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A Review of Recent Advances in *Leishmania* Nanovaccines With Emphasis on Liposomal Nanovaccines

Ahmad Mehravaran[1], **Javad Akhtari**[2, 3*], **Navid Kalkali**[1]

1. Infectious Diseases and Tropical Medicine Research Center, Research Institute of Cellular and Molecular Sciences in Infectious Diseases, Zahedan University of Medical Sciences, Zahedan, Iran.
2. Toxoplasmosis Research Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.
3. Department of Medical Nanotechnology, School of Advanced Technologies in Medicine, Mazandaran University of Medical Sciences, Sari, Iran.

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ABSTRACT

Background: Leishmaniasis remains a significant global health challenge, with no approved human vaccine available. Recent advances in nanotechnology have opened new avenues for vaccine development, offering improved antigen delivery, enhanced immunogenicity, and targeted immune responses.

Materials and Methods: This review provides a comprehensive overview of recent progress in *Leishmania* nanovaccines, with a particular focus on liposomal formulations.

Results: We discuss the immunological basis for vaccine design, the limitations of conventional vaccines, and the advantages of nano-carrier systems. Liposomal nanovaccines are highlighted for their versatility, biocompatibility, and ability to co-deliver antigens and adjuvants, leading to potent Th1-biased immune responses. We also examined other promising nano-platforms, including polymeric nanoparticles, solid lipid nanoparticles (SLNs), emulsions, and virus-like particles (VLPs). Despite promising preclinical results, challenges remain in scalability, stability, and clinical translation.

Conclusion: This review underscores the potential of nanovaccines to overcome existing barriers and accelerate the development of effective vaccines against leishmaniasis.

*** Corresponding Author:**
Javad Akhtari, Associate Professor.
Address: *Toxoplasmosis Research Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.*
Phone: *+98 (912) 8015865*
E-mail: *javad.akhtari@gmail.com*

Introduction

Leishmaniasis, a neglected tropical disease, is caused by protozoan parasites from the genus *Leishmania* and transmitted via the bite of infected sandflies. It presents in several clinical forms, such as cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL), and visceral leishmaniasis (VL) with the visceral form being fatal without treatment. Existing control measures depend largely on chemotherapy, which faces limitations due to toxicity, expense, and growing drug resistance. Vaccination is considered the most enduring solution for prevention; however, no human vaccine has been approved to date [1, 2]

Several obstacles have hindered leishmaniasis vaccine development, such as substantial antigenic diversity, intricate host parasite interplay, and the necessity for a strong Th1-mediated immune response. Conventional vaccine platforms, such as whole killed parasites, live attenuated strains, and recombinant subunit vaccines have demonstrated limited success in clinical settings. While adjuvants are crucial for directing immune responses, many established options either provide insufficient protection or raise safety issues [3, 4].

Nanotechnology presents novel strategies to address these limitations. Nano-carriers are capable of shielding antigens from degradation, improving their uptake by antigen-presenting cells (APCs), stimulating cross-presentation, and enabling the coordinated delivery of immunomodulators. Various nanoparticle-based delivery systems, including liposomes, archaeosomes, micelles, polymersomes, and immune-stimulating complexes (ISCOMs) have emerged as powerful tools in biomedical research and infectious disease therapy, including for parasitic diseases, like leishmaniasis [5–7].

Within this category, liposomal nanovaccines have become a prominent platform because of their biocompatibility, adaptability, and demonstrated capacity to induce protective immunity in preclinical studies. This review intends to consolidate recent progress in *Leishmania* nanovaccines, placing particular emphasis on liposomal designs. We will discuss their formulation principles, modes of action, and advances in preclinical and clinical evaluation, while also addressing other nano-formulations and prospective research pathways in this area.

Immunological Basis for *Leishmania* Vaccine Design

Host immune response to *Leishmania*

Immunity against leishmaniasis is largely dependent on a Th1-type response, marked by the secretion of interferon-gamma (IFN-γ), interleukin-12 (IL-12), and tumor necrosis factor-alpha (TNF-α). These cytokines stimulate macrophages to clear intracellular parasites by generating nitric oxide (NO) and reactive oxygen species (ROS). Conversely, a Th2 response, promoted by cytokines, such as IL-4, IL-10, and IL-13 correlates with disease advancement and sustained parasite survival [8].

CD4+ T cells play a pivotal role in protective immunity against Leishmania. CD8+ T cells also contribute through cytotoxic functions and cytokine release. However, the involvement of CD8+ T cells differs according to the *Leishmania* species and the specific infection setting. This variability underscores the challenges in devising a broadly effective vaccine [9].

