

Leishmaniasis of the New World: Current Concepts and Implications for Future Research

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INTRODUCTION	230
BIOLOGICAL AND MOLECULAR CHARACTERISTICS OF <i>LEISHMANIA</i> SPP.	230
TAXONOMIC STATUS OF THE NEW WORLD LEISHMANIAL PARASITES	231
HOST RESPONSE AND IMMUNITY TO LEISHMANIAL INFECTION	232
THE HUMAN LEISHMANIASIS	233
Clinical Forms and Etiology	233
Immunological Features.....	233
Pathology	237
Laboratory Diagnosis	237
Treatment	238
EPIDEMIOLOGIC FEATURES OF AMERICAN LEISHMANIASIS	239
Prevalence and Geographic Distribution.....	239
Sand Fly Vectors and Vertebrate Reservoirs	240
PREVENTION AND CONTROL	240
Vector Control	240
Elimination of Reservoir Hosts.....	240
Surveillance and Treatment of Human Cases	240
Personal Protection.....	240
Immunization	241
CONCLUDING REMARKS	241
ACKNOWLEDGMENTS.....	241
REFERENCES	241

INTRODUCTION

Leishmaniasis is endemic in many areas of tropical and subtropical America (at least 24 countries), where it constitutes a significant public health problem (103). The disease in this region is basically a zoonosis; humans are only incidental hosts in the life cycle of the various human pathogenic parasite species. Control of leishmaniasis in the New World is complicated by the variety of different *Leishmania* species and their diverse clinical manifestations and by the fact that each parasite species has a unique epidemiologic pattern. Furthermore, in many regions of endemicity in the Americas, two or more parasite species are often sympatric.

The outcome of leishmanial infection in humans depends largely on the immune responsiveness of the host and the virulence of the infecting parasite strain. The protozoa in this genus are capable of producing a broad spectrum of disease in humans, ranging from asymptomatic infection to horribly disfiguring forms of mucosal leishmaniasis (ML; espundia) or the potentially fatal visceral form of the disease (kala-azar).

The more benign self-healing forms of leishmanial infection usually result in protection against reinfection, and cell-mediated immunity is involved in this protection. These observations suggest that leishmaniasis might be controlled by immunization. However, considering the genetic polymorphism and biological diversity of the parasites, develop-

ment of effective vaccines may be a formidable task. Until such vaccines are available, more conventional measures such as vector and reservoir control and aggressive epidemiologic surveillance will continue to be the best options for prevention and containment of the disease.

BIOLOGICAL AND MOLECULAR CHARACTERISTICS OF *LEISHMANIA* SPP.

Parasitic protozoa of the genus *Leishmania* (Kinetoplastida:Trypanosomatidae) are a biologically diverse group of microorganisms. They possess a unique mitochondrial or kinetoplastic DNA (kDNA) and appear to be among the most highly diverged eukaryotic cells known (291). In nature, all *Leishmania* spp. are transmitted by the bite of infected phlebotomine sand flies (Diptera:Psychodidae). These parasites have two distinct stages in their life cycle: a motile flagellated promastigote stage that lives extracellularly within the alimentary tract of the sand fly vector (305–310) and a nonmotile amastigote stage that resides within macrophages of the vertebrate host (58).

Sophisticated laboratory models involving well-characterized cell lines, cloned parasites, genetically defined animals, and colony-reared sand flies are now available for studying experimental leishmaniasis. Many of these parasites can also be grown in vitro in defined or semidefined liquid media as either promastigotes (119) or axenic amastigotes (224). Consequently, considerable progress in defining the molecular and biological characteristics of these microorganisms, as

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well as the immunological mechanisms involved in host protection, has recently been made.

The following aspects of parasite virulence are now recognized: (i) infectivity varies even among clones of a given *Leishmania* strain (111, 137); (ii) amastigotes are generally more infective than promastigotes (58); (iii) actively motile promastigotes in the stationary phase of growth are more infective than the longer and slender forms in log-phase growth (265, 269); (iv) promastigotes often lose infectivity after long periods of *in vitro* cultivation (58); (v) the changes in virulence that are observed in different growth phases or after prolonged cultivation parallel the development of biochemical and antigenic changes in the parasites (266, 267); and (vi) during the differentiation process from infective (metacyclic) promastigote to amastigote, there is an increase in the expression of certain genes that probably preadapts the parasite to survival in the hostile environment of the macrophage phagolysosomes (57, 267). Spontaneous or experimentally generated genetic variants of differing infectivities have also been selected to define the molecular determinants of parasite virulence and to study possible drug or vaccine targets (35, 142, 143, 181).

Leishmania spp. multiply by binary fission in both their invertebrate and vertebrate hosts; however, a sexual process may also exist in this genus (158). Although it is generally believed that *Leishmania* spp. depend primarily on mutation for their variability (152), there is recent evidence of hybrid formation (41, 71, 81), gene amplification-deletion, and possible genetic exchange between distinct organisms (222), suggesting that genetic recombination may also be involved in the evolution of these parasites.

Recent genetic studies have revealed many interesting aspects of the molecular biology of leishmanial parasites. A number of nuclear genes have been cloned and identified; these genes are potential drug or vaccine targets (47, 173, 210), and they appear to be responsible for parasite virulence or developmentally regulated adaptation in the vertebrate host (57, 141, 161, 286). The *Leishmania* genome is relatively small, approximately 50,000 kb (163), and is diploid at most loci (69, 128). The various *Leishmania* species contain 25 to 30 small chromosomes that are readily separated by pulsed-field electrophoresis (90, 282). Molecular karyotypic analyses among leishmanial stocks have demonstrated chromosome size polymorphisms and a high degree of plasticity in the genome (29, 222, 275). In addition, current data suggest that a variety of chromosomal alterations involving amplification of certain genes may be a common characteristic among *Leishmania* spp. (36, 90, 128, 222). Several repeated gene families also have been demonstrated among *Leishmania* spp. (47, 127, 157, 161, 163, 173, 198, 286). These and other examples of gene amplification may play an important role in shaping the parasite's genome and providing new substrates for its evolution (35).

TAXONOMIC STATUS OF THE NEW WORLD LEISHMANIAL PARASITES

Taxonomic studies of leishmanial isolates from the New World indicate tremendous diversity within this genus. A number of new *Leishmania* species from sylvan areas of the Neotropics have been described recently. Some of those parasites are associated with disease in humans; others appear to be restricted to lower orders of mammals, such as rodents and edentates (100, 102, 103, 117, 146, 150, 152–154, 212, 287, 290). However, some of the latter parasites may yet

be shown capable of causing human disease, particularly in persons with altered cellular immune responses.

Because of differences in virulence and in the response to chemotherapeutic agents among the various *Leishmania* species, correct parasite identification is essential in any clinical study of leishmaniasis. Except for minor differences in size, all species of *Leishmania* are morphologically similar. For this reason, the initial criteria for identification and classification of the parasites associated with human disease were based on extrinsic characteristics (e.g., clinical manifestations, geographic and epidemiologic factors, and a variety of other biologic criteria). However, it is now recognized that these extrinsic characteristics can be quite variable, depending on such things as the immune status of the human host or the species and behavior of the vector. Consequently, they are not reliable criteria for establishing taxonomic status. The development of newer genetic, biochemical, and immunologic techniques has provided more precise taxonomic markers based on the intrinsic characteristics of the parasites themselves. Among the molecular tools currently in use are isoenzyme electrophoresis, analysis of minicircle kDNA heterogeneity, and species-specific monoclonal antibodies or DNA probes. All of these techniques can be used to identify leishmanial isolates by comparison to reference strains. For more detailed reviews of methods for the characterization of *Leishmania* spp., see Lainson and Shaw (152), Grimaldi et al. (103), and Rioux (251).

