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Chapter 6

Vaccines for Visceral Leishmaniasis: Hopes and Hurdles

Eltahir Awad Gasim Khalil

Additional information is available at the end of the chapter

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Abstract

The leishmaniases are vector-borne parasitic diseases with multiple disease phenotypes that range from self-healing cutaneous ulcers to disfiguring post-kala-azar dermal leishmaniasis and fatal visceral leishmaniasis (VL). Infected individuals can develop subclinical infections or overt disease. Current treatments are toxic and expensive. The only successful control measure is case detection and drug treatment. Resistance to anti-leishmanial drugs are increasing with few drugs in the pipeline. The *Leishmania* parasites are good candidates for vaccine development, with no change in its antigenic coat and extensive cross-reactivity between species. First-generation vaccines are safe, immunogenic with inconclusive efficiency. These vaccines presented the leishmanin skin test (LST) as a potentially good surrogate marker of immunogenicity/protection that can help in future vaccine studies. First-generation vaccines are the only leishmaniasis vaccines that progressed to phase III. Second-generation vaccines are safe and immunogenic, but none progressed to phase III. Third-generation vaccines recently entered human testing. Alternative approaches include in silico prediction of immunogenic *Leishmania* epitopes with *in vitro* immunogenicity testing. New adjuvants can help in the quest to develop efficacious leishmaniasis vaccines. Failure of second- and third-generation vaccines to reach phase III, rising drug resistance and continued VL pandemics make it a necessity to revisit first-generation vaccines.

Keywords: visceral leishmaniasis, first/second/third-generation vaccines, adjuvants

1. Introduction

The leishmaniases are vector-borne, widely prevalent parasitic diseases that are transmitted by phlebotomine sand flies. The transmission is either zoonotic or anthroponotic. Together with malaria they constitute the most commonly prevalent neglected parasitic diseases. The leishmaniases are among the most commonly neglected tropical diseases. The parasite is a

unicellular organism that leads to multiple disease phenotypes that range from benign self-healing cutaneous ulcers to a markedly disfiguring diffuse cutaneous/post-kala-azar dermal leishmaniasis and fatal visceral leishmaniasis (VL, kala-azar). Cutaneous leishmaniasis (CL) is caused by *L. tropica*, *L. aethiopica*, and *L. major* in the Old World and *L. mexicana*, *L. guyanensis*, *L. amazonensis*, and *L. braziliensis* in the New World. Visceral leishmaniasis is a fatal form of the leishmaniasis if not treated. VL is a major health problem and is caused by *L. donovani* and *L. infantum* that are particularity prevalent in East Africa, the Indian subcontinent, Mediterranean Basin, and Latin America [1–9]. The HIV pandemic aggravated further the leishmaniasis morbidity and mortality. Drug treatment with sodium stibogluconate/paromomycin, miltefosine, or liposomal amphotericin B is expensive and carries major risks of toxicities. Current control measures that include case detection, drug treatment, and insecticide-impregnated bed nets are failing as evidenced by repeated epidemics especially in East Africa. In addition, increasing drug resistance and geographical expansion of the leishmaniases due to global warming and wars make the search for vaccines for the leishmaniases a necessity [2, 10–15].

1.1. Immunity against visceral leishmaniasis

Visceral leishmaniasis is characterized by immune suppression manifesting as pancytopenia and anergy to some antigens like *Leishmania* antigens and purified protein derivative (PPD). Following successful drug treatment, a state of immune reconstitution ensues which is characterized by a dermatosis affecting most Sudanese patients known as post-kala-azar dermal leishmaniasis (PKDL). Macrophages, $CD4^+$ T cells, $CD8^+$, NK cells, and dendritic cells are known to be involved in the immune responses against *Leishmania* infections. Infection with *L. donovani* parasite can follow two different scenarios: susceptible individuals develop overt disease with dissemination of *Leishmania* parasites through infected macrophages with secretion of IL-4 and IL-10, and nonspecific stimulation of B cells and secretion of large amounts of antileishmanial antibodies [Th_2 immune response]. Alternatively, individuals can develop protective immune responses [subclinical infection] with secretion of parasite antigen-specific IFN-γ, TNF-α by stimulated $CD4^+$ T cells [Th_1 immune response], and conversion in the leishmanin skin test (LST). Eliciting an exact immune response is an important VL vaccine requirement that should simulate those induced by natural infection. An important feature of an efficacious *Leishmania* vaccine should be to induce a parasite-specific Th_1 immune response with sufficient amounts of IFN-γ and LST reactivity that should last for life. Induction of antileishmanial antibodies by a vaccine should be taken against it, taking into consideration that patients with overt diseases secrete large amounts of non-neutralizing antibodies. These antibodies have been shown to facilitate the internalization of the *Leishmania* parasites into macrophages [2, 16–19].

