Article published online: 2021-06-26

Review of Development of Live Vaccines against Leishmaniasis

Mohammad Hossein Feiz Haddad[1,2] Jalal Lomei[3] Azar Shokri[4] Habib Habibpour[1,2] Hossein Rezvan[5] Alireza Nourian[5] Mohammad Reza Mahmoudi[6]

[1] Cellular and Molecular Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[2] Department of Parasitology, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[3] Division of Integrative Physiology, Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden
[4] Vector-borne Disease Research Center, North Khorasan University of Medical Sciences, Bojnurd, Iran
[5] Department of Pathobiology, Faculty of Veterinary Science, Bu-Ali Sina University, Hamedan, Iran
[6] Cellular and Molecular Research Center, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran

Address for correspondence Habib Habibpour, PhD, Department of Parasitology, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran (e-mail: h.habibpour59@gmail.com).

J Child Sci 2021;11:e178–e184.

Abstract

Leishmaniasis is a serious public health problem in both tropical and temperate regions, caused by protozoan parasites of the genus *Leishmania*. Cutaneous leishmaniasis is the most common form of leishmaniasis worldwide. After recovery from the initial infection in most of the patients, a long-lasting natural immunity will be established. In individuals with HIV infection or in immune deficient patients, the more dangerous forms can occur. Despite many attempts, there is no efficient vaccine for leishmaniasis. The main concern for live-attenuated vaccines is the possibility of returning to the virulent form. Therefore, the safety is an important point in designing a successful vaccine. Nonvirulent parasites as vaccine candidates are achievable through gamma-irradiation, long-term culture, random mutations induced by chemical agents, and temperature-sensitive mutations. The type of change(s) in such parasites is not known well and drawbacks such as reversion to virulent forms was soon realized. *Leishmania tarentolae* with capacity of adaptation to mammalian system has a potential to be used as nonpathogenic vector in vaccine programs. Due to its nonpathogenic intrinsic property, it does not have the ability to replace with the pathogen form. Moreover, the main problems are associated with the production of live vaccines, including lyophilization, storage, standards, and quality control that must be considered. In this review, we focused on the importance of different approaches concerning the development of a live vaccine against leishmaniasis.

Keywords
- *Leishmania*
- leishmaniasis
- live-attenuated
- vaccine

received
August 29, 2020
accepted after revision
May 14, 2021

DOI https://doi.org/10.1055/s-0041-1731336.
ISSN 2474-5871.

Georg Thieme Verlag KG, Rüdigerstraße 14, 70469 Stuttgart, Germany

Introduction

Leishmaniasis, caused by an intracellular protozoan parasites of the genus *Leishmania*, is prevalent in subtropical and tropical regions all around the world.[1–3] The disease is considered as neglected tropical diseases with the incidence of 1.5 million new cases annually[4,5] which leads to a considerable mortality and morbidity.[6] There are three main forms of the disease, namely, cutaneous leishmaniasis (CL), visceral leishmaniasis (VL), and mucocutaneous leishmaniasis (MCL).[7] VL, also known as kala-azar, is caused by *Leishmania donovani* (anthroponotic form) and *L. infantum* (zoonotic form). VL is the most important and severe form of leishmaniasis with 500,000 new cases annually. In this form of the disease, the parasites invade in internal organs such as liver and spleen. VL can lead to death in untreated cases and there is no effective vaccine for it.[8] MCL is mainly caused by *L. braziliensis* complex in mucosal tissues of nose and mouth and can result in massive tissue damage and permanent disfiguration.[9] CL is self-healing disease[10] caused mostly by the *L. tropica* and *L. major* in the Central Asia and Middle East[11] and *L. braziliensis* and *L. mexicana* complexes in the Americas.[12] CL is the most common form of leishmaniasis in the world, and 90% of all CL cases occur only in seven countries: Syria, Iran, Saudi Arabia, Algeria, Peru, Brazil, and Afghanistan.[1] CL can lead to skin deformities, and in immune compromised patients giving rise to the diffuse cutaneous leishmaniasis.[13] The host immune response has a crucial role in leishmaniasis, both in efficacy of treatment and the clinical patterns of the disease.[14] Success in development of vaccine depends on selecting an appropriate vaccine candidate and the immune biology of pathogen/host interactions.[15] Cell-mediated immunity particularly CD4 T cells are critical in leishmaniasis and protection against the disease.[16] The host defense mainly relies on response of Th1 cells which were activated through interleukin (IL)-12. Antigen presenting cells (APCs) and T cells, as they produce IL-12 and interferon gamma (IFN-γ), are crucial for limiting the numbers of leishmania parasites.[17] In contrast, Th2 cells produce cytokines, mainly IL-4, IL-5, and IL-13 and anti-inflammatory cytokines that have suppressive effect on host immunity which leads to parasite survival during leishmaniasis.[17,18] These effects are exclusively studied in CL and murine models.[7,19] Generation of proper immunologic memory is the main challenge in designing a vaccine that can cause effective immunity. Different studies revealed that long-lasting immunity against leishmaniasis needs to involve the function of both regulatory T cells (Tregs) and Th1-mediated immune response.[20,21] Currently, treatment of any form of leishmaniasis is remarkably dependent on pentavalent antimonials as first-line and amphotericin B as second-line drugs.[22] This treatment is usually adequate but long duration of treatment, high costs, and side effects are still challenging. Reversion to virulence form has been reported in host with weak immune system, especially in HIV infected individuals.[23] Furthermore, strains which are resistant to available drugs created the need for an effective vaccine.[24] Currently, there is no impermissible vaccine against human leishmaniasis, even several vaccines have developed to clinical trials, most are still in early research phases and need more develop (►**Table 1**).[25–27] From 2004, four vaccines have been licensed against canine leishmaniosis, two in Brazil (Leishmune, license was withdrawn in 2014, and Leish-Tec) and two in Europe (CaniLeish and LetiFend).[28] Efforts for finding an effective vaccine against leishmaniasis is continuing worldwide.[9,29] Finally, development of an effective vaccine is one of the most cost-effective ways to eliminate and/or control CL and VL. This review aims to discuss the importance of different approaches concerning the development of a live-attenuated vaccine against leishmaniasis.

