An overview on *Leishmania* vaccines: A narrative review article

Hossein Rezvan*, Mohammad Moafi

Department of Laboratory Sciences, Faculty of Para-Veterinary Sciences, Bu-Ali Sina University, Hamedan, Iran.

**Abstract**

Leishmaniasis is one of the major health problems and categorized as a class I disease (emerging and uncontrolled) by World Health Organization (WHO), causing highly significant morbidity and mortality. Indeed, more than 350 million individuals are at risk of *Leishmania* infection, and about 1.6 million new cases occur causing more than 50 thousands death annually. Because of the severe toxicity and drug resistance, present chemotherapy regimen against diverse forms of *Leishmania* infections is not totally worthwhile. However, sound immunity due to natural infection, implies that vigor cellular immunity against *Leishmania* parasites, via their live, attenuated or killed forms, can be developed in dogs and humans. Moreover, genetically conserved antigens ([in most of *Leishmania* species], and components of sand fly saliva confer potential immunogenic molecules for *Leishmania* vaccination. Vaccines successes in animal studies and some clinical trials clearly justify more researches and investments illuminating opportunities in suitable vaccine designation.

© 2015 Urmia University. All rights reserved.

**Key words:** Adjuvant, Leishmaniasis, Vaccines

*Correspondence:
Hossein Rezvan. PhD
Department of Laboratory Sciences, Faculty of Para-Veterinary Sciences, Bu-Ali Sina University, Hamedan, Iran.
E-mail: hrezvan@gmail.com
Introduction

Leishmaniasis is a vector-borne protozoan disease spread by female sand flies, second to malaria in its prevalence, and it is currently amongst the six endemic diseases considered as high priorities worldwide. World Health Organization (WHO) clarifies that approximately 0.2 to 0.4 and 0.7 to 1.2 million cases of visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL), respectively, occur each year, amongst a susceptible population of 350 million in 88 countries on five continents. The parasite is transmittable through the bite of two Phlebotomine genera (Phlebotomus in the old world, and Lutzomyia in the new world) in a zoonotic (from infected animals such as dogs or rodents) or anthropoponic model.

Diagnosis is based on clinical criteria manifested in humans, histopathology of lesions, detection and isolation of parasites from the lesions through biopsy (either by microscopy or culture methods), employment of soluble Leishmania protein in enzyme-linked immunosorbent assay (ELISA) method, and analysis of the small subunit ribosomal RNA genes employing the polymerase chain reaction (PCR). The current treatment is based on chemotherapy which relies on administration of drugs with high expenditure of purchase and serious side effects such as, toxicity, poor compliance and rapid induction of resistance in endemic areas.

Vaccination remains the most appropriate opportunity for the prevention and safe treatment of all forms of the disease so development of a safe, effective and affordable anti-Leishmania vaccine is one of the main global public health priorities and remains the most promising approach. This article provides the latest information regarding saliva, first generation, second generating, third generation, and live vaccines, respectively.

1. Saliva vaccine. Immunization of mice either with Phlebotomus papatasi saliva or plasmid DNA comprising genes of P. papatasi or Lutzomyia longipalpis suggested that salivary molecules could be investigated as components of a vaccine against Leishmaniasis, hence, a cross sectional descriptive study aiming characterization of the antibody response to the saliva of P. papatasi in people living in endemic areas of CL was carried out in Tunisia. The results showed that the salivary proteins triggered the production of various antibody isotypes. Moreover, the immunodominant antigen called PpSP30 was recognized by all IgG subclasses, whereas PpSP12 was not by IgG4. In addition, scientists say that the dose of salivary gland extract (SGE) used in immunization can significantly affect cellular immune response. To elaborate, one of Balb/c inoculated once by SGE presented a substantial augmentation in IL-10 production, whereas, the other group inoculated three times by the same preparation showed an increase in IFN-γ production in the draining lymph nodes of the inoculated mice.

2. First generation vaccines (Whole killed parasite or Leishmania fractions). Vaccines composed of whole killed parasites have been administered in randomized clinical trials (RCT) for prophylactic purposes. However, the results of administration of these vaccines had been associated with inconsistent efficacy due to application of variable criteria such as adjuvant doses and rout of the vaccine administration. For example, Iranian researchers in Razi institute investigated efficacy of autoclaved L. major (ALM) different in their formulation. The mentioned investigations substantiated ALM plus Bacillus Calmette Guerine (BCG) could not promote immunity rather than BCG alone. Nevertheless Soudi and colleagues showed that co-administration of BCG and ALM rectally induced protective type 1 immune responses against L. major infection in comparison with ALM alone.

