

Leishmaniasis Vaccination: Targeting the Source of Infection

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In the history of vaccine development, key observations have gradually led to our ability to take rational approaches toward the induction of protective immunity to microbial pathogens. The earliest efforts consisted of inoculating lesion material into healthy individuals resulting in the then inexplicable prevention of the development of similar disease. Centuries later, after researchers found that microscopic forms were responsible for the major human diseases such as tuberculosis and smallpox, vaccines consisting of inoculating weakened or attenuated organisms were developed. The discovery of the cell populations that mediate antibody and cellular immune responses, followed by the identification of the cytokine mediators of cellular immunity and the ability to identify, isolate, purify, and produce antigenic molecules of various pathogens has permitted a rational approach toward vaccine development. Perhaps no disease model has been more widely used to understand the roles of cell populations and cytokines in mediating disease susceptibility and resistance than the infection of inbred mice with the *Leishmania* parasite (1). For example, BALB/c mice infected subcutaneously with *Leishmania major* develop nonhealing, disseminated, fatal infections, whereas C57BL mice similarly infected are more resistant, and heal their infections. In addition to susceptibility factors determined by host genetics, it has recently been appreciated that the insect that transmits leishmania to humans may play a key role in the establishment of infection. The study by Valenzuela et al. in this issue (2) exploits this information in what may prove to be a unique approach to vaccination.

Leishmania are protozoan parasites of macrophages, transmitted to humans and mammalian reservoirs by small biting insects known as sand flies. Several species of the parasite cause human disease, manifested in a variety of forms ranging from a single cutaneous lesion to fatal disseminated infection of lymphoid and hematopoietic tissues. There are two basic forms of the parasite: the promastigote, that develops and is transmitted by the sand fly, and the amastigote that multiplies inside of macrophages. An effective immune response is one that limits parasite replication within macrophages, limiting disease progression. This can be accomplished by prior immunization with the parasite or selected

parasite antigens. However, the sand fly itself may influence the infection process by enhancing the ability of the promastigote to establish the initial macrophage infection. The identification of components of the sand fly vector that are capable of exacerbating leishmanial infections was made more than a decade ago by Titus and Ribeiro, who demonstrated that extracts of sand fly salivary glands increased infectivity in mice (3). If vector components are indeed necessary for establishing infection in the mammalian host, then an indirect approach to immunizing against *Leishmania* could be directed against relevant antigens of the insect rather than those of the parasite. The study by Valenzuela et al. identifies an insect component that may be used to induce an immune response against the parasite.

The immunological basis for the increased infectivity of *Leishmania* in association with salivary gland homogenate from sand flies remains to be understood. Although the induction of proinflammatory molecules is associated with homogenate components, their role in the infection process is not yet defined. However, some components of sand fly salivary glands are amongst the most potent vasodilators (4), which induce a large influx of nascent macrophage/monocytic cells, the host cell for the parasite. *Leishmania* are susceptible to complement-mediated lysis, and it is to the parasites' advantage to enter the macrophage in an expedient manner. It is known that increased production of TGF- β and IL-10 are closely associated with disease susceptibility in humans and in animal models (5–8). *Leishmania* are obligate intracellular parasites of macrophages, and each of these cytokines is associated with increasing virulence of this and other pathogens. It is possible that induction of one or both of these molecules by vector saliva components could increase macrophage infection. Conversely, IL-12 and IFN- γ are cytokines associated with resistance to leishmaniasis (9, 10). In a previous study by Sacks et al., it was demonstrated that a powerful delayed-type hypersensitivity (DTH) response was induced in mice by prior exposure to sand fly bites (11). The DTH response was characterized by a massive cellular infiltrate, with T cells that produced IFN- γ , the effector cytokine most closely associated with limiting parasite replication in macrophage, and was correlated with reduced infections when the mice were subsequently bitten by infected sand flies. The study is reminiscent of one conducted in guinea pigs, in which Behin et al. elicited DTH reactions by either chemical sensitization or bacille Cal-

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mette-Guérin (BCG) immunization and found that such reactions could effectively decrease the infectivity of *Leishmania* at the DTH site (12).

The study by Valenzuela et al. tested components of the sand fly saliva for their ability to induce a DTH response after a sand fly bite. One such protein, termed SP15, was capable of inducing a strong DTH response and protection against leishmaniasis in the absence of antibody, while other fractions of sand fly saliva appeared to be less effective. Histologically, the DTH response induced by immunization with SP15 was characterized by massive infiltrates of neutrophils and eosinophils, similar to the response induced by the bite itself. This is unlike the typical DTH induced by bacterial antigens, such as the tuberculin reaction, which is predominantly a mononuclear cell infiltrate, and calls into question the immunological basis for the anti-leishmanial activity observed. It has long been recognized, for example, that several histological forms of DTH exist (13, 14). The argument against the role of antibody in the protection induced by immunization with SP15 comes from the observation that protection was achieved in B cell-deficient mice. Further characterization of sand fly components and perhaps passive transfer experiments could test the authors' hypothesis that the protective DTH can be induced by any protein in the saliva, or if there is a potentially important role for antigen specificity such as in the classic DTH reaction induced by mycobacteria (15).

