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Vaccination in Leishmaniasis: A Review Article

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ABSTRACT

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

Keywords: Immune response, Leishmaniasis, Vaccination

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INTRODUCTION

Leishmaniasis is a vector-borne disease caused by more than 30 species of *Leishmania* parasites. The disease has a broad clinical picture, ranging from skin lesions to fatal visceral infections[1]. Leishmaniasis is endemic to four continents and more than 98 countries[2]. According to the WHO, 350 million people are at risk for leishmaniasis[2]. Leishmaniasis is found in humans in two main forms: CL and VL. Approximately 58,000 VL cases and 220,000 CL cases are reported annually[2]. The CL is divided into cutaneous, mucocutaneous, and diffused cutaneous types[2]. *L. tropica* and *L. major* are the main causes of CL, while *L. infantum* and *L. donovani* are the main causes of VL. Different species of rodents in various parts of Iran act as a reservoir for rural CL. These species include *Rhombomys opimus* and *Meriones libycus* found in the central and northeast, *M. libycus*, *M. persicus*, and *M. hurrianae* in the south, as well as *Tatera indica* and *Nesokia indica* in the west and southwest[3,4]. The *Leishmania* parasite is transmitted in the Old World, including Europe, Africa, and Asia, by the bite of the female sandfly of the genus *Phlebotomus*, and in the New World, including America, by *Lutzomyia*. The

List of Abbreviations:
BT1, biopterin transporter; **CC**, complete cure; **CFA,** complete Freund's Adjuvant; **CL,** cutaneous leishmaniasis; **CP,** cysteine protease; ***C. parvum*;** *Cryptosporidium parvum*; **CPA,** cysteine proteinase Type II; **CPB,** cysteine proteinase Type I; **CPB^{-CTE},** CPB without its unusual C-terminal extension; **DC,** dendritic cellsl; ***DHFR-TS*,** dihydrofolate reductase-thymidylate synthase; **DT,** double transfectants; **DTH,** delayed-type hypersensitivity; **i.d.,** intradermal; **i.m.,** intramuscular; **i.v.**, intravenous; **MDP,** muramyldipetide; **MPL-A,** monophosphoryl lipid A; **MVA,** modified vaccinia Ankara; **NO,** nitric oxide; **PBMC,** peripheral blood mononuclear cells; **ODN**, oligodeoxynucleotides; ***P. orientalis,*** *Platanus orientalis*; **S.C.,** subcutaneous; **SIR2,** silent information regulatory; **ST,** single transfectants; ***S. typhimurium*,** *Salmonella typhimurium*; **TSA,** thermal shift assay; **VL,** visceral leishmaniasis

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main hosts are vertebrates, and the most commonly infected hosts include humans, dogs, and rodents[5]. The sandfly family consists of five genera and 700 species, of which about 30 species are involved in the transmission of the *Leishmania* parasite[6]. Table 1 shows the main species of *Leishmania* that cause human disease. Over the years, many types of research have been conducted on the *Leishmania* vaccine. In each of these studies, candidate antigens were produced using improved laboratory techniques and various experimental models were examined. An overview of the results from the past to the present investigations can provide a fruitful research strategy for researchers. Meanwhile, such studies have shown that different vaccine administration routes can affect protective immunity. Despite the large number of preclinical vaccine candidates, and approaches designed to emulate this protective response[7], the successful transition of *Leishmania* vaccines into human trials has remained elusive, though considerable efforts are underway[8,9]. Therefore, the purpose of this article is to provide a more comprehensive review of the current advances in leishmania vaccine development.

Immunity against leishmaniasis

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

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

Table 1. The main species of *Leishmania* that cause human disease

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

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

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

Vaccination concepts in leishmaniasis

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

Challenges of efficient vaccine design

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

Immunization methods against CL

Leishmanization

Adler observed that Lebanese children whose arms have been exposed to infected mosquitoes by their

mothers will be protected against severe forms of the disease in the future[34]. This process was not followed because it caused uncontrolled growth of skin lesions and also led to a high prevalence of the disease in people with suppressed immune systems, particularly those with HIV and organ transplants[35,36]. The first method of immunization against leishmaniasis known as "leishmanization" was developed in 1940 and has been used in various countries for several years[37]. This vaccine was discontinued due to its lack of safety and is now limited to the vaccine registered in Uzbekistan and the vaccine used in clinical trials in Iran[38,39]. In this procedure, live and active *L. major* promastigotes are injected intradermally into the anatomical position of the deltoid muscle. An active ulcer then develops and eventually heals on its own. The result of this method is long-term immunity against rural and urban leishmaniasis. Tables 2 and 3 shows leishmanization experiments in Iran and USSR countries.

First-generation vaccines

These vaccines contain the whole body of the parasite with or without adjuvant[39]. First-generation vaccines replaced leishmanization, and the vaccine is now used in some human trials. These categories include killed, live attenuated, and fractionated vaccines[40]. Table 4 lists the first-generation vaccines with full specifications.

Killed vaccines

This type of vaccine was developed and evaluated by Mayrink *et al.* in Brazil[41,42]. The result of the leishmanin skin test was satisfactory, but the vaccine had only a 50% protective effect. In Venezuela, Sharples *et al.*[43] used a mixture of killed *L. amazonensis*, *L. mexicana*, and *Bacillus Calmet Guerin* to treat CL, resulting in a 95% improvement and activation of Th1 immunity[43-45]. Studies in Brazil have shown that a mixture of killed *L. amazonensis* with half a dose of meglumine antimoniate is very effective in treating CL[46]. According to a study conducted in Ecuador, a proportion of *L. brasiliensis*, *L. guianensis*, and *L. amazonensis* provided favorable protection against CL[47-49]. Two studies in Iran have shown that autoclaved *L. major* vaccine with BCG is safe but does not provide promising immunity against CL[50,51]. The results of a study by Mahmoodi *et al.*[52] revealed that cases who received the ALM + BCG vaccine had a higher stimulation index and IFN-γ levels than those who received BCG alone or in the control group. The results of this study showed that the induction of Th1 immune response in volunteers who received the vaccine was much lower than those with or without a previous history of leishmaniasis, and it was assumed that these individuals became immune[52]. Th1 is activated in *L. major* infection, but *L. amazonsensis* can remain active in the presence of Th1 and can reduce the T cell response. Therefore, the vaccine made for *L. major* is neither effective for another leishmaniasis nor VL. In general, vaccination with killed *Leishmania* promastigotes could be considered as a safe and economical treatment; nevertheless, further trials aiming at the evaluation of different adjuvants potentially pave the way for more efficient vaccines[53].

Live attenuated vaccine

These vaccines are currently the gold standard. In attenuated live vaccines, the parasite is both nonpathogenic and superior to killed vaccines[54]. Methods of preparing attenuated live parasites include long-term *in vitro* culture[55], use of temperature sensitivity[56], gamma radiation[57], chemical mutagenesis[58], and culture with gentamicin[59]. Titus and co-workers[60] developed a live attenuated vaccine by knocking down certain *Leishmania* genes. Examples in this regard are the *DHFR-TS*[60] and the *lp2* gene, which encodes an enzyme, transports guanosine diphosphate mannose to the Golgi apparatus[61-63], the *lpg2* mutant from *L. mexicana*[64], the CP (*cpa* and *cpb*) from *L. mexicana*[65,66], the *SIR2* from *L. infantum*[67], and the BT1 gene from *L. donovani*[68].

Suicidal cassettes

Muyombwe *et al.*[69] followed a method of producing a vaccine against leishmaniasis, which was to induce suicide genes. This method is performed by inducing drug-sensitive genes. They used a combination of thymidine kinase and gancyclovir against *L. major* and finally using gancyclovir treatment, partial to complete protection was achieved[70,71]. Besides, the susceptible strain of *L. major*, which contained the altered thymidine kinase *HSV-1* (*tk*) gene and the cytosine deaminase gene from *Saccharomyces cerevisiae* (*cd*), increased susceptibility to gancyclovir and 5-fluorocytosine. *L. major* infection recovered within two weeks of treatment with either drug alone or in combination with ganciclovir and 5-fluorocytosine[70,71].

Fractionated vaccine

This kind of vaccine is advantageous due to its high purity and yield. Several molecules, either membrane proteins, such as HASPB1 and A2 protein, or soluble fractions of the parasite, i.e. PDI, TPI, elF-2, aldolase, enolase, P45, tryhpanothione reductase, and

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Table 2. Leishmanization experiments in Iran

Year	Study place	No.of individuals	*Leishmania* species	Infected with disease (%)	Comment	Ref.
1946	Tehran	120	*L. tropica major*	90	Cross protection against *L.tropica minor*	111
1977	Isfahan	250	*L. major*	47	The incidence rate of CL in leishmanized children was one-sixth to one-seventh to control group.	112,113
1982-86	Isfahan and Dezful	160,000	*L. major*	89.5	Under 1% of new cases of CL were among leishmanized people.	112,113
1983-1989	On army recruits and revolutionary guard	1800,000 and 6000 refugees	*L. major*	56.7–90	Reduction of the incidence rate of CL by Leishmanization among leishmanized people between one-sixth to one-eighth of its original level	113,114
2005	Tehran	28	*L.major*	100	Total protection was seen in 100% (11/11) of volunteers.	115
1989	Individuals receiving NLCV (no. 27)	unvaccinated individuals (no.30)	*L. major*	61.5% in vaccinated and 90% in unvaccinated individuals	With 27% protection in the NLCV group	116
2001	Isfahan Province	200	Deep-freeze promastigote forms of *L. major*	40–45	Production of *L. major* under good manufacturing practices condition at Razi Institute	unpublished

NLCV, nonliving crud vaccine

recombinant F14, among others have been used as a potential target for vaccination, both against cutaneous and VL. Also, some polyproteins have been tested with some degrees of success (Q protein, Leish-111f, 110f etc.)[72].

Second-generation vaccines

Second-generation vaccines are based on synthetic or recombinant subunits and genetically modified *Leishmania* strains, recombinant bacteria, or viruses carrying *Leishmania* antigen genes[73-75]. A summary of these vaccines against *Leishmania* is given in Table 5.

Vaccines based on nonpathogenic *Leishmania*

In 2015, Katebi *et al.*[76] showed that vaccination with *L. tarentolae*-PpSP15 in combination with CpG as a prime-boost modality confers strong protection against *L. major* infection, which was superior to other vaccination methods discussed in the present study. This approach represents a novel and promising strategy for vaccination against Old World CL. In 2014, Zahedifard *et al.*[77] demonstrated the effect of a novel combination of protective parasitic antigens created by *L. tarentolae*, together with sandfly salivary antigen as a vaccine strategy against *L. major* infection. The immunogenicity and protective effect of different DNA/Live and Live/Live prime-boost vaccination with live *L. tarentolae* expressing CPs (type I and II, CPA/CPB) and PpSP15 from *Phlebotomus papatasi*, were tested in BALB/c and C57BL/6 mice. Both humoral and cellular immune responses were assessed before challenge and at 3 and 10 weeks after *Leishmania* infection. In both strains of mice, the strongest protective effect was observed when the mice primed with PpSP15 DNA and then received PpSP15 DNA and live recombinant *L. tarentolae* as a booster[77]. In 2015, Shahbazi *et al.*[78] vaccinated outbreed dogs with a prime-boost regimen based on recombinant *L. tarentolae* expressing the *L. donovani* A2 antigen, along with CP genes (CPA and CPB^{-CTE}) and evaluated its immunogenicity and protective immunity against *L. infantum* infectious challenges.

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Table 3. Early leishmanization experiments in USSR countries[117]

Year	Inoculum	Number	Infected with disease (%)	Comment	Ref.
1942-1968	1.5×10^6	647	60-90	Used infected hamster tissue	118
1972	$1.0\ \ 10^6$	65	100	A new isolate replaced older ineffective strain	119
1978	2×10^6	475	14-100	High level of nodules	118
1979	4×10^6	39	100	Pretest of frozen vaccine	118
1968	0.8×10^6	2245	98	93.2% of ulcers <2 cm at 2 months	120
1968	$0.1\text{-}1.2 \times 10^6$	12500	90	Found little influence of culture age, medium or number	121
2018	-	9500	96-100	-	118

They showed that vaccinated animals produced significantly higher levels of IgG2, but not IgG1, as well as IFN-γ and TNF-α, but low IL-10 levels, before and after challenge as compared to control animals. Protection in dogs was also associated with a strong DTH response and a low parasite burden in the vaccinated group. Overall, immunization with recombinant *L. tarentolae* A2-CPA-CPB^{-CTE} proved to be immunogenic and induced partial protection in dogs, hence representing a promising live vaccine candidate against canine VL[78]. In 2013, Saljoughian *et al.*[79] used a tri- gene fusion recombinant *L. tarentolae* expressing the *L. donovani* A2 antigen, along with CPs, as a live vaccine. Their results showed that immunization with both prime-boost A2-CPA-CPB^{-CTE}-recombinant *L. tarentolae* protects BALB/c mice against *L. infantum* challenge. This protective immunity is associated with the Th1 immune response due to the high levels of IFN-γ production before the challenge, leading to a significant increase in the IFN-γ/IL-10 ratio compared to the control groups. In addition, this immunization induced an elevated level of IgG1 and IgG2a humoral immune responses. Protection in mice was also associated with a high NO production and low parasite burden. Altogether, these results indicate the potential of the A2-CPA-CPB^{-CTE}-recombinant *L. tarentolae* as a safe live vaccine candidate against VL[79].

Lactococcus lactis as a tool for *Leishmania* vaccination

L. lactis is a well-defined, food-grade lactic acid bacterium commonly known as generally recognized as safe status. A better understanding of this bacterium at a molecular level has led to the development of unprecedented genetic tools that enable the expression of heterologous proteins. Consequently, the ability of *L. lactis* to express and deliver these proteins to eukaryotic hosts offers a promising approach to achieve potent treatments for various diseases. Currently, 13 genera have been classified under the lactic acid bacterium group, including *Lactococcus*, *Lactobacillus*, *Streptococcus*, *Pediococcus*, *Paralactobacillus*, *Enterococcus*, *Carnobacterium*, *Lactosphaera*, *Leuconostoc*, *Oenococcus*, *Tetragenococcus*, *Weisella*, *and* Vagococcus[80]. In 2012, Hugentobler *et al.*[81] described the generation of *L. lactis*(*alr*-) strain as the vector expression of the protective *Leishmania* antigen, LACK, in the cytoplasm, secreted or anchored to the bacterial cell wall or co-expressing mouse IL-12. They showed that oral immunization using live *L. lactis*, secreting both LACK and IL-12, was the only regimen that partially protected BALB/c mice against the next *L. major* challenge. This issue highlights the importance of temporal and physical proximity of the delivered antigen and adjuvant for optimal immune priming by oral immunization. In 2019, Torkashvand *et al.*[82] expressed F1S1 fusion protein, including the N-terminal region of S1 subunit of PT and FHA type1 immunodominant domain by *L. lactis*, and evaluated its immunogenicity. Based on their results, mice immunized with LL-F1S1 produced significant levels of specific IFN-γ compared to controls and DTaP-immunized mice. The F1S1-specific IgG antibody response was lower in LLF1S1-immunized mice, while the IgG2a/IgG1 ratio was higher in this group compared to the DTaP-immunized mice. In 2020, Davarpanah and co-workers[83] explained that PpSP15 is an immunogenic salivary protein from *P. papatasi*. Immunization with *Lactococcus lactis* expressing sand fly PpSP15 salivary protein has been shown to protect against *L. major* infection. In their study, BALB/c mice were challenged with *L. major* plus *P. papatasi* salivary gland homogenate. Evaluation of footpad thickness and parasite burden displayed a delay in disease development and reduced the number of parasites in PpSP15 vaccinated animals as compared to the control group. In addition, vaccinated mice exhibited Th1 type immune responses. Importantly, immunization with *L. lactis*-PpSP15-EGFP^{cwa} enhanced long-term memory in mice, which lasted for at least six months.