In innovative approaches sub-nits of Leishmania antigens (such as membrane antigens of L. donovani promastigotes or LAg) with novel adjuvant such as monosphoryl lipid A (MPL-A), a non-toxic derivative product of the lipopolysaccharide (LPS) of Salmonella minnesota, have shown better prophylaxis in comparison with whole killed Leishmania cells. For example, Ravindar et al. administered L. donovani promastigote antigens associated with BCG, MPL-A, and cationic liposome in Leishmania antigen vaccine formulations against murine VL as a comparative study. This survey resulted in high levels of protection in mice immunized with BCG + LAg and MPL + LAg, though, the highest level of protection was exhibited by the liposomal Lag immunized group. Moreover, another sub-unit antigen, fucose mannose ligand (FML) antigen, could be able to show 43.3% protection against VL in mice. Furthermore, Brazilian scientists documented that usage of the FML vaccine in dogs has induced 92.0% and 95.0% protection in naturally exposed vaccinated dogs.

3. Second generation vaccines. Most vaccine studies pursue the sub-unite vaccines comprising of recombinant proteins or poly-proteins produced by DNA cloning. Second generation of refined vaccines, such as recombinant proteins associated with adjuvant or expressed in other microbial vectors, showed more feasible application for mass vaccination. Recombinant nature of vaccines implies that they facilitate their approachability in large scale, and cost effective production. Moreover, responses derived upon second generation vaccines can be strengthened and refined by relevant adjuvant. However, second generation vaccines with different adjuvant meet differing potencies and confinements. In this part, a list of defined peptide vaccine approaches being under development is provided and their potential strengths and weaknesses is summarized.

3.1. Surface expressed glycoprotein (gp63) or leishmaniolysin. Leishmania surface leishmaniolysin (gp63) is a surface expressed glycoprotein implementing
catalytic activity as a metalloproteinase which may protect *Leishmania* in macrophages.\(^{36,37}\) This protein was delivered by a plethora of immunization regimens, nonetheless, a suspicious discoveries from animal models were continued by mostly negative T cell responses in humans.\(^{29,38,39}\) For example, immunogenicity of gp63 subunit vaccine was analyzed either in its native or recombinant forms. Human T lymphocyte responses to gp63 derived from *L. amazonensis* (in its native or recombinant form) were evaluated in individuals with active or cured CL, mucocutaneous leishmaniasis (MCL) or VL. In this study it was substantiated that delivering of native gp63 failed to elicit the proliferation of T cell responses, whilst recombinant gp63 (rgp63) produced in *Escherichia coli* (*E. coli*) accomplished in T cell line stimulation. As a result, it was documented that rgp63 was efficient for human T cell elicitation in patients with active or cured *Leishmania* infection.\(^{40}\) On the contrary in one another study it was substantiated, peripheral blood leukocytes (PBL) neither proliferated nor produced any IFN-\(\gamma\) following *in vitro* stimulation with the gp63, nonetheless, it could be an eligible safe candidate in the presence of appropriate adjuvant.\(^{41}\)

In recent years, liposome application, as potent adjuvant, in gp63 *Leishmania* vaccine was analyzed in new studies. For example, it was authenticated that distearoylphosphatidylcholine (DSPC) liposome used as vaccine adjuvant with the immunodominant gp63 of *L. donovani* promastigotes were able to promote significant protection against progressive VL in susceptible Balb/c mice. To paraphrase, control of disease progression and parasitic burden in mice vaccinated with gp63 in cationic DSPC liposomes was promoted via IFN-\(\gamma\) augmentation and down regulation of IL-4. In addition, CD8 T-cell responses was elicited by gp63 protein associated by DSPC.\(^{41}\)

In another study, the administration of rgp63-based vaccines derived from *L. donovani* with MPL-A (in a cationic liposome) has resulted in an increased number of IFN-\(\gamma\) producing effector T cells.\(^{42}\)

Furthermore, scientists has evaluated the protective and durable immunity of gp63 protein cloned into mammalian expressive vector (pcDNA3.1) by strategies comprised of DNA/DNA, DNA prime/protein-boost, protein/protein in the susceptible Balb/c mice against experimental VL using CpG-ODN as adjuvant. The researchers substantiated that vaccination based on gp63 DNA elicited immune responses and conferred protection.\(^{43}\)