1.2. Feasibility of vaccines for the leishmaniases

A vaccine against the *Leishmania* parasite is a real feasibility, because unlike the plasmodium and other parasites, *Leishmania* rarely changes its antigenic coat. *Leishmania* infections induce lifelong immunity with extensive cross-reactivity between different species of leishmania. Therefore, a single vaccine can be potentially effective against many forms of the leishmaniases.

Although the exchange of genetic material between distant *Leishmania* strains [*L. major* and *L. donovani*] has recently been raised, this may have some implications for drug treatment, but not leishmaniasis vaccine development [20, 21].

1.3. Vaccine biomarkers of immunogenicity, susceptibility, and protection

The ability of vaccines to induce antibody production, Th_1 (IFN-γ) or Th_2 (IL-4, IL-10) immune response, can be objectively measured for phase II studies. Based on published data, we believe that the leishmanin skin test (LST) can be used as a surrogate marker for induction of cell-mediated immunity/protection against visceral leishmaniasis in phase II/III studies [22–25].

1.4. The leishmanin skin test (LST)

LST is an in vivo skin test that marks *Leishmania* antigen-specific T-cell responses. The brand of LST reagent used in East Africa is an *L. major* suspension that is manufactured under GMP conditions in Pasteur Institute, Iran. The LST has been shown to be a potentially good diagnostic aide for the diagnosis of African visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL) in all age groups in endemic areas. LST reactivity indicates sustained cell-mediated immunity, which is nonreactive in patients with VL and becomes reactive 6 months after cure. In addition, LST reactivity is a lifelong phenomenon [2, 11, 26]. Individuals with LST reactivity do not develop VL as was shown in a two decades follow-up period among the large numbers of LST reactive individuals in VL endemic areas in Sudan [2, 27] (Khalil et al., personal communication). Evaluation of LST reactivity in endemic areas in East Africa and India as reported previously included small sample size and did not specify the duration between cure and LST testing. Bern et al. [28] in Bangladesh demonstrated that the frequency of LST reactivity increased with increasing duration following cure using *L. infantum* antigen. The Bangladesh study mentioned loss of LST reactivity, but did not show any data about population movement that we specifically look at when evaluating LST reactivity from year to year. The questions of LST standardization, sensitivity, potency, stability of the *Leishmania* antigens, and longevity of the skin reaction were addressed satisfactory by Weigle and colleagues in 1991 [29]. Combination of different *Leishmania* strains can markedly improve the specificity of the LST as was shown previously [28–32]. In conclusion, different *Leishmania* strains in the LST reagent, the inadequate technique (subcutaneous rather than intradermal injection), and the time of test reading can greatly affect the outcome and interpretation of results of LST. LST is a potentially good surrogate marker of immunogenicity/protection that can be useful in future VL vaccine studies.

2. Leishmanization

Leishmanization is a true predecessor of leishmaniasis vaccines; the procedure was practiced in Central Asia and the Middle East for times deep in history. Although leishmanization is still practiced in some areas, it is considered unsafe and cannot be standardized. Recently, it

has been used as a method of evaluation for candidate vaccines [33]. Leishmanization gave way to killed or live-attenuated first-generation *Leishmania* vaccines. Leishmanization like first-generation and third-generation vaccines that use genetically modified *Leishmania* parasites or use bacteria and viruses that carry *Leishmania* genes is daunted by the issue of standardization [34].