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Table 4. Types of first-generation vaccines against *Leishmania*

Antigen	Vaccine form/ adjuvant/del. system	Animal model	Targeted disease (*Leishmania* spp.)	Summary of the experimental system	Result	Another outcome	Ref.
L. major	Pathogenic 10^4 live promastigotes	C57BL/6	CL/*L. major*	Immunized through the ear (i.d.) and footpad (s.c.). Challenged 7 weeks later with 10^3 promastigotes	Protection	s.c. route more effective enhanced IFN-γ and IL-10 levels in s.c. and i.d. immunization, respectively.	38
L. major	Nonpathogenic live promastigotes	C57BL/6 BALB/c	CL/*L. major*	Immunized by intraperitoneal or subcutaneous injection. Challenged with pathogenic promastigotes	Protection	Complete protection in C57BL/6 mice while partial in BALB/c mice	55
L. braziliensis	Avirulent *L. braziliensis*	BALB/c	CL/*L. major*	Immunization with Nmethyl-N′-methyl-N′-nitro-N-nitrosoguanidine treated promatigotes	Protection	Immunity conferred and transferred by Lyt-1+ cells	56
L. major	γ-irradiated *L. major*	CBA	CL/*L. major*	Immunized through subcutaneous injection. Challenged with two strains *of L. major*	Protection	LN cells activated infected macrophages *in vitro* to kill the parasite	57
L. major	LPG deficient avirulent *L. major*	BALB/c	CL/*L. major*	Vaccination with $CD4^+$ T-cell line derived from avirulent promastigote immunized mice. Challenged with a virulent strain	Protection	Enhanced TNF and IL-2 production, suppressed IL-4, negative DTH	58
L. mexicana L. major	Long-term culture of 5×10^6 promastigotes with gentamycin	BALB/c	CL/*L. mexicana/L. major*	Immunization with s.c. injection followed by challenge with 5×10^6 wild type promastigotes	Protection	Lesion size reduced by 80%, significantly reduced infected macrophages	59
L. donovani L. infantum	Long-term culture of promastigotes with gentamycin	BALB/c	VL/*L. donovani/L. infantum*	Immunized subcutaneously followed by challenge with wild type promastigotes	Protection	Percentage of infected macrophages reduced by 91–99%	59

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Antigen	Vaccine form/ adjuvant/del. system	Animal model	Targeted disease (*Leishmania* spp.)	Summary of the experimental system	Result	Another outcome	Ref.
L. chagasi	Attenuated 10^7 promastigotes	BALB/c	VL/*L. chagasi*	Challenge with virulent promastigotes	No protection	----	122
L. chagasi	10^7 live promastigotes	BALB/c	VL/*L. chagasi*	Immunization (s.c.) and challenged both with 10^7 live promastigotes	Protection	88% parasite reduction, increased IFN-γ, IL-10, and IL-4 levels, low TGF-β level	122
L. chagasi	10^2 or 10^4 live promastigotes	BALB/c	VL/*L. chagasi*	Immunization (s.c.) with 10^2 or 10^4 promastigotes and challenged with 10^7 live promastigotes	Intermediate protection	No protection in 10^2 doses, low IFN-γ, high TGF-β levels, no effect on IL-10 and IL-4 production as compared to control	122
L. major L. chagasi	10^2 and 10^7 live promastigotes	BALB/c	VL/*L. chagasi*	Challenged with 10^6 *L. chagasi* promastigotes	No protection	----	122
L. chagasi L. donovani L. major	DHFR-TS knock-out Promastigotes	BALB/c	VL/*L. chagasi*	Challenged with 10^7 virulent *L. chagasi*	No protection	A negligible amount of IFN-γ Release	122
L. major	DHFR-TS knock-out promastigotes	BALB/c BALB/c (nu/nu) CBA/T6	CL/*L. major*	Immunization through s.c., i.v. and i.m. routes. Challenged with 10^6 virulent promastigotes	Protection	i.v. route, parasite burden reduced by158–1990 fold in BALB/c mice, i.m. and s.c. the route also produces protection in CBA mice but not in BALB/c mice.	60
L. major	DHFR-TS knock-out promastigotes 10^4, 10^6, and 10^8 dose	BALB/c C57BL/6	CL/*L. amazonensis*	Immunization through i.v. and s.c. routes	Partial protection	10^8 dose developed 40–75% and 49–57% smaller lesion size in BALB/c and C57BL/6 mice, respectively	123
L. major	DHFR-TS knock-out 10^8 promastigotes	Monkey	CL/*L. major*	Immunization subcutaneously and challenged with 10^7 promastigotes	No protection	Positive proliferative response (79%), no IFN-γ production, negative DTH response	124

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Antigen	Vaccine form/ adjuvant/del. system	Animal model	Targeted disease (*Leishmania* spp.)	Summary of the experimental system	Result	Another outcome	Ref.
L. major	Live promastigotes Different doses	BALB/c	CL/*L. major*	Immunization with 10^6, 3×10^3, 10^3, 3.3×10^2, 1.1×10^2, or 3.7×10^1 dose. Challenge with 10^6 promastigotes	Protection only in 1.1×10^2 Borderline disease in half of the 3×10^3 dose no protection in other doses	Enhanced IFN-γ production with low IgG1/IgG2a ratio in protected mice, Th1/Th2 response (both IFN-γ and IL-4 levels high) in borderline disease mice, and Th2 response in progressive disease mice.	61
L. major	lpg2−mutant promastigotes	BALB/c	CL/*L. major*	Immunization (s.c.) with 5×10^6 promastigotes and challenged with wild type 2×10^6 parasites	Protection	Suppressed IL-10 and IL-4 production, low IFN-γ level, negative DTH response	62
L. major	Δlpg2−mutant promastigotes + CpG oligonucleotides	C57BL/6	CL/*L. major*	Immunization with Δlpg2 with a single dose of CpG ODN (50 µg)	Protection	100 fold parasite reduction, no IFN-γ production, no DTH response	125
L. mexicana	CP mutant promastigotes	BALB/c C57BL/6 CBA/Ca	CL/*L. mexicana*	Immunization (s.c.) with 5×10^6 Δcpa or Δcpb or both. Challenged with 10^6 wild type promastigotes	Protection	Increased IFN-γ and IL-2 levels with low IL-4, no difference in IL-5, IL-10, and IL-12 levels, high IgG2a/ IgG1 ratio	65
L. mexicana	CP deficient promastigote	Hamster	CL/*L. mexicana*	Immunization (i.d.) with 10^3 Δcpb or Δcpa/cpb promastigotes and challenged with wild type *L. mexicana*	Protection	High IFN-γ, no difference in IL-10 while TGF-β, IL-4, and IL-12 p40 not detected	66
L. infantum	SIR2 deficient	BALB/c	VL/*L. infantum*	Immunization (i.p.) with 10^8 promastigotes and challenged with 10^8 wild type promastigotes	Protection	Enhanced NO level, high IFN-γ/IL-0 ratio, no difference in IL-4 and IL-2 levels, high IgG1 and IgG2a titer	67

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Antigen	Vaccine form/ adjuvant/del. system	Animal model	Targeted disease (*Leishmania* spp.)	Summary of the experimental system	Result	Another outcome	Ref.
L. donovani	BT1 knock-out promastigotes	BALB/c	VL/*L. donovani*	Immunization (i.v.) with 5 × 10^7 mutant promastigotes. Challenged with 5×10^7 luciferase-expressing virulent promastigotes	Protection	Infection rate reduced by 75%, increased IFN-γ level, no IL-4 production	68
L. tarentolae	Nonpathogenic *L. tarentolae* promastigotes	BALB/c	VL/*L. donovani*	Immunization (i.p.) with 5 × 10^6 promastigotes and challenged with 5×10^7 virulent *L. donovani* promastigotes	Protection	80-85% parasite reduction, enhanced IFN-γ production, no IL4, spleen cell proliferation increased by 17 fold	126
L. major	Suicide system of promastigotes with thymidine kinase gene of HSV-1	BALB/c	CL/*L. major*	Mice infected by tk-transfected or wild type promastigotes and treatment given by ganciclovir	Partial to complete	-----	69
L. major	tk-cd$^{+/+}$ transfected promastigotes	BALB/c	CL/*L. major*	Mice infected with tk-cd$^{+/+}$ transfected and wild-type promastigotes. Treatment is given by ganciclovir and 5-fluorocytosine	Protection	Mice infected with transfected promastigotes were completely cured by either or both drugs.	71
L. amazonensis	Porphyrogenic (DT) and non-porphyrogenic (ST) transfectants	Hamster	VL/*L. donovani*	Photodynamic vaccination with DT + ALA, DT - ALA, ST + ALA, or ALA. Challenged with 10^7 amastigotes	Protection	99% parasite reduction, increased DTH, and lymphoproliferative response, high IFN-γ, iNOS, and IL-12 expression, high IgG2a titer	127
L. infantum		Human and animal	CL	injecting one milliliter of the fraction intracutaneously in four different points of the skin. These were people who had been ill for at least three months	Protection		128

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Table 5. Second-generation vaccines against *Leishmania*

Antigen	Adjuvant/delivery system	Animal model	Targeted disease (Leishmania spp.)	Result	Other outcomes	Ref.
gp63	*S. typhimurium*	CBA, BALB/c	CL/*L. major*	Protection	Protection only in CBA mice, 67–78% parasite reduction, activated CD4+ T cells which secret IFN-γ and IL-2 but not IL-4, negative DTH response	129
gp63	Alone and along with BCG or *C. parvum* or MDP	CBA, BALB/c	CL/*L. major*	Protection	Antigen alone reduced the lesion size comparable to those of gp63 + BCG, protection induced by gp63 + adjuvant varied depending on the site of vaccination relative to that of the challenge	130
rgp63	*C. parvum*	BALB/c	CL.	No protection	----	131
rgp63	*E. coli*	Monkeys	CL/*L. major*	Partial protection	Positive DTH response, no IFN-γ production, high IgG antibody level	132
gp63	Liposomes liposomes + CFA	CBA	CL/*L. mexicana*	protection	The protection conferred only by gp63 + liposomes	133
rgp63	*S. typhimurium*	BALB/c	CL/*L. major*	protection	Activated T cells secreted IFN-γ and IL-2 but not IL-4, high IgG2a levels, no IgG1, negative DTH response.	90
rgp63	*S. typhimurium*	BALB/c	CL and VL/*L. major* or *L. donovani*	Protection	Protection induced against both species, high IFN-γ level, IL-2, and IL-4 not detectable, negative DTH response.	134
rgp63	*S. typhimurium*	F1 (BALB/c C57BL/6)	CL/*L. mexicana*	Protection	High IFN-γ and IL-2 mRNA expression but not IL-4 and IL-10	135
rgp63	Transfected BCG	BALB/c CBA/J	CL/*L. mexicana* or *L. major*	Protection	Protection against *L. mexicana* and *L. major* in both mouse strains, strong lymphoproliferative response.	136,137
gp63	Cationic liposomes	BALB/c	VL/*L. donovani*	Protection	86% and 81% parasite reduction in liver and spleen respectively, high IFN-γ and IgG2a levels even after challenge, low IL-4 production, positive DTH response.	139

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Antigen	Adjuvant/delivery system	Animal model	Targeted disease (Leishmania spp.)	Result	Other outcomes	Ref.
gp63 or rgp63	*E. coli*	Human	CL/VL	Protection	strong proliferative response to both species, high IFN-γ production in PBMC culture upon antigen stimulation.	140
Peptide PT3 of gp63	Poloxamer or CFA or DC pulsed	BALB/c	CL/*L. major*	Protection	Protection only by PT3 (p154–168), enhanced IL-2 but not IL-4 production, no lesion in the second study while reduced lesion development in the third study	141-144
rgp63	Transfected L929 cells with CD40L + gp63	BALB/c C57BL/6	CL/*L. major* or *L. amazonensis*	Protection	Both strains of mice protected against both parasite species, high IL-12 production	145
M-2	*C. parvum* Saponin CFA	CBA BALB/c C57BL/6	CL/*L. amazonensis*	Variable protection	*C. parvum* gave better results, followed by saponin, complete protection in CBA, partial in BALB/c, and no protection in C57BL/6, protection correlated with increased IgG1 and IgG2	146
GP46/M-2	Vaccinia virus	BALB/c	CL/*L. amazonensis*	Protection	IL-2, IFN-γ, and IL-4 production, high IgG1, IgG2a, and IgM with low IgG3 and IgG2b	147
PSA-2	*C. parvum*	C3H/He	CL/*L. major*	Protection	100-fold parasite reduction, predominant IgG1 with IgG2a and IgG2b before the challenge, high IFN-γ but no IL-4 level	148
rPSA-2	Transfected *E. coli* + *C. parvum* ISCOM	C3H/He	CL/*L. major*	No protection	High IFN-γ production, high IgG1, IgG2a, IgG2b, and weak IgG3	149
LACK/rp24	IL-12	BALB/c	CL/*L. major*	Protection	Upregulation of IFN-γ and downregulation of IL-4 transcripts	150
rLACK	rIL-12	BALB/c	CL/*L. major*	Protection	Mice protected only when challenged after two weeks of last immunization, not protected when challenged after 12 weeks of immunization, high IFN-γ (after two weeks)	87

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Antigen	Adjuvant/delivery system	Animal model	Targeted disease (Leishmania spp.)	Result	Other outcomes	Ref.
rLACK	rIL-12	BALB/c	CL/*L. amazonensis*	Protection	After the challenge, the IFN-γ level decreased to the levels of IL-10 and IL-4, high anti-LACK and parasite-specific antibodies	151
rLACK	-----	BALB/c	CL/*L. amazonensis*	No protection	A slight increase in IFN-γ level, IL-10, and IL-4 levels comparable to PBS control.	152
FML	Saponin	BALB/c	VL/*L. donovani*	Protection	84.4% reduction in liver parasite burden, 79.1% and 89.1% increase in proliferative and antibody responses respectively, high antibody level.	153
FML	Saponin	BALB/c	VL/*L. donovani*	Protection	94.7% liver parasite reduction, no change in IFN-γ level while significant decrease in IL-10 production, high DTH response, increase in IgG, IgM, IgG1, IgG2a, and IgG2b anti-FML antibodies	154
FML	Saponin	Swiss albino	VL/*L. donovani*	Protection	85.5% reduction in liver parasite burden, 80% increase in the antibody response	155
FML	Saponin aluminum hydroxide	Swiss albino	VL/*L. donovani*	Protection	85% and 88% liver parasite reduction in FML + saponin and FML + Al(OH)3 group respectively, increased IgG2a level in the former group, similar IgG2b, and IgG3 in both vaccines	156
FML	Saponin	Hamster	VL/*L. donovani*	Protection	Positive DTH response, high anti-FML antibodies.	157
FML	Saponins (Riedel De Haen(R), QuilA, Qs21), IL-12	Swiss Albino	VL/*L. donovani*	Protection	High anti-FML IgG1, IgG2a, and IgG2b, positive DTH response, 73%, 93%, and 79.2% liver parasite reduction in R-FML, QuilA-FML, and Qs21-FML vaccinees respectively, high IFN-γ level in QS21-FML and R-FML vaccines	158
FML	Fractions of Riedel De Haen—QS21 and deacylsaponins	Swiss Albino	VL/*L. chagasi*	Protection	95% and 86% liver parasite reduction in QS21-FML and deacylsaponins-FML vaccinees respectively, positive DTH response, high IFN-γ production, high IgG, IgG1, IgG2a, IgG2b, and IgG3 in QS21-FML vaccinees but not in deacylsaponins	159
GP36	Saponin	BALB/c	VL/*L. donovani*	Protection	68.1% liver parasite reduction, high IgG2a, IgG2b, and IgG1 antibodies, positive DTH response	160

