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Review Article

Leishmania Vaccines: A Narrative Review on Last Decade Developments

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Abstract

Leishmaniasis is a vector-borne protozoan disease transferred by the sting of a female sandfly. Currently, both cutaneous and visceral forms of leishmaniasis (VL) are observed in Iran. Cutaneous leishmaniasis (CL) is reported in 18 provinces in the country in rural (zoonotic) and urban (anthroponotic) forms. VL is endemic in some areas of Iran. Various drugs are used in the treatment of leishmaniasis, but due to created challenges such as high toxicity, side effects, prolongation, and drug resistance, the process of curing will face problems. The prevention of infection is also costly. One of the best ways to control the disease is to find an effective vaccine. The efficient vaccine will cause long-term immunity and simultaneously breaks the disease transmission chain. This study aimed to review different types of existing vaccines, along with those in trials. It was found that multi anti-gene vaccines created the best immunity against the parasite and could be more successful. Developments in genetic engineering and release systems have raised hope to achieve the vaccines soon.

Keywords: Leishmania, Vaccine, Leishmaniasis, Prevention

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Introduction

Leishmaniasis is a wide-spectrum disease, which consists of various clinical syndromes, including wounds with self-cure, the contention of mucous, and visceral disease. In addition, the disease has a wide range of species of pathogenic agents, repositories, and carriers.

Leishmania is a vector-borne disease which is transmitted to humans by the sting of a female sand fly. The agent of the disease is a protozoan flagellate observed in two forms, the flagellates called promastigote (leptomonad) and the non-flagellate amastigote form (leishman body). Flagellate types are observed in the body of the carrier insect, and in the artificial cultivation media, non-flagellate types exist in vertebrate host cells such as humans having an intercellular life cycle (1). Currently, 53 species of *Leishmania* are determined in humans and different animals. Twenty species of them are known as pathogenic in humans (2).

Leishmania parasite, after being located in the skin through the bit of a female sand fly, will cause three different clinical forms of the disease, including cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (diffuse CL), and visceral leishmaniasis (VL).

CL creates ulcerative wounds in which scars remain for a lifetime. Muco-cutaneous type causes severe injuries in nasal and oral mucous and occasionally complete destruction in infected tissues. Replacement and increase of protozoa in the tissue cells of the liver, spleen, lymph nodes, and bone marrow create VL with systemic symptoms and thus cause death in the patient if not cured. Unfortunately, due to non-diagnosed cases, the disease is treated insignificantly.

Leishmaniasis is in the context of neglected tropical diseases, especially in underdeveloped and developing countries. According to the World Health Organization (WHO), leishmania infections occur annually in 98 endemic tropical and subtropical countries and 310 million people are vulnerable to infection. In addition, 0.7-1 million new cases of leishmaniasis have been reported globally (1).

Considering that leishmaniasis is an unmanageable disease in some geographical areas and is increasing in endemic regions, the most significant indices in managing the disease are diagnosis, treatment, and prevention (3,4).

Leishmaniasis is currently reported in both cutaneous and visceral forms In Iran. CL is reported in rural (zoonotic) and urban (anthroponotic) forms in 18 provinces and 143 cities. It is estimated that the incidence of CL is 23 cases per 100 000 population in Iran. According to registered cases in health centers, CL is the most common form of the disease, and 20 000 cases are reported annually. The real cases are estimated to reach many times higher than the reported ones (5).

VL or Kala-azar disease in Iran is the Mediterranean

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type. *Leishmania infantum* is the agent, and canids are the reservoirs of the disease. Kala-azar disease is reported as a sporadic infection from all over Iran, except for the major parts in Sistan and Baloochestan. However, in 4 provinces and 30 cities, it is reported endemic, and its estimated incidence is 0.092 in 100 thousand population (2,5).

Using a direct agglutination test in the dogs revealed 12.5% VL in 20 provinces of the country. The highest prevalence (14%) was reported in Azerbaijan and Ardabil, in the northwest of Iran (6,7).

Rapid and accurate diagnosis of the disease via microscopic, culture, serological, and molecular methods, along with proper and on-time treatment is important and can be helpful in its prevention and control. Effective treatment may prevent a poor prognosis of the disease. Misdiagnosis of the disease is due to the non-availability of effective diagnostic methods, limited resources in endemic regions, and the lack of experienced physicians and laboratory-trained staff (8,9).

Various drugs have been used in the treatment of Leishmaniasis, but due to many challenges created by these drugs, including high toxicity, side effects, long-term cure, and drug resistance causing the recurrence of the disease, the treatment process is challenging (10,11).

