Using Humans to Make a Human Leishmaniasis Vaccine

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Abstract

The cellular immune response to peptide pools from conserved *Leishmania* antigens in leishmaniasis-immune individuals identified epitopes for a human DNA vaccine (Das *et al.*, this issue).

Leishmaniasis is a vector-borne infectious disease affecting the poorest communities around the world and has been designated by the World Health Organization as one of the 17 neglected tropical diseases. Environmental changes such as global warming or human migrations have affected the vector-reservoir-host triad and led to an expansion of the leishmaniasis geographical range (1). Leishmaniasis comprises diseases of varied etiologies that are caused by approximately 20 different *Leishmania* parasite species and are transmitted by nearly 30 medically important sand fly vector species (1). Mammals including rodents, canids, and in some cases humans are reservoirs of the disease.

Despite the availability of well-characterized animal models that have resulted in a good understanding of the immune response to *Leishmania* and recognition of correlates of protection from leishmaniasis (2), an effcacious human vaccine has eluded us thus far (3). In this issue of *Science Translational Medicine*, Das *et al.* (4) used human cells to select components for a next-generation DNA vaccine, representing a new strategy to make leishmaniasis vaccines for humans.

TOWARD A LEISHMANIASIS VACCINE

Besides the lackluster economic outlook for a leishmaniasis vaccine, there are other inherent challenges facing its development. An efficacious vaccine for human leishmaniasis must take into account that *Leishmania* parasites are transmitted by the bite of an insect vector and therefore bypass the skin barrier and its innate protective mechanisms and are also intracellular and inaccessible to antibodies. Unlike most established vaccines, an efficacious leishmaniasis vaccine must rely on the induction of a robust T cell immunity that overcomes the human and parasite intrinsic variability (2). This immunity depends on efficient presentation of specific epitopes to T cells by diverse human leukocyte antigen I (HLA-I) and HLA-II molecules present on the surface of antigen-presenting cells. Moreover, to

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ensure wide coverage, a leishmaniasis vaccine must also take into account the antigenic variability resulting from the existence of multiple *Leishmania* species and numerous strains.

Despite these challenges, there is consensus in the leishmaniasis scientific community that a prophylactic vaccine against human leishmaniasis is a viable option (3). Interestingly, in endemic areas, the resolution of cutaneous leishmaniasis (CL) results in life-long protection against the disease. This notion led to the practice of "leishmanization," in which controlled inoculation of virulent parasites in discrete parts of the body was used as a live vaccine (3). Historically and to date, this has been the most effective way to "immunize" people living in a leishmaniasis-endemic area. However, logistics—including scalability, reproducibility, and transport—combined with the likelihood of adverse events inherent to a live virulent vaccine limits the practicality and acceptability of leishmanization.

Another major limitation facing the field of leishmaniasis vaccines has been the inability to move vaccine candidates forward to clinical trials. One main obstacle concerns product development of a vaccine candidate. Despite the existence of a large pool of vaccine candidates against *Leishmania*, only a few have made it to human clinical trials (3). These include first-generation killed *Leishmania* vaccines and two second-generation vaccines, Leish-111f (NCT00121862) and LEISH-F3 (NCT01484548). Unfortunately, the majority of candidates will face the challenge of transitioning from basic research to product implementation, termed the "valley of death" for vaccine development. This involves multiple technological and financial barriers, including issues of scalability, reproducibility, and cost.

THE MAKING OF LEISHDNAVAX

In this issue of *Science Translational Medicine*, Das *et al.* addressed the abovementioned challenges in a new strategy for the development of a human leishmaniasis vaccine (4). This approach began with the selection of seven parasite-derived vaccine candidates known for their ability to induce T cell responses in rodents: KMP11, TSA, CPA, CPB, P74, HASPB, and A2. These antigens were further screened for their homology in four Old World parasite species and strains, mostly from human isolates collected from Europe, Africa, and Asia (Fig. 1). Das and colleagues then tested peripheral blood mononuclear cells (PBMCs) from immune individuals living in *Leishmania* endemic areas to down-select and refne these vaccine candidates (4). The premise for this approach is that individuals cured from visceral leishmaniasis (VL) and CL, or that are asymptomatic for VL, should display a protective T helper 1 (T$_H$1) immune response toward *Leishmania* parasites.

This strategy simultaneously tackled parasite variability and variation of the human T cell immune response to parasite antigens (Fig. 1).

