Leishmaniasis vaccines: past, present and future

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ARTICLE INFO

Keywords:
Vaccine
Leishmaniasis
Prophylaxis
Immunotherapy
Immunochemotherapy
Emerging infectious disease

ABSTRACT

No vaccine exists against any form of leishmaniasis. Because recovery from infection is usually accompanied by a strong immunity and because it is possible to protect experimental animals against live challenge, hope for the development of a vaccine for humans has been high. However, leishmaniasis is a disease of the poor and the market for a vaccine is very limited. Until a few years ago, with minimal resources, only a pragmatic approach was possible for testing the first-generation vaccines (i.e. killed whole parasites). Recently, funding has become available for developing defined second-generation vaccines, including recombinant proteins and DNA constructs. With new adjuvants also being developed there is new hope, and several new vaccines are in development against leishmaniasis.

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1. Background

The leishmaniases are a group of parasitic diseases caused by intracellular protozoa belonging to the genus *Leishmania*. The manifestations of leishmaniasis range from a self-healing cutaneous lesion (cutaneous leishmaniasis, CL) to a lethal visceral form of the disease (visceral leishmaniasis, VL; also known as kala azar). VL is a major public health problem in the Indian subcontinent and East Africa. However, VL also occurs in the Mediterranean region and Latin America, where dogs are the primary reservoir of infection. Parasites are transmitted to humans from infected dogs or humans by the bite of sand flies. CL is widespread in central and western Asia, North Africa and Latin America. The reservoir may be infected humans or wild animals.

Patients who have been cured of leishmaniasis are usually protected by a strong immune response induced by the infection, giving rise to the belief that development of a vaccine should be easily achievable. Several candidate vaccines that can protect animal models of leishmaniasis against a live challenge have been identified [1]. Despite these, the availability of live challenge (see Section 7) and the wealth of knowledge about the immunology of leishmaniasis from experimental models and parasite biology there is no vaccine available against any form of leishmaniasis. Here, reasons for the lack of a vaccine, experiences with first-generation vaccines and current activities in preclinical or clinical development of leishmaniasis vaccines will be reviewed.

2. Why a vaccine is not available

2.1. Cost of development

The current cost of developing a vaccine has been estimated to be hundreds of millions of dollars; 60–80% of this is allotted to preclinical and even more so to clinical development of the vaccine (Fig. 1). However, research on the biology of the parasite, its immunology (from experimental leishmaniasis) and genetics (the genome of *Leishmania* has been sequenced) have attracted most of the funding from national and international agencies, whereas funding for human vaccine development has been minimal until very recently, except for one candidate vaccine funded by the Bill and Melinda Gates Foundation (Fig. 1).

2.2. Political involvement

About 90% of the burden of VL is present in five countries (India, Bangladesh, Sudan, Ethiopia and Brazil). Those affected in these countries are among the poorest of the poor, and hence there is not a large enough market for pharmaceutical companies to invest a considerable amount of time and money to develop a vaccine. Therefore a strong political will and considerable resources are required from the emerging market economies. Brazil has contributed to testing the first-generation vaccines; however, more needs to be done.

In some countries around the Persian Gulf and the Mediterranean Sea CL is a public health problem and no good drug exists for its treatment. These countries spend considerable amounts of funds every year on leishmaniasis, but the problem persists. Development of an affordable, safe and efficacious vaccine would be a very good investment in the long term for the affected countries, and in Europe and the Americas for travellers.

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3. How many vaccines are needed?

All species of Leishmania share an enormous number of antigens, so serological differentiation of the causative agent is impossible. Hence it may be possible to design a universal leishmaniasis vaccine composed of several conserved and common antigens that would protect against different species of Leishmania.

4. Cost-effectiveness of vaccines

If a universal vaccine were to be developed it would not be cost-effective to use it in all epidemiological situations. For example, the incidence of VL in the Indian subcontinent is around 0.1–0.2% (i.e. 1 case per 1000 population) and to protect 1000 cases 1 million individuals would have to be vaccinated. At present, with the high cost of VL treatment of around US$120–140/patient, to treat 1000 patients would cost US$120–140,000, but this is considerably less than the US$1 million required if there were a vaccine that was 100% protective at US$1/0 dose (very difficult to achieve). However, for many foci of CL or VL in Africa, where the annual incidence in a community can reach up to 4–5% and for which no efficacious treatment exists or no other control system is in place, a vaccine would be the best method of control. One possibility is to identify only high-risk populations in low endemic foci and only vaccinate those people. This requires a considerable infrastructure and vigilance programme, which would be expensive to maintain and is often non-existent for poor populations.

