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# *Leishmania* and Human Immunodeficiency Virus Coinfection: the First 10 Years

JORGE ALVAR,[1]* CARMEN CAÑAVATE,[1] BEATRIZ GUTIÉRREZ-SOLAR,[1] MARIBEL JIMÉNEZ,[1] FERNANDO LAGUNA,[2] ROGELIO LÓPEZ-VÉLEZ,[3] RICARDO MOLINA,[1] AND JAVIER MORENO[1]

*Laboratorio de Referencia de Leishmaniasis, Servicio de Parasitología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, 28220 Majadahonda, Madrid,[1] Servicio de Enfermedades Infecciosas, Centro de Investigaciones Clínicas, Instituto de Salud Carlos III, C/Sinesio Delgado s/n, 28029 Madrid,[2] and Unidad de Medicina Tropical y Parasitología Clínica, Servicio de Enfermedades Infecciosas, Hospital Ramón y Cajal, 28034 Madrid,[3] Spain*



## INTRODUCTION

The protozoan *Leishmania* spp. are present in the macrophages of a wide variety of vertebrates. When hematophagous insects of the Psychodidae family (phlebotomes and lutzomyias) feed on them, they are infected with parasitized circulating or skin macrophages; when these sand flies feed on blood again, they can transmit the parasite once it has reached its infective stage (metacyclogenesis). That is, the biological cycle fits the description of the most typical metaxenous diseases: parasitized vertebrate reservoir, insect vector, susceptible vertebrate host.

Depending on the virulence factors of the parasite itself and on the immune response established by the host, in this case humans, a spectrum of diseases known as leishmaniasis can appear. The symptoms include cutaneous lesions and/or visceral involvement. The former resolve spontaneously after a few months but, depending on the causative *Leishmania* species, can evolve into diffuse cutaneous, relapsing cutaneous, or mucocutaneous leishmaniasis. Once established, the clinical course of untreated visceral leishmaniasis (VL) leads to death

* Corresponding author. Mailing address: Laboratorio de Referencia de Leishmaniasis, Servicio de Parasitología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, 28220-Majadahonda, Madrid, Spain. Phone: 34-1-509 79 01. Fax: 34-1-509 79 66.

in the majority of patients. Among the visceral forms of infection, those produced by *Leishmania donovani*, result, in a variable percentage, in post-kala-azar dermal leishmaniasis, despite treatment. The immunological bases that determine the development of one form or another are not the subject of the present review, but they have been extensively studied (160).

Leishmaniasis, in its visceral and cutaneous forms, is distributed throughout at least 88 countries, 21 in the New World and 67 in the Old World, including Africa, America, Asia, and Europe (83). More than 90% of the cutaneous cases appear in Afghanistan, Saudi Arabia, Algeria, Brazil, Iran, Iraq, Syria, and Sudan. A similar percentage of the visceral cases appear in India and Sudan. Approximately 350 million persons live in areas of active parasite transmission. From the epidemiological point of view, infections are zoonotic when animals serve as the reservoir and anthroponotic when humans serve as the reservoir. Transmission is rural and periurban for zoonotic leishmaniasis and urban for the anthroponotic forms. More than 25 species of *Leishmania* are capable of producing disease in humans, but only 2 can maintain the anthroponotic human-human cycle, always via the bite of the sand fly; these species are *L. tropica* for cutaneous leishmaniasis (CL) and *L. donovani* for the visceral forms (299).

The World Health Organization (WHO) estimates that 17 million persons are infected by human immunodeficiency virus (HIV) worldwide and that one-third of them live in the zones of endemic leishmaniasis infection (301).

### World Impact: the Mediterranean Basin

In Mediterranean countries, leishmaniasis is hypoendemic (for example, in Spain there is 0.3 case per 100,000 inhabitants), transmission is rural and periurban, and the infection is zoonotic, with the dog serving as the principal reservoir (83). On the other hand, up to October 1995, 146,453 accumulated AIDS cases were reported in Europe; 104,259 of these were in southwestern European countries. The majority of cases of *Leishmania*-HIV coinfection are found in southern Europe (Italy, France, Spain, and, at a distance, Portugal). The WHO Division for Control of Tropical Disease has recorded, for the period up to November 1995, 858 cases of coinfection throughout the world; the distribution of these cases is shown in Table 1.

