Cutaneous Leishmaniasis: Update on Vaccine Development

Davian C Whyte and Rachel Zufferey

Department of Biological Sciences, St. John's University, Queens, NY, USA.

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 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 works[4–7]). 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 al[8]). 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

Table 1. Summary of CL vaccines.

VACCINATION TYPE	ANTIGENS
Live vaccination	Live *L major*
Whole-killed vaccines	Whole-killed *L major, L guyanensis, L braziliensis*, or *L amazonensis* (alone or in combination[20]) Merthiolate-killed *L amazonensis* (Leishvacin)[21]
Live attenuated vaccines	Null mutants: *dhfr-ts*$^{-/-}$,[22–24] *hsp70-II*$^{-/-}$,[25] *ldcen*$^{-/-}$,[26] *ldp27*$^{-/-}$,[26,27] CP$^{-/-}$[28] "Suicidal" parasites: *lmtkcd*$^{+/+}$,[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] *Le*IF[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 *Phlebotomus papatasi*,[55,56] PpSP15,[57] saliva of *L longipalpis*,[58] *L intermedia* 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; *Le*IF, *L braziliensis* elongation and initiation factor; SLA, soluble *Leishmania* antigen.

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 Scott[18]). 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 al[20]). 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 *La*Ag (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 irradiation[69] 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, *ldcen*$^{-/-}$ 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-aminolevulinate 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 Uzonna[6]), 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 antigen[74] 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 non-pathogenic strain *L tarentolae* in combination with CpG as a prime boost conferred resistance to *L major* infection in mice.[57] Similarly, immunization with recombinant *Ljm*11 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 Scott[18]). 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 of 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 al[97]). 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 al[99]), 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 stain 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.

Author Contributions

DCW and RZ wrote, reviewed, and approved the final manuscript.

REFERENCES

1.   WHO. Status of vaccine research and development of vaccines for leishmaniasis. http://www.who.int/immunization/research/meetings_workshops/ Leishmaniasis_vaccineRD_Sept2014.pdf. Published 2014.

2. David CV, Craft N. Cutaneous and mucocutaneous leishmaniasis. *Dermatol Ther*. 2009;22:491–502.

3. Kaye P, Scott P. Leishmaniasis: complexity at the host-pathogen interface. *Nat Rev Microbiol*. 2011;9:604–615.

4. Carlsen ED, Liang Y, Shelite TR, Walker DH, Melby PC, Soong L. Permissive and protective roles for neutrophils in leishmaniasis. *Clin Exp Immunol*. 2015;182:109–118.

5. Tacchini-Cottier F, Zweifel C, Belkaid Y, et al. An immunomodulatory function for neutrophils during the induction of a CD4+ Th2 response in BALB/c mice infected with *Leishmania major*. *J Immunol*. 2000;165:2628–2636.

6. Okwor I, Uzonna J. Vaccines and vaccination strategies against human cutaneous leishmaniasis. *Hum Vaccin*. 2009;5:291–301.

7. Korbel DS, Finney OC, Riley EM. Natural killer cells and innate immunity to protozoan pathogens. *Int J Parasitol*. 2004;34:1517–1528.

8. Feijo D, Tiburcio R, Ampuero M, Brodskyn C, Tavares N. Dendritic cells and *Leishmania* infection: adding layers of complexity to a complex disease. *J Immunol Res*. 2016;2016:3967436.

9. Gorak PM, Engwerda CR, Kaye PM. Dendritic cells, but not macrophages, produce IL-12 immediately following *Leishmania donovani* infection. *Eur J Immunol*. 1998;28:687–695.

10. Leon B, Lopez-Bravo M, Ardavin C. Monocyte-derived dendritic cells formed at the infection site control the induction of protective T helper 1 responses against *Leishmania*. *Immunity*. 2007;26:519–531.

11. Konecny P, Stagg AJ, Jebbari H, English N, Davidson RN, Knight SC. Murine dendritic cells internalize *Leishmania major* promastigotes, produce IL-12 p40 and stimulate primary T cell proliferation in vitro. *Eur J Immunol*. 1999;29:1803–1811.

12. Marovich MA, McDowell MA, Thomas EK, Nutman TB. IL-12p70 production by *Leishmania major*-harboring human dendritic cells is a CD40/CD40 ligand-dependent process. *J Immunol*. 2000;164:5858–5865.

13. Liew FY, Li Y, Millott S. Tumor necrosis factor-alpha synergizes with IFN-gamma in mediating killing of *Leishmania major* through the induction of nitric oxide. *J Immunol*. 1990;145:4306–4310.