Table 1 Development status of current vaccine candidates

Candidate name/identifier	Preclinical	Phase I	Phase II	Reference
LEISH-F2			X	11,25
LEISH-F3		X		11,26
Various *Lutzomyia* and fly antigens	X			11,27
Various second-generation protein-based vaccines	X			11,27
Various third-generation DNA-based and heterologous prime-boost vaccines	X			11,27

Live Active Vaccines

Leishmanization (LZ) was the first effort in using live parasite for human immunization, which was used in some countries for immunization against CL. There is a constant concern about revision to virulent type. This method was used in last century in several countries particularly in the Middle East, but it countered severe barriers such as local lesions in 2 to 3% of cases. The program was stopped due to some problems such as spreading the HIV infection, increasing the use of immunosuppressive drugs, permanent skin lesions, dissemination of lesions, ethical reasons, and problems in quality control of inoculant and long-lasting parasite persistence. Uzbekistan is one of the endemic countries, which LZ is licensed and its efficacy in human trials was proven. Efforts for improving the safety of this practice is continuing as this program can lead to an efficient vaccine against CL. It is declared that under controlled condition, it is achievable to elicit quick immune responses using the killed parasites and adjuvants.[30]

Live-Attenuated Vaccine

Several methods such as long-term in vitro cultures,[31] attenuation with temperature,[32] chemical mutagenesis,[33,34]

culture under drug pressure,[35] and γ-attenuation have been used to develop an attenuated *Leishmania* strains for many years. Recently, live-attenuated mutants through the gene alteration have revealed ability to induce proper immune responses in experimental hosts (►**Table 2**). This helps researchers to identify genes which are necessary for *Leishmania* parasite's survival and/or for their virulence.[33,49–54]

In summary, it is believed that live-attenuated parasites can be considered suitable vaccine candidate as they present antigens to the APCs and activate them to induce immunity through mimicking natural infection and lead to the highest rate of CD4+ T cells polarization.[55] Also, it evaluates the memory of the immune system due to delivery of whole antigens instead of its subunits, and finally, they cause antigen constancy through long time subclinical infection. Therefore, memory cells and subsequently antigen-specific responses will be produced and react to reinfection.[56] A significant protection by attenuated parasites in murine models have been approved, but there is forever reversion capability that makes them inefficient for using them in

Table 2 Defined and undefined genetic alteration live-attenuated vaccines against leishmaniasis