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Antigen	Adjuvant/delivery system	Animal model	Targeted disease (Leishmania spp.)	Result	Other outcomes	Ref.
FML	------	Dogs	VL	Protection	92% protection achieved after two years, vaccinees showed positive DTH response.	161
FML	QuilA	Dogs	VL	Protection	95% protection achieved, positive DTH response	162
FML	QuilA	Dogs	VL/*L. donovani*	Protection	60% dogs protected, high anti-FML IgG, IgG2	163
FML	Saponin	Dogs	VL	Protection	90% dogs protected, 79–95% positive DTH response, high IgG2 than IgG1	162
FML	Saponin	Dogs	VL	Protection	Highanti-FMLantibodies, 82.7% positive DTH response, increase in $CD8^+$ TandCD21$^+$ Bcells	164
FML	Saponin	Dogs	VL	Protection	Act as a transmission-blocking vaccine, high IFN-γ, NO. and IgG2 production, high $CD8^+$ T cell proliferation	165-168
LiESA	MDP	Dogs	VL/*L. infantum*	Protection	92% vaccine efficacy, high IgG2 level, enhanced IFN-γ and no production while no change in IL-4 level	169,170
LiESA	MDP	Dogs	VL/*L. infantum*	Protection	Increased IFN-γ and anti-LiESA IgG2. level, positive DTH response	171
Recombinant CP (rCP5)	IL-12	C57BL/6	CL/*L. mexicana*	Protection	-----	172
CP	CFA	BALB/c	CL/*L. major*	Protection	Enhanced splenocyte proliferation and IFN-γ level, no IL-5 production.	173
rCPA rCPB	Poloxamer 407	BALB/c	CL/*L. major*	Partial protection	Only by rCPB, enhanced IFN-γ level, equal IgG1, and IgG2a antibody levels	174
rCPA/rCPB	Fused hybrid in pET23a	BALB/c	CL/*L. major*	Partial protection	High IgG2a, enhanced IFN-γ production with little IL-5	175
Peptide I of CP	----	CBA	CL/*L. amazonensis*	Protection	Enhanced IFN-γ, IL-4, and NO production, Proliferation of $CD8^+$ T-cell subsets	176

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Antigen	Adjuvant/delivery system	Animal model	Targeted disease (Leishmania spp.)	Result	Other outcomes	Ref.
rGRP78	CFA	C57BL/6	CL/*L. major*	Protection	83% mice protected.	178
78 kDa	-----	BALB/c	VL/*L. donovani*	-----	Increase in IgG2a levels, low IgG1	179
78 kDa	MPL-A, liposomal encapsulation, rIL-12, ALD, CFA	BALB/c	VL/*L. donovani*	Protection	92%, 93.4%, and 98% liver parasite reduction by 78 kDa+MPL-A or liposomal encapsulation or rIL-12 vaccinees, enhanced IFN-γ and IL-2 levels with low IL-4 and IL-10, positive DTH response, high IgG2a level	180
P4 P8 A2	*C. parvum*	BALB/c	CL/*L. pifanoi/L. amazonensis*	Protection	Only P4 and P8 gave protection and P8 gave cross-protection, high IFN-γ level while no change in IL-2 level	181
P4	*P. acnes*	BALB/c	CL/*L. pifanoi*	Protection	$CD4^+$ T-cell related protection, high IFN-γ, MIF, TNF-α mRNA expression, high IL-2 level, and no change in IL-4 level	182
P8	------	Dogs	VL/*L. infantum*	----	High IFN-γ and TNF-α expression in P8-stimulated PBMC, low IL-4 but no IL-10 level	183
P4 P8	----	Human	CL	----	Enhanced IFN-γ and IL-2 levels in respective antigen-stimulated PBMC culture, extremely low IL-4 level	184
P4	----	Human	CL	-----	Enhanced IFN-γ level in P4-stimulated PBMC culture, IL-4 detectable	185
rA2	*P. acnes*	BALB/c	VL/*L. donovani*	Protection	89% liver parasite reduction, enhanced IFN-γ level, no change in IL-4 level, high IgG1, IgG2a, IgG2b, and IgG3	186
rA2	------	BALB/c	VL/*L. chagasi*	Protection	High IFN-γ production, enhanced CTL activity mediated by $CD8^+$ T cells, low antibody response	187
rA2	Saponin	Dogs	VL/*L. chagasi*	Partial protection	Enhanced IFN-γ while low IL-10 production, increased IgG and IgG2 but not IgG1	188
rHASPB1	IL-12	BALB/c	VL/*L. donovani*	Protection	91% liver and 70–90% splenic parasite reduction in rHASPB1 vaccinees, increased IL-12 production by DC, exclusive IgG1 response, increased IFN-γ producing $CD8^+$ T cells.	189

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Antigen	Adjuvant/delivery system	Animal model	Targeted disease (Leishmania spp.)	Result	Other outcomes	Ref.
rHASPB1	Montanide	Dogs	VL/*L. infantum*	Partial protection	50% dogs asymptomatic, high anti-HASPB1 antibody titer.	190
rLcr1	CFA Ribi adjuvant	BALB/c C3H	VL/*L. chagasi*	Partial protection	In infected mice, high IFN-γ production in both mice, detectable IL-10 but not IL-5 levels in splenocytes to Lcr1 stimulation.	191
rLcr1	BCG expressing Lcr1	BALB/c	VL/*L. chagasi*	Protection	High IFN-γ and reduced IL-10 production, no detectable IL-4.	192
rH1	Montanide	Monkeys	CL/*L. major*	Partial protection	High antibody levels, positive DTH response.	193
rH1 peptides of H	IL-12 IFA	BALB/c	CL/*L. major*	Partial protection	Partial protection even in absence of adjuvants, LP1-3 also gave partial protection.	89
rORFF	CFA	BALB/c	VL/*L. donovani*	Partial protection	Detectable anti-ORFF antibody titer, the proliferation of spleen cells	194
rORFF	CpG ODN	BALB/c	VL/*L. donovani*	Protection	84% liver parasite reduction, enhanced IFN-γ and IgG2a production, NO production dose-dependent.	195
rORFF	----	BALB/c	VL/*L. donovani*	Partial protection	45–60% parasite reduction, low IgG2a/IgG1 ratio, high IFN-γ, and IL-12 as compared to controls.	196
rORFF	IL-12 DNA	BALB/c	VL/*L. donovani*	Protection	82% parasite reduction, enhanced IFN-γ, IL-12, and IgG2a production, no change in IL-4 level, enhanced splenocyte proliferation.	197
rLiP0	CpG ODN	C57BL/6 BALB/c	CL/*L. major*	Protection	Complete protection only in C57BL/6 mice, partial in BALB/c, 150-fold parasite reduction, high IFN-γ, and IgG2a production	198
Ribosomal proteins (LRP)	CpG ODN	BALB/c C57BL/6	VL/*L. major*	Protection	Protection in both strains, 3 fold parasite reduction, high IFN-γ level and IgG2a/IgG1 ratio, no increase in IL-4, detectable IL-10	199
rKMP-11	ts-mutant expressing KMP-11	BALB/c	CL/*L. major*	Partial protection	-----	200

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Antigen	Adjuvant/delivery system	Animal model	Targeted disease (Leishmania spp.)	Result	Other outcomes	Ref.
rKMP-11	Hybrid cell vaccine	BALB/c	VL/*L. donovani*	Protection	Enhanced IFN-γ, IL-4, and IL-13 expression but not IL-10	201
rPFR-2	FIA	Hamster	CL/*L. panamensis/L. mexicana*	Protection	Only female hamster protected against *L. panamensis*, positive DTH response, no protection against *L. Mexicana*	202
Protein Q	BCG	Dogs	VL/*L. infantum*	Protection	90% protection, positive DTH response,	203
Protein Q	CpG ODN	BALB/c	VL/*L. infantum*	Protection	99% reduction in liver and splenic parasite burden, high IgG2a/IgG1 ratio, high IFN-γ with low IL-4 production	204
rTSA	IL-12	BALB/c	CL/*L. major*	Protection	Protection only in rTSA-IL12 vaccinees, induce human PBMC proliferation.	205
TSA LmSTI1 TSA+LmSTI1	IL-12	BALB/c	CL/*L. major*	Protection	The protection conferred in all three vaccinees group when adjuvant is used, significant protection by LmSTI1 + IL-12 and TSA + LmSTI1 + IL-12, partial by TSA + IL-12	206
TSA+LmSTI1	rhIL-12 + alum	Monkeys	CL/*L. major*	Protection	No lesion development even on rechallenge after 4 months of first challenge.	206
rLMSTI1	Encapsulation in liposomes	BALB/c	CL/*L. major*	Protection	High IgG level and IgG2a/IgG1 ratio	207
rLMSTI1	Encapsulation of antigen with CpG-ODN	BALB/c	CL/*L. major*	Protection	High IgG titer and IgG2a/IgG1 ratio	208
rLeish-111f	MPL-SE	BALB/c	CL/*L. major*	Protection	Enhanced IFN-γ and IgG2a production, low IL-4 level	209
rLeish-111f	MPL-SE rmIL-12	BALB/c	CL/*L. major*	Protection	Enhanced IFN-γ production, no detectable IL-4, mixed IgG1, and IgG2a response	109

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Antigen	Adjuvant/delivery system	Animal model	Targeted disease (Leishmania spp.)	Result	Other outcomes	Ref.
rLeish-111f	MPL-SE	BALB/c C57BL/6 Syrian hamster	VL/*L. infantum*	Protection	91.7% and 99.6% splenic parasite reduction in mice and hamster respectively, enhanced IFN-γ, IL-2, TNF production with low IL-4 level in mice	210
TSA+LmSTI1 + LeIF+Lbhsp83	GM-CSF	Human	MCL	Protection	83% of patients showed complete clinical cure (CC) after nine months, all were CC after a five-year follow-up	211
rTSA rLeIF rLbSTI1 rLACK	CpG ODN	BALB/c	CL/*L. braziliensis*	No Protection	Enhanced IFN-γ production in response to TSA or LeIF or LACK stimulation, high IgG1/IgG2a ratio	212
rTSA+rLeIF+ rLmSTI1	MPL-SE AdjuPrime	Dogs	VL/*L. chagasi*	-----	Induce Th1 response, specific IgG response to all three antigens, high IgG2a/IgG1 ratio when MPL-SE is used as compared to AdjuPrime	213
rMML	MPL-SE AdjuPrime	Dogs	VL/*L. infantum*	No protection	87% cumulative incidence in vaccines even after two years of vaccination	103
rLeish-110f+ Glucantime	MPL-SE	Dogs	VL/*L. chagasi*	protection	83.3% and 66.6% survival rate by immunochemotherapy and chemotherapy respectively, high proliferative response, high antibody titer in immunotherapy as compared to immunochemotherapy	197

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Third-generation vaccines

DNA vaccines

These vaccines contain plasmid DNA, which, after injection, encodes foreign proteins, leading to the synthesis of endogenous proteins and the production of specific immune responses[84]. DNA vaccines promote both cellular and humoral immunity[85,86]. DNA vaccines can come in many forms, including recombinant proteins[87-97], single vaccines[89,90,93,96,98-100], or multigene forms[92-95,101]. These vaccines were tested in mice against CL and VL[84,85,86,91,94,95,99,101], in hamsters against VL[102,103], and dogs against VL[104-107]. DNA vaccines are made up of heterologous DNA (usually a plasmid) that produces antigenic proteins. These DNAs are supplied by vectors that allow them to be expressed in eukaryotic cells[84]. Advantages of DNA vaccines include (1) fast, simple, and cheap large-scale production, (2) no need for low temperature, transportation, and storage, and (3) the ability to provide long-term protection against multiple strains of *Leishmania*. The main concern with these vaccines is the risk of parasite DNA entering the mammalian genome. This problem carries the potential risk of cancer and autoimmune diseases[84]. A summary of DNA vaccines is given in Table 6 and the best recombinant salivary candidates is shown in Table 7.

Vaccine products for potential licensing

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

DISCUSSION

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

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

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Table 6. Third-generation vaccines against *Leishmania*

Antigen	Adjuvant/delivery system Animal model	system	Targeted disease (*Leishmania* spp.)	Result	Other outcomes	Ref.
gp63	pCMV	BALB/c	CL/*L. major*	Protection	Enhanced IL-12 and IFN-γ production, no detectable IL-4	90, 214
gp63	pCMV3ISS or pcDNA3	BALB/c	CL/*L. major*	Partial protection	30% of mice protected, enhanced IFN-γ protection but not IL-4	94, 215
gp63 or gp46	VR1012	BALB/c	CL/*L. mexicana*	Partial protection	100-fold parasite and 30% reduction in lesion size, mixed IgG2a and IgG1 response, high IgG2a/IgG1 in gp46 vaccinee	216,217
gp63 + gp46 + CPb	VR1012	BALB/c	CL/*L. mexicana*	Protection	80% and 1,000-fold reduction in lesion size and parasite burden respectively	216,217
ORFF	pcDNA3.1	BALB/c	VL/*L. donovani*	Protection	78–80% and 58–60% reduction in liver and spleen parasites respectively, enhanced IFN-γ expression but no change in IL-4 expression	99
PSA-2	pCI-neo	C3H/He	CL/*L. major*	Protection	Enhanced IFN-γ production as compared to control, no detectable IL-4 and IL-5, high IgG2a/IgG1 ratio	218,219
A2	pcDNA3	BALB/c	CL/VL *L. amazonensis/L. chagasi*	Protection	Protection against both species enhanced IFN-γ with low IL-4 and IL-10 production	220
LACK	pCI-neo	BALB/c	VL/*L. chagasi*	Protection	Increased IFN-γ and IL-4 production with low IL-10 and TNF-α level	221
LACK	pCI-neo	BALB/c	VL/*L. chagasi*	No protection	Increased IFN-γ and IL-10 production with no IL-4	222
LACK	pCI-neo	BALB/c	VL/*L. chagasi*	No protection	Enhanced IFN-γ with no IL-4 production	223
LACK	pCMV3ISS	BALB/c	CL/*L. major*	Partial to complete protection	Partial protection by LACK vaccine while complete in LACKp24 vaccinees	94
LACK	MIDGE or MIDGE-NLS	BALB/c	CL/*L. major*	Protection	Enhanced IFN-γ production with no IL-4, high IgG2a/IgG1 ratio	224

