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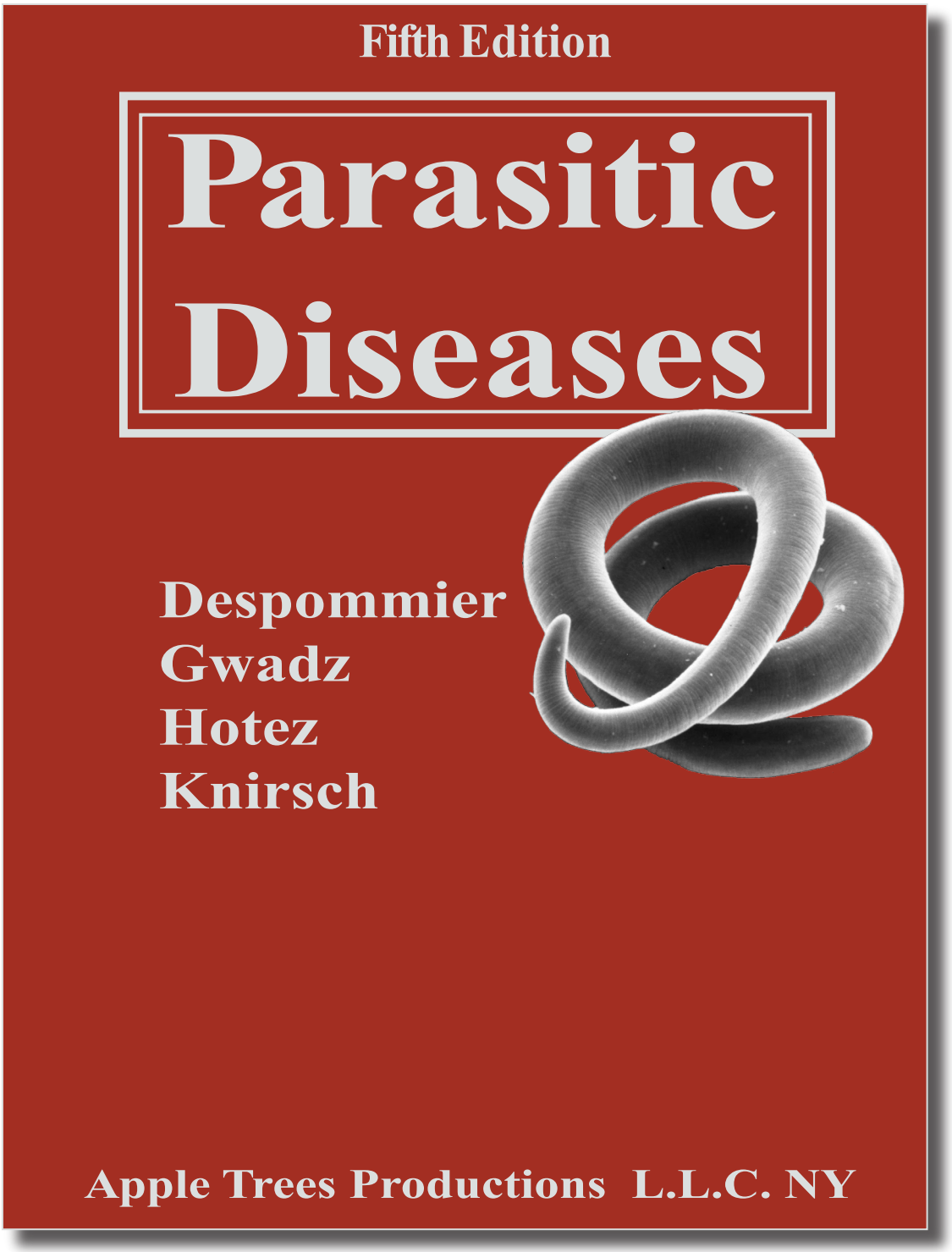

2. Introduction to the Leishmania

The genus Leishmania comprises a genetically diverse group of vector-borne haemoflagellate parasites.[1, 2] *Leishmania spp.* are transmitted from host to host by the bite of sand flies (Fig. 2.1). *Phlebotomous spp.* are vectors of Old World Leishmaniasis, while sand flies in the genus Lutzomyia transmit Leishmaniasis throughout the Western Hemisphere.

