Review of Development of Live Vaccines against Leishmaniasis

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

Keywords
► Leishmania
► leishmaniasis
► live-attenuated
► vaccine

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**Introduction**

Leishmaniasis, caused by an intracellular protozoan parasites of the genus *Leishmania*, is prevalent in subtropical and tropical regions all around the world.1–3 The disease is considered as neglected tropical diseases with the incidence of 1.5 million new cases annually4,5 which leads to a considerable mortality and morbidity.6 There are three main forms of the disease, namely, cutaneous leishmaniasis (CL), visceral leishmaniasis (VL), and mucocutaneous leishmaniasis (MCL).7 VL, also known as kala-azar, is caused by *Leishmania donovani* (anthroponotic form) and *L. infantum* (zoonotic form). VL is the most important and severe form of leishmaniasis with 500,000 new cases annually. In this form of the disease, the parasites invade in internal organs such as liver and spleen. VL can lead to death in untreated cases and there is no effective vaccine for it.8 MCL is mainly caused by *L. braziliensis* complex in mucosal tissues of nose and mouth and can result in massive tissue damage and permanent disfiguration.9 CL is self-healing disease10 caused mostly by the *L. tropica* and *L. major* in the Central Asia and Middle East11 and *L. braziliensis* and *L. mexicana* complexes in the Americas.12 CL is the most common form of leishmaniasis in the world, and 90% of all CL cases occur only in seven countries: Syria, Iran, Saudi Arabia, Algeria, Peru, Brazil, and Afghanistan.1 CL can lead to skin deformities, and in immune compromised patients giving rise to the diffuse cutaneous leishmaniasis.13 The host immune response has a crucial role in leishmaniasis, both in efficacy of treatment and the clinical patterns of the disease.14 Success in development of vaccine depends on selecting an appropriate vaccine candidate and the immune biology of pathogen/host interactions.15 Cell-mediated immunity particularly CD4 T cells are critical in leishmaniasis and protection against the disease.16 The host defense mainly relies on response of Th1 cells which were activated through interleukin (IL)-12. Antigen presenting cells (APCs) and T cells, as they produce IL-12 and interferon gamma (IFN-γ), are crucial for limiting the numbers of leishmania parasites.17 In contrast, Th2 cells produce cytokines, mainly IL-4, IL-5, and IL-13 and anti-inflammatory cytokines that have suppressive effect on host immunity which leads to parasite survival during leishmaniasis.17,18 These effects are exclusively studied in CL and murine models.7,19 Generation of proper immunologic memory is the main challenge in designing a vaccine that can cause effective immunity. Different studies revealed that long-lasting immunity against leishmaniasis needs to involve the function of both regulatory T cells (Tregs) and Th1-mediated immune response.20,21 Currently, treatment of any form of leishmaniasis is remarkably dependent on pentavalent antimonials as first-line and amphotericin B as second-line drugs.22 This treatment is usually adequate but long duration of treatment, high costs, and side effects are still challenging. Reversion to virulence form has been reported in host with weak immune system, especially in HIV infected individuals.23 Furthermore, strains which are resistant to available drugs created the need for an effective vaccine.24 Currently, there is no impermissible vaccine against human leishmaniasis, even several vaccines have developed to clinical trials, most are still in early research phases and need more develop (Table 1).25–27 From 2004, four vaccines have been licensed against canine leishmaniosis, two in Brazil (Leishmune, license was withdrawn in 2014, and Leish-Tec) and two in Europe (CaniLeish and LetiFend).28 Efforts for finding an effective vaccine against leishmaniasis is continuing worldwide.9,29 Finally, development of an effective vaccine is one of the most cost-effective ways to eliminate and/or control CL and VL. This review aims to discuss the importance of different approaches concerning the development of a live-attenuated vaccine against leishmaniasis.