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Antigen	Adjuvant/delivery system	system Animal model	Targeted disease (*Leishmania* spp.)	Result	Other outcomes	Ref.
CPa or CPb CPa + CPb	pCB6	BALB/c	CL/*L. major*	Protection	Protection only in CPa + CPb vaccines increased IFN-γ level, but no IL-5	92
CPb	VR1012	BALB/c	CL/*L. mexicana*	Partial protection	100-fold parasite and 50% reduction in lesion size	216,217
KMP-11	pCMV-LIC	Hamster	VL/*L. donovani*	Protection	Inducemixed Th1/Th2 response, enhanced IFN-γ, TNF-α, IL-12, iNOS expression including IL-4, low IL-10 level, high IgG2a, and IgG1 titer	225
KMP-11	pCMV-LIC	BALB/c	VL/*L. donovani*	Protection	96.7% and 98.7% reduction in splenic and liver parasite respectively, enhanced IFN-γ and IL-4 production, suppressed IL-10 level	226
KMP-11	pCMV-LIC+IL-12	BALB/c	CL/*L. major*	Protection	93% reduction in lesion size, enhanced IFN-γ with suppressed IL-4 and IL-10 production	226
P4	pcDNA3+IL-12 or HSP70	BALB/c	CL/*L. amazonensis*	Partial to complete protection	Complete protection with enhanced IFN-γ and TNF-α, low IL-10 production in P4 + IL-12 vaccines while partial with mixed IFN-γ and IL-10 response in P4 + HSP70 vaccines	101
NH36	VR1012	BALB/c	VL/*L. chagasi*	Protection	91% liver parasite reduction, increased IFN-γ with reduced IL-10 and IL-4 levels, positive DTH response, high IgG2b titer	227
papLe22	pcDNA3.1	Hamster	VL/*L. infantum*	Partial protection	Parasite circulation reduced by 50%, produce high anti-pepLe22 but low anti-*Leishmania* antibody titer	91
NH	VR1012	BALB/c	CL/VL *L. amazonensis/L. chagasi*	No protection	Enhanced IFN-γ, IL-4, and IL-10 production	228
NH36	VR1012	BALB/c	CL/VL *L. chagasi/L. mexicana*	Protection	88% and 65% reduction in *L. chagasi* parasite burden and *L. mexicana* infected lesion size respectively, 2–5 fold increase in IFN-γ producing $CD4^+$ T cells, low antibody response, positive DTH response to *L. donovani*	96

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Antigen	Adjuvant/delivery system	system Animal model	Targeted disease (*Leishmania* spp.)	Result	Other outcomes	Ref.
LeIF PSA-2	pCMV3ISS	BALB/c	CL/*L. major*	No protection	---------	94
TSA LmSTI1 TSA+LmSTI	pcDNA3	BALB/c	CL/L. *major*	Protection	Protection induced by all three vaccines enhanced IFN-α production with no IL-4, high IgG2a titer	93
H2A+H2B+ H3+H4	pcDNA3	BALB/c	CL/*L. major*	Protection	Enhanced IFN-γ with little IL-4 production, low antibody response dominated by IgG2a	95
KMPII+TRYP+ LACK+gp63	pMOK	Dogs	VL/*L. infantum*	No protection	Increased anti-*Leishmania* IgG, IgA, and IgM	97
LACK-PB	pcDNA3-vaccinia virus	BALB/c	CL/*L. major*	Protection	1,000 fold and 70% decrease in parasite burden and lesion size respectively, increased IFN-γ level with low IL-10 and IL-4 levels	229
Heterologous prime-boost vaccine						
LACK-PB	pCI-neo—vaccinia virus	Dogs	VL/*L. infantum*	Protection	60% of dogs protected, enhanced IFN-γ and IL-4 expression, high IgG2a/IgG1 ratio	106
LACK-PB	pcDNA3.1 + IL-12 DNA or IL-18 DNA—vaccinia virus	BALB/c	CL/*L. major*	Protection	Enhanced IFN-γ production, high IgG2a/IgG1 ratio	230
LACK-PB	pCI-neo—MVA	BALB/c	CL/*L. major*	Protection	65–92% reduction in lesion size, increased IFN-γ and TNF-α levels	231
LACK-PB	MVA	BALB/c	VL/*L. infantum*	Protection	144–244, 6–9, and 9–30 fold parasite reduction in the lymph node, spleen, and liver respectively, increased IFN-γ and TNF-α levels	232
LACK-PB	pcDNA3—*Salmonella enterica* serovar *Typhimurium*	BALB/c	CL/*L. major*	Protection	Increased IFN-γ level with low IL-10, high IgG2a titer	233

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Antigen	Adjuvant/delivery system	system Animal model	Targeted disease (*Leishmania* spp.)	Result	Other outcomes	Ref.
LACK-PB	pCI-neo—MVA	Dogs	VL/*L. infantum*	Protection	Increased IFN-γ expression with low IL-10 and IL-4 transcripts, high IgG2 titer	220
CPa+CPb-PB	pCB6 + CpG ODN + Montanide 720	Dogs	VL/*L. infantum*	Protection	High IFN-γ/IL-10 ratio, increased IgG, IgG2 but not IgG1 titer, positive DTH response	107
CPa+CPb-PB	pCB6 + CpG ODN + Montanide 720	BALB/c	VL/*L. infantum*	Protection	Increased IFN-γ, high IFN-γ/IL-5 ratio, high IgG and IgG2a titer, low IgG1	234
CTE of CPb-PB	CpG ODN + Montanide 720	BALB/c	VL/*L. infantum*	No protection	Increased IL-5 level, high IL-5/IFN-γ ratio, high IgG2a/IgG1 ratio	235
CPc-PB	pcDNA3.1 + DHFR + CpG ODN+ Montanide 720	BALB/c	VL/*L. infantum*	Protection	Enhanced IFN-γ and NO production, high IgG2a/IgG1 ratio	236
LiP0-PB	pcDNA3	BALB/c	CL/*L. major*	Protection	84.8–99.1% parasite reduction, enhanced IFN-γ production, mixed IgG2a/IgG1 response	237

Table 7. The best recombinant salivary candidates as antigens for detection of anti-saliva antibodies

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

Lu. Longipalpis, *Lutzomyia longipalpis*

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

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

CONFLICT OF INTEREST. None declared.

REFERENCES

1. Mcgwire BS, Satoskar AR. Leishmaniasis: clinical syndromes and treatment. *QJM* 2014; **107**(1): 7-14.
2. Alvar J, Ve´lez ID, Bern C, Herrero M, Desjeux P, Cano J, Jannin J, den Boer M. Leishmaniasis worldwide and global estimates of Its incidence. *PloS one* 2012; **7**(5): e35671.
3. Mohebali M, Yaghoobi-Ershadi MR, Akhavan AA, Hajjaran H, Abaei MR. Characterization of Leishmania infection in rodents from endemic areas of the islamic republic of Iran. *Eastern mediterranean health journal* 2004; **10**(4-5): 591-599.
4. Mirzaei A, Rouhani S, Taherkhani H,Farahmand M, Kazemi B, Hedayati M, Baghaei A, Davari B, Parvizi P. Isolation and detection of Leishmania species among naturally infected Rhombomis opimus, a reservoir host of zoonotic cutaneous leishmaniasis in Turkemen Sahara, North East of Iran. *Experimental parasitology* 2011; **129**(4): 375-380.
5. Quinnell RJ, Courtenay O. Transmission, reservoir hosts and control of zoonotic visceral leishmaniasis. *Parasitology* 2009; **136**(14): 1915-1934.
6. Desjeux P. Leishmaniasis public health aspects and control. *Clinics in dermatology* 1996; **14**(5): 417-423.
7. Costa CH, Peters NC, Maruyama SR, Brito Jr EC, Santos IKF. Vaccines for the leishmaniases: pro-posals for a research agenda. *Plos neglected tropical diseases* 20011; **5**(3): e943.
8. Osman M, Mistry A, Keding A, Gabe R, Cook E, Forrester S, Wiggins R, Di Marco S, Colloca S, Siani L, Cortese R, Smith DF, Aebischer T, Kaye PM, Lacey CJ . A third generation vaccine for human visceral leishmaniasis and post kala azar dermal leishmaniasis: First-in-human trial of ChAd63-KH. *PloS neglected tropical diseases* 2017; **11**(5): e0005527.
9. Duthie MS, Hoeven NV, MacMillen Z , Picone A, Mohamath R, Erasmus J, Hsu F-C, Stinchcomb DT, Reed SG. Heterologous immunization with defined RNA and subunit vaccines enhances T cell responses that protect against Leishmania donovani. *Frontiers in immunology* 2018; **9**: 2420.
10. Hide M, Bucheton B, Kamhawi S, Bras-Gonçalves R, Sundar S, Lemesre JL, Banuls AL. Understanding human leishmaniasis: the need for an integrated approach. *Encyclopedia of infectious diseases* 2007: 87-123.
11. Tripathi P, Singh V, Naik S. Immune response to leishmania: paradox rather than paradigm. *FEMS immunology and medical microbiology* 2007; **51**(2): 229-242.
12. Scott P. Development and regulation of cell-mediated immunity in experimental leishmaniasis. *Immunologic research* 2003; **27**(2-3): 489-498.
13. Sengupta S, Chowdhury S, BoseDasgupta S, Wright CW, Majumder HK. Cryptolepine-induced cell death of Leishmania donovani promastigotes is augmented by inhibition of autophagy. *Molecular biology international* 2011; **2011**: 187850.
14. Roy S, Dutta D, Satyavarapu EM, Yadav PK, Mandal C, Kar S, Mandal Ch. Mahanine exerts in vitro and in vivo antileishmanial activity by modulation of redox homeostasis. *Scientific reports* 2017; **7**(1): 4141.
15. Aghaei M, KhanAhmad H, Aghaei S, Nilforoushzadeh MA, Mohaghegh MA, Hejazi SH. The role of Bax in the apoptosis of Leishmania-infected macrophages. *Microbial pathogenesis* 2020; **139**: 103892.
16. Aghaei M, Khanahmad H, Aghaei S, Hosseini SM, Farahmand M, Hejazi SH. Evaluation of transgenic Leishmania infantum expressing mLLO-BAX-SMAC in the apoptosis of the infected macrophages in vitro and in vivo. *Parasite immunology* 2020; **42**(11): 12726.
17. Basmaciyan L, Azas N, Casanova M. A potential acetyltransferase involved in Leishmania major metacaspase-dependent cell death. *Parasites and vectors* 2019; **12**(1): 266.

[DOR: 20.1001.1.1028852.2022.26.1.8.9]
[DOI: 10.52547/ibj.26.1.35]

18. Nagill R, Kaur S. Vaccine candidates for leishmaniasis: a review. *International mmunopharmacology* 2011; **11**(10): 1464-1488.
19. Thomaz-Soccol V, Ferreira da Costa ES, Karp SG, Junior Letti LA, Soccol FT, Soccol CR. Recent advances in vaccines against Leishmania based on patent applications. *Recent patents on biotechnology* 2018; **12**(1): 21-32.
20. Lachaud L, Dedet JP, Marty P, Faraut F, Buffet P, Gangneux JP, Ravel C, Bastien P, Working group for the notification of human Leishmanioses in France. *Euro surveillance* 2013; **18**(29): 20534.
21. Chamaillé L, Tran A, Meunier A, Bourdoiseau G, Ready P, Dedet JP. Environmental risk mapping of canine leishmaniasis in France. *Parasites and vectors* 2010; **3**(1): 1-8.
22. Kedzierski L. Leishmaniasis vaccine: where are we today? *Journal of global infectious diseases* 2010; **2**(2): 177
23. Mayrink W, Genaro O, Silva JCF, d Costa RT, Tafuri WL, Toledo VPC, d Silva AR, Reis AB, Williams P, d Costa CA. Phase I and II open clinical trials of a vaccine against Leishmania chagasi infections in dogs. *Memórias do instituto oswaldo cruz 1996;* **91** (6): 695-697.
24. Lasri S, Sahibi H, Sadak A, Jaffe CL, Rhalem A. Immune responses in vaccinated dogs with autoclaved Leishmania major promastigotes. *Veterinary research* 1999; **30** (5):441-450.
25. Giunchetti RC, Corrêa-Oliveira R, Martins-Filho OA, Teixeira-Carvalho A, Roatt BM, de Oliveira Aguiar-Soares RD, De Souza JV, das Dores Moreira N, Malaquias LCC, e Castro LLM. Immunogenicity of a killed Leishmania vaccine with saponin adjuvant in dogs. *Vaccine* 2007; **25** (44): 7674-7686.
26. Uzonna JE, Wei G, Yurkowski D, Bretscher P. Immune elimination of Leishmania major in mice: implications for immune memory, vaccination, and reactivation disease. *Journal of immunology* 2001; **167**: 6967-74.
27. 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. *Nature medicine* 2004; **10**(10): 1104-1110.
28. Das A, Ali N. Correction: combining cationic liposomal delivery with MPL-TDM for cysteine protease cocktail vaccination against Leishmania donovani: evidence for antigen synergy and protection. *PloS neglected tropical diseases* 2015; **9**(10): e0004185.
29. Badiee A, Heravi Shargh V, Khamesipour A, Jaafari MR. Micro/nanoparticle adjuvants for antileishmanial vaccines: present and future trends. *Vaccine* 2013; **31**(5): 735-749.
30. McMahon-Pratt D, Alexander J. Does the Leishmania majorparadigm of pathogenesis and protection hold for new world cutaneous leishmaniases or the visceral disease? *Immunological reviews* 2004; **201**: 206-224.
31. Spath GF, Epstein L, Leader B, Singer SM, Avila HA, Turco SJ, Beverley SM. Lipophosphoglycan is a virulence factor distinct from related glycoconjugates in the protozoan parasite Leishmania major. *Proceedings of the National Academy of Sciences of the United States of America* 2000; **97**(16): 9258-9263.
32. Ilg T, Stierhof YD, Craik D, Simpson R, Handman E, Bacic A. Purification and structural characterization of a filamentous, mucin-like proteophosphoglycan secreted by Leishmania parasites. *The journal of biological chemistry* 1996; **271**(35): 21583-21596.
33. Soong L, Chang CH, Sun J, Longley BJ, Jr, Ruddle NH, Flavell RA, McMahon-Pratt D. Role of $CD4^+$ T cells in pathogenesis associated with Leishmania amazonensis infection. *Journal of immunology* 1997; **158**(11): 5374-5383.
34. Adler S, Theodor O. The distribution of sandflies and leishmaniasis in Palestine, Syria and Mesopotamia. *Annals of tropical medicine and parasitology* 1929; **23**(2): 269-306.
35. Dunning N. Leishmania vaccines: From leishmanization to the era of DNA technology. *Bioscience horizons* 2009; **2**(1): 73-82.
36. Noazin S, Modabber F, Khamesipour A, Smith PG, Moulton LH, Nasseri K, Sharifi I, Khalil EAG, Bernal IDV, Antunes CMF, Kieny MP, Tanner M. First generation leishmaniasis vaccines: A review of field efficacy trials. *Vaccine* 2008; **26**(52): 6759-6767.
37. Sundar S, Singh B. Identifying vaccine targets for anti-leishmanial vaccine development. *Expert review of vaccines* 2014; **13**(4): 489-505.
38. Tabbara KS, Peters NC, Afrin F, Mendez S, Bertholet S, Belkaid Y, Sacks DL. Conditions influencing the efficacy of vaccination with live organisms against Leishmania major infection. *Infection and immunity* 2005; **73**(8): 4714-4722.
39. Khamesipour A, Rafati S, Davoudi N, Mahboudi F, Modabber F. Leishmaniasis vaccine candidate for development: a global overview. The Indian journal of medical research 2006; **123**(3): 423-438.
40. Modabber F. Vaccines against leishmaniasis. *Annals of tropical medicine and parasitology* 1995; **89**(Suppl1): 83-88.
41. Mayrink W, Da Costa CA, Magalhães PA, Melo MN, Dias M, Lima AO, Michalick MS, Williams P. A field trial of a vaccine against American dermal Leishmaniasis. *Transactions of the royal society of tropical medicine and hygiene* 1979*;* **73**(4): 385-387.
42. Mayrink W, Williams P, Da Costa CA, Magalhaes PA, Melo MN, Dias M, Lima AO, Michalick MS, Carvalho EF, Barros GC, Sessa PA. An experimental vaccine against American dermal Leishmaniasis: experience in the State of Espirito Santo, Brazil. *Annals of tropical medicine and parasitology* 1985; **79**(3): 259-269.
43. Sharples CE, Shaw MA, Castes M, Convit J, Blackwell JM. Immune response in healthy volunteers vaccinated with BCG plus killed leishmanial promastigotes: antibody responses to mycobacterial and Leishmanial antigens. *Vaccine* 1994; **12**(15): 1402-1412.
44. Convit J, Rondon A, Ulrich M, Bloom B, Castellanos P, Pinardi M, Castes M, Garcia L. Immunotherapy versus chemotherapy in localised cutaneous Leishmaniasis. *Lancet* 1987; **329**(8530): 401-405.