5. First-generation vaccines

5.1. Prophylaxis

As for many other diseases, the first approach to developing a leishmaniasis vaccine was to use killed organisms. The ease of growing Leishmania in culture media made it possible to use promastigotes grown in vitro. Some studies reported moderate success in the 1930s and 1940s, but it was not until the 1980s that several controlled trials were conducted in Brazil by Mayrink and colleagues. One in particular showed 53.3% efficacy in those who responded to the vaccination by cellular immunity measured by the leishmanin skin test [2]. The vaccine was eventually tested in a phase 3 trial in Colombia [3] and was safe but not sufficiently efficacious (Table 1). Other trials were done in Latin America but they could not be repeated [4] or were inconclusive, either because of the low incidence of the disease during the trials or due to flooding and El Niño, which made follow-up impossible.

The vaccine produced from L. major (Razi Serum and Vaccine Institute, Iran) was tested in several phase 1–3 trials against CL in Iran caused by L. major [5] or L. tropica [6] and against VL in Sudan [7]. The observation originally reported by Antunes et al. [2] was confirmed in the studies when the skin test conversion after vaccination was >30% [4,6], as shown in Table 1.

A comprehensive review of published prophylactic vaccine trials has been published by Noazin et al. [8].

5.2. Immunotherapy

The same vaccines that were ineffective for prophylaxis showed encouraging results as adjuncts to chemotherapy, either by allowing dose reduction of drug treatment of CL in Brazil [9] or by achieving cure in cases of post-kala azar dermal leishmaniasis (PKDL) in Sudan [10]. A trial was done to assess whether the addition of Mayrink’s vaccine to sodium stibogluconate (SSG), the first-line drug for VL, could reduce by half the dose of SSG (i.e. 8 mg/kg/day) to minimize side effects but still achieve cure. Of the 47 patients in the experimental group, all were cured after four rounds of treatment, compared with only 4 of 49 (8.2%) in the control group (P<0.001). Each round consisted of 10 daily treatments followed by 10 days of no treatment [9].

It is estimated that up to 50% of treated VL patients in Sudan may develop PKDL, of whom about half do not cure spontaneously even after many years. These patients require 2–3 months of daily injection of SSG to be cured. It is important to cure PKDL patients because they can contribute to the reservoir of infection. In a pilot trial 87% (13/15) of the patients receiving both SSG and vaccine were cured by day 60, compared with 53% (8/15) in the group treated with SSG alone [10]. By day 90 of follow-up there had been two relapses in the group treated with SSG alone and none in the group treated with SSG and vaccine. After 6 months follow-up all patients treated with SSG and vaccine were cured, giving a final cure rate of 100% vs. 40% for those treated with SSG alone (P<0.004).

The reasons for the difference in effectiveness of the vaccine when used for prophylaxis and treatment are not known. From mouse studies it seems that the vaccine can divert the already ongoing mixed immune response to a predominantly CD8+ T-cell response in the infected host, but it is not immunogenic enough.
### Table 1

Leishmaniasis vaccines used in prophylaxis and immunochemotherapy.

<table>
<thead>
<tr>
<th>Form of disease</th>
<th>Vaccine used</th>
<th>No. of vaccine doses</th>
<th>Efficacy range (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prophylaxis</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Zoonotic CL caused by <em>L. major</em> (Iran)</td>
<td>ALM + BCG vs. BCG</td>
<td>1, 2 or 3</td>
<td>Overall 12–25%</td>
<td>[1,5]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>In LST+ 17–35%</td>
<td></td>
</tr>
<tr>
<td>Anthroponotic CL caused by <em>L. tropica</em> (Iran)</td>
<td>ALM + BCG vs. BCG</td>
<td>1</td>
<td>Overall 7.4%</td>
<td>[6]</td>
</tr>
<tr>
<td>Zoonotic CL (Brazil)</td>
<td>Mayrink's initial vaccine vs. placebo</td>
<td>3</td>
<td>Overall 17–21%</td>
<td>[2]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>In LST+ 53.3%</td>
<td></td>
</tr>
<tr>
<td>Zoonotic CL (Brazil)</td>
<td>Mayrink's BioBras vs. placebo</td>
<td>3</td>
<td>Overall 13.2%</td>
<td>[3]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>In LST+ 6.5%</td>
<td></td>
</tr>
<tr>
<td>Zoonotic CL (Colombia)</td>
<td>ALM + BCG vs. BCG</td>
<td>2</td>
<td>Overall 6.5%</td>
<td>[7]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>In LST+ 43.3%</td>
<td></td>
</tr>
<tr>
<td>Zoonotic CL (Brazil)</td>
<td>ALM + BCG + low-dose SSG vs. placebo</td>
<td>40</td>
<td>100% vs. 8.2%</td>
<td>[9]</td>
</tr>
<tr>
<td>Immunotherapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL (Brazil)</td>
<td>Mayrink's BioBras + low-dose SSG vs. low-dose SSG alone</td>
<td>4</td>
<td>Overall 100%</td>
<td>[10]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>In LST+ 40%</td>
<td></td>
</tr>
</tbody>
</table>