14. Novais FO, Santiago RC, Bafica A, et al. Neutrophils and macrophages cooperate in host resistance against *Leishmania braziliensis* infection. *J Immunol*. 2009;183:8088–8098.

15. Bhattacharya P, Ali N. Involvement and interactions of different immune cells and their cytokines in human visceral leishmaniasis. *Rev Soc Bras Med Trop*. 2013;46:128–134.

16. Costa DL, Carregaro V, Lima-Junior DS, et al. BALB/c mice infected with antimony treatment refractory isolate of *Leishmania braziliensis* present severe lesions due to IL-4 production. *PLoS Negl Trop Dis*. 2011;5:e965.

17. Zaph C, Uzonna J, Beverley SM, Scott P. Central memory T cells mediate long-term immunity to *Leishmania major* in the absence of persistent parasites. *Nat Med*. 2004;10:1104–1110.

18. Glennie ND, Scott P. Memory T cells in cutaneous leishmaniasis. *Cell Immunol*. 2016;309:50–54.

19. WHO. Control of Leishmaniasis. Report by the Secretariat, Sixtieth World Health Assembly A60/10 Provisional agenda item. http://apps.who.int/iris/bitstream/10665/22438/1/A60_10-en.pdf. Published 2007.

20. Noazin S, Khamesipour A, Moulton LH, et al. Efficacy of killed whole-parasite vaccines in the prevention of leishmaniasis: a meta-analysis. *Vaccine*. 2009;27:4747–4753.

21. Velez ID, Gilchrist K, Arbelaez MP, et al. Failure of a killed *Leishmania amazonensis* vaccine against American cutaneous leishmaniasis in Colombia. *Trans R Soc Trop Med Hyg*. 2005;99:593–598.

22. Titus RG, Gueiros-Filho FJ, de Freitas LA, Beverley SM. Development of a safe live *Leishmania* vaccine line by gene replacement. *Proc Natl Acad Sci U S A*. 1995;92:10267–10271.

23. Veras P, Brodskyn C, Balestieri F, et al. A dhfr-ts- *Leishmania major* knockout mutant cross-protects against *Leishmania amazonensis*. *Mem Inst Oswaldo Cruz*. 1999;94:491–496.

24. Khamesipour A, Rafati S, Davoudi N, Maboudi F, Modabber F. Leishmaniasis vaccine candidates for development: a global overview. *Indian J Med Res*. 2006;123:423–438.

25. Carrion J, Folgueira C, Soto M, Fresno M, Requena JM. *Leishmania infantum* HSP70-II null mutant as candidate vaccine against leishmaniasis: a preliminary evaluation. *Parasit Vectors*. 2011;4:150.

26. Dey R, Natarajan G, Bhattacharya P, et al. Characterization of cross-protection by genetically modified live-attenuated *Leishmania donovani* parasites against *Leishmania mexicana*. *J Immunol*. 2014;193:3513–3527.

27. Selvapandiyan A, Dey R, Nylen S, Duncan R, Sacks D, Nakhasi HL. Intracellular replication-deficient *Leishmania donovani* induces long lasting protective immunity against visceral leishmaniasis. *J Immunol*. 2009;183:1813–1820.

28. Saravia NG, Escorcia B, Osorio Y, et al. Pathogenicity and protective immunogenicity of cysteine proteinase-deficient mutants of *Leishmania mexicana* in non-murine models. *Vaccine*. 2006;24:4247–4259.

29. Sah JF, Ito H, Kolli BK, Peterson DA, Sassa S, Chang KP. Genetic rescue of *Leishmania* deficiency in porphyrin biosynthesis creates mutants suitable for analysis of cellular events in uroporphyria and for photodynamic therapy. *J Biol Chem*. 2002;277:14902–14909.

30. Ghaffarifar F, Jorjani O, Mirshams M, Miranbaygi MH, Hosseini ZK. Photodynamic therapy as a new treatment of cutaneous leishmaniasis. *East Mediterr Health J*. 2006;12:902–908.

31. Olobo JO, Anjili CO, Gicheru MM, et al. Vaccination of vervet monkeys against cutaneous leishmaniosis using recombinant Leishmania "major surface glycoprotein" (gp63). *Vet Parasitol*. 1995;60:199–212.