Species	Attenuation process	Model	Outcome of immunization	References
L. tropica, *L. major*	Long-term in vitro culture	BALB/c and C57BL/6	Persistent low-grade cutaneous disease, BALB/c: partially protection C57BL/6: completely resistant	31
L. major	Long-term in vitro culture	BALB/c	Protection	36
L. chagasi	Long-term in vitro culture	BALB/c	No protection	37
L. amazonensis	Long-term in vitro culture	C57BL/6	Decrease parasite load, increased IFN-γ, smaller lesions	38
L. braziliensis	Temperature sensitivity	BALB/c	Protection	32
L. major	Radio attenuated	CBA	Resistance to subsequent infection with *L. mexicana*	39
L. major	Gamma irradiation	BALB/c and CBA	Protection against heterologous and homologs challenge	33
L. infantum	In vitro under gentamicin pressure	Dog	In the endemic area against canine visceral leishmaniosis gentamicin-attenuated *L. infantum* induced a strong and significant protective effect	40–42
L. infantum	KHARON1 (KH1) null mutants (ΔLikh1)	BALB/c Mice	Δ*Likh1*-immunized mice presented reduced parasite burden upon challenging with virulent *L. infantum*, when compared with naïve mice. An effect associated with increased IL-17 production and *Li* SLA-specific IgG serum levels	43
L. infantum	*HSP70-II* genes null mutant (LiΔHSP70-II)	BALB/c and C57BL/6	LiΔHSP70-II attenuated line activates mammalian immune system for inducing moderate proinflammatory responses	44
L. donovani	LdCen($^{-/-}$) centrin deleted	Mice and hamster	LdCen$^{-/-}$ live-attenuated vaccine induced a strong antibody production, a selectively CD4+ and CD8 + T cell activation in addition to prominent type 1 immune response that contributed to a remarkable reduction in bone marrow parasite load, even 24 months post-*L. infantum* infection	45
L. donovani	Arabino-1, 4-lactone oxidase (ΔALO) null mutants	BALB/c Mice	Live-attenuated ΔALO parasites are induce protective immunity, safe, and can provide sustained protection against *L. donovani*	46
L. donovani	*p27* gene-deleted (Ldp27$^{-/-}$), Ldp27episomal add-back (Ldp27$^{-/-}$AB), *Centrin1* gene deleted (LdCen$^{-/-}$), and LdCen episomal add-back (LdCen$^{-/-}$AB) lines of *L. donovani*	BALB/c mice	Attenuated parasite-infected mice induced higher IL-2 and IFN-γ but significantly less IL-10 production by ovalbumin-specific CD4+ T cells, resulting in the proliferation of Th1 cells	47
L. donovani	*Centrin1* and *p27* genes deleted live-attenuated *Leishmania* parasites (*Ld*Cen1$^{-/-}$ and *Ld*p27$^{-/-}$)	Human macrophages	*Ld*Cen1$^{-/-}$ and *Ld*p27$^{-/-}$ strongly stimulated production of proinflammatory cytokines including, IL-12, IFN-γ, TNF-α, IL-2, IL-6, and IL-17 in the PBMCs	48

Abbreviations: IFN-γ, interferon gamma; IgG, immunoglobulin G; IL, interleukin; TNF-α, tumor necrosis factor α.

human vaccination programs. There is a great risk about consequent reactivation in HIV/*Leishmania* coinfected individuals along with increasing the asymptomatic cases. Additionally, attenuation may lead to poor immune response as the strains are not able to induce subclinical infection or they are not able to express necessary antigenic epitopes. Despite of satisfactory experimental results, the important safety points are needed for attention before using genetically attenuated parasitic vaccines. For achieving an effective memory response against *Leishmania* parasites, persistence of antigen by producing subclinical infections is worthwhile. Reactivation of *Leishmania* is shown in immunocompromised patients. This proves the importance of careful investigation of live-attenuated parasites which can cause subclinical infection.[30]

Based on the used attenuation procedure, live-attenuated parasites are divided in two groups: defined and undefined genetically modified (►**Table 2**).[57] In defined attenuation, by specific mutagenesis, the parasites lost the ability of encoding one or more essential virulence genes. Lazer irradiation or chemical mutagenesis or long-term in vitro culture can produce undefined attenuated parasites. In clinical trial performed in Iran with live vaccine, the researchers used stabilates which were made from stationary phase of parasites. They performed two trials in adults and followed them till healing the leishmanial lesions.[15,58] There is a great challenge in using of live-attenuated *Leishmania* in the regions with high risk of HIV infection. Nowadays, the approved live-attenuated vaccines for clinical trials are made from *L. major*[59] and that means the vaccine will be prepared soon and will be able to help protect masses against the other coexistence species in endemic areas.[6]

