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Cutaneous Leishmaniasis: Update on Vaccine Development

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Abstract

Leishmaniasis is an important disease mediated by the protozoan parasite *Leishmania* via the bite of the female sandfly insect vector. Leishmaniasis is endemic in the tropical and subtropical regions. The most common form of the disease is cutaneous leishmaniasis, which affects more than 10 million people worldwide and includes at least 1.5 million new cases every year. So far, treatment of the disease relies on unsatisfactory chemotherapy that can be complicated by the rising appearance of drug-resistant parasites. Furthermore, it is challenging to achieve solid control of the insect vector and animal reservoir. Therefore, the development of a safe and effective vaccine is urgently needed for the treatment and prevention of leishmaniasis. This review focuses on the recent advances in the development of a safe vaccine that could be used for prevention and treatment of cutaneous leishmaniasis. A short outlook for future research efforts is also presented.

Keywords

Cutaneous leishmaniasis; vaccine; immunotherapy

Introduction

Leishmaniasis represents an important global health problem in tropical and subtropical areas, affecting at least 12 million people worldwide. Each year, 2 million new cases arise and 350 million humans are at risk of contracting this disease in more than 88 countries.¹ The World Health Organization still considers leishmaniasis as one of the emerging uncontrolled diseases affecting mainly poor regions. Transmission of the disease is achieved through the injection of single-celled parasites by infected female sandflies of the genus *Phlebotomus* in the Old World and *Lutzomyia* in the New World. The most common form of leishmaniasis experienced worldwide, as well as in the United States, is the cutaneous form of leishmaniasis (CL), which is caused by approximately 20 species of *Leishmania*. Cutaneous form of leishmaniasis is considered a zoonotic disease as it is typically passed on

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from vertebrate animals to humans, who are accidental hosts. Various forms of CL exist. The localized cutaneous form is characterized by a self-healing lesion at the site of the bite and is caused primarily by *Leishmania major*, *Leishmania tropica*, and *Leishmania aethiopica* in the Old World, and *Leishmania amazonensis* and *Leishmania mexicana* in the New World. *Leishmania braziliensis*, *Leishmania panamensis*, and *Leishmania guyanensis* account for the more severe form of CL called mucocutaneous leishmaniasis, which is only prevalent in the New World and affects the mouth, nose, and occasionally the ear tissues.^{2,3} Mucocutaneous leishmaniasis is hard to treat and is often associated with secondary infections that can be lethal. Diffuse cutaneous leishmaniasis is caused primarily by *L mexicana* and *L amazonensis* and is characterized by lesions that spread from the site of infection and may cover the whole body. *Leishmania donovani* and *Leishmania infantum* are the causative agents of the most severe form of the disease, visceral leishmaniasis, which is lethal if not treated. Occasionally, patients cured of *L donovani* infection exhibit a syndrome called post kala-azar dermal leishmaniasis.

Leishmania infection predominantly triggers a T-cell-mediated immune response. Shortly after infection, *Leishmania* parasites are phagocytosed by neutrophils, macrophages, and dendritic cells (DCs). Although neutrophils are among the first cells recruited to the site of infection, their role in disease progression and control is controversial and depends on the strain of the parasite and mice (reviewed in previous works⁴⁻⁷). Dendritic cell functions primarily in antigen processing and presentation for T-cell priming, leading to CD4+ or CD8+ polarization (reviewed in the study by Feijo et al⁸). Dendritic cell also secretes most of the cytokine interleukin 12 (IL-12), which is necessary for the induction of a protective helper T type 1 (T_H1) response characterized by the production of IFN- γ and tumor necrosis factor α (TNF- α).⁹⁻¹² On activation with IFN- γ and/or TNF- α , macrophages efficiently kill most parasites by inducing the generation of nitric oxide (NO) and reactive oxygen species.^{13,14} In contrast, a helper T type 2 (T_H2)-type response leads to disease progression and is associated with the production of interleukin 10 (IL-10), interleukin 2 (IL-2), and interleukin 4 (IL-4).^{15,16} Lasting protection against *Leishmania* is mediated by several subsets of memory T cells (T effector, CD4+) and involves the production of cytokines IL-2, IL-4, and IFN- γ .^{17,18}

The treatment of CL relies primarily on inadequate, expensive chemotherapeutic drugs that display several undesirable side effects and can be sometimes difficult to administer.¹⁹ In addition, the rising appearance of drug-resistant parasites complicates the drug treatment of leishmaniasis, and controlling the sandflies' and/or animal reservoirs represents a real challenge. These compelling facts combined with the rising occurrence of CL make the development of a safe, effective vaccine a necessity for the prevention and treatment of CL.