The taxonomic chronology of all *Leishmania* species, including their nomenclature and classification, also has been reviewed in detail (88, 152, 323). Since the original description of these parasites and the creation of the genus *Leishmania* by Ross in 1903, the number of recognized species has continually increased, and several different taxa or classification schemes have been proposed. In 1979, a systematic regrouping of the taxa was carried out by Lainson and Shaw (151), who recognized the distinct developmental patterns of some parasite species in the sand fly gut as having evolutionary, taxonomic, and biologic importance. More recently, the same authors proposed a further subdivision of the genus *Leishmania* (152), with the peripylarian parasites assigned to a new subgenus, designated *Viannia* Lainson and Shaw 1987, and the suprapylarian groups assigned to the subgenus *Leishmania* Saf'anova 1982. The latter classification system depends on extrinsic characteristics of the parasite, namely, where it develops in the sand fly gut. Preliminary studies (7, 305) have suggested that this is not an all-or-none phenomenon and that the site(s) of parasite development in the insect gut may be variable, depending on the sand fly species and other external factors. Further application of molecular techniques at the genomic level, which would be useful for studying molecular evolution and phylogenetic relationships, is required to clarify this proposed taxonomic distinction. In more modern classification systems, biochemical analyses and Adansonian systematics of the parasites rather than their biologic, clinical, or epidemiologic features are the major criteria (100, 102, 145–147, 152). Numerical taxonomy based on phenetic, choristic, and cladistic analyses of isoenzyme data also has been used to define the molecular evolution of and phylogenetic relationships among leishmanial parasites (102, 251, 252). The next stage in the classification of the *Leishmania* spp. probably will be based on their molecular genetics, and a broad assemblage of methodologies, including analyses of restriction fragment length polymorphisms of nuclear DNA and kDNA, molecular karyotyping of chromosomal DNA

(37, 38), and DNA sequencing of phylogenetically informative nuclear genes, such as rRNA and dihydrofolate reductase-thymidylate synthase sequences, will be used (303). This information will help define the fundamental mechanisms involved in species identification and taxonomic divergence among these microorganisms. It may also be useful in vaccine development and in clinical and epidemiological studies.

HOST RESPONSE AND IMMUNITY TO LEISHMANIAL INFECTION

Clinical and experimental evidence indicates that vector, parasite, and host factors all influence the evolution and outcome of leishmanial infection. The failure of the vertebrate host to control the infection is apparently related to two major factors: (i) the ability of some strains of *Leishmania* spp. to resist the microbicidal effect of activated macrophages and (ii) the breakdown of host-protective cellular immune responses. As obligate intracellular parasites, these microorganisms have evolved complex strategies for evading host defense mechanisms that occur before (the complement-mediated killing process), during (the toxic effect of oxygen metabolites produced during the macrophage-induced respiratory burst), and after (the nonoxidative killing effect of lysosomal hydrolases or nitric oxide from L-arginine) entry into host cells (58, 73, 110, 167, 169). Recent studies of several membrane surface enzymes and other macromolecules synthesized by leishmanial parasites have provided new insights about their functions in the establishment of infection (73, 92, 93, 136, 137, 169, 191, 210, 265). However, the various mechanisms developed by leishmanial parasites for evading host defenses are still not well defined. New evidence suggests that the parasite virulence determinants which are responsible for evasion of such defenses are often developmentally regulated, allowing these organisms to survive in the immunologically hostile environment of the host (57, 80, 110, 265). Recent experimental studies also have shown that the saliva of some sand fly species contains one or more substances that enhance leishmanial infection in animals (164, 276, 300). Salivary gland material from *Lutzomyia longipalpis* has an inhibitory effect on the abilities of macrophages to present parasite antigens to specific T cells and can down-regulate the ability of these cells to produce hydrogen peroxide in response to an activating stimulus from gamma interferon (IFN- γ) (297).

Although a number of antibody-mediated effects against leishmanial infection have been demonstrated, cell-mediated immunity, rather than humoral immunity, is considered the main protective immune response in leishmaniasis. Earlier studies indicated a good correlation between delayed-type hypersensitivity (DTH) and resistance to clinical and experimental leishmaniasis (186, 234). More recent data have shown that, although the tuberculin type of DTH may be protective, the Jones-Mote reaction is correlated with disease exacerbation (77, 298). However, DTH per se does not seem to be a disease-promoting mechanism in the susceptible murine host (122).

The innate or acquired capacities of macrophages for killing intracellular parasites appear ultimately to eliminate or control leishmanial infection (31, 40, 186, 234). The current hypothesis is that T-cell-mediated macrophage activation is the major protective mechanism against leishmanial infection. After having contact with parasite antigens that are displayed on the infected host cell membrane (34), T cells are stimulated to secrete IFN- γ . This substance, together

with other cytokines elaborated by T cells or macrophages (e.g., interleukin-2 [IL-2], granulocyte-macrophage colony-stimulating factor, and tumor necrosis factor alpha), leads to the local activation of macrophages to kill amastigotes (96, 171). These lymphokines act as macrophage-activating cofactors and provide the second signal for IFN- γ in the induction of resistance to infection mediated by nitric oxide from L-arginine (30, 31, 75, 96, 168, 169).

Although macrophage functions are necessary for the immunological control of leishmaniasis, the suppressive responses of these cells or of their parasite-induced functions (62, 63, 199, 243, 244) can also play important roles in determining the susceptibility to infection. Barral-Netto et al. (27) recently reported that one way in which parasites can survive the hostile environment within macrophages is by inducing the cells to produce large amounts of transforming growth factor β . Injections of this substance rendered mice susceptible to leishmanial infection, while injections of antibodies that block transforming growth factor β helped mice clear preexisting leishmanial infections.

The use of murine models for the identification of T-cell subsets secreting defined patterns of cytokines that lead to strikingly different effector functions has greatly improved our understanding of the regulation of the immune response (205) and of the cellular immune mechanisms involved in host resistance and susceptibility to leishmanial infection. Inoculation of genetically resistant inbred strains of mice with *Leishmania major* results in control and resolution of cutaneous infection. Resistance of the host is associated with selective activation and differentiation of effective CD4⁺ helper T (T_H) cells, the T_H1 subset, which are characterized by a very distinct cytokine secretion pattern (e.g., IL-2, IFN- γ , and lymphotoxin). In contrast, the progressive and fatal infections in more susceptible mouse strains are correlated with activation of the T_H2 CD4⁺ cell response, which expresses IL-4, IL-5, IL-6, and IL-10 among other interleukins (118, 122, 125, 166, 171, 172, 270, 284, 285). Protective helper-inducer T cells, which produced IFN- γ but were slow to proliferate in response to parasite antigen in vitro, were also isolated from BALB/cJ mice chronically infected with *Leishmania donovani* (122). IFN- γ seems to play a critical role in the early immune response that both optimally controls *L. donovani* infection and induces the tissue granulomatous response (294). However, the differential production of T_H1- and T_H2-derived cytokines does not seem to determine the genetically controlled or vaccine-induced rate of cure in murine visceral leishmaniasis (VL) (138).