Genetically Live-Attenuated Vaccines

Genetically modified vaccines have been developed in recent years and are powerful tools for creating immunity.[59] For achieving a protective vaccine, modifying some of *Leishmania* genes through deleting virulent or necessary genes seems to be successful.[21] The transfection technology through modifying one or more genes in *Leishmania* parasite leads to a great affect. The DNA fragments could be transferred through physical methods such as electroporation to the parasite's nuclei.[60] Antibiotic-resistant genes in a linear structure need to be combined with the genome through homologous recombination for removing a gene. Antibiotic-resistant genes in a linear form should be constructed and need to integrated by homolog recombining for removing a gene. This leads to transferring the sequence of DNA in genome of *Leishmania* parasite.[60] To achieve an absolute knockout, there should be another construct to produce antibiotic-resistant gene by *Leishmania* parasite to set the alleles of the second gene. This manipulation leads to parasites new phenotype and features which hereditary transfer to the next generation. The main concern in gene targeting is the new gene location control as it is important that it should not interfere with genes normal function. Although the genome of *Leishmania* parasite is easily manipulated, the site of entered gene is important and should be confirmed by molecular genetics assays. Another important issue after gene transfer is the phenotype alterations and need to be studied more. In this regard, one of the famous experiments was performed by Titus et al who decided to vaccinate mice against virulent *L. major* parasite[49] with dihydrofolate reductase-thymidylate synthase null mutant of the parasite, which they achieved through gene targeting. Unfortunately, it did not show persistent protection affect in susceptible mouse strains and in Rhesus monkeys[61] but revealed the resistant phenotype of *L. major* parasite.[49] Another attempt on mice and hamster, using mutants of manipulated *L. Mexicana* which lacked cysteine proteinase genes, showed promising results such as low parasite burden, smaller lesions, and delay in disease onset in animals were observed.[52,62] These findings show that the protective immune response is achievable through genetic attenuation of live *Leishmania* parasite. In a study performed by Uzonna et al, they found that very susceptible mice were protected after vaccination with phosphoglycan (PG)-deficient *L. major* without considerable Th1 response.[54] Experiments with BT1 null mutant of *L. donovani* in infecting mice in comparison to wild type were promising and the susceptible individuals were able to attain protective immunity against the parasite.[53] Other researchers proved the efficacy of live genetically attenuated *Leishmania* vaccines in inducing immunity as Silvestre et al demonstrated the production of IFN-γ/ IL-10 in SIR2-deficient *L. infantum* that seems to be protective.[63] Other researchers tried to attain an effective vaccine with elimination of all PGs through deleting the lipophosphoglycan (*LPG*) gene in *L. major*. This leads to creating a nonpathogen parasite, which obtained life ability at low level for more than 2 years in mice. Thus, immunization with *lpg2*$^{-}$ parasites in very susceptible BALB/c mice induced protection against virulent *L. major* parasites.[12] In another research performed by Kedzierski et al, vaccination with a virulent *L. major* with absence of phosphomannomutase in susceptible BALB/c mice induced a protective response. They declared that increase in Th cells and their role in lymph nodes during the infection and low levels of IL-13 and IL-10 are the main reason of protection against *Leishmania* parasites.[64] In a 2009 study, it was revealed that by using of centrin gene (LdCen1$^{-/-}$) disrupted *L. donovani* parasites were able to protect susceptible BALB/c mice and immune-deficient mice and hamsters against infection with *L. braziliensis*.[65] Fiuza et al showed that vaccination of dogs with LdCen$^{-/-}$ vaccine leads to Type 1 polarization and Type 2 inhibition along with production of antibody.[62] It is shown by Dey et al that knockout of protein 27 (Ldp27$^{-/-}$) in amastigotes of *L. donovani* can induce protection against other *Leishmania* species.[47] Mutant of *L. infantum* which lacks both HSP70-II (DHSP70-II) alleles can protect BALB/c mice of infection with *L. major*, resulting in production of NO and type 1 immune responses with immunoglobulin G subclass analyses as reported by Carrion et al.[66] New generation of live-attenuated vaccine from *L. major* strain (LmCen$^{-/-}$) for LZ has been explained by Zhang et al as they suggested effective protection against leishmaniasis

without causing lesion. It seems the gene-free vaccine can be considered for human clinical trials in phase I.[7] Some limitations including concern about probability of reversion to virulent form and using them in immune-deficient people and manufacturing problems make the use of these vaccine more difficult.

Nonpathogenic *Leishmania* (*Leishmania tarentolae*)

In a study conducted by Breton et al, the use of nonpathogenic *Leishmania* strain (*L. tarentolae*) as a vector for designing a vaccine for VL is suggested.[67] The strain is harmless for human and in vitro evaluations revealed that the parasites are able to infect both human and murine phagocytic cells specially dendrite cells (DCs). Also, the parasites are able to promote DC maturation through monitoring their surface activation markers such as MHC-II, CD40, and CD83.[67,68] The results obtained from experiments of *L. tarentolae* are comparable with the results of *L. major* infection in recruiting immune cells and producing inflammatory cytokines. Analysis of genome sequence has shown the similarity between pathogenic species including *L. major*, *L. braziliensis*, and *L. infantum* and 19% similarity between the shared genes in all species.