TABLE 1. Number of *Leishmania*-HIV coinfections recorded by the WHO[a]

| Continent and country | No. of cases |
|---|---|
| Africa | |
| Algeria | 2 |
| Cameroon | 1 |
| Ethiopia | 29 |
| Guinea Bissau | 1 |
| Kenya | 25 |
| Malawi | 1 |
| Sudan | 3 |
| Tunisia | 28 |
| Asia | |
| India | 5 |
| America | |
| Brazil | 25 |
| Guadalupe | 1 |
| Panama | 1 |
| Peru | 1 |
| United States | 5 |
| Venezuela | 1 |
| Europe | |
| France | 127 |
| Italy | 130 |
| Portugal | 22 |
| Spain | 450 |

[a] Data up to November 1995. Data provided by the WHO Division for Control of Tropical Disease.

The possible overlap in the rural and periurban transmission of *Leishmania* spp. with urban and periurban transmission of HIV in countries in which both infections are highly endemic, such as Brazil and India, is a problem that worries the WHO. This situation becomes more acute with the now-established rural transmission of HIV and urbanization of *Leishmania* spp., even for typically rural forms, such as *Leishmania chagasi* which causes VL in Brazil (111).

Classic VL in immunocompetent individuals in Mediterranean countries is found primarily in children (43), although in recent years an increase in the number of cases in adults has been observed. This has changed the pattern of presentation by age groups, although the AIDS pandemic is not the only cause. In the south of France, fewer than half of the VL cases in adults are currently found in HIV-positive patients (82, 178), although in Spain this proportion is higher. Thus, until 1985, when the first case of coinfection was diagnosed (80), 70% of leishmaniasis cases were found in children under the age of 15 years. At present, 75% of cases are seen in adults, 50 to 60% of whom are HIV positive (11). To establish the prevalence of coinfection, essentially four studies have been performed. In 111 febrile HIV-positive patients from Madrid and Palma de Mallorca, 17% had amastigotes in bone marrow (14). Other authors have determined a frequency of 7 and 14% in 57 and 50 febrile HIV positive patients, respectively (39, 197). Finally, of 107 HIV-positive subjects in Seville, a prevalence of 13% was found, and 5.6% of the subjects had subclinical VL (230). In a second group of 121 HIV-positive patients from Granada who exhibited symptoms related to VL, the prevalence was 16% (230). In spite of these differences, it is striking that *Leishmania infantum* is situated in third place among the parasitic diseases most frequently found in HIV-positive individuals, after *Toxoplasma gondii* and *Cryptosporidium parvum*. In fact, the WHO estimates that between 2 and 9% of all AIDS patients in southern Europe will develop VL (301). Almost all cases of *Leishmania* coinfection with HIV have been described in patients with HIV-1, but one has also been diagnosed in a patient with HIV-2 (257).

The profile of patients suffering from VL is not especially different from that of patients with other HIV-associated opportunistic infections. In southern European countries, the age of patients coinfected with *Leishmania* and HIV ranges from 29 to 33 years, and 80 to 85% are male; coinfection has also been described in HIV-positive children (127). The prevalence of drug addiction in these southern countries is different from that in northern Europe or America, Africa, or Asia. The number of drug addicts per million inhabitants in Spain is 5 to 7 times greater than the mean for European countries and 1.6 times greater than in Italy. In 1993, this habit provoked an incidence of AIDS three times higher in Spain than in other European countries and almost double that in Italy or France. Thus, when risk groups for HIV infection are analyzed in Spain, intravenous drug addicts (IVDU) represent 66% of the total whereas homosexuals (15%), heterosexuals (7%), individuals infected by vertical transmission (2%), and other groups (10%; hemoderivatives and unknown) are far behind the first group. The numbers are the exact reverse of those for other parts of the world, in which IVDU represent only 15% of the risk population (18).

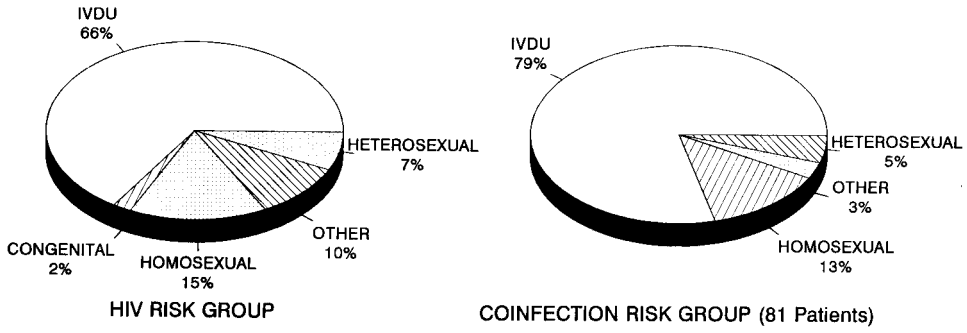


FIG. 1. Percentage of patients in risk groups for HIV and coinfection with HIV and *Leishmania* spp.