The mechanisms leading to preferential induction and/or expansion of distinct T_H cell subsets are still not well understood. Evidence suggests that several factors may affect this process during leishmanial infection: (i) the cytokine environment present during the initial events of cell differentiation; (ii) the interaction with regional antigen-presenting cells, which can preferentially present different classes of antigens; (iii) the influence of other costimulatory signals; and (iv) the differential signalling mechanisms used by the T_H cell subsets after the engagement of the T-cell receptor (3, 85, 171, 205, 207). Evidence of cross-regulation of T_H cell subsets also exists (205), but it is still unclear how or to what extent the protective and the disease-promoting T-cell subpopulations interact during leishmanial infection (118, 124, 125, 166, 171, 270, 271). Contributions by other lymphocyte populations, such as CD8⁺ (83, 122, 270, 292, 299) and gamma-delta T cells (200), to the mediation of protective immunity against leishmanial infection have been

shown but are less well understood. Natural killer cells have been implicated in immunity against visceral *L. donovani* infection but not in resistance against cutaneous *L. major* infection in mice (144, 190). In another study, the spontaneous cytotoxicity of macrophage precursors from spleens of *L. donovani*-infected mice was defined as a cooperative effect of antibody-dependent cellular cytotoxicity and macrophage colony-stimulating factor activation (123). In addition, three different patterns of genetic control, influenced by both *H-2* (class II) and non-*H-2* genes, for the immune response to *Leishmania mexicana* antigens in mice have been distinguished (175).

THE HUMAN LEISHMANIASSES

Clinical Forms and Etiology

The currently recognized *Leishmania* species associated with human disease in the New World are listed in Table 1. In addition, other parasites (e.g., *Leishmania garnhami* and *Leishmania pifanoi*) that were originally described as distinct species on the basis of their unique clinical and epidemiologic features now, in view of more recent molecular studies, appear to be simply geographic variants of already recognized species. Moreover, a number of other leishmanial isolates have been associated with human disease but are still not fully characterized. The available data suggest that some of these isolates represent additional new species (103, 152).

The clinical manifestations and prognosis of infection with the various human pathogenic leishmanial parasites in the Americas have been reviewed recently (101, 312). A wide variety of clinical manifestations have been associated with most of the New World *Leishmania* species; many of the parasites are capable of producing in the human host a spectrum of disease rather than a single clinical form as was thought previously. The leishmaniasis of the New World can be grouped into two broad clinical categories: American visceral leishmaniasis (AVL) and American cutaneous leishmaniasis (ACL). The latter category includes a variety of forms that are commonly referred to by their characteristic clinical and pathologic features: cutaneous leishmaniasis (CL), which may heal spontaneously; ML, the hyperergic form; and diffuse cutaneous leishmaniasis (DCL), the anergic form. AVL is usually caused by *Leishmania chagasi*, although occasionally *Leishmania amazonensis* has been isolated from patients with visceral disease or with post-kala-azar dermal leishmaniasis (26). Likewise, *L. chagasi* has been associated with cutaneous lesions, apparently without visceral involvement (220, 233). In contrast, ACL is usually caused by parasite species belonging to the *Leishmania braziliensis* or *L. mexicana* complex; however, a few cases of ACL from Brazil (203), Ecuador, and Venezuela (117) have been associated with a parasite similar to the Old World *L. major*. Self-healing ulcerative CL accompanied by cellular immune responses has been associated with all of the New World dermatotropic *Leishmania* species. In contrast, DCL, the progressive, anergic, nonulcerative form of the disease that is accompanied by defective cellular immune responses, has been observed only with parasite species of the *L. mexicana* complex. ML, the hyperergic invasive ulcerative form that progresses in the absence of any apparent cellular defect, is most frequently associated with *L. braziliensis* (103), but *Leishmania panamensis* (278), *Leishmania guyanensis* (213, 277), and members of the *L. mexicana* complex (26, 313) can also cause this mutilating form of

the disease. In addition, a few human cases of mixed parasite infections (*L. braziliensis* and *L. amazonensis* or *L. braziliensis* and *L. chagasi*) have been described (221, 289). Cases of CL apparently due to naturally occurring hybrid parasites (*L. braziliensis* × *L. panamensis* and *L. braziliensis* × *L. guyanensis*) have also been reported recently from Nicaragua (71) and Venezuela (41), respectively. Recently, VL and disseminated CL due to *L. braziliensis* complex parasites have been reported in persons with human immunodeficiency virus type 1 infection (5, 6, 32, 70, 74, 94, 179, 204, 219, 228, 231, 255, 280) or in those being treated with immunosuppressant drugs (13). In summary, it is becoming increasingly apparent that the clinical expression of leishmanial infection in the human host is dependent on a number of different factors, of which the parasite species is only one. Most of the New World *Leishmania* species appear capable of producing a spectrum of disease manifestations, depending on the immune status of the host and other external factors.

Immunological Features

Research on the clinical immunology of leishmaniasis was reviewed recently (101, 186). If human infection is analogous to the murine model, then genetic or racial differences also may play a role in determining some of the variations observed among patients in the course of leishmanial infection (314). Both cell-mediated immunity and humoral immune responses are induced by leishmanial infections in humans. Although strong DTH may coexist with some nonhealing forms of the disease, such as ML, healing does not occur in its absence. On the other hand, the available clinical data clearly exclude an essential protective role for humoral antibody response. A lymphocytic response to *Leishmania* antigen usually develops during both CL and ML but is absent in DCL and AVL. Conversely, anti-*Leishmania* antibody titers are generally low in the sera of patients with CL or ML but moderate to high in patients with DCL or AVL. These data suggest that T_H1 - and T_H2 -like immune profiles may exist in human leishmaniasis, as they occur in tissues or peripheral blood of patients with other diseases (257). Recovery and the development of long-lasting resistance to reinfection appear to be the rule in CL. Some observations suggest, however, that immunity conferred by prior self-resolving leishmanial infection may not always be complete (91, 279). In the case of ML, there is a partial protective immunity against the parasite, as reflected by a resolution of the primary lesion, but the disease later recurs, causing destructive lesions of the mucocutaneous tissues. In both CL and ML, a direct correlation exists among DTH, level of lymphoproliferative response, and duration of the disease (52, 54, 193). When tested in vitro, peripheral lymphocytes from patients with CL or ML also produce high levels of IFN- γ in response to parasite antigen (50). The parameters of some lymphocyte and macrophage functions evaluated in patients with CL or ML were comparable except for the enhanced parasite-specific lymphoproliferative response observed in patients with ML (52-54). No apparent difference in levels of specific peripheral blood lymphocyte subpopulations (T and B cells, OKT_8^+ cells, OKT_4^+ cells, OKT_4/OKT_8 ratio) was observed in CL and ML patients (52). In both CL and ML lesions, there is also a predominance of T memory cells (CD4[CD45RO]) compared with naive cells (9CD[CD45RA]), but the percentages of cells containing IFN- γ mRNA are equivalent in both types of lesions (232).