Control and prevention proceeding is highly difficult due to a wide spectrum of vectors and animal reservoirs; it is almost not successful and requires a high budget. According to many studies, the best and the most economical solution to control this disease is to find an efficient vaccine. A wide range of evidence confirms the efficiency of vaccines not only in creating long-term prevention but also in a cure for Leishmaniasis. In any way, vaccines can produce long-term prevention against the disease and cut the transition chain of the disease (12).

Trial and clinical evidence showing the management of Leishmaniasis can indicate two major witnesses to strengthen the possibility of producing a vaccine against Leishmaniasis. The first one is the positive Montenegro cutaneous test which shows an active cellular immunity system against parasites in patients previously infected with Leishmania.

The other one is the use of the live promastigotes from *Leishmania major*, which is injected into the individual (Leishmanization). The injection contains promastigotes grown in culture media in the selected area of the body such as arm. These individuals will be immunized against infection caused by cutaneous Leishmania (producing a self-healing lesion) after the disease period (13,14).

The present study aimed at gathering and reviewing new and available data on leishmania vaccines and their efficiency.

Vaccine

Vaccine is a biological component that provides active acquired immunity to a special infectious disease. Vaccination strategy is one of the economic methods for controlling infectious diseases (15,16). There are several types of vaccines in use, which can mention such as killed (inactivated), attenuated, toxoid, subunit, recombinant, semi-virus particles, and DNA or RNA (genetic) vaccines (12).

The characteristics of an ideal vaccine for leishmania are immunization against the majority of strains of the parasite, minimum repetition with holding long-term immunity against disease strains, capability to use for therapeutic and prophylactic purposes, low cost, and easy transportation. Nowadays, it has been found that to achieve these goals, multi anti-gene vaccines should create best immunity against the parasite in order to be more successful.

Historically, 10 countries are considered pioneers in leishmania vaccine research among which the United States of America has gained first place for conducting 692 studies in 1980-2015. India and Brazil stand in the second and third places, and Iran has the fourth place by performing 191 research in this regard (17).

Clinical studies indicated that different leishmania vaccines are used for therapeutic and prophylactic purposes. Leishmania vaccines are divided into live vaccines, as well as first-, second-, and third-generation vaccines.

Live Vaccine

In this type of vaccine, which was the subject of research for many years, low doses of live protozoa of *L. major* are used for immunization.

The intradermal inoculation of live *Leishmania* (usually *L. major*) promastigote to induce an artificial CL lesion in a selected part of the body such as the arm to protect against further natural CL has been named Leishmanization .

This vaccine produces more than 90% immunity against the re-infection of parasites in middle-east countries and the Soviet Republic (ex-Russia). During the Iran-Iraq war, leishmanization was performed in a high population of soldiers before dispatching to the front. This method could significantly reduce the incidence rate of CL in soldiers during this war by one-seventh (2). Unfortunately, in rare cases, the possibility to develop a lesion, which does not heal during the expected period of time, was found in leishmanization; this type of lesion is the main drawback of leishmanization that was mainly developed in individuals who participated in mass leishmanization. This method is not popular anymore since skin lesions left for months. Therefore, other methods were studied for prophylactic purposes without skin lesions.

The second meeting of the expert committee on the control of leishmaniasis, in Geneva (1989) recommended that at present time, leishmanization must be stopped

and used only when all other control measures have failed or been found impossible; leishmanization must be used as a last resort of Cl control (2). Nowadays, leishmanization could be employed as a tool to protect against leishmaniasis in emergency conditions such as war and to evaluate candidate vaccines.

First Generation Vaccines

Killed Leishmania Vaccines Alone or With Adjuvant

Salles Gomes is the pioneer in studies about killed leishmania vaccines, he injected a patient having the skin lesions extract of killed promastigote and observed different immune responses. According to observations, he concluded that by this method, he can reach a vaccine for leishmaniasis (14,18).

In the first mid of 20th century, many researchers from Latin America attempted to find different antigens to be used in vaccine production. Mayrink et al used killed *L. amazonensis* promastigotes (autoclaved) in Brazil (19). Similarly, Convit et al employed killed *L. Mexicana* promastigotes with bacille Calmette-Guérin (BCG) vaccine as an adjuvant for prophylaxis and immunotherapy in Venezuela and reported a significant reduction in the incidence rate of infection (17). In Ecuador, killed leishmania promastigote was used as a vaccine afterward. Moreover, in Iran and Sudan, autoclaved *L. major*, along with BCG as an adjuvant was applied against Old World leishmaniasis (19).

These types of vaccines are known as prophylaxis containing killed protozoa, along with alum adjuvant or BCG. These vaccines will stimulate the cell immune system of the patient while not leading to the production of a suitable and effective anti-body for protection.