[DOR: 20.1001.1.1028852.2022.26.1.8.9]
[DOI: 10.52547/ibj.26.1.35]

45. Convit J, Ulrich M, Zerpa O, Borges R, Aranzazu N, Valera M, Villarroel H, Zapata Z, Tomedes I. Immunotherapy of American cutaneous leishmaniasis in Venezuela during the period 1990–1999. *Transactions of the royal society of tropical medicine and hygiene* 2003; **97**(4): 469-472.
46. Machado-Pinto J, Pinto J, Da Costa CA, Genaro O, Marques MJ, Modabber F, Mayrink W. Immuno-chemotherapy for cutaneous Leishmaniasis: a controlled trial using killed Leishmania (Leishmania) amazonensis vaccine plus antimonial. *International journal of dermatology* 2002; **41**(2): 73-78.
47. Armijos RX, Weigel MM, Aviles H, Maldonado R, Racines J. Field trial of a vaccine against New World cutaneous leishmaniasis in an at-risk child population: safety, immunogenicity, and efficacy during the first 12 months of follow-up.*The journal of infection diseases* 1998; **177**(5): 1352-1357.
48. Armijos RX, Weigel MM, Romero L, Garcia V, Salazar J. Field trial of a vaccine against new world cutaneous leishmaniasis in an at-risk child population: how long does protection last? *The journal of infection diseases* 2003; **187**(12): 1959-1961.
49. Armijos RX, Weigel MM, Calvopina M, Hidalgo A, Cevallos W, Correa J. Safety, immunogenecity, and efficacy of an autoclaved Leishmania amazonensis vaccine plus BCG adjuvant against New World cutaneous leishmaniasis. *Vaccine* 2004; **22**(9-10): 1320-1326.
50. Momeni AZ, Jalayer T, Emamjomeh M, Khamesipour A, Zicker F, Ghassemi RL, Dowlati Y, Sharifi I, Aminjavaheri M, Shafiei A, Alimohammadian M H, Hashemi-Fesharki R, Nasseri K, Godal T, Smith P G, Modabber F. A randomised, double blind, controlled trial of a killed *L. major* vaccine plus BCG against zoonotic cutaneous leishmaniasis in Iran. *Vaccine* 1998; **17**(5): 466-472.
51. Sharifi I, Fe Kri AR, Aflatonian MR, Khamesipour A, Nadim A, Mousavi MRA, Momeni A Z, Dowlati Y, Godal T, Zicker F, Smith P G, Modabber F. Randomized vaccine trial of single dose of killed Leishmania major plus BCG against anthroponotic cutaneous leishmaniasis in Bam, Iran. *Lancet* 1998; **351**: 1540-1544.
52. Mahmoodi M, Khamesipour A, Dowlati Y, Rafati S, Momeni AZ, Emamjomeh M, Hejazi H, Modabber F. Immune response measured in human volunteers vaccinated with autoclaved Leishmania major vaccine mixed with low dose of BCG. *Clinical and experimental immunology* 2003; **134**(2): 303-308.
53. Kedzierski L, Zhu Y, Handman E. Leishmania vaccines: progress and problems. *Parasitology* 2006; **133**(S2): S87.
54. Selvapandiyan A, Duncan R, Debrabant A, Lee N, Sreenivas G, Salotra P, et al. Genetically modified live attenuated parasites as vaccines for leishmaniasis. *Indian journal of medical research* 2006;**123**:455-66.
55. Mitchell G.F, Handman E, Spithill T.W. Vaccination against cutaneous Leishmaniasis in mice using nonpathogenic cloned promastigotes of Leishmania major and importance of route of injection. *Australian journal of experimental biology and medical science* 1994; **62**(2): 145-153
56. Gorczynski RM. Immunization of susceptible BALB/c mice against Leishmania braziliensis: II. Use of temperature-sensitive avirulent clones of parasite for vaccination purposes. *Cellular immunology* 1985; **94**(1): 11-20.
57. 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 immunology* 1993; **15**(2): 35-46.
58. 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 *Infection and immunity* 1993; **61**(12): 5205-2513.
59. Daneshvar H, Coombs GH, Hagan P, Phillips RS. Leishmania mexicana and Leishmania major: attenuation of wild-type parasites and vaccination with the attenuated lines. *The journal of infectious diseases* 2003; **187**(10): 1662-1668.
60. Titus RG, Gueiros-Filho FJ, De Freitas LA, Beverley SM. Development of a safe live Leishmania vaccine line by gene replacement. *Proceedings of the National Academy of Sciences* 1995; **92**(22): 10267-10271.
61. Uzonna JE, Wei G, Yurkowski D, Bretscher P. Immune elimination of Leishmania major in mice: implications for immune memory, vaccination, and reactivation disease. *Journal of immunology* 2001; **167**: 6967-6974.
62. 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. *The journal of immunology* 2004; **172**(6): 3793-3797.
63. Späth GF, Lye LF, Segawa H, Sacks DL, Turco SJ, Beverley SM. Persistence without pathology in phosphoglycan-deficient Leishmania major. *Science* 2003; **301**(5637): 1241-1243.
64. Ilg T, Demar M, Harbecke D. Phosphoglycan repeat-deficient Leishmania mexicanaparasites remain infectious to macrophages and mice. *Journal of biological chemistry* 2001; **276**(7): 4988-4997.
65. Alexander J, Coombs GH, Mottram JC. Leishmania mexicana cysteine proteinase-deficient mutants have attenuated virulence for mice and potentiate a Th1 response. *The journal of immunology* 1998; **161**(12): 6794-6801.
66. Saravia NG, Escorcia B, Osorio Y, Valderrama L, Brooks D, Arteaga L, Coombs G, Mottram J, Travi BL. Pathogenicity and protective immunogenicity of cysteine proteinase-deficient mutants of Leishmania mexicana in non-murine models. *Vaccine* 2006; **24**(19): 4247-4259.
67. 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

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correlates with protection. *The journal of immunology* 2007; **179**(5): 3161-3170.
68. Papadopoulou B, Roy G, Breton M, Kündig C, Dumas C, Fillion I, Singh AK, Olivier M, Ouellette M. Reduced infectivity of a Leishmania donovani biopterin transporter genetic mutant and its use as an attenuated strain for vaccination. *Infection and immunity* 2002; **70**(1): 62-68.
69. Muyombwe A, Olivier M, Harvie P, Bergeron MG, Ouellette M, Papadopoulou B. Protection against Leishmania major challenge infection in mice vaccinated with live recombinant parasites expressing a cytotoxic gene. *Journal of infectious diseases* 1998; **177**(1): 188-195.
70. Davoudi N, Mahboudi F, Azizi M, Adeli A, McMaster RW. Introduction of three independent selection markers in Leishmania. *Iranian biomedical journal* 2003; **1**: 13- 18.
71. Davoudi N, Tate CA, Warburton C, Murray A, Mahboudi F, McMaster WR. Development of a recombinant Leishmania major strain sensitive to ganciclovir and 5-fluorocytosine for use as a live vaccine challenge in clinical trials. *Vaccine* 2005; **23**(9): 1170-1177.
72. Joshi S, Rawat K, Yadav NK, Kumar V, Siddiqi MI, Dube A. Visceral leishmaniasis: advancements in vaccine development via classical and molecular approaches. *Frontiers in immunology* 2014; **5**: 380.
73. Palatnik de Sousa CB, Borojevic R, Previato JO, Mendonca-Previato L. Inhibition of Leishmania donovani promastigote internalization into murine macrophages by chemically defined parasite glycoconjugate. *Infection and immunity* 1989; **57**(3): 754-763.
74. Jardim A, Tolson DL, Turco SJ, Pearson TW, Olafson RW. The Leishmania donovani lipophosphoglycan T lymphocyte reactive component is a tightly associated protein complex. *Journal of immunology* 1991; **147**(10): 3538-3544.
75. Rachamim N, Jaffe CL. Pure protein fromLeishmania donovani protects mice against both cutaneous and visceral leishmaniasis. *Journal of immunology* 1993; **150**(6): 2322–2331.
76. Katebi A, Gholami E, Taheri T, Zahedifard F, Habibzadeh S, Taslimi Y, Shokri F, Papadopoulou B, Kamhawi S, Valenzuela JG, Rafati S. Leishmania tarentolae secreting the sand fly salivary antigen PpSP15 confers protection against Leishmania major infection in a susceptible BALB/c mice model. *Molecular immunology* 2015; **67**(2): 501-511.
77. Zahedifard F, Gholami E, Taheri T, Taslimi Y, Doustdari F, Seyed N, Torkashvand F, Meneses C, Papadopoulou B, Kamhawi S, Valenzuela JG. Enhanced protective efficacy of nonpathogenic recombinant Leishmania tarentolae expressing cysteine proteinases combined with a sand fly salivary antigen. *Plos neglected tropical diseases* 2014; **8**(3): e2751.
78. Shahbazi M, Zahedifard F, Taheri T, Taslimi Y, Jamshidi S, Shirian S, Mahdavi N, Hassankhani M, Daneshbod Y, Zarkesh-Esfahani SH, Papadopoulou B. Evaluation of live recombinant nonpathogenic Leishmania tarentolae expressing cysteine proteinase and A2 genes as a candidate vaccine against experimental canine visceral leishmaniasis. *Plos one* 2015; **10**(7): e0132794.
79. Saljoughian N, Taheri T, Zahedifard F, Taslimi Y, Doustdari F, Bolhassani A, Doroud D, Azizi H, Heidari K, Vasei M, Namvar Asl N. Development of novel prime-boost strategies based on a tri-gene fusion recombinant L. tarentolae vaccine against experimental murine visceral leishmaniasis. *PloS neglected tropical diseases* 2013; **7**(4): e2174.
80. Stiles ME, Holzapfel WH. Lactic acid bacteria of foods and their current taxonomy. *International journal of food microbiology* 1997; **36**(1): 1-29.
81. 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**(39): 5726-5732.
82. Torkashvand A, Bahrami F, Adib M, Ajdary S. Subcutaneous immunization with recombinant Lactococcus lactis expressing F1S1 fusion protein induces systemic and mucosal immune responses in BALB/C mice. *Reports of biochemistry and molecular biology* 2019; **7**(2): 196.
83. Davarpanah E, Seyed N, Bahrami F, Rafati S, Safaralizadeh R, Taheri T. Lactococcus lactis expressing sand fly PpSP15 salivary protein confers long-term protection against Leishmania major in BALB/c mice. *PLoS neglected tropical diseases* 2020; **14**(1): e0007939.
84. Liu MA, Wahren B, Hedestam GB. DNA vaccines: recent developments and future possibilities. *Human gene therapy* 2006; **17**(11): 1051-61.
85. Alarcon JB, Waine GW, McManus DP. DNA vaccines: technology and application as anti-parasite and anti-microbial agents. *Advances in parasitology* 1999; **42**: 343-410.
86. Restifo NP, Ying H, Hwang L, Leitner WW. The promise of nucleic acid vaccines. *Gene therapy* 2000; **7**(2): 89-92.
87. Gurunathan S, Prussin C, Sacks DL, Seder RA. Vaccine requirements for sustained cellular immunity to an intracellular parasitic infection. Nature medicine 1998; **4**(12): 1409-15.
88. Ghosh A, Zhang WW, Matlashewski G. Immunization with A2 protein results in a mixed Th1/Th2 and a humoral response which protects mice against Leishmania donovani infections. *Vaccine* 2001; **20**(1-2): 59-66.
89. Solioz N, Blum-Tirouvanziam U, Jacquet R, Rafati S, Corradin G, Mauël J, Fasel N. The protective capacities of histone H1 against experimental murine cutaneous leishmaniasis. *Vaccine* 1999; **18**(9-10): 850-85.
90. Xu DU, Liew FY. Protection against leishmaniasis by injection of DNA encoding a major surface glycoprotein, gp63, of *L. major*. *Immunology* 1995; **84**(2): 173.
91. Fragaki K, Suffia I, Ferrua B, Rousseau D, Le Fichoux