TABLE 1. Currently recognized *Leishmania* species causing human disease in the New World

<i>Leishmania</i> sp.	Proven or suspected vector(s) (<i>Lutzomyia</i> spp.)	Proven or suspected reservoir(s)	Known geographic distribution	Reference(s)
Subgenus <i>Viannia</i> <i>L. braziliensis</i>	<i>L. wellcomei</i> ^a	Rodentia	Argentina, Belize, Bolivia, Brazil, Colombia, Ecuador, Costa Rica, Guatemala, Honduras, Nicaragua, Panama, Paraguay, Peru, Venezuela	10, 82, 99, 102, 103, 147, 245, 263
	<i>L. yucumensis</i> ^a	<i>Akodon arviculoides</i>		
	<i>L. llanos martinsi</i> ^a	<i>Rattus rattus</i>		
	<i>L. spinicrassa</i> ^a	<i>Oryzomys nigripes</i>		
	<i>L. whitmani</i> ^a	<i>Oryzomys capito</i>		
	<i>L. carrerai</i> ^a	<i>Oryzomys concolor</i>		
	<i>L. intermedia</i>	<i>Rhipidomys leucodactylus</i>		
	<i>L. migonei</i>	<i>Proechimys</i> spp. Marsupialia		
	<i>L. squamiventris</i>	<i>Didelphis marsupialis</i>		
	<i>L. ovallesi</i>	Edentata		
	<i>L. lichi</i>	<i>Choloepus didactylus</i> Carnivora <i>Canis familiaris</i> Perissodactyla <i>Equus caballus</i> <i>Equus asinus</i> <i>Equus caballus</i> × <i>Equus asinus</i>		
<i>L. peruviana</i>	<i>L. peruensis</i>	Carnivora	Peru	10, 99, 226, 258
	<i>L. verrucarum</i>	<i>Canis familiaris</i> Marsupialia <i>Didelphis albiventris</i>		
<i>L. guyanensis</i>	<i>L. umbratilis</i> ^a	Edentata	Brazil, Colombia, Ecuador, French Guiana, Guyana, Peru, Surinam, Venezuela	11, 99, 102, 103, 147, 151, 263
	<i>L. anduzei</i> ^a	<i>Choloepus didactylus</i> ^a		
	<i>L. whitmani</i> ^a	<i>Tamandua tetradactyla</i> ^a Marsupialia		
	<i>L. ovallesi</i>	<i>Didelphis marsupialis</i> Rodentia <i>Proechimys guyanensis</i>		
<i>L. panamensis</i>	<i>L. trapidoi</i> ^a	Edentata	Colombia, Costa Rica, Ecuador, Honduras, Nicaragua, Panama, Venezuela	99, 103, 147, 151, 99, 103, 147, 151
	<i>L. gomezi</i> ^a	<i>Choloepus hoffmanni</i> ^a		
	<i>L. ylephiletor</i> ^a	<i>Bradypus griseus</i> ^a		
	<i>L. panamensis</i> ^a	<i>Bradypus infuscatus</i> Rodentia <i>Heteromys dermarestianus</i> Carnivora <i>Bassaricyon gabii</i> <i>Nasua nasua</i> <i>Potos flavus</i>		
<i>L. lainsoni</i>	<i>L. ubikitolis</i>	Rodentia <i>Agouti paca</i>	Brazil	155, 288

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TABLE 1—Continued

<i>Leishmania</i> sp.	Proven or suspected vector(s) (<i>Lutzomyia</i> spp.)	Proven or suspected reservoir(s)	Known geographic distribution	Reference(s)
<i>L. naiffi</i>	<i>L. isquamiventris</i> ^a <i>L. paraensis</i> <i>L. ayrozai</i>	Edentata <i>Dasypus novemcinctus</i> ^a	Brazil	102, 154, 212
<i>L. colombiensis</i>	<i>L. hartmanni</i> <i>L. gomezi</i> <i>L. panamensis</i>	Edentata Choloepus hoffmanni	Colombia, Panama, Venezuela	146
<i>L. shawi</i>	<i>L. whitmani</i>	Primates Cebus apella Chiropotes satanus Edentata Choloepus didactylus Bradypus tridactylus Carnivora Nasua nasua	Brazil	287
Subgenus <i>Leishmania</i> <i>L. mexicana</i> (syn. <i>L. pifanoi</i>)	<i>L. olmeca olmeca</i> ^a <i>L. diabolica</i> <i>L. christophei</i> <i>L. ayacuchensis</i>	Rodentia Ototylomys phyllotis ^a Heteromys desmaretianus Nyctomys sumichrasti Sigmodon hispidus Heteromys anomalus Ototylomys spp. Heteromys spp. Nyctomys spp. Sigmodon spp. Carnivora Canis familiaris Felis domesticus	Belize, Colombia, Costa Rica, Dominican Republic, Ecuador, Guatemala, Honduras, Mexico, Panama, United States, Venezuela	99, 103, 117, 145, 147, 202
<i>L. amazonensis</i> (syn. <i>L. garnhami</i>)	<i>L. flaviscutellata</i> ^a <i>L. olmeca nociva</i> <i>L. reducta</i> <i>L. youngi</i>	Rodentia Proechimys guyanensis ^a Proechimys cuvieri Oryzomys capito Oryzomys goeldii Dasyprocta prymnolopha Sciurus vulgaris Marsupialia Marmosa murina Marmosa cinerea Metachirus nudicaudatus Didelphis marsupialis Didelphis albiventris Philander opossum Carnivora Cerdocyon thous Potos flavus Edentata Tamandua tetradactyla Primates Aotus trivirgatus Sanguinus geoffroyi	Bolivia, Brazil, Colombia, Costa Rica, Ecuador, French Guiana, Panama, Peru, Venezuela	11, 103, 148, 201, 252

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TABLE 1—Continued

<i>Leishmania</i> sp.	Proven or suspected vector(s) (<i>Lutzomyia</i> spp.)	Proven or suspected reservoir(s)	Known geographic distribution	Reference(s)
<i>L. venezuelensis</i>	<i>L. olmeca bicolor</i> <i>L. trinidadensis</i>	Carnivora <i>Felis domesticus</i>	Venezuela	41
<i>L. chagasi</i> (syn. <i>L. infantum</i>)	<i>L. longipalpis</i> ^a <i>L. ovalesi</i> <i>L. evansi</i>	Carnivora <i>Cerdocyon thous</i> ^a <i>Lycalopex vetulus</i> ^a <i>Canis familiaris</i> ^a Marsupialia <i>Didelphis albiventris</i> <i>Didelphis marsupialis</i>	Argentina, Bolivia, Brazil, Colombia, Ecuador, El Salvador, Guadelupe, Guatemala, Honduras, Martinique, Mexico, Nicaragua, Paraguay, Suriname, United States, Venezuela	4, 37, 38, 68, 103, 147, 152, 202, 233, 302, 319
<i>L. major</i> -“like” (syn. <i>L. major</i>)	?	?	Brazil, Ecuador, Venezuela	117, 203

^a Proven vector or reservoir.

The characteristic immunologic feature in DCL is an antigen-specific impairment of DTH in the presence of circulating antibodies (66). A parasite-specific defective lymphoproliferative response of blood lymphocytes is observed, although the cells do respond to mitogens or unrelated antigens (53, 230). Peripheral lymphocytes from a patient with DCL caused by *L. mexicana* also failed to secrete IFN- γ when tested with leishmanial antigen, but the patient's cells did produce this lymphokine when they were challenged by mitogen or unrelated antigen (209). This specific impairment of the cell-mediated immune response has been attributed to the suppressive effect of mononuclear phagocytes (230). The available evidence also suggests that promastigotes derived from patients with DCL express epitopes that preferentially stimulate suppressor activities in those patients; in contrast, these determinants appear to be expressed less, if at all, by parasites derived from CL patients. Conversely, the antigen-specific suppression in patients with DCL can be abrogated by drug treatment, which suggests that antigen-induced T regulatory cells are most likely responsible for the nonresponsiveness seen during the active disease (3). Although *L. amazonensis* isolates causing DCL appear to be closely related antigenically, they also possess some strain-related antigenic differences (162).

VL is associated with antigen-specific immunosuppression during the acute phase of the disease that appears to be induced by a cell-mediated response (49). The mechanism of this immunosuppression is still unclear. It has been suggested that decreased numbers of T cells in peripheral blood (49, 50, 211, 246), the absence of antigen-reactive peripheral lymphocytes (268), or the presence of suppressor cells (49) might account for the diminished responsiveness. Cocultivation experiments with frozen autologous mononuclear cell populations obtained from peripheral blood of VL patients before and after chemotherapy reduced the reactivity re-

sponses of posttreatment cells to leishmanial antigens by 80%. However, depletion of mononuclear cell preparations of adherent cells, high-avidity Fc⁺ cells, OKT₄⁺ cells, and OKT₈⁺ cells failed to restore lymphocyte reactivity to leishmanial antigens (49). Moreover, a serum-mediated suppression of mitogen-driven lymphocyte proliferative capacity has been demonstrated in patients with VL (25); this effect was accompanied by high levels of a soluble IL-2 receptor in the serum (28). A soluble factor that suppresses IFN-dependent macrophage activation (80) may also be produced by human monocytes infected with *L. donovani*.