The development of a CL vaccine has been met by several challenges. Specifically, varying genetic characteristics of individual hosts and parasites and, more importantly, the varying immune responses caused by different *Leishmania* species make the development of CL vaccine incredibly complex. An effective vaccination geared toward combating the disease must not only be safe and easily accessible but also be capable of efficiently sustaining the prolonged induction of CD4+ and CD8+ memory T cells (reviewed in the study by Glennie and Scott¹⁸). This induction is essential and allows the immune system to efficiently respond

to a pathogen previously encountered, contributing to a lifelong protection against CL. The various vaccination strategies are described in the following sections and summarized in Table 1.

Live Vaccination

Attempts to contrive an effective vaccine date back to the early 20th century when live parasites were first inoculated in healthy individuals through a process known as leishmanization. This procedure led to a lifelong immunity and provided the rational proof that vaccination against leishmaniasis may be possible. Due to safety concerns and problem in standardization, leishmanization was later discontinued in most countries.⁶⁰ However, the traditional practice of leishmanization is making a strong comeback in certain endemic regions because it mimics a natural infection.⁶¹ Due to its potential efficacy, efforts to develop a safer leishmanization process by concomitant stimulation of the immune system to control the growth of the parasite are currently underway.^{62–65}

Whole-Killed Vaccines

Whole-killed parasites of strains *L. major*, *L. guyanensis*, *L. braziliensis*, and *L. amazonensis* (alone or in combination) were tested in human trials but were ineffective in mediating protection. These parasites provided poor antigens and thus did not trigger a robust immune response, even in the presence of adjuvants (summarized in the study by Noazin et al²⁰). The main advantages of using whole-killed parasite vaccines are their safety and easiness in mass production. Unfortunately, intramuscular vaccination of Balb/C mice with merthiolate-killed *L. amazonensis* antigens *LaAg* (Leishvacin) enhanced susceptibility to cutaneous leishmaniasis due to overproduction of transforming growth factor β (TGF- β).⁶⁶ Phase 3 trial showed that 3-time intramuscular injection of the Leishvacin formula failed to mediate protection in human subjects.²¹ However, more recent studies demonstrated that intranasal vaccination using the same antigens provided protection against *L. amazonensis* and *L. braziliensis* in a mouse and hamster model of infection, respectively, proving that the route of administration plays a critical role in the efficacy of a vaccine.^{67,68}

Live Attenuated Parasites

Due to the inefficacy of the whole-killed vaccines, there has been a consequent shift toward “live attenuated” vaccines, which seem to provide a more advantageous substitute. By mimicking the actions of the naturally occurring *Leishmania* infection, the live attenuated parasites can present a wide variety of antigens to the antigen-presenting cells, leading to a more effective immune response and a better overall defensive result. Nonvirulent microorganisms were generated by knocking out specific virulence genes or alternatively by subjecting the parasites to irradiation⁶⁹ or long-term in vitro cultivation.

Null mutants as vaccine candidates

The first null mutant strains used as vaccine candidates were developed in the 1990s when genetic manipulation of the parasite became possible.⁷⁰ Vaccination with a null mutant was originally achieved with dihydrofolate reductase thymidylate synthase (*dhfr-ts*^{-/-}) knockout

parasites, which induced substantial protection against both *L major* and *L amazonensis* infections in mice but failed to prevent infection in monkeys.^{22–24} Null mutant of *linJhsp70-II*^{−/−} (heat shock protein 70-II) of *L infantum* protected Balb/C mice against *L major* infection, induced NO production, and triggered a T_H1 immune response. In addition, this strain failed to form a lesion in immunodeficient mice, suggesting that it is a safe vaccine candidate.²⁵ More recently, *Idcen*^{−/−} and *ldp27*^{−/−}, lacking *CENTRIN* or *P27* gene, respectively, were tested against *L mexicana* infection and found to be effective in protecting Balb/C mice.²⁶ However, *LdCen*^{−/−} conferred only partial protection against *L braziliensis*.²⁷ Cysteine proteinase-deficient mutants of *L mexicana* (CP-deficient *L mexicana*) were effective in protecting hamsters against homologous challenge by eliciting significantly lower levels of T_H2-associated cytokines IL-10 and TGF-β than the corresponding wild type.²⁸ None of these null mutant strains has been tested in other animal models yet. Despite encouraging results, null mutants as vaccine candidates may revert to a virulent form and thus create a true concern regarding their safety.⁷¹