**Live Active Vaccines**

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

**Live-Attenuated Vaccine**

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

<table>
<thead>
<tr>
<th>Candidate name/identifier</th>
<th>Preclinical</th>
<th>Phase I</th>
<th>Phase II</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEISH-F2</td>
<td>X</td>
<td></td>
<td>11,25</td>
<td></td>
</tr>
<tr>
<td>LEISH-F3</td>
<td>X</td>
<td>11,26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Various <em>Lutzomyia</em> and fly antigens</td>
<td>X</td>
<td>11,27</td>
<td></td>
<td></td>
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<tr>
<td>Various second-generation protein-based vaccines</td>
<td>X</td>
<td></td>
<td></td>
<td>11,27</td>
</tr>
<tr>
<td>Various third-generation DNA-based and heterologous prime-boost vaccines</td>
<td>X</td>
<td></td>
<td></td>
<td>11,27</td>
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</table>
culture under drug pressure, and γ-attenuation have been used to develop an attenuated Leishmania strains for many years. Recently, live-attenuated mutants through the gene alteration have revealed ability to induce proper immune responses in experimental hosts. This helps researchers to identify genes which are necessary for Leishmania parasite’s survival and/or for their virulence.

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

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

<table>
<thead>
<tr>
<th>Species</th>
<th>Attenuation process</th>
<th>Model</th>
<th>Outcome of immunization</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. tropica, L. major</td>
<td>Long-term in vitro culture</td>
<td>BALB/c and C57BL/6</td>
<td>Persistent low-grade cutaneous disease, BALB/c: partially protection C57BL/6: completely resistant</td>
<td>31</td>
</tr>
<tr>
<td>L. major</td>
<td>Long-term in vitro culture</td>
<td>BALB/c</td>
<td>Protection</td>
<td>36</td>
</tr>
<tr>
<td>L. chagasi</td>
<td>Long-term in vitro culture</td>
<td>BALB/c</td>
<td>No protection</td>
<td>37</td>
</tr>
<tr>
<td>L. amazonensis</td>
<td>Long-term in vitro culture</td>
<td>C57BL/6</td>
<td>Decrease parasite load, increased IFN-γ, smaller lesions</td>
<td>38</td>
</tr>
<tr>
<td>L. braziliensis</td>
<td>Temperature sensitivity</td>
<td>BALB/c</td>
<td>Protection</td>
<td>32</td>
</tr>
<tr>
<td>L. major</td>
<td>Radio attenuated</td>
<td>CBA</td>
<td>Resistance to subsequent infection with L. mexicana</td>
<td>39</td>
</tr>
<tr>
<td>L. major</td>
<td>Gamma irradiation</td>
<td>BALB/c and CBA</td>
<td>Protection against heterologous and homologous challenge</td>
<td>33</td>
</tr>
<tr>
<td>L. infantum</td>
<td>In vitro under gentamicin pressure</td>
<td>Dog</td>
<td>In the endemic area against canine visceral leishmaniasis gentamicin-attenuated L. infantum induced a strong and significant protective effect</td>
<td>40–42</td>
</tr>
<tr>
<td>L. infantum</td>
<td>KHARON1 (KH1) null mutants</td>
<td>BALB/c</td>
<td>∆Likh1-immunized mice presented reduced parasite burden upon challenging with virulent L. infantum, when compared with naïve mice. An effect associated with increased IL-17 production and Li SLA-specific IgG serum levels</td>
<td>43</td>
</tr>
<tr>
<td>L. infantum</td>
<td>HSP70-II genes null mutant</td>
<td>BALB/c and C57BL/6</td>
<td>LiΔHSP70-II attenuated line activates mammalian immune system for inducing moderate proinflammatory responses</td>
<td>44</td>
</tr>
<tr>
<td>L. donovani</td>
<td>LdCen(-/-) centrin deleted</td>
<td>Mice and hamster</td>
<td>LdCen-/- live-attenuated vaccine induced a strong antibody production, a selectively CD4+ and CD8 + T cell activation in addition to prominent type 1 immune response that contributed to a remarkable reduction in bone marrow parasite load, even 24 months post-L. infantum infection</td>
<td>45</td>
</tr>
<tr>
<td>L. donovani</td>
<td>Arabino-1, 4-lactone oxidase (∆ALO) null mutants</td>
<td>BALB/c</td>
<td>Live-attenuated ∆ALO parasites are induce protective immunity, safe, and can provide sustained protection against L. donovani</td>
<td>46</td>
</tr>
<tr>
<td>L. donovani</td>
<td>p27 gene-deleted (Ldp27-/-), Ldp27episomal add-back (Ldp27-/- AB), Centrin1 gene deleted (LdCen-/-), and LdCen episomal add-back (LdCen-/- AB) lines of L. donovani</td>
<td>BALB/c mice</td>
<td>Attenuated parasite-infected mice induced higher IL-2 and IFN-γ but significantly less IL-10 production by ovalbumin-specific CD4+ T cells, resulting in the proliferation of Th1 cells</td>
<td>47</td>
</tr>
<tr>
<td>L. donovani</td>
<td>Centrin1 and p27 genes deleted live-attenuated Leishmania parasites (LdCen1-/- and Ldp27-/-)</td>
<td>Human macrophages</td>
<td>LdCen1-/- and Ldp27-/- strongly stimulated production of proinflammatory cytokines including, IL-12, IFN-γ, TNF-α, IL-2, IL-6, and IL-17 in the PBMcs</td>
<td>48</td>
</tr>
</tbody>
</table>