Leishmania spp. are primarily zoonotic in nature,[3] infecting a wide range of vertebrates throughout the tropical and subtropical world. All possess a well-characterized kinetoplast and live as obligate intracellular parasites within macrophages and other phagocytic cells of the reticuolendothelial system. All species of Leishmania share many features of their genetics, mode of transmission, biochemistry, molecular biology, immunobiology, and susceptibility to drugs. Differences at all the above levels exist between the cutaneous and visceralizing species of Leishmania.

The number of humans suffering from Leishmaniasis is unknown, but a conservative guess is that the prevalence is several million. More than 350 million people live within an area of transmission (see www.who.org). Leishmaniasis occurs in 88 countries located in Southern Europe, Africa, Asia, South Asia, and South and Central America. The subgenus Leishmania is distributed throughout the Old and the New World, whereas the subgenus Viannia is only found in the New World. In the Western Hemisphere, no less than fifteen species regularly infect people*: Leishmania (Leishmania) amazonensis, Leishmania (Viannia) braziliensis, L. (V.) colombiensis, L. (L.) donovani, L. (L.) garnhami, L. (V.) guyanensis, L. (L.) infantum chagasi, L. (V.) lainsoni, L. (V.) lindenbergi, L. (L.) mexicana, L. (V.) naiffi, L. (V.) panamensis, L. (L.) pifanoi, L. (V.) shawi,* and *L. (L.) venezualensis*. In the Eastern Hemisphere, there are significantly fewer species that infect humans: *L. (L.) donovani, L. (L.) infantum*, *L. (L.) aethiopica*, *L. (L.) major,* and *L. (L.) tropica*.

Figure 2.1. Sand fly taking a blood meal.

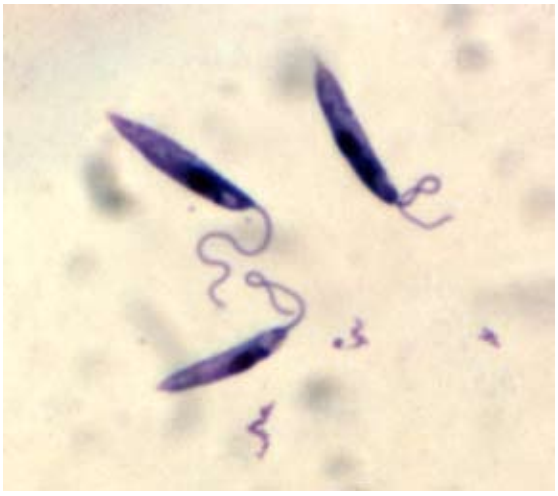

Figure 2.2. Promastigotes of *Leishmania spp.,* as seen in culture.

As might be expected, clinical conditions caused by *Leishmania spp*. vary greatly, depending upon the species of Leishmania and the immune status of the host. Disease can present as cutaneous lesions that resolve over time, or as systemic disease of the reticuloendothelial system often resulting in death of the host if left untreated. Fortunately, there are fewer clinical entities than the number of species of pathogens that cause them; cutaneous, muco-cutaneous, and visceral Leishmaniasis.

This introductory chapter will summarize the biology and molecular biology of the entire group, with the tacit assumption that they all behave similarly in their intracellular environment and within their sand fly vectors. Exceptions will be presented whenever they relate to a disease process applicable only to that species. The Leishmania Genome Project is based on the genome of *Leishmania major* and is essentially completed (see www.sanger.ac.uk/Projects/L_major/).