The progression of VL is related to markedly reduced lymphocyte proliferation and decreased IL-2 and IFN- γ production by peripheral lymphocytes in response to leishmanial antigen (50, 51). In contrast, the IFN- γ levels in children with asymptomatic and subclinical self-healing *L. chagasi* infections were significantly higher than those observed in children with subclinical infections progressing to VL (51). These reduced cellular responses return to normal after successful chemotherapy (50, 64). The levels of CD4 (and CD8) T cells are within the normal range, but there is a significant decrease in UCHL-1 T cells (helper-inducer) in the peripheral blood mononuclear cells of these patients that is involved with antigen-specific IL-2 production (268). However, the level of these cells during active disease also increased to normal after treatment (64). An imbalance of endogenous cytokine production also exists in patients with VL, resulting in predominance of IL-4 over IFN- γ (328). In addition, while natural killer cell activity was markedly deficient in some of the VL patients tested, in the absence of exogenous IFN- γ this cell-mediated immunity effector mechanism was detectable in all cases in the presence of recombinant IFN- γ (115).

In contrast to depression of the cellular immune response, there is a marked humoral response during active disease, with elevated nonspecific immunoglobulin levels, mostly of

the immunoglobulin G and M classes, causing a reversal of the albumin/globulin ratio. Relatively high titers of anti-*Leishmania* antibodies, however, are also a common finding in patients with the disease. Thus, hypergammaglobulinemia, rheumatoid factors, and circulating immune complexes, suggesting polyclonal B-cell activation, are characteristic features of VL (48, 86, 225).

Pathology

Successful mammalian infection by *Leishmania* spp. depends on the abilities of the parasite to evade nonspecific host defenses, to attach to and be ingested by the host cell, and to survive within the phagolysosome of macrophages. The intracellular parasitism of macrophages by *Leishmania* spp. can stimulate different types of inflammatory reactions, and in this manner it gives rise to the various clinical and pathological patterns of the disease. The histopathologies of all clinical forms of American leishmaniasis have been reviewed recently (101, 249). Basically, there is a histopathologic spectrum ranging from anergic forms of infection with heavily parasitized macrophages (e.g., DCL and VL) to hypersensitive or allergic forms (e.g., ML) with scanty organisms and a tuberculoid response. In addition, the last two forms may develop immunopathological alterations (e.g., fibrinoid necrosis of vascular walls or of the connective matrix) that produce extensive tissue damage. Although little is known about the immunopathology of the progressive and destructive form of ML, some underlying causes of its hyperergic response might include the following factors: (i) the resistance of some parasite strains to elimination and the persistence of "allergic" antigen that evokes hyperergic hypersensitivity inflammatory responses and (ii) autoimmune phenomena related to antigens cross-reactive between leishmanial parasites and host tissues (86, 249, 301).

The various geographically distinct groups of CL were histologically compared to determine whether the nature and intensities of tissue responses gave any indication of the likely outcome of infections (248). Although there was no simple or unified pattern, a five-stage histologic classification was defined for Brazilian patients with CL and ML; this system was found to have some clinicoprognostic value (250). More recently, a correlation among histopathology, immune response, clinical presentation, and evolution in *L. braziliensis* infection in Colombia has been shown; variations in the histopathologic features that were associated with the presence or absence of parasites in the lesions were found to be related to response to treatment and the ultimate prognosis (109).

Laboratory Diagnosis

A presumptive diagnosis of leishmaniasis can be made on the basis of results of laboratory tests in conjunction with clinical and epidemiologic data. However, a definitive diagnosis of the disease still requires demonstration of the parasite. In chronic cases of CL and ML, a definitive diagnosis is sometimes difficult because of the paucity of parasites in lesions (101, 180, 312).

The diagnosis of AVL is complicated by the fact that the clinical signs and symptoms of the disease resemble those of a number of other infectious diseases (180, 242). The insidious and nonspecific nature of AVL, as well as serological cross-reactivity, may confound the diagnosis (18, 114, 238, 241, 242). Therefore, a definitive diagnosis of VL also depends on detection of parasites by examination of smears

of bone marrow, lymph node, or splenic aspirate (101, 180, 242). Although results of noninvasive diagnostic procedures such as the enzyme-linked immunosorbent assay (ELISA) and the direct agglutination test compare favorably with direct parasite detection (60, 79, 196, 326, 327), these serological tests cannot differentiate between past or subclinical infection and active VL (326). More recently, a simple immunoblot assay, using colloidal gold conjugated to protein A, was developed for the rapid serodiagnosis of active visceral disease (283). Field trials of the immunoblot assay are necessary for further evaluation, however.

The various clinical laboratory procedures used for diagnosing leishmaniasis have been extensively reviewed (101, 180, 223). The classical methods used for direct demonstration of the parasite in tissues or skin lesions include the following: (i) examination of stained smears or histologic sections; (ii) inoculation of hamsters with aspirates from infected tissues or with triturated tissue fragments; and (iii) in vitro culture of tissue homogenates or aspirates in biphasic media. A combination of these direct techniques is still the most commonly used method for the diagnosis of leishmaniasis, especially the cutaneous form (101, 216). However, newer immunologic and molecular techniques are now available. In situ detection of amastigotes with characterization of the infecting parasite species can be done in CL by immunocytochemical procedures, using either polyclonal (274) or monoclonal (8, 178) antibodies. Rapid in situ diagnosis of leishmaniasis is also possible by examining dot blots of fluid aspirates or touch blots of infected tissues on nitrocellulose paper. The dot blots are subsequently tested, employing monoclonal antibodies (113) or kDNA- (13, 319) or genomic (126) DNA-labelled probes. Both radioisotope (22, 23) and nonisotope (23, 318) detection systems have been described. An alternative approach is in situ hybridization, using kDNA probes for the detection of individual parasites in imprint smears of aspirates from lesions (22-24).

The use of total kDNA probes or selective cloning of species-specific kDNA fragments for direct identification of parasites in biopsy material (23, 39, 95) has been under investigation since the 1980s, but such probes have not been used enough with clinical material to evaluate their efficacy. The success of hybridization identification has been shown in many laboratories and is due mainly to the correct selection of clones containing highly repeated sequences and probes for *Leishmania* complex discrimination. Greater discrimination within and between members of the major *Leishmania* complexes requires recombinant DNA selection of specific sequences (23). Specific recombinant probes for *L. major* (140, 229, 237), *Leishmania tropica* (185), *Leishmania aethiopica* (159), *L. donovani* (72, 89, 95, 130, 174), and *L. braziliensis* (215) species complexes have now been reported, and there are increasing numbers of recombinant probes which may be able to distinguish geographically isolated parasite strains (23, 72, 130, 320). kDNA hybridization can also be employed for identification of *Leishmania* spp. within sand flies (130, 159, 256); alternatively, both the sand fly and parasite species can be confirmed in the vector by using double probes on squash blots (237). More recently, the polymerase chain reaction technique has also been used to amplify kDNA sequences and to detect *Leishmania* parasites in biopsy material from patients and from suspected animal reservoirs as well as from dry sand fly specimens (76, 176, 254, 293). Synthetic oligonucleotide primers specific for *L. donovani* (293) and *L. braziliensis* species complexes (76, 176) now have been thoroughly evaluated under field conditions. The polymerase chain

reaction technique is a rapid and highly sensitive method for the diagnosis of VL and appears to be capable of distinguishing between past and current infections (39). Therefore, this technique might also be useful for monitoring treatment since resolution of the clinical features does not necessarily imply elimination of the parasites or cure (259).