Immune evasion strategies of *Leishmania*

Leishmania parasites evade host immunity through multiple strategies, including disrupting phagolysosome maturation, altering Toll-like receptor (TLR) signaling, down-modulating MHC class II expression to impair antigen presentation, and promoting the secretion of immunosuppressive cytokines, such as IL-10 and TGF-β. To be successful, a vaccine must counter these evasion strategies by generating robust and durable memory T-cell immunity, encompassing both systemic responses and localized tissue-resident memory T cells, along with potent effector T-cell populations [10].

Implications for Vaccine Design

An effective leishmaniasis vaccine must elicit a robust and long-lasting Th1-polarized response, engaging both arms of cellular immunity by activating CD4+ and CD8+ T cells. It should ideally recapitulate key aspects of natural infection to establish concomitant immunity and employ adjuvants that sufficiently enhance immunogenicity while minimizing undue inflammatory reactions. Nano-carriers are uniquely positioned to fulfill these criteria through precise targeting of APCs, controlled antigen release, and the coordinated delivery of antigen together with immunostimulatory agents [11].

Current Landscape of *Leishmania* Vaccines

First-generation vaccines: Whole-killed and live-attenuated parasites

Initial vaccine strategies, exemplified by formulations, like autoclaved *Leishmania major* combined with BCG, have demonstrated only partial efficacy in field studies. Although live-attenuated candidates, such as centrin-knockout strains, often provide stronger immunogenicity, their potential for reversion to a pathogenic state presents significant safety considerations.

Second-generation vaccines: Recombinant subunits and DNA vaccines

Vaccines based on recombinant proteins, including LEISH-F1 and LEISH-F2, have progressed to clinical evaluation but typically depend on strong adjuvants to achieve protective immunity. DNA vaccines, like ChAd63-KH, have shown potential in generating CD8+ T-cell responses; however, their development is constrained by challenges related to delivery efficiency and overall immunogenic potency.

Third-generation vaccines: Nucleic acid and nanoparticle-based platforms

In recent years, mRNA and nanovaccines have emerged as advanced platforms. While they are often classified as third-generation vaccines—following first-generation (whole pathogen) and second-generation (subunit) vaccines—some sources consider them part of a distinct next-generation or even fourth-generation category due to their unique mechanism and rapid adaptability. These platforms are particularly promising due to their rapid manufacturability, scalability, and inherent capacity to elicit potent cellular immune responses [12].

Nanovaccine

Leishmaniasis, a neglected tropical disease caused by protozoan parasites of the genus Leishmania, represents a persistent global health challenge for which no licensed human vaccine exists. Protective immunity against leishmaniasis is critically dependent on robust cell-mediated immunity (CMI), specifically a potent Th1-type response characterized by IFN-γ production and cytotoxic $CD8^+$ T cell activation. Conventional subunit vaccines often fail to elicit this durable and polarized CMI, highlighting the need for advanced delivery platforms that can enhance antigen immunogenicity and direct appropriate immune polarization.

Nanoparticle-based vaccine strategies have emerged as a promising avenue to address these challenges. Their tunable properties, such as size, surface charge, and controlled antigen release, enable improved dendritic cell (DC) targeting, enhanced antigen presentation, and co-delivery of antigens with molecular adjuvants. However, the selection of nanocarrier is crucial, as not all platforms successfully drive the required Th1 bias. For instance, vaccination with chitosan nanoparticles loaded with soluble leishmanial antigen (SLA) induced a mixed Th1/Th2 response that proved non-protective in murine models of CL, underscoring the necessity of careful adjuvant and formulation design.

Promising preclinical data have been generated using various nanoformulations. Cationic liposomes, especially when co-formulated with adjuvants, like monophosphoryl lipid A (MPLA), have demonstrated a strong capacity to promote Th1 polarization. They enhance DC maturation, drive the production of IL-12 and IFN-γ, and generate polyfunctional $CD4^+$ and $CD8^+$ T cell responses, correlating with significant protection against VL. Other platforms, including poly (lactic-co-glycolic acid) (PLGA) nanoparticles loaded with autoclaved parasite antigens, solid lipid nanoparticles (SLNs) for DNA vaccine delivery, and virus-like particles (VLPs) presenting fusion proteins, have also shown encouraging immunogenicity [13].