Using PBMCs from individuals from India, where *L. donovani* causes VL, and from Tunisia, where *L. major* and *L. tropica* cause CL and *L. infantum* causes VL, the authors stimulated cells with peptide pools derived from five of the seven vaccine candidates (Fig. 1). As expected, the T cell immune response to peptide pools varied among different donors. What was unexpected was the absence of dominant immunogenic peptides in any of the five

antigens. Instead, the study cohort recognized peptides that spanned the majority of each antigen, likely reflecting the immunogenicity of the vaccine candidates and the individuality of the human immune response. This observed variability of the human T cell immune response within a single population brings into focus the pitfalls of vaccine candidate selection solely on the basis of the immunogenically and protective efficacy of an antigen in rodent studies.

In partnership with the company MOLOGEN AG (www.mologen.com), Das *et al.* constructed five MIDGE (minimalistic immunogenically defined gene expression)–T_H1 vectors, each containing a sequence enriched for human T cell epitopes from one of the five selected vaccine candidates (KMP11, TSA, CPA, truncated CPB, and P74), creating LEISHDNAVAX. The MIDGE-T_H1 platform is a commercially available next-generation DNA technology. This vector is optimized for better transfection of cells and better induction of T cell immune responses and has demonstrated superior protection in a rodent model of leishmaniasis as compared with that of a conventional DNA plasmid (5). Importantly, the modularity of the LEISHDNAVAX strategy permits prompt and low-cost modification of the vaccine through the removal of or addition to any of the antigen-encoding MIDGE-T_H1 vectors.

By electing to use the MIDGE-T_H1 vector, Das and colleagues have improved the likelihood of success in transitioning LEISHDNAVAX through product development. DNA vaccine production is advantageous owing to its simplicity, reproducibility, adaptability, and low-cost production compared with recombinant proteins. Additionally, DNA vaccination has been shown to be safe in humans (6).

REVERSE TRANSLATION: FROM HUMANS TO MICE

Having designed LEISHD-NAVAX for immunogenicity in humans, Das *et al.* returned to animal models—in a sense, reverse-translating their research—by testing the efficacy of LEISHD-NAVAX in a mouse model of VL (Fig. 1). Immunization of BALB/c mice with LEISHD-NAVAX induced cellular and humoral immune responses to all five antigens present in the vaccine. Moreover, compared with controls, vaccinated animals displayed a significant reduction in spleen and liver parasitemia 1 month and up to 90 days after intravenous challenge with 10 million *L. donovani* parasites. The parasite burden reduction was dose-dependent, indicating the specificity of the response to LEISHDNAVAX. Upon infection with *Leishmania*, vaccinated animals showed a signifcant increase in the proinfammatory T_H1 cytokines interferon-γ and tumor necrosis factor–α but maintained the same level of the anti-inflammatory cytokines interleukin-10 (IL-10) and IL-4. This demonstrates the induction of a potent T_H1 immune response by LEISHDNAVAX.

LINGERING QUESTIONS

The vaccine design adopted by Das *et al.* (Fig. 1) brings us one step closer to the development of an efficacious human leishmaniasis vaccine. Importantly, although LEISHDNAVAX is targeted to parasites from the Old World, it has the potential to become a pan-*Leishmania* vaccine.

Nevertheless, some aspects require further exploration of LEISHDNAVAX. One question is whether it will induce long-term memory. In the study by Das *et al.* (4), the animals were challenged 10 days after the last immunization, precluding the evaluation of a memory response, which is a prerequisite to the function of an efficacious vaccine. Ideally, LEISHDNAVAX should be tested in a long-term protection study.

Another question is whether LEISH-DNAVAX will demonstrate protective efficacy in a more relevant animal model that displays disease pathology similar to the one observed in humans, such as hamsters (7). The authors elected to use BALB/c mice to test protection against VL. BALB/c mice are considered by many to be a poor animal model for VL because they control the infection and do not show clinical signs of disease (8). We also have to keep in mind that leishmaniasis is a vector-borne disease and that the contribution of the vector in disease transmission and vaccine development has to be considered. Will LEISHD-NAVAX protect against sand fy–initiated models of infection? Recent studies have highlighted the virulence of vector-transmitted *Leishmania* parasites that abrogates protection after needle challenge of vaccinated animals (9). Such models deliver a more physiological parasite dose and co-deposit the *Leishmania* into the skin along-side established virulence factors present in the infected vector (10), thus better mimicking what occurs naturally.

REFLECTIONS

Currently, there are no vaccines available for prevalent parasitic diseases with high morbidity and mortality such as malaria, tuberculosis, and many others. We can certainly benefit from the fresh look that Das *et al.* adopted, using humans to make a human vaccine. LEISHDNAVAX came to fruition through the efforts of a consortium that brought together major researchers from countries endemic and nonendemic for leishmaniasis with expertise in a variety of disciplines and representing academic institutions and private enterprises. These partnerships created the critical mass required for such an undertaking. We hope that these partnerships are promoted to develop solutions for other neglected infectious diseases.

