target CL and MCL, this formulation was tested across various forms of leishmaniasis. Mice immunized with Leish-111f were protected against CL, showing enhanced humoral and cell-mediated responses when administered with Ribi 529, whereas MPL[69] primarily induced a humoral response. When tested against *L. infantum* infection, the vaccine also elicited a strong Th1-type immune response in immunized mice, resulting in up to a 99.6 per cent reduction in parasitic burden MPL[37,69]. In canine trials, Leish-111f + MPL-SE proved effective in mild cases of VL but showed limited efficacy in dogs with severe disease[70]. Despite this, Leish-111f remains a promising candidate for treating both canine and human populations.

LiESP/QA-21: The LiESP/QA-21 vaccine commercially available in Europe is formulated for canines from the excreted-secreted proteins (LiESP) of *L. infantum*. During trials, naive dogs vaccinated with LiESP/QA-21 were monitored for serological humoral responses. Some vaccinated dogs experienced local swelling with minor pain, and a few unrelated deaths occurred during the transfer and vaccination period; these dogs were excluded from the final analysis[31]. Overall, the vaccine demonstrated 68.4 per cent efficacy and 92.7 per cent protection. Serological studies indicated that vaccinated dogs developed antibodies against ESP and PSA, two main vaccine antigens, with 96 per cent showing an IgG2 response against PSA, and 89 per cent showing an IgG1 response against ESP[23]. Additional studies also showed cell-mediated immune responses induced by this vaccine in dogs[71,72], supporting its promising commercial use.

Q protein/LetiFend: An effort to provide immunity against *L. infantum* in canines involved administering a chimeric multi-component Q protein in either single or double doses. Results indicated that vaccinated dogs exhibited no or only minor clinical symptoms. Serum analysis revealed high levels of anti-Q antibodies, along with increased globulin levels in both single- and double-dose Q-vaccinated dogs. Furthermore, spleen and lymph node examinations confirmed an absence of parasitic burden. A delayed-type hypersensitivity (DTH) response and nitric oxide (NO) production were also observed[28]. This work led to the development of the commercial canine vaccine LetiFend® by LETI Pharma, Barcelona[29], for use in Europe. The overall efficacy of LetiFend® in preventing confirmed cases of *L. infantum* visceral leishmaniasis in dogs within high endemic areas was reported to be 72 per cent.

Leish-Tec: The A2 protein, mentioned previously[5,21-25,50], has been shown to protect mice, monkeys[24], and dogs[25] against *Leishmania* infection. Studies indicate that vaccinated dogs exhibit enhanced humoral immunity, with increased IgG, particularly IgG1 and IgG2, compared to placebo and untreated groups, in the

vaccinated dogs compared to the placebo and untreated ones. The efficacy of the vaccine was reported at 71.4 per cent, with a protection rate of 96.4 per cent in vaccinated animals[73]. These findings led to the development of a commercial canine vaccine in Brazil. Formulated with recombinant A2 protein and saponin as an adjuvant, this vaccine, marketed as Leish-Tec® by CevaSanté Animale[73], effectively protects canines from VL.

DNA-based *Leishmania* vaccine candidates

DNA antigens from *Leishmania*, designed to stimulate cellular immunity, are also relevant to this discussion. Leishmune®, an FML-saponin-based vaccine, was the first licensed veterinary vaccine in Brazil to protect against canine VL and also helped reduce parasite transmission to humans in endemic regions. With preclinical and clinical trials demonstrating 92-95 per cent protection and 76-80 per cent efficacy, Leishmune® proved highly effective[11]. The FML component, a glycoprotein fraction present on the parasite's surface throughout its life cycle, is known to inhibit the penetration of promastigotes and amastigote[11] forms into host cells. The success of this vaccine underscored its importance in leishmaniasis control, offering substantial canine protection and contributing to lower infection rates in human populations. However, due to the lack of blinded evaluation during clinical trials, Leishmune® was not approved for commercial use, leading to the termination of its marketing license in 2014[26].

Pleish-dom: Pleish-dom is a DNA vaccine combining four protein-encoding genes. Antigenic regions of the LACK, TSA, KMP11, and LmSTI1 proteins were cloned into the vector pcDNA 3.1, creating a chimeric construct administered to mice. Results demonstrated extensive protection against infection in vaccinated mice, with a strong Th1-mediated immune response[12]. It remains unclear whether this protection is due to synergy or the additive effect of the genes.

Genetically attenuated live *Leishmania* recombinant parasite vaccines

Genetically attenuated live *Leishmania* vaccines present a promising approach for combating leishmaniasis by utilizing genetically modified parasites rendered non-pathogenic[74-76]. These vaccines are designed to stimulate robust and long-lasting immunity by exposing presenting the immune system to live parasites that mimic natural infection without causing disease. Genetic modifications focus on deleting or inactivating specific genes, particularly those expressed in the amastigote stage[77,78] that are essential for parasite virulence, thus ensuring host safety and enhancing vaccine efficacy. Both conventional homologous recombination[79] and the more advanced CRISPR Cas9 approach[77] have been used to delete the virulence genes in *Leishmania* strains associated with VL or CL. These genetically attenuated parasites have been tested in various animal models, including the industry-grade GLP parasites, confirming their safety and protective efficacy[38-40,80-83]. Notably, the centrin gene-deleted *L. major* knockout parasite, developed through CRISPR as a marker-free gene deletion and originating from a CL-causing strain, protects against both homologous and heterologous strains of *Leishmania*.