In southern Europe, the cases of coinfection are distributed among the following groups (301): IVDU represent 50 to 92%, sexual transmission (homo-, bi-, or heterosexual) represents 5 to 40%, hemoderivatives represent 4 to 13%, and other unknown causes represent 3% (9, 31, 33, 46, 189, 205, 206, 243, 254, 259). The frequency of coinfection among IVDU is thus 15 to 25% greater than HIV frequency for the same risk group (Fig. 1). These data have given rise to hypotheses that the drug addiction habit plays a specific role in *Leishmania* transmission (13, 15).

### Reactivation or Primary Infection

The question about coinfection is whether it represents a latent infection reactivated by immune system suppression or whether it is a primary *Leishmania* infection taking advantage of the lymphocyte reduction caused by the HIV infection. In immunocompetent patients, infection by *Leishmania* spp. is not always followed by disease, since parasitized but completely healthy persons have been detected (219). In addition, a considerable group of parasitized subjects have oligosymptomatic infections that resolve on their own (23). It is estimated that only 1 in every 5 or 10 immunocompetent persons infected by *Leishmania* spp. subsequently suffers from clinical VL (84). The *Leishmania* infection/disease ratio in HIV-positive patients is unknown.

The first hypothesis about coinfection is based on the prevalence of positive intradermal skin reactions in epidemiological studies in countries such as Italy (37, 118, 220, 221) and France (177, 191). These individuals would be asymptomatic carriers of *L. infantum* who, during a later episode of immunodepression, would develop a clinical case of VL (12, 108, 171, 217). This would be a typical example of an opportunistic parasite. The literature also describes individuals from *Leishmania*-free areas, such as Australia, who are infected by the parasite when visiting zones of endemic infection and who express it only years later, after a bout of immune system depression (166). In this manner, HIV infection would unmask the true endemicity of *Leishmania* infection. In the case of HIV infection, reactivation is also known to occur.

Because a T-lymphocyte-mediated immune response is needed to control *Leishmania* infection (160), an additional possibility is that the parasite takes advantage of the immunodepression in HIV patients at the time of primary infection and establishes itself to produce a clinical syndrome. This hypothesis might also explain why strains that are poorly pathogenic in immunocompetent hosts and even apparently nonpathogenic flagellates cause disease in these patients.

The opportunistic behavior of *Leishmania* spp. resembles that of *Mycobacterium tuberculosis*-HIV coinfection (Table 2). As with tuberculosis, a cutaneous test (with leishmanin) can identify individuals who have had previous contact with the parasite. Since it is rare that a radical cure is achieved in advanced stages of HIV infection, it would be of interest to perform, in a prospective manner, a leishmanin test (together with control tests for cutaneous cellular immunity) on all HIV-positive patients in the early stages of disease, so that once the predictive value of the test has been determined, a prophylactic strategy or early treatment can be undertaken.

The second hypothesis takes into account the transmission of protozoa to produce primary infection. *Leishmania* transmission to HIV-positive patients occurs naturally by infection through the bite of parasitized sand flies, as it does in the general population; that is, in the rural or periurban environment, the latter of which is commonly used by IVDU for drug and syringe sharing.

Moreover, in light of the epidemiological data, the information derived from the microbiological markers, the frequency with which parasitized macrophages are found in the peripheral blood of coinfected patients, and certain entomological data, we suggest that transmission can also be achieved mechanically and have proposed a second cycle complementary to the conventional one (Fig. 2). This alternative cycle would be

TABLE 2. Comparative characteristics of tuberculosis and VL in HIV-positive patients

| Characteristic | Manifestation in patients with: | |
|---|---|---|
| | Tuberculosis | VL |
| Association with HIV | Frequent in HIV-positive patients; appears at any time but mainly in patients with CD4 cell counts of $<200/mm^3$ | Frequent in HIV-positive patients; appears almost exclusively in patients with CD4 cell counts of $<200/mm^3$ |
| Means of acquisition | Primary infection or reactivation | Primary infection or reactivation |
| Cutaneous test indicative of previous exposure | PPD; when positive, chemoprophylaxis is required | Leishmanin; test is of unknown predictive value |
| Response to treatment | Acceptable | Frequent relapses |