Y, Kubar J. Immunisation with DNA encoding Leishmania infantum protein papLe22 decreases the frequency of parasitemic episodes in infected hamsters. *Vaccine* 2001; **19**(13-14): 1701-1709.
92. Rafati S, Salmanian AH, Taheri T, Vafa M, Fasel N. A protective cocktail vaccine against murine cutaneous leishmaniasis with DNA encoding cysteine proteinases of Leishmania major. *Vaccine* 2001; **19**(25-26): 3369-3375.
93. Campos-Neto A, Webb JR, Greeson K, Coler RN, Skeiky YA, Reed SG. Vaccination with plasmid DNA encoding TSA/LmSTI1 leishmanial fusion proteins confers protection against Leishmania major infection in susceptible BALB/c mice. *Infection and immunity* 2002; **70**(6): 2828-2836.
94. Ahmed SB, Bahloul C, Robbana C, Askri S, Dellagi K. A comparative evaluation of different DNA vaccine candidates against experimental murine leishmaniasis due to L. major. *Vaccine* 2004; **22**(13-14): 1631-1639.
95. Iborra S, Soto M, Carrión 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**(29-30): 3865-3876.
96. Aguilar-Be I, da Silva Zardo R, de Souza EP, Borja-Cabrera GP, Rosado-Vallado M, Mut-Martin M, del Rosario García-Miss M, de Sousa CB, Dumonteil E. Cross-protective efficacy of a prophylactic Leishmania donovani DNA vaccine against visceral and cutaneous murine leishmaniasis. *Infection and immunity* 2005; **73**(2): 812-819.
97. Rodríguez-Cortés A, Ojeda A, López-Fuertes L, Timón M, Altet L, Solano-Gallego L, Sánchez-Robert E, Francino O, Alberola J. Vaccination with plasmid DNA encoding KMPII, TRYP, LACK and GP63 does not protect dogs against Leishmania infantum experimental challenge. *Vaccine* 2007; **25**(46): 7962-7971.
98. Gurunathan S, Sacks DL, Brown DR, Reiner SL, Charest H, Glaichenhaus N, Seder RA. Vaccination with DNA encoding the immunodominant LACK parasite antigen confers protective immunity to mice infected with Leishmania major. *The journal of experimental medicine* 1997; **186**(7): 1137-1147.
99. Sukumaran B, Tewary P, Saxena S, Madhubala R. Vaccination with DNA encoding ORFF antigen confers protective immunity in mice infected with Leishmania donovani. *Vaccine* 2003; **21**(11-12): 1292-1299.
100. Borja-Cabrera GP, Santos FN, Miyashiro LM, Santos FB, Palatnik de Sousa CB. Nucleoside hydrolase DNA vaccine against visceral leishmaniasis. *Procedia in vaccinology* 2009; **1**(1): 104-109.
101. Campbell K, Diao H, Ji J, Soong L. DNA immunization with the gene encoding P4 nuclease of Leishmania amazonensis protects mice against cutaneous leishmaniasis. *Infection and immunity* 2003; **71**(11): 6270-6278.
102. Kumari S, Samant M, Misra P, Khare P, Sisodia B, Shasany AK, Dube A. Th1-stimulatory polyproteins of soluble Leishmania donovani promastigotes ranging from 89.9 to 97.1 kDa offers long-lasting protection against experimental visceral leishmaniasis. *Vaccine* 2008; **26**(45): 5700-5711.
103. Gradoni L, Manzillo VF, Pagano A, Piantedosi D, De Luna R, Gramiccia M, Scalone A, Di Muccio T, Oliva G. Failure of a multi-subunit recombinant leishmanial vaccine (MML) to protect dogs from Leishmania infantum infection and to prevent disease progression in infected animals. *Vaccine* 2005; **23**(45): 5245-5251.
104. Perrin P, Jacob Y, Aguilar-Setien A, Loza-Rubio E, Jallet C, Desmezieres E, Aubert M, Cliquet F, Tordo N. Immunization of dogs with a DNA vaccine induces protection against rabies virus. *Vaccine* 1999; **18**(5-6): 479-486.
105. Saldarriaga OA, Travi BL, Park W, Perez LE, Melby PC. Immunogenicity of a multicomponent DNA vaccine against visceral leishmaniasis in dogs. *Vaccine* 2006; **24**(11): 1928-1940.
106. Ramiro MJ, Zárate JJ, Hanke T, Rodriguez D, Rodriguez JR, Esteban M, Lucientes J, Castillo JA, Larraga V. Protection in dogs against visceral leishmaniasis caused by Leishmania infantum is achieved by immunization with a heterologous prime-boost regime using DNA and vaccinia recombinant vectors expressing LACK. *Vaccine* 2003; **21**(19-20): 2474-2484.
107. Rafati S, Nakhaee A, Taheri T, Taslimi Y, Darabi H, Eravani D, Sanos S, Kaye P, Taghikhani M, Jamshidi S, Rad MA. Protective vaccination against experimental canine visceral leishmaniasis using a combination of DNA and protein immunization with cysteine proteinases type I and II of L. infantum. *Vaccine* 2005; **23**(28): 3716-3725.
108. 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.
109. Skeiky YA, Coler RN, Brannon M, Stromberg E, Greeson K, Crane RT, Campos-Neto A, Reed SG. Protective efficacy of a tandemly linked, multi-subunit recombinant leishmanial vaccine (Leish-111f) formulated in MPL adjuvant. *Vaccine* 2002; **20**(27-28): 3292-3303.
110. Okwor I, Uzonna J. Vaccines and vaccination strategies against human cutaneous leishmaniasis. *Human vaccines* 2009; **5**(5): 291-301
111. Ansari N. Culture Et Isolement De Leishmania Tropica. Leishmanisation Prophyactique. *Archive de institut hesarak* 1964; **11**(2): 31-35.
112. Nadim A, Javadian E, Mohebali M. The experience of leishmanization in the Islamic Republic of Iran. *Eastern mediterranean health journal* 1997; **3**(2): 284-289.
113. Nadim A, Javadian E, Tahvildar-Bidruni G, Ghorbani M.. Effectiveness of leishmanization in the control of cutaneous leishmaniasis. *Bulletin de la société de pathologie exotique et de ses filiales* 1983; **76**(4) : 377-383.
114. Mohebali M, Mehrabi Tavana A, Javadian E, Esfahani A, Hajjaran H, Akhoundi B. Preparation and standardization of Leishmania suspension and its evaluation for leishmaniazation. *Experimental*

parasitology 1987; **64**: 147-156.
115. Khamesipour A, Dowlati Y, Asilian A, Hashemi-Fesharki R, Javadi A, Noazin S, Modabber F. Leishmanization: use of an old method for evaluation of candidate vaccines against leishmaniasis. *Vaccine* 2005; **23**(28): 3642-3648
116. Mohebali M, Javadian EH, Fesharakl R, Mohammadzadeh M, Nadlm A, Mesdaghinia A. Trial of a non-living crude vaccine against zoonotic cutaneous leishmaniasis. *Orginal articles* 1995; **8**(4): 211-215.
117. Mohebali M, Nadim A, Khamesipour A. An overview of leishmanization experience: A successful control measure and a tool to evaluate candidate vaccines. *Acta tropica* 2019; **200**: 105173.
118. Sergiev VP. Control and prophylaxis of cutaneous leishmaniasis in the middle Asia republics of the former USSR. *Bulletin de la société française de parasitologie* 1992; **10**(2): 183-187.
119. Dubrovsky YA. Some Data on the Spatial Structure of Area of Natural Nidality of Cutaneous Leishmaniasis. Russia: Research on Medical Geography, Moscow Branch of the USSR Geography Society; 1973.
120. Dubrovsky YA. Materials on natural focality of cutaneous leishmaniasis in the USSR subzone of northern deserts. *Meditsinskaia parazitologiia parazitarnye bolezni* 1973; **42** (6): 646-655.
121. Lysenko AJ, Lubova VV. Epidemiology and Geography of the Visceral Leishmaniasis in USSR. International Symposium on Leishmaniasis Ecology. Montpellier, France, 1974.
122. Streit JA, Recker TJ, Gueiros Filho F, Beverley SM, Wilson ME. Protective immunity against the protozoan Leishmania chagasi is induced by subclinical cutaneous infection with virulent but not avirulent organisms. *The journal of immunology* 2001; **166**(3): 1921-1929.
123. Veras PS, Brodskyn CI, Balestieri FM, De Freitas LA, Ramos AP, Queiroz AR, Barral A, Beverley SM, Barral-Netto M. A dhfr-ts-Leishmania major knockout mutant cross-protects against Leishmania amazonensis. *Memorias do instituto oswaldo cruz* 1999; **94**(4): 491-496.
124. Amaral VF, Teva A, Oliveira-Neto MP, Silva AJ, Pereira MS, Cupolillo E, Porrozzi R, Coutinho SG, Pirmez C, Beverley SM, Grimaldi Jr G. 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. *Memorias do instituto oswaldo cruz* 2002; **97**(7): 1041-1048.
125. Kébaïer C, Uzonna JE, Beverley SM, Scott P. Immunization with persistent attenuated Δlpg2 Leishmania major parasites requires adjuvant to provide protective immunity in C57BL/6 mice. *Infection and immunity* 2006; **74**(1): 777-7780.
126. Breton M, Tremblay MJ, Ouellette M, Papadopoulou B. Live nonpathogenic parasitic vector as a candidate vaccine against visceral Leishmaniasis. *Infection and immunity* 2005; **73**(10): 6372-6382.
127. Kumari S, Samant M, Khare P, Misra P, Dutta S, Kolli B.K, Sharma S, Chang K.P, Dube A. Photodynamic vaccination of hamsters with inducible suicidal mutants of Leishmania amazonensis elicits immunity against visceral leishmaniasis. *European journal of immunology* 2009; **39**(1): 178-191.
128. Button LL, McMaster WR. Molecular cloning of the major surface antigen of leishmania. *Journal of experimental medicine* 1988; **167**(2): 724-729.
129. Yang DM, Fairweather N, Button LL, McMaster WR, Kahl LP, Liew FY. Oral Salmonella typhimurium (AroA-) vaccine expressing a major Leishmanial surface protein (gp63) preferentially induces T helper 1 cells and protective immunity against Leishmaniasis. *The journal of immunology* 1990; **145**(7): 2281-2285.
130. Rivier D, Bovay P, Shah R, Didisheim S, Mauel J. Vaccination against Leishmania major in a CBA mouse model of infection: role of adjuvants and mechanism of protection. *Parasite immunology* 1999; **21**(9): 461.
131. Handman E, Button LL, McMaster WR. Leishmania major: production of recombinant gp63 its antigenicity and immunogenicity in mice. *Experimental parasitology* 1990; **70**(4): 427-435.
132. Olobo JO, Anjili CO, Gicheru MM, Mbati PA, Kariuki TM, Githure JI, Koech DK, McMaster WR. Vaccination of vervet monkeys against cutaneous leishmaniosis using recombinant Leishmania 'major surface glycoprotein'(gp63). *Veterinary parasitology* 1995; **60**(3-4): 199-212.
133. Russell DG, Alexander J. Effective immunization against cutaneous Leishmaniasis with defined membrane antigens reconstituted into Liposomes. *The journal of immunology* 1988; **140**(4): 1274-1279.
134. Mcsorley SJ, Xu D, Liew F. Vaccine efficacy of Salmonella strains expressing glycoprotein 63 with different promoters. *Infection and immunity* 1997; **65**(1): 171-178.
135. González CR, Noriega FR, Huerta S, Santiago A, Vega M, Paniagua J, Ortiz-Navarrete V, Isibasi A, Levine MM. Immunogenicity of a Salmonella typhi CVD 908 candidate vaccine strain expressing the major surface protein gp63 of Leishmania mexicana mexicana. *Vaccine* 1998; **16**(9-10): 1043-1052.
136. Connell ND, Medina-Acosta E, McMaster WR, Bloom BR, Russell DG. Effective immunization against cutaneous Leishmaniasis with recombinant bacille Calmette-Guerin expressing the Leishmania surface proteinase gp63. *Proceedings of the National Academy of Sciences* 1993; **90**(24): 11473-11477.
137. Abdelhak S, Louzir H, Timm J, Blel L, Benlasfar Z, Lagranderie M, Gheorghiu M, Dellagi K, Gicquel B. Recombinant BCG expressing the Leishmania surface antigen Gp63 induces protective immunity against Leishmania major infection in BALB/c mice. *Microbiology* 1995; **141**(7): 1585-1592.
138. Jaafari MR, Ghafarian A, Farrokh-Gisour A, Samiei A, Kheiri MT, Mahboudi F, Barkhordari F, Khamesipour A, McMaster WR. Immune response and protection assay of recombinant major surface glycoprotein of Leishmania (rgp63) reconstituted with liposomes in BALB/c mice. *Vaccine* 2006; **24**(29-30): 5708-5717.
139. Bhowmick S, Ravindran R, Ali N. gp63 in stable

cationic liposomes confers sustained vaccine immunity to susceptible BALB/c mice infected with Leishmania donovani. *Infection and immunity* 2008; **76**(3): 1003-1015.
140. Russo DM, Burns JM, Carvalho EM, Armitage RJ, Grabstein KH, Button LL, McMaster WR, Reed SG. Human T cell responses to gp63, a surface antigen of Leishmania. The journal of immunology 1991; **147**(10): 3575-3580.
141. Jardim A, Alexander J, Teh HS, Ou D, Olafson RW. Immunoprotective Leishmania major synthetic T cell epitopes. *The journal of experimental medicine* 1990 ; **172**(2): 645-648.
142. Soares LR, Sercarz EE, Miller A. Vaccination of the Leishmania major susceptible BALB/c mouse. I. The precise selection of peptide determinant influences $CD4^+$ T cell subset expression. *International immunology* 1994; **6**(5): 785-794.
143. Spitzer N, Jardim A, Lippert D, Olafson RW. Long-term protection of mice against Leishmania major with a synthetic peptide vaccine. *Vaccine* 1999; **17**(11-12): 1298-1230.
144. Tsagozis P, Karagouni E, Dotsika E. Dendritic cells pulsed with peptides of gp63 induce differential protection against experimental cutaneous Leishmaniasis. *International journal of immuno-pathology and pharmacology* 2004; **17**(3): 343-352.
145. Chen G, Darrah PA, Mosser DM. Vaccination against the intracellular pathogens Leishmania major and L. amazonensis by directing CD40 ligand to macrophages. *Infection and immunity* 2001; **69**(5): 3255-3263.
146. Champsi J, McMahon-Pratt D. Membrane glycoprotein M-2 protects against Leishmania amazonensis infection. *Infection and immunity* 1988; **56**(12): 3272-3279.
147. McMahon-Pratt D, Rodriguez D, Rodriguez JR, Zhang Y, Manson K, Bergman C, Rivas L, Rodriguez JF, Lohman KL, Ruddle NH. Recombinant vaccinia viruses expressing GP46/M-2 protect against Leishmania infection. *Infection and immunity* 1993; **61**(8): 3351-3359.
148. Handman E, Symons FM, Baldwin TM, Curtis JM, Scheerlinck JPY. Protective vaccination with promastigote surface antigen 2 from Leishmania major is mediated by a TH1 type of immune response. *Infection and immunity* 1995; **63**(11): 4261-4267.
149. Sjölander A, Baldwin TM, Curtis JM, Bengtsson KL, Handman E. Vaccination with recombinant parasite surface antigen 2 from Leishmania major induces a Th1 type of immune response but does not protect against infection. *Vaccine* 1998; **16**(20): 2077-2084.
150. Mougneau E, Altare F, Wakil AE, Zheng S, Coppola T, Wang ZE, Waldmann R, Locksley RM, Glaichenhaus N. Expression cloning of a protective Leishmania antigen. *Science* 1995; **268**(5210): 563-566.
151. Ferraz colho EA, Pereira Tavares CA, Amorim Carvalho FA, Chaves KF, Teixeira KN, Rodriguez RC, Charest H, Matlashewski G, Tostes Gazzinelli R, Fernandes P. Immune responses induced by the Leishmania (Leishmania) donovani A2 antigen but not by the LACK antigen are protective against experimental Leishmania (Leishmania) amazoensis infection. *Infection immunity* 2003; **71**(7): 3988-3994.
152. Pinto EF, Pinheiro RO, Rayol A, Larraga V, Rossi-Bergmann B. Intranasal vaccination against cutaneous Leishmaniasis with a particulated Leishmanial antigen or DNA encoding LACK. *Infection and immunity* 2004; **72**(8): 4521-4527.
153. Palatnik-de-Sousa CB, Paraguai-de-Souza E, Gomes EM, Borojevic R. Experimental murine Leishmania donovani infection: immunoprotection by the fucose-mannose ligand (FML). *Brazilian journal of medical and biological research* 1994; **27**(2): 547.
154. Santos WR, Aguiar IA, de Souza EP, de Lima VM, Palatnik M, Palatnik-de-Sousa CB. Immunotherapy against murine experimental visceral Leishmaniasis with the FML-vaccine. *Vaccine* 2003; **21**(32): 4668-4676.
155. Palatnik-de-Sousa CB, Gomes EM, Paraguai-de-Souza E, Doa Sontis WR, De Macedo SR, De Medeiros LV, Luz K. the FML (fucose-mannose ligand) of leishmanial donovani. A new tool diagnosis prognosis trans fusional control and vaccination against human kala azar. *Revista de sociedade brasileira medicina tropical* 1996; **29**(2): 153-163.
156. Santos WR, de Souza EP, Palatnik M, de Sousa CB. Vaccination of Swiss Albino mice against experimental visceral Leishmaniasis with the FML antigen of Leishmania donovani. *Vaccine* 1999; **17**(20-21): 2554-2561.
157. Palatnik-de-Sousa CB, Moreno M, Paraguai-de-Souza E, Borojevic R. The FML vaccine (fucose-mannose ligand) protects hamsters from experimental kala-azar. *Ciênc. cult.(säo paulo)* 1994; **64**(4): 290-296.
158. Santos WR, De Lima VMF, De Sousa EP, Bernardo RR, Palatnik M, de Sousa CBP. Saponins, IL12 and BCG adjuvant in the FML-vaccine formulation against murine visceral leishmaniasis. *Vaccine* 2002; **21**(1–2): 30-43.
159. Oliveira-Freitas E, Casas CP, Borja-Cabrera GP, Santos FN, Nico D, Souza LO, Tinoco LW, Da Silva BP, Palatnik M, Parente JP, Palatnik-de-Sousa CB. Acylated and deacylated saponins of Quillaja saponaria mixture as adjuvants for the FML-vaccine against visceral Leishmaniasis. *Vaccine* 2006; **24**(18): 3909-3920.
160. Paraguai de Souza E, Bernardo RR, palatnik M, palatnik de Souza CB. Vaccination of Bal/C mice against experimental visceral Leishmaniasis with the GP36 glycoprotein antigen of Leishmanial donovani. *Vaccine* 2001; **19**(23-24): 3104-3115.
161. da Silva VO, Borja-Cabrera GP, Pontes NN, de Souza EP, Luz KG, Palatnik M, de Sousa CB. A phase III trial of efficacy of the FML-vaccine against canine kala-azar in an endemic area of Brazil (Sao Goncalo do Amaranto, RN). *Vaccine* 2000; **19**(9-10): 1082-1092.
162. Borja-Cabrera GP, Mendes AC, Paraguai-de-Souza E, Okada LYH, Trivellato FADA, Kawasaki JYA, Cerqueira Costa A, Barbosa Reis A, Genaro O, Maria Melo Batista L, Palatnik M, Beatriz Palatnik-de-Sousa C. Effective immunotherapy against canine visceral Leishmaniasis with the FML vaccine. *Vaccine* 2004;