A key advancement lies in the strategic incorporation of TLR agonists (e.g. CpG, MPLA) within nanoparticles. This approach synergistically potentiates the adjuvant effect, promoting DC activation, MHC class I cross-presentation, and the critical Th1-skewed immunity necessary to combat intracellular *Leishmania* parasites. Despite these advances, significant hurdles remain in translating nanovaccines from preclinical models to clinical application. These include ensuring stability, scalable good manufacturing practice (GMP) production, achieving consistent, safe, and potent Th1 immunity without reactogenicity. Consequently, ongoing research focuses on optimizing nanoparticle composition, antigen-adjuvant combination, and delivery routes to develop an effective vaccine against this complex parasitic disease [14].

Liposomal Nanovaccines: Principles and Advantages

Liposomes

Liposomes are versatile nanocarriers that enhance subunit vaccine efficacy by serving both as delivery vehicles and intrinsic adjuvants. Their biocompatible and biode-

gradable phospholipid bilayers protect peptide antigens from degradation and facilitate controlled release. Liposomes can be engineered in size, charge, and surface composition to target APCs, such as DCs, promoting uptake and activation. Cationic liposomes, for example, improve the APC internalization and induce strong Th1 immune responses, crucial against intracellular pathogens, like Leishmania. Liposomal formulations (e.g. niosomes with autoclaved *L. major*) delay lesion development in murine models by enhancing antigen-specific immunity. By co-delivering antigens and immunostimulants, liposomes promote MHC class I and II presentation, activating both CD4+ and CD8+ T cells. Their adaptability allows mucosal, subcutaneous, or intramuscular administration, each optimizing distinct immune pathways. Overall, liposome-based nanovaccines represent a promising strategy for leishmaniasis by improving antigen stability, targeting, and immunogenicity [15].

Structure and composition

Liposomes are spherical, phospholipid bilayer vesicles capable of encapsulating hydrophilic antigens within their aqueous interior or integrating hydrophobic antigens directly into the lipid membrane. Their structural and chemical composition can be precisely engineered to modulate critical properties, such as stability, surface charge, and specific interactions with immune cells [16].

Mechanisms of immune enhancement

Liposomal platforms enhance vaccine efficacy through multiple synergistic mechanisms. They protect encapsulated antigens from premature enzymatic degradation, thereby ensuring delivery in an intact form. Their structure facilitates efficient uptake by APCs, such as DCs and macrophages, primarily via endocytosis. Certain formulations, particularly cationic or pH-sensitive liposomes, can promote antigen cross-presentation on MHC class I molecules, which is essential for activating CD8+ T-cell responses. Furthermore, liposomes enable the co-delivery of antigens and immunostimulatory adjuvants—such as TLR agonists, including CpG ODN or MPL—creating a potent, localized stimulus that synergistically amplitates the immune response [17].

Key design parameters

The immunological performance of liposomal vaccines is dictated by several critical design parameters. Particle size is a primary determinant; vesicles smaller than 200 nm typically drain efficiently to lymph nodes, while larger particles (exceeding 400 nm) tend to be retained at the injection site and can preferentially promote a Th1-skewed response. Surface charge also plays a crucial role, with cationic formulations generally enhancing APC uptake and favoring Th1 immunity. Additionally, surface functionalization with specific ligands, such as mannose or antibodies, can be employed to actively target receptors on APCs, thereby increasing the precision and efficiency of vaccine delivery [18].

Preclinical and Clinical Progress in Liposomal *Leishmania* Vaccines

Preclinical studies

Extensive preclinical research utilizing murine and hamster infection models has validated the protective potential of liposomal vaccines. Key findings include:

Cationic liposomes carrying soluble *Leishmania* antigen (SLA) have successfully stimulated robust Th1 immunity and conferred protection against *L. major* challenge.

Formulations that co-encapsulate SLA together with the TLR9 agonist CpG ODN have shown enhanced DC activation and superior parasite clearance.

Surface functionalization with mannose residues has improved APC-targeting via mannose receptor engagement, resulting in significantly heightened vaccine immunogenicity.

Clinical trials

Although no liposomal anti-leishmanial vaccine has achieved regulatory approval to date, several candidates have progressed to early-stage human testing. Early-phase trials, for example, have assessed the safety and immunogenic profile of liposomal systems delivering recombinant antigens, such as LEISH-F1 combined with the adjuvant MPL-SE, both in healthy volunteers and in patients with CL. Liposomes and their derivatives represent one of the most extensively studied and versatile nanoparticulate platforms for leishmaniasis vaccine development. Their biocompatibility, ability to encapsulate diverse cargo (from soluble antigens to DNA), and inherent adjuvant properties make them a cornerstone of nanovaccine research in this field.