“Suicidal” parasites as vaccination tools

“Suicidal” parasites are transgenic lines of *Leishmania* that are designed so they can be killed either by physical methods or by application of a specific drug. Therefore, their growth within a host can be precisely controlled, making these strains safer than their live virulent counterparts. Delta-aminolevulinic dehydratase and porphobilinogen deaminase are absent in *Leishmania*, and thus, expression of these enzymes render the transgenic parasites sensitive to UV irradiation.²⁹ Vaccination with such a transgenic line led to a 99% reduction in parasitic load.³⁰ Another study demonstrated that transgenic parasites *lmtkcd*^{+/+}, expressing thymidine kinase and cytosine deaminase, become sensitive to the drugs ganciclovir and 5-fluorocytosine. Balb/C mice lesions were cured in 2 weeks in the presence of these drugs, and the transgenic line mediated complete protection when wild-type *L major* was injected 8 days after vaccination.⁷² However, development of drug resistance is a plausible risk associated with the latter vaccination protocol.

Purified Antigens and Recombinant Subunits

In recent studies, more than 30 different *Leishmania* recombinant subunits and purified antigens have been identified and tested in animal models (reviewed in the study by Okwor and Uzonna⁶), but most of these models were assessed against *L donovani*, the causative agent of visceral leishmaniasis, the most severe form of the disease. Recombinant subunits or antigens are very safe and relatively easy to produce in large quantities but need to be co-injected with an adjuvant to stimulate the immune system. In addition, several injections (boosts) may be required to induce a satisfactory immune response. Recombinant proteins are typically expressed using a heterologous microbial system, whereas others, known as synthetic vaccines, are produced in vitro as short polypeptides that are predicted to be immunogenic. Synthetic vaccines are considered much safer than vaccines originating from a parasite. Purified antigens originate from the parasite, and their isolation protocol may be difficult to upscale or may contain contaminants. Regarding purified antigens, much work has been done with parasite cell surface metalloprotease GP63, which conferred only partial protection in monkeys but mediated robust protection in mice against challenge with both *L*

mexicana and *L. major*.^{31–33} *Leishmania* homolog of receptors for activated C kinase (LACK) has also attracted much interest as a vaccine candidate because it is expressed in both insect and vertebrate host form of the parasite.³⁴ Mice vaccinated with LACK became resistant to *L. major* infection.³⁵ Other examples of antigens are *L. major* H2B histone protein and its divergent N-terminal region, which were tested for their ability to protect against CL and visceral leishmaniasis in the presence of the adjuvant CpG.³⁶ Immunization with sterol 24-C-methyltransferase sterol methyl transferase of *L. infantum*, formulated with monophosphoryl lipid A as adjuvant, cross-protected mice against CL caused by *L. major*.³⁷

Examples of recombinant antigens include histone H1, which was tested in vervet monkeys in the presence of the Montanide ISA 720 adjuvant and showed reduced lesion formation after *L. major* infection that self-healed with time,³⁸ suggesting a good potential for human vaccination. Fusion protein made of CP A and B from *L. major* and cysteine protease from *L. pifanoi* mediated only partial protection in mice.^{39,40} KSAC is a recombinant protein made of KMP-11, SMT, A2 and CPB that when injected with the toll-like receptor (TLR)-4 agonist glucopyranosyl lipid A protected against cutaneous disease following sandfly transmission of *L. major* in susceptible Balb/C mice.⁴¹ With the idea to develop a pan-*Leishmania* vaccine, *L. major* recombinant ribosomal proteins L3 and L5 combined with CpG-oligo-deoxynucleotides conferred protection against *L. major* and *L. braziliensis* challenge in Balb/C mice by inducing a T_H1 response.⁴² Leish-111F, an antigen made of 3 fused proteins (*L. major* thiol-specific antioxidant thiol-specific antioxidant [TSA], *L. major* stress-inducible protein-1 [STI1], and *L. braziliensis* elongation and initiation factor) rendered mice resistant to *L. major* infection.^{43,44} This antigen, combined with the adjuvant monophosphoryl lipid A plus squalene (MPL-SE), was the first defined vaccine candidate that was tested in human phase 1 and 2 clinical trials and was found to be safe and immunogenic.⁴⁵ It is still unclear whether Leish-111F confers protection in humans; however, optimization of Leish-111F system is currently underway.¹ The C-terminal and N-terminal domains of *L. donovani* nucleoside hydrolase vaccines also decreased the footpad lesion formation caused by *L. amazonensis*.⁷³ Although numerous purified and recombinant antigens have been tested successfully in animals, no human trials have yet been attempted. It is encouraging to observe that cross-species reactivity exists with several species-specific antigens, opening the possibility of a “pan” anti-*Leishmania* vaccine. Although there is no lack of antigen candidates, the challenge lies in identifying the proper adjuvant(s) that will induce a robust protective immunity.

