Mini Review Article

Current Approaches to Develop a Live Vaccine against *Leishmania major*

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Abstract

Leishmaniasis is an infectious disease that is endemic in 88 countries. Most of the patients after recovery from the infection develop a long-lived natural immunity against re-infection. Reactivation of leishmaniasis subsequent to suppression of the immune system due to HIV infection or administration of systemic immunosuppressive drugs, underscores the importance of developing new drugs and effective vaccine. Despite the many efforts that have been done, there is still no effective vaccine. Up to now, many candidate vaccines from three generations of the vaccine, including Live/killed vaccines, subunit vaccines, and DNA vaccines have been developed and studied. However the sophisticated vaccines, such as prime-boost DNA vaccines are introduced, the best results are obtained from live vaccines. As safety is the most important obstacle to the use of live vaccines, many different approaches have been used to enhance the safety of live vaccine candidates. In this short review, these approaches are summarized.

Keywords: Leishmaniasis, Leishmanization, live-attenuated vaccine

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Introduction

Leishmaniasis is a parasitic disease caused by an obligate protozoan transmitted to hosts such as human, rodents, and canids through the bite of infected female sand fly. More than 50 Leishmania species have been identified, and over 20 leishmania species have been described that are pathogenic for human[1]. Leishmaniasis is endemic in 88 countries. Currently, 350 million people are at the risk of this disease, and 1.5 million new cases are infected annually. Leishmaniasis, schistosomiasis, and hookworm infection impose the highest disease burden among the tropical diseases, and World Health Organization considered them as neglected diseases[2–4]. The spectrum of clinical manifestations of the disease is wide, ranging from simple cutaneous leishmaniasis (CL) and mucosal/mucocutaneous infection to serious visceral leishmaniasis[5,6]. CL is the most common form of leishmaniasis, with lowest mortality rate among all clinical forms, and is responsible for 50–75% of all new cases[2]. Leishmania species that causes cutaneous form of the disease are *Leishmania major*, *L. tropica*, *L. aethiopica*, *L. mexicana*, *L. braziliensis*, *L. guyanensis*, *L. panamensis*, *L. peruviana, and L. amazonensis*[7].

Nowadays, treatment of all clinical forms of leishmaniasis is highly dependent on chemotherapeutic agents, including Pentavalent Antimonials and Amphotericin B[8]. This therapy is

usually effective; however, factors such as side effects, duration of treatment, and high costs are the concerns that make the therapy challeng. Additionally, reports on the emergence of drug resistant strains further highlights the need for an effective vaccine[9]. Reactivation of asymptomatic leishmania infections in immunocompromised patients, especially in HIV infected patients, accelerates the progression of the disease, providing another rationale for the need to develop effective vaccines and drugs[10].

A good understanding of immunity, which is generated against Leishmania, is a key element to develop an efficient vaccine. Resistance to infection depends on cell-mediated immune responses. Previous studies on C57BL/6 mice revealed that a high level production of interferon gamma via T helper1 (Th1) cells promotes macrophages to deviate to M1 classical phenotype, which can promote phagocytosis, and then kill the parasites effectively using NO and other free radicals[11,12]. The generation of immunological memory is a fundamental requirement of effective immunity and vaccination. Results of studies showed that long-lasting immunity against leishmaniasis requires the involvement of both a Th1-mediated immune response and regulatory function of $CD4^+$ $CD25^+$ T cells[13,14].

Strategies to develop vaccine against *Leishmania major*

History of vaccination against leishmaniasis goes back to ancient times, however, no effective vaccine has been introduced[15]. The finding that most individuals who have been infected naturally, and then recovered, develop resistance to later re-infection due to long-lived immunity, supports the notion of the possibility of developing an effective vaccine[16,17]. Inoculation of the live *L.major* in a non-visible part of the skin induces a usually benign infection with spontaneous healing after 6–9 months. This vaccine is called leishmanization and was the first strategy to develop immunity against leishmaniasis. During the 1980s more than two million people in Iran were immunized by leishmanization; this program reduced the incidence of the disease[18]. As mentioned, CL caused by leishmanization, usually produces a self-healing lesion. Although rarely, in some cases, the sore remained for a long time and increased the risk of disseminated leishmaniasis in immunocompromised individuals[19,20]. In addition, due to the increase in the prevalence of HIV infection and growth in the use of immunosuppressive drugs, the risk attributed to leishmanization is increased. Nowadays, leishmanization is no longer used because of serious concerns about its safety and standardization[20]. Although, still is used in Uzbekistan[21,22].

Besides leishmanization, several approaches have been used to develop a safe and effective vaccine against leishmaniasis, including killed vaccines, subunit vaccines, and DNA vaccines[23–25]. Despite the great efforts have been done to develop a vaccine by using these approaches, none of them reached the clinical trials. In recent years, the use of live leishmania has been suggested again. Live vaccines are sufficiently potent to stimulate proper immune responses. Additionally, the results of previous studies showed that to sustain immunological memory, persistence of a small number of live parasites is essential (concomitant immunity)[13,14,26]. Therefore, live vaccine can be considered as the gold standard for protection against leishmaniasis. The problem of non-healing lesions that appeared in some cases followed by leishmanization is the main obstacle in the way of using live parasite as a vaccine. To conquer this problem, different solutions have been employed such as using live attenuated organisms, non-pathogen organism, and live pathogenic Leishmania with an appropriate adjuvant to reduce the pathological effects of live vaccines[27–30] (Figure 1).