The indirect methods currently in use for diagnosing leishmanial infection are based on several serologic techniques, including ELISA (16–18, 60, 79, 87, 114, 196, 241), direct agglutination test (79, 196, 197, 326, 327), immunoblot (196, 238), and immunofluorescence (101, 114, 312), which detect anti-*Leishmania* antibodies. The leishmanin skin (Montenegro) test, which measures the cutaneous DTH or cellular responses to *Leishmania*-derived antigens, is still frequently used for clinical diagnosis of the disease and in epidemiological surveys on the prevalence of leishmanial infection (101, 180, 223). However, the skin test fails to distinguish between current and past leishmanial infection (180, 311, 316). At present, there is no standardized antigen preparation available for the determination of DTH responses in leishmaniasis patients, and whole killed promastigotes are still used (180). As immunologic techniques are refined and as highly specific antigens and antibodies become available, they may prove useful in the development of improved skin tests (19, 239, 240, 316, 317) and serological assays (131, 133, 241, 264, 283) for clinical diagnosis and epidemiological surveys.

Treatment

Comprehensive reviews of both the clinical and the experimental chemotherapy of leishmaniasis have been published (33, 43, 120, 217). Treatment of the disease is usually based on the use of leishmanicidal drugs, principally injections of pentavalent antimony (Sb^5) compounds, which despite their toxic properties still remain the treatment of choice (120, 321, 322). The biochemical basis for the antileishmanial activity of antimonial drugs is still not well understood, although it may involve inhibition of ATP synthesis (33). The two Sb^5 compounds in common use, sodium stibogluconate (Pentostam; Burroughs Wellcome) and meglumine antimoniate (Glucantime; Rhone Poulenc), give similar therapeutic results. Treatment trials of these drugs in cases of CL, ML, and VL have been done in diverse patient populations with different parasite infections and different dosage regimens; consequently, the results and recommended therapies have been variable (44, 45, 61, 84, 107, 156, 214, 215, 273, 295). In the 1980s, use of 20 mg of Sb^5 per kg per day was recommended for treating VL (321). Subsequently, this dose was recommended for CL and ML as well (120, 323), but a maximum daily dose of 850 mg was specified. Recent data indicate that the response to Sb^5 is better with higher daily doses of the drug and with longer treatment but the side effects (arthralgias, myalgias, and hepatic, cardiac, and renal toxicity) are also greater. Other considerations in using the higher and longer dosage schedule in areas of endemicity include cost and the logistics of administration (20, 21, 45, 84, 156). A regimen of 20 mg of sodium stibogluconate per kg per day, without an upper limit on the daily dose, is now recommended; CL cases are treated for 20 consecutive days, while 28 days of continuous therapy are recommended for ML and VL. Determination of cure varies according to the clinical form of the disease; patients with AVL should be seen at 1.5, 3, 6, and 12 months after completion of therapy for clinical and laboratory follow-up. In the case of ML, the initial response to therapy should be determined 3 months

after the end of treatment, and patients should be monitored for several years for indication of relapse. Antimonial therapy should be discontinued if the patient develops significant arrhythmias, prolongation of the corrected QT interval (to >0.50 s), or concave ST segments (120) on electrocardiogram. Response to treatment with antimonial drugs varies considerably depending on the parasite strain involved (104–106), the patient's immune status, and the clinical form or stage of the disease. Treatment is generally effective for both CL and AVL, but patients with severe mucosal disease are less responsive (43, 84, 120, 182, 218, 273). DCL is often the most difficult to cure because of the defective host immunity which usually occurs with this form of the disease (15, 43).

As antimony refractoriness has increased in the various clinical forms of leishmaniasis (44, 104, 106, 129, 192, 253, 273, 295), therapeutic regimens have been modified in both dose and duration of therapy (45, 84, 120) and by the addition of other antileishmanial compounds. Other systemic drugs with proven efficacy in human leishmaniasis are amphotericin B (43, 182), pentamidine (45, 295), itraconazole or miconazole (2, 42, 272), and the orally administered agent allopurinol ribonucleoside (61, 78, 107, 183, 184). However, most of the reported studies supporting the use of these various alternatives have been preliminary; consequently, there are insufficient data to select one drug over another (120). Furthermore, these alternative drugs may not be equally effective with all *Leishmania* species (43, 45, 107). Also, the associated high toxicity with some of these alternative drugs is a limiting factor in their systemic use in CL (45, 182). In view of the relatively high self-healing rate in ACL, all studies of potentially new therapeutic regimens must include observations on untreated patients with similar lesions as well (107). The failure of many studies to include untreated control subjects has made it difficult to evaluate the efficacy of different treatment regimens.

Although the mode of action of Sb^5 is still not well understood, clinical and experimental studies have demonstrated the following: (i) *Leishmania* spp. readily acquire resistance to Sb^5 under drug pressure in vivo and in vitro (104–106, 129); (ii) in vitro induced Sb^5 resistance is stable in *Leishmania* spp.; (iii) parasite virulence does not decrease under in vitro Sb^5 drug pressure (105, 106); (iv) there are significant differences in susceptibility to Sb^5 among clones of a single clinical isolate obtained from an untreated patient (105); (v) in vitro susceptibility of *Leishmania* isolates to Sb^5 showed 89 and 86% correlations with clinical outcome after Pentostam and Glucantime treatment, respectively; (vi) parasite strains from Sb^5 -treated patients who failed to complete a course of Pentostam were as refractory to this drug as were in vitro induced Sb^5 drug-resistant *Leishmania* clones; (vii) moderate resistance to Sb^5 exists among *Leishmania* strains in nature, and some isolates are innately less susceptible to this drug than others (106); (viii) with Sb^5 pressure from undermedication, those parasites that are inherently most drug resistant are favored; (ix) accumulation of Pentostam in Sb^5 -susceptible *Leishmania* clones is two to five times greater than in Sb^5 -resistant clones; and (x) in vitro induced, drug-resistant clones of *Leishmania* spp. exhibit cross-resistance that is characteristic of the human multidrug resistance phenomenon observed in cancer cells (104).

In addition to systemic therapy, several forms of local treatment have also been tested with cutaneous forms of the disease; these include (i) topical application of drugs, (ii) curettage, (iii) irradiation, (iv) heat, and (v) freezing. However, conclusive studies with adequate controls have not been done with any of these methods (43). Liposome-

entrapped or receptor-mediated drug delivery to *Leishmania*-infected macrophages is another promising new area for the treatment of leishmaniasis, as demonstrated recently in experimental models (206, 217). Such delivery systems, which rely on drug uptake by macrophages, appear to enhance the effectiveness of normal chemotherapy.

Studies of metabolic differences between leishmanial parasites and their vertebrate hosts have identified other new targets for drug development (33, 56, 92, 93, 139). Because of the cost and toxicity of the current antimony-based drugs, intense efforts are now being made to develop more effective antileishmanial compounds on the basis of knowledge of these potential drug targets (33, 217, 235, 322). A major therapeutic problem is that leishmanial parasites have the capacity for gene amplification, a complex mechanism that allows the parasites to amplify genes that are resistant to a wide variety of chemical compounds. The basic biochemical mechanisms associated with resistance are still not well defined (35, 104); however, amplified DNAs can now be modified to produce DNA transfection vectors for some *Leishmania* species (135, 160). This technique has been employed to develop stable genetic transformation systems for directly identifying drug resistance genes (69); it has important implications for drug and vaccine development.

