Vaccines to prevent leishmaniasis

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Leishmaniasis is a parasitic disease that encompasses a range of clinical manifestations affecting people in tropical and subtropical regions of the world. Epidemiological and experimental data indicate that protection from disease can be achieved in most people. In addition, we know how the host immune system must respond to infection in order to control parasite growth. However, there is still no vaccine for use in humans. Here, we review our understanding of host immunity following Leishmania infection and also discuss recent advances in the development of vaccines to prevent leishmaniasis, highlighting a new promising approach that targets the parasite hemoglobin receptor.

Keywords: immunity; leishmania; parasites; vaccines
vaccine. People cured of *Leishmania* infections develop lifelong immunity. Therefore, prevention of leishmaniasis through prophylactic vaccination is feasible. Advances in our understanding of *Leishmania* infection pathogenesis and the generation of host-protective immunity, together with completed *Leishmania* genome sequences, has opened new avenues for vaccine research. However, major challenges remain, including the translation of ideas from animal models to clinical settings, and the transition of products from the laboratory to the field. This review will highlight recent advancements in the development of vaccines to prevent and/or treat leishmaniasis, and discuss future prospects.

**PROTECTIVE IMMUNE RESPONSES IN THE HOST**

A good understanding of immunity generated against pathogens is important for developing an effective vaccine. Our current understanding of host immune responses generated against *Leishmania* parasites is mainly based on the studies in animal models. Studies in mice show that protective immunity to *Leishmania* infection requires the development of interleukin-12-dependent, parasite-specific Th1 responses, characterized by interferon-γ and tumor necrosis factor production by CD4+ T cells. These inflammatory cytokines are required for the generation of reactive oxygen and nitrogen species by infected macrophages that enables killing of intracellular parasites. Recent advances have also been made in understanding immunoregulatory mechanisms that suppress parasite-specific CD4+ T-cell responses in human VL patients. These include the discovery that interleukin-10 produced by CD4+ T cells is a potent, autocrine inhibitor of interferon-γ production and promotes parasite persistence in spleen tissue from VL patients. Thus, interleukin-10 has been identified as a potential therapeutic target for use in combination with drug therapy or to improve therapeutic vaccine efficacy.

The generation of immunological memory is a requirement of effective vaccination. Studies on the generation of effector and central memory CD4+ T cells indicate that central memory T cells mediate long-term immunity to *L. major* infection, even in the absence of persistent parasites. Thus, defining the requirements and understanding the conditions for central memory CD4+ T-cell formation and maintenance will be helpful in vaccine design.

Our knowledge that the majority of individuals infected with *Leishmania* parasites control parasite growth without causing serious disease, combined with our understanding about the types of immune responses required for killing parasites and those that suppress this immunity, means that developing vaccines against leishmaniasis is a realistic goal.

**WHY DO WE NEED A VACCINE TO PREVENT AND/OR TREAT LEISHMANIASIS?**

Treatment of leishmaniasis is dependent on chemotherapy. The most commonly used drugs are pentavalent antimonials, oral miltefosine, amphotericin B, liposomal amphotericin B and paramomycin. A major problem is that these drugs are associated with problems of cost, toxicity, length and duration of treatment, route of injection (for example, intravenous infusion) and the development of parasite drug resistance. Pentavalent antimonials were the first line of treatment for many years, but increasing parasite resistance in endemic regions has limited their use. In the state of Bihar in India, almost 60% of cases are refractory to treatment with this drug. Consequently, amphotericin B is now used as the main drug to treat VL patients. However, this drug is also associated with toxicity and there are reports of drug-resistant parasites. Miltefosine was developed as an oral drug and showed an early promise; however, there are now increasing incidences of relapse in patients treated with this drug. Recently, a single dose of ambisome (lipid formulation of amphotericin B) was shown to be effective in treating VL patients, with a lower incidence of toxicity, compared with conventional treatment, in a multicentre clinical trial. Nevertheless, there are concerns that this type of drug-treatment protocol may promote the development of drug-resistant parasites. Therefore, combination drug therapy is being actively developed for use in endemic regions. However, studies in a mouse model suggest that the *L. donovani* can develop resistance to drugs, even when they are used in combination. Therefore, despite advances in chemotherapeutic options, it is unlikely that chemotherapy alone will enable disease elimination, and hence there is an urgent need for an effective vaccine if long-term goals to controlling and eliminating this disease are to be achieved.