Active immunization against leishmaniasis has been going on for centuries. The original version of a human vaccine consisted of inoculating lesion material into a naive individual using a thorn. The procedure was designed to produce a mild disease to prevent more severe disfigurement after subsequent natural exposure. During the past two decades, a significant amount of work has been focused on prophylactic vaccine approaches designed to prevent disease progression in mice infected with *Leishmania*. Approaches have included the use of whole attenuated parasites, parasite extracts, and of leishmanial antigens delivered as genes or proteins. Such approaches have met with moderate to spectacular success in mouse models (16, 17). Human vaccine trials using whole parasite preparations have met with partial success (18), indicating that an effective human vaccine for leishmaniasis may be possible once the appropriate immune response targets are identified. Until now, the effort has been focused on antigens of the *Leishmania* parasite.

Although the vast majority of vaccines have been directed against antigens of the infectious agent transmitted by the arthropod vector, other vaccine approaches have been directed to the arthropod itself. For example, success with vaccines against tick saliva has been achieved in cattle, and a vaccine against fleas has been developed for dogs. However, the concept of using nonparasite components to induce a specific immune response designed to attack the parasite and decrease infection through nonspecific means is a new and potentially exciting approach toward vaccination against vector-borne pathogens. The identification of a specific salivary gland component capable of inducing the

tissue reaction that leads to decreased infection is an important finding and will aid in our understanding of host-vector interactions at the molecular level. If this component is conserved among multiple species of sand fly, one could envision the development of a vaccine capable of protecting against multiple *Leishmania* species by immunizing with a single vector protein. Persons living in endemic regions would be repeatedly boosted when bitten by both infected and uninfected insects. However, although it is possible that such an approach could limit the severity of disease, it would not likely be totally protective. In hyperendemic areas, there is often a high degree of exposure to bites from uninfected sand flies, yet disease incidence is high. Nonetheless, it is possible that sand fly molecules could be considered as components of a vaccine, together with parasite antigens. However, any approach using sand fly proteins as vaccine components must take into consideration a vaccine formulation that will induce a nonpathologic DTH response. The cellular infiltrate observed after immunization with SP15 suggests elements of an allergic nature, with potential implications of provoking adverse reactions in individuals with atopic reaction through this type of immunization approach.

An effective leishmaniasis vaccine will be one that prevents the development of disease. The distinction between vaccines designed to prevent establishment of infection versus those designed to prevent disease is an important one. A decrease in infection, such as was achieved through the prior exposure to sand fly bites or injection of purified protein, will not necessarily confer immunity to the parasite itself or prevent subsequent disease progression. In the guinea pig study of Behin et al. (12), the decreased infectivity induced by a DTH response did not eliminate infection, as metastatic lesions developed later in initially protected animals, nor did it confer immunity to subsequent challenge given in the absence of a DTH response. This latter problem may not occur in nature, where the only exposure to infection that humans receive is in conjunction with sand fly proteins. Thus, a DTH response would theoretically accompany each exposure to infection. The debate of infection versus disease is reminiscent of that surrounding the malaria sporozoite vaccine. As the sporozoite is the infective form transmitted by the mosquito, an effective immune response against it confers immunity in mice. However, should sporozoites escape this immune response, disease will occur, as no immunity has been induced against the tissue stages of the parasite. Similarly, *Leishmania* has a tissue stage that is responsible for pathology and is distinct from the promastigotes transmitted by the sand fly. Thus, vaccines that only prevent infection and do not establish immunity to tissue parasites may bear a risk. On the other hand, it is possible that immunizing against sand fly components could produce a low grade infection, with a degree of immunity to the amastigote tissue stage sufficient to prevent disease. Such a state of immunity is the norm in humans infected with leishmania; most infections are subclinical. Perhaps limiting the number of surviving parasites after exposure via a DTH reaction is why there are so many asymptomatic human in-

fections in certain endemic foci. Furthermore, there is the unexplained observation that humans living in endemic areas often have milder lesions than those who come into endemic areas from outside the region. This comparative resistance could be explained by a recall response to vector components. Ideally, through a combination of vaccine components directed against both vector and pathogen targets, safe, effective, and long-lasting immunity can be achieved. Consideration of the vector and the pathogen as an integrated unit in the infectious process represents a change in our thinking with regard to vaccine development.