32. Abdelhak S, Louzir H, Timm J, et al. Recombinant BCG expressing the leishmania surface antigen Gp63 induces protective immunity against *Leishmania major* infection in BALB/c mice. *Microbiology*. 1995;141:1585–1592.

33. Gonzalez CR, Noriega FR, Huerta S, et al. Immunogenicity of a *Salmonella typhi* CVD 908 candidate vaccine strain expressing the major surface protein gp63 of *Leishmania mexicana mexicana*. *Vaccine*. 1998;16:1043–1052.

34. Mougneau E, Altare F, Wakil AE, et al. Expression cloning of a protective *Leishmania* antigen. *Science*. 1995;268:563–566.

35. Gurunathan S, Sacks DL, Brown DR, et al. Vaccination with DNA encoding the immunodominant LACK parasite antigen confers protective immunity to mice infected with *Leishmania major*. *J Exp Med*. 1997;186:1137–1147.

36. Chenik M, Louzir H, Ksontini H, Dilou A, Abdmouleh I, Dellagi K. Vaccination with the divergent portion of the protein histone H2B of *Leishmania* protects susceptible BALB/c mice against a virulent challenge with *Leishmania major*. *Vaccine*. 2006;24:2521–2529.

37. Goto Y, Bhatia A, Raman VS, et al. *Leishmania infantum* sterol 24-c-methyl-transferase formulated with MPL-SE induces cross-protection against *L. major* infection. *Vaccine*. 2009;27:2884–2890.

38. Masina S, Gicheru M, Demotz SO, Fasel NJ. Protection against cutaneous leishmaniasis in outbred vervet monkeys using a recombinant histone H1 antigen. *J Infect Dis*. 2003;188:1250–1257.

39. Soong L, Duboise SM, Kima P, McMahon-Pratt D. *Leishmania pifanoi* amastigote antigens protect mice against cutaneous leishmaniasis. *Infect Immun*. 1995;63:3559–3566.

40. Zadeh-Vakili A, Taheri T, Taslimi Y, Doustdari F, Salmanian AH, Rafati S. Immunization with the hybrid protein vaccine, consisting of *Leishmania major* cysteine proteinases Type I (CPB) and Type II (CPA), partially protects against leishmaniasis. *Vaccine*. 2004;22:1930–1940.

41. Peters NC, Bertholet S, Lawyer PG, et al. Evaluation of recombinant *Leishmania* polyprotein plus glucopyranosyl lipid A stable emulsion vaccines against sand fly-transmitted *Leishmania major* in C57BL/6 mice. *J Immunol*. 2012;189:4832–4841.

42. Ramirez L, Santos DM, Souza AP, et al. Evaluation of immune responses and analysis of the effect of vaccination of the *Leishmania major* recombinant ribosomal proteins L3 or L5 in two different murine models of cutaneous leishmaniasis. *Vaccine*. 2013;31:1312–1319.

43. Coler RN, Skeiky YA, Bernards K, et al. Immunization with a polyprotein vaccine consisting of the T-cell antigens thiol-specific antioxidant, *Leishmania major* stress-inducible protein 1, and *Leishmania* elongation initiation factor protects against leishmaniasis. *Infect Immun*. 2002;70:4215–4225.

44. Skeiky YA, Coler RN, Brannon M, et al. Protective efficacy of a tandemly linked, multi-subunit recombinant leishmanial vaccine (Leish-111f) formulated in MPL adjuvant. *Vaccine*. 2002;20:3292–3303.

45. Duthie MS, Raman VS, Piazza FM, Reed SG. The development and clinical evaluation of second-generation leishmaniasis vaccines. *Vaccine*. 2012;30:134–141.

46. Xu D, Liew FY. Protection against leishmaniasis by injection of DNA encoding a major surface glycoprotein, gp63, of L. major. *Immunology*. 1995;84:173–176.

47. Xu D, McSorley SJ, Chatfield SN, Dougan G, Liew FY. Protection against *Leishmania major* infection in genetically susceptible BALB/c mice by gp63 delivered orally in attenuated *Salmonella typhimurium* (AroA- AroD-). *Immunology*. 1995;85:1–7.

48. Walker PS, Scharton-Kersten T, Rowton ED, et al. Genetic immunization with glycoprotein 63 cDNA results in a helper T cell type 1 immune response and protection in a murine model of leishmaniasis. *Hum Gene Ther*. 1998;9: 1899–1907.