Leishmania first-generation vaccines were produced from completely killed parasites and were of interest because of ease of use and low cost production. These vaccines are the only type entered the third phase of clinical studies for many years and are still under study. However, it is demonstrated that autoclaving lowers the immunogenicity of the parasite by destroying most of the proteins. Therefore, these types of vaccines do not mimic natural infection and are less immunogenic.

The first clinical phase of these vaccines against visceral leishmania was performed in Sudan in 2006. The results were not convincing and urged for further research, which unfortunately was left incomplete. Research was necessary and continued on the prophylactic purposes of the vaccine to create cell immunity (14).

In Iran, a strain of *L. major* applied in leishmanization was used in the production process of the killed vaccine by the Razi institute in Hesarak, and clinical trial phases 1 and 2 started in 1991. However, according to researchers' observations, the produced leishmania antigen was unstable and though kept at -70°C and could not prevent proteolytic activities (14,20).

Vaccines Containing Live-attenuated Strains of Leishmania

Currently, several live-attenuated vaccines containing strains of L. braziliensis and normally weakened strains of *L. donovani* have been used in leishmaniasis vaccine production, but unfortunately, none of them have been successful (14,21).

Second-Generation Vaccines

They are recombined vaccines. In fact, recombined immunogenic protein particles of the leishmania parasite are used in these vaccines. The recombined proteins, along with adjuvants are employed for strengthening the immune response against them or those in microbial vectors proved to be appropriate for general vaccination (14,22,23).

Various proteins have been candidates and tested as possible vaccines. Surface and non-membrane antigens of leishmania spp. have been used in producing the vaccine. Among the above-mentioned antigens are H1, LPG, LeiF2, LmSTII, Gp63, HASPB1, LCR1, Gp46, TSA, LD1, P/36 KACK, SP15, PSA-2, and cysteine proteinase (14).

Lipophosphoglycan

Lipophosphoglycan (LPG) is the major macromolecule (25%) on the surface of the promastigote stage and is produced by all Leishmania species. In addition, LPG is thought to play a role in complement activation and resistance to complement-mediated lysis in the attachment and entry of promastigotes into mammalian macrophages. Using a purified form of LPG of *L. major* and *L. mexicana* resulted in relative immunity in mice, but the stimulation of the mechanism of T-cells is not recognized yet (2,14).

Thiol Specific Antioxidant

Thiol specific antioxidant (TSA) is a highly conserved antigen among the old and new world Leishmania genus. It was isolated from the supernatant of *L. major* culture media. When it was inoculated to the mice model, along with interleukin-2 (IL-12) as an adjuvant, it could induce excellent protection against infection in the murine model of Old World CL (2).

Glycoprotein63

This superficial glycoprotein of leishmania is a zinc-metalloprotease that is a virulence factor and has a significant role in the parasite entering phagosome. Glycoprotein63 (Gp63) is the major surface protein on the promastigote form of leishmania spp. and is highly conserved among all *Leishmania* species. Its recombined type is called rgp63, which represents the lack of a sugar molecule and has a molecular weight of 54-58 kD. When inoculated to murine models in the form of a liposome, Gp63 could create 75% immunity, and the immunity

response of type 1 T helper (Th1) was significant (2,14).

Leishmania Derived Recombinant Poly Protein (Leish-111f)

This poly antigen vaccine contains SA, LmSTII, and LeLF antigens, is highly effective, and is simultaneously used in the prevention and cure of drug-resistance species of leishmania. This vaccine was successful in the first phase of the clinical trial in the United States. This type is the only leishmania vaccine, which is entered into the second phase of clinical trials. This vaccine is efficient in the significant increase of CD4+T-cells, the production of interferon-gamma (INF-γ) and IL-12, and the induction of a Th1 immunity response (14).

Leishmania major Amastigote Class I Nuclease

Leishmania parasite is incapable of synthesis nuclease purine by itself; thus, it needs this enzyme for purine synthesis. *L. major* amastigote class I nuclease (LmaCIN) is highly expressed in the amastigote stage of *L. major* and can induce the immunity response of type Th1. A vaccine including this nuclease could be an appropriate candidate against Old World CL (14).

Recombinant P4 Antigen

P4 is another Leishmania surface antigen, which creates relative immunity in mice against *L. pifanoi* and *L. amazonensis* if used in pure form. In addition, this antigen induces the Th1 immunity response in patients infected with the American type of CL (24,25). Since 1997, some studies have been performed on the P4 antigen in Iran, and significant results have been reported in this respect (26).