A prominent and successful strategy involves the use of cationic liposomes, particularly those formulated with 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP). The positive surface charge enhances interaction

with and uptake by APCs. For instance, DOTAP-based nanoliposomes encapsulating SLA are highly effective against CL. This formulation, with an encapsulation efficiency of ~70%, induced a powerfully Th1-polarized immune response in BALB/c mice, characterized by high levels of IFN-γ and IgG2a antibodies, alongside low IL-4. Immunization led to a significant reduction in both footpad lesion size and splenic parasite burden, establishing cationic nanoliposomes as a potent delivery system. This approach has been successfully adapted for DNA vaccines, where cationic liposomes co-delivering the *pc-LACK* antigen gene and the *pc-IL-12* gene synergistically enhanced a protective Th1 response and reduced disease pathology [19, 20].

The liposomal platform has proven equally effective for delivering recombinant multi-epitope protein antigens designed for VL. Encapsulation of the chimeric protein ChimeraT within liposomes elicited a robust Th1-type response—marked by high levels of IFN-γ, IL-12, and IgG2a—and provided substantial protection, reducing parasite load across multiple organs. Importantly, this liposomal adjuvant performed comparably to the potent but less translatable saponin adjuvant. Similarly, a cationic liposome formulation containing the LiChimera protein not only promoted a strong Th1 cytokine profile (IFN-γ, TNF-α) and memory T cell generation but also uniquely modulated detrimental T cell subsets, leading to significant reductions in hepatic and splenic parasite burdens [21, 22].

Further refinement includes the co-encapsulation of immunomodulatory adjuvants. A study utilizing DSPC liposomes loaded with both SLA and the TLR7 agonist imiquimod demonstrated that the combined delivery enhanced the Th1 response and conferred significant protection, highlighting how liposomes can integrate antigen and adjuvant in a single carrier for improved efficacy [23].

However, the literature also presents important nuances and limitations. Not all liposomal formulations guarantee protection, underscoring that the specific composition and resulting immune polarization are critical. For example, cationic nanoparticle immune stimulating complexes (PLUSCOMs) containing SLA and Quil A induced a mixed Th1/Th2 response with elevated IFN-γ and IgG2a, but this profile was insufficient to reduce lesion size or parasite burden upon challenge [6]. Similarly, research exploring the effect of surface charge using DOTAP (positive) and DSPC (negative) nanoparticles within an ISCOMATRIX system found that, while charge modulated specific immune parameters (e.g. IgG2a levels or splenic load), neither formulation successfully skewed the response toward a purely protective Th1 phenotype or achieved significant protective immunity [24].

In summary, liposome-based nanovaccines have demonstrated remarkable success, particularly when formulating cationic carriers with defined antigens to drive a decisive Th1-polarized immunity. The platform's flexibility allows for the incorporation of recombinant multi-epitope proteins, DNA, and small molecule adjuvants. However, contrasting outcomes emphasize that successful vaccination depends on a complex interplay between lipid composition, antigen properties, and the precise immune profile elicited, thereby guiding the rational design of future formulations. A number of these studies, along with their details and results, are summarized in Table 1.

Other Promising Nano-carrier Platforms

Polymeric nanoparticles

Biodegradable polymers, such as PLGA and chitosan are versatile platforms that offer controlled antigen release and are particularly suited for mucosal vaccine delivery due to their biocompatibility and tunable degradation rates. For instance, PLGA nanoparticles co-loaded with autoclaved *L. major* antigen and CpG ODN have demonstrated significant protective efficacy in murine models by promoting a sustained Th1-dominant immune response and reducing parasitic burden.

Poly lactic-co-glycolic acid

Poly lactic-co-glycolic acid (PLGA) nanoparticles have emerged as a highly versatile and effective delivery platform for subunit vaccines against VL, primarily by promoting a potent and durable Th1-polarized cellular immune response essential for parasite clearance. One prominent strategy involves the encapsulation of rationally designed multi-epitope peptides. For instance, a nanovaccine containing the HisDTC peptide, encompassing T-cell epitopes from four *L. infantum* histones, was developed. When encapsulated in PLGA nanoparticles, it induced a robust and long-lasting Th1 response in BALB/c mice, characterized by strong delayed-type hypersensitivity, elevated IFN-γ and TNF-α levels, suppressed IL-10, and the generation of central memory $CD4^+$ and $CD8^+$ T cells. This response resulted in a significant and durable reduction in hepatic and splenic parasite burdens, surpassing the commercial vaccine LetiFend®. Notably, co-encapsulation of TLR2/3 ago-