49. Montalvo-Alvarez AM, Folgueira C, Carrion J, Monzote-Fidalgo L, Canavate C, Requena JM. The *Leishmania* HSP20 is antigenic during natural infections, but, as DNA vaccine, it does not protect BALB/c mice against experimental *L. amazonensis* infection. *J Biomed Biotechnol*. 2008;2008:695432.

50. Zanin FH, Coelho EA, Tavares CA, et al. Evaluation of immune responses and protection induced by A2 and nucleoside hydrolase (NH) DNA vaccines against *Leishmania chagasi* and *Leishmania amazonensis* experimental infections. *Microbes Infect*. 2007;9:1070–1077.

51. Campos BL, Silva TN, Ribeiro SP, et al. Analysis of iron superoxide dismutase-encoding DNA vaccine on the evolution of the *Leishmania amazonensis* experimental infection. *Parasite Immunol*. 2015;37:407–416.

52. Iborra S, Soto M, Carrion J, Alonso C, Requena JM. Vaccination with a plasmid DNA cocktail encoding the nucleosomal histones of *Leishmania*

confers protection against murine cutaneous leishmaniosis. *Vaccine*. 2004;22:3865–3876.

53. Carneiro MW, Santos DM, Fukutani KF, et al. Vaccination with *L. infantum chagasi* nucleosomal histones confers protection against new world cutaneous leishmaniasis caused by *Leishmania braziliensis*. *PLoS ONE*. 2012;7:e52296.

54. Dominguez-Bernal G, Horcajo P, Orden JA, et al. HisAK70: progress towards a vaccine against different forms of leishmaniosis. *Parasit Vectors*. 2015;8:629.

55. Kamhawi S, Belkaid Y, Modi G, Rowton E, Sacks D. Protection against cutaneous leishmaniasis resulting from bites of uninfected sand flies. *Science*. 2000;290:1351–1354.

56. Morris RV, Shoemaker CB, David JR, Lanzaro GC, Titus RG. Sandfly maxadilan exacerbates infection with *Leishmania major* and vaccinating against it protects against *L. major* infection. *J Immunol*. 2001;167:5226–5230.

57. Katebi A, Gholami E, Taheri T, et al. *Leishmania tarentolae* secreting the sand fly salivary antigen PpSP15 confers protection against *Leishmania major* infection in a susceptible BALB/c mice model. *Mol Immunol*. 2015;67:501–511.

58. Gomes R, Oliveira F, Teixeira C, et al. Immunity to sand fly salivary protein LJM11 modulates host response to vector-transmitted leishmania conferring ulcer-free protection. *J Invest Dermatol*. 2012;132:2735–2743.

59. de Moura TR, Oliveira F, Novais FO, et al. Enhanced *Leishmania braziliensis* infection following pre-exposure to sandfly saliva. *PLoS Negl Trop Dis*. 2007;1:e84.

60. Greenblatt CL. The present and future of vaccination for cutaneous leishmaniasis. *Prog Clin Biol Res*. 1980;47:259–285.

61. McCall LI, Zhang WW, Ranasinghe S, Matlashewski G. Leishmanization revisited: immunization with a naturally attenuated cutaneous *Leishmania donovani* isolate from Sri Lanka protects against visceral leishmaniasis. *Vaccine*. 2013;31:1420–1425.

62. Laabs EM, Wu W, Mendez S. Vaccination with live *Leishmania major* and CpG DNA promotes interleukin-2 production by dermal dendritic cells and NK cell activation. *Clin Vaccine Immunol*. 2009;16:1601–1606.

63. Huang L, Hinchman M, Mendez S. Coinjection with TLR2 agonist Pam3CSK4 reduces the pathology of leishmanization in mice. *PLoS Negl Trop Dis*. 2015;9:e0003546.

64. Wu W, Weigand L, Belkaid Y, Mendez S. Immunomodulatory effects associated with a live vaccine against *Leishmania major* containing CpG oligodeoxynucleotides. *Eur J Immunol*. 2006;36:3238–3247.

65. Mendez S, Tabbara K, Belkaid Y, et al. Coinjection with CpG-containing immunostimulatory oligodeoxynucleotides reduces the pathogenicity of a live vaccine against cutaneous Leishmaniasis but maintains its potency and durability. *Infect Immun*. 2003;71:5121–5129.

66. Pinheiro RO, Pinto EF, Lopes JR, Guedes HL, Fentanes RF, Rossi-Bergmann B. TGF-beta-associated enhanced susceptibility to leishmaniasis following intramuscular vaccination of mice with *Leishmania amazonensis* antigens. *Microbes Infect*. 2005;7:1317–1323.