Cysteine Proteases II and I

Recombinant vaccine from *L. major* cysteine protease I and II, along with Poloxamer 407 adjuvant-induced immunity response related to the secretion level of INF-γ from CD8+cells in mice models (27,28). Using the C-terminal part of cysteine protease I of *L. infantum* as a vaccine and its injection in different doses created relative immunity in dogs (29).

In some studies, a non-pathogenic species of leishmania was employed as a vector for the gene expression of specific antigens; for instance, *L. tarantula* was used as a live vaccine or a vector for cysteine protease gene expression, and the A2 gene of *L. infantum* which induced immunity in dogs (30). Additionally, the A2 gene expression of *L. donovani* in non-pathogenic *L. tarantula* was applied as a vaccine and yielded remarkable outcomes (31).

Another study investigated peptide vaccine production against six epitopes of *L. major,* including CPB, CPC, msTI-1, TSA, Lelf, and LPG3, and epitopes LPG3 and msTI-1 were effective immunogens (28).

Third-Generation Vaccines

The newest and most promising leishmania vaccines in the recent decade are the ones manufactured from DNA, expressing antigens through injecting DNA plasmids into cell vectors, leading to the stimulation of the immune system. These vaccines are easily produced and bear low costs, but their use is restricted due to the large size of DNA and unknown consequences of the DNA-entering genome. Choosing appropriate and efficient vectors is another impediment in producing third-generation vaccines.

Leishmania Homologue for the Receptors of Activated C-kinase (LACK)

One of the DNA vaccines for leishmania is LACK. In fact, the LACK gene is a plasminogen-binding protein in *L. major*. The gene is an analogue from RACK proteins (a receptor for activated C-kinase) in eukaryotes, which has a role in stabilizing the active form of protein kinase C and acts as a receptor for multi-complex proteins participating in signal routes. Previous evidence has confirmed the role of this gene in the survival of parasites and their resistance. LACK gene vaccine, which is designed as an accompanying liposome and creates a rapid immune response of gene product against the parasite, is one of the most discussed vaccines in murine models. This vaccine induces both cellular and humoral immune responses (14,31,32).

ChAd63-KH

ChAd63-KH is a simian adenovirus-vectored vaccine, which is made from Kinetoplastid membrane protein-11 (KMP-11 and surface protein B of acetylated hydrophil from the gene HASPB of Leishmania, which is combined in Chimpanzee's virus named Adeno-63. The vaccine is under clinical trial (2). The ChAd63-KH vaccine is used to treat patients with persistent post-kala-azar dermal leishmaniasis.

P4 Gene

Campbell et al entered a gene encoding P4 nuclease alone and along with various adjuvants such as IL-12 and HASP70 to *L. amazonensis* in a mouse model and infected the mouse to *L. amazonensis* and *L. major*. They found that gene P4 along with HASP70 adjuvant can cure the species of Old and New World Leishmaniasis, and nominated it as the DNA vaccine (33-35).

Other DNA vaccines inducing immunity in mice are gene expression cysteine proteases with cationic solid lipid nanoparticles (CSLN) and could create polytope HLA-A201 transgenic vaccine, resulting in a remarkable immunity in mice (30,36).

Different carrier systems are used in DNA vaccines, including electroporation and CSLN, and these carriers will increase the immune response of vaccines (29).

A combination of DNA vaccines and cysteine protease I and II vaccines was used in dogs against *L. infantum* and resulted in remarkable prevention in these animals (37,38).

Other Vaccines

Vaccines Against Sand fly Salivary Glands

One of the most significant characteristics of these types of vaccines is that they lack antigens of parasites. Investigation on Phlebotomus papatasi salivary glands led to the recognition of a 15-kDa molecule called SP15, showing high immunity against leishmania after injection to the mice model.

Producing a vaccine with the above-mentioned contents raises the subject of the possibility of immunity against all species of leishmania due to human immune responses to vector salivary glands (14,39).

Conclusion

Vaccination could play a remarkable role in the treatment and prevention of leishmaniasis. One of the most important hygiene priorities is to produce a safe and economical vaccine that is efficient for different parasites. In the recent decade, many efforts have been made in the field of production of *Leishmania* vaccine, and there were many challenges raised from the lack of sufficient knowledge on the pathogens of parasites, immunity system reactions, and parasite escape from the immune system.

Thus, researchers should attempt to produce multi anti-gene vaccines to create the best immunity against Leishmaniasis. Nowadays, using the DNA of vaccines has opened a new era in this field. Developments in genetic engineering and releasing systems have raised hope to achieve vaccines in the near future. Iran is one of the pioneered countries in Leishmaniasis studies and can achieve remarkable success in the field of producing vaccines and related research.

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