In a new approach, Rezvan *et al.* analyzed immunogenic peptides derived from *L. mexicana* in HLA-A2.1 transgenic (HHDII) and Balb/c mouse models. They have shown that none of the peptides predicted for Balb/c mouse MHC class I elicited CTL activity or significantly up-regulated the IFN-\(\gamma\).\(^{44}\) However, the immunogenicity of peptides derived from gp63 and restricted to HLA-DR1 was evaluated by FVB/N-DR1 transgenic mice model. The scientists evidenced that AAR peptides, which is derived from gp63 and restricted to HLA-DR1, can be a potent candidate in *Leishmania* infections due to its ability in IFN-\(\gamma\) and TH-1 induction.\(^{45}\)

### 3.2. *Leishmania* homologue for receptors of activated C kinase (LACK)

LACK protein belongs to a large family of WD 40 repeat (a short structural motif of approximately forty amino acids) proteins confined to eukaryotes. LACK gene expressed by both the promastigote and amastigote forms demonstrated polymorphic characteristic making it an appropriate molecule for genotyping *Leishmania* strains.\(^{27,46}\) In addition, LACK could be exploited along with other proteins (such as A2, NH, and K39) in ELISA with amended sensitivity.\(^{47}\)

Recently, considerable interest has been focused on LACK antigen as a potential vaccine candidate for leishmaniasis which is a result of its immuno-pathogenic role in murine *L. major* infection. To elaborate, it is authenticated that LACK antigen is potenttoenhance c-10 production, but down regulate of IFN-\(\gamma\) production, nevertheless, vaccination with recombinant LACK (rLACK) antigen in the presence of recombinant IL-12 (rIL-12) evokes CD4\(^+\) T cell inducing protection in mice against *L. major* infection (This protection correlated with augmentation of IFN-\(\gamma\) and reduction of IL-4).\(^{48,49}\) In one another study, researchers analyzed the ability of *Lactococcus lactis* expressing LACK and IL-12 gene in *Leishmania* protection in Balb/c mice. These scientists demonstrated that the preceded live vaccine could expand antigen-specific multifunctional TH1 CD4\(^+\) and CD8\(^+\) T cells and a systemic LACK-specific TH1 immune response.\(^{50,51}\) Moreover, researchers of Molecular Center in Spain demonstrated that immunization with modified Vaccina Ankara Virus expressing LACK antigen (extracted from *L. infantum*) stimulated CD4\(^+\) and CD8\(^+\) effector T cells.\(^{52}\)

### 3.3. Hydrophilic acylated surface proteins (HASP).

Family of HASP comprising extensive and variant amino acid repeats which not only is expressed at the plasma membrane of intracellular (amastigote) of all Old World *Leishmania* species, but also HASP is presented in infective extracellular stage of *L. major*. However, some sub-genus of *L. avanianna* have lost HASP genes.\(^{53}\) On one hand, some important enigmas remained unsolved about this plasma membrane protein. To elaborate, not only the pathway of HASP secretion is mysterious, but also the exact biological function of this protein remains obscure.\(^{27}\) On the other hand, some novel findings which might be useful for *Leishmania* vaccine designation has been revealed. For example, HASPB1 induced protection or immunity does not need an adjuvant. This seems reasonable to assume that its mechanism of immunity is similar to DNA vaccine.\(^{53,54}\)

### 3.4. *Leishmania*-derived recombinant poly-protein (Leish-111f) or LEISH-F1

Currently, few products enrolled as second generation vaccine have entered randomized clinical trials (RCTs) or veterinary testing. Just a single
product LEISH-F1 (formerly known as Leish-111f) being a fusion protein of three relatively Leishmania proteins formulated with MPL-SE, has entered phase II clinical human testing. LEISH-F1 is a single-polyprotein composed of three integrated molecules: L. major homologue of eukaryotic thiol-specific antioxidant (TSA), L. major stress-inducible protein-1 (LmSTI1) and the L. braziliensis elongation and initiation factor (LeIF).

In one study, researchers analyzed the safety and immunogenicity of the LEISH-F1 plus MPL-SE adjuvant when the protein was used in combination with sodium stibogluconate for the treatment of both mucosal and cutaneous leishmaniasis (MCL and CL respectively). These scientists proved that most of volunteers demonstrated IgG antibody and T-cell responses being specific to the LEISH-F1 antigen four weeks after the last injection of vaccine.

