Progress towards a *Leishmania* vaccine

*Khaled S. Tabbara, PhD.*

**ABSTRACT**

Leishmaniasis is a vector-born protozoan disease. Approximately 12 million individuals are affected worldwide with an estimated annual incidence of 1.5-2 million. Two clinical manifestations are recognized, cutaneous, and visceral, both of which are common in the Middle East. In both forms, infection is chronic, with potential deformities, persistence following cure, and lifelong risk of reactivation. Attempts to develop an effective human *Leishmania* vaccine have not yet succeeded. Leishmanization, a crude form of live vaccination historically originated in this part of the world. Experimental vaccination has been extensively studied in model animals in the past 2 decades. In this review, major human killed vaccine trials are surveyed, and modern trends in *Leishmania* vaccine development, including subunit vaccines, naked DNA vaccines, and transmission blocking vaccines are explored. Recent findings of a link between persistence of live parasites, and maintenance of long-term immunity suggest live vaccination with attenuated strains, as a future vaccination strategy.

Saudi Med J 2006; Vol. 27 (7): 942-950

Vaccination is the single most effective measure for the control and prevention of infectious diseases. The majority of epidemic contagious bacterial, and viral infections have been controlled in the developed world during the second half of the twentieth century, mainly through mass vaccination, to the point where smallpox has been totally eradicated as a human disease, and polio is on its way to extinction through the efforts of the World Health Organization (WHO). Innumerable vaccines are available today for the prevention of various bacterial and viral diseases. Yet, despite this success, science has not yet been able to develop a single effective human vaccine against any of the parasitic diseases. Despite the time, research effort, and funds dedicated, the drive for the development of vaccines against parasitic diseases has been wrought with disappointment. Diseases such as malaria, leishmaniasis, and schistosomiasis continue to affect millions of people in developing, and underdeveloped countries resulting in significant mortality, and morbidity.

**Leishmaniasis.** Leishmaniasis is a vector-born protozoan disease, second to malaria in its prevalence. The WHO estimates approximately 12 million affected individuals with an estimated annual incidence of 1.5-2 million, among a susceptible population of approximately 350 million in 88 different countries on 5 continents. The parasite is transmitted by phlebotomine sand flies of the genera *Phlebotomus* and *Lutzomyia* in a zoonotic (from the affected animals- dogs and rodents) or anthroponotic fashion (from the other humans, as is the case in parts of India, Afghanistan and Iran).

The life cycle of this kinetoplastid parasite involves alternating life forms in the vector, and the mammalian host. The vector takes up the amastigote (nonmotile form of the parasite) from the host during...
Leishmaniasis, in both the Old and New worlds, are prevalent along with its cutaneous and visceral forms, is prevalent in our area of the world, including Gulf Cooperative Council countries, Yemen, Iraq, Iran, and the Eastern Mediterranean. Although, no local transmission is noted in Bahrain, one case of suspected local transmission was reported in 1982. A number of imported cases are seen yearly in Bahrain. In Saudi Arabia both cutaneous [Leishmania major (L. major), L. tropica], and visceral [L. donovani and L. infantum] leishmaniasis are reported. Cutaneous leishmaniasis is prevalent throughout Saudi Arabia, while visceral disease is mainly localized to the southwestern region. In the southwest, annual incidence rates of cutaneous leishmaniasis range between 12 and 38 cases while those of visceral are in the order of 6-8 cases per 10,000. Seropositivity among dogs, the main reservoir of L. infantum in the southwest is reported to be 19.3% while rodents, with infection rates reaching 93% are the main reservoir for cutaneous species prevalent in the eastern region. The same 3 species prevail in Iraq and Iran while in Yemen 2 species from the Donovania complex, namely, L. donovani and L. infantum are prevalent along with the cutaneous L. tropica, while Oman reported cases of cutaneous L. tropica and visceral L. infantum. A single report of visceral leishmaniasis, by an unidentified species, was reported in Dubai. In the Eastern Mediterranean countries of Lebanon and Syria, cutaneous species belong mainly to L. tropica with minor cases of L. major while L. infantum is the major visceral species. In Jordan, L. major is hyperendemic along the Jordan Valley and Wadi Araba, while L. tropica is endemic in the Mediterranean influenced north. Rodents, such as Psammomys obesus, are the main reservoir for L. major and dogs for L. infantum in that geographical region. Several cases of cutaneous and viscerotrophic (L. tropica) leishmaniasis have been reported in American servicemen involved in desert storm.

