CLINICAL IMPLICATIONS OF BASIC RESEARCH

Leishmania — A Parasitized Parasite

Phillip Scott, Ph.D.

Alerting the host to an infection is a critical step in the development of an immune response, and identifying the pathogen-specific molecules that sound the alarm has preoccupied immunologists for more than 20 years. A recent study by Ives et al.[1] shows that infection of the protozoan parasite leishmania with an endogenous virus augments the immune response — which perhaps is not such a good thing for the patient.

Leishmaniasis is caused by many different species of intracellular protozoan parasites, and it is prevalent in southern Europe, the Middle East, India, parts of Africa, Central America, and South America. Infection can lead to either a visceral and potentially fatal disease or a cutaneous lesion that may resolve over time. However, cutaneous leishmanial lesions often do not resolve quickly and may metastasize to other cutaneous sites. In South America, a particularly severe form of the disease, termed mucocutaneous leishmaniasis, develops after resolution of a primary lesion in a small number of people. Paradoxically, the development of mucocutaneous leishmaniasis does not stem from a weak immune response but is instead due to an unregulated inflammatory response that leads to a massive infiltration of inflammatory cells. This infiltration results in severe destruction of the nasopharyngeal mucosa, despite the effective reduction of the number of parasites. At present, there is no way to predict in whom this more severe form of the infection will develop.

Leishmania parasites invade macrophages and multiply within an endosomal compartment. Toll-like receptors (TLRs) — pattern-specific recognition receptors that detect infectious organisms at an early stage of infection — cover the cell surface and line endosomal compartments; these receptors have the opportunity to interact with the parasite at either site. To determine whether mucocutaneous leishmaniasis–derived *Leishmania guyanensis* differentially activates macrophages, Ives et al. infected macrophages with clones that promoted metastasis and clones that did not. Their results were dramatic and clear — metastasis-promoting clones induced much higher levels of proinflammatory cytokines and chemokines than did clones that did not promote metastasis. Using various TLR pathway–mutant mice, the authors went on to show that this response was dependent on the presence of the endosomally localized TLR 3 gene product (TLR3), which recognizes double-stranded RNA, and its downstream adaptor protein, toll–interleukin-1–receptor domain-containing adapter-inducing interferon-beta (TRIF). Aware that some South American leishmania parasites contain a double-stranded RNA virus (LRV1), which was first described almost 20 years ago,[2] Ives et al. asked whether the presence of the virus influenced parasite-induced macrophage responses. Importantly, metastasis-promoting clones had higher levels of LRV1, and LRV1 double-stranded RNA alone promoted high levels of inflammatory cytokines; this finding supports the hypothesis that LRV1-bearing parasites might augment disease through TLR3-dependent pathways in a subgroup of patients with mucocutaneous leishmaniasis (Fig. 1).

So, how might the virus influence the pathogenesis of leishmaniasis? Obviously, an increased immune response would normally be associated with efficient parasite clearance and less disease. However, in mucocutaneous leishmaniasis, the immune response is not down-regulated once the parasites are controlled, thereby exacerbating disease. The hypothesis is that the presence of the virus maintains an active immune response, leading to severe immunopathologic conditions. Activation of macrophages is unlikely to be completely dependent on the presence of this virus, since leishmania molecules are known to bind at least five different TLRs.[3] However, the data presented in this study suggest that LRV1 may greatly amplify the responses of macrophages after interaction with leishmania parasites. How this effect on macrophages promotes metastasis has yet to be determined.

To date, the distribution of leishmania strains containing LRV1 has been limited to specific re-