The main goal in live vaccine research is the prevention of lesion formation, and at the same time the development of long lasting immunity. There is evidence from a preliminary clinical study in Uzbekistan that killed promastigotes delivered with live vaccine reduced the size and duration of active lesions. Another approach to reach the goal is inoculation of virulent *L. major* with an appropriate adjuvant in order to induce an immune response similar to natural infection, to make the response more rapid and robust, so as to promote earlier control of parasite growth and healing of cutaneous lesions. Mendez S. et al. successfully developed a leishmanization protocol by mixing live *L. major* with CpG-containing immunostimulatory

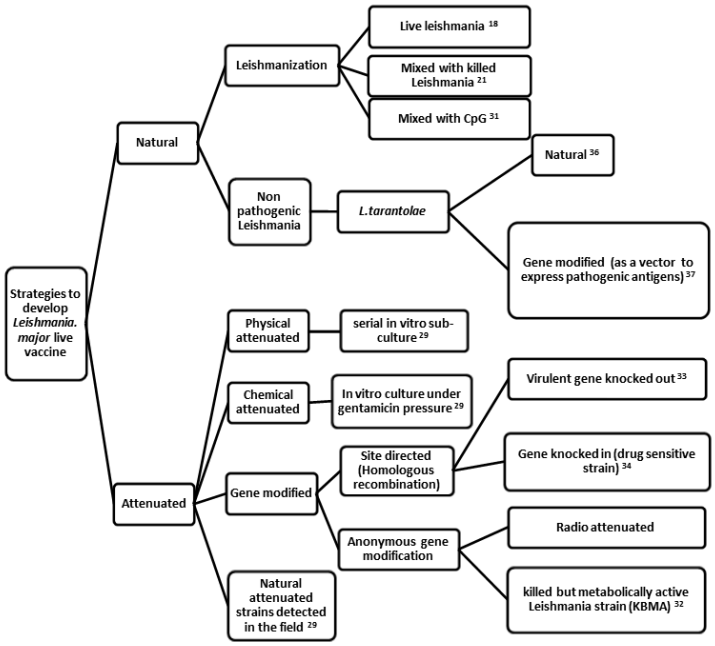

Figure 1. Classification of strategies used in researches to develop *Leishmania. major* live vaccines.

oligodeoxynucleotides to minimizes pathology while maintaining long-term protection in a mouse model[31].

Another method which is used to reduce pathological effects of *L. major* inoculation is attenuation of virulence. Several procedures have been used to develop a live attenuated *Leishmania* vaccine, including chemical mutagenesis, serial *in vitro* cultures, temperature sensitivity, and irradiation. Datta S. et al. used Ultraviolet-A radiation and psoralen compound for production of an attenuated viable Leishmania called killed but metabolically active Leishmania strain (KBMA). Results showed that KBMA leishmania induced strong Th1 responses in susceptible mice and could be considered as candidate vaccine[32].

Targeted gene disruption of both alleles have been used previously to produce live attenuated *L. major*. The first *L. major* modified gene was a dihydrofolate reductase thymidylate synthase gene, gave disappointing results during further studies in monkeys[33]. Although, safety concerns regarding conversion back to virulence form make them questionable for human use. Another approach is the addition of the suicide cassettes to the genome of parasites that could provide suitable candidates for leishmanization, to guarantee an effective treatment of non-resolving lesions. The suicide cassette is translated in response to external stimuli, and kills the parasite. An example of this method is the gene modified *L. major*, which is sensitive to 5-fluorocytosine or Ganciclovir[34]. Despite good results in animal studies, no such formulation has reached even in pre-clinical stage as yet.

In spite of the investigations aimed at artificially attenuated wild *Leishmania* spp., some researchers focused on live natural non-pathogenic *Leishmania* strains. Breton M *et al.* recently introduced lizard protozoan parasite *L. tarentolae*, that is nonpathogenic to human, as a vaccine candidate against leishmaniasis[35]. It should be highlighted that *L. tarentolae* infection is cleared rapidly, but cannot induce the "concomitant immunity". Recently, a group of researchers have begun efforts to use *L. tarentolae* in a novel live-vaccination strategy. In this regard, Zahedifard F et al. immunized BALB/c mice with a live recombinant *L. tarentolae* expressing sand fly saliva antigens together with two types of cysteine proteinases from *L. major*. The results of this study showed that vaccination induced a strong parasites specific T_H1 response and conferred protection against *L. major* infection[36]. It should be considered that *L. tarentolae* disappears quickly by macrophages[35], therefore, use of agents that increase the asymptomatic persistent infections of this parasites, may promote the immunological memory

formation. Additionally, utilization of other leishmania spp. with the lower pathogenicity and also higher persistency in macrophages, may increase the efficiency of vaccination.

Conclusion

Over the years, great efforts have been done, and many advances have been achieved, nonetheless, there is no vaccine to insure sufficient level of protection against leishmania infection yet. However, properties of a successful vaccine against CL are known. Effective CL vaccine should primes a robust parasite-specific TH1-cell responses to induce an appropriate phagocytic function, as well as, induction of adequate specific regulatory response to support long-lasting memory T cell formation, thise property have not been included in the attempts to produce leishmaniasis vaccine before. In addition, a successful vaccine should be safe. It is important to consider that live attenuated parasites may return to virulence form; hence, non-pathogenic leishmania-based vaccines are powerful tools to conquer Leishmania infection. Moreover, main problems associated with the production of live vaccines, including standards and quality control - which existed from the beginning - must be considered too.

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Conflict of Interest

The authors further declare that, they have no conflict of interest.

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