This suggests that some of these genes are related to the capacity of pathogenicity in this strain which makes it harmless to human. For instance, the presence of high copy number of amastin family in pathogenic species of *Leishmania* and presence of just two copies of *L. tarentolae*[69] and this is the reason of *L. tarentolae*'s inability for application in mammalian macrophages. A protective immune response against *L. donovani* in susceptible BALB/c mice has been induced by experimental intraperitoneal administration of *L. tarentolae* that leads to a conclusion that this was caused through presentation of enhanced antigen and effective response of Th1 cells.[67] By considering the ability of gene manipulation and producing transgenic *L. tarentolae* which can be immune dominant antigens of *Leishmania* parasite, it would be considered as an effective vector in antileishmanial vaccines. The plan is using this strain of *Leishmania* as a special delivering and expressing leishmanial antigens system in host. Also, the A2 protein which is expressed by *L. donovani* complex and promotes visceralization of parasite is not present in *L. tarentolae*. Recent studies have demonstrated that the absence of virulence genes including *LPG3*, *CPB*, *GP63*, and *amastin* is not related to the lack of pathogenicity in *L. tarentolae*.[70] In a study, administration of A2-recombinant *L. tarentolae* vaccine was induced protection against *L. infantum* challenge in BALB/c mice and the protection was associated with production of high levels of IFN-γ before and after challenge.[71] As *L. tarentolae* is able to induce T-cells-mediated protection, it could be considered as a safe vector for live vaccines against *Leishmania* parasites and possibly other intracellular pathogens. Also, infection with *L. tarentolae* can elicit maturation of DC which are responsible for parasite phagosytose.[72] Salehi et al designed and evaluated a live vaccine by using recombinant E7 protein which was expressed by *L. tarentolae* for protection of mice against human papillomavirus–associated tumors. Interestingly, the vaccine showed the best protection against TC-1-induced tumors[73]. Comparative studies on *L. tarentolae* can be helpful for understanding the pathogenicity of *Leishmania* and its relationships with the host.

Conclusion

Despite the efforts of achieving an effective vaccine against leishmaniasis, there is still a long way to meet the vaccine development goals. Easy adaptation of *Leishmania tarentolae* and its safety to mammalian cells is promising to use it as a vector in live vaccines. However, it is important to take in considerations the major problems associated with production of live vaccines, including lyophilization, storage, standards, and quality control.

Note

The results described in this article were part of PhD student thesis of H.H.

Funding

This study was financially supported by Ahvaz Jundishapur University of Medical Sciences (grant no. CMRC-9820).

Conflict of Interest

None declared.

References

1 Torres-Guerrero E, Quintanilla-Cedillo MR, Ruiz-Esmenjaud J, Arenas R. Leishmaniasis: a review. F1000 Res 2017;6:750–750
2 Feiz Haddad MH, Ghasemi E, Maraghi S, Tavala M. Identification of *Leishmania* species isolated from human cutaneous leishmaniasis in Mehran, Western Iran using nested PCR. Iran J Parasitol 2016; 11(01):65–72
3 Hamoon NavardS, Rezvan H, Feiz Haddad MH, et al. Therapeutic effects of mesenchymal stem cells on cutaneous leishmaniasis lesions caused by *Leishmania major*. J Glob Antimicrob Resist 2020;23:243–250
4 Hotez PJ, Alvarado M, Basáñez M-G, et al. The global burden of disease study 2010: interpretation and implications for the neglected tropical diseases. PLoS Negl Trop Dis 2014;8(07): e2865
5 Vos T, Barber RM, Bell B, et al; Global Burden of Disease Study 2013 Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 2015;386(9995):743–800
6 Zabala-Peñafiel A, Todd D, Daneshvar H, Burchmore R. The potential of live attenuated vaccines against cutaneous leishmaniasis. Exp Parasitol 2020;210:107849
7 Zhang WW, Karmakar S, Gannavaram S, et al. A second generation leishmanization vaccine with a markerless attenuated *Leishmania major* strain using CRISPR gene editing. Nat Commun 2020;11 (01):3461
8 Khatoon N, Pandey RK, Prajapati VK. Exploring *Leishmania* secretory proteins to design B and T cell multi-epitope subunit vaccine using immunoinformatics approach. Sci Rep 2017;7(01):8285
9 Mohammed ASA, Tian W, Zhang Y, Peng P, Wang F, Li T. *Leishmania* lipophosphoglycan components: a potent target for synthetic

