Review

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The immunological, environmental, and phylogenetic perpetrators of metastatic leishmaniasis

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Cutaneous leishmaniases have persisted for centuries as chronically disfiguring parasitic infections affecting millions of people across the subtropics. Symptoms range from the more prevalent single, self-healing cutaneous lesion to a persistent, metastatic disease, where ulcerations and granulomatous nodules can affect multiple secondary sites of the skin and delicate facial mucosa, even sometimes diffusing throughout the cutaneous system as a papular rash. The basis for such diverse pathologies is multifactorial, ranging from parasite phylogeny to host immunocompetence and various environmental factors. Although complex, these pathologies often prey on weaknesses in the innate immune system and its pattern recognition receptors. This review explores the observed and potential associations among the multifactorial perpetrators of infectious metastasis and components of the innate immune system.

An ancient and emerging disease

Leishmaniases have persisted for centuries as life-threatening and disfiguring parasitic diseases affecting millions of people across the subtropics. Currently, 98 countries are listed as having endemic disease, amounting to an estimated 12 million cases with 2 million more each year [1]. Human disease is caused by sp. of *Leishmania* protozoan parasites and is cycled among hosts through the bite of a female sand fly vector. Symptoms range from single self-healing cutaneous lesions to fatal visceralization or chronic metastatic dissemination throughout the skin. However, despite its prevalence, persistence, and conspicuous symptoms, the disease remains largely uncontrolled, with few new treatment options and no comprehensively effective vaccine. Migration and densification of populations in subtropical regions are compounding with global warming and a growing HIV-positive (immunodeficient) demographic to class leishmaniasis as a serious emerging global threat [2]. Further, growing local and international instability has fuelled major outbreaks in new populations that spread quickly among the vulnerable of conflict zones, living in densely packed and poorly insulated shelters. These unsettled populations pose a risk of widening leishmanial geography during resettlement, as was the case after the Sudanese Civil War, the Gulf and Iraq wars, and currently among Syrian refugees [3,4].

The centuries of geographically isolated evolution have allowed each *Leishmania* spp. to develop intricate pathways

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Glossary

Damage- (or Danger-) associated molecular patterns (DAMPs): molecules that are generally present due to a noninfectious threat to cellular integrity and are capable of initiating signaling cascades, through PRRs such as NLRs and TLRs. Some examples are proteins released during DNA damage such as cytosolic DNA and RNA fragments, or nucleotides such as ATP. Fragments of damaged tissue such as hyaluronan, uric acid, and heparin sulfate have also been described as potent DAMPs.
Highly active antiretroviral therapy (HAART): a combination of at least three drugs proven to suppress HIV replication, thus prolonging and improving the quality of life for individuals with HIV. A combination is used to avoid the development of drug resistance.
Immune reconstitution inflammatory syndrome (IRIS): a condition developing during immune recovery from a major immunosuppressive event (e.g., HIV infection, or VL) in which the immune system responds to previously acquired antigens with an overwhelming level of inflammation that paradoxically worsens the disease.
Kala-azar (KA): a Hindi term for 'Black Fever', describing the unexplained cutaneous discoloration associated with end-stage VL, where parasites fatally (if left untreated) infest the liver, spleen, and bone marrow.
***Leishmania* RNA virus (LRV):** a cytoplasmic double-stranded RNA (dsRNA) virus residing within some strains of the *Leishmania* parasite, which may act as a virulence factor in metastatic leishmaniasis.
Nucleotide-binding oligomerization domain receptors (NLRs): also called Nod-like receptors, are intracellular sensors of PAMPS and DAMPs able to cooperate with TLRs to regulate or potentiate the inflammatory and apoptotic response.
Pathogen-associated molecular patterns (PAMPs): small molecular motifs common among certain pathogen groups that are recognized by PRRs in cells of the innate immune system and generally produce a cytokine signaling cascade. Some examples include bacterial lipopolysaccharide (LPS, specific to TLR4), flagellin (TLR5), dsRNA (TLR3), and unmethylated CpG DNA (TLR9).
Retinoic acid-inducible gene 1 like receptors (RLRs): also called RIG-1-like receptors, are cytoplasmic RNA helicase enzymes, which recognize viruses by binding their dsRNA. RIG-1, MDA5, and LGP2 are the currently described members of this PRR family.
T helper cell subsets (Th1/Th2/Th17/Treg): subsets of a CD4 T cell lineage, which promote various types of immune response. Th1: cell mediated cytotoxic response (via IFN-γ). Th2: B cell mediated antibody response (via IL-4, IL-5, and IL-13). Th17: antifungal response (via IL-17A). Treg: anti-inflammatory response (via IL-10).
Toll-like receptors (TLRs): are PRRs able to detect a range of pathogenic patterns on the plasma membrane (TLR1, TLR2, TLR4, TLR5, and TLR6) or within the endosomal compartment (TLR3, TLR7/8, and TLR9).

of immune evasion, creating various symptomatic outcomes, and enabling parasites to persist under astounding immunological pressure, even existing as life-long infections after symptomatic resolution [5].