### Table 1 - Major species of *Leishmania* causing human disease and their geographic distribution.

<table>
<thead>
<tr>
<th>Species</th>
<th>Region</th>
<th>Distribution</th>
<th>Manifestation</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Leishmania major</em></td>
<td>Old world</td>
<td>Middle East, Africa, Asia</td>
<td>Cutaneous</td>
</tr>
<tr>
<td><em>Leishmania tropica</em></td>
<td>Old world</td>
<td>Mediterranean basin</td>
<td>Cutaneous</td>
</tr>
<tr>
<td><em>Leishmania aethiopica</em></td>
<td>Old world</td>
<td>Ethiopia and Kenya</td>
<td>Cutaneous/Mucocutaneous</td>
</tr>
<tr>
<td><em>Leishmania infantanum</em></td>
<td>Old world</td>
<td>Mediterranean basin</td>
<td>Visceral</td>
</tr>
<tr>
<td><em>Leishmania donovani</em></td>
<td>Old world</td>
<td>Africa, India, Arabian Peninsula</td>
<td>Visceral</td>
</tr>
<tr>
<td><em>Leishmania amazonensis</em></td>
<td>New world</td>
<td>North, Central and South America</td>
<td>Cutaneous</td>
</tr>
<tr>
<td><em>Leishmania mexicana</em></td>
<td>New world</td>
<td>South America</td>
<td>Cutaneous</td>
</tr>
<tr>
<td><em>Leishmania braziliensis</em></td>
<td>New world</td>
<td>South America</td>
<td>Cutaneous/Mucocutaneous</td>
</tr>
<tr>
<td><em>Leishmania chagasi</em></td>
<td>New world</td>
<td>Central and South America</td>
<td>Visceral</td>
</tr>
</tbody>
</table>

Two primary clinical manifestations are recognized in leishmaniasis, cutaneous, and visceral. Nine major species of *Leishmania* involved in human disease are grouped into old world and new world species (Table 1). Cutaneous leishmaniasis, the milder form of the disease, is by far the most prevalent, while visceral leishmaniasis is associated with a high mortality rate approaching 90% in untreated cases. In both forms of disease, the infection is chronic, with potential deformities, persistence following cure, and lifelong risk of reactivation. Reactivation of disease takes several forms. These include destructive mucocutaneous leishmaniasis (espundia), prevalent in South America, which develops months or years following healing of new world cutaneous leishmaniasis, *Leishmania* recidivans, the reactivation of a localized dormant skin lesion, and post-kalaazar dermal leishmaniasis following cure of visceral leishmaniasis. An increase in the incidence of visceral, and cutaneous leishmaniasis has been noted in recent years. This trend is associated with the rise of the HIV pandemic, increased travel to endemic areas, and as a function of massive relocation of refugees to camps as a result of wars, such as in Afghanistan and Iraq.
Historical background of vaccine development. Due to the mild consequences of the cutaneous form of the disease, vaccination trials, in general, have mainly centered on the use of cutaneous species for vaccination against both cutaneous and visceral disease. Leishmanization, historically the earliest form of vaccination practiced by Arab Bedouins, consisted of controlled infection by the introduction of exudates from cutaneous lesions, in a “Jennerian” approach, using a sharp thorn, at discrete sites of the body, to avoid the development of deforming scars at visible sites in young girls.