Because of the high cost of antimonial drugs and their toxicity and increasing parasite resistance, immunotherapy recently has received attention as an alternative approach to the treatment of leishmaniasis (322). Preliminary studies in Venezuela suggest that immunotherapy, using killed leishmanial parasites plus *Mycobacterium bovis* BCG as adjuvant, is as effective in treating active ACL as classical antimonial therapy; furthermore, it lacks the toxic side effects (65). These studies must be confirmed, but if they can be verified, immunotherapy would drastically simplify and reduce the cost of treatment. Another immunotherapeutic approach is based on the apparent role of IFN- γ in protective immunity to *Leishmania* infection (208). Badaro et al. (14) reported that the effect of pentavalent antimony against VL was enhanced by its combination with recombinant IFN- γ . In limited studies with this combination, some patients who were previously unresponsive to several courses of antimonial therapy were cured (14, 15, 177). Intradermal application of IFN- γ or the combination of tumor necrosis factor alpha and IFN- γ has shown promising results in the treatment of CL (30, 96, 116, 168).

EPIDEMIOLOGIC FEATURES OF AMERICAN LEISHMANIASIS

The term American leishmaniasis is actually a misnomer, since it does not refer to a single disease entity; rather, it denotes a variety of different diseases. As noted before, at least 13 distinct *Leishmania* species are recognized as causing human illness in the Americas (Table 1). Each of these parasites has a unique life cycle, with different sand fly vectors, different animal reservoirs, and a different geographic distribution. It is important to realize that these 13 *Leishmania* species are as different from each other in their epidemiology as are the four *Plasmodium* species that cause human malaria, but it is not the objective of this article to review in detail the life cycle of each of the New World *Leishmania* species. That subject has been covered thoroughly in other recent reviews (101, 103, 146, 151, 152, 154, 287, 290).

However, the various leishmaniasis in the Americas have some common epidemiologic features: (i) they are confined

largely to tropical and subtropical regions; (ii) they are acquired from the bite of infected phlebotomine sand flies; (iii) each of them is maintained in a zoonotic cycle involving wild or domestic animals or both (people are not essential for maintenance of these parasites and are usually dead-end hosts); (iv) most of them occur in persons residing in rural areas or having contact with sylvan habitats; and (v) because of their zoonotic character, changes in human behavior or alterations in the environment or both can have a major impact on their prevalence and transmission patterns.

The importance of ecologic and demographic changes in the epidemiology of the various New World leishmaniasis has only recently been appreciated. The epidemiology of each of these diseases is extremely complex and can be altered by changes at any point in the "epidemiologic triangle" that is formed by humans, the reservoir host(s), and the sand fly vector(s) (148). Most of the environmental factors affecting the epidemiology of the various leishmaniasis are still poorly understood; nonetheless, the available data suggest that some of the parasites and their vectors can adapt to ecologic changes such as deforestation and urbanization (148, 149). As the parasites and vectors adapt to these altered environmental conditions, the epidemiology of the diseases associated with them also changes.

For example, AVL due to *L. chagasi* is now much more common than it was 50 years ago. The massive destruction of primary forests in the Neotropics and the concomitant development of farmland and rural settlements have led to conditions that support large populations of *Lutzomyia longipalpis*, the principal sand fly vector, and of dogs and foxes, the principal reservoir hosts of *L. chagasi* (149). As a consequence, AVL now occurs in many parts of Latin America where it had not been found previously (103). Furthermore, the prevalence of AVL associated with *L. chagasi* appears to be increasing in the suburban areas of major Brazilian cities such as Fortaleza, Teresina, Sao Luis, and Rio de Janeiro, where dogs alone now seem to be the major reservoir of the parasite (103, 322).

Another change in the epidemiology of AVL may occur as a result of the global AIDS epidemic. Until now, AVL has been mainly a disease of young malnourished children; adult cases of this form of the disease have been rare. However, as the prevalence of human immunodeficiency virus type 1 infection increases in tropical America, it is probable that AVL will appear more frequently as an opportunistic infection in adults with human immunodeficiency virus type 1 infection and AIDS. This has been the pattern observed in southern Europe with *Leishmania infantum* infection (5, 32, 229).

A change also has been observed in the epidemiology of ACL caused by *L. braziliensis* (103, 149). Infection with this parasite is often observed in persons in forested areas, where *L. braziliensis* is maintained in an enzootic cycle involving various wild animals and sylvan sand flies. However, following deforestation and human colonization, the parasite appears capable of adapting to the resulting ecologic changes by switching to a cycle involving peridomestic sand fly species and domestic animals such as dogs, horses, and mules (1, 149, 150). These are just a few examples of the plasticity of the leishmanial parasites and of their ability to adapt to changing ecologic conditions.

Prevalence and Geographic Distribution

Leishmaniasis is extremely common in many regions of Latin America. Although recognition of the geographic

distributions of the various parasites and their prevalence has increased during recent years, the disease is still grossly underreported (67). Furthermore, some leishmanial infections are asymptomatic or subclinical; frank disease represents only a proportion of total infections (12, 16, 17). This is especially true with infections caused by *L. chagasi* (16, 17).

Table 1 lists the 13 *Leishmania* species currently recognized as human pathogens in the Americas as well as their proven or suspected vectors, animal reservoirs, and geographic distribution. For more specific information on their epidemiology, see recent reviews by Grimaldi et al. (103), the World Health Organization (322), Lainson (148), and Lainson and Shaw (152).

Sand Fly Vectors and Vertebrate Reservoirs

The human pathogenic *Leishmania* spp. are all transmitted by the bites of infected phlebotomine sand flies (Diptera: Psychodidae). More than 350 different sand fly species from the Americas are known (325), but only 32 of these have been implicated as proven or suspected vectors of human leishmaniasis (Table 1). In contrast, a wide variety of wild and domestic mammals have been implicated as reservoir hosts of New World *Leishmania* species. In general, a close ecologic relationship exists between the sand fly vector(s) of a given parasite and its animal reservoir(s). In some cases, the same phlebotomine and mammalian species serve as vectors and reservoirs of a given *Leishmania* species throughout its geographic range; with other parasites, several different sand fly and animal species are involved in different ecologic and geographic regions.

The phlebotomine vectors of New World leishmaniasis are usually included in the genus *Lutzomyia*. There is some confusion in the scientific literature on this point, since some authors (236) have referred to *Psychodopygus* as a second genus in the Americas. However, the consensus among sand fly taxonomists (165, 296) is that *Psychodopygus* is simply a subgenus within the genus *Lutzomyia*.

Several studies of the developmental biology of New World leishmanial parasites in natural and unnatural sand fly vectors have been reported (305–310). The life cycles of these parasites in their invertebrate hosts have the following common features. Amastigote forms of the parasite are ingested by the sand fly when it takes a blood meal from an infected mammalian host. Within 24 h after ingestion, the amastigotes transform into promastigotes, which then undergo rapid multiplication within the blood meal. The ingested blood initially goes to the anterior midgut of the insect, where it becomes encased within the peritrophic matrix (306). After about 3 days, the peritrophic matrix disintegrates and the promastigotes migrate to the hindgut (in the case of peripylarian parasites) or to the foregut and midgut (suprapylarian parasites), where further multiplication and differentiation occur. After about 7 days, the parasites move anteriorly to the esophagus-pharynx-stomodaeal valve region of the alimentary tract, where they attach to the cuticular lining by flagellar hemidesmosomes (305, 306, 308–310). Transmission of the parasites to the next mammalian host occurs when the infected fly takes another blood meal; in the process of feeding, the insect regurgitates infective promastigotes into the host (281). As noted before, sand fly saliva contains a number of different substances that enhance the infectivity of the parasites and also assist the insect in bloodfeeding (247, 297, 300). The interactions of the various *Leishmania* species with their natural reservoir (vertebrate) hosts is less well studied, but a well-balanced

host-parasite relationship is the general rule; infection is often asymptomatic, with little pathology (148, 150, 151).