Table 1. Overview of liposomal nanovaccine formulations against *leishmania* in preclinical studies

Nanocarrier (Composition)	Size (nm)/Zeta Potential (mV)	Antigen	Animal Model	Key Immune Response	Protective Outcome/Effect	Ref.
Cationic nanoliposome (DOTAP: CHOL)	Size:100 ZP: 52.8±2.9	SLA (*L. major*)	BALB/c mouse	Strong Th1 response: High IFN-γ & IgG2a; low IL-4.	Significant reduction in parasite burden (footpad & spleen) and footpad swelling.	[19]
Cationic Liposome (DOTAP/DOPE/ Cholesterol)	Size: 207.2±0.26 ZP: 41.6±6.46	pc-LACK & pc-IL-12 (DNA)	BALB/c mouse	Protective Th1 response: Marked increase in IFN-γ & IgG2a; decrease in IL-4.	Reduced splenic parasite burden and footpad lesion size post-challenge.	[20]
Liposome (DPPC: CHOL)	Size: 435.6±19.5 ZP: 3.85±0.48	ChimeraT (multi-epitope protein)	BALB/c mouse	Strongly polarized Th1 response: High levels of IFN-γ, IL-12, GM-CSF, & IgG2a; low IL-4 & IL-10.	Marked reduction in parasite burden in spleen, liver, lymph nodes, and bone marrow. Performance comparable to saponin adjuvant.	[21]
Cationic liposome (DDAB: CHOL: OA)	Size: 457.1±14.9 ZP: 54.4 ±7.7	LiChimera (Multi-epitope chimeric protein)	BALB/c mouse	Sustained Th1 cellular response: High production of IFN-γ, IL-2, TNF-α; generation of multifunctional memory $CD4^+$ & $CD8^+$ T cells.	Significant reduction in parasite burden in the spleen and liver.	[22]
Liposome (DSPC: CHOL)	Size: 215±11 ZP: −6.5 ±2	SLA + Imiquimod (TLR7 adjuvant)	BALB/c mouse	Th1 response: Elevated IFN-γ and IgG2a.	Reduced lesion severity and splenic parasite burden compared to controls.	[23]
Cationic nanoparticle immune stimulating complex (PLUSCOM: DOTAP/cholesterol/Quil A)	Size: 163.8±36.4 ZP: 35.3±6.8	SLA (*L. major*)	BALB/c mouse	Mixed Th1/Th2 response: Significantly elevated IFN-γ and specific IgG2a antibodies; lower IL-4 levels.	No protective efficacy: No significant reduction in lesion size or parasite burden was observed.	[6]
ISCOMATRIX Nanoparticles (positive: DOTAP; negative: DSPC)	Size: Positive: 93.7±10.8 Negative: 110.8±22 ZP: Positive: 37.2±5.4 Negative: 14±2.8	SLA (*L. major*)	BALB/c mouse	Mixed Th1/Th2 response by both: Elevated IFN-γ and IL-4; increased antigen-specific IgG. Negative particles induced higher IgG2a.	No significant protective immunity: Neither formulation provided significant protection against challenge infection, despite modulating immune parameters.	[24]

nists did not provide additional benefit over the peptide-loaded PLGA nanoparticles alone [25].

The rational design of antigens is frequently enhanced by immunoinformatics and reverse vaccinology. Margaroni et al. designed a universal chimeric protein (LeishChim) from conserved epitopes across three pathogenic *Leishmania* species. To overcome weak immunogenicity, LeishChim was co-encapsulated with the adjuvant MPLA in PLGA nanoparticles. This rationally designed nanovaccine successfully elicited a strong, specific Th1 cellular immune response in preclinical models, demonstrating the efficacy of a pipeline from in silico prediction to nanoparticulate formulation [26].

Beyond single proteins, PLGA nanoparticles are effective for the co-delivery of combined antigenic elements. Tosyali et al. engineered PLGA nanoparticles to co-deliver lipophosphoglycan (LPG) with either soluble or autoclaved leishmanial antigen (SLA/ALA). These ~250-300 nm particles provided sustained release and, critically, selectively stimulated Th1 immunity in vitro (as evidenced by high NO, IFN-γ, and IL-12 levels without IL-4/IL-10). In vivo, immunization with these dual-antigen nanoparticles conferred approximately 80% protection in challenged mice, linked to elevated IFN-γ and IL-12 levels and a sharp reduction in parasite load [27].