There are no commercially available vaccines

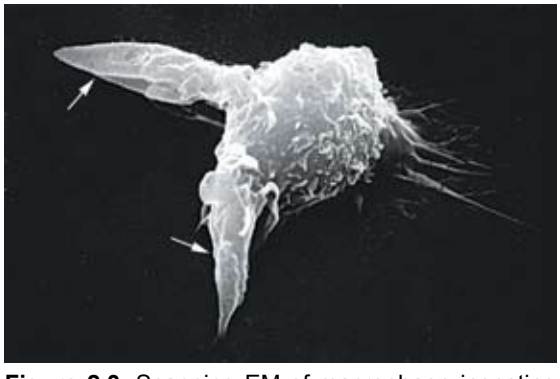

Figure 2.3. Scanning EM of macrophage ingesting two promastigotes (arrows). Photo by K-P Chang.

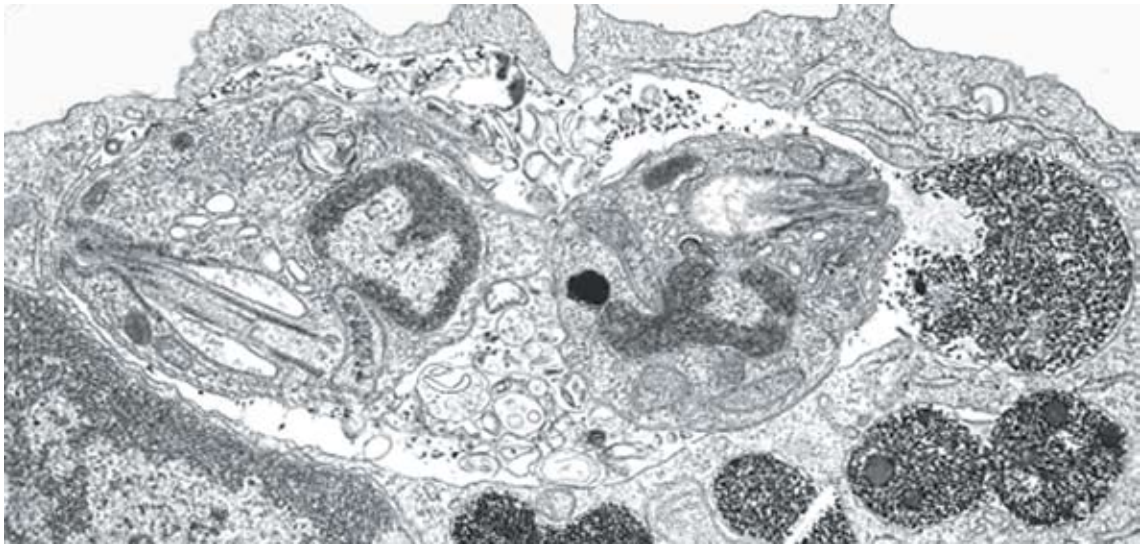

Figure 2.4. Electron micrograph of two amastigotes of *Leishmania spp.* Photo by K-P Chang.

as of yet, but infection with many of the species of Leishmania results in permanent immunity to reinfection with the same species.[4] Perhaps data derived from the genome project will hasten the development of an effective, cheap, easy-to-administer vaccine against the most dangerous forms of Leishmaniasis.

Life Cycle

The sand fly

Infection begins when the insect obtains blood from an infected mammal. As it does so it injects saliva containing numerous well-characterized bioactive components, many of which are peptides or proteins.[7, 8] One such protein, maxadilan, is a potent vasodilator,[9] a 7 kDa peptide believed essential to the taking of a blood meal by the fly. Maxadilan's primary mode of action is to reduce intracellular calcium in the host at the site of the bite wound through a cAMP-dependent mechanism, causing arterial dilation.[10, 11] Blood can then easily be drawn up by the sand fly. The receptor for maxadilan is the pituitary adenylate cyclase-activating polypeptide, a membrane-bound protein found on many cell types in the body, including smooth muscle cells and macrophages. During feeding, sand flies become maximally filled with blood and cannot regurgitate the excess, due to the inhibition of the emptying reflex by a parasite-specific peptide that interacts with myosin to prevent contraction of stomach muscle.[12] This enhances the chances for the sand fly to become infected and to remain so throughout the period that the parasite needs (i.e., 1-2 weeks) in order to develop into the infectious stage for a mammalian host.