DNA Vaccines

DNA vaccines, also referred to as third-generation vaccines, are the newest approach in vaccine development. The main advantage of DNA vaccines is that they induce a stronger immune response against the encoded antigen⁷⁴ by providing a constant source of antigen in its native configuration. Furthermore, they are safe, relatively easy to administer, and preferentially induce a T_H1 immune response.⁷⁵ Similar to purified or recombinant antigens, DNA vaccines may require the co-injection of adjuvants and several boosts to induce a satisfactory protective immune response.

The gene coding for surface metalloprotease GP63 was the first DNA vaccine developed against leishmaniasis. Expression of GP63 in mice mediated solid protection against *L major* infection when DNA was injected or when GP63 was expressed in attenuated *Salmonella typhimurium*.^{46–48} *Leishmania* homolog of receptors for activated C kinase antigen is the most extensively studied DNA vaccine against *Leishmania*. In clinical trials, inclusion of IL-12 increased the protection of LACK compared with LACK alone.³⁵ DNA-encoding A2 protein mediated protection against *L amazonensis* infection in mice in contrast to heat shock protein 20 (HSP20) and surface protein 2.^{49,50} More recently, the TSA-based DNA vaccine was successful in controlling *L major* challenge via a T_H1 immune response.⁷⁶ Iron superoxide dismutase of *L donovani* protected Balb/C mice against *L amazonensis* infection by inducing IFN- γ production which led to reduced parasitism.⁵¹ Further studies that involved vaccination with plasmid pcDNA3H3H4 expressing *L major* histone proteins H3 and H4 resulted in partial resistance to *L major* challenge associated with the development of mixed T_H1/T_H2-type response and a reduction in the number of parasite-specific regulatory T cells at the site of infection.⁷⁷ Vaccination with DNA-encoding *L infantum* histone genes H2A, H2B, H3, and H4 also controlled both *L major* and *L braziliensis* infections in Balb/C mice.^{52,53} Addition of KMP-11 (kinetoplastid membrane protein-11), A2, and HSP70 genes to H2A, H2B, H3, and H4 in the form of HisAK70 DNA vaccine was successful in clearing parasites from the liver in a mouse model of visceral leishmaniasis and resulted in 100% inhibition of parasite visceralization in the CL model.⁵⁴ Therefore, the enhanced DNA vaccine provided cross-protection against both CL and visceral leishmaniasis (*L major* and *L infantum*).⁵⁴ The overall efficacy of this vaccine was attributed to the ability of the immunized mice to control key factors such as IFN- γ , IL-10, and IL-4 activity. The promising nature of the HisAK70 once again reinforces the common belief that development of an effective antileishmanial vaccine is entirely possible, and that HisAK70 may play an integral role in such development. Although the results were more than promising in the mouse model, such success has yet to be translated in primates and humans.

Sandfly Saliva Components

During the infection process, a sandfly injects parasites as well as saliva components, which have been shown to help the establishment of infection.⁷⁸ Similar to immunization with parasite antigens, immunization with sandfly saliva components is very safe. In addition, previous studies have examined sandfly saliva as a transmission blocking vaccine candidate. Pre-exposure to the saliva of the *P papatasi* sandfly mediated protection against *L major* challenge by inducing strong IFN- γ production.^{55,56} Furthermore, *P papatasi* PpSP15, a component of the sandfly saliva, when expressed and secreted by the nonpathogenic strain *L tarentolae* in combination with CpG as a prime boost conferred resistance to *L major* infection in mice.⁵⁷ Similarly, immunization with recombinant *Ljml1* or salivary gland extracts from *L longipalpis* saliva mediated protection against *L major* infected sandfly bites and *L braziliensis*, respectively, via induction of IFN- γ .⁵⁸ In contrast, *L intermedia* salivary gland sonicate failed to control *L braziliensis* infection and even increased disease progression due to low IFN- γ to IL-4 ratio.⁵⁹ These conflicting results suggest that (1) salivary gland components have the ability to change the immune response of mice by either increasing susceptibility or resistance and (2) use of sandfly saliva components may not be a

suitable strategy for all strains of *Leishmania*. Because leishmanization seems to be an effective procedure that does not involve the sandfly, sandfly components may not be essential to the development of an effective *Leishmania* vaccine but may still be useful against certain strains of *Leishmania*.