Further optimizing cellular targeting and activation, Athanasiou et al. developed a sophisticated system using HLA-A2.1-restricted chimeric peptides from three *L. infantum* proteins (CPA, H1, and KMP-11). Encapsulating these peptides in PLGA nanoparticles and incorporating MPLA or surface-modifying with a targeting p8 octapeptide significantly enhanced (DC) maturation and IL-12 production in vitro. The MPLA-incorporating formulation (Mix B) proved most effective, driving a protective $CD8^+$ T cell response in HLA-transgenic mice and reducing hepatic and splenic parasite burden by ~73% and 62%, respectively, one-month post-challenge [28].

Similarly, a focused study on a single multi-epitope peptide from cysteine protease A (CPA_{160189}), co-delivered with MPLA in PLGA, demonstrated the platform's ability to induce DC maturation, a peptide-specific Th1/$CD8^+$ T cell response, and a balanced IgG2a/IgG1 profile. This response correlated with a 90% reduction in splenic and a 48% reduction in hepatic parasite burden at one month. While splenic protection waned at four months, the sustained control in the liver highlighted the induction of partial but significant vaccine-mediated immunity [29].

Collectively, these studies validate PLGA nanoparticles as a robust platform for delivering defined leishmanial epitopes and antigens. They effectively promote antigen presentation and DC activation, especially when combined with adjuvants, like MPLA, leading to the critical Th1 and cytotoxic T-cell responses necessary for protection against VL.

Polylactide

Polylactide (PLA)-based nanoparticles have also demonstrated significant efficacy as antigen delivery systems for leishmaniasis vaccines. Ayari-Riabi et al. developed surfactant-free poly (D, L-lactide) nanoparticles (PLA-NPs) to adsorb the recombinant *L. major* histone H2B antigen. This formulation induced a Th1-biased immune response (as evidenced by a protective IgG2a/IgG1 profile) and provided substantial protection in a murine CL model, significantly reducing both lesion size and parasite burden [30].

For VL, Ottino et al. formulated PLA nanoparticles loaded with *Leishmania braziliensis* crude antigen. Two particle sizes were tested (388 nm and 570 nm) in the golden hamster model. The smaller nanoparticles (LB-PSmP, 388 nm) proved particularly effective, showing a favorable safety profile, inducing sustained humoral responses, and reducing splenic parasite load by 90%, alongside ameliorated hepatic and splenic pathology [31].

Further enhancing the platform, a study engineered chitosan-coated PLA submicrometric particles (SMPs) for antigen delivery. These coated nanoparticles promoted local inflammatory recruitment and proinflammatory cytokine production (e.g. TNF-α) without significant tissue damage, while effectively modulating systemic T and B cell populations. This underscores the potential of surface-modified PLA nanoparticles to safely enhance antigen immunogenicity [32].

Poly (methylmethacrylate)

Poly (methylmethacrylate) (PMMA) nanoparticles have also been explored as an adjuvant system for DNA vaccines against leishmaniasis. In one study, a DNA plasmid encoding the *L. major* TSA (thiol-specific antioxidant) antigen was adsorbed onto PMMA nanoparticles. This PMMA-based nanovaccine induced a robust and balanced immune response in mice, characterized by enhanced lymphocyte proliferation, a Th1-biased cytokine profile (elevated IFN-γ), and strong humoral immunity with high total IgG and both IgG1/IgG2a subclasses. Critically, immunization with this formulation led to a significant reduction in splenic parasite burden post-challenge, outperforming the DNA vaccine alone. This demonstrates PMMA's utility as an effective adjuvant platform that enhances both arms of the immune response for improved protection [33].

Chitosan

Chitosan nanoparticles represent another biodegradable polymeric platform effectively used for leishmanial antigen delivery. In a key study, the antigen superoxide dismutase B1 (SODB1) was loaded into chitosan nanoparticles via ionic gelation. This formulation functioned as both a sustained-release carrier and an intrinsic adjuvant. Immunization in mice induced a strongly Th1-polarized immune response, evidenced by a significant increase in IgG2a levels and a high IgG2a/IgG1 ratio. Notably, a single-dose administration elicited a response comparable to a triple-dose schedule, highlighting the platform's potential for simplified vaccination. This underscores chitosan's role in enhancing antigen immunogenicity and stability for a potent cellular immune response against leishmaniasis [34]. In the context of *Leishmania* nanovaccines, chitosan has emerged as a promising biocompatible polymer for antigen delivery and adjuvantity. Recent studies have explored chitosan nanoparticles for vaccine development against leishmaniasis, though with

varying outcomes. For instance, whole *Leishmania lysate* antigens (WLL) or soluble antigens (SLA) encapsulated in chitosan nanoparticles elicited a mixed Th1/Th2 response in BALB/c mice, which is suboptimal for protection. In contrast, chitosan nanoparticles loaded with recombinant *Leishmania* superoxide dismutase (SODB1) induced a significantly increased IgG2a/IgG1 ratio, indicative of a Th1-polarized cellular immune response critical for combating intracellular *Leishmania* parasites. These findings highlight the potential of chitosan-based nanocarriers, particularly when combined with well-defined antigens, to enhance immunogenicity and drive protective Th1 immunity, positioning chitosan as a versatile platform for developing effective needle-free nanovaccines against leishmaniasis [13].