The parasite undergoes a complex series of developmental changes inside the gut tract of the sand fly,[13] and progresses to the flagellated metacyclic stage after about a week following ingestion. It first attaches to the wall of the gut tract by non-specific hydrophobic interactions between the surface of the parasite's flagella and the insect stomach cell membrane.[14] Attachment to other regions of the insect intestinal tract later on during the differentiation to the metacyclic promastigote stage is mediated, in part, by specific insect galectins (e.g., PpGalec),[15, 16] and the parasite cell surface multipurpose molecule, lipophosphoglycan. The release of infectious stage organisms, a necessary final step in their development, is mediated by arabinosyl capping of LPG scGal residues upon differentiation to the metacyclic stage.[17] The leptomonad stage (heretofore unrecognized) locates to the anterior region of the gut and secretes a gel-like substance that blocks the digestive tract of the sand fly, causing the infected insect to regurgitate its complement of infectious metacyclic promastigotes into the host's subcutaneous tissues during feeding.[13] This is similar to a strategy employed by the plague bacillus while inside the rat flea.

The mammalian host

The flagellated metacyclic promastigote stage (Fig. 2.2) resides in the anterior midgut and thorax and is injected into the host along with the dipteran's salivary secretions. In addition to aiding the parasite to establish infection in the sand fly (see above),

some of those same salivary proteins aid in Leishmania's ability to colonize the mammalian host.[5, 6] At least one salivary product interferes with production of IL-10.[18] Maxadilan induces negative effects on host immune cell function, including inhibition of the release of TNF-α, upregulation of IL-6 synthesis in macrophages, and stimulation of prostaglandin E2 production.[19] Presumably, some or all of these altered host responses play a role in aiding the parasite to establish itself in the host, although their precise mechanisms have not yet been determined.

The promastigotes deposited in the extracellular matrix at the site of the bite adhere there, aided by lipophosphoglycan and a surface membrane laminin receptor protein on their surface.[20] The promastigotes induce the production of antibodies and become opsonized. As the result, the C3 component of complement attaches to the parasite cell surface.[21] They are then able to attach to red cells or platelets and become engulfed by dendritic cells[22] or macrophages (Fig. 2.3). Many would-be pathogens are unable to survive this step and are digested by inclusion into phagolysosomal vacuoles. In contrast, Leishmania are able to avoid digestion and are free to differentiate into amastigotes to begin the intracellular phase of their life cycle.

It is at this point in the life cycle that differences between species of Leishmania become obvious. Those that cause only cutaneous lesions remain at the site throughout the infection, while those that cause visceral or mucocutaneous lesions somehow manage to find their way to the appropriate site in the body. The host and parasite factors resulting in these different biologies are still being sorted out in the research laboratory. For example, dendritic cells increase in number in the draining lymph nodes of experimentally infected mice infected with *L. (L.) tropica*, but infected dendritic cells do not appear to migrate to the lymph nodes.[23] How the parasites reach the draining lymphoid tissue remains to be demonstrated.

The mechanism of Leishmania survival inside the macrophage involves alteration of the phagolysosome, and comes about as the result of host cell interaction with lipophosphoglycan.[24] Infected phagocytes display abnormal maturation of the phagolysosome due to lipophosphoglycan's interference with F-actin, an essential component of the process of fusion of lysosomes with the phagocytic vacuole.[25] This lack of fusion, in part, enables the parasite to evade digestion. Amastigotes divide inside their host cells (Fig. 2.4) and can remain at the site of injection, resulting in the clinical condition known as a cutaneous Leishmaniasis. Alternatively, they can be carried by the phagocytes to mucocutaneous junctions, or to the reticuloendothelial tissues, resulting in mucocutaneous or visceral Leishmaniasis, respectively.