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

Based on the used attenuation procedure, live-attenuated parasites are divided in two groups: defined and undefined genetically modified (►Table 2). In defined attenuation, by specific mutagenesis, the parasites lost the ability of encoding one or more essential virulence genes. Lazer irradiation or chemical mutagenesis or long-term in vitro culture can produce undefined attenuated parasites. In clinical trial performed in Iran with live vaccine, the researchers used stabilates which were made from stationary phase of para-

Genetically Live-Attenuated Vaccines

Genetically modified vaccines have been developed in recent years and are powerful tools for creating immunity. For achieving a protective vaccine, modifying some of Leishmania genes through deleting virulent or necessary genes seems to be successful. The transfection technology through modifying one or more genes in Leishmania parasite leads to a great affect. The DNA fragments could be transferred through physical methods such as electroporation to the parasite’s nuclei. Antibiotic-resistant genes in a linear structure need to be combined with the genome through homologous recombination for removing a gene. Antibiotic-resistant genes in a linear form should be constructed and need to integrated by homolog recombinating for removing a gene. This leads to transferring the sequence of DNA in genome of Leishmania parasite. To achieve an absolute knockout, there should be another construct to produce antibiotic-resistant gene by Leishmania parasite to set the alleles of the second gene. This manipulation leads to parasites new phenotype and features which hereditary transfer to the next generation. The main concern in gene targeting is the new gene location control as it is important that it should not interfere with genes normal function. Although the genome of Leishmania parasite is easily manipulated, the site of entered gene is important and should be confirmed by molecular genetics assays. Another important issue after gene transfer is the phenotype alterations and need to be studied more. In this regard, one of the famous experiments was performed by Titus et al who decided to vaccinate mice against virulent L. major parasite with dihydrofolate reductase-thymidylate synthase null mutant of the parasite, which they achieved through gene targeting. Unfortunately, it did not show persistent protection affect in susceptible mouse strains and in Rhesus monkeys but revealed the resistant phenotype of L. major parasite. Another attempt on mice and hamster, using mutants of manipulated L. Mexicana which lacked cysteine proteinase genes, showed promising results such as low parasite burden, smaller lesions, and delay in disease onset in animals were observed. These findings show that the protective immune response is achievable through genetic attenuation of live Leishmania parasite. In a study performed by Uzonna et al, they found that very susceptible mice were protected after vaccination with phosphoglycan (PG)-deficient L. major without considerable Th1 response. Experiments with BT1 null mutant of L. donovani in infecting mice in comparison to wild type were promising and the susceptible individuals were able to attain protective immunity against the parasite. Other researchers proved the efficacy of live genetically attenuated Leishmania vaccines in inducing immunity as Silvestre et al demonstrated the production of IFNγ/IL-10 in SIR2-deficient L. infantum that seems to be protective. Other researchers tried to attain an effective vaccine with elimination of all PGs through deleting the lipophosphoglycan (LPG) gene in L. major. This leads to creating a nonpathogen parasite, which obtained life ability at low level for more than 2 years in mice. Thus, immunization with lpg2− parasites in very susceptible BALB/c mice induced protection against virulent L. major parasites. In another research performed by Kedzierski et al, vaccination with a virulent L. major with absence of phosphomannomutase in susceptible BALB/c mice induced a protective response. They declared that increase in Th cells and their role in lymph nodes during the infection and low levels of IL-13 and IL-10 are the main reason of protection against Leishmania parasites. In a 2009 study, it was revealed that by using of centrin gene (LdCen1−/−) disrupted L. donovani parasites were able to protect susceptible BALB/c mice and immune-deficient mice and hamsters against infection with L. braziliensis. Fiuza et al showed that vaccination of dogs with LdCen1−/− vaccine leads to Type 1 polarization and Type 2 inhibition along with production of antibody. It is shown by Dey et al that knockout of protein 27 (Ldp27−/−) in amastigotes of L. donovani can induce protection against other Leishmania species. Mutant of L. infantum which lacks both HSP70-II (DHSP70-II) alleles can protect BALB/c mice of infection with L. major, resulting in production of NO and type 1 immune responses with immunoglobulin G subclass analyses as reported by Carrion et al. New generation of live-attenuated vaccine from L. major strain (LmCen1−/−) for LZ has been explained by Zhang et al as they suggested effective protection against leishmaniasis...
without causing lesion. It seems the gene-free vaccine can be considered for human clinical trials in phase I. Some limitations including concern about probability of reversion to virulent form and using them in immune-deficient people and manufacturing problems make the use of these vaccine more difficult.