Dendrimer

Dendrimers have been investigated as nano-adjuvants for DNA vaccines against leishmaniasis. In one study, a DNA plasmid encoding the TSA antigen was combined with a biodegradable poly (ethylene glycol)-citrate G2 dendrimer. This formulation, along with a PMMA-based counterpart, was used to immunize mice. Both nano-adjuvants successfully promoted a Th1-polarized immune response, marked by significant lymphocyte proliferation, elevated IFN-γ production, and a strong IgG2a antibody response. Consequently, vaccination with these formulations led to a significant reduction in splenic parasite burden after challenge. This demonstrates the potential of dendrimer nanostructures to effectively adjuvant DNA vaccines, enhancing protective cellular immunity against *L. major* infection [35].

Solid lipid nanoparticles

Solid lipid nanoparticles (SLNs) provide a stable, biocompatible matrix that efficiently encapsulates and delivers both genetic material and protein antigens while protecting them from degradation. Cationic SLNs formulated with genes encoding cysteine proteinases have successfully elicited a protective Th1-polarized immunity in mouse models, highlighting their potential as a versatile and effective DNA vaccine delivery system for leishmaniasis [36].

Emulsions and micelles

Oil-in-water nanoemulsions, such as the adjuvant system GLA-SE, function as potent immunostimulants that robustly enhance cellular immunity by promoting antigen uptake and inflammatory cytokine secretion. The combination of the recombinant polyprotein LEISH-F3 with GLA-SE has shown promising results in preclinical studies, leading to its advancement into clinical trials to evaluate safety and immunogenicity in humans [37].

Virus-like particles and immunostimulating complexes

Virus-like particles (VLPs), which are non-infectious nanostructures mimicking native viruses, are highly immunogenic and capable of stimulating strong humoral and cellular immune responses due to their repetitive antigen display. Immunostimulating complexes (ISCOMs), composed of saponins and phospholipids, form cage-like structures that effectively deliver antigens to the immune system but require further refinement to optimize their stability and specific immunomodulatory profile for leishmaniasis applications [5, 24].

Adjuvants in leishmaniasis vaccine development: Current strategies and future directions

The lack of an approved human vaccine for leishmaniasis highlights the essential function of adjuvants in boosting the immunogenicity of vaccine candidates. These components are vital for amplifying and steering the host immune reaction, specifically toward a protective Th1-polarized cell-mediated response necessary for clearing intracellular parasites. Adjuvants are generally divided into two classes: Particulate delivery systems and non-particulate immunostimulants. Particulate systems encompassing aluminum salts, liposomes, niosomes, virosomes, SLNs, and biodegradable polymers, such as PLGA—act primarily as carriers. They enhance antigen stability, promote uptake by APCs, and can create a depot effect. Non-particulate immunostimulants, which include cytokines (e.g. IL-12), bacterial derivatives (e.g. BCG, MPL), TLR agonists (e.g. CpG ODN), and saponins (e.g. QS-21), work by directly activating immune signaling pathways to provoke strong innate and adaptive immunity [38].

Choosing a suitable adjuvant is a fundamental factor in determining vaccine success. Preclinical studies have shown encouraging outcomes with various formulations, such as antigens encapsulated in liposomes, recombinant proteins paired with cytokines, like IL-12, and antigen-polymer conjugates. Nevertheless, obstacles persist concerning safety profiles, formulation stability, scalable manufacturing, and the ability to induce durable protection without undesirable side effects. Emerging investigative efforts are increasingly directed at hybrid strategies that merge the precise delivery advantages of particulate carriers with the powerful immune stimu-

lation of molecular adjuvants. These integrated approaches, together with the development of innovative platforms, like virosomes and next-generation nanomaterials, offer considerable potential for creating an effective and widely applicable leishmaniasis vaccine [39].