A common route of entry – widely different outcomes

Leishmania is generally transmitted through the bite of an infected sand fly. However, from this common origin, the same sp. can cause widely different outcomes. In most instances, disease is 'asymptomatic', without any obvious pathology, although still able to support life-long infection. The presence of persistent parasites in asymptomatic infections is a double-edged sword – on the one hand, potentially conferring immunity to superinfection, but on the other hand, creating the dangerous likelihood of reactivation, which is often associated with a more severe symptomatic outcome. In infections for which pathology is overt, outcomes can again vary widely. Localized cutaneous leishmaniasis (LCL) occurs in many cases, which can persist as chronic open lesions or resolve into hyperpigmented scars. For the more severe forms of leishmaniasis, pathology is not limited to the infection site but instead progresses in various ways that can be divided into metastatic leishmaniasis, diffuse CL (DCL) or a systemic visceralization (VL) that has an important cutaneous complication, post-kala-azar dermal leishmaniasis (PKDL). These forms can also appear following seemingly 'asymptomatic' infections without a prior cutaneous presentation.

Surprisingly, little is known about the basic mechanisms of symptomatic divergence. This review aims to assemble the current knowledge on the immunological, environmental, and phylogenetic perpetrators of persistent and metastatic outcomes, which significantly complicate the diagnosis, treatment, and control of leishmaniasis. We also use this opportunity to propose new potential risk factors that are supported by anecdotal evidence with the hope to stimulate much-needed further research.

Symptomatic outcomes of cutaneous leishmaniasis

Human infections are generally caused by species of two major *Leishmania* subgenera, namely *Leishmania* (*Leishmania*) and *L. (Viannia)*. Although *L. (Leishmania)* is found worldwide, the majority of infections occur in the Paleotropics (Eurasia and Africa), where common infecting species are *Leishmania major*, *Leishmania tropica*, *Leishmania aethiopica*, and *Leishmania donovani*. Species of the *Viannia* subgenus, by contrast, are exclusively endemic in the Neotropics (the Americas), with common infections being caused by *Leishmania braziliensis*, *Leishmania panamensis*, and *Leishmania guyanensis*. Depending on the infecting species and the immune response in a susceptible host, *Leishmania* parasites can induce two major pathologies: VL or CL.

Although VL, or 'kala-azar' (see Glossary), is the most serious form of the disease, it is relatively rare, contributing to only 10% of all leishmaniasis worldwide. *Leishmania* parasites are mostly dermotropic, where even VL is often followed by the diffuse and difficult-to-treat cutaneous lesions of PKDL. This review focuses on the various forms of persistent CL.

Localized cutaneous leishmaniasis

CL is endemic in numerous regions of the subtropics. It is most frequently caused by *L. major*, *L. tropica*, and *L. aethiopica* in the Paleotropics, whereas in the Neotropics it is caused by *Leishmania mexicana* and *Leishmania amazonensis*, or *L. (Viannia) braziliensis*, *L. (Viannia) panamensis*, and *L. (Viannia) guyanensis* [6]. Although CL generally manifests as a lesion localized at the inoculation site (LCL), its various physical presentations and immunopathologies have complicated diagnosis and scuttled attempts of forming a universal therapeutic or vaccination strategy. Globally, lesions can vary from a small self-healing ulceration to granulomatous nodules and large, seeping, erythematous wounds. Chronic infection and inflammation can last for several months or years and often leads to significant tissue damage and permanent, hyperpigmented scarring. In certain cases, the infection metastasizes to sites beyond the inoculation and can be referred to as metastatic leishmaniasis, by analogy to tumor cell metastasis.

Metastatic leishmaniasis

Metastatic complications occur across all regions where leishmaniasis is endemic but are particularly prevalent and aggressive in *L. (Viannia)* infections of the Neotropics [7]. They may also present with various symptoms, seemingly dependent on differences in the immune response elicited by the various metastatic parasite species (Box 1). Particular symptomatic outcomes can be grouped geographically (Figure 1), where, for example, the Paleotropics hosts many forms of nonulcerative, papular, and herpetiform leishmaniasis spreading within a small radius of the primary lesion, whereas the Neotropics is better known for large ulcerative and granulomatous lesions, which often occur at sites distant from the primary lesion. Indeed, certain Neotropical parasites have a specific tropism for the delicate mucosal tissues of the nose and face, creating a particularly disfiguring disease known as mucosal leishmaniasis. The inflammation in the nasal mucosa is inordinately potent when contrasted with the sometimes undetectably low number of parasites. Lesion biopsies often reveal no infection. This phenomenon emphasizes the major role of the immune response in disease pathology and the potential of immunomodulatory agents in antileishmanial therapy. However, the immune involvement is diverse and opposing responses have been blamed for the various forms of infectious metastasis. For example, the mostly Paleotropical recidivans CL is described as a symptomatic reactivation of infection within the scars of a healed lesion; it is characterized by a potent cell mediated response in multiple nodules, which spread and coalesce to form significant tissue damage. Conversely, a total lack of a cell mediated response has been seen in DCL, where infection diffuses into hundreds of immunologically anergic nodules throughout the skin [8].

Post-kala-azar dermal leishmaniasis

PKDL is an important dermal complication of Paleotropical VL, occurring in 10–20% of VL patients in India and up to 60% in Eastern African states such as Sudan and Ethiopia [9]. Although treatment is essential in Indian