PREVENTION AND CONTROL

In theory, control of leishmaniasis should be possible by interruption of the transmission cycle. Four approaches have been used, although to date these have had only limited success. The basic problem is that the New World leishmaniasis are zoonotic diseases that are maintained in natural cycles involving wild animals and insects.

Vector Control

Attempts at vector control have focused mainly on adult sand flies, since the larval breeding sites of most species are unknown. Insecticides are quite useful in controlling these insects in domestic and peridomestic situations, and to date, resistance has not been a serious problem (304, 315, 322). In contrast, the use of insecticides in forested areas, by either local application or aerial spraying, has not been very effective (322, 325). The clearing of forests around villages and settlements has been useful in reducing the abundance of some sylvan vectors by eliminating their breeding and daytime resting sites (325). Sand flies have a relatively short flight range and do not travel long distances, so local insecticide application or environmental management can be helpful in controlling the insects in defined areas. However, until more is learned about the biology and natural history of some of the important vector species, attempts to control sand flies and the diseases associated with them will have only limited success.

Elimination of Reservoir Hosts

Control of AVL due to *L. chagasi* has been achieved by the elimination of infected dogs (322), but canine surveillance programs are labor-intensive and expensive, and they require constant vigilance to be effective. Elimination of potential wild animal reservoirs is really neither feasible nor ecologically sound. Furthermore, as noted before with the example of *L. braziliensis*, some parasite species appear able to infect domestic animals and utilize them as alternative reservoir hosts when their natural sylvan hosts are displaced (1, 148, 149).

Surveillance and Treatment of Human Cases

Surveillance, detection, and treatment of human leishmaniasis are effective in reducing the prevalence of active disease; however, these activities by themselves do little to reduce the incidence of new cases. As noted previously, humans are not involved in the basic maintenance cycles of the various New World leishmaniasis, so treating infected people does little to interrupt parasite transmission in nature.

Personal Protection

Individual protective measures by persons exposed to sand fly bites are quite effective. Outdoor measures include use of repellents such as diethyltoluamide, protective clothing, and avoidance (i.e., staying out of forested areas at night). Indoor protection can be obtained by the use of fine-mesh screens, bed nets, and mosquito coils (323). These measures may all be effective in reducing an individual's risk of leishmanial infection, but they are not particularly useful

on a community-wide basis because of their cost, the discomfort they cause in warm climates, and cultural resistance.

Immunization

To date, no vaccine against any form of leishmaniasis has been shown conclusively to be effective (322). Nonetheless, current research suggests that the development of unit vaccines against leishmaniasis may be feasible. Two approaches to developing immunoprophylactic methods against the disease have been adopted. One approach is the induction of protection by using whole parasites (either attenuated, killed, or disrupted). The other approach is subcellular fractionation of the parasites with the aim of identifying, isolating, and inducing protection with purified antigens.

CL caused by *L. major* has been the target of most human vaccination attempts. A live-promastigote vaccine has been evaluated with this clinical form of the disease (97, 98, 108). Although the vaccine confers resistance to infection, a significant percentage of persons receiving it developed cutaneous lesions. Furthermore, this type of immunization can be used only with *Leishmania* species that produce benign self-healing lesions. Killed vaccines are still only in the experimental stage of development. Preliminary vaccination trials against CL with phenol-killed promastigotes have given conflicting results (97, 227). The first field trial evaluating the efficacy of a killed-promastigote vaccine in Brazil was inconclusive (187). In a second trial, only 30% of vaccinees showed skin test conversion; of those people that did convert, there was about a 70% decrease in the incidence of natural disease compared with the incidence in the control group (188). In a third trial in the Amazon region of Brazil, there were also reductions (67.3 and 85.7%) in the annual incidence of ACL among vaccinees developing a positive leishmanin skin test. However, when the skin test-positive and skin test-negative vaccinees in that study were combined, the difference between the vaccinated and control groups was not significant (9). Results with killed vaccines in the Old World have also given inconclusive or negative results (121).

Nonetheless, complete or appreciable levels of protection against CL and VL have been achieved in mice by using more defined antigens such as the major promastigote surface glycoprotein gp63, either incorporated into liposomes (260) or expressed in *Salmonella* spp. (324), and other glycoconjugates in conjunction with adjuvants (55, 112, 132, 189). By using synthetic peptides corresponding to the primary structure of gp63, it has been possible to characterize murine T-cell epitopes that can specifically activate either T_H1 cells, which induce immunoprotection against CL (134), or 347T_H2 cells, which enhance disease progression (170). Recent emphasis has turned to the evaluation of these and more defined leishmanial antigens to identify epitopes that induce appropriate T-cell responses in humans (46, 194, 195, 240). For instance, it has been shown that gp63 elicited strong T-cell proliferative responses (CD4⁺ T-cell subset) and IFN- γ production in leishmaniasis patients (194, 261). Recombinant gp63, which is produced in *Escherichia coli*, also stimulated T cells from these patients (261). In addition, stimulation of T lymphocytes isolated from leishmaniasis patients was also possible with *Leishmania* lipophosphoglycan-associated proteins (194, 262). Although still very preliminary, these results are encouraging and suggest that it

may be possible to develop safe protective vaccines against leishmaniasis.

CONCLUDING REMARKS

During the last decade, the application of new biomedical technologies to the study of leishmanial parasites has had a substantial impact on our understanding of these microorganisms and of the diseases caused by them. Considerable progress has been made in characterization of the genomic structures of the leishmanial parasites as well as in the identification of molecular determinants responsible for their virulence. In addition, the compositions of antigens and other macromolecules that are potentially capable of inducing protective immune responses or of being the targets for specific therapeutic procedures have been determined. Although many advances in understanding some of the cellular immune responses generated during leishmanial infections have been made, there are still many unanswered questions about the complex immunologic mechanisms involved in the control of human leishmaniasis. New techniques have also provided a number of novel approaches that can be applied to more practical issues such as the specific diagnosis of the disease or the molecular epidemiology of each *Leishmania* species.

Although still largely in the experimental stage, vaccination may prove to be the easiest and most effective intervention method for the prevention of leishmaniasis at a population level. The vaccines currently in use are still of the conventional type (attenuated or killed forms of the pathogen itself), but the development of a new generation of safe and effective subunit vaccines (using either recombinant or synthetic peptides or infectious recombinant vectors) is now within our reach. However, until such vaccines are available, an integrated control approach that uses more traditional methods (vector reduction, elimination of infected reservoirs, personal protection, surveillance, and treatment) is the only option.

Finally, compared with our current knowledge about the biology of leishmanial parasites and the response of animals and humans to infection with these microorganisms, relatively little is known about the insect vector. A better understanding of sand fly biology might also yield new insights into improved methods for the control of leishmaniasis. In view of the limited effectiveness of the traditional leishmaniasis control methods, studies on the basic biology of sand flies should be another research priority.

ACKNOWLEDGMENTS

This work was supported in part by grants from the National Institutes of Health (AI-21049 and AI-28528), the MacArthur Foundation (Molecular Parasitology and Vector Biology Programs), and the Brazilian National Council of Scientific Development and Technology (CNPq).

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