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Figure 1 Life cycle and transmission of *Leishmania* parasites. The promastigote form of *Leishmania* parasites responsible for human disease (VL, CL and MCL) are injected into the skin as a female sand fly takes a blood meal (1), and are then taken up by host macrophages (2). Promastigotes convert to the non-flagellated, amastigote form inside macrophages (3) and then divide by binary fission (4). The amastigotes are released by the rupture of macrophages (5) and can then be taken up by a female sand fly during another blood meal. The amastigote form then converts in the promastigote form in the midgut of the sand fly and can then again be transmitted to another human (anthroponotic transmission) or to animals that act as reservoirs (zoonotic transmission) (6).
**PAST AND PRESENT VACCINE CANDIDATES**

Despite different *Leishmania* species causing a range of clinical manifestations, genomic analysis indicates a large degree of sequence homology between species, suggesting it may be possible to generate broadly effective vaccines against different clinical diseases. An effective vaccine against leishmaniasis has existed in the past. This involved inoculation with live, virulent parasites, in a process called leishmanization. It was practiced successfully in the former Soviet Union, Middle East and Israel. However, it was abandoned in most countries because of logistical problems and safety concerns, due to some individuals developing non-healing lesions and immune suppression.

Whole-killed (autoclaved) *Leishmania* promastigotes were also tested as vaccines against CL and VL. Testing of killed parasite vaccines took place in Brazil in the early 1940s, and was then tested either alone or in combination with adjuvant in phase-I, II and III trials. Clinical trials with autoclaved *Leishmania*, adjuvanted with BCG, showed that this approach could reduce the incidence of CL by 18–78%. Similar trials were conducted in Iran, Sudan and Ecuador with variable safety and efficacy. Unfortunately, the autoclaved parasites showed decreasing potency with time, although studies with thimerosal preserved and non-autoclaved preparations have shown reduced effects of storage. However, concerns remain regarding the feasibility of developing killed, whole-parasite vaccines, including the variation in results obtained from different field and clinical trial sites in the past, and potential difficulties in producing such a product to good clinical manufacturing standards.

Various attenuated parasites have also been tested in animal models. These parasites are generally taken up by host cells in a similar way to virulent parasites, and persist for some time without replicating. This allows the host to mount robust immune responses against parasite antigens. Radio-attenuated and biochemically altered parasites have proven to confer good protection in mice and hamsters without adjuvant, although concerns regarding conversion back to virulence make the latter option questionable for human use. However, targeted elimination of virulence genes may overcome this problem and could produce attractive vaccine candidates against leishmaniasis. Genetically modified *Leishmania* parasites lacking essential genes like dihydrofolate reductase, biopterin reductase or cystein proteases have been shown to stimulate protection against challenge with virulent parasite strains.

The use of drug-sensitive *Leishmania* mutants alone or with adjuvant has been proposed as a mechanism to induce anti-leishmanial immunity, as has the use of non-pathogenic *Leishmania* species like *L. tarantulae*, which can stimulate protection against virulent *L. donovani* strains. However, the main problem with using killed or attenuated parasites are the concerns relating to safety and feasibility for large-scale use in the field.

