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Research Article

Vaccine Candidates, Immuno-Dominant Antigens and Potent Vaccine Adjuvants for Preventing Cutaneous Leishmaniasis: A Systematic Review

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ABSTRACT

Cutaneous leishmaniasis (CL) is the most common clinical form of leishmaniasis that causes skin disease. Currently, there is no licensed prophylactic vaccine for CL, as the mechanisms of healing and memory T-cell responses that develop after infection with CL are far from fully understood. A review of the published articles identifying CL vaccine candidates, immuno-dominant antigens and potent vaccine adjuvants is needed to provide comprehensive information. Therefore, we aimed to review vaccine candidates, immuno-dominant antigens and potent vaccine adjuvants for preventing cutaneous leishmaniasis.

First-generation vaccine candidates showed complete protection of the specified animal model. They induced strong T-cell mediated and antibody-mediated humoral immune responses (e.g. Curdlan dectin-1, Total Leishmania Antigen (TLA) and *L. infantum* heat shock proteins (LiΔHSP70-II)). Almost all second and third-generation vaccine candidates and the immuno-dominant antigens of the parasite and the host enhance T cell-mediated and antibody-mediated immune responses. We also reviewed potent vaccine adjuvants such as Myrrh Silver Nanoparticles (MSNPs) and Imiquimod, which play an important role in enhancing immune responses against Leishmania antigens. The T-cell mediated immune response was significantly induced in various experimental models (e.g. IFN-γ and TNF-α response) and also the humoral arm in some instances (e.g. IgG2). This review thus provides comprehensive information on the efficacy and induction of protective immunity of vaccine candidates, antigenic molecules and vaccine adjuvants against CL. However, there is still a need for a comprehensive understanding of the immuno-pathogenesis of the disease upon vaccination.

Keywords: Cutaneous leishmaniasis; Vaccine candidates; Immuno-dominant antigens; Potent vaccine adjuvants

Abbreviations: AMPs: Antimicrobial Peptides; APCs: Antigen Presenting Cells; CL: Cutaneous Leishmaniasis; CLR: C-type Lectin Receptors; CNPs: Copper Nano-Particles; CRAMP: Cathelicidin-related Antimicrobial Peptides; DC: Dendritic Cells; DCL: Diffuse Cutaneous Leishmaniasis; DTH: Delayed Type Hypersensitivity; GP63: Glycoprotein-63; HASPB: Hydrophilic Acylated Surface Protein B; JBI: Joanna Briggs Institute's; KMP11: Kinetoplastid Membrane Protein 11; LACK: *Leishmania* Homolog of Receptors for Activated C-kinase; LiHSP70-11: *L. infantum* Heat Shock Protein 70-11; LPG: Lipo-Phsphoglycan; MCL: Mucocutaneous Leishmaniasis; MSNPs: Myrrh Silver Nanoparticle; NO: Nitric Oxide; PEPCK: *Leishmania* Phospho-Enol-Pyruvate Carboxy-Kinase; PKDL: Post Kalazar

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Dermal Leishmaniasis; PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses; PROSPERO: Prospective Register of Systematic Reviews; PRR: Pattern Recognition Receptor; ROS: Reactive Oxygen Species; SDLNs: Skin-Draining Lymph Nodes; SNPs: Silver Nanoparticles; STI1: Stress-Inducible Protein-1; TH1: T helper 1 cells; TLA: Total *Leishmania* Antigen; TLR: Toll Like Receptor; TSA: Thiol-Specific Antioxidant; VL: Visceral Leishmaniasis

INTRODUCTION

Cutaneous leishmaniasis (CL) is the most prevalent form of leishmaniasis and is characterized by skin lesions that can ulcerate and leave disfiguring scars that lead to discrimination in affected communities [1]. CL encompasses a spectrum of self-healing and chronic skin diseases [2]. The clinical presentation varies depending on the parasite load and host immune response [3]. Common signs of CL are skin papules and ulcerations whereas symptoms may include: Breathing difficulty, skin sores that develop into a slow-healing skin ulcer, closed nose, runny nose, nosebleeds and swallowing difficulty [4].