The successful in vitro cultivation of *Leishmania* in 1908 paved the way for scientific vaccination trials using cultured promastigotes. Vaccination trials were undertaken by various groups in the years that followed including 2 large scale trials in the Soviet Union and Israel. However, observed ill-consequences in a percentage of vaccinated individuals, including development of uncontrolled lesions, exacerbation of psoriasis, and other skin diseases, and diminished responses to the triple diphtheria-pertussis-tetanus vaccine, lead to the abandonment of live-vaccination efforts.

Killed vaccines, similar to many bacterial and viral agents, would appear to be the natural alternate route. However, early trials in the 1940s were abandoned following conflicting results. Interest in experimental killed vaccines shifted in the 1980s to the use of the mouse model. Early studies showed excellent protection using irradiated promastigotes, however, only intravenous and intraperitoneal, but not subcutaneous vaccination was protective. Regardless, large scale human trials were conducted in Iran, and South America using subcutaneously injected killed vaccines. The recognition of the importance of cell-mediated immunity in protection prompted the incorporation of bacille Calmette-Guérin (BCG) as an adjuvant, and delayed type hypersensitivity (DTH) responses, using leishmanial antigens, as a surrogate marker of protection (Leishmanin or Montenegro test).

Protective immunity against *Leishmania*. Following the definition in the mid 1980s of 2 functionally distinct subpopulations of T helper cells, T helper1 (Th1) and T helper2 (Th2), experimental *L. major* (the causative agent of old-world cutaneous leishmaniasis) infection in the mouse gained prominence as the model for the study of the interplay of Th1/Th2 in protection against infection. Inbred mouse strains are divided into 2 categories based on their resistance or susceptibility to experimental *L. major* infection. Resistance against *L. major* was shown to be dependent on the development of a protective Th1 response, while susceptible strains develop a skewed Th2 response. The mechanisms of, and the factors controlling, the polarization of T helper responses was worked out utilizing this *L. major* animal model.

Helper T lymphocyte subpopulations (Th1/Th2) are defined based on the cytokines produced, and the type of responses associated with each subpopulation. The Th1 responses are associated with the production of proinflammatory cytokines [interleukin (IL)-2, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ], and mediate DTH, while Th2 responses are associated with the production of anti-inflammatory cytokines (IL-4, IL-5, IL-6 and IL-10) and result in the production of immunoglobulin E class antibodies and eosinophilia. Cross-regulation is an additional feature of T helper responses, where IFN-γ produced by Th1 cells down-regulates the development of Th2 responses while IL-4 exerts a similar effect on the Th1 response. The mechanism of resistance is dependent on IFN-γ-activation of infected macrophages resulting in the upregulation of inducible nitric oxide synthase leading to the intracellular production of nitric oxide radicals, which are toxic to the intracellular amastigotes.

Human vaccine trials. Killed *Leishmania* vaccine is the only type of vaccine used in human vaccination trials. Clinical trials, in various stages of phase I (safety), II (reactivity), and III (efficacy), have been conducted in 3 main geographic areas, namely, Sudan, Iran, and South America. In Sudan, the second main focus of old world visceral leishmaniasis following India, phase I, II, and III clinical trials were conducted using autoclaved *L. major* (ALM) with adjuvants, including BCG and aluminum hydroxide (Alum). Side effects were minimal and localized to the site of infection. A number of individuals converted to leishmanin skin test (LST) reactive, and lymphocytes from a number of vaccinated volunteers produced INF-γ in response to *L. major* proteins. The addition of BCG to the vaccine was shown to significantly increase the LST conversion rate, in comparison with ALM alone, and LST converted individuals were significantly protected against visceral leishmaniasis, however, the overall efficacy of the vaccine was only 6%.