Challenges and future perspectives

Scalability and manufacturing

A significant translational hurdle lies in scaling up the production of nano-vaccines under stringent GMP conditions. The synthesis of nanoparticles with consistent and reproducible characteristics, such as particle size, surface charge, polydispersity index, and precise antigen loading is inherently complex and costly. Establishing standardized, robust, and cost-effective manufacturing processes is therefore essential to move promising candidates from the laboratory to clinical and commercial production, ensuring batch-to-batch uniformity and meeting regulatory requirements for purity and safety.

Stability and storage

The long-term stability of many nano-formulations presents a major logistical challenge, particularly for deployment in leishmaniasis endemic regions, which often have limited infrastructure. Most lipid- and polymer-based systems require uninterrupted cold-chain storage to prevent physicochemical degradation or aggregation of the nanoparticles. Consequently, a critical research priority is the development of thermostable formulations, potentially through lyophilization (freeze-drying), spray-drying, or the incorporation of stabilizing excipients, to create vaccines that retain potency under diverse and demanding field conditions.

Regulatory and safety considerations

The regulatory pathway for nanovaccines is still evolving, as agencies worldwide work to establish specific guidelines for these complex products. A primary concern is the limited long-term safety data on novel nano-adjuvants and carriers, including their biodistribution, potential for accumulation, and chronic effects in vivo. Comprehensive toxicological studies and well -defined characterization protocols are needed to clarify these profiles, enabling the development of clear regulatory frameworks that ensure both the safety and efficacy of nanovaccines before they can be approved for widespread human use.

Integration with novel technologies

The future of leishmaniasis vaccinology likely lies in the strategic integration of nanocarriers with other cutting-edge technologies. Combining nano-delivery systems with mRNA platforms could leverage the strengths of both rapid development and potent immunogenicity. Similarly, nanotechnology could enhance vaccines based on CRISPR-engineered live-attenuated parasites by improving their targeted delivery. Furthermore, integrating nanovaccines with viral vectors or other prime-boost regimens could synergistically enhance the magnitude, quality, and durability of the protective immune response.

Personalized and combination approaches

Advancing beyond a one-size-fits-all model, future strategies may involve personalized vaccines tailored to the predominant Leishmania species in a given region or to specific host genetic factors influencing immune response. Additionally, combination approaches that pair prophylactic vaccines with immunotherapeutic agents or existing drugs could be pivotal, especially for vulnerable populations, such as individuals co-infected with HIV and Leishmania, where restoring immune competence is as crucial as inducing a specific anti-parasitic response.

Conclusion

Liposomal nanovaccines constitute a highly promising strategy to address the shortcomings of traditional leishmaniasis vaccines. By functioning as both sophisticated delivery vehicles and intrinsic adjuvants, they significantly enhance the stability, bioavailability, and targeted presentation of diverse antigenic payloads from soluble parasite extracts to DNA-encoded proteins. Their core advantage lies in the synergistic co-delivery of antigens and immunomodulators directly to APCs, a mechanism that reliably promotes robust, Th1-polarized immunity, as evidenced by elevated IFN-γ and protective antibody isotypes in preclinical models.

Despite consistent demonstrations of superior immunogenicity and protective efficacy compared to conventional formulations, outcomes are critically dependent on precise nanocarrier design, including parameters, like size, surface charge (e.g. cationic DOTAP systems), and lipid composition. While certain designs excel at inducing Th1 responses, others may elicit mixed profiles insufficient for full protection. The ongoing integration of molecular adjuvants, such as cytokines, further refines this cellular immunity. Although challenges in manu-

facturing, long-term stability, and clinical translation persist, the inherent versatility and safety profile of liposomal platforms solidify their role as a cornerstone of next-generation vaccine development. Future progress hinges on the continued optimization of these formulations, rigorous clinical validation, and their strategic integration into comprehensive disease control initiatives.

Despite these promising preclinical results, several limitations and research gaps must be acknowledged. The majority of studies have been conducted in diverse murine models with varying protocols, making direct comparisons challenging. Furthermore, the field currently lacks clinical trials to evaluate the safety, immunogenicity, and efficacy of liposomal nanovaccines in humans. Formulation challenges, including scalability, long-term stability, and sterilization processes, also remain significant hurdles that need to be addressed to facilitate the translation of these experimental vaccines from the bench to the bedside.

Ethical Considerations

Compliance with ethical guidelines

This article is a review study with no human or animal sample.

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Authors contribution's

Conceptualization and methodology: Ahmad Mehravaran; Resources and formal analysis: Navid Kalkali; Validation, visualization, project administration, and writing: Javad Akhtari.

Conflict of interest

The authors declared no conflict of interest

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