Discussion

Recombinant vaccines represent a promising strategy for inducing immunity against leishmaniasis. These vaccines aim to generate protective immunity by using specific antigens derived from the parasite, produced through genetic engineering techniques[77]. These antigens are recognized by the immune system, triggering a targeted response. The key players in this response are CD4 and CD8 T cells: CD4 T cells activate immune cells like macrophages, dendritic cells, and B cells, while CD8 T cells directly kill infected cells, helping to control the parasite[42]. This immune response can be either humoral or cell-mediated, involving T-helper (Th) and Cytotoxic T (Tc) cells. Tc cells can kill infected cells directly and produce cytokines that support pathogen elimination by other immune mechanisms (Figure).

The response is mediated mainly by two types of T-helper cells, Th1 and Th2, depending on antigen interaction. Th2 responses primarily generate a humoral response marked by antibody production, while Th1 responses activate both cellular and humoral immunity. Th1 cells are essential for activating macrophages and producing cytotoxic T lymphocytes, which work to eliminate intracellular pathogens. They also stimulate the production of antigen-specific antibodies, enhancing the overall immune response[84]. Additionally, memory T-cells are crucial for long-term immunity against leishmaniasis, enabling rapid and effective responses upon re-infection. Various vaccine platforms, including live attenuated, recombinant protein, DNA, mRNA, and viral vector vaccines,

are designed to harness the potential of these cells by promoting robust T-cell activation and memory formation.

Incorporating an appropriate adjuvant is crucial, as it enhances the body's immune response to the vaccine[69]. Adjuvants can increase the strength and duration of the immune response, ensure proper activation of immune cells, and help direct the immune response toward a more effective pathway, such as promoting Th1 cell-mediated immunity, which is essential for combating intracellular pathogens like *Leishmania*.

Utilizing a multi-valent antigen cocktail to induce a robust immune response involving CD4 and CD8 T cells[54] is a highly effective strategy for vaccine development. This approach enhances immunization by presenting multiple epitopes from various antigens, thereby stimulating a broader and more potent immune response[55]. Multi-valent vaccines can target different aspects of the pathogen's lifecycle, increasing the chances of effective immunity.

Delivery vectors play a vital role in ensuring that antigens reach the appropriate cells and tissues in the body. Various delivery methods, such as viral, bacterial, and nanoparticle-based systems, can enhance the uptake and presentation of antigens to the immune system. These vectors also facilitate the sustained release of antigens, providing prolonged exposure to the immune system and enhancing the overall efficacy of vaccines.

Live attenuated *Leishmania* vaccine candidates would allow the parasites to replicate within the host, triggering both humoral and cellular immune responses. This approach typically leads to longer-lasting immunity and may reduce the need for multiple immunizations[42,83]. In contrast, dead parasite vaccines or other single antigen immunizations usually induce primarily a humoral immune response, often requiring multiple doses and resulting in shorter-lasting immunity. By eliciting strong and sustained CD4 and CD8 T cell responses, live attenuated vaccines have the potential to achieve higher levels of efficacy and long-lasting immunity, ultimately contributing to better control and eradication of the disease.

Conclusion

The lack of a single effective vaccine against leishmaniasis after decades of research stems from a combination of scientific, immunological, economic, and logistical challenges. The complexity of the *Leishmania* parasite, its immune evasion mechanisms, the diverse forms of the disease, and the socio-economic context in which leishmaniasis occurs have all contributed to the slow progress. However, recombinant vaccines represent a significant potential in combating this disease. Continued research and development are crucial to addressing current challenges and delivering effective vaccines to those in need.

By targeting specific antigens and employing innovative delivery systems, recombinant vaccines could become critical tools for preventing and managing leishmaniasis. Nonetheless, a major challenge remains in the partial protection offered by a few formulations. Other obstacles include regional variability in protection, lower efficacy rates, and potential side effects. CRISPR-based approaches for creating live attenuated vaccines represent a promising advancement in the field of vaccine development, particularly for diseases like leishmaniasis caused by *Leishmania* parasites. While the precision, efficiency, and potential for rapid development are significant advantages, researchers must navigate challenges related to delivery, safety, stability, and ethical considerations. As this field continues to evolve, ongoing research will be crucial to addressing these challenges and harnessing the full potential of CRISPR technology in the fight against leishmaniasis. Also, utilizing nanoparticles as delivery vehicles for antigens or adjuvants can improve the stability and targeting of vaccine components. This approach enhances antigen presentation to the immune system and can be tailored to promote specific immune responses. The use of mRNA and DNA vaccine platforms allows for a rapid response to emerging strains of *Leishmania*.

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