Similarly, trials were undertaken in Iran on ALM vaccine with BCG, using single or multiple injections. Two large single dose protection trials included approximately 6000 subjects. In the first study of schoolchildren, no difference was observed in the 2-year incidence of anthroponotic cutaneous leishmaniasis between vaccinated (ALM+BCG), and...
control (BCG alone) groups, however, a sex-stratified analysis showed a protective efficacy in boys of 18% and 78% for the first and second years following vaccination. In the second study, cumulative 2-year incidence rates were similar in both control, and vaccinated groups, however, within 80 days of vaccination LST converters had a significantly lower incidence rate. A study of immune responses in volunteers receiving a single dose ALM plus BCG vaccine compared with responses of individuals recovered from clinical cutaneous leishmaniasis demonstrated that natural infection conferred far superior in vitro proliferative and IFN-γ production responses as compared with vaccination. It was apparent that single dose injection was safe, but not effective. Multiple injections of ALM plus BCG in a phase II trial showed no ill effects from multiple BCG vaccination. While the addition of BCG to boosters significantly increased the frequency, and magnitude of LST, it did not increase the proliferative or IFN-γ responses.

In the new world, human vaccination trials were conducted in Brazil, Columbia, Ecuador, and Venezuela. Strains used for vaccination included *L. amazonensis*, *L. braziliensis*, or a cocktail combination of *L. amazonensis*, *L. guyanensis*, and *L. braziliensis*. Vaccination trials were conducted to asses immunogenicity and protection, or therapeutic potential in infected individuals. Phase I/II trials with multiple autoclaved *L. amazonensis* vaccine injected intramuscularly without BCG were shown to be safe, resulted in significant LST conversions and IFN-γ production one year following vaccination compared with the placebo group.

Multiple intradermal vaccinations with *L. amazonensis* plus BCG resulted in a significantly higher rate of LST conversion, but a positive LST did not correlate with protection as the vaccinated subjects had a similar rate of infection to those receiving placebo. The use of 2 intradermal injections of a cocktail of 3 strains (*L. braziliensis*, *L. guyanensis*, and *L. amazonensis*) plus BCG offered better protection against cutaneous leishmaniasis with an efficacy of 72.9%, and children who received the vaccine had one-fourth the risk of developing the disease.

The therapeutic use of *Leishmania* vaccines has shown great promise in simple cutaneous, severe mucocutaneous, and diffuse forms of American cutaneous leishmaniasis. Multiple injections of a killed *L. amazonensis* promastigote vaccine with half the normal dose of meglumine antimonite cured 100% of 47 patients with simple cutaneous leishmaniasis as compared with 8.2% of the placebo group receiving the same dose of meglumine without the vaccine.

In another study with a small number of patients with severe mucocutaneous, and diffuse forms of American cutaneous leishmaniasis, multiple intradermal injections of pasteurized *L. braziliensis* plus viable BCG achieved complete recovery in all patients following 9-10 monthly injections. Despite this curative success, protective vaccination of the susceptible 350 million individuals resident in endemic regions remains elusive.

**Modern trends in Leishmania vaccine development.** The advent of molecular technology in the past 20 years has influenced trends of vaccine development, including that against *Leishmania*. Gene cloning has made it possible to produce larger quantities of pure proteins in vitro, without the need for tedious isolation. Thus, vaccine production moved into an era of subunit vaccines instead of the use of whole organisms. In addition, the success of the introduction of small DNA sequences coding for specific proteins as a means of correcting defective genes opened up possibilities for the use of DNA vaccines coding for critical immunogenic components of microorganisms as an alternate approach to the direct introduction of microbial proteins. Other, more recent, strategic approaches in the development of vaccines against vector-transmitted infections include the development of transmission-blocking vaccines. Our deeper understanding of the mechanisms underlying pathogenesis of disease, and the requirements for attaining protective immunity increased the search for more effective adjuvants, which induce the appropriate set of cytokines to direct the immune response towards protective mechanisms.

**Subunit vaccines.** The single guiding principle for an effective subunit vaccine is the identification of parasite proteins with T cell epitopes that stimulate IFN-γ producing Th1 lymphocytes. Protective immunity against a parasite with alternating life forms, and hundreds of proteins, however, poses a complicated situation, where protective immunity cannot be reduced to a single epitope or protein. It is more likely that a group of epitopes from a various proteins expressed in the *Leishmania* promastigotes, and amastigotes are collectively responsible for protective immunity.