In addition, different RCT substantiatied that LEISH-F1 + MPL-SE vaccine induced IFN-γ production. These interventional studies demonstrated this formulation is safe and immunogenic in healthy individuals with and without history of previous infection with L. donovani.

Furthermore, LEISH-F1 application was not just associated with MPL. For example, Sakai et al. in Japan analyzed the above-mentioned antigen with cholera toxin as a different adjuvant. Their trial showed that intranasal immunization with LEISH-F1 augments IFN-γ production and protects mice from Leishmaniasis in L. major infection.

4. Third generation vaccines (Naked DNA vaccines).

Though recombinant protein-based vaccines have achieved some degrees of protection in mice and dogs, they have faced complicated problems in the process of getting marketing authorization particularly in human medicine due to indispensable need to an adjuvant. Naked DNA vaccines are extremely safe since they do not contain any pathogenic organism that may revert in virulence and they have also achieved considerable success especially by gene gun in rodents. Nonetheless, they have often been proved to be insufficient for providing protection in non-murine models.

In order to increase their immunogenicity, heterologous prime-boost (HPB) strategy can be implemented for DNA vaccine amendment. This strategy selectively expands memory T cells being specific for the vaccine antigen. Prime-boost assays analyzed against Leishmania with the LACK antigen accomplished protection in mice, nonetheless, it only conferred moderate protection in dogs. On the contrary, prime-boost vaccination using cysteine proteinase A and cysteine proteinase B antigens was highly protective in both mice and dogs, as a rare successful DNA vaccine in non-murine models.

Furthermore, scientists profess that a cocktail of different conserved antigens would probably provide the best protection against the parasite. The Researchers of the Pasteur Institute demonstrated that mice vaccinated with a cocktail DNA vaccine encoding cysteine proteinases type I, II and III with solid lipid nanoparticles were protected successfully against L. major infection. In one different study, Ahmed et al. analyzed the immunity of DNA vaccines encoding LACKp24, TSA, LmSTI1 and CPa in Balb/c mice. They demonstrated that the cocktail DNA vaccine succeeded optimal protection when low parasite dose administered in the dermis of the ear.

However, there are some critical impediments jeopardizing DNA vaccine promotion in clinical trials. For example, human cells may become cancerous due to insertion of foreign DNA into their genomes.

5. Leishmanization and live-attenuated Leishmania.

The inoculation of live and virulent L. major leading to a single lesion is called leishmanization (LZ). The LZ lesion upon cure prevents future natural infection which might be numerous lesions in sites, which might be important in the aspect of cosmetic issues.

On one hand, CL usually produces a self-healing lesion. Although rarely the lesion remains for a long time. Thus, scientists are targeting to develop other strategies aiming to exploit live Leishman’s bodies whilst the probability of refractory Leishmania lesions could be excluded.

On the other hand, live attenuated Leishmania vaccines have become an attractive field because it has been cleared that complete Leishmania cDNA expression library injected into mice is more protective than any sub-pools of the library plasmids or a subunit.

Up to this time, several targeted gene eliminations have been analyzed to develop Leishmania-attenuated vaccine strains against CL. For example, researchers have administered dihydrofolate reductase thymidylate synthase (DHFR-TS) knockout parasites derived from L. major. They professed that this vaccine can produce durable protection in mice but application of this vaccine was not successful in rhesus monkeys. Moreover, scientists of Manitoba University demonstrated that L. major strains being deficient for phosphoglycan (PG) gene (lpg2-) were able to confer protection against virulent L. major challenge, whereas, these attenuated strains are not capable of inducing IFN-γ production.

Furthermore, the administration of live attenuated vaccine emerges as a promising vaccine strategy within the scope of VL. To demonstrate, scientists tested the ability of a L. infantum deletion mutant, lacking both HSP70-II alleles (ΔHSP70-II), to provide protection against Leishmania infection in Balb/c mice. This interventional study showed that the vaccine (ΔHSP70-II) would be safe as immune deficient SCID mice. Hamsters (Mesocricetus auratus) infected with mutant parasites did not develop any sign of pathology.

In addition scientists have exploited a novel radioactive strategy aiming at attenuated vaccine production. For example, Ultraviolet-A radiation and psoralen compound
were administered for production of viable *Leishmania* called killed but metabolically active *Leishmania* strain (KBMA). The KBMA *Leishmania* strains derived from either *L. major* or *L. infantum* were developed into the amastigote form inside macrophages. Furthermore, splenocytes from the mice vaccinated with either live *L. infantum chagasi* or KBMA *L. infantum chagasi* displayed similar cytokine patterns in *vitro*. These results suggest that KBMA technology is a potentially safe and effective novel vaccine strategy against the intracellular protozoa.