neoglycoproteins as a vaccine candidate for leishmaniasis. Carbohydr Polym 2020;237:116120
10 Bennis I, De Brouwere V, Belrhiti Z, Sahibi H, Boelaert M. Psychosocial burden of localised cutaneous leishmaniasis: a scoping review. BMC Public Health 2018;18(01):358
11 Gillespie PM, Beaumier CM, Strych U, Hayward T, Hotez PJ, Bottazzi ME. Status of vaccine research and development of vaccines for leishmaniasis. Vaccine 2016;34(26):2992–2995
12 de Vasquez AM, Saenz RE, Petersen JL, Christensen HA, Johnson CM. *Leishmania mexicana* complex: human infections in the Republic of Panama. Am J Trop Med Hyg 1990;43(06):619–622
13 Desjeux P. Leishmaniasis: current situation and new perspectives. Comp Immunol Microbiol Infect Dis 2004;27(05):305–318
14 Khalili G, Dobakhti F, Mahmoudzadeh-Niknam H, Khaze V, Partovi F. Immunotherapy with imiquimod increases the efficacy of glucantime therapy of *Leishmania major* infection. Iran J Immunol 2011;8(01):45–51
15 Srivastava S, Shankar P, Mishra J, Singh S. Possibilities and challenges for developing a successful vaccine for leishmaniasis. Parasit Vectors 2016;9(01):277
16 Bogdan C. Mechanisms and consequences of persistence of intracellular pathogens: leishmaniasis as an example. Cell Microbiol 2008;10(06):1221–1234
17 Maspi N, Abdoli A, Ghaffarifar F. Pro- and anti-inflammatory cytokines in cutaneous leishmaniasis: a review. Pathog Glob Health 2016;110(06):247–260
18 Hurdayal R, Brombacher F. The role of IL-4 and IL-13 in cutaneous leishmaniasis. Immunol Lett 2014;161(02):179–183
19 Kaye P, Scott P. Leishmaniasis: complexity at the host-pathogen interface. Nat Rev Microbiol 2011;9(08):604–615
20 Belkaid Y, Piccirillo CA, Mendez S, Shevach EM, Sacks DL. CD4+CD25+ regulatory T cells control *Leishmania major* persistence and immunity. Nature 2002;420(6915):502–507
21 Mendez S, Reckling SK, Piccirillo CA, Sacks D, Belkaid Y. Role for CD4(+) CD25(+) regulatory T cells in reactivation of persistent leishmaniasis and control of concomitant immunity. J Exp Med 2004;200(02):201–210
22 Tiuman TS, Santos AO, Ueda-Nakamura T, Filho BP, Nakamura CV. Recent advances in leishmaniasis treatment. Int J Infect Dis 2011;15(08):e525–e532
23 Bentwich Z. Concurrent infections that rise the HIV viral load. J HIV Ther 2003;8(03):72–75
24 Croft SL, Sundar S, Fairlamb AH. Drug resistance in leishmaniasis. Clin Microbiol Rev 2006;19(01):111–126
25 Duthie MS, Raman VS, Piazza FM, Reed SG. The development and clinical evaluation of second-generation leishmaniasis vaccines. Vaccine 2012;30(02):134–141
26 Nagill R, Kaur S. Vaccine candidates for leishmaniasis: a review. Int Immunopharmacol 2011;11(10):1464–1488
27 Rezvan H, Moafi M. An overview on *Leishmania* vaccines: a narrative review article. Vet Res Forum 2015;6(01):1–7
28 Velez R, Gállego M. Commercially approved vaccines for canine leishmaniosis: a review of available data on their safety and efficacy. Trop Med Int Health 2020;25(05):540–557
29 Bezerra IPS, Costa-Souza BLS, Carneiro G, Ferreira LAM, de Matos Guedes HL, Rossi-Bergmann B. Nanoencapsulated retinoic acid as a safe tolerogenic adjuvant for intranasal vaccination against cutaneous leishmaniasis. Vaccine 2019;37(28):3660–3667
30 Saljoughian N, Taheri T, Rafati S. Live vaccination tactics: possible approaches for controlling visceral leishmaniasis. Front Immunol 2014;5:134
31 Mitchell GF, Handman E, Spithill TW. Vaccination against cutaneous leishmaniasis in mice using nonpathogenic cloned promastigotes of *Leishmania major* and importance of route of injection. Aust J Exp Biol Med Sci 1984;62(Pt 2):145–153