Immunotherapy

Leishmania is an intracellular parasite; thus, control of its infection is T-cell mediated. Both CD4+ and CD8+ T cells are important for primary immunity against *L major*, even though their contributions vary depending on the strain of *Leishmania* (reviewed in the study by Glennie and Scott¹⁸). CD4+ T_H1 cells produce IFN- γ and TNF- α that activate macrophages, resulting in parasite elimination in resistant mice.¹³ In contrast, the early production of IL-4 promotes differentiation and proliferation of T_H2 cells, resulting in disease progression in susceptible mice.⁷⁹ The amount of IL-12 produced by DC at the initial phase of infection determines the outcome of the infection. Low levels of IL-12 lead to a T_H2 immune response, whereas high amounts of IL-12 result in a T_H1 immune response.⁸⁰ However, IL-4 and IL-13 synergize to mediate susceptibility to *L major* infection.^{81,82} Other important cytokines that regulate the disease progression are IL-10 and IL-17, which favor parasite survival and disease progression.^{83,84}

Recovery from *Leishmania* infection leads to infection-induced resistance, which is the underlying principle of leishmanization and by extension, of lifelong immunity. A thorough understanding of the molecular processes involved in infection-induced immunity is critical for vaccine development. In mice, infection-induced immunity is characterized by IFN- γ producing CD4+ T_H1 cells. Stimulation and maintenance of T_H1 cells are mediated by IL-12, which is secreted by antigen-presenting cells such as DCs. Because CD8+ cells can also produce IFN- γ , they are believed to contribute to *L major* immunity by suppressing the early CD4+ T_H2 cell development. IL 12 promotes a T_H1 response in a mouse model of CL for long-term immunity.^{85,86} Consistent with this concept, inclusion of IL-12 as part of a DNA vaccine cocktail improved protection against *L major* challenge.^{87–89} However, administration of IL-12 in humans is toxic; thus, this strategy is not suitable for human vaccination.^{90,91}

Several studies have demonstrated that complete clearance of the parasite by a T_H1 immune response, which is desirable for the safety of a patient, is, however, not sufficient in mediating long-term immunity.^{6,92} It is also well accepted that sustained controlled stimulation of IFN- γ -producing long-lived memory CD4+ T cells is necessary to confer long-term immunity.¹⁸ This can be accomplished by having a small population of persistent parasites or by “boosting” several times to maintain protection.^{93–95} Alternatively, adjuvants need to be added to the vaccine cocktail to elicit the proper immune response.

Targeting TLRs for vaccine development

Toll-like receptors are a collection of 13 eleven-transmembrane proteins that recognize structurally conserved molecules derived from pathogens and play a role in innate immune system. Many adjuvants are TLR antagonists and thus amplify the response of the immune system. Adjuvants improve the efficacy of *Leishmania* vaccine candidates by triggering high

levels of IL-12 and IFN- γ expression, both of which play vital roles in long-term immunological memory.^{85,86,96} The TLR7 agonist Aldara and TLR9 agonist CpG DNA exhibited therapeutic antileishmanial properties (reviewed in the study by Raman et al⁹⁷). In contrast, TLR2 agonist Pam3CSK4 led to conflicting results depending on the mouse model used.^{63,98} These encouraging results support the idea that targeting the proper TLRs for vaccine development is a feasible strategy.