Other approaches include using immunogenic surface antigens of *Leishmania* parasites as vaccine candidates. Several of these have been tested in mouse models and canine VL with data suggesting that protection against leishmaniasis can be achieved with defined candidate proteins. A saponin formulation of fucosamine mannose ligand that is expressed throughout the life cycle of parasite, was found to be safe, protective and immunogenic in an experimental mouse and hamster models. This formulation has now become the Leishmune veterinary vaccine, licensed after a series of canine VL field studies. Lipid formulations of soluble leishmania antigen from *L. donovani* were also tested as vaccine candidates in a hamster model of *L. donovani* infection, and this conferred protection with increased delayed type hypersensitive reactions in response to parasite antigen, enhanced parasite-specific antibody responses and improved parasite-specific T-cell responses. Liposomal soluble leishmania antigen (from *L. major*) incorporated with phosphorothioate CpG ODN (PS CpG) or phosphodiaster CpG ODN (PO CpG) has also been tested in a mouse model of CL, and generated significant levels of protection. The excretory/secretory proteins isolated from culture supernatants of *L. infantum* and adjuvanted with muramyl dipeptide were tested in dogs experimentally infected with *L. infantum*. This vaccine, termed LIESAP-MDP, induced significant, long-lasting protection against canine VL in a field trial in an endemic area of France with naturally infected dogs. However, a major hurdle with these fractionated vaccines for human applications is their production to good clinical manufacturing standards, as well as gene variation and polymorphisms in field isolates.

Recombinant proteins, either alone or combined with adjuvant or with bacteria/recombinant virus as a delivery vehicles, have also been tested as vaccines in preclinical studies. There have been significant efforts in recent time to identify recombinant antigens that can protect against *Leishmania* infection in experimental models. Some of these antigens include kinetoplastid membrane protein-11, sterol 24-c-methyltransferase, amastigote specific protein A2, cysteine proteinase B, *L. braziliensis* elongation and initiation factor, *K26/HASPB*, *Leishmania*-activated C kinase, promastigote surface antigen 2, nucleoside hydrolase and surface expressed glycoprotein gp63. Although most of these recombinant antigens have been tested in animal models for their immunogenicity and protective efficacy, only a few have progressed to clinical trials in non-human primates, dogs or in preclinical human studies. A multisubunit recombinant *Leishmania* vaccine, Leish-111F, containing a *L. major* homolog of eukaryotic thiole-specific antioxidant, *L. major* stress inducible protein-1 and *L. braziliensis* elongation and initiation factor, in formulation with MPL-SE, has been shown to provide protection in mouse models of CL and VL but failed to prevent canine VL caused by natural *L. infantum* infection. Nevertheless, Leish-111F/MPL-SE is the first defined vaccine candidate to progress to human phase-I and phase-II clinical trials in healthy volunteers in South America, CL and VL patients in Brazil and Peru and patients cured of VL in India. As with all subunit vaccines, potential problems include variations in immunogenicity, based on human lymphocyte antigen expression in individuals, gene variation and polymorphisms in parasites, as well as the potential to drive selective pressure of parasites away from the molecules used in vaccines.

Finally, DNA vaccines to prevent leishmaniasis are also undergoing development and testing. This approach is not new but has several advantages, such as low costs of production, stability of materials, sustained expression of relevant antigens and efficient generation of effector and memory immune responses. In addition, more than one antigen can be produced by a single construct. The non-methylated CpG motif of bacterial DNA provides the further advantage of activating innate immune cells to produce interleukin-12, which can prime CD4+ T cells to develop into Th1 cells. A list of vaccine antigen candidates being tested in DNA vaccines for CL and VL is shown in Table 1.

Therefore, despite many years of effort in identifying immunogenic parasite antigens and advances in vaccine technologies, there does not yet appear to be a vaccine candidate capable of delivering the level of protection needed for a disease elimination program. However, a significant advance was recently described in a study by Guha et al., where they targeted the parasite hemoglobin receptor (HbR) using a DNA vaccine approach and tested it in an experimental model of VL. *Leishmania* parasites require heme for various metabolic activities; however, they lack an endogenous heme synthesis pathway, thus
making them dependent on the host. HbR is expressed on the cell surface of the parasite and is conserved among different species. This receptor is not only important for hemoglobin endocytosis, but also has hexokinase activity, distinct from host hexokinase, which is important for regulating glycolysis. These important properties of parasite HbR led Guha and colleagues to test this molecule as a DNA vaccine candidate.