22(17-18): 2234-2243.
163. Borja-Cabrera GP, Coreia Pontes NN, De Silva VO, Paraguay De Souza E, Santos WR, Gomes M, Luz GK, Palatnik M, Palatnik de Sousa CB. long lasting protection against caning kala azar using the FML-QuilA saponin vaccine in the endemic area of Brazil (Sao Gonsalo do Amarante RN). *Vaccine* 2002; **20**(27-28): 3277-3284.
164. Borja-Cabrera GP, Santos FN, Bauer FS, Parra LE, Menz I, Morgado AA, Soares IS, Batista LM, Palatnik-de-Sousa CB. Immunogenicity assay of the Leishmune vaccine against canine visceralL in Brazil. *Vaccine* 2008; **26**(39): 4991-4997.
165. Araújo MS, de Andrade RA, Vianna LR, Mayrink W, Reis AB, Sathler-Avelar R, Teixeira-Carvalho A, Andrade MC, Mello MN, Martins-Filho OA. Despite Leishvaccine and Leishmune trigger distinct immune profiles, their ability to activate phagocytes and $CD8^+$ T-cells support their high-quality immunogenic potential against canine visceral Leishmaniasis. *Vaccine* 2008; **26**(18): 2211-2224.
166. Araújo MS, de Andrade RA, Sathler-Avelar R, Teixeira-Carvalho A, Andrade MC, Vianna LR, Mayrink W, Reis AB, Malaquias LC, Mello MN, Martins-Filho OA. T-cell-derived cytokines, nitric oxide production by peripheral blood monocytes and seric anti-Leishmania (Leishmania) chagasi IgG subclass patterns following immunization against canine visceral Leishmaniasis using leishvaccine and leishmune. *Vaccine* 2009; **27**(7): 1008-1017.
167. Nogueira FS, Moreira MA, Borja-Cabrera GP, Santos FN, Menz I, Parra LE, Xu Z, Chu HJ, Palatnik-de-Sousa CB, Luvizotto MC. Leishmune vaccine blocks the transmission of canine visceral leishmaniasis: absence of Leishmania parasites in blood, skin and lymph nodes of vaccinated exposed dogs. *Vaccine* 2005; **23**(40): 4805-4810.
168. Saraiva EM, de Figueiredo Barbosa A, Santos FN, Borja-Cabrera GP, Nico D, Souza LO, de Oliveira Mendes-Aguiar C, De Souza EP, Fampa P, Parra LE, Menz I. The FML-vaccine (Leishmune) against canine visceral leishmaniasis: a transmission blocking vaccine. *Vaccine* 2006; **24**(13): 2423-2431.
169. Lemesre JL, Holzmuller P, Cavaleyra M, Goncalves RB, Hottin G, Papierok G.Protection against Lemesre JL, Holzmuller P, Cavaleyra M, Gonçalves RB, Hottin G, Papierok G. Protection against experimental visceral leishmaniasis infection in dogs immunized with purified excreted secreted antigens of Leishmania infantum promastigotes. *Vaccine* 2005; **23**(22): 2825-2840.
170. Lemesre JL, Holzmuller P, Gonçalves RB, Bourdoiseau G, Hugnet C, Cavaleyra M, Papierok G. Long-lasting protection against canine visceral leishmaniasis using the LiESAp-MDP vaccine in endemic areas of France: double-blind randomised efficacy field trial. *Vaccine* 2007; **25**(21): 4223-4234.
171. Bourdoiseau G, Hugnet C, Gonçalves RB, Vézilier F, Petit-Didier E, Papierok G, Lemesre JL. Effective humoral and cellular immunoprotective responses in Li ESAp-M.DP vaccinated protected dogs. *Veterinary immunology and immunopathology* 2009; **128**(1-3): 71-78.
172. Aebischer T, Wolfram M, Patzer SI, Ilg T, Wiese M, Overath P. Subunit vaccination of mice against new world cutaneous leishmaniasis: comparison of three proteins expressed in amastigotes and six adjuvants. *Infection and immunity* 2000; **68**(3): 1328-1336.
173. Rafati S, Baba AA, Bakhshayesh M, Vafa M. Vaccination of BALB/c mice with Leishmania major amastigote-specific cysteine proteinase. *Clinical and experimental immunology* 2000; **120**(1): 134-138.
174. Rafati S, Kariminia A, Seyde-Eslami S, Narimani M, Taheri T, Lebbatard M. Recombinant cysteine proteinases-based vaccines against Leishmania major in BALB/c mice: the partial protection relies on interferon gamma producing CD8+ T lymphocyte activation. *Vaccine* 2002; **20**(19-20): 2439-2447.
175. 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**(15-16): 1930-1940.
176. Alves CR, Benévolo-de-Andrade TC, Alves JL, Pirmez C. Th1 and Th2 immunological profile induced by cysteine proteinase in murine leishmaniasis. *Parasite immunology* 2004; **26**(3): 127-135.
177. Ferreira JH, Gentil LG, Dias SS, Fedeli CE, Katz S, Barbiéri CL. Immunization with the cysteine proteinase Ldccys1 gene from Leishmania (Leishmania) chagasi and the recombinant Ldccys1 protein elicits protective immune responses in a murine model of visceral leishmaniasis. *Vaccine* 2008; **26**(5): 677-685.
178. Jensen AT, Curtis J, Montgomery J, Handman E, Theander TG. Molecular and immunological characterisation of the glucose regulated protein 78 of Leishmania donovani. *Biochimica et biophysica acta* 2001; **1549**(1): 73-87.
179. Mukherjee M, Bhattacharyya A, Duttagupta S. Serodiagnostic and immunoprophylactic potential of a 78kDa protein of Leishmania donovani of Indian origin. *Medical science monitor* 2002; **8**(4): BR117-122.
180. Nagill R, Kaur S. Enhanced efficacy and immunogenicity of 78 kDa antigen formulated in various adjuvants against murine visceral leishmaniasis. *Vaccine* 2010; **28**(23): 4002-4012.
181. Soong L, Duboise SM, Kima P, McMahon-Pratt D. Leishmania pifanoi amastigote antigens protect mice against cutaneous leishmaniasis. *Infection and immunity* 1995; **63**(9): 3559-3566.
182. Kar S, Metz C, McMahon-Pratt D. CD4+ T cells play a dominant role in protection against New World leishmaniasis induced by vaccination with the P-4 amastigote antigen. *Infection and immunity* 2005; **73**(6): 3823-3827.
183. Carrillo E, Ahmed S, Goldsmith-Pestana K, Nieto J, Osorio Y, Travi B, Moreno J, McMahon-Pratt D. Immunogenicity of the P-8 amastigote antigen in the experimental model of canine visceral leishmaniasis. *Vaccine* 2007; **25**(8): 1534-1543.

184. Coutinho SG, Oliveira MP, Da-Cruz AM, De Luca PM, Mendonça SC, Bertho AL, Soong L, McMahon-Pratt D. T-cell responsiveness of American cutaneous leishmaniasis patients to purified Leishmania pifanoiamastigote antigens and Leishmania braziliens is promastigote antigens: Immunologic patterns associated with cure. *Experimental parasitology* 1996; **84**(2): 144-155.
185. Haberer JE, Da-Cruz AM, Soong L, Oliveira-Neto MP, Rivas L, McMahon-Pratt D, Coutinho SG. Leishmania pifanoi amastigote antigen P-4: epitopes involved in T-cell responsiveness in human cutaneous leishmaniasis. *Infection and immunity* 1998; **66**(7): 3100-3105.
186. Ghosh A, Zhang WW, Matlashewski G. Immunization with A2 protein results in a mixed Th1/Th2 and a humoral response which protects mice against Leishmania donovani infections. *Vaccine* 2001; **20**: 59-66.
187. Resende DM, Caetano BC, Dutra MS, Penido ML, Abrantes CF, Verly RM, Resende JM, Piló-Veloso D, Rezende SA, Bruna-Romero O, Fernandes AP. Epitope mapping and protective immunity elicited by adenovirus expressing the Leishmania amastigote specific A2 antigen: correlation with IFN-γ and cytolytic activity by CD8+ T cells. *Vaccine* 2008; **26**(35): 4585-4593.
188. Fernandes AP, Costa MM, Coelho EA, Michalick MS, de Freitas E, Melo MN, Tafuri WL, de Melo Resende D, Hermont V, de Freitas Abrantes C, Gazzinelli RT. Protective immunity against challenge with Leishmania (Leishmania) chagasi in beagle dogs vaccinated with recombinant A2 protein. *Vaccine* 2008; **26**(46): 5888-5895.
189. Stäger S, Smith DF, Kaye PM. Immunization with a recombinant stage-regulated surface protein from Leishmania donovani induces protection against visceral leishmaniasis. *The journal of immunology* 2000; **165**(12): 7064-7071.
190. Moreno J, Nieto J, Masina S, Cañavate C, Cruz I, Chicharro C, Carrillo E, Napp S, Reymond C, Kaye PM, Smith DF. Immunization with H1, HASPB1 and MML Leishmania proteins in a vaccine trial against experimental canine leishmaniasis. *Vaccine* 2007; **25**(29): 5290-5300.
191. Wilson ME, Young BM, Andersen KP, Weinstock JV, Metwali A, Ali KM, Donelson JE. A recombinant Leishmania chagasi antigen that stimulates cellular immune responses in infected mice. *Infection and immunity* 1995; **63**(5): 2062-2069.
192. Streit JA, Recker TJ, Donelson JE, Wilson ME. BCG expressing LCR1 of Leishmania chagasi induces protective immunity in susceptible mice. *Experimental parasitology* 2000; **94**(1): 33-41.
193. Masina S, M. Gicheru M, Demotz SO, Fasel NJ. Protection against cutaneous leishmaniasis in outbred vervet monkeys, using a recombinant histone H1 antigen. *The journal of infectious diseases* 2003; **188**(8): 1250-1257.
194. Dole VS, Raj VS, Ghosh A, Madhubala R, Myler PJ, Stuart KD. Immunization with recombinant LD1 antigens protects against experimental leishmaniasis. *Vaccine* 2000; **19**(4-5): 423-430.
195. Tewary P, Sukumaran B, Saxena S, Madhubala R. Immunostimulatory oligodeoxynucleotides are potent enhancers of protective immunity in mice immunized with recombinant ORFF leishmanial antigen. *Vaccine* 2004; **22**(23-24): 3053-3060.
196. Tewary P, Jain M, Sahani MH, Saxena S, Madhubala R. A heterologous prime-boost vaccination regimen using ORFF DNA and recombinant ORFF protein confers protective immunity against experimental visceral leishmaniasis. *Journal of infectious diseases* 2005; **191**(12): 2130-2137.
197. Tewary P, Saxena S, Madhubala R. Co-administration of IL-12 DNA with rORFF antigen confers long-term protective immunity against experimental visceral leishmaniaisis. *Vaccine* 2006; **24**(13): 2409-2416.
198. Iborra S, Carrión J, Anderson C, Alonso C, Sacks D, Soto M. Vaccination with the Leishmania infantum acidic ribosomal P0 protein plus CpG oligodeoxynucleotides induces protection against cutaneous leishmaniasis in C57BL/6 mice but does not prevent progressive disease in BALB/c mice. *Infection and immunity* 2005; **73**(9): 5842-5852.
199. Iborra S, Parody N, Abánades DR, Bonay P, Prates D, Novais FO, Barral-Netto M, Alonso C, Soto M. Vaccination with the Leishmania major ribosomal proteins plus CpG oligodeoxynucleotides induces protection against experimental cutaneous leishmaniasis in mice. *Microbes and infection* 2008; **10**(10-11): 1133-1141.
200. Ramírez JR, Gilchrist K, Robledo S, Sepúlveda JC, Moll H, Soldati D, Berberich C. Attenuated toxoplasma gondii ts-4 mutants engineered to express the Leishmania antigen KMP-11 elicit a specific immune response in BALB/c mice. *Vaccine* 2001; **20**(3-4): 455-461.
201. Basu R, Bhaumik S, Haldar AK, Naskar K, De T, Dana SK, Walden P, Roy S. Hybrid cell vaccination resolves Leishmania donovani infection by eliciting a strong $CD8^+$ cytotoxic T-lymphocyte response with concomitant suppression of interleukin-10 (IL-10) but not IL-4 or IL-13. *Infection and immunity* 2007; **75**(12): 5956-5966.
202. Saravia NG, Hazbon MH, Osorio Y, Valderrama L, Walker J, Santrich C, Cortazar T, Lebowitz JH, Travi BL. Protective immunogenicity of the paraflagellar rod protein 2 of Leishmania mexicana. *Vaccine* 2005; **23**(8): 984-995.
203. Molano I, Alonso MG, Miron C, Redondo E, Requena JM, Soto M, Nieto CG, Alonso C. A Leishmania infantum multi-component antigenic protein mixed with live BCG confers protection to dogs experimentally infected with L. infantum. *Veterinary immunology and immunopathology* 2003; **92**(1-2): 1-3.
204. Parody N, Soto M, Requena JM, Alonso C. Adjuvant guided polarization of the immune humoral response against a protective multicomponent antigenic protein (Q) from Leishmania infantum. A CpG + Q mix protects BALB/c mice from infection. *Parasite immunology* 2004; **26**(6-7): 283-293.