Leishmania spp. have salvage pathways[25, 28] for nucleic acid synthesis. The enzymes reside within the glycosome,[26] a specialized organelle unique to the kinetoplastidae. Cutaneous lesions form in most instances, allowing sand flies access to infected host cells at the raised margin. Circulating macrophages in blood-harboring amastigotes can also be taken up by the vector.

Cellular and Molecular Pathogenesis

Virulence factors and pathogenesis

The cell and molecular biology of *Leishmania spp.* has been reviewed.[27, 28] Most of what is known regarding the biology of Leishmania is derived from murine models and in vitro cell culture using various species of Leishmania.[29] The following summary of pathogenic mechanisms is derived from both types of experimental approaches. The turning-on of heat shock genes,[30] as well as cassettes of other developmentally regulated genes,[31] occurs as the parasite makes the transition from an environment dependent upon ambient temperature (sand fly) to the homeothermic essential niche inside the mammalian host cell. The amastigote downregulates IL-12, which delays the onset of cell-mediated protective immune responses.[32, 33] Amastigotes of *L. mexicana* interfere with antigen presentation by macrophages, employing cysteine protease B.[34] The amastigote stage also possesses potent cysteine protease inhibitors,[35] which it presumably uses to modify host cysteine protease activity during intracellular infection.

Replication of amastigotes is dependent upon host cyclophillins,[36] since division is inhibited by cyclosporin A.[37] The membrane of the promastigote contains a zinc protease, leishmanolysin,[38] a 63 kDa glycoprotein whose crystalline structure has been determined.[39] Current evidence favors a role for leishmanolysin in migration of parasites through the intracellular matrix by digestion of collagen type IV after their release from infected cells.[40] Induction of the chemokine MIP-1β by neutrophils harboring amastigotes attracts macrophages to the site of infection. Macrophages then engulf infected neutrophils, thus acquiring the infection.[41]

Protective immune mechanism(s)

The mechanism(s) of protective immunity vary with clinical types of Leishmania.[42] The cutaneous forms typically induce well-defined Th-1 responses, which are T-cell-mediated, and play a critical role in controlling and finally eliminating the organism.[43] Permanent immunity to reinfection with cutaneous Leishmania causing organisms is the rule, and depends upon inducing high levels of CD4+ T-cell memory.[44] In addition, Langerhans cells are thought to play a major role in antigen presentation and in the induction of IL-12[45, 46] and IL-27.[47] The main effector mechanism involves CD4+ T cell-dependent macrophage activation and subsequent killing of amastigotes by nitric oxide.[48] Chemokines are also important for immunity,[49, 50] and include MIP-3beta and INF-δ.[50] Antibodies appear to play no role in immunity to cutaneous Leishmaniasis, and probably aid the parasite in gaining entrance into the macrophage.[51]

Protective immune mechanisms induced by infection with visceral Leishmaniasis (*L. (L.) donovani and L. (L.) infantum*), include IL-12 and INF-δ. Immunity is suppressed by IL-10 and TGF-β.[43]

To further complicate the clinical spectrum of diseases caused by Leishmania, one has to be reminded of the fact that *Leishmania spp*. have been around a long time, and have, within the last 165 million years, begun to diverge evolutionarily due to continental drift. Organisms in the New World must, by necessity, behave somewhat differently from their ancestor species that still continue to infect mammals in the Old World. The same is true for its hosts, including humans. Thus, when considering the type of disease and the immune responses to them, there exist many exceptions to the above summaries. For an excellent review on this aspect of the biology of Leishmania, see McMahon-Pratt and Alexander.[52]

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