67. Pratti JE, Ramos TD, Pereira JC, et al. Efficacy of intranasal LaAg vaccine against *Leishmania amazonensis* infection in partially resistant C57Bl/6 mice. *Parasit Vectors*. 2016;9:534.

68. da Silva-Couto L, Ribeiro-Romao RP, Saavedra AF, et al. Intranasal vaccination with leishmanial antigens protects golden hamsters (*Mesocricetus auratus*) against Leishmania (*Viannia*) Braziliensis infection. *PLoS Negl Trop Dis*. 2015;9: e3439.

69. Rivier D, Shah R, Bovay P, Mauel J. Vaccine development against cutaneous leishmaniasis. Subcutaneous administration of radioattenuated parasites protects CBA mice against virulent *Leishmania major* challenge. *Parasite Immunol*. 1993;15:75–84.

70. Turco S, Descoteaux A, Ryan K, Garraway L, Beverley S. Isolation of virulence genes directing GPI synthesis by functional complementation of *Leishmania*. *Braz J Med Biol Res*. 1994;27:133–138.

71. Spath GF, Lye LF, Segawa H, Turco SJ, Beverley SM. Identification of a compensatory mutant (lpg2-REV) of *Leishmania major* able to survive as amastigotes within macrophages without LPG2-dependent glycoconjugates and its significance to virulence and immunization strategies. *Infect Immun*. 2004;72:3622–3627.

72. Davoudi N, Khamesipour A, Mahboudi F, McMaster WR. A dual drug sensitive *L. major* induces protection without lesion in C57BL/6 mice. *PLoS Negl Trop Dis*. 2014;8:e2785.

73. Nico D, Gomes DC, Alves-Silva MV, et al. Cross-protective immunity to *Leishmania amazonensis* is mediated by CD4+ and CD8+ epitopes of *Leishmania donovani* nucleoside hydrolase terminal domains. *Front Immunol*. 2014;5:189.

74. Liu MA, Wahren B, Karlsson Hedestam GB. DNA vaccines: recent developments and future possibilities. *Hum Gene Ther*. 2006;17:1051–1061.

75. Dumonteil E. DNA vaccines against protozoan parasites: advances and challenges. *J Biomed Biotechnol*. 2007;2007:90520.

76. Tabatabaie F, Mahdavi M, Faezi S, et al. Th1 platform immune responses against *Leishmania major* induced by Thiol-specific antioxidant-based DNA vaccines. *Jundishapur J Microbiol*. 2014;7:e8974.

77. Carrion J. Mechanisms of immunity to *Leishmania major* infection in mice: the contribution of DNA vaccines coding for two novel sets of histones (H2A-H2B or H3-H4). *Comp Immunol Microbiol Infect Dis*. 2011;34:381–386.

78. Titus RG, Ribeiro JM. Salivary gland lysates from the sand fly *Lutzomyia longipalpis* enhance *Leishmania* infectivity. *Science*. 1988;239:1306–1308.

79. Reiner SL, Locksley RM. The regulation of immunity to *Leishmania major*. *Annu Rev Immunol*. 1995;13:151–177.

80. Himmelrich H, Parra-Lopez C, Tacchini-Cottier F, Louis JA, Launois P. The IL-4 rapidly produced in BALB/c mice after infection with *Leishmania major* down-regulates IL-12 receptor beta 2-chain expression on CD4+ T cells resulting in a state of unresponsiveness to IL-12. *J Immunol*. 1998;161:6156–6163.

81. Matthews DJ, Emson CL, McKenzie GJ, Jolin HE, Blackwell JM, McKenzie AN. IL-13 is a susceptibility factor for *Leishmania major* infection. *J Immunol*. 2000;164:1458–1462.

82. Hurdayal R, Brombacher F. The role of IL-4 and IL-13 in cutaneous Leishmaniasis. *Immunol Lett*. 2014;161:179–183.

83. Kane MM, Mosser DM. The role of IL-10 in promoting disease progression in leishmaniasis. *J Immunol*. 2001;166:1141–1147.

84. Lopez Kostka S, Dinges S, Griewank K, Iwakura Y, Udey MC, von Stebut E. IL-17 promotes progression of cutaneous leishmaniasis in susceptible mice. *J Immunol*. 2009;182:3039–3046.

85. Pakpour N, Zaph C, Scott P. The central memory CD4+ T cell population generated during *Leishmania major* infection requires IL-12 to produce IFN-gamma. *J Immunol*. 2008;180:8299–8305.