205. Webb JR, Campos-Neto A, Ovendale PJ, Martin TI, Stromberg EJ, Badaro R, Reed SG. Human and murine immune responses to a novel Leishmania major recombinant protein encoded by members of a multicopy gene family. *Infection and immunity* 1998; **66**(7): 3279-3289.
206. Campos-Neto A, Porrozzi R, Greeson K, Coler RN, Webb JR, Seiky YA, Reed SG, Grimaldi G. Protection against cutaneous leishmaniasis induced by recombinant antigens in murine and nonhuman primate models of the human disease. *Infection and immunity* 2001; **69**(6): 4103-4108.
207. Badiee A, Jaafari MR, Khamesipour A. Leishmania major: immune response in BALB/c mice immunized with stress-inducible protein 1 encapsulated in liposomes. *Experimental parasitology* 2007; **115**(2): 127-134.
208. Badiee A, Jaafari MR, Samiei A, Soroush D, Khamesipour A. Coencapsulation of CpG oligodeoxynucleotides with recombinant Leishmania major stress-inducible protein 1 in liposome enhances immune response and protection against leishmaniasis in immunized BALB/c mice. *Clinical and vaccine immunology* 2008; **15**(4): 668-667.
209. Coler RN, Skeiky YA, Bernards K, Greeson K, Carter D, Cornellison CD, Modabber F, Campos-Neto A, Reed SG. 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. *Infection and immunity* 2002; **70**(8): 4215-4225.
210. Coler RN, Goto Y, Bogatzki L, Raman V, Reed SG. Leish-111f, a recombinant polyprotein vaccine that protects against visceral Leishmaniasis by elicitation of CD4+ T cells. *Infection and immunity* 2007; **75**(9): 4648-54.
211. Badaro R, Lobo I, Munos A, Netto EM, Modabber F, Campos-Neto A, Coler RN, Reed SG. Immunotherapy for drug-refractory mucosal leishmaniasis. *The journal of infectious diseases* 2006; **194**(8): 1151-1159.
212. Salay G, Dorta ML, Santos NM, Mortara RA, Brodskyn C, Oliveira CI, Barbieri CL, Rodrigues MM. Testing of four Leishmania vaccine candidates in a mouse model of infection with Leishmania (Viannia) braziliensis, the main causative agent of cutaneous leishmaniasis in the New World. *Clinical and vaccine immunology* 2007; **14**(9): 1173-1181.
213. Fujiwara RT, Vale AM, da Silva JC, da Costa RT, da Silva Quetz J, Martins Filho OA, Reis AB, Oliveira RC, Machado-Coelho GL, Bueno LL, Bethony JM. Immunogenicity in dogs of three recombinant antigens (TSA, LeIF and LmSTI1) potential vaccine candidates for canine visceral leishmaniasis. *Veterinary research* 2005; **36**(5-6): 827-838.
214. Xu DU, Liew FY. Protection against leishmaniasis by injection of DNA encoding a major surface glycoprotein, gp63, of L. major. *Immunology* 1995; **84**(2): 173.
215. Walker PS, Scharton-Kersten T, Rowton ED, Hengge U, Bouloc A, Udey MC, Vogel JC. Genetic immunization with glycoprotein 63 cDNA results in a helper T cell type 1 immune response and protection in a murine model of leishmaniasis. *Human gene therapy* 1998; **9**(13): 1899-1907.
216. Dumonteil E, Andrade-Narvarez F, Escobedo-Ortegon J, Ramirez-Sierra MJ, Valencia-Pacheco G, Flores-Serrano A, Canto-Lara S, Arjona-Torres A. Comparative study of DNA vaccines encoding various antigens against Leishmania mexicana. *Developments in biological* 2000; **104**: 135-141.
217. Dumonteil E, Jesus RS, Javier EO, del Rosario GM. DNA vaccines induce partial protection against Leishmania mexicana. *Vaccine* 2003; **21**(17-18): 2161-2168.
218. Handman E, Noormohammadi AH, Curtis JM, Baldwin T, Sjölander A. Therapy of murine cutaneous leishmaniasis by DNA vaccination. *Vaccine* 2000; **18**(26): 3011-3017.
219. Noormohammadi AH, Hochrein H, Curtis JM, Baldwin TM, Handman E. Paradoxical effects of IL-12 in leishmaniasis in the presence and absence of vaccinating antigen. *Vaccine* 2001; **19**: 4043-4052.
220. Ramos I, Alonso A, Marcen JM, Peris A, Castillo JA, Colmenares M, Larraga V. Heterologous prime-boost vaccination with a non-replicative vaccinia recombinant vector expressing LACK confers protection against canine visceral leishmaniasis with a predominant Th1-specific immune response. *Vaccine* 2008; **26**(3): 333-344.
221. Gomes DC, Pinto EF, De Melo LD, Lima WP, Larraga V, Lopes UG, Rossi-Bergmann B. Intranasal delivery of naked DNA encoding the LACK antigen leads to protective immunity against visceral leishmaniasis in mice. *Vaccine* 2007; **25**(12): 2168-2172.
222. Marques-da-Silva EA, Coelho EA, Gomes DC, Vilela MC, Masioli CZ, Tavares CA, Fernandes AP, Afonso LC, Rezende SA. Intramuscular immunization with p36 (LACK) DNA vaccine induces IFN-γ production but does not protect BALB/c mice against Leishmania chagasi intravenous challenge. *Parasitology research* 2005; **98**(1): 67-74.
223. Melby PC, Yang J, Zhao W, Perez LE, Cheng J. Leishmania donovani p36 (LACK) DNA vaccine is highly immunogenic but not protective against experimental visceral leishmaniasis. *Infection and immunity* 2001; **69**(8): 4719-4125.
224. Lopez-Fuertes L, Perez-Jimenez E, Vila-Coro AJ, Sack F, Moreno S, Konig SA, et al. DNA vaccination with linear minimalistic (MIDGE) vectors confers protection against Leishmania major infection in mice. *Vaccine* 2002; **21**: 247-257.
225. Basu R, Bhaumik S, Basu JM, Naskar K, De T, Roy S. Kinetoplastid membrane protein-11 DNA vaccination induces complete protection against both pentavalent antimonial-sensitive and-resistant strains of Leishmania donovani that correlates with inducible nitric oxide synthase activity and IL-4 generation: evidence for mixed Th1-and Th2-like responses in visceral leishmaniasis. *The journal of immunology* 2005;

174(11): 7160-7171.
226. Bhaumik S, Basu R, Sen S, Naskar K, Roy S. KMP-11 DNA immunization significantly protects against L. donovani infection but requires exogenous IL-12 as an adjuvant for comparable protection against L. major. *Vaccine* 2009; **27**(9): 1306-1316.
227. Gamboa-León R, de Souza EP, Borja-Cabrera GP, Santos FN, Myashiro LM, Pinheiro RO, Dumonteil E, Palatnik-de-Sousa CB. Immunotherapy against visceral leishmaniasis with the nucleoside hydrolase-DNA vaccine of Leishmania donovani. *Vaccine* 2006; **24**(22): 4863-4873.
228. Zanin FHC, Coelho EAF, Tavares CAP, Marques-da-Silva EA, Silva Costa MM, Rezende SA, Gazzinelli RT, Fernandes AP. Evaluation of immune responses and protection induced by A2 and nucleoside hydrolase (NH) DNA vaccines against Leishmania chagasi and Leishmania amazonensis experimental infections. *Microbes and infection* 2007; **9**(9): 1070-1077.
229. Gonzalo RM, del Real G, Rodriguez JR, Rodriguez D, Heljasvaara R, Lucas P, Larraga V, Esteban M. A heterologous prime–boost regime using DNA and recombinant vaccinia virus expressing the Leishmania infantum P36/LACK antigen protects BALB/c mice from cutaneous leishmaniasis. *Vaccine* 2002; **20**(7-8): 1226-1231.
230. Tapia E, Pérez-Jiménez E, López-Fuertes L, Gonzalo R, Gherardi MM, Esteban M. The combination of DNA vectors expressing IL-12^{+} IL-18 elicits high protective immune response against cutaneous leishmaniasis after priming with DNA-p36/LACK and the cytokines, followed by a booster with a vaccinia virus recombinant expressing p36/LACK. *Microbes and infection* 2003; **5**(2): 73-84.
231. Perez-Jimenez E, Kochan G, Gherardi MM, Esteban M. MVA-LACK as a safe and efficient vector for vaccination against leishmaniasis. *Microbes and infection* 2006; **8**(3): 810-822.
232. Dondji B, Pérez-Jimenez E, Goldsmith-Pestana K, Esteban M, McMahon-Pratt D. Heterologous prime-boost vaccination with the LACK antigen protects against murine visceral leishmaniasis. *Infection and immunity* 2005; **73**(8): 5286-5289.
233. Lange UG, Mastroeni P, Blackwell JM, Stober CB. DNA-Salmonella enterica serovar Typhimurium primer-booster vaccination biases towards T helper 1 responses and enhances protection against Leishmania major infection in mice. *Infection and immunity* 2004; **72**(8): 4924-4928.
234. Rafati S, Zahedifard F, Nazgouee F. Prime-boost vaccination using cysteine proteinases type I and II of Leishmania infantum confers protective immunity in murine visceral leishmaniasis. *Vaccine* 2006; **24**(12): 2169-2175.
235. Rafati S, Zahedifard F, Azari MK, Taslimi Y, Taheri T. Leishmania infantum: prime boost vaccination with C-terminal extension of cysteine proteinase type I displays both type 1 and 2 immune signatures in BALB/c mice. *Experimental parasitology* 2008; **118**(3): 393-401.
236. Khoshgoo N, Zahedifard F, Azizi H, Taslimi Y, Alonso MJ, Rafati S. Cysteine proteinase type III is protective against Leishmania infantum infection in BALB/c mice and highly antigenic in visceral leishmaniasis individuals. *Vaccine* 2008; **26**(46): 5822-5829.
237. Iborra S, Soto M, Carrión J, Nieto A, Fernández E, Alonso C, Requena JM. The Leishmania infantum acidic ribosomal protein P0 administered as a DNA vaccine confers protective immunity to Leishmania major infection in BALB/c mice. *Infection and immunity* 2003; **71**(11): 6562-6572.
238. Teixeira C, Gomes R, Collin N, Reynoso D, Jochim R, Oliveira F, Seitz A, Elnaiem D-E, Caldas A, Paula de Souza A, Brodskyn C, Indiani de Oliveira C, Mendonca I, Costa CHN, Volf P, Barral A, Kamhawi S, Valenzuela JG. Discovery of markers of exposure specific to bites of Lutzomyia longipalpis, the vector of Leishmania infantum chagasi in Latin America. *PloS neglected tropical diseases* 2010; **4**(3): e638.
239. Souza AP, Andrade BB, Aquino D, Entringer P, Miranda JC, Alcantara R, Ruiz D, Soto M, Teixeira CR, Valenzuela GJ, Indiani de Oliveira C, Brodskyn CI, Barral-Netto M, Barral A. Using recombinant proteins from Lutzomyia longipalpis saliva to estimate human vector exposure in visceral Leishmaniasis endemic areas. *PloS neglected tropical diseasess* 2010; **4**(3): e649.
240. Soares BR, Souza AP, Prates DB, de Oliveira CI, Barral-Netto M, Miranda JC, Barral A. Seroconversion of sentinel chickens as a biomarker for monitoring exposure to visceral leishmaniasis. *Scientific reports* 2013; **3**: 2352.
241. Marzouki S, Abdeladhim M, Abdessalem CB, Oliveira F, Ferjani B, Gilmore D, Louzir H, Valenzuela JG, Ben Ahmed M. Salivary antigen SP32 is the immunodominant target of the antibody response to Phlebotomus papatasi bites in humans. *PloS neglected tropical diseasess* 2012; **6**(11): e1911.
242. Marzouki S, Kammoun-Rebai W, Bettaieb J, Abdeladhim M, Hadj Kacem S, Abdelkader R, GritliS, Chemkhi J,Aslan H, Kamhawi S, Ben Salah A, Louzir H, Valenzuela JG, Ben Ahmed M. Validation of recombinant salivary protein PpSP32 as a suitable marker of human exposure to Phlebotomuspapatasi, the vector of Leishmania major in Tunisia. *Plos neglected tropical diseasess* 2015; **9**(9): e0003991.
243. Mondragon-Shem K, Al-Salem WS, Kelly-Hope L, Abdeladhim M, Al-Zahrani MH, Valenzuela JG, et al. Severity of old world cutaneous leishmaniasis is influenced by previous exposure to sandfly bites in Saudi Arabia. *PLoS neglected tropical diseases* 2015; **9**(2):e0003449.
244. Sima M, Ferencova B, Warburg A, Rohousova I, Volf P. Recombinant salivary proteins of Phlebotomus orientalis are suitable antigens to measure exposure of domestic animals to sand fly bites. *Plos neglected tropical diseasess* 2016; **10**(3): e0004553.
245. Drahota J, Martin-Martin I, Sumova P, Rohousova I, Jimenez M, Molina R, Volf P. Recombinant antigens from Phlebotomus perniciosus saliva as markers of canine exposure to visceral leishmaniases Vector. *PloS*

neglected tropical diseasess 2014; **8**(1): e2597.
246. MartõÂn-MartõÂn I, Molina R, RohousÏovaÂ I, Drahota J, Volf P, JimeÂnez M. High levels of anti-Phlebotomus perniciosus saliva antibodies in different vertebrate hosts from the re-emerging leishmaniosis focus in Madrid, Spain. *Veterinary parasitology* 2014; **202**(3-4): 207-216.
247. Kostalova T, Lestinova T, Sumova P, Vlkova M, Rohousova I, Berriatua E, Oliva G, Fiorentino E, Scalone A, Gramiccia M, Gradoni L, Volf P. Canine antibodies against salivary recombinant proteins of Phlebotomus perniciosus: A longitudinal study in an endemic focus of canine leishmaniasis. *PloS neglected tropical diseasess* 2015; **9**(6): e0003855.
248. Kostalova T, Lestinova T, Maia C, Sumova P, Vlkova M, Willen L, Polanska N, Fiorentino E, Scalone A, Oliva G, Veronesi F Cristóvão JM, Courtenay O, Campino L, Gradoni L, Gramiccia M, Volf P. The recombinant protein rSP03B is a valid antigen for screening dog exposure to Phlebotomus perniciosus across foci of canine leishmaniasis. *Medical and veterinary* entomology 2017; **31**(1): 88-93.
249. MartõÂn-MartõÂn I, Molina R, JimeÂnez M. Kinetics of anti-Phlebotomus perniciosus saliva antibodies in experimentally bitten mice and rabbits. *PloS one* 2015; **10**(11): e0140722.

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