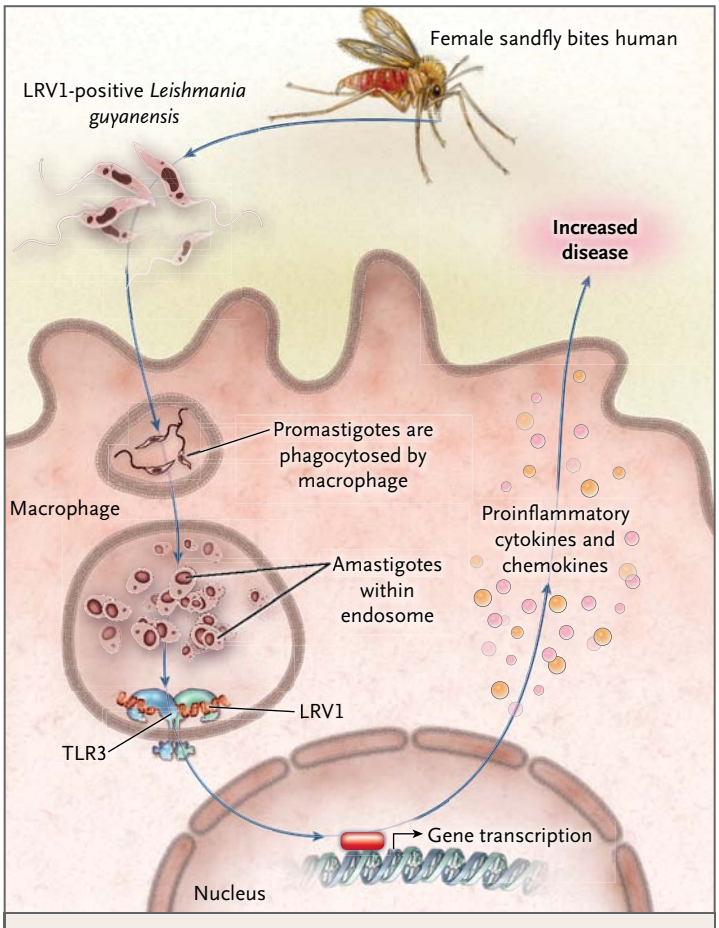

Figure 1. Leishmania and LRV1.

Leishmania parasites are transmitted to the mammalian host by a sandfly. Once inoculated into the skin, the parasites in the promastigote stage infect macrophages and transform into the amastigote form. The parasites live and divide within an endosomal compartment of the macrophage. A recent study by Ives et al.[1] shows that some leishmania parasites contain an RNA virus, termed LRV1, which promotes the production of proinflammatory molecules such as tumor necrosis factor α, interleukin-6, CXCL10, and CCL5. The response to the virus is mediated by the TLR 3 gene product (TLR3), an RNA receptor that is expressed within the same endosomal compartment as the parasites. Ives et al. hypothesize that viral RNA is released by dead parasites soon after infection; the binding of viral RNA to TLR3 results in the production of cytokines and chemokines that enhance inflammatory responses and thus exacerbate disease.

gions in South America,[4] and a larger number of strains must be surveyed to support the hypothesis that those containing the virus are associated with the subsequent development of mucocutaneous leishmaniasis. Moreover, it will be important to determine whether the virus influences primary leishmania infection. For example, early after infection with *L. braziliensis*, some patients have an exaggerated immune response that is manifested by dramatic lymphadenopathy before the development of a cutaneous lesion.[5] Could this exaggerated response be due to the presence of the LRV1, and could it promote early dissemination of the parasite? Finally, if the presence of LRV1 is indeed linked to an increased incidence of severe leishmania-induced disease, screening for the virus could become an important part of diagnosis. Currently, the standard of care in Brazil for cutaneous leishmaniasis is treatment with pentavalent antimony. However, other available treatments, such as liposomal amphotericin, may be more effective. If the authors' hypothesis is borne out, patients infected with strains of leishmania containing LRV1 might be treated more aggressively at the time of diagnosis to prevent the development of mucocutaneous disease.

Disclosure forms provided by the author are available with the full text of this article at NEJM.org.

From the School of Veterinary Medicine, University of Pennsylvania, Philadelphia.

1. Ives A, Ronet C, Prevel F, et al. Leishmania RNA virus controls the severity of mucocutaneous leishmaniasis. Science 2011;331:775-8.
2. Stuart KD, Weeks R, Guilbride L, Myler PJ. Molecular organization of Leishmania RNA virus 1. Proc Natl Acad Sci U S A 1992;89:8596-600.
3. Liese J, Schleicher U, Bogdan C. The innate immune response against Leishmania parasites. Immunobiology 2008;213:377-87.
4. Salinas G, Zamora M, Stuart K, Saravia N. Leishmania RNA viruses in Leishmania of the Viannia subgenus. Am J Trop Med Hyg 1996;54:425-9.
5. Barral A, Barral-Netto M, Almeida R, et al. Lymphadenopathy associated with Leishmania braziliensis cutaneous infection. Am J Trop Med Hyg 1992;47:587-92.