DCs containing vaccines

Dendritic cells are one of the first phagocytic and antigen-presenting cells that phagocytose *Leishmania* parasites shortly after the inoculation. Due to their unique ability to initiate and moderate immune responses, typically in the generation of a protective T_H1 cell immune response (reviewed in the study by Bagirova et al⁹⁹), DC may serve as a central target for the eventual development of proficient vaccines against *Leishmania*. This idea was exploited by several laboratories, and results from these studies have been summarized in the recent review by Bagirova et al.⁹⁹ In a typical DC vaccination protocol, DCs are isolated and stimulated with the antigens of interest (soluble *Leishmania* antigen, subunit, recombinant proteins, or DNA vaccine) before being injected into an animal followed by parasite challenge. Dendritic cell vaccination, with most antigens tested, provided protection against *L. major* in a mouse model of infection.⁹⁹ However, DC vaccine is not applicable against *L. amazonensis* and *L. mexicana* as these species poorly activate DCs.⁹⁹ Instead, *L. amazonensis* parasites activate natural killer (NK) cells, which promote IL-12 secretion similar to DCs.¹⁰⁰ Thus, modulation of NK cells may offer an alternative vaccine strategy against this strain of *Leishmania*. Dendritic cell vaccines are extremely safe. Their success depends on the choice of antigen (native versus denatured antigens and recombinant proteins) as certain antigens exacerbate the disease rather than mediate protection.¹⁰¹ In addition, the inclusion of a suitable adjuvant is critical in optimizing the efficiency of such protocol, as well as the use of proper subtypes of DC.^{102,103}

Conclusions

Cutaneous form of leishmaniasis remains a serious global health problem. Currently, no effective vaccines exist against this disease despite much effort from numerous research groups over several decades. Various approaches have been tested, from live whole parasites to attenuated cell lines and from the use of individual antigens/recombinant proteins to DNA vaccines. Several suitable antigens have been identified so far and delivered promising results in animal models. One of the main challenges is to transfer results from animal model studies to humans. More recently, immunotherapy has presented itself as a promising strategy for *Leishmania* vaccination. However, a better knowledge of the CL immunology is needed to uncover suitable points of intervention. This will provide a platform for the identification of suitable adjuvants, reagents, and methodologies needed to induce the maturation and proliferation of the proper memory T cells that are “pretrained” to recognize and clear *Leishmania* parasites on an ulterior infection. Overcoming these challenges will lead to the development of an effective vaccine for prevention and treatment of not only CL but also the most severe visceral leishmaniasis.

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Table 1

Summary of CL vaccines.

VACCINATION TYPE	ANTIGENS
Live vaccination	Live <i>L. major</i>
Whole-killed vaccines	Whole-killed <i>L. major</i> , <i>L. guyanensis</i> , <i>L. braziliensis</i> , or <i>L. amazonensis</i> (alone or in combination ²⁰) Merthiolate-killed <i>L. amazonensis</i> (Leishvacin) ²¹
Live attenuated vaccines	Null mutants: <i>dhfr-ts</i> ^{-/-} , ²²⁻²⁴ <i>hsp70-II</i> ^{-/-} , ²⁵ <i>ldcen</i> ^{-/-} , ²⁶ <i>ldp27</i> ^{-/-} , ^{26,27} <i>CP</i> ^{-/-} ²⁸ “Suicidal” parasites: <i>lmtkcd</i> ^{+/+} , ²⁹ δ -aminolevulinate dehydratase and porphobilinogen deaminase transgenics ³⁰
Purified antigens	GP63, ³¹⁻³³ LACK, ^{34,35} H2B histone, ³⁶ sterol 24-C-methyltransferase ³⁷
Recombinant subunits	Histone 1, ³⁸ CP A and B, ^{39,40} KSAC, ⁴¹ ribosomal proteins L3 and L5, ⁴² Leish-111F, ⁴³⁻⁴⁵ LeIF ^{43,44}
DNA vaccines	GP63, ⁴⁶⁻⁴⁸ LACK, ³⁵ A2, ^{49,50} iron superoxide dismutase, ⁵¹ histone proteins H2A, H2B, H3 and H4, ^{52,53} MKP-11, ⁵⁴ HisAK70 ⁵⁴
Sandfly saliva components	Saliva of <i>Phlebotomus papatasi</i> , ^{55,56} PpSP15, ⁵⁷ saliva of <i>L. longipalpis</i> , ⁵⁸ <i>L. intermedia</i> salivary gland extract ⁵⁹
DC-based vaccines	SLA, protein subunits, recombinant proteins, DNA vaccine

Abbreviations: CL, cutaneous form of leishmaniasis; CP, cysteine proteinase; DC, dendritic cell, LACK, *Leishmania* homolog of receptors for activated C kinase; LeIF, *L. braziliensis* elongation and initiation factor; SLA, soluble *Leishmania* antigen.