During a blood meal, flagellated promastigotes deposited in the dermis are ingested by phagocytes such as neutrophils and macrophages, where they transform into amastigotes, a stage of the parasite that copes better with changes in temperature and pH. Amastigotes multiply within the phagocyte until they rupture the cell and infect other tissues. The rapid recruitment of neutrophils and inflammatory monocytes after infection with *Leishmania* influences the course of the disease [5]. Neutrophils can have both a protective and a damaging function, while inflammatory monocytes kill Leishmania parasites and differentiate into monocyte-derived dendritic cells that promote the development of protective $CD4^+$ T helper 1 (TH1) cells [6]. Control of *Leishmania* infection depends on the production of IFN-γ by $CD4^+$ TH1 cells, resulting in enhanced killing by macrophages due to the production of reactive oxygen species and nitric oxide [7].

The immunological spectrum observed in CL patients ranges from individuals with a strong T-cell response characterized by delayed-type hypersensitivity (DTH) and high IFN-γ levels to individuals who have no DTH response but possibly high antibody levels [8]. *Leishmania* species are killed by IFN-γ-activated macrophages and not neutralized by antibodies, individuals with a strong DTH have few parasites in their lesions, while those with only a humoral response are unable to control the parasite load [8,9]. Patients without a T-cell response are expected to develop severe disease, termed Diffuse Cutaneous Leishmaniasis (DCL). At the other end of the spectrum, patients with an exaggerated immune response due to immunopathology also develop a severe disease phenotype, Mucocutaneous Leishmaniasis (MCL). Most MCL patients have lesions on the lip and around the nose where the skin and mucosa meet [4]. Between these extremes are patients who develop lesions that can heal themselves or become chronic, with intermediate levels of T cells and antibody responses [10].

Cutaneous leishmaniasis lesions self-heal without treatment in over 70% of patients, depending on the immune response and the species of *Leishmania* [11]. Intralesional or systemic antimonial is the gold standard for treating CL; other therapeutic options (e.g. parmomycin, Imiquimod ointments and cryotherapy) appear promising [12]. At the moment, vector control and rational treatment are the only ways to treat and control but, there aren't plenty of medicines on the market and the available ones are either very expensive, have harmful side effects, or are ineffective. However, the vector control techniques are not as effective [13].

A better understanding of the immune response in the pathogenesis of CL is still needed, taking into account the different species that cause different clinical manifestations of the disease [14]. The fact that we are still far from fully understanding the mechanisms of healing and memory responses that form after infection with CL and how to evaluate these responses, is one of the factors contributing to a failure to develop a vaccine for CL [15]. Although studies have been conducted on vaccine trials for CL in different study settings, there has been no fully licensed effective vaccine for human usage [16,17]. The development of a leishmaniasis vaccine that is safe, effective and reasonably priced is desperately needed. Moreover, there is a need to know the overall status of vaccines candidates, immuno-dominant antigens and potent vaccine adjuvants to devise appropriate interventional measures in improving the statuses of vaccine trials for CL. Thus, this review aimed to provide an overview of published articles on vaccine candidates, immuno-dominant antigens and potent vaccine adjuvants for preventing CL.

MATERIALS AND METHODS

Reporting and registration of the protocol

This systematic review was conducted to compile the most recent pieces of evidence using published and gray literature on vaccine candidates, immuno-dominant antigens and potent vaccine adjuvants for preventing CL. The protocol for this review was registered on the Prospective Register of Systematic Reviews (PROSPERO) international database (protocol registration number: CRD42022360929). This review followed the protocol of the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines (Table S1) [18].

Literature review

A comprehensive systematic literature search was conducted by using different databases from 20 October, 2023 to 10 December, 2023, there had been no systematic reviews done before. Databases were searched for recently published studies done on vaccine candidates, immuno-dominant antigens and potent vaccine adjuvants for CL. Restriction was applied on the year of publication and language, no restriction was applied on the study subjects (experimental model) as well as the study setting. For the searching strategy, the following key terms were used in combination with the Boolean operators "AND" and "OR". The basic search terms and phrases were "cutaneous leishmaniasis", "vaccine candidates" and "vaccine adjuvants". To fit advanced search in databases in terms or phrases using Boolean operators, "AND" and "OR" were used. For database searching, the following advanced search strategies were used: (Vaccine candidates) (All Fields) OR (vaccine adjuvants) (All Fields) OR (potent vaccine targets) (All Fields) and (Cutaneous leishmaniasis) (All Fields). Advanced search strategy was applied

(Cutaneous leishmaniasis) with all of the words and vaccine candidates OR vaccine adjuvants with at least one of the words and Boolean operators were also applied (cutaneous leishmaniasis) and (vaccine candidates OR vaccine adjuvants) and only journal article and published articles were included from all databases. In addition to the electronic database search, gray literature was searched. Reference lists (bibliographies) of the included studies were also searched to obtain additional articles.