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References

1. Reiner, S.L., and R.M. Locksley. 1995. The regulation of immunity to *Leishmania major*. *Annu. Rev. Immunol.* 13:151–177.
2. Valenzuela, J.G., Y. Belkaid, M.K. Garfield, S. Mendez, S. Kamhawi, E.D. Rowton, D.L. Sacks, and J.M.C. Ribeiro. 2001. Toward a defined anti-*Leishmania* vaccine targeting vector antigens: characterization of a protective salivary protein. *J. Exp. Med.* 194:331–342.
3. Titus, R.G., and J.M. Ribeiro. 1988. Salivary gland lysates from the sand fly *Lutzomyia longipalpis* enhance *Leishmania* infectivity. *Science*. 239:1306–1308.
4. Lanzaro, G.C., A.H. Lopes, J.M. Ribeiro, C.B. Shoemaker, A. Warburg, M. Soares, and R.G. Titus. 1999. Variation in the salivary peptide, maxadilan, from species in the *Lutzomyia longipalpis* complex. *Insect Mol. Biol.* 8:267–275.
5. Barral-Netto, M., A. Barral, C.E. Brownell, Y.A.W. Skeiky, L.R. Ellingsworth, D.R. Twardzik, and S.G. Reed. 1992. Transforming growth factor- β in leishmanial infection: an important parasite escape mechanism. *Science*. 257:545–548.
6. Heinzl, F.P., M.D. Sadick, S.S. Multha, and R.M. Locksley. 1991. Production of IFN- γ , interleukin 2, interleukin 4, and interleukin 10 by CD4⁺ lymphocytes in vivo during healing and progressive murine leishmaniasis. *Proc. Natl. Acad. Sci. USA*. 88:7011–7015.
7. Ghalib, H.G., M.R. Piuvezam, Y.A.W. Skeiky, M. Siddig, F.A. Hashim, A.M. El-Hassan, D.M. Russo, and S.G. Reed. 1993. Interleukin 10 production correlates with pathology in human *Leishmania donovani* infections. *J. Clin. Invest.* 92:324–329.
8. Reed, S.G., and P. Scott. 1993. T cell and cytokine responses in leishmaniasis. *Curr. Opin. Immunol.* 5:524–531.
9. Scott, P. 1991. IFN- γ modulates the early development of Th1 and Th2 responses in a murine model of cutaneous leishmaniasis. *J. Immunol.* 146:3149–3155.
10. Scharton-Kersten, L.C., C. Alfonso, M. Wysocka, G. Trinchieri, and P. Scott. 1995. IL-12 is required for natural killer cell activation and subsequent T helper cell development in experimental leishmaniasis. *J. Immunol.* 154:5320–5330.
11. Kamhawi, S., Y. Belkaid, G. Modi, E. Rowton, and D. Sacks. 2000. Protection against cutaneous leishmaniasis resulting from bites of uninfected sand flies. *Science*. 290:1351–1354.
12. Behin, R., J. Mael, and D.S. Rowe. 1977. Mechanisms of protective immunity in experimental cutaneous leishmaniasis of the guinea-pig. III. Inhibition of leishmanial lesion in the guinea-pig by delayed hypersensitivity reaction to unrelated antigens. *Clin. Exp. Immunol.* 29:320–325.
13. Dienes, L., and T. Mallory. 1932. Histological studies of hypersensitive reaction. Part I. The contrast between the histological responses in the tuberculin (allergic) type and the anaphylactic type of skin reactions. *Am. J. Pathol.* 8:689.
14. Raffel, S., and J.M. Newel. 1958. Delayed hypersensitivity induced by antigen-antibody complexes. *J. Exp. Med.* 108:823.
15. Coler, R.N., Y.A.W. Skeiky, P.J. Ovendale, T.S. Vedvick, L. Gervassi, J. Guderian, S. Jen, S.G. Reed, and A. Campos-Neto. 2000. Cloning of a *Mycobacterium tuberculosis* gene encoding a PPD protein that elicits strong tuberculosis specific delayed type hypersensitivity. *J. Infect. Dis.* 182:224–233.
16. Mendez, S., S. Gurunathan, S. Kamhawi, Y. Belkaid, M.A. Moga, Y.A.W. Skeiky, A. Campos-Neto, S.G. Reed, R.A. Seder, and D. Sacks. 2001. The potency and durability of DNA- and protein-based vaccines against *Leishmania major* evaluated using low-dose, intradermal challenge. *J. Immunol.* 166:5122–5128.
17. Gurunathan, S., C. Prussin, D.L. Sacks, and R.A. Seder. 1998. Vaccine requirements for sustained cellular immunity to an intracellular parasite infection. *Nat. Med.* 4:1409–1415.
18. Sharifi, I., A.R. FeKri, M.R. Aflatonian, A. Khamesipour, A. Nadim, M.R. Mousavi, A.Z. Momeni, Y. Dowlati, T. Godal, F. Zicker, et al. 1998. Randomised vaccine trial of single dose of killed *Leishmania major* plus BCG against anthroponotic cutaneous leishmaniasis in Bam, Iran. *Lancet*. 351:1540–1543.