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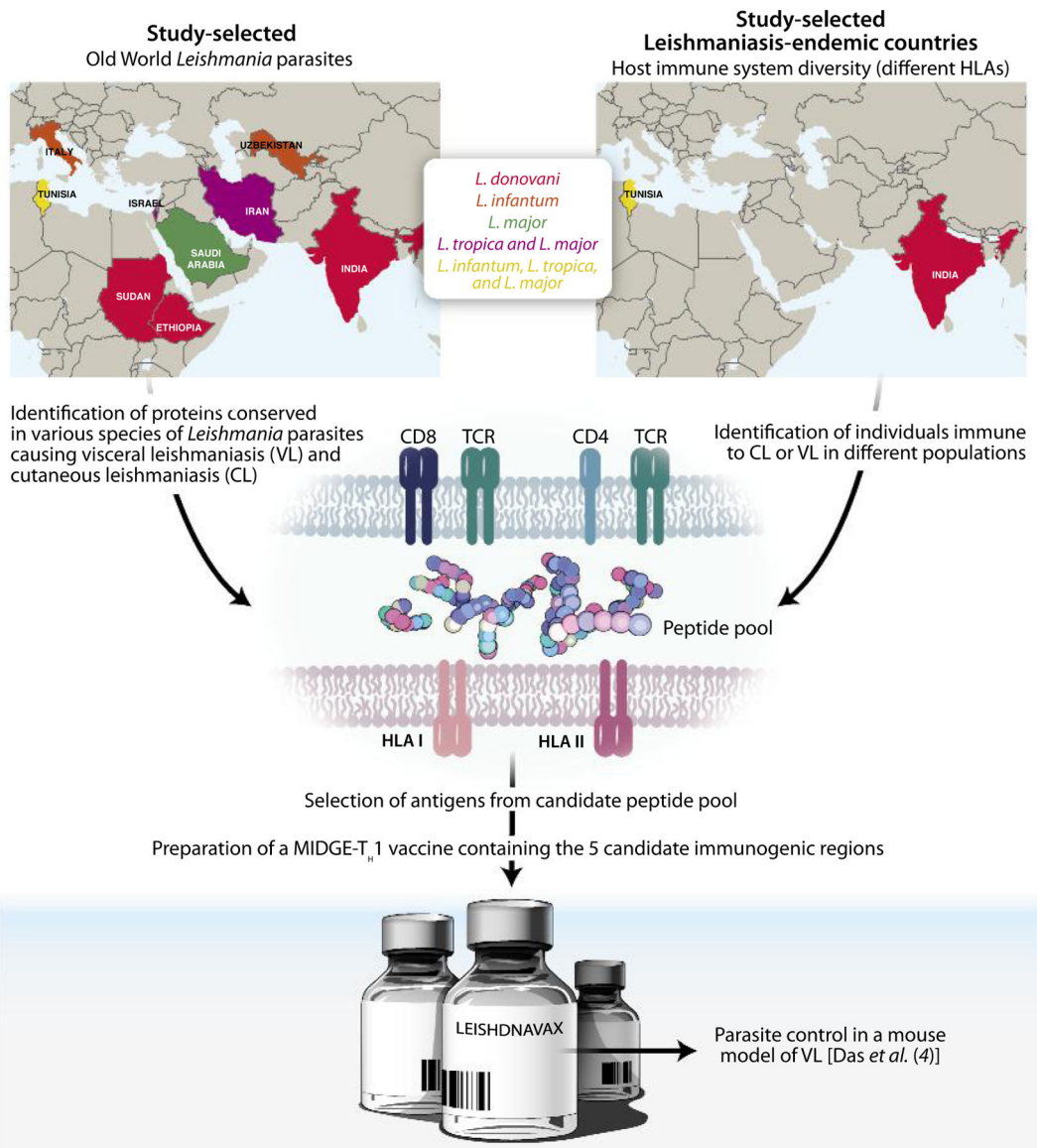
Fig. 1. LEISHDNAVAX: A T$_H$1-inducing *Leishmania* DNA vaccine.
Leishmaniasis is a neglected infectious disease caused by the protozoan parasite *Leishmania*.
Immunity and protection from leishmaniasis requires induction of a T$_H$1 cellular immunity.
This poses a challenge due to diversity of the human HLA as well as diversity of
immunogenic antigens on different *Leishmania* species. To overcome these obstacles, Das *et al.* (4) used PBMCs from immune individuals residing in Tunisia and India, countries
endemic for different forms of leishmaniasis, to identify sequences containing T$_H$1-inducing
epitopes conserved across Old World *Leishmania* species. LEISHDNAVAX was made using
five MIDGE-T$_H$1 vectors and was tested for protective effcacy against visceral leishmaniasis
(VL) in mice.