In spite of the investigations aimed at artificially attenuated wild *Leishmania* spp., some researchers focused on naturally attenuated *Leishmania* strains. McCall et al. demonstrated that Immunization with a naturally attenuated cutaneous *L. donovani* isolated from Sri Lanka protected Balb/c mice against VL.

### Summary and conclusions

In order to implement a successfully proposed strategies and actions, a long-term *Leishmania* vaccine plan needs to be developed. Through this review article, it was clear that many scientists with various approaches are dedicated to implement researches that have efficacy against Leishmaniasis, nonetheless, it was also proved that under the conditions of this study the majority of tests performed were not accomplished for WHO validation. However, we may be getting closer to develop a safe and effective leishmaniasis vaccine(s) as a result of newer vaccine approaches, which have evolved from whole irradiated live parasites, or the use of defined antigens such as LEISH-F, either in the form DNA vaccines or microbial vectors.

### References

1. Veland N, Boggild AK, Valencia C, et al. *Leishmania* (Viannia) species identification on clinical samples from cutaneous leishmaniasis patients in Peru: Assessment of a molecular stepwise approach. J Clin Microbiol 2012; 50:495-498.

2. Marlow MA, da Silva Mattos M, Makowiecky ME, et al. Divergent profile of emerging cutaneous leishmaniasis in subtropical Brazil: New Endemic areas in the southern frontier. PLoS One 2013; 8:e56177.

3. Karami M, Doudi M, Setorki M. Assessing epidemiology of cutaneous leishmaniasis in Isfahan, Iran. J Vector Borne Dis 2013;50(1):30-37.

4. Alvar J, Vélez ID, Bern C, et al. Leishmaniasis worldwide and global estimates of its incidence. PLoS One 2012; 7:e35671.

5. Dujardin JC, Decuyper S. Drug resistance in leishmania parasites. New York, USA: Springer 2013; 65-83.

6. Rezvan H, Rees RC, Ali SA. Development of A peptide-based sub-unit vaccine by selecting peptides from the essential surface glycoproteins of *Leishmania* parasites, Gp63, Using transgenic mouse model. In proceedings: 12th Iranian reserchers conference in Europe. Manchester, UK: 2003; 52.

7. Nagayoshi Y, Miyazaki T, Minematsu A, et al. P245 Unexpected effects of the monoamine oxidase A inhibitor clorgyline on antifungal susceptibility of *Candida glabrata*. Int J Antimicrob Agents 2013; 42:S119-S120.

8. Jiménez M, González E, Iriso A, et al. Detection of *Leishmania infantum* and identification of blood meals in *Phlebotomus perniciosus* from a focus of human leishmaniasis in Madrid, Spain. Parasitol Res 2013;112(7):2453-2459.

9. Tuckow AP, Temeyer KB, Brake DK, et al. Acetylcholinesterase of the sand fly, *Phlebotomus papatasi* (Scopoli): cDNA Sequence, baculovirus expression, and biochemical properties. Parasit Vectors 2013; 6: 31.

10. Campino I, Cortes S, Dionisio L, et al. The first detection of *Leishmania major* in naturally infected Sergentomyia minut in Portugal. Mem Inst Oswaldo Cruz. 2013; 108(4): 516-518.

11. McCarthy CB, Santini MS, Pimenta PF, et al. First comparative transcriptomic analysis of wild adult male and female *Lutzomyia longipalpis*, vector of visceral leishmaniasis. PLoS One 2013; 8:e58645.

12. Afsahi A, Aein Z, Rezvan H, et al. Studies on using cattle and sheep hydrid cyst fluid instead of the fetal calf serum (FCS) in leishmania culture. Zahedan J Res Med Sci 2013; 15(12): 9-12.

13. Ali SA, Rezvan H, Khodadadi A, et al. A mouse model to evaluate the role of CTLs in *Leishmania* DNA vaccines. In proceedings: Spring trypanosomiasis/leishmaniasis and malaria meetings. Newcastle, UK: 2008; 129.

14. Cruz I, Millet A, Carrillo E, et al. An approach for interlaboratory comparison of conventional and real-time PCR assays for diagnosis of human leishmaniasis. Exp Parasitol 2013;134(3):281-289.