Recent vaccines under consideration, referred to as second-generation vaccines, are recombinant proteins produced by DNA cloning. Some are life cycle stage specific, while others may be shared between promastigotes and amastigotes, or may be conserved across species. These include the membrane-bound protease gp63, promastigote surface antigen (PSA)-2, and the leishmanial Eukaryotic ribosomal protein
**Leishmania initiation factor (LeIF),**\(^76\) and **Leishmania** (homologue of the receptor for ) activated C kinase (LACK), the **Leishmania** homologue of the receptor for activated C kinase. Others are nonproteins such as **Leishmania** lipophosphoglycan. These subunit vaccines are at their early stages of development, and have been used exclusively in experimental mouse models and in monkeys,\(^77\) either singly or in a cocktail containing several proteins.\(^78\) In the human context, these subunit vaccines have been useful to identify potentially protective molecules using in vitro stimulation assays of lymphocytes from immune individuals recovered from clinical leishmaniasis.\(^79\)

**Naked DNA vaccines.** Immunization with naked DNA (third generation vaccines) is a new approach to vaccination that has potentially far-reaching effects in the field of vaccination, not just parasitic, but all infectious diseases.\(^80\) In this approach, genes encoding the target proteins are cloned into a mammalian expression vector, and the DNA is directly injected intradermally or intramuscularly.\(^81\) Plasmid DNA is taken up by cells, where it is transcribed into mRNA and translated into their respective proteins. The attractiveness of DNA vaccination lies in the stability of DNA in comparison with proteins, the relative ease of its production and purification, and its long-term survival in the host with continued production of the relevant proteins over a long period. DNA vaccines against several infectious diseases are in clinical trials.\(^80\),\(^82\) Experimental animal vaccination with DNA encoding leishmanial gp63, LACK and PSA-2 were shown to be protective in susceptible mouse strains.\(^83\),\(^84\) However, clinical trials have not yet commenced.

**Transmission blocking vaccines.** Sand flies do not merely mechanically transmit the leishmanial promastigotes when they bite their victims. The sand fly may actually play a role in influencing the course of infection by modulating the immune responses of the host to the transmitted promastigotes. Titus and Ribeiro\(^86\) demonstrated that sand fly salivary gland extracts increased infectivity in mice. Thus, if components of sand fly saliva contributed to infectivity, it would be possible to protect against infection by stimulating a response against these components. Kamhawi et al\(^87\) showed the potential of such an approach by demonstrating protection of mice against sand fly transmitted infection following prior exposure of mice to bites of uninfected sand flies. Valenzuela et al\(^88\) later identified sand fly salivary proteins, which can potentially be used to induce an immune response limiting the infectivity of **Leishmania**. One protein, termed SP15, induced a strong DTH response and protection against leishmaniasis in the absence of antibodies. Thus, the potential for such vaccines is real, and is being further pursued as an alternate strategy for protection against naturally transmitted leishmaniases.

**Role of adjuvants in vaccines.** Adjuvants are “helpers” for immune reactivity which are used to modulate the immune response in quality or amplitude.\(^90\) In protective immunity against **Leishmania**, the objective is to induce a strong Th1-mediated DTH response associated with the production of IFN-\(\gamma\). Although the mechanisms of action of adjuvants have remained mysterious, we now have a better understanding of the mechanism of immune initiation involving “professional” antigen presenting cells (APC), namely, the dendritic cells. The activation of these APC, thus is a prerequisite for an effective immune response.\(^90\) The ability of bacteria and some of their products to stimulate the immune response have been exploited as effective adjuvants. Among these, BCG is used as an adjuvant in a variety of situations requiring a robust immune response, and has been extensively exploited in human vaccines against leishmaniasis.\(^90\) Newer strategies under investigation in experimental situations are the incorporation of type 1 cytokine genes including IL-12,\(^91\) IFN-\(\gamma\),\(^92\) and TNF-\(\alpha\),\(^93\) into bacterial vehicles, used as adjuvants. Certain bacterial DNA sequences containing unmethylated dinucleotides (CpG motifs) have been shown to activate B lymphocytes and dendritic cells, to stimulate the production of type 1 cytokines by macrophages, and to be effective adjuvants for experimental **Leishmania** vaccines in the mouse.\(^94\) Unfortunately, currently identified CpGs, however, are not as effective in the human context,\(^95\) and time might be required to identify motifs, which are stimulatory for humans. An old adjuvant under trial in human vaccines against leishmaniases is Alum. Alum is routinely used in many human protein vaccine preparations against a variety of infectious diseases.\(^96\) A newer adjuvant under consideration, a derivative from *Salmonella* lipopolysaccharide cell wall, monophosphoryl lipid A, is safe and stimulates a durable Th1 response.\(^97\) Other bacteria and bacterial products have also been used as experimental adjuvants with variable effects.\(^98\)-\(^100\)