**Nonpathogenic Leishmania (Leishmania tarentolae)**

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

This suggests that some of these genes are related to the capacity of pathogenicity in this strain which makes it harmless to human. For instance, the presence of high copy number of amastin family in pathogenic species of Leishmania and presence of just two copies of L. tarentolae and this is the reason of L. tarentolae’s inability for application in mammalian macrophages. A protective immune response against L. donovani in susceptible BALB/c mice has been induced by experimental intraperitoneal administration of L. tarentolae that leads to a conclusion that this was caused through presentation of enhanced antigen and effective response of Th1 cells. By considering the ability of gene manipulation and producing transgenic L. tarentolae which can be immune dominant antigens of Leishmania parasite, it would be considered as an effective vector in antileishmanial vaccines. The plan is using this strain of Leishmania as a special delivering and expressing leishmanial antigens system in host. Also, the A2 protein which is expressed by L. donovani complex and promotes visceralization of parasite is not present in L. tarentolae. Recent studies have demonstrated that the absence of virulence genes including LPC3, CPB, GP63, and amastin is not related to the lack of pathogenicity in L. tarentolae. In a study, administration of A2-recombinant L. tarentolae vaccine was induced protection against L. infantum challenge in BALB/c mice and the protection was associated with production of high levels of IFN-γ before and after challenge. As L. tarentolae is able to induce T-cells-mediated protection, it could be considered as a safe vector for live vaccines against Leishmania parasites and possibly other intracellular pathogens. Also, infection with L. tarentolae can elicit maturation of DC which are responsible for parasite phagostyose. Salehi et al designed and evaluated a live vaccine by using recombinant E7 protein which was expressed by L. tarentolae for protection of mice against human papillomavirus–associated tumors. Interestingly, the vaccine showed the best protection against TC-1-induced tumors. Comparative studies on L. tarentolae can be helpful for understanding the pathogenicity of Leishmania and its relationships with the host.

**Conclusion**

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

**Note**

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**Conflict of Interest**

None declared.

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