15. Rahi AA, Nsaif S, Hassoni JJ, et al. Comparison of diagnostic methods in Cutaneous Leishmaniasis in Iraq. Am J BioSci 2013; 1:1-5.

16. Souza AP, Soto M, Costa JM, et al. Towards a more precise serological diagnosis of human tegumentary leishmaniasis using leishmania recombinant proteins. PLoS One 2013; 8:e66110.

17. Soto J, Rojas E, Guzman M, Vet al. Intraleisonsal anthimony for single lesions of Bolivian cutaneous leishmaniasis. Clin Infect Dis 2013;56(9):1255-1260.

18. Zucca M, Scutera S, Savoia D. New chemotherapeutic strategies against malaria, leishmaniasis and trypanosomiasis. Curr Med Chem 2013; 20:502-526.

19. Sarkar J, Pal S, Bhattacharya S, Bet al. *In vitro* Antileishmanial activity of *Pleuromia pudica* leaf extracts on *Leishmania donovani* promastigotes. AmEurasian J Sci Res 2013, 8:68-71.
20. Mayrink W, Mendonca MA, de Paula JC, et al. Cluster randomised trial to evaluate the effectiveness of a vaccine against cutaneous leishmaniasis in the Caratinga microregion, south-east Brazil. Trans R Soc Trop Med Hyg 2013;107(4):212-219.
21. Garcia BM, Barrio AB, Parodi RC, et al. Immunological correlates of cure in the first American cutaneous leishmaniasis patient treated by immunotherapy in Argentina. Invest Clin 2011;52(4):365-375.
22. BorjaCabrera G, Santos F, Santos F, et al. Immunotherapy with the saponin enriched-Leishmune vaccine versus immunochemothry in dogs with natural canine visceral leishmaniasis. Vaccine 2010; 28(3): 597-603.
23. Rezvan H. Studies on immunology of leishmania mexicana. PhD Thesis. Nottingham Trent University, Nottingham, UK: 2007.
24. de Moura TR, Oliveira F, Carneiro MW, et al. Functional transcriptomics of wild-caught Lutzomyia intermedia salivary glands: Identification of a protective salivary protein against Leishmania braziliensis infection. PLoS Negl Trop Dis 2013;7(5):e2242.
25. Marzouki S, Ahmed MB, Boussofara T, et al. Characterization of the antibody response to the saliva of Phlebotomus papatasi in people living in endemic areas of cutaneous leishmaniasis. Am J Trop Med Hyg 2011; 84:653-661.
26. Carregaro V, Costa DL, Brodskyn C, et al. Dual effect of Lutzomyia longipalpis saliva on Leishmania braziliensis infection is mediated by distinct saliva-induced cellular recruitment into Balb/c mouse ear. BMC Microbiol 2013; 13:102.
27. Alvar J, Croft SL, Kaye P, et al. Case study for a vaccine against leishmaniasis. Vaccine 2013; 31:B244-B249.
28. Soudi S, Hosseini A, Hashemi S. Co-administration of rectal BCG and autoclaved Leishmania major induce protection in susceptible Balb/c mice. Parasite Immunol 2011;33:561-571.
29. Kedzierski L. Leishmaniasis vaccine: Where are we today? J Glob Infect Dis 2010;2(2): 177-185.
30. Ravindran R, Bhowmick S, Das A, et al. Comparison of BCG, MPL and cationic liposome adjuvant systems in leishmanial antigen vaccine formulations against murine visceral leishmaniasis. BMC Microbiol 2010; 10:181.
31. Wang W, Singh M. Selection of adjuvants for enhanced vaccine potency. World J Vaccines 2011; 1:33-78.
32. Ravindran R, Maji M, Ali N. Vaccination with liposomal leishmanial antigens adjuvanted with monophosphoryl lipid-trehalose dicorynomycolate (mpl-tdm) confers long-term protection against visceral leishmaniasis through a human administrable route. Mol Pharm 2011; 9:59-70.
33. Mutisso JM, Macharia JC, Kiio MN, et al. Development of Leishmania vaccines: Predicting the future from past and present experience. J Biomed Res 2013, 27:85.
34. Noazin S. Evaluation of first generation vaccines against human leishmaniasis and the implication of leishmanin skin test (LST) response in disease prevalence. PhD Thesis. University of Basel, Switzerland 2008.