The success of any vaccine depends on many factors, including the generation of effective antigen-specific antibody responses, the priming and maintenance of parasite-specific T-cell responses and the generation of T cells with appropriate effector functions. Guha et al. found that patients with active VL produced reactive antibodies against HbR, and that these antibodies were able to inhibit parasite growth in a complement dependent manner in vitro. They also showed that HbR-DNA vaccination of mice stimulated the production of antigen-specific IgG2a antibodies and promoted the generation of antigen-specific T-cell responses that were able to produce multiple Th1-related cytokines simultaneously (that is, a polyfunctional T-cell response). Moreover, immunization with this DNA vaccine enabled sterile cure in hamsters and mice following challenge with virulent L. donovani (Figure 2). This is remarkable. These results were obtained in the absence of adjuvant, and thus highlight the potential of HbR as a DNA vaccine candidate for human use. However, further testing, including independent validation of efficacy, must be performed. In addition, and as mentioned earlier, DNA vaccines have shown great promise in animal models, but have not yet proven their utility in humans. There have been no clinical trials beyond phase-II to test DNA vaccines in humans. Thus, a major challenge for DNA vaccine candidates, such as parasite HbR, remains the demonstration of safety and efficacy in humans in both clinical trial and field settings.

Table 1 Leishmania vaccine antigens being tested as candidate DNA vaccines

| Candidate antigen | Models | Disease | Species | Reference |
|-------------------|--------|---------|---------|-----------|
| LACK              | Dog, mice | VL, CL | L. donovani, L. chagasi, L. major, L. infantum | 86-90 |
| gp63              | Mice, dogs | CL, VL | L. major, L. infantum | 88,90 |
| KMP11             | Mice | CL, VL | L. major, L. donovani | 91-93 |
| CPB               | Dogs, mice | VL, CL | L. infantum, L. major | 94,95 |
| ORFF              | Mice | VL | L. donovani | 96 |
| NH 36             | Mice | VL, CL | L. chagasi, L. maxicana | 97,98 |
| TRYP              | Dogs | VL | L. infantum | 90 |
| PSA-2             | Mice | CL | L. major | 88 |

Abbreviations: CPB, cysteine proteinase B; CL, cutaneous leishmaniasis; gp63, glycoprotein 63, KMP11, kinetoplastid membrane protein-11; LACK, Leishmania-activated C kinase; NH36, nucleoside hydrolase 36; ORFF, open reading frame F; PSA-2, promastigote surface antigen 2; TRYP, tryparedoxin peroxidase; VL, visceral leishmaniasis.

CONCLUDING REMARKS: PROBLEMS AND FUTURE DIRECTION

Vaccination is the most cost-effective way of controlling infectious diseases. The success of vaccine development depends upon understanding the immunobiology of pathogen/host interactions, selection of appropriate vaccine candidates and choosing the right adjuvant or delivery vehicle. In addition, the vaccine must be able to generate long-lasting immunity, the best immune correlates of protection must be identified so vaccine efficacy can be efficiently evaluated and it must be able to transition from preclinical testing to human trials. However, despite a better understanding of immune regulatory
pathways established following infection or vaccination, we still have a limited capacity to modulate these to clinical advantage with available adjuvants or drugs. Ideally, the vaccine should also be effective against all causative agents for a particular disease. This would allow significant saving in product development and testing, which will be an important consideration in future vaccine development programs. The development of a vaccine against leishmaniasis has been slow. However, increased knowledge gained in recent years in all of the areas is paving the way for renewed efforts to make and test new vaccines aimed at preventing and/or treating leishmaniasis. If funding sources can be identified and commit to the long road of vaccine development, we are confident this is one parasitic disease that can ultimately be controlled.

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