32 Gorczynski RM. Immunization of susceptible BALB/c mice against *Leishmania braziliensis*. II. Use of temperature-sensitive avirulent clones of parasite for vaccination purposes. Cell Immunol 1985;94(01):11–20
33 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(02):75–84
34 Kimsey PB, Theodos CM, Mitchen TK, Turco SJ, Titus RG. An avirulent lipophosphoglycan-deficient *Leishmania major* clone induces CD4+ T cells which protect susceptible BALB/c mice against infection with virulent *L. major*. Infect Immun 1993;61(12):5205–5213
35 Daneshvar H, Coombs GH, Hagan P, Phillips RS. *Leishmania mexicana* and *Leishmania major*: attenuation of wild-type parasites and vaccination with the attenuated lines. J Infect Dis 2003;187(10):1662–1668
36 Segovia M, Artero JM, Mellado E, Chance ML. Effects of long-term in vitro cultivation on the virulence of cloned lines of *Leishmania major* promastigotes. Ann Trop Med Parasitol 1992;86(04):347–354
37 Streit JA, Recker TJ, Filho FG, Beverley SM, Wilson ME. Protective immunity against the protozoan *Leishmania chagasi* is induced by subclinical cutaneous infection with virulent but not avirulent organisms. J Immunol 2001;166(03):1921–1929
38 Gualdieri L, Rinaldi L, Petrullo L, et al. Intestinal parasites in immigrants in the city of Naples (southern Italy). Acta Trop 2011;117(03):196–201
39 Alexander J. A radioattenuated *Leishmania major* vaccine markedly increases the resistance of CBA mice to subsequent infection with *Leishmania mexicana*. Trans R Soc Trop Med Hyg 1982;76(05):646–649
40 Chettri JK, Deshmukh S, Holten-Andersen L, Jafaar RM, Dalsgaard I, Buchmann K. Comparative evaluation of administration methods for a vaccine protecting rainbow trout against *Yersinia ruckeri* O1 biotype 2 infections. Vet Immunol Immunopathol 2013;154(1-2):42–47
41 Daneshvar H, Molaei MM, Kamiabi H, Burchmore R, Hagan P, Stephen Phillips R. Gentamicin-attenuated *Leishmania infantum*: cellular immunity production and protection of dogs against experimental canine leishmaniasis. Parasite Immunol 2010;32(11-12):722–730
42 Daneshvar H, Sedghy F, Dabiri S, et al. Alteration in mononuclear cell subpopulations in dogs immunized with gentamicin-attenuated *Leishmania infantum*. Parasitology 2012;139(13):1689–1696
43 Santi AMM, Lanza JS, Tunes LG, et al. Growth arrested live-attenuated *Leishmania infantum* KHARON1 null mutants display cytokinesis defect and protective immunity in mice. Sci Rep 2018;8(01):11627
44 Solana JC, Ramírez L, Corvo L, et al. Vaccination with a *Leishmania infantum* HSP70-II null mutant confers long-term protective immunity against *Leishmania major* infection in two mice models. PLoS Negl Trop Dis 2017;11(05):e0005644
45 Fiuza JA, Gannavaram S, Santiago HdaC, et al. Vaccination using live attenuated *Leishmania donovani* centrin deleted parasites induces protection in dogs against *Leishmania infantum*. Vaccine 2015;33(02):280–288
46 Anand S, Madhubala R. Genetically engineered ascorbic acid-deficient live mutants of *Leishmania donovani* induce long lasting protective immunity against visceral leishmaniasis. Sci Rep 2015;5:10706
47 Dey R, Dagur PK, Selvapandiyan A, Mccoy JP, Salotra P, Duncan R, et al. Live attenuated Leishmania donovani p27 gene knockout parasites are nonpathogenic and elicit long-term protective immunity in BALB/c mice. J Immunol 2013;190:2138–2149. Doi: 10.4049/jimmunol.1202801a
48 Avishek K, Kaushal H, Gannavaram S, et al. Gene deleted live attenuated *Leishmania* vaccine candidates against visceral