86. Stobie L, Gurunathan S, Prussin C, et al. The role of antigen and IL-12 in sustaining Th1 memory cells in vivo: iL-12 is required to maintain memory/effector Th1 cells sufficient to mediate protection to an infectious parasite challenge. *Proc Natl Acad Sci U S A*. 2000;97:8427–8432.

87. Maspi N, Ghaffarifar F, Sharifi Z, Dalimi A. Codelivery of DNA vaccination encoding LeIF gene and IL-12 increases protection against *Leishmania major* infection in BALB/c mice. *Parasite Immunol*. 2016;38:228–235.

88. Hugentobler F, Di Roberto RB, Gillard J, Cousineau B. Oral immunization using live *Lactococcus lactis* co-expressing LACK and IL-12 protects BALB/c mice against *Leishmania major* infection. *Vaccine*. 2012;30:5726–5732.

89. Hugentobler F, Yam KK, Gillard J, Mahbuba R, Olivier M, Cousineau B. Immunization against *Leishmania major* infection using LACK- and IL-12-expressing *Lactococcus lactis* induces delay in footpad swelling. *PLoS ONE*. 2012;7:e30945.

90. Cohen J. IL-12 deaths: explanation and a puzzle. *Science*. 1995;270:908.

91. Wright AK, Briles DE, Metzger DW, Gordon SB. Prospects for use of interleukin-12 as a mucosal adjuvant for vaccination of humans to protect against respiratory pneumococcal infection. *Vaccine*. 2008;26:4893–4903.

92. Amaral VF, Teva A, Oliveira-Neto MP, et al. Study of the safety, immunogenicity and efficacy of attenuated and killed *Leishmania* (*Leishmania*) *major* vaccines in a rhesus monkey (*Macaca mulatta*) model of the human disease. *Mem Inst Oswaldo Cruz*. 2002;97:1041–1048.

93. Tubo NJ, Jenkins MK. CD4+ T cells: guardians of the phagosome. *Clin Microbiol Rev*. 2014;27:200–213.

94. Belkaid Y, Piccirillo CA, Mendez S, Shevach EM, Sacks DL. CD4+CD25+ regulatory T cells control *Leishmania major* persistence and immunity. *Nature*. 2002;420:502–507.

95. Uzonna JE, Wei G, Yurkowski D, Bretscher P. Immune elimination of *Leishmania major* in mice: implications for immune memory, vaccination, and reactivation disease. *J Immunol*. 2001;167:6967–6974.

96. Sakai S, Takashima Y, Matsumoto Y, Reed SG, Hayashi Y, Matsumoto Y. Intranasal immunization with Leish-111f induces IFN-gamma production and protects mice from *Leishmania major* infection. *Vaccine*. 2010; 28:2207–2213.

97. Raman VS, Duthie MS, Fox CB, Matlashewski G, Reed SG. Adjuvants for *Leishmania* vaccines: from models to clinical application. *Front Immunol*. 2012;3:144.

98. Pandey SP, Chandel HS, Srivastava S, et al. Pegylated bisacyloxypropylcysteine, a diacylated lipopeptide ligand of TLR6, plays a host-protective role against experimental *Leishmania major* infection. *J Immunol*. 2014; 193:3632–3643.

99. Bagirova M, Allahverdiyev AM, Abamor ES, et al. Overview of dendritic cell-based vaccine development for leishmaniasis. *Parasite Immunol*. 2016;38:651–662.

100. Sanabria MX, Vargas-Inchaustegui DA, Xin L, Soong L. Role of natural killer cells in modulating dendritic cell responses to *Leishmania amazonensis* infection. *Infect Immun*. 2008;76:5100–5109.

101. Tsagozis P, Karagouni E, Dotsika E. Dendritic cells pulsed with peptides of gp63 induce differential protection against experimental cutaneous leishmaniasis. *Int J Immunopathol Pharmacol*. 2004;17:343–352.

102. Carrion J, Nieto A, Soto M, Alonso C. Adoptive transfer of dendritic cells pulsed with *Leishmania infantum* nucleosomal histones confers protection against cutaneous leishmaniosis in BALB/c mice. *Microbes Infect*. 2007;9:735–743.

103. Remer KA, Apetrei C, Schwarz T, Linden C, Moll H. Vaccination with plasmacytoid dendritic cells induces protection against infection with *Leishmania major* in mice. *Eur J Immunol*. 2007;37:2463–2473.