ALM + BCG, killed *L. major* vaccine produced by Razi Serum and Vaccine Institute, Iran, mixed with Bacillus Calmette-Guérin produced by Pasteur Institute, Iran; Alum-ALM, alum-adsorbed ALM; CL, cutaneous leishmaniasis; LST+, those who converted their leishmanin skin test from negative to >5 mm; Mayrink's initial vaccine, mixture of several species of *Leishmania*; Mayrink's BioBras, killed *L. amazonensis* produced by BioBras, Brazil; SSG, sodium stibogluconate, the first-line drug in many countries; VL, visceral leishmaniasis.


6. Second-generation vaccines in clinical development

Only one second-generation vaccine is in clinical development. This is the LEISH-F1 + MPL-SE vaccine (previously called Leish-111F + MPL-SE) of Reed and co-workers [11], consisting of three recombinant *Leishmania* polyprotein LEISH-F1 antigens (TSA-LmSTI1-LeIF), together with the adjuvant monophosphoryl lipid and squalene in a stable emulsion (MPL-SE). After satisfactory completion of preclinical development and approval from the FDA, an initial phase 1 safety and immunogenicity trial was conducted in the USA followed by several studies initiated in Latin America [12] and recently in India (S. Reed, Personal communication, IDRI, Seattle, USA).

Three other vaccines are in preclinical development in Europe. These are the synthetic vaccine RAPSODI [13] and two DNA vaccines. One of these, based on a viral vector, is being developed at York University, UK by Paul Kaye and colleagues (P. Kaye, Personal communications) and another, LEISHDNAVAX [14], is being developed by Mologen (Berlin, Germany) using new technology (linear vector minimalistic immunogenetically defined gene expression; MIDGE) to deliver selected *Leishmania* antigens. These constructs may be used alone or in combination with a synthetic adjuvant (double stem loop immunomodulator; dSLIM) (Fig. 2).

7. Opportunities for testing new vaccines

Historically, protection against CL has been achieved through deliberate infection using live parasites at a preferred body site—a procedure known as leishmanisation. This method, using *L. major* promastigotes (the form of parasite in the sand fly and cell-free cultures), was adopted in Iran, Israel and Uzbekistan several decades ago. Although highly protective, this form of vaccination is not practical since it produces a lesion that can last several months and the delivery of a standardised inoculum to large populations is not...
trivial. However, leishmanisation provides a powerful tool to test vaccine candidates against CL.

Leishmanisation can only be employed in volunteers with a very low risk (or no risk) of HIV infection and a high risk of CL. Since the best prophylactic measure for CL is leishmanisation, unlike some other live challenge systems used for vaccine evaluation (malaria, cholera), all volunteers in the trial would benefit by becoming immune, either by the experimental vaccines or by leishmanisation.

One of the problems of leishmanisation is the variability of virulence (take rates) of the promastigotes used, which started at very high levels but have dropped to about 30–40%. Recently, for the purpose of standardising the inoculates, the Razi Institute (Iran) has produced a seed bank from a well-characterised L. major strain and many seed lots have been kept in liquid nitrogen. From these, frozen samples preserved in glycerol ready for injection have been produced and kept frozen until use. Two pilot studies conducted by Khamesipour et al. showed reproducibility of infection and 100% protection against a second challenge with leishmanisation [15].

Leishmanisation provides a powerful tool for evaluating a new vaccine since the read-out is short (few months) and the sample size is small (30–50/arm), hence it is possible to search for biomarkers of immunity. Moreover, the study would cost much less than field efficacy trials, which require thousands of volunteers (usually children in an endemic foci) and can take years to deliver final results.

Acknowledgments

I thank Dr. Federica Giovannini for assistance in preparing the manuscript.

Funding: FM was an invited speaker at the International Conference on Emerging Zoonotic Diseases, Cairo, Egypt, 14–17 October 2009; support from the International Society of Chemotherapy is acknowledged. This work was supported in part by the LEISH-NAVAX project of European Community FP-7.

Competing interests: This is a review of material in the public domain. The author declares that there is no conflict of interest.

Ethical approval: The author declares that all clinical trials mentioned here with which the author was associated received ethical clearance from the required authorities (institutional, national and WHO) as applicable.

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