Eligibility criteria

Inclusion criteria: Articles that met the following predetermined inclusion criteria were included in this systematic review.

- Types of studies that are employed *in vivo* pre-clinical and clinical trial studies.
- Study subjects that are conducted in all types of study subjects (human and animal models). Published and unpublished studies in any period (the study period was not restricted for inclusion). Studies reported in the English language up to December, 2023 were included.

Exclusion criteria: Articles that met the following predetermined exclusion criteria were included in this systematic review.

- *In vitro* pre-clinical trial studies (studies conducted on culture media)
- *In silico* analysis studies were also excluded
- In addition, despite the above-mentioned preset eligibility criteria, articles that were not fully accessible after three or more personal email contacts with the corresponding author and articles without an abstract and/or full text were all excluded.

Outcome of interest measurement

The primary outcome of this systematic review was to determine the efficacy of vaccine candidate, immuno-dominant antigens and effective vaccine adjuvants in different experimental models. The secondary outcome of this review was to identify induced immune response such as cell-mediated immune response and antibody-mediated humoral immune responses upon vaccination.

Assessment of the methodological quality of the studies

The JBI quality appraisal tool for experimental studies was used to assess the quality of included articles and the risk of bias in each study [19]. The assessment tool contains seven criteria:

- Clear inclusion and exclusion criteria
- Was true randomization used for the assignment of study subjects to treatment groups
- Was allocation to treatment groups concealed
- Was there a control group
- Clear cause and effect relationship
- Outcome measured in a reliable way
- Risk of bias.

It was evaluated using the JBI critical appraisal checklist options of "yes," "no," "unclear," and "not applicable." The risks for biases were classified as low (total score, 5 to 8) and high (total score, 0 to 4). The study scored 50% or higher on all quality-assessed items, which were considered low-risk and included in this review. Disagreements during the full-text quality assessment were resolved through discussion (Table 1).

Data extraction and management

The differences between the three review authors were solved with discussion. Any discrepancies were resolved through a review by the other author. From each study, the following details were extracted from each study: Author, year of publication, study setting, study design, study subjects/experimental model, sample size, age, sex, vaccine target, target antigen, target disease and protection status and vaccine type were extracted. A detailed description of the characteristics of individual studies is provided in (Tables 2-4).

Table 1: A descriptive summary of thirty-two studies reporting vaccine candidates, immuno-dominant antigens and potent vaccine adjuvants for preventing cutaneous leishmaniasis included in this systematic review.

Author	Clear inclusion and exclusion criteria	Was true randomization used for the assignment of study subjects to treatment groups?	Was allocation to treatment groups concealed?	Was there a control group?	Clear cause and effect relationship?	Outcome measured in a reliable way?	Risk of bias (quality status)
Rostamian et al. [23]	Yes	Yes	Yes	Yes	Yes	Yes	Low risk
Albalawi et al. [24]	Yes	Yes	Yes	Yes	Yes	Yes	Low risk
Azizi et al. [25]	Yes	Yes	Yes	Yes	Yes	Yes	Low risk
Hojatizade et al. [26]	Yes	Yes	Yes	Yes	Yes	Yes	Low risk
Biari et al. [27]	Yes	Yes	Yes	Yes	Yes	Yes	Low risk
Thacker et al. [29]	Yes	Yes	Yes	Yes	Yes	Yes	Low risk
Emami et al. [30]	Yes	Yes	Yes	Yes	Yes	Yes	Low risk
Germano et al. [33]	Yes	Yes	Yes	Yes	Yes	Yes	Low risk
Pratti et al. [36]	Yes	Yes	Yes	Yes	Yes	Yes	Low risk
Jorjani et al. [41]	Yes	Yes	Yes	Yes	Yes	Yes	Low risk

Gholami et al. [50]	Yes	Yes	Yes	Yes	Yes	Yes	Low risk
Zahedifard et al. [51]	Yes	Yes	Yes	Yes	Yes	Yes	Low risk
Germano et al. [52]	Yes	Yes	Yes	Yes	Yes	Yes	Low risk
Zimara et al. [53]	Yes	Yes	Yes	Yes	Yes	Yes	Low risk
Soto et al. [54]	Yes	Yes	Yes	Yes	Yes	Yes	Low risk
Feiz-Barazandeh et al. [55]	Yes	Yes	Yes	Yes	Yes	Yes	Low risk
Asadi et al. [56]	Yes	Yes	Yes	Yes	Yes	Yes	Low risk
Giraud et al. [57]	Yes	Yes	Yes	Yes	Yes	Yes	Low risk
Rodriguez et al. [58]	Yes	Yes	Yes	Yes	Yes	Yes	Low risk
Sharma et al. [59]	Yes	Yes	Yes	Yes	Yes	Yes	Low risk
Fernandez et al. [60]	Yes	Yes	Yes	Yes	Yes	Yes	Low risk
Keshavarzian et al. [61]	Yes	Yes	Yes	Yes	Yes	Yes	Low risk
Louis et al. [62]	Yes	Yes	Yes	Yes	Yes	Yes	Low risk
Dalimi et al. [63]	Yes	Yes	Yes	Yes	Yes	Yes	Low risk
Reyes et al. [64]	Yes	Yes	Yes	Yes	Yes	Yes	Low risk
Younis et al. [65]	Yes	Yes	Yes	Yes	Yes	Yes	Low risk
Salari et al. [66]	Yes	Yes	Yes	Yes	Yes	Yes	Low risk
Lajevardi et al. [67]	Yes	Yes	Yes	Yes	Yes	Yes	Low risk
Awad et al. [68]	Yes	Yes	Yes	Yes	Yes	Yes	Low risk
Maciel et al. [69]	Yes	Yes	Yes	Yes	Yes	Yes	Low risk
Mehravaran et al. [70]	Yes	Yes	Yes	Yes	Yes	Yes	Low risk
Rostamian et al. [71]	Yes	Yes	Yes	Yes	Yes	Yes	Low risk

Table 2: First-generation (killed and live attenuated) vaccine trials with their study characteristics.

Author	Year of publication	Study setting	Study design	Study subjects/ Ex. model	Sample size	Age	Sex	Vaccine target	Target antigen	Target disease and protection status	Vaccine type
Germano et al. [33]	2020	Argentina	Pre-clinical trial	BALB/c mice	18	6-8 Weeks	Female	*L. amazonensis*	sTLA and sTLA+Poly (I:C)	CL/ partial protection,	Whole organism vaccines
Pratti et al. [36]	2019	Brazil	Pre-clinical trial	C57BL/6 WT and Tlr9-/- C57BL/6 mice	15	6-8 Weeks	Female	*L. amazonensis*	LaAg	CL/protection on WT mice but not for TLR9-/- mice	Whole organism vaccine
Germano et al. [52]	2022	Argentina	Pre-clinical trial	BALB/c mice	15	6-8 Weeks	Female	*L. amazonensis*	TLAs, TLA with Poly (I:C) and Montanide ISA 763	CL/ protection with TLA with Poly (I:C) and Montanide ISA 763	Whole organism protein vaccines
Zimara et al. [53]	2018	Germany	Pre-clinical trial	BALB/c mice and C57BL/6 mice	57	6-8 Weeks	Female	*L. major*	Curdlan Dectin-1	CL/ protection	Whole organism vaccine
Soto et al. [54]	2021	Spain	Pre-clinical trial	BALB/ c mice	26	6 Weeks	Female	*L. amazonensis*	*LiΔHSP70-II* and PBS	CL/ protection	Live attenuated vaccines

Table 3: Second and third-generation vaccine trials and immuno-dominant antigens with their study characteristics.

Author	Year of publication	Study setting	Study design	Study subjects/ Ex. model	Sample size	Age	Sex	Vaccine target	Target antigen	Target disease and protection status	Type of vaccine
Gholami et al. [50]	2019	Iran	Pre-clinical	BALB/c mice	30	6-8 Weeks	Female	*L. tropica*	PsSP9, whole Ph. sergenti SGH	CL/protection but not with Ph. sergenti SGH	Recombinant DNA vaccine
Zahedifard et al. [51]	2019	Iran	Pre-clinical	BALB/c mice	5	6-8 Weeks	Female	*L. major* promastigotes	Brevinin 2R and lauric acid conjugate	CL/protection	Recombinant vaccine
Jorjani et al. [41]	2018	Iran	Pre-clinical trial	BALB/c mice	98	6-8 Weeks	Female	*L. major*	pcLACK, pcLACK+ pcTSA+ pCAGGS-IL12	CL/ protection,	DNA vaccine
Feiz-Barazandeh et al. [55]	2020	Canada	Pre-clinical trial	BALB/c and C57LB/6 mice	20	6-8 Weeks	Female	*L. major* metacyclic promastigotes	PEPCK	CL/ no protection. PEPCK increase virulence.	Recombinant vaccine
Asadi et al. [56]	2020	Iran	Pre-clinical trial	BALB/c mice	16	6-8 Weeks	Inbred	*L. major* promastigotes	CRAMP	CL/ protective role	DNA vaccine
Giraud et al. [57]	2019	France	Pre-clinical trial	C57BL/6 and DBA mice	39	6-8 Weeks	Female	*L. amazonensis* promastigotes	Osteopontin	CL/ no protection, shape the host immune response towards the parasites.	Recombinant vaccine
Rodriguez et al. [58]	2020	USA	Pre-clinical trial	BALB/c mice	5	6-8 Weeks	Female	*L. major* metacyclic promastigotes	MetAP1 LM inhibitors (OJT006, OJT007, OJT008)	CL/ protection with only OJT008	Recombinant vaccine
Sharma et al. [59]	2022	Brazil	Pre-clinical trial	BALB/c mice	10	6-8 Weeks	Female	L. braziliensis	Centrin	CL/ no protection, centrin deficient parasite doesn't cause disease	DNA vaccine
Fernandez et al. [60]	2018	Spain	Pre-clinical trial	Hamsters	32	12 Weeks	Male	*L. infantum*	LACK	CL and VL/ protection	Recombinant DNA vaccine
Keshavarzian et al. [61]	2020	Iran	Pre-clinical trial	BALB/c mice	20	6-8 Weeks	Female	*L. major*	ILL+CpG	CL/ protection,	Recombinant vaccine
Louis et al. [62]	2019	USA	Pre-clinical trial	C57BL/6 mice	5	6-8 Weeks	Female	*L. major*	PEPCK	CL/ protection for mice injected ID, not for IM	Synthetic DNA vaccine
Dalimi et al. [63]	2020	Iran	Pre-clinical trial	BALB/c mice	15	6 Weeks	Female	*L. major* promastigotes	KMP+GP96	CL/ protective,	Recombinant DNA vaccine
Reyes et al. [64]	2021	Mexico	Pre-clinical trial	BALAB/c mice	30	4-6 Weeks	Female	*L. mexicana*	LmxMBA:PVAX1	CL/ protection	Recombinant DNA vaccine

Younis et al. [65]	2021	Sudan	RCT	Humans	24	18-50 years	Both sex	PKDL	Chad63-KH	PKDL/ protection, only 5/23 patients showed partial improvement	Chad63-KH vaccine
Salari et al. [66]	2020	Iran	Pre-clinical trial	BALB/c mice	100	6-8 Weeks	Female	*L. major*	KMP11 and LACK with CPG ODN	CL/ protection, with LACK/ KMP11/ EGFP with CpG but not with LACK/ KMP11/ EGFP+non-CpG	Recombinant vaccine
Lajevardi et al. [67]	2022	Iran	Pre-clinical	BALB/c mice	28	6-8 Weeks	Female	*L. major* and *L. tropica*	PpSP15, PsSP9	CL/ protection	Recombinant protein vaccine

Table 4: Potent vaccine adjuvants with their study characteristic.

Author	Year of publication	Study setting	Study design	Study subjects/ Ex. model	Sample size	Age	Sex	Vaccine target	Target disease and protection status	Adjuvants
Rostamian et al. [23]	2018	Iran	Pre-clinical trial	BALB/c mice	105	5-7 Weeks	Female	*L. tropica* (LtSLA and recombinant LtSTI1)	CL/protection with MPL and STI1 but not with R848 and SLA	MPL and R848
Albalawi et al. [24]	2021	Egypt	Pre-clinical trial	BALB/c mice	48	6-8 Weeks	Male	*L. major* amastigotes	CL/ protection	CUNPs alone or with MA
Azizi et al. [25]	2019	Iran	Pre-clinical	BALB/c mice	24	70-80 days	Female	*L. major*	CL/protection with SLA plus CMP groups	CMP
Hojatizade et al. [26]	2019	Iran	Pre-clinical	BALB/c mice	70	6-8 Weeks	Female	*L. major* (SLA)	CL/no protection	Cationic DOTAP/ DOPE/CHOL Liposomes
Biari et al. [27]	2020	Iran	Pre-clinical	BALB/c mice	50	6-8 Weeks	Female	*L. major* whole *Leishmania* lysate antigens (WLL)	CL/ no protection, used when TH-2 immune response is desired	Sphingomyelin (SM) Liposomes
Thacker et al. [29]	2020	USA	Pre-clinical	Rhesus monkey (macaque)	4	6-11 Years	Both	*L. major* or *L. amazonensis*	CL/protection	CpG ODN D35
Emami et al. [30]	2018	Iran	Pre-clinical	BALB/c mice	130	6-8 Weeks	Female	*L. major* SLA	CL/protection	MPL and IMQ
Awad et al. [68]	2021	S. Arabia	Pre-clinical trial	BALB/c mice	8	6-8 Weeks	Female	*L. major*	CL/ protection, with only MSNPs, and only partial protection with Pentostam and CNPs	MSNPs
Maciel et al. [69]	2021	Brazil	Pre-clinical trial	C57BL/6 and BALB/c mice	5	6-8 Weeks	Female	*L. amazonensis* whole antigens	CL/ only minimal protection,	MPLA/ AddaVax

Mehravaran et al. [70]	2020	Iran	Pre-clinical trial	BALB/c mice	10	6-8 Weeks	Female	*L. major* SLA	CL/ protection	Lip+Imiquimod
Rostamian et al. [71]	2020	Iran	Pre-clinical	BALB/c mice	150	5-7 Weeks	Female	*L. major* SLA and *L. tropica* SLA, LtSTI1	CL/ protection	MPLA

Data analysis

Data were extracted using a Microsoft Excel 2010 spreadsheet. A qualitative synthesis of publications has been performed and results were reported.

RESULTS

The search of the different databases is provided a total of 661 studies. After adjustment by year of publication within the past 5 years a total of 163 remained. 66 studies were discarded as their full text was not available. Nine duplicate articles were discarded. One article written in another language has also been discarded. Of the remaining 87 articles, 14 review articles were discarded. After the reviews of their title and abstracts, 38 articles were discarded in which they did not meet the inclusion criteria. The full texts of the remaining 35 studies were reviewed in detail. Three studies were discarded after the full text had been reviewed, since they did not address much of the needed information. The remaining 32 eligible studies were included for the systematic review of vaccine candidates, immuno-dominant antigens and potent vaccine adjuvants after quality assessment using the JBI quality assessment critical appraisal checklist.

Description of included studies

Thirty-two articles were conducted on vaccine candidates, immuno-dominant antigens and potent vaccine adjuvants for preventing CL, of these, 11 studies focused on the identification of vaccine adjuvants [20-26]. Only one clinical trial study was conducted on human subjects [27-31]. 20 first, second and third-generation vaccine studies were conducted in different experimental animal models (e.g. mice and hamsters) [32-36]. The studies were conducted in different study settings. Study subjects included in the studies could be of any type (animal or human model). All the studies were experimental (pre-clinical and clinical trials) on different CL species such as *L. major; L. amazonensis, L. mexicana* and *L. infantum* and then first, second and third-generation vaccines were identified. As well, studies included in this review were assessed for the provision of complete data about the efficacy and immune response induction of the candidate vaccine, immuno-dominant antigens and potent vaccine adjuvants [37-42]. Independent evaluators re-assessed all the articles before any analysis and the studies were fit in terms of their quality [43-45]. The description of included studies is presented (Table 1).

DISCUSSION

Most first-generation vaccine trials showed protection of the immunized animal model; among these, the C-Type Lectin Receptors (CLR), which belong to the Pattern Recognition Receptors (PRR), predominantly recognize carbohydrates and non-carbohydrates [46-51]. The CLR dectin-1 recognizes β-glucans, which occur naturally in the cell wall of fungi [52-58]. All human monocyte populations as well as Macrophages, Dendritic Cells (DCs), Neutrophils, Eosinophils and B cells show high dectin-1 expression in this review [58-63]. It has been shown that the function of human dectin-1 is comparable to that of mouse dectin-1 [64-69]. Dectin-1 in particular and CLRs in general can be regarded as important checkpoints for adaptive immune responses [70-72]. Dectin-1$^+$ myeloid DCs could be interesting targets for a Curdlan-based immunotherapy or vaccination strategy in humans; as such expansion of DC subsets may also occur at the site of infection and in the Skin-Draining Lymph Nodes (SDLNs) of CL patients [73]. This is particularly intriguing given that dectin-1$^+$ myeloid DCs are thought to decrease disease-promoting TH2-type responses [74].

In this review, the potent antigenic molecules have been highlighted with their ability to serve as vaccine targets for CL. As part of the second-generation vaccine, these molecules can be categorized based on the pathways/origin of the antigenic proteins. Secreted and excreted antigens such as Thiol-Specific Antioxidant (TSA), intracellular antigens such as Stress-Inducible Protein-1 (STI1), membrane/surface antigens such as Kinetoplastid Membrane protein-11 (KMP-11), lipo-phsphoglycan (LPG), glycoprotein-63 (gp63) and salivary proteins. Among the antigenic molecules, cathelicidin is an important type of antimicrobial peptide (AMP) found in varying amounts in all living organisms [75]. Compared to intact cells, Leishmania-infected macrophages express the gene for Cathelicidin-Related Antimicrobial Peptides (CRAMP) faster. In a cell culture medium, human type 1 macrophages express more human cathelicidin than type 2 and this report further shows that similar to bacterial infections, *Leishmania* infection can cause skin cells to express CRAMP [76].

The antigen of *Leishmania* homolog of receptors for activated c-kinase (lack) is a 36 kDa protein that is expressed in both stages of the Leishmania parasite life cycle. The *lack* gene, which comprises 939 base pairs, is located on chromosome 28 [77]. By redirecting IL-4 responses to protective TH-1 responses, this protein is protective when administered as a DNA vaccine in mice [78]. However, LACK vaccination requires IL-12 as an adjuvant to be effective against *Leishmania* [79]. *Leishmania* Phospho-Enol-Pyruvate Carboxy-Kinase (PEPCK), an enzyme essential for gluconeogenesis, is a class II-restricted immuno-dominant antigen [80]. Vaccination against *L. major* with PEPCK protein, peptides or DNA can lead to significant protection. In addition, PEPCK is a component of numerous *Leishmania* species, including those that cause both CL and Visceral Leishmaniasis (VL). Thus, it is plausible to envision a single vaccine that could provide comprehensive protection against a variety of Leishmania parasite strains, as indicated in our review PEPCK induces strong immune responses such as induction of skin-resident T cells [81].

A complex protein with a strong association with the LPG of *Leishmania* promastigotes is known as KMP-11. The LPG exhibits strong antigenicity for both human and murine T lymphocytes [78]. In this review, the KMP-11 protein showed three different immunological properties: Activation of B cells, induction of lymphocyte proliferation and cytotoxic response and generation of

protective immunity and IFN-γ in animal models [82].

A third-generation randomized controlled trial in humans demonstrating the safety and immunogenicity of the ChAd63-KH vaccine in patients with Post-Kalazar Dermal Leishmaniasis (PKDL) represents an important milestone in the development of a therapeutic vaccine as an adjunct treatment for PKDL patients. ChAd63-KH is based on a well-characterized simian adenovirus backbone. ChAd63-KH encodes two *Leishmania* antigens, KMP-11 and Hydrophilic Acylated Surface Protein B (HASPB), both of which are therapeutically effective as monovalent vaccines in preclinical animal models. KMP-11 is expressed in promastigotes and amastigotes of all *Leishmania* and is rich in $CD8^+$ T cell epitopes. HASPB is expressed by amastigotes and has conserved N- and C-termini flanking polymorphic repeats [83]. Previous studies have shown that ChAd-mediated vaccination strongly stimulates human $CD8^+$ and $CD4^+$ T cell responses and antibodies [84], with patients showing no unusual vaccine-induced responses. In general, this phase IIa clinical trial ChAd63-KH vaccine is safe and immunogenic in Sudanese PKDL patients, setting the way for additional research to assess its effectiveness in a therapeutic setting.

Most of the adjuvants in this review showed significant protection in immunized mice when administered in combination with various *Leishmania* antigens. Adjuvants are chemicals that modulate or enhance a successful immune response to the antigens of the vaccine. The formulations are usually emulsions and vesicles that can be used as vehicles for the administration of antigen vaccine components and allow the gradual release of vaccine antigen components over time. Adjuvants can also support the immunogenicity of the antigen by enhancing local inflammation and uptake of the antigen by Antigen-Presenting Cells (APCs), facilitating their migration to the lymph nodes and improving the efficacy of the leishmaniasis vaccine. Ideally, adjuvants would also help to reduce the amount of antigen or the number of immunizations required for vaccination [85].

Commiphora molmol (myrrh), a medicinal plant, is used in some ointments and healing balms for cuts and other minor skin conditions and is used as an adjuvant [86]. In this review, it is shown that MSNPs were used to Produce Silver Nanoparticles (SNPs), which were then tested *in vivo* for their efficacy in treating lesions in BALB/c mice. SNPs are thought to be non-enzymatic and difficult to inhibit by parasites, making them suitable for the generation of Reactive Oxygen Species (ROS). In addition to their ability to generate large amounts of ROS, SNPs are also thought to have special properties that can be used to treat CL. This is consistent with studies on metabolic activity, which showed that parasites treated with MSNPs multiplied less frequently than parasites in the Copper Nanoparticle (CNP) group [87].

The adjuvant Imiquimod can stimulate monocytes to produce antiviral cytokines such as IL-1β, TNF and IFN-α. It could also stimulate the release of IFN-γ and IL-12 from macrophages, which could enhance the TH-1 immune response. Imiquimod therapy of macrophages infected with *L. donovani* has also been shown to eradicate intracellular amastigotes, depending on nitric oxide (NO) production by treated macrophages. Previous studies have shown that Imiquimod, which has been used against *L. major* challenge, can trigger the TH-1 immune response by promoting the release of IFN-γ from macrophages [88]. In addition, cytosine triphosphate Deoxy-nucleotides (CpG ODNs), a potent vaccine adjuvant, are critical for TH-17 cell development and IL-17 production. They facilitate the recruitment of leukocytes, especially neutrophil granulocytes, to the target site and enhance the TH-1 immune response in the early phase [89].

The efficacy of the combination of the potent adjuvant poly (I: C) and the Montanide ISA 763 with *L. amazonensis* total antigens obtained by freeze-thaw cycles or sonication in protecting immunized mice was determined. Although, a Toll- Like Receptor (TLR-3) agonist, poly (I:C), triggers innate and adaptive TH-1 immunity, they are unstable due to nuclease break down because they are nucleic acid-based adjuvants. Therefore, the use of particulate formulations, such as emulsions, could be a good way to solve this problem [90]. A water-in-oil emulsion called Montanide ISA 763 forms a depot at the site of inoculation, which allows a gradual release of the antigen. In addition, this emulsion increases antigen uptake by APCs, modifies the electrical charge of the antigen to make it more immunogenic and protects against enzymatic degradation. In the development of leishmaniasis vaccines, adjuvants should be considered as sophisticated agents that can significantly affect a wide range of immune responses. The study of adjuvants is the study of the factors that regulate the expression of various immune responses.

CONCLUSION

In this review vaccine candidates, potent antigenic molecules and some effective vaccine adjuvants were demonstrated and showed protective immunity on different experimental models. A recent clinical trial in Sudan evaluated the immunogenicity and safety of the ChAd63-KH vaccine. This is the only third-generation vaccine against CL in humans currently in clinical development. In addition, numerous clinical vaccine trials are planned and underway, as well as the development of new adjuvants. The complexity of the immune responses required for protection, the high cost of vaccine research and the limited understanding of the immuno-pathogenesis of the disease have made the development of a safe and effective vaccine for CL a difficult task, however, as outlined in this review, given the rapid advances in the field of parasite immunology and genetics, a robust vaccine against CL should be possible sooner rather than later. Moreover, the CL burden is concentrated in countries with limited resources and the problem is exacerbated by a lack of political will and charitable commitment. Therefore, there is a clear need for increased investment in research and development. In the end, it is also important to establish an auxiliary overview of CL vaccine candidates to raise awareness of the progress made in CL vaccine development.

AUTHORS' CONTRIBUTIONS

Mohammed Adem and Abiy Ayele Angelo are participated in collecting relevant articles from the databases. After articles had been collected, Abiy Ayele Angelo, Mohammed Adem, Gashaw Adane and Yeshambel Belyhun extracted data and prepared the draft manuscript. Then, Abiy Ayele Angelo, Bisrat Birke Teketelew, Dereje Mengesha Berta, Elias Chane, Negesse Cherie, Mesele Nigus, Getu Girmay, Mebratu Tamir and Mohammed Adem revised the manuscript. Finally, Abiy Ayele Angelo and Mohammed Adem finalized the manuscript and Abiy Ayele Angelo communicated with the journal. All authors read and approved the final manuscript.Three authors (Abiy Ayele Angelo, Mohammed Adem and Yeshambel Belyhun) independently screened articles by their title, abstract and full text to identify eligible articles using predetermined inclusion and exclusion criteria. All the retrieved

primary studies were then combined, exported and managed using reference manager software by three authors (Abiy Ayele Angelo, Mohammed Adem and Yeshambel Belyhun). After the exclusion of duplicate studies, titles and abstracts were independently screened for inclusion in full-text appraisal, which was done by three of the authors (Abiy Ayele Angelo, Mohammed Adem and Yeshambel Belyhun) and the disagreement between authors that arose during data extraction and selection is solved based on evidence-based discussion and by the involvement of a fourth author (Gashaw Adane).

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