In conclusion, the quest for protective vaccines against parasitic diseases continues in many laboratories around the world, foremost among which are those against malaria, leishmaniasis, and schistosomiasis. Protection following natural disease attests to the viability of vaccination for protection. However, the fact that vaccines against parasitic diseases have remained elusive in comparison with the readily achievable vaccines against viral and bacterial diseases must point to a fundamental
difference between them. In the case of *Leishmania*, recent experimental evidence points to the importance of the persistence of a small number of viable organisms following recovery from disease for the preservation of long-lasting immunity (concomitant immunity).\(^{101}\) This persistence is under the control of regulatory CD4+ CD25+ T lymphocytes.\(^{102}\) Human clinical trials have pointed to the failure of killed vaccines in achieving efficacious protection, as compared with recovery from clinical disease. It appears that controlled infection (leishmanization) might be the only efficacious method of vaccination. However, the risk of reactivation, particularly at this age with the HIV pandemic, and the fact that areas of prevalence of leishmaniasis coincide with hot HIV foci, precludes the mass use of live vaccination. Instead, it appears that ethical considerations, and international licensing would favor the use of live attenuated mutants of *Leishmania* for vaccination. Two such mutant, dhf-ts-, a knockout mutant for dihydrofolate reductase-thymidylate synthase,\(^{103}\) and lgp2-, a phosphoglycan-deficient mutant\(^{104}\) may offer a viable alternate for live vaccination with virulent strains. The dhf-ts- mutant can only undergo a limited number of replication cycles in macrophages without producing disease,\(^{103,105}\) while the phosphoglycan-deficient mutant are not able to survive in sand flies or macrophages, but retained the ability to persist indefinitely in the mammalian host without producing disease.\(^{105}\) Vaccination with these mutants has been shown to be protective in experimental animals.\(^{104,105}\) It may be that such studies will pave the way to effective vaccination against the leishmaniasis.

**Acknowledgments.** The author wishes to thank Professor Fazal Dar, and Drs. Abdulrahman Ismail and Abiola Senok (College of Medicine, Arabian Gulf University) for their valuable advice and contributions. Special thanks are also due to Dr. Shaden Kamhawi (National Institute of Allergy and Infectious Diseases, NIH, USA) for critically reading the manuscript, and for her expert opinion and supplementary references regarding epidemiology of leishmaniasis in the Middle East.

**References**

Leishmania vaccine ... Tabbara

43. Sokolova AN. Preventive vaccination with the living parasites of cutaneous leishmaniasis. Trans Turkmek Cutan-Venereol Inst Ashkhabad 1940: 11: 44.
Leishmania vaccine ... Tabbara


60. Swain SL, McKenzie DT, Weinberg AD, Hancock W. Characterization of T helper 1 and 2 cell subsets in normal mice. Helper T cells responsible for IL-4 and IL-5 production are present as precursors that require priming before they develop into lymphokine-secreting cells. J Immunol 1988; 141: 3445-3455.


Leishmania vaccine ... Tabbara