leishmaniasis elicit pro-inflammatory cytokines response in human PBMCs. Sci Rep 2016;6:33059
49 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(22):10267–10271
50 Dumas C, Ouellette M, Tovar J, et al. Disruption of the trypanothione reductase gene of *Leishmania* decreases its ability to survive oxidative stress in macrophages. EMBO J 1997;16(10):2590–2598
51 Hübel A, Krobitsch S, Hörauf A, Clos J. *Leishmania major* Hsp100 is required chiefly in the mammalian stage of the parasite. Mol Cell Biol 1997;17(10):5987–5995
52 Alexander J, Coombs GH, Mottram JC. *Leishmania mexicana* cysteine proteinase-deficient mutants have attenuated virulence for mice and potentiate a Th1 response. J Immunol 1998;161(12):6794–6801
53 Papadopoulou B, Roy G, Breton M, et al. Reduced infectivity of a *Leishmania donovani* biopterin transporter genetic mutant and its use as an attenuated strain for vaccination. Infect Immun 2002;70 (01):62–68
54 Uzonna JE, Späth GF, Beverley SM, Scott P. Vaccination with phosphoglycan-deficient *Leishmania major* protects highly susceptible mice from virulent challenge without inducing a strong Th1 response. J Immunol 2004;172(06):3793–3797
55 Spörri R, Reis e Sousa C. Inflammatory mediators are insufficient for full dendritic cell activation and promote expansion of CD4+ T cell populations lacking helper function. Nat Immunol 2005;6(02): 163–170
56 Foulds KE, Wu CY, Seder RA. Th1 memory: implications for vaccine development. Immunol Rev 2006;211(01):58–66
57 Silvestre R, Cordeiro-da-Silva A, Ouaissi A. Live attenuated *Leishmania* vaccines: a potential strategic alternative. Arch Immunol Ther Exp (Warsz) 2008;56(02):123–126
58 Khamesipour A, Rafati S, Davoudi N, Maboudi F, Modabber F. Leishmaniasis vaccine candidates for development: a global overview. Indian J Med Res 2006;123(03):423–438
59 Alvar J, Croft SL, Kaye P, Khamesipour A, Sundar S, Reed SG. Case study for a vaccine against leishmaniasis. Vaccine 2013;31 (Suppl 2):B244–B249
60 Papadopoulou B, Dumas C. Parameters controlling the rate of gene targeting frequency in the protozoan parasite *Leishmania*. Nucleic Acids Res 1997;25(21):4278–4286
61 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(07):1041–1048
62 Pulcini C, Massin S, Launay O, Verger P. Factors associated with vaccination for hepatitis B, pertussis, seasonal and pandemic influenza among French general practitioners: a 2010 survey. Vaccine 2013;31(37):3943–3949
63 Silvestre R, Cordeiro-Da-Silva A, Santarém N, Vergnes B, Sereno D, Ouaissi A. SIR2-deficient *Leishmania infantum* induces a defined IFN-γ/IL-10 pattern that correlates with protection. J Immunol 2007;179(05):3161–3170
64 Kedzierski L, Curtis JM, Doherty PC, Handman E, Kedzierska K. Decreased IL-10 and IL-13 production and increased CD44hi T cell recruitment contribute to *Leishmania major* immunity induced by non-persistent parasites. Eur J Immunol 2008;38(11): 3090–3100
65 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(03):1813–1820
66 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. Doi: 10.1186/1756-3305-4-150
67 Breton M, Tremblay MJ, Ouellette M, Papadopoulou B. Live nonpathogenic parasitic vector as a candidate vaccine against visceral leishmaniasis. Infect Immun 2005;73(10):6372–6382
68 Taylor VM, Muñoz DL, Cedeño DL, Vélez ID, Jones MA, Robledo SM. *Leishmania tarentolae*: utility as an in vitro model for screening of antileishmanial agents. Exp Parasitol 2010;126(04):471–475
69 Raymond F, Boisvert S, Roy G, et al. Genome sequencing of the lizard parasite *Leishmania tarentolae* reveals loss of genes associated to the intracellular stage of human pathogenic species. Nucleic Acids Res 2012;40(03):1131–1147
70 Azizi H, Hassani K, Taslimi Y, Najafabadi HS, Papadopoulou B, Rafati S. Searching for virulence factors in the non-pathogenic parasite to humans *Leishmania tarentolae*. Parasitology 2009;136 (07):723–735
71 Mizbani A, Taheri T, Zahedifard F, et al. Recombinant *Leishmania tarentolae* expressing the A2 virulence gene as a novel candidate vaccine against visceral leishmaniasis. Vaccine 2009;28(01): 53–62
72 Breton M, Zhao C, Ouellette M, Tremblay MJ, Papadopoulou B. A recombinant non-pathogenic *Leishmania* vaccine expressing human immunodeficiency virus 1 (HIV-1) Gag elicits cell-mediated immunity in mice and decreases HIV-1 replication in human tonsillar tissue following exposure to HIV-1 infection. J Gen Virol 2007;88(Pt 1):217–225
73 Salehi M, Taheri T, Mohit E, Zahedifard F, Seyed N, Taslimi Y, et al. Recombinant *Leishmania tarentolae* encoding the HPV type 16 E7 gene in tumor mice model. Immunotherapy 2012;4:1107–1120. Doi: 10.2217/imt.12.110