35. Rezvan H, Feiz Haddad MH, Asteal F, et al. Evaluation of immunogenicity of peptides derived from Leishmania major gp63 with high affinity to human MHC I molecules using HLA. A2.1 HHID II transgenic mouse model. In proceedings: The sixth national and the first regional congress on parasitology and parasitic diseases. Karaj, Iran: 2008.
36. Sinha S, Sundaram S, Singh AP, et al. A gp63 based vaccine candidate against visceral leishmaniasis. Bioinformation 2011, 5:320.
37. Isnard A, Shio MT, Olivier M. Impact of leishmania metalloprotease gp63 on macrophage signaling. Front Cell Infect Microbiol 2012;2:72. doi: 10.3389/fcimb. 2012.00072.
38. Rezvan H, Ali SA, Rees R. Leishmania parasite subunit vaccine in HLA-A2 transgenic mouse model. Iran J Public Health 2005;34(Sup):50-51.
39. Rezvan H, Feiz Haddad MH, Asteal F, et al. Evaluation of CTL activity in mice immunised with L mexicana gp63 cDNA. In proceedings: The sixth national and the first regional congress on parasitology and parasitic diseases. Karaj, Iran: 2008.
40. Basyoni MM. Leishmania vaccines updates. Parasitol United J 2011;5(1):1-10.
41. Bhowmick S, Ravindran R, Ali N. Gp63 in stable cationic liposomes confers sustained vaccine immunity to susceptible Balb/c mice infected with Leishmania donovani. Infect Immun 2008;76(3):1003-1015.
42. Mazumder S, Maji M, Ali N. Potentiating effects of MPL on DSPC bearing cationic liposomes promote recombinant GP63 vaccine efficacy: High immunogenicity and protection. PLoS Negl Trop Dis 2011; 5:e1429.
43. Mazumder S, Maji M, Das A, et al. Potency, efficacy and durability of DNA/DNA, DNA/protein and protein/protein based vaccination using gp63 against Leishmania donovani in Balb/c mice. PLoS One 2011; 6:e14644.
44. Rezvan H, Rees R, Ali S. Immunogenicity of MHC class I peptides derived from Leishmania mexicana gp63 in HLA-A2. 1 transgenic (HHDII) and Balb/c Mouse models. Iran J Parasitol 2012; 7:27.
45. Rezvan H. Immunogenicity of HLA-DR1 restricted peptides derived from leishmania major gp63 using FVB/N-DR1 transgenic mouse model. Iran J Parasitol 2013; 8:273-279.
46. Zhang CY, Zhou J, Ding B, et al. Phylogenetic analysis of LACK gene sequences for 22 Chinese leishmania isolates. Infect Genet Evol 2013;17:79-86.
47. Costa MM, Penido M, dos Santos MS, et al. Improved canine and human visceral leishmaniasis immuno-diagnosis using combinations of synthetic peptides in
enzyme-linked immunosorbent assay. PLoS Negl Trop Dis 2012; 6:e1622.
48. Soto M, Ramirez L, Pineda MA, et al. Searching genes encoding leishmania antigens for diagnosis and protection. Scholarly Research Exchange 2009; doi:10.3814/2009/173039.
49. Nagill R, Kaur S. Vaccine candidates for leishmaniasis: A review. Int Immuno pharmacol 2011; 11:1464-1488.
50. Hugentobler F, Yam KK, Gillard J, et al. Immunization against Leishmania major infection using LACK-and IL-12-expressing Lactococcus lactis induces delay in footpad swelling. PLoS One 2012; 7:e30945.
51. Hezarjaribi HZ, Ghaffarifar F, Dalimi A, et al. Effect of IL-22 on DNA vaccine encoding LACK gene of Leishmania major in Balb/c mice. Exp Parasitol 2013;134(3):341-348.
52. Sanchez-Sampedro L, Gomez CE, Mejias-Perez E, et al. High quality long-term CD4+ and CD8+ effector memory populations stimulated by DNA-LACK/MA-LACK regimen in Leishmania major Balb/c model of infection. PLoS One 2012; 7:e38859.
53. Depledge DP, MacLean LM, Hodgkinson MR, et al. Leishmania-specific surface antigens show sub-genus sequence variation and immune recognition. PLoS Negl Trop Dis 2010; 4:e829.
54. Zand M, Narasu ML. Vaccination against leishmaniasis. Annals Biol Res 2013; 4:170-174.
55. Beaumier CM, Gillespie PM, Hotez PJ, et al. New vaccines for neglected parasitic diseases and dengue. Transl Res 2013; 162(3):144-155.
56. Kaye P, Aebischer T. Visceral leishmaniasis: Immunology and prospects for a vaccine. Clin Microbiol Infect 2011; 17(10):1462-1470.