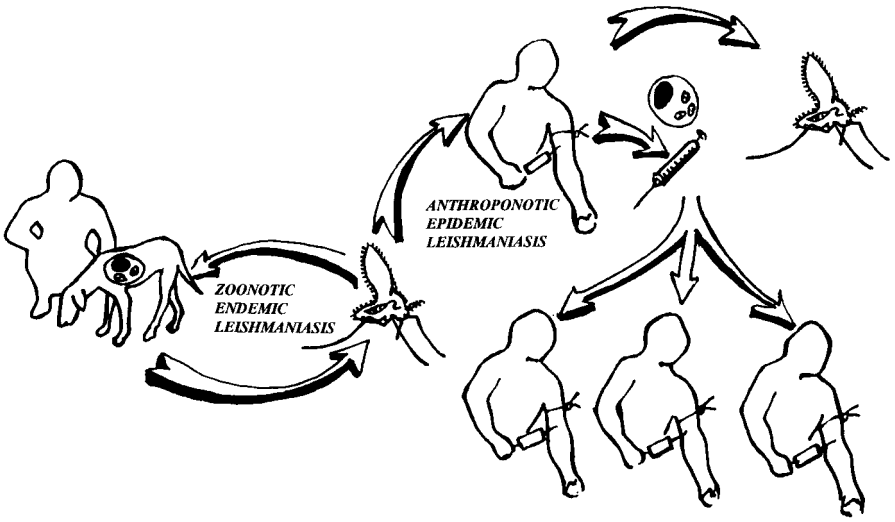


FIG. 2. Zoonotic and anthroponotic *L. infantum* cycles. Reprinted from reference 15 with permission of the publisher.

maintained among intravenous drug addicts who share syringes (13, 14), a risk group that 10 years ago in southern Europe included up to 80% of all those infected by HIV and which today includes 66%.

### An Alternative Cycle: Artificial, Epidemic, and Anthroponotic

The alternative cycle has been defined as artificial, epidemic, and anthroponotic: artificial because syringes substitute for sand flies and metacyclogenesis is unnecessary because amastigotes are transmitted; epidemic because of the form of presentation and the number of cases; and anthroponotic because the drug addicts act as parasite reservoirs (15).

The indicative epidemiological data referring to drug addiction in the Mediterranean countries have been enumerated in depth. Moreover, the information gathered from 81 coinfected patients diagnosed in our laboratory showed that 79 of them were IVDUs. Up to January 1995, 29,520 AIDS cases were recorded in Spain, 66% in the IVDU risk group (18). Although there is no information on the probability of contracting leishmaniasis in non HIV-IVDUs, analysis of the figures for the above coinfected patients indicates that there is a statistical significance for IVDUs becoming coinfected ($\chi^2$ $P = 0.0177$, two tailed, 95% confidence intervals) when compared with other HIV risk groups. Other indirect data that support this anthroponotic transmission are reviewed here. Biochemical variants of *L. infantum* which do not appear in immunocompetent patients with leishmaniasis or among strains isolated from dogs have been isolated from coinfected patients (13, 122, 139, 233). More evidence is that between 50 and 53% of coinfected patients have amastigotes in peripheral blood monocytes (when smears containing 0.3 ml of blood are prepared) (174, 190) and that this percentage increases to 67% if buffy coat is cultured in Novy-Nicolle-McNeal (NNN) medium (163). There is no information of the efficiency of blood staining as a tool for the diagnosis of VL in immunocompetent patients due to the scarcity of circulating parasitized macrophages, although it has been tried in the case of *L. infantum* infection (174). Finally, entomological information also suggests human-to-human transmission: volumes of infected blood as small as 0.3 to 0.5 μl, habitually ingested by a sand fly in the normal feeding process, are sufficient to initiate parasitization by these insects (202, 203). It is common for IVDUs to share 0.3 ml of blood via syringes, an amount that would ensure parasite transmission and explain the high prevalence of VL among HIV-positive individuals. The possibility of this type of transmission was reinforced recently by the experimental transmission of leishmaniasis to hamsters transfused with 0.1 ml of parasitized blood (218).

The ease with which sand flies can be infected with the blood of coinfected patients in the laboratory suggests that these immunodepressed subjects serve as true secondary reservoirs of leishmaniasis in the natural environment (202). The real epidemