**Vaccination in Leishmaniasis: A Review Article**

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**ABSTRACT**

Leishmaniasis is caused by protozoan Leishmania parasites that are transmitted through female sandfly bites. The disease is predominantly endemic to the tropics and semi-tropics and has been reported in more than 98 countries. Due to the side effects of anti-Leishmania drugs and the emergence of drug-resistant isolates, there is currently no encouraging prospect of introducing an effective therapy for the disease. Hence, it seems that the key to disease control management is the introduction of an effective vaccine, particularly against its cutaneous form. Advances in understanding underlying immune mechanisms are feasible using a variety of candidate antigens, including attenuated live parasites, crude antigens, pure or recombinant Leishmania proteins, Leishmania genes encoding protective proteins, as well as immune system activators from the saliva of parasite vectors. However, there is still no vaccine against different types of human leishmaniasis. In this study, we review the works conducted or being performed in this field. DOI: 10.52547/ibj.26.1.35

**Keywords**: Immune response, Leishmaniasis, Vaccination

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**INTRODUCTION**

Leishmaniasis is a vector-borne disease caused by more than 30 species of *Leishmania* parasites. The disease has a broad clinical picture, ranging from skin lesions to fatal visceral infection.[¹] Leishmaniasis is endemic to four continents and more than 98 countries.[²] According to the WHO, 350 million people are at risk for leishmaniasis.[³] Leishmaniasis is found in humans in two main forms: CL and VL. Approximately 58,000 VL cases and 220,000 CL cases are reported annually.[²] The CL is divided into cutaneous, mucocutaneous, and diffused cutaneous types.[²] *L. tropica* and *L. major* are the main causes of CL, while *L. infantum* and *L. donovani* are the main causes of VL. Different species of rodents in various parts of Iran act as a reservoir for rural CL. These species include *Rhombomys opimus* and *Meriones libycus* found in the central and northeast, *M. libycus*, *M. persicus*, and *M. hurrianae* in the south, as well as *Tatera indica* and *Nesokia indica* in the west and southwest.[³⁴] The *Leishmania* parasite is transmitted in the Old World, including Europe, Africa, and Asia, by the bite of the female sandfly of the genus *Phlebotomus*, and in the New World, including America, by *Lutzomyia*. The

List of Abbreviations:

- BT1, biotin transporter
- CC, complete cure
- CFA, complete Freund’s Adjuvant
- CL, cutaneous leishmaniasis
- CP, cysteine protease
- C. parvum, *Cryptosporidium parvum*
- CPA, cysteine proteinase Type II
- CPB, cysteine proteinase Type I
- CPB*STE*, CPB without its unusual C-terminal extension
- DC, dendritic cells
- DHFR-TS, dihydrofolate reductase-thymidylate synthase
- DT, double transfectants
- DTH, delayed-type hypersensitivity
- i.d., intradermal
- i.m., intramuscular
- i.v., intravenous
- MDP, muramyldipeptide
- MPL-A, monophosphoryl lipid A
- MVA, modified vaccinia Ankara
- NO, nitric oxide
- PBMC, peripheral blood mononuclear cells
- ODN, oligodeoxynucleotides
- P. orientalis, *Platannus orientalis*
- S.C., subcutaneous
- SIR2, silent information regulatory
- ST, single transfectants
- S. typhimurium, *Salmonella typhimurium*
- TSA, thermal shift assay
- VL, visceral leishmaniasis
main hosts are vertebrates, and the most commonly infected hosts include humans, dogs, and rodents.[5] The sandfly family consists of five genera and 700 species, of which about 30 species are involved in the transmission of the *Leishmania* parasite.[6] Table 1 shows the main species of *Leishmania* that cause human disease. Over the years, many types of research have been conducted on the *Leishmania* vaccine. In each of these studies, candidate antigens were produced using improved laboratory techniques and various experimental models were examined. An overview of the results from the past to the present investigations can provide a fruitful research strategy for researchers. Meanwhile, such studies have shown that different vaccine administration routes can affect protective immunity. Despite the large number of preclinical vaccine candidates, and approaches designed to emulate this protective response,[7] the successful transition of *Leishmania* vaccines into human trials has remained elusive, though considerable efforts are underway[8,9]. Therefore, the purpose of this article is to provide a more comprehensive review of the current advances in leishmania vaccine development.

### Immunity against leishmaniasis

Macrophages are the primary hosts for *Leishmania*, but their role in preventing or progressing the disease has been described in T-cell-dependent behavior; however, the fate of the infected macrophages before T cell presence is not well-known.[10] Because specieilized T cells apeare late in the infection, the parasite is able to regulate disease progression in the host.

Parasites can manipulate killing mechanisms of macrophages, at the time of their entry, and stimulate the production of IL-4 and certain disease-stimulating factors by T cells, leading to the progress of the disease and survival of the parasite[11]. As soon as the parasite diverts the CD40 signaling pathway to the pre-parasitic pathway in macrophages, the interaction between the CD40 ligand presented on activated T cells surfaces and CD40 receptors of infected macrophages cannot activate the anti-parasitic pathway, and probably reaction of T cell-macrophage does not maintain the host[12]. In addition to the host apoptosis, stimulation of parasite apoptosis can be one of the therapeutic goals to increase the effectiveness of antiparasitic drugs. For instance, the study of Sengupta

| *Leishmania* species | Disease form in humans | Geographical distribution | Reservoir | Vectors |
|----------------------|------------------------|--------------------------|----------|---------|
| *L. aethiopa* **     | Localized CL, Diffuse CL | Ethiopia, Kenya          | Rock hyraxes | *P. longipes*; *P. pedifer* |
| *L. major* **        | Localized CL           | North Africa, the Middle East and Central Asia, Sub-Saharan Africa and Sahel belt, Sudan, North India, and Pakistan | Rodents | *P. papatasi* and *P. duboscqi* |
| *L. mexicana**       | Localized CL           | Central America          | Forest rodents | *Lutzomyia olmeca* |
| *Leishmania amazonensis** | Localized CL     | South America, north of the Amazon | Forest rodents | *L. flaviscutellata* |
| *L. braziliensis**   | Localized CL            | South America, Central America and Mexico | Forest rodents, peridomestic animals | *Psychodopygus*; *Lutzomyia spp.* |
| *L. peruviana**      | Localized CL           | West Andes of Peru., Argentine highlands | Dog | *L. verrucarum*, *L. pvmenis* |
| *L. infantum* **     | VL                     | Mediterranean basin; Middle East and Central Asia to Pakistan; China; Central and South America, southern Europe, northwest Africa | Dogs, cats, foxes, and jackals | *P. perniciosus* and *P. arias* |
| *L. donovani* **     | VL                     | Ethiopia, Sudan, Kenya, India, China, Bangladesh, Burma | Human anthroponosis, Rodents Sudan, canines | *Phlebotomus argentipes*, *P. orientalis*, and *Pseudostomatella martini* |

**Old World species; " New World species; *P., Phlebotomus; L., Lutzomyia*

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Table 1. The main species of *Leishmania* that cause human disease
et al.\textsuperscript{13} showed that the natural indoloquinoline alkaloid cryptolepine causes a decrease in the cell viability of L. donovani AG83 promastigotes in both time- and concentration-dependent manners by increasing ROS and lipid peroxidation production and decreasing cellular glutathione levels. The results of Roy et al.'s\textsuperscript{14} study also indicated that the plant carbazole alkaloid exerts \textit{in vitro} and \textit{in vivo} antileishmanial activity by the modulation of reduct homeostasis. Furthermore, about inducing host apoptosis, researches have demonstrated the integration of expressional cassettes containing pro-apoptotic genes in \textit{Leishmania} by transgenic method or downregulating antiapoptotic molecule by miRNA could accelerate the apoptosis process of infected macrophages, restrict the possibility of differentiation and induce more proliferation of \textit{Leishmania}. These events would result in the expansion of the disease, and the appearance of the lesion\textsuperscript{15}. A study by Aghaei et al.\textsuperscript{16} signified that the transgenic \textit{L. infantum} expressing mLLO-BAX-SMAC proteins can accelerate the apoptosis of infected macrophages compared to wild-type \textit{Leishmania}. It means that transgenic \textit{Leishmania} is proved to increase the rate of apoptosis in infected macrophages compared to intact strain. Since metacaspases are the key regulators of death or life of parasites, and these proteins do not exist in mammals, they can be considered as targets for fighting against parasitic infections in the future\textsuperscript{17}.

Vaccination concepts in leishmaniasis

There are some facts to support the possibility of developing an effective vaccine against CL. However, due to the increased resistance to first-line drugs and the toxicity of second-line drugs, the development of an effective vaccine against the disease is very desirable. The use of vaccines is advantageous over chemotherapy as they induce long-lasting effects and can be administered both in prophylactic and therapeutic modes. Also, the vaccine will not counter the problem of resistance as in the case of chemotherapy\textsuperscript{18}. As stated in a study published by Thomaz-Soccol et al.\textsuperscript{19} in 2018, the number of patents for leishmaniasis vaccines is 74 in the United States and 36 in Brazil. In Brazil, 20,000 cases of leishmaniasis and more than 3,000 cases of VL, and in India, 8,000 cases of VL are reported annually\textsuperscript{20}. Spain and France are still endemic for VL. In France, for example, the prevalence of VL is 0.22 per 100,000 population in the endemic regions\textsuperscript{21}. Therefore, vaccination against leishmaniasis is essential in these areas. Moreover, the highest number of patents was reported in that study to be related to the private sector (94 cases), and the lowest was related to cooperation between universities and companies (11 cases); however, universities and nondeneducational public institutions had 65 and 13 patent cases, respectively\textsuperscript{21}. Therefore, the need for more cooperation between public and private institutions seems to be necessary.

Challenges of efficient vaccine design

To date, many attempts have been made to test clinically prepared vaccines in various human trials, but they have been ineffective. It is widely believed that this problem arises from economic and financial pressures\textsuperscript{22}. Some studies have shown that using the whole parasite leads to inefficient antigen presentation and anti-\textit{Leishmania} memory cell development, thus reducing immunity\textsuperscript{23-25}. Also, preserving central memory T cells does not require the presence of parasites\textsuperscript{26}. There may not have been a suitable human adjuvant system for testing these vaccines\textsuperscript{27-29}. Vaccination provides long-term protection in the absence of attenuated strains such as LdCEN\textsuperscript{6} (centrin mutant) or PMMA (phosphomannosumetase). This finding was performed in a mouse model and not in humans. Injection of protective antigens in different models or immunotherapy has helped to find the factors involved in increasing anti-\textit{Leishmania} immunity. One of the major problems facing the vaccine against CL is the fact that despite causing cutaneous disease, the Old and New World parasites, \textit{L. major} and \textit{L. mexicana}/\textit{L. amazonensis}, respectively, are significantly different\textsuperscript{30}. There are differences in virulence factors between these species, as well as in the immune responses induced by them. For instance, LPG is a virulence factor for \textit{L. major}\textsuperscript{31}, but not for \textit{L. Mexicana}\textsuperscript{32}. During \textit{L. major} infection, the protective role of Th1 responses has been established, but \textit{L. amazonensis} can persist in the presence of Th1 responses and cause minimal disease in the complete absence of T cells\textsuperscript{33}. These findings show major, but not well-understood, differences in the immunobiology of parasites that appear to cause the same disease. This matter may have implications for the vaccine development process as the anti-CL vaccine may have different needs for the Old and New World leishmaniasis. Therefore, a vaccine against CL caused by \textit{L. major} might not necessarily be effective against the New World spectrum of diseases, including mucocutaneous and diffuse cutaneous forms. Another challenge for the vaccine is to obtain protection against VL even if it is efficacious against varied forms of CL.

Immunization methods against CL

\textbf{Leishmanization}

Adler observed that Lebanese children whose arms have been exposed to infected mosquitoes by their
mothers will be protected against severe forms of the disease in the future. This process was not followed because it caused uncontrolled growth of skin lesions and also led to a high prevalence of the disease in people with suppressed immune systems, particularly those with HIV and organ transplants. The first method of immunization against leishmaniasis known as "leishmanization" was developed in 1940 and has been used in various countries for several years. This vaccine was discontinued due to its lack of safety and is now limited to the vaccine registered in Uzbekistan and the vaccine used in clinical trials in Iran. In this procedure, live and active L. major promastigotes are injected intradermally into the anatomical position of the deltoid muscle. An active ulcer then develops and eventually heals on its own. The result of this method is long-term immunity against rural and urban leishmaniasis. Tables 2 and 3 shows leishmanization experiments in Iran and USSR countries.

First-generation vaccines

These vaccines contain the whole body of the parasite with or without adjuvant. First-generation vaccines replaced leishmanization, and the vaccine is now used in some human trials. These categories include killed, live attenuated, and fractionated vaccines. Table 4 lists the first-generation vaccines with full specifications.

Killed vaccines

This type of vaccine was developed and evaluated by Mayrink et al. in Brazil. The result of the leishmanin skin test was satisfactory, but the vaccine had only a 50% protective effect. In Venezuela, Sharples et al. used a mixture of killed L. amazonensis, L. mexicana, and Bacillus Calmette Guerin to treat CL, resulting in a 95% improvement and activation of Th1 immunity. Studies in Brazil have shown that a mixture of killed L. amazonensis with half a dose of meglumine antimoniate is very effective in treating CL. According to a study conducted in Ecuador, a proportion of L. brasilensis, L. guianensis, and L. amazonensis provided favorable protection against CL. Two studies in Iran have shown that autoclaved L. major vaccine with BCG is safe but does not provide promising immunity against CL. The results of a study by Mahmoodi et al. revealed that cases who received the ALM + BCG vaccine had a higher stimulation index and IFN-γ levels than those who received BCG alone or in the control group. The results of this study showed that the induction of Th1 immune response in volunteers who received the vaccine was much lower than those with or without a previous history of leishmaniasis, and it was assumed that these individuals became immune.

Th1 is activated in L. major infection, but L. amazonensis can remain active in the presence of Th1 and can reduce the T cell response. Therefore, the vaccine made for L. major is neither effective for another leishmaniasis nor VL. In general, vaccination with killed Leishmania promastigotes could be considered as a safe and economical treatment; nevertheless, further trials aiming at the evaluation of different adjuvants potentially pave the way for more efficient vaccines.

Live attenuated vaccine

These vaccines are currently the gold standard. In attenuated live vaccines, the parasite is both nonpathogenic and superior to killed vaccines. Methods of preparing attenuated live parasites include long-term in vitro culture, use of temperature sensitivity, gamma radiation, chemical mutagenesis, and culture with gentamicin. Titus and co-worker developed a live attenuated vaccine by knocking down certain Leishmania genes. Examples in this regard are the DHFR-TS and the 6p2 gene, which encodes an enzyme, transports guanosine diphosphate mannose to the Golgi apparatus, the lpg2 mutant from L. mexicana, the CP (cpa and cpb) from L. mexicana, the SIR2 from L. infantum, and the BT1 gene from L. donovani.

Suicidal cassettes

Myxombe et al. followed a method of producing a vaccine against leishmaniasis, which was to induce suicide genes. This method is performed by inducing drug-sensitive genes. They used a combination of thymidine kinase and gancyclovir against L. major and finally using gancyclovir treatment, partial to complete protection was achieved. Besides, the susceptible strain of L. major, which contained the altered thymidine kinase HSV-1 (tk) gene and the cytosine deaminase gene from Saccharomyces cerevisiae (cd), increased susceptibility to gancyclovir and 5-fluorocytosine. L. major infection recovered within two weeks of treatment with either drug alone or in combination with gancyclovir and 5-fluorocytosine.

Fractionated vaccine

This kind of vaccine is advantageous due to its high purity and yield. Several molecules, either membrane proteins, such as HASPB1 and A2 protein, or soluble fractions of the parasite, i.e. PDI, TPI, elf-2, aldolase, enolase, P45, trypanothione reductase, and...
recombinant F14, among others have been used as a potential target for vaccination, both against cutaneous and VL. Also, some polypeptides have been tested with some degrees of success (Q protein, Leish-111f, 110f etc.).

Second-generation vaccines

Second-generation vaccines are based on synthetic or recombinant subunits and genetically modified Leishmania strains, recombinant bacteria, or viruses carrying Leishmania antigen genes. A summary of these vaccines against Leishmania is given in Table 5.

Vaccines based on nonpathogenic Leishmania

In 2015, Katebi et al. demonstrated the effect of a novel combination of protective parasitic antigens created by L. tarentolae, together with sandfly salivary antigen as a vaccine strategy against L. major infection. The immunogenicity and protective effect of different DNA/Live and Live/Live prime-boost vaccination with live L. tarentolae expressing CPs (type I and II, CPA/CPB) and PpSP15 from Phlebotomus papatasi, were tested in BALB/c and C57BL/6 mice. Both humoral and cellular immune responses were assessed before challenge and at 3 and 10 weeks after Leishmania infection. In both strains of mice, the strongest protective effect was observed when the mice primed with PpSP15 DNA and then received PpSP15 DNA and live recombinant L. tarentolae as a booster. In 2015, Shahbazi et al. vaccinated outbred dogs with a prime-boost regimen based on recombinant L. tarentolae expressing the L. donovani A2 antigen, along with CP genes (CPA and CPB-CTE) and evaluated its immunogenicity and protective immunity against L. infantum infectious challenges.

Table 2. Leishmanization experiments in Iran

| Year | Study place | No. of individuals | Leishmania species | Infected with disease (%) | Comment | Ref. |
|------|-------------|--------------------|-------------------|--------------------------|---------|-----|
| 1946 | Tehran      | 120                | L. tropica major  | 90                       | Cross protection against L. tropica minor | 111  |
| 1977 | Isfahan     | 250                | L. major          | 47                       | The incidence rate of CL in leishmanized children was one-sixth to one-seventh to control group. | 112,113 |
| 1982-86 | Isfahan and Dezful | 160,000        | L. major          | 89.5                     | Under 1% of new cases of CL were among leishmanized people. | 112,113 |
| 1983-1989 | On army recruits and revolutionary guard | 1800,000 and 6000 refugees | L. major          | 56.7-90                  | Reduction of the incidence rate of CL by Leishmanization among leishmanized people between one-sixth to one-eighth of its original level | 113,114 |
| 2005 | Tehran      | 28                 | L. major          | 100                      | Total protection was seen in 100% (11/11) of volunteers. | 115  |
| 1989 | Individuals receiving NLCV (no. 27) | 199 | L. major          | 61.5% in vaccinated and 90% in unvaccinated individuals | With 27% protection in the NLCV group | 116  |
| 2001 | Isfahan Province | 200               | Deep-freeze promastigote forms of L. major | 40-45 | Production of L. major under good manufacturing practices condition at Razi Institute | unpublished |

NLCV, nonliving crude vaccine

In 2014, Zahedifard et al. demonstrated the effect of a novel combination of protective parasitic antigens created by L. tarentolae, together with sandfly salivary antigen as a vaccine strategy against L. major infection. The immunogenicity and protective effect of different DNA/Live and Live/Live prime-boost vaccination with live L. tarentolae expressing CPs (type I and II, CPA/CPB) and PpSP15 from Phlebotomus papatasi, were tested in BALB/c and C57BL/6 mice. Both humoral and cellular immune responses were assessed before challenge and at 3 and 10 weeks after Leishmania infection. In both strains of mice, the strongest protective effect was observed when the mice primed with PpSP15 DNA and then received PpSP15 DNA and live recombinant L. tarentolae as a booster. In 2015, Shahbazi et al. vaccinated outbred dogs with a prime-boost regimen based on recombinant L. tarentolae expressing the L. donovani A2 antigen, along with CP genes (CPA and CPB-CTE) and evaluated its immunogenicity and protective immunity against L. infantum infectious challenges.
They showed that vaccinated animals produced significantly higher levels of IgG2, but not IgG1, as well as IFN-γ and TNF-α, but low IL-10 levels, before and after challenge as compared to control animals. Protection in dogs was also associated with a strong DTH response and a low parasite burden in the vaccinated group. Overall, immunization with recombinant L. tarentolae A2-CPA-CPB-CTE proved to be immunogenic and induced partial protection in dogs, hence representing a promising live vaccine candidate against VL.

In 2018, Abdellahi et al. described the generation of L. lactis(adr-) strain as the vector expression of the protective Leishmania antigen, LACK, in the cytoplasm, secreted or anchored to the bacterial cell wall or co-expressing mouse IL-12. They showed that oral immunization using live L. lactis, secreting both LACK and IL-12, was the only regimen that partially protected BALB/c mice against the next L. major challenge. This issue highlights the importance of temporal and physical proximity of the delivered antigen and adjuvant for optimal immune priming by oral immunization. In 2019, Torkashvand et al. expressed FIS1 fusion protein, including the N-terminal region of S1 subunit of PT and FHA type1 immunodominant domain by L. lactis, and evaluated its immunogenicity. Based on their results, mice immunized with LL-FIS1 produced significant levels of specific IFN-γ compared to controls and DTaP-immunized mice. The FIS1-specific IgG antibody response was lower in LLF1S1-immunized mice, while the IgG2a/IgG1 ratio was higher in this group compared to the DTaP-immunized mice. In 2020, Davarpanah and co-workers explained that PpSP15 is an immunogenic salivary protein from P. papatasi. Immunization with Lactococcus lactis expressing sand fly PpSP15 salivary protein has been shown to protect against L. major infection. In their study, BALB/c mice were challenged with L. major plus P. papatasi salivary gland homogenate. Evaluation of footpad thickness and parasite burden displayed a delay in disease development and reduced the number of parasites in PpSP15 vaccinated animals as compared to the control group. In addition, vaccinated mice exhibited Th1 type immune responses. Importantly, immunization with L. lactis-PpSP15-EGFP enhanced long-term memory in mice, which lasted for at least six months.

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### Table 3. Early leishmanization experiments in USSR countries

| Year   | Inoculum | Number | Infected with disease (%) | Comment                                                                 | Ref. |
|--------|----------|--------|---------------------------|-------------------------------------------------------------------------|------|
| 1942-1968 | 1.5 × 10⁷ | 647    | 60-90                     | Used infected hamster tissue                                            | 118  |
| 1972    | 1.0 × 10⁷ | 65     | 100                       | A new isolate replaced older ineffective strain                         | 119  |
| 1978    | 2.0 × 10⁷ | 475    | 14-100                    | High level of nodules                                                   | 118  |
| 1979    | 4.0 × 10⁷ | 39     | 100                       | Pretest of frozen vaccine                                               | 118  |
| 1968    | 0.8 × 10⁶ | 2245   | 98                        | 93.2% of ulcers <2 cm at 2 months                                       | 120  |
| 1968    | 0.1-1.2 × 10⁶ | 12500 | 90                        | Found little influence of culture age, medium or number                 | 121  |
| 2018    | -        | 9500   | 96-100                    | -                                                                       | 118  |

*In 2012, Hugentobler et al. expressed FIS1 fusion protein, including the N-terminal region of S1 subunit of PT and FHA type1 immunodominant domain by L. lactis, and evaluated its immunogenicity. Based on their results, mice immunized with LL-FIS1 produced significant levels of specific IFN-γ compared to controls and DTaP-immunized mice. The FIS1-specific IgG antibody response was lower in LLF1S1-immunized mice, while the IgG2a/IgG1 ratio was higher in this group compared to the DTaP-immunized mice. In 2020, Davarpanah and co-workers explained that PpSP15 is an immunogenic salivary protein from P. papatasi. Immunization with Lactococcus lactis expressing sand fly PpSP15 salivary protein has been shown to protect against L. major infection. In their study, BALB/c mice were challenged with L. major plus P. papatasi salivary gland homogenate. Evaluation of footpad thickness and parasite burden displayed a delay in disease development and reduced the number of parasites in PpSP15 vaccinated animals as compared to the control group. In addition, vaccinated mice exhibited Th1 type immune responses. Importantly, immunization with L. lactis-PpSP15-EGFP enhanced long-term memory in mice, which lasted for at least six months.*
### Table 4. Types of first-generation vaccines against *Leishmania*

| Antigen                  | Vaccine form/adjuvant/del. system | Animal model | Targeted disease (Leishmania spp.) | Summary of the experimental system                                                                 | Result | Another outcome                                                                 | Ref. |
|--------------------------|----------------------------------|--------------|-----------------------------------|------------------------------------------------------------------------------------------------------|--------|---------------------------------------------------------------------------------|------|
| *L. major*               | Pathogenic 10⁷ live promastigotes | C57BL/6      | CL/ L. major                      | Immunized through the ear (i.d.) and footpad (s.c.). Challenged 7 weeks later with 10⁶ promastigotes | Protection | s.c. route more effective enhanced IFN-γ and IL-10 levels in s.c. and i.d. immunization, respectively. | 38   |
| *L. major*               | Nonpathogenic live promastigotes | C57BL/6, BALB/c | CL/ L. major                      | Immunized by intraperitoneal or subcutaneous injection. Challenged with pathogenic promastigotes      | Protection | Complete protection in C57BL/6 mice while partial in BALB/c mice                | 55   |
| *L. braziliensis*        | Avirulent *L. braziliensis*      | BALB/c       | CL/ L. major                      | Immunization with Nmethyl-N′-methyl-N′-nitro-N-nitosoguanidine treated promastigotes                   | Protection | Immunity conferred and transferred by Lyt-1+ cells                             | 56   |
| *L. major*               | γ-irradiated *L. major*          | CBA          | CL/ L. major                      | Immunized through subcutaneous injection. Challenged with two strains of *L. major*                 | Protection | LN cells activated infected macrophages *in vitro* to kill the parasite         | 57   |
| *L. major*               | LPG deficient avirulent *L. major* | BALB/c      | CL/ L. major                      | Vaccination with CD4⁺ T-cell line derived from avirulent promastigote immunized mice. Challenged with a virulent strain | Protection | Enhanced TNF and IL-2 production, suppressed IL-4, negative DTH                  | 58   |
| *L. mexicana*            | Long-term culture of 5 × 10⁷ promastigotes with gentamycin | BALB/c      | CL/ *L. mexicana*/ L. major       | Immunization with s.c. injection followed by challenge with 5 × 10⁶ wild type promastigotes         | Protection | Lesion size reduced by 80%, significantly reduced infected macrophages          | 59   |
| *L. donovani*            | Long-term culture of promastigotes with gentamycin | BALB/c      | VL/ *L. donovani*/ L. infantum    | Immunized subcutaneously followed by challenge with wild type promastigotes                        | Protection | Percentage of infected macrophages reduced by 91–99%                           | 59   |
| Antigen                          | Vaccine form/adjvant/del. system | Animal model | Targeted disease (Leishmania spp.) | Summary of the experimental system                                                                                     | Result                                      | Another outcome                                                                 | Ref.  |
|---------------------------------|---------------------------------|--------------|------------------------------------|----------------------------------------------------------------------------------------------------------------------|---------------------------------------------|---------------------------------------------------------------------------------|-------|
| *L. chagasi*                    | Attenuated 10^7 promastigotes    | BALB/c       | VL/L. chagasi                      | Challenge with virulent promastigotes                                                                                 | No protection                              |                                                                                  | 122   |
| *L. chagasi*                    | 10^7 live promastigotes          | BALB/c       | VL/L. chagasi                      | Immunization (s.c.) and challenged both with 10^7 live promastigotes                                                  | Protection                                 | 88% parasite reduction, increased IFN-γ, IL-10, and IL-4 levels, low TGF-β level | 122   |
| *L. chagasi*                    | 10^2 or 10^4 live promastigotes  | BALB/c       | VL/L. chagasi                      | Immunization (s.c.) with 10^2 or 10^4 promastigotes and challenged with 10^7 live promastigotes                    | Intermediate protection                    | No protection in 10^2 doses, low IFN-γ, high TGF-β levels, no effect on IL-10 and IL-4 production as compared to control | 122   |
| *L. major L. chagasi*           | 10^2 and 10^7 live promastigotes | BALB/c       | VL/L. chagasi                      | Challenged with 10^6 *L. chagasi* promastigotes                                                                     | No protection                              |                                                                                  | 122   |
| *L. chagasi* L. donovani L. major* | DHFR-TS knock-out Promastigotes  | BALB/c       | VL/L. chagasi                      | Challenged with 10^7 virulent *L. chagasi*                                                                        | No protection                              | A negligible amount of IFN-γ Release                                             | 122   |
| *L. major*                      | DHFR-TS knock-out promastigotes  | BALB/c       | CL/L. major                        | Immunization through s.c., i.v. and i.m. routes. Challenged with 10^6 virulent promastigotes                      | Protection                                 | i.v. route, parasite burden reduced by 158–1990 fold in BALB/c mice, i.m. and s.c. the route also produces protection in CBA mice but not in BALB/c mice. | 60    |
| *L. major*                      | DHFR-TS knock-out promastigotes  | BALB/c       | C57BL/6                            | Immunization through i.v. and s.c. routes                                                                         | Partial protection                         | 10^8 dose developed 40–75% and 49–57% smaller lesion size in BALB/c and C57BL/6 mice, respectively | 123   |
| *L. major*                      | DHFR-TS knock-out promastigotes  | Monkey       | CL/L. major                        | Immunization subcutaneously and challenged with 10^5 promastigotes                                                 | No protection                              | Positive proliferative response (79%), no IFN-γ production, negative DTH response | 124   |
| Antigen         | Vaccine form/ adjuvant/del. system | Animal model | Targeted disease (Leishmania spp.) | Summary of the experimental system                                                                 | Result                                                                 | Another outcome                                                                 |
|----------------|------------------------------------|--------------|-----------------------------------|------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------|---------------------------------------------------------------------------------|
| *L. major*     | Live promastigotes                 | BALB/c       | CL/L. major                       | Immunization with $10^6$, $3 \times 10^1$, $10^2$, $3.3 \times 10^2$, $1.1 \times 10^3$, or $3.7 \times 10^3$ promastigotes | Protection only in $1.1 \times 10^2$ borderline disease in half of the $3 \times 10^3$ dose no protection in other doses | Enhanced IFN-γ production with low IgG1/IgG2a ratio in protected mice. Th1/Th2 response (both IFN-γ and IL-4 levels high) in borderline disease mice, and Th2 response in progressive disease mice. |
| *L. major*     | lpg2−mutant promastigotes          | BALB/c       | CL/L. major                       | Immunization (s.c.) with $5 \times 10^6$ promastigotes and challenged with wild type $2 \times 10^6$ parasites | Protection                                                              | Suppressed IL-10 and IL-4 production, low IFN-γ level, negative DTH response   |
| *L. major*     | Δlpg2−mutant promastigotes + CpG oligonucleotides | C57BL/6 | CL/L. major                       | Immunization with Δlpg2 with a single dose of CpG ODN (50 µg)                                       | Protection                                                              | 100 fold parasite reduction, no IFN-γ production, no DTH response               |
| *L. mexicana*  | CP mutant promastigotes            | BALB/c       | C57BL/6/CBA/Ca                    | Immunization (s.c.) with $5 \times 10^6$ Δcpa or Δcpb or both. Challenged with $10^6$ wild type promastigotes | Protection                                                              | Increased IFN-γ and IL-2 levels with low IL-4, no difference in IL-5, IL-10, and IL-12 levels, high IgG2a/IgG1 ratio |
| *L. mexicana*  | CP deficient promastigote          | Hamster      | CL/L. mexicana                    | Immunization (i.d.) with $10^3$ Δcpb or Δcpa/cpb promastigotes and challenged with wild type *L. mexicana* | Protection                                                              | High IFN-γ, no difference in IL-10 while TGF-β, IL-4, and IL-12 p40 not detected |
| *L. infantum*  | SIR2 deficient                     | BALB/c       | VL/L. infantum                    | Immunization (i.p.) with $10^8$ promastigotes and challenged with $10^8$ wild type promastigotes      | Protection                                                              | Enhanced NO level, high IFN-γ/IL-0 ratio, no difference in IL-4 and IL-2 levels, high IgG1 and IgG2a titer |

*Ref.* 61: Abdellahi et al. *Vaccine and Leishmania Iran. Biomed. J.* 26 (1): 1-35

*Ref.* 62: L. major lpg2−mutant promastigotes

*Ref.* 65: L. mexicana CP mutant promastigotes

*Ref.* 66: L. mexicana CP deficient promastigote

*Ref.* 67: L. infantum SIR2 deficient
| Antigen      | Vaccine form/adjuvant/del. system | Animal model | Targeted disease (Leishmania spp.) | Summary of the experimental system                                                                 | Result                                         | Another outcome                                                                 | Ref. |
|--------------|----------------------------------|--------------|-----------------------------------|------------------------------------------------------------------------------------------------------|-----------------------------------------------|--------------------------------------------------------------------------------|------|
| *L. donovani*| BT1 knock-out promastigotes       | BALB/c       | VL/JL. *donovani*                 | Immunization (i.v.) with $5 \times 10^7$ mutant promastigotes. Challenged with $5 \times 10^7$ luciferase-expressing virulent promastigotes | Protection                                    | Infection rate reduced by 75%, increased IFN-γ level, no IL-4 production     | 68   |
| *L. tarentolae* | Nonpathogenic *L. tarentolae* promastigotes | BALB/c           | VL/JL. *donovani*                 | Immunization (i.p.) with $5 \times 10^7$ promastigotes and challenged with $5 \times 10^7$ virulent *L. donovani* promastigotes | Protection                                    | 80-85% parasite reduction, enhanced IFN-γ production, no IL4, spleen cell proliferation increased by 17 fold | 126  |
| *L. major*   | Suicide system of promastigotes with thymidine kinase gene of HSV-1 | BALB/c       | CL/JL. *major*                    | Mice infected by tk-transfected or wild type promastigotes and treatment given by ganciclovir         | Partial to complete                           | -----                                                                 | 69   |
| *L. major*   | tk-cd<sup>+</sup> transfected promastigotes | BALB/c       | CL/JL. *major*                    | Mice infected with tk-cd<sup>+</sup> transfected and wild-type promastigotes. Treatment is given by ganciclovir and 5-fluorocytosine | Protection                                    | Mice infected with transfected promastigotes were completely cured by either or both drugs. | 71   |
| *L. amazonensis* | Porphyrogenic (DT) and non-porphyrogenic (ST) transfectants | Hamster      | VL/JL. *donovani*                 | Photodynamic vaccination with DT + ALA, DT - ALA, ST + ALA, or ALA. Challenged with $10^7$ amastigotes | Protection                                    | 99% parasite reduction, increased DTH, and lymphoproliferative response, high IFN-γ, iNOS, and IL-12 expression, high IgG2a titer | 127  |
| *L. infantum* | Human and animal                  | CL.          |                                   | injecting one milliliter of the fraction intracutaneously in four different points of the skin. These were people who had been ill for at least three months | Protection                                    | -----                                                                 | 128  |
Table 5. Second-generation vaccines against *Leishmania*

| Antigen | Adjuvant/delivery system | Animal model | Targeted disease (Leishmania spp.) | Result | Other outcomes | Ref. |
|---------|--------------------------|--------------|----------------------------------|--------|----------------|------|
| gp63    | *S. typhimurium*         | CBA, BALB/c  | CL/L. major                      | Protection | Protection only in CBA mice, 67–78% parasite reduction, activated CD4+ T cells which secret IFN-γ and IL-2 but not IL-4, negative DTH response | 129  |
| gp63    | Alone and along with BCG or *C. parvum* or MDP | CBA, BALB/c  | CL/L. major                      | Protection | Antigen alone reduced the lesion size comparable to those of gp63 + BCG, protection induced by gp63 + adjuvant varied depending on the site of vaccination relative to that of the challenge | 130  |
| rgp63   | *C. parvum*              | BALB/c       | CL                               | No protection | ---- | 131 |
| rgp63   | *E. coli*                | Monkeys      | CL/L. major                      | Partial protection | Positive DTH response, no IFN-γ production, high IgG antibody level | 132 |
| gp63    | Liposomes liposomes + CFA | CBA          | CL/L. mexicana                   | Protection | The protection conferred only by gp63 + liposomes | 133 |
| rgp63   | *S. typhimurium*         | BALB/c       | CL/L. major                      | Protection | Activated T cells secreted IFN-γ and IL-2 but not IL-4, high IgG2a levels, no IgG1, negative DTH response. | 134 |
| rgp63   | *S. typhimurium*         | BALB/c       | CL and VL/L. major or *L. donovani* | Protection | Protection induced against both species, high IFN-γ level, IL-2, and IL-4 not detectable, negative DTH response. | 135 |
| rgp63   | *S. typhimurium*         | F1 (BALB/c C57BL/6) | CL/L. mexicana                   | Protection | High IFN-γ and IL-2 mRNA expression but not IL-4 and IL-10 | 136, 137 |
| rgp63   | Transfected BCG          | BALB/c CBA/J | CL/L. mexicana or L. major       | Protection | Protection against *L. mexicana* and *L. major* in both mouse strains, strong lymphoproliferative response. | 136, 137 |
| gp63    | Cationic liposomes       | BALB/c       | VL/L. donovani                   | Protection | 86% and 81% parasite reduction in liver and spleen respectively, high IFN-γ and IgG2a levels even after challenge, low IL-4 production, positive DTH response. | 139 |
| Antigen       | Adjuvant/delivery system | Animal model | Targeted disease (Leishmania spp.) | Result                                                                 | Other outcomes                                                                 | Ref. |
|---------------|--------------------------|--------------|-----------------------------------|------------------------------------------------------------------------|--------------------------------------------------------------------------------|------|
| gp63 or rgp63 | *E. coli*                | Human        | CL/VL                             | Protection                                                             | Strong proliferative response to both species, high IFN-γ production in PBMC culture upon antigen stimulation. | 140  |
| Peptide PT3 of gp63 | Poloxamer or CFA or DC pulsed | BALB/c       | CL/L. major                       | Protection                                                             | Protection only by PT3 (p154–168), enhanced IL-2 but not IL-4 production, no lesion in the second study while reduced lesion development in the third study | 141-144 |
| rgp63         | Transfected L929 cells with CD40L + gp63 | BALB/c C57BL/6 | CL/L. major or L. amazonensis    | Protection                                                             | Both strains of mice protected against both parasite species, high IL-12 production | 145  |
| M-2           | *C. parvum* Saponin CFA | CBA BALB/c C57BL/6 | CL/L. amazonensis                | Variable protection                                                   | *C. parvum* gave better results, followed by saponin, complete protection in CBA, partial in BALB/c, and no protection in C57BL/6, protection correlated with increased IgG1 and IgG2 | 146  |
| GP46/M-2      | Vaccinia virus           | BALB/c       | CL/L. amazonensis                | Protection                                                             | IL-2, IFN-γ, and IL-4 production, high IgG1, IgG2a, and IgM with low IgG3 and IgG2b | 147  |
| PSA-2         | *C. parvum*              | C3H/He       | CL/L. major                       | Protection                                                             | 100-fold parasite reduction, predominant IgG1 with IgG2a and IgG2b before the challenge, high IFN-γ but no IL-4 level | 148  |
| rPSA-2        | Transfected *E. coli* + *C. parvum* ISCOM | C3H/He       | CL/L. major                       | No protection                                                         | High IFN-γ production, high IgG1, IgG2a, IgG2b, and weak IgG3 | 149  |
| LACK/rp24     | IL-12                    | BALB/c       | CL/L. major                       | Protection                                                             | Upregulation of IFN-γ and downregulation of IL-4 transcripts | 150  |
| rLACK         | rIL-12                   | BALB/c       | CL/L. major                       | Protection                                                             | Mice protected only when challenged after two weeks of last immunization, not protected when challenged after 12 weeks of immunization, high IFN-γ (after two weeks) | 87   |
| Antigen | Adjuvant/delivery system | Animal model | Targeted disease (Leishmania spp.) | Result | Other outcomes |
|---------|--------------------------|--------------|-----------------------------------|--------|---------------|
| rLACK   | rIL-12                   | BALB/c       | CL/L. amazonensis                | Protection | After the challenge, the IFN-γ level decreased to the levels of IL-10 and IL-4, high anti-LACK and parasite-specific antibodies. | 151 |
| rLACK   | -----                    | BALB/c       | CL/L. amazonensis                | No protection | A slight increase in IFN-γ level, IL-10, and IL-4 levels comparable to PBS control. | 152 |
| FML     | Saponin                  | BALB/c       | VL/L. donovani                   | Protection | 84.4% reduction in liver parasite burden, 79.1% and 89.1% increase in proliferative and antibody responses respectively, high antibody level. | 153 |
| FML     | Saponin                  | BALB/c       | VL/L. donovani                   | Protection | 94.7% liver parasite reduction, no change in IFN-γ level while significant decrease in IL-10 production, high DTH response, increase in IgG, IgM, IgG1, IgG2a, and IgG2b anti-FML antibodies. | 154 |
| FML     | Saponin                  | Swiss albino | VL/L. donovani                   | Protection | 85.5% reduction in liver parasite burden, 80% increase in the antibody response | 155 |
| FML     | Saponin                    | Swiss albino | VL/L. donovani                   | Protection | 85% and 88% liver parasite reduction in FML + saponin and FML + Al(OH)3 group respectively, increased IgG2a level in the former group, similar IgG2b, and IgG3 in both vaccines | 156 |
| FML     | Saponin                  | Hamster      | VL/L. donovani                   | Protection | Positive DTH response, high anti-FML antibodies. | 157 |
| FML     | Saponins (Riedel De Haen(R), QuilA, Qs21), IL-12 | Swiss Albino | VL/L. donovani                   | Protection | High anti-FML IgG1, IgG2a, and IgG2b, positive DTH response, 73%, 93%, and 79.2% liver parasite reduction in R-FML, QuilA-FML, and Qs21-FML vaccinees respectively, high IFN-γ level in Qs21-FML and R-FML vaccines | 158 |
| FML     | Fractions of Riedel De Haen—QS21 and deacylsaponins | Swiss Albino | VL/L. chagasi                   | Protection | 95% and 86% liver parasite reduction in QS21-FML and deacylsaponins-FML vaccinees respectively, positive DTH response, high IFN-γ production, high IgG, IgG1, IgG2a, IgG2b, and IgG3 in Qs21-FML vaccinees but not in deacylsaponins | 159 |
| GP36    | Saponin                  | BALB/c       | VL/L. donovani                   | Protection | 68.1% liver parasite reduction, high IgG2a, IgG2b, and IgG1 antibodies, positive DTH response | 160 |
| Antigen | Adjuvant/delivery system | Animal model | Targeted disease (Leishmania spp.) | Result | Other outcomes | Ref. |
|---------|--------------------------|--------------|-----------------------------------|--------|---------------|------|
| FML     | -----                    | Dogs         | VL                                | Protection | 92% protection achieved after two years, vaccinees showed positive DTH response. | 161  |
| FML     | QuilA                    | Dogs         | VL                                | Protection | 95% protection achieved, positive DTH response. | 162  |
| FML     | QuilA                    | Dogs         | VL/L. donovant                    | Protection | 60% dogs protected, high anti-FML IgG, IgG2 | 163  |
| FML     | Saponin                  | Dogs         | VL                                | Protection | 90% dogs protected, 79–95% positive DTH response, high IgG2 than IgG1 | 162  |
| FML     | Saponin                  | Dogs         | VL                                | Protection | High anti-FML antibodies, 82.7% positive DTH response, increase in CD8+ TandCD21+ Bcells | 164  |
| FML     | Saponin                  | Dogs         | VL                                | Protection | Act as a transmission-blocking vaccine, high IFN-γ, NO. and IgG2 production, high CD8+ T cell proliferation | 165-168 |
| LiESA   | MDP                      | Dogs         | VL/L. infantum                    | Protection | 92% vaccine efficacy, high IgG2 level, enhanced IFN-γ and no production while no change in IL-4 level | 169, 170 |
| LiESA   | MDP                      | Dogs         | VL/L. infantum                    | Protection | Increased IFN-γ and anti-LiESA IgG2. level, positive DTH response | 171  |
| Recombinant CP (rCP5) | IL-12                    | C57BL/6      | CL/L. mexicana                    | Protection | ----- | 172  |
| CP      | CFA                      | BALB/c       | CL/L. major                       | Protection | Enhanced splenocyte proliferation and IFN-γ level, no IL-5 production. | 173  |
| rCPA/rCPB | Poloxamer 407           | BALB/c       | CL/L. major                       | Partial protection | Only by rCPB, enhanced IFN-γ level, equal IgG1, and IgG2a antibody levels | 174  |
| rCPA/rCPB | Fused hybrid in pET23a   | BALB/c       | CL/L. major                       | Partial protection | High IgG2a, enhanced IFN-γ production with little IL-5 | 175  |
| Peptide I of CP | ----                    | CBA          | CL/L. amazonensis                | Protection | Enhanced IFN-γ, IL-4, and NO production, Proliferation of CD8+ T-cell subsets | 176  |
| Antigen | Adjuvant/delivery system | Animal model | Targeted disease (Leishmania spp.) | Result | Other outcomes |
|---------|--------------------------|--------------|----------------------------------|--------|----------------|
| rGRP78  | CFA                      | C57BL/6      | CL/L. major                      | Protection | 83% mice protected. |
| 78 kDa  | -----                    | BALB/c       | VL/L. donovani                   | -----   | Increase in IgG2a levels, low IgG1 |
| 78 kDa  | MPL-A, liposomal encapsulation, rIL-12, ALD, CFA | BALB/c | VL/L. donovani | Protection | 92%, 93.4%, and 98% liver parasite reduction by 78 kDa + MPL-A or liposomal encapsulation or rIL-12 vaccinees, enhanced IFN-γ and IL-2 levels with low IL-4 and IL-10, positive DTH response, high IgG2a level |
| P4      | C. parvum                | BALB/c       | CL/L. pifanoi/L. amazonensis    | Protection | Only P4 and P8 gave protection and P8 gave cross-protection, high IFN-γ level while no change in IL-2 level |
| P8      | -----                    | Dogs         | VL/L. infantum                  | -----   | High IFN-γ and TNF-α expression in P8-stimulated PBMC, low IL-4 but no IL-10 level |
| P4      | P. acnes                 | BALB/c       | CL/L. pifanoi                   | Protection | CD4⁺ T-cell related protection, high IFN-γ, MIF, TNF-α mRNA expression, high IL-2 level, and no change in IL-4 level |
| P8      | -----                    | Dogs         | VL/L. infantum                  | -----   | Enhanced IFN-γ and IL-2 levels in respective antigen-stimulated PBMC culture, extremely low IL-4 level |
| P4      | -----                    | Human        | CL                               | -----   | Enhanced IFN-γ level in P4-stimulated PBMC culture, IL-4 detectable |
| P4      | -----                    | Human        | CL                               | -----   | Enhanced IFN-γ level in P4-stimulated PBMC culture, IL-4 detectable |
| rA2     | P. acnes                 | BALB/c       | VL/L. donovani                   | Protection | 89% liver parasite reduction, enhanced IFN-γ level, no change in IL-4 level, high IgG1, IgG2a, IgG2b, and IgG3 |
| rA2     | -----                    | BALB/c       | VL/L. chagasi                    | Protection | High IFN-γ production, enhanced CTL activity mediated by CD8⁺ T cells, low antibody response |
| rA2     | Saponin                  | Dogs         | VL/L. chagasi                    | Partial protection | Enhanced IFN-γ while low IL-10 production, increased IgG and IgG2 but not IgG1 |
| rHASPB1 | IL-12                    | BALB/c       | VL/L. donovani                   | Protection | 91% liver and 70–90% splenic parasite reduction in rHASPB1 vaccinees, increased IL-12 production by DC, exclusive IgG1 response, increased IFN-γ producing CD8⁺ T cells. |
| Antigen       | Adjuvant/delivery system | Animal model | Targeted disease (Leishmania spp.) | Result               | Other outcomes                                                                 | Ref. |
|---------------|--------------------------|--------------|-----------------------------------|----------------------|-------------------------------------------------------------------------------|------|
| rHASPB1       | Montanide                | Dogs         | VL/L. infantum                    | Partial protection   | 50% dogs asymptomatic, high anti-HASPB1 antibody titer.                        | 190  |
| rLcr1         | CFA                      | BALB/c       | VL/L. chagasi                     | Partial protection   | In infected mice, high IFN-γ production in both mice, detectable IL-10 but not IL-5 levels in splenocytes to Lcr1 stimulation. | 191  |
| rLcr1         | Ribi adjuvant            | C57BL/6      | VL/L. chagasi                     | Protection           | High IFN-γ and reduced IL-10 production, no detectable IL-4.                  | 192  |
| rLcr1         | BCG expressing Lcr1      | BALB/c       | VL/L. chagasi                     | Protection           | High IFN-γ and reduced IL-10 production, no detectable IL-4.                  | 192  |
| rH1           | Montanide                | Monkeys      | CL/L. major                       | Partial protection   | High antibody levels, positive DTH response.                                 | 193  |
| rH1           | peptides of H            | BALB/c       | CL/L. major                       | Partial protection   | Partial protection even in absence of adjuvants, LP1-3 also gave partial protection. | 89   |
| rORFF         | CFA                      | BALB/c       | VL/L. donovani                    | Partial protection   | Detectable anti-ORFF antibody titer, the proliferation of spleen cells      | 194  |
| rORFF         | CpG ODN                  | BALB/c       | VL/L. donovani                    | Protection           | 84% liver parasite reduction, enhanced IFN-γ and IgG2α production, NO production dose-dependent. | 195  |
| rORFF         | ----                     | BALB/c       | VL/L. donovani                    | Partial protection   | 45–60% parasite reduction, low IgG2α/IgG1 ratio, high IFN-γ, and IL-12 as compared to controls. | 196  |
| rORFF         | IL-12 DNA                | BALB/c       | VL/L. donovani                    | Protection           | 82% parasite reduction, enhanced IFN-γ, IL-12, and IgG2α production, no change in IL-4 level, enhanced splenocyte proliferation. | 197  |
| rLiP0         | CpG ODN                  | C57BL/6      | CL/L. major                       | Protection           | Complete protection only in C57BL/6 mice, partial in BALB/c, 150-fold parasite reduction, high IFN-γ, and IgG2α production | 198  |
| Ribosomal proteins (LRP) | CpG ODN              | BALB/c       | VL/L. major                       | Protection           | Protection in both strains, 3 fold parasite reduction, high IFN-γ level and IgG2α/IgG1 ratio, no increase in IL-4, detectable IL-10 | 199  |
| rKMP-11       | ts-mutant expressing KMP-11 | BALB/c     | CL/L. major                       | Partial protection   | -----                                                                        | 200  |
| Antigen        | Adjuvant/delivery system | Animal model | Targeted disease (Leishmania spp.) | Result                  | Other outcomes                                                                                           | Ref. |
|---------------|--------------------------|--------------|-----------------------------------|-------------------------|---------------------------------------------------------------------------------------------------------|------|
| rKMP-11       | Hybrid cell vaccine      | BALB/c       | VL/L. donovani                     | Protection              | Enhanced IFN-γ, IL-4, and IL-13 expression but not IL-10                                                  | 201  |
| rPFR-2        | FIA                      | Hamster      | CL/L. panamensis/L. mexicana       | Protection              | Only female hamster protected against *L. panamensis*, positive DTH response, no protection against *L. Mexicana* | 202  |
| Protein Q     | BCG                      | Dogs         | VL/L. infantum                    | Protection              | 90% protection, positive DTH response,                                                                 | 203  |
| Protein Q     | CpG ODN                  | BALB/c       | VL/L. infantum                    | Protection              | 99% reduction in liver and splenic parasite burden, high IgG2a/IgG1 ratio, high IFN-γ with low IL-4 production | 204  |
| rTSA          | IL-12                    | BALB/c       | CL/L. major                       | Protection              | Protection only in rTSA-IL12 vaccinees, induce human PBMC proliferation.                                   | 205  |
| TSA LmSTI1    |                          |              |                                   |                         |                                                                                                         |      |
| TSA+LmSTI1    | IL-12                    | BALB/c       | CL/L. major                       | Protection              | The protection conferred in all three vaccinees group when adjuvant is used, significant protection by LmSTI1 + IL-12 and TSA + LmSTI1 + IL-12, partial by TSA + IL-12 | 206  |
| TSA+LmSTI1    | rhIL-12 + alum           | Monkeys      | CL/L. major                       | Protection              | No lesion development even on rechallenge after 4 months of first challenge.                               | 206  |
| rLMSTI1       | Encapsulation in liposomes| BALB/c     | CL/L. major                       | Protection              | High IgG level and IgG2a/IgG1 ratio                                                                    | 207  |
| rLMSTI1       | Encapsulation of antigen with CpG-ODN | BALB/c | CL/L. major                       | Protection              | High IgG titer and IgG2a/IgG1 ratio                                                                    | 208  |
| rLeish-111f   | MPL-SE                   | BALB/c       | CL/L. major                       | Protection              | Enhanced IFN-γ and IgG2a production, low IL-4 level                                                    | 209  |
| rLeish-111f   | MPL-SE + rmIL-12         | BALB/c       | CL/L. major                       | Protection              | Enhanced IFN-γ production, no detectable IL-4, mixed IgG1, and IgG2a response                           | 109  |

*Ref.* 109, 201, 202, 203, 204, 205, 206, 207, 208, 209

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| Antigen         | Adjuvant/delivery system | Animal model          | Targeted disease (Leishmania spp.) | Result                       | Other outcomes                                                                 |
|-----------------|--------------------------|-----------------------|-----------------------------------|------------------------------|--------------------------------------------------------------------------------|
| rLeish-111f     | MPL-SE                   | BALB/c C57BL/6 Syrian hamster | VL/L. infantum                   | Protection                   | 91.7% and 99.6% splenic parasite reduction in mice and hamster respectively, enhanced IFN-γ, IL-2, TNF production with low IL-4 level in mice |
| TSA+LmSTI1 + LeIF+Lbhsp83 | GM-CSF                  | Human                 | MCL                              | Protection                   | 83% of patients showed complete clinical cure (CC) after nine months, all were CC after a five-year follow-up |
| rTSA rLeIF rLbSTI1 rLACK | CpG ODN                | BALB/c                | CL/L. braziliensis                | No Protection                | Enhanced IFN-γ production in response to TSA or LeIF or LACK stimulation, high IgG1/IgG2a ratio |
| rTSA+rLeIF+ rLmSTI1 | MPL-SE AdjuPrime        | Dogs                  | VL/L. chagasi                    | ----                         | Induce Th1 response, specific IgG response to all three antigens, high IgG2a/IgG1 ratio when MPL-SE is used as compared to AdjuPrime |
| rMML            | MPL-SE AdjuPrime         | Dogs                  | VL/L. infantum                   | No protection                | 87% cumulative incidence in vaccines even after two years of vaccination |
| rLeish-110f+ Glucantime | MPL-SE                 | Dogs                  | VL/L. chagasi                    | protection                  | 83.3% and 66.6% survival rate by immunochemotherapy and chemotherapy respectively, high proliferative response, high antibody titer in immunotherapy as compared to immunochemotherapy |
Third-generation vaccines

DNA vaccines
These vaccines contain plasmid DNA, which, after injection, encodes foreign proteins, leading to the synthesis of endogenous proteins and the production of specific immune responses[84]. DNA vaccines promote both cellular and humoral immunity[88,89]. DNA vaccines can come in many forms, including recombinant proteins[87-89], single vaccines[89,90,93,96-100], or multigene forms[92,95,101]. These vaccines were tested in mice against CL and VL[84,85,86,91,94-95,99,101], in hamsters against VL[102,103], and dogs against VL[104-107]. DNA vaccines are made up of heterologous DNA (usually a plasmid) that produces antigenic proteins. These DNAs are supplied by vectors that allow them to be expressed in eukaryotic cells[84]. Advantages of DNA vaccines include (1) fast, simple, and cheap large-scale production, (2) no need for low temperature, transportation, and storage, and (3) the ability to provide long-term protection against multiple strains of Leishmania. The main concern with these vaccines is the risk of parasite DNA entering the mammalian genome. This problem carries the potential risk of cancer and autoimmune diseases[84]. A summary of DNA vaccines is given in Table 6 and the best recombinant salivary candidates is shown in Table 7.

Vaccine products for potential licensing
There are no licensed products yet, but potential candidates could be as follows[108]: (1) a mixture of recombinant proteins (Leish F1, Leish F2, and Leish F3), designed by Infectious Disease Research Institute (Seattle, USA), is currently in the second phase of a clinical trial; (2) recombinant proteins from Leishmania and sandfly saliva (phlebotomus) antigens, designed by Sabin product development partnership (Washington, USA)[19], is now in the preclinical phase. FML-QuilA (Leishmune®), a protein vaccine, was the first approved vaccine in Brazil in 2003. However, the license to produce and sell the vaccine was suspended in 2014, and its production was stopped by factories. The reason for discontinuation was the incompleteness of the third phase of the trial. There are presently two vaccines against canine VL: A2 Leishmanial Ag from Brazil and Li ESP/QA-21 from France[19].

DISCUSSION

Vaccines are undoubtedly the most effective way to control diseases. For this reason, the development of safe and cost-effective vaccines, particularly for the diseases with no available vaccine (e.g., leishmaniasis) is an important global public health priority. A major barrier to the development of an effective vaccine is related to the discrepancies between the animal models and human diseases, as well as the transition of the research from the laboratory to the field. Additionally, many questions related to the immune responses and maintenance of immunological memory during an active Leishmania infection have not yet been extensively studied or answered. This article tried to focus on the latest information related to antileishmanial vaccine development and also major problems with vaccine development and implementation. Candidates for the Leishmania vaccines include leishmanization, as well as the first-, second-, and third-generation vaccines. The development of an effective Leishmania vaccine poses many challenges, mainly related to the complexity of the immune responses to Leishmania, insufficient knowledge of Leishmania pathogenesis, and the discrepancy between the Old and New World parasites. It appears that a successful vaccine will most likely be composed of several antigens rather than a single one, which suggests that combination vaccines and well-developed adjuvants, such as Leish-111f and MPL-SE, have the best chances of success. Further clinical trials provide more information on the success of these combination vaccines. In addition, the poor efficacy of the killed and subunit vaccines makes the use of live-attenuated vaccines the next best alternative[109]. Many questions about antileishmanial immunity in humans have not yet been answered. It is not clear whether parasite persistence is required to maintain immunity in humans. Although parasite persistence in humans is unknown, it is worth noting that an experimental mouse model has revealed the persistence of the parasite following infection[110]. A study has been shown that the absence of parasites leads to the loss of immunity, implying that continuous antigen presence is needed for complete protection[22].

In contrast, another study in a mouse model has revealed that the maintenance of memory T-cells is independent of parasite persistence, and therefore vaccination with non-persistent strains and non-persistent, attenuated strains such as LdCEN or ΔPMM results in long-term protection[22]. In general, due to the complex nature of the immune response to Leishmania, it is crucial to better understand the determinants of T-cell for long-term immunity and the immunity factors affecting antileishmanial immunity before the development of an effective vaccine. Our understanding of the determinants of T cells is required for long-term protective immunity, although there are still many unknowns. It is hoped that new strategies will be developed to produce effective T-cell vaccines.
Table 6. Third-generation vaccines against *Leishmania*

| Antigen | Adjuvant/delivery system | Animal model | Targeted disease (Leishmania spp.) | Result | Other outcomes | Ref. |
|---------|---------------------------|--------------|----------------------------------|--------|----------------|------|
| gp63    | pCMV                      | BALB/c       | CL/L. major                      | Protection | Enhanced IL-12 and IFN-γ production, no detectable IL-4 | 94, 214 |
| gp63    | pCMV3ISS or pcDNA3        | BALB/c       | CL/L. major                      | Partial protection | 30% of mice protected, enhanced IFN-γ protection but not IL-4 | 94, 215 |
| gp63 or gp46 | VR1012               | BALB/c       | CL/L. mexicana                   | Partial protection | 100-fold parasite and 30% reduction in lesion size, mixed IgG2a and IgG1 response, high IgG2a/IgG1 ratio in gp46 vaccinee | 216, 217 |
| gp63 + gp46 + CPb | VR1012               | BALB/c       | CL/L. mexicana                   | Protection | 80% and 1,000-fold reduction in lesion size and parasite burden respectively | 216, 217 |
| ORFF    | pcDNA3.1                  | BALB/c       | VL/L. donovarsi                  | Protection | 78–80% and 58–60% reduction in liver and spleen parasites respectively, enhanced IFN-γ expression but no change in IL-4 expression | 99 |
| PSA-2   | pCI-neo                   | C3H/He       | CL/L. major                      | Protection | Enhanced IFN-γ production as compared to control, no detectable IL-4 and IL-5, high IgG2a/IgG1 ratio | 218, 219 |
| A2      | pcDNA3                    | BALB/c       | CL/VL L. amazonensis/L. chagasi  | Protection | Protection against both species enhanced IFN-γ with low IL-4 and IL-10 production | 220 |
| LACK    | pCI-neo                   | BALB/c       | VL/L. chagasi                    | Protection | Increased IFN-γ and IL-4 production with low IL-10 and TNF-α level | 221 |
| LACK    | pCI-neo                   | BALB/c       | VL/L. chagasi                    | No protection | Increased IFN-γ and IL-10 production with no IL-4 | 222 |
| LACK    | pCI-neo                   | BALB/c       | VL/L. chagasi                    | No protection | Enhanced IFN-γ with no IL-4 production | 223 |
| LACK    | pCMV3ISS                  | BALB/c       | CL/L. major                      | Partial to complete protection | Partial protection by LACK vaccine while complete in LACKp24 vaccinees | 94 |
| LACK    | MIDGE or MIDGE-NLS        | BALB/c       | CL/L. major                      | Protection | Enhanced IFN-γ production with no IL-4, high IgG2a/IgG1 ratio | 224 |
| Antigen | Adjuvant/delivery system | system | Targeted disease (Leishmania spp.) | Result | Other outcomes | Ref. |
|---------|--------------------------|--------|----------------------------------|--------|---------------|------|
| CPa or CPb | pCB6 | BALB/c | CL/L. major | Protection | Protection only in CPa + CPb vaccines increased IFN-γ level, but no IL-5 | 92 |
| CPb | VR1012 | BALB/c | CL/L. mexicana | Partial protection | 100-fold parasite and 50% reduction in lesion size | 216, 217 |
| KMP-11 | pCMV-LIC | Hamster | VL/L. donovani | Protection | Induced mixed Th1/Th2 response, enhanced IFN-γ, TNF-α, IL-12, iNOS expression including IL-4, low IL-10 level, high IgG2a, and IgG1 titer | 225 |
| KMP-11 | pCMV-LIC | BALB/c | VL/L. donovani | Protection | 96.7% and 98.7% reduction in splenic and liver parasite respectively, enhanced IFN-γ and IL-4 production, suppressed IL-10 level | 226 |
| KMP-11 | pCMV-LIC+IL-12 | BALB/c | CL/L. major | Protection | 93% reduction in lesion size, enhanced IFN-γ with suppressed IL-4 and IL-10 production | 226 |
| P4 | pcDNA3+IL-12 or HSP70 | BALB/c | CL/L. amazonensis | Partial to complete protection | Complete protection with enhanced IFN-γ and TNF-α, low IL-10 production in P4 + IL-12 vaccines while partial with mixed IFN-γ and IL-10 response in P4 + HSP70 vaccines | 101 |
| NH36 | VR1012 | BALB/c | VL/L. chagasi | Protection | 91% liver parasite reduction, increased IFN-γ with reduced IL-10 and IL-4 levels, positive DTH response, high IgG2b titer | 227 |
| papLe22 | pcDNA3.1 | Hamster | VL/L. infantum | Partial protection | Parasite circulation reduced by 50%, produce high anti-pepLe22 but low anti-Leishmania antibody titer | 91 |
| NH | VR1012 | BALB/c | CL/VL L. amazonensis/L. chagasi | No protection | Enhanced IFN-γ, IL-4, and IL-10 production | 228 |
| NH36 | VR1012 | BALB/c | CL/VL L. chagasi/L. mexicana | Protection | 88% and 65% reduction in L. chagasi parasite burden and L. mexicana infected lesion size respectively, 2–5 fold increase in IFN-γ producing CD4+ T cells, low antibody response, positive DTH response to L. donovani | 96 |
| Antigen | Adjuvant/delivery system | Animal model | Targeted disease (Leishmania spp.) | Result | Other outcomes | Ref. |
|---------|-------------------------|--------------|-----------------------------------|--------|---------------|------|
| LeIF PSA-2 | pCMV3ISS | BALB/c | CL/L. major | No protection | Protection induced by all three vaccines enhanced IFN-α production with no IL-4, high IgG2a titer | 94 |
| TSA LmSTI1 | pcDNA3 | BALB/c | CL/L. major | Protection | Enhanced IFN-γ with little IL-4 production, low antibody response dominated by IgG2a | 93 |
| TSA+LmSTI | pcDNA3 | BALB/c | CL/L. major | Protection | Enhanced IFN-γ with little IL-4 production, low antibody response dominated by IgG2a | 95 |
| H2A+H2B+H3+H4 | pcDNA3 | BALB/c | CL/L. major | Protection | Enhanced IFN-γ with little IL-4 production, low antibody response dominated by IgG2a | 97 |
| KMPH+TRYP+LACK+gp63 | pMOK | Dogs | VL/L. infantum | No protection | Increased anti- Leishmania IgG, IgA, and IgM | 97 |
| LACK-PB | pcDNA3-vaccinia virus | BALB/c | CL/L. major | Protection | 1,000 fold and 70% decrease in parasite burden and lesion size respectively, increased IFN-γ level with low IL-10 and IL-4 levels | 229 |

### Heterologous prime-boost vaccine

- **LACK-PB** | pCI-neo—vaccinia virus | Dogs | VL/L. infantum | Protection | 60% of dogs protected, enhanced IFN-γ and IL-4 expression, high IgG2a/IgG1 ratio | 106 |
- **LACK-PB** | pcDNA3.1 + IL-12 DNA or IL-18 DNA—vaccinia virus | BALB/c | CL/L. major | Protection | Enhanced IFN-γ production, high IgG2a/IgG1 ratio | 230 |
- **LACK-PB** | pCI-neo—MVA | BALB/c | CL/L. major | Protection | 65–92% reduction in lesion size, increased IFN-γ and TNF-α levels | 231 |
- **LACK-PB** | MVA | BALB/c | VL/L. infantum | Protection | 144–244, 6–9, and 9–30 fold parasite reduction in the lymph node, spleen, and liver respectively, increased IFN-γ and TNF-α levels | 232 |
- **LACK-PB** | pcDNA3—Salmonella enterica serovar Typhimurium | BALB/c | CL/L. major | Protection | Increased IFN-γ level with low IL-10, high IgG2a titer | 233 |
Table 7. The best recombinant salivary candidates as antigens for detection of anti-saliva antibodies

| Recombinant protein | Protein family | Sandfly species | Host species | Reference |
|---------------------|----------------|-----------------|--------------|-----------|
| LJM17               | YRP            | Lu. longipalpis | dog, fox, human | 238,239 |
| LJM11               | YRP            | Lu. longipalpis | human, dog, chicken | 238,240 |
| LJM17+LJM11         | YRP            | Lu. longipalpis | human        | 238       |
| rPpSP32             | SP32-like      | Phlebotomus papatasi | human    | 241-243   |
| rPorSP24            | YRP            | P. orientalis   | sheep, goat, dog | 244       |
| rSP03B              | YRP            | P. perniciosus  | mouse, dog, hare, rabbit | 245-248   |
| rSP01               | apyrase        | P. perniciosus  | mouse, dog   | 245       |
| rSP01B              | apyrase        | P. perniciosus  | mouse, dog, hare, rabbit | 245,246,249 |

Lu. Longipalpis, Lutzomyia longipalpis
The most important thing to consider before making a *Leishmania* vaccine is to determine the best immunity correlations, as well as to develop efficient delivery systems and improved adjuvants. According to advanced research in parasite immunology and genetic engineering, an effective anti-*Leishmania* vaccine not far away. In this study, data extraction was performed by two researchers, which may result in errors. Searching for English language and scientific articles in other languages, which may have valuable information from Africa, the Middle East, and Asia, were limited. Despite these limitations, the present study attempted to review the content of credible articles that lead to clear and up-to-date information on the performance and effectiveness of various vaccines designed against leishmaniasis.

Given the global importance of leishmaniasis, decisive measures must be taken to prevent this disease with social impacts. It seems that one of the effective ways to control leishmaniasis is immunization of people living in endemic areas of the disease. In this review, it was found that an effective vaccine against leishmaniasis is not yet available, and scientists in this field have chosen different methods to produce such a vaccine. The results of these efforts have been the production of three different generations of *Leishmania* vaccines. In any case, summarizing the results of these studies and trying to clarify as much as possible the ambiguities in the immunity of leishmaniasis and especially the interaction of the parasite with host cells will help to advance in the right direction. Understanding more about the unknown mechanisms of the behavior of the parasites inside the host body will persuade us to produce an effective vaccine against the disease.

**CONFLICT OF INTEREST.** None declared.

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