57. Silva A, Tavares J, Silvestre R, et al. Characterization of Leishmania infantum thiol-dependent reductase 1 and evaluation of its potential to induce immune protection. Parasite Immunol 2012; 34:345-350.
58. Llanos-Cuentas A, Calderón W, Cruz M, et al. A clinical trial to evaluate the safety and immunogenicity of the LEISH-F1+ MPL-SE vaccine when used in combination with sodium stibogluconate for the treatment of mucosal leishmaniasis. Vaccine 2010; 28:7427-7435.
59. Nascimento E, Fernandes DF, Vieira EP, et al. A clinical trial to evaluate the safety and immunogenicity of the LEISH-F1+ MPL-SE vaccine when used in combination with meglumine antimoniate for the treatment of cutaneous leishmaniasis. Vaccine 2010; 28:6581-6587.
60. Chakravarty J, Kumar S, Trivedi S, et al. A clinical trial to evaluate the safety and immunogenicity of the LEISH-F1+ MPL-SE vaccine for use in the prevention of visceral leishmaniasis. Vaccine 2011; 29:3531-3537.
61. Sakai SI, Takashima Y, Matsumoto Y, et al. Intranasal immunization with Leish-111f induces IFN-γ production and protects mice from Leishmania major infection. Vaccine 2010; 28:2207-2213.
62. Khan KH. DNA vaccines: Roles against diseases. Germs 2013; 3: 26-35.
63. Ali S, Rezvan H, McArdle S, et al. CTL responses to Leishmania mexicana gp63-cDNA vaccine in a murine model. Parasite Immunol 2009; 31:373-383.
64. Rezvan H, Rees R, Ali S. Leishmania mexicana Gp63 cDNA using gene gun induced higher immunity to L. mexicana infection compared to soluble leishmanial antigen in Balb/c. Iran J Parasitol 2011; 6(4): 60-75.
65. Todoli F, Rodriguez-Cortés A, del Carmen Nñeiz M, et al. Head-to-head comparison of three vaccination strategies based on DNA and raw insect-derived recombinant proteins against leishmaniasis. PLoS One 2012; 7:e51181.
66. Doroud D, Zahedifard F, Vatanara A, et al. Delivery of a cocktail DNA vaccine encoding cysteine proteinases type I, II and III with solid lipid nanoparticles potentiate protective immunity against Leishmania major infection. J Control Release 2011; 153:154-162.
67. Ahmed SBH, Touhihi L, Chhtourou Y, et al. DNA based vaccination with a cocktail of plasmids encoding immunodominant Leishmania major antigens confers full protection in Balb/c mice. Vaccine 2009; 27(1):99-106.
68. Khamesipour A, Abbasi A, Firooz A, et al. Treatment of cutaneous lesion of 20 years' duration caused by leishmanization. Indian J Dermatol 2012; 57:123-125.
69. Palatnikde Sousa CB. Vaccines for canine leishmaniasis. Front Immunol 2012; 3:69.
70. Selvapandian A, Dey R, Gannavaram S, et al. Immunity to visceral leishmaniasis using genetically defined live-attenuated parasites. J Trop Med 2013; 2013:1-8.
71. Liu D, Okwor I, Mou Z, et al. Deficiency of leishmania phosphoglycans influences the magnitude but does not affect the quality of secondary (memory) anti-leishmania immunity. PLoS One 2013; 8:e66058.
72. Carrión J, Folgueira C, Soto M, et al. Leishmania infantum HSP70-II null mutant as candidate vaccine against leishmaniasis: A preliminary evaluation. Parasit Vectors 2011; 4:150. doi: 10.1186/1756-3305-4-150.
73. Datta S, Adak R, Chakraborty P, et al. Radio-attenuated leishmanial parasites as immunoprophylactic agent against experimental murine visceral leishmaniasis. Exp Parasitol 2012; 130:39-47.
74. Bruhn KW, Birnbaum R, Haskell J, et al. Killed but metabolically active Leishmania infantum as a novel whole-cell vaccine for visceral leishmaniasis. Curr Vaccine Immunol 2012; 19(4):490-498.
75. McCall LI, Zhang WW, Matlashewski G. Leishmanization revisited: Immunization with a naturally attenuated cutaneous Leishmania donovani isolate from Sri Lanka protects against visceral leishmaniasis. Vaccine 2013; 31(10):1420-1425.