3. First-generation vaccines

First-generation vaccines as whole parasite killed/attenuated were tested in animals and humans for cutaneous and visceral leishmaniases. Human studies have to be commended despite raised points of standardization and licensure purposes. First-generation vaccines are less costly and easy to manufacture. In addition, first-generation vaccines are the only human prophylactic VL vaccines that went on to phase III. Khalil and colleagues conducted the first human phase III VL vaccine study that was followed by a number of extended phase II studies on vaccines against visceral leishmaniasis [27]. Although the vaccine was not efficaciously different from BCG, important conclusions came out of this study: firstly, the leishmanin skin test (LST) is a first potentially good surrogate marker for immunogenicity/protection in humans. Secondly, modulation of whole parasite vaccines with strong adjuvants like alum markedly improved the immunogenicity of whole parasite vaccine as shown in phase II/extended phase II studies. Lack of funds under the pretense of poor standardization and lack of licensure potentials prevented progression of alum-precipitated *Leishmania* vaccines to phase III [22–25, 35–37].

The future of VL control is bleak based on frequent VL pandemics that kill thousands of people in developing countries, increasing drug resistance, lack of new antileishmanial drugs in the pipeline, and failure of second-generation vaccines to make it to phase III. In view of all the above and the current regulations that prohibit the wide use of whole parasites/antigen vaccines, standardization of whole parasite/antigens has to be addressed objectively.

Important points have to be highlighted when revisiting first-generation vaccines: the *Vaccinia* [smallpox] vacine which is the first vaccine that helped to eradicate small pox has been a whole virus. Furthermore, the control and near elimination of poliomyelitis is successful due to the blessing of an attenuated whole virus. Since the above vaccines are considered fit for human use, whole parasite vaccines have to be given a similar standing especially in the era of existing strong adjuvants. Furthermore, the success of immunochemotherapy of post-kala-azar dermal leishmaniasis using alum-precipitated autoclaved *L. major* vaccine further supports giving a second chance for first-generation VL vaccines. The inconclusive results that were obtained from first-generation vaccine meta-analysis and put it into disrepute are probably due to the fact that the analysis included studies for cutaneous as well as visceral diseases in the same basket. It has to be clearly stated that these disease phenotypes are different with different immune responses and different endpoints of evaluation of efficacy [12, 13, 23, 25, 27, 35, 36, 38–41].

4. Second-generation vaccines

Second-generation vaccines are recombinant *Leishmania* antigens (single peptides/polypeptides) that are highly purified, amenable to standardization/large-scale production, reproducibility, and cost-effective production. Safety and immunogenicity have been assured in phase I and II studies. But, it is clear that strong adjuvants are needed for these subunit vaccines to be satisfactorily immunogenic. Recently, our group tried an alternative cheaper way where an in silico approach was employed to predict immunogenic epitopes/peptides of *Leishmania* parasite antigenic coat. The predicted peptides were manufactured commercially and tested in an in vitro whole blood system and were shown to be immunogenic [IFN-γ production; no IL-10 production]. It was concluded that these peptides can be taken further for prophylactic leishmanin vaccine development. Further studies are underway to combine these peptides with known and potential adjuvants to increase their immunogenicity [42–49]. In conclusion, second-generation VL vaccines will succeed when the mechanisms by which macrophages select the most suitable epitopes to induce the appropriate immune response are known.

5. Third-generation vaccines

DNA vaccines came into existence with advances in molecular biology and biotechnology, and the injection of small circle of DNA encoding potentially immunogenic proteins became a reality [50]. A number of experimental third-generation vaccines have been studied with demonstrated immunogenicity and healing abilities. The first human study for a third-generation therapeutic vaccine for visceral leishmaniasis and PKDL was carried out on healthy volunteers in the United Kingdom using CHAd63-KH vaccine. The CHAd63-KH vaccine is a replication-defective simian adenovirus expressing a novel synthetic gene (KH) encoding two *Leishmania* proteins KMP-11 and HASPB. The vaccine was shown to be safe and immunogenic [51–53].

6. Adjuvants for *Leishmania* vaccines

A plethora of adjuvants, live organisms (BCG), cytokines, oligonucleotide (CpG), minerals and particulate lipids, and polymer-based adjuvants are under investigations for *Leishmania* vaccine. Although there is an urgent need for studies on adjuvants in disease-endemic areas, access to potent adjuvants is the main hurdle for investigators and researchers in leishmaniasis-endemic countries [54–61].

7. Conclusion

Failure of second- and third-generation vaccines to reach phase III, rising drug resistance, and continued devastating VL pandemics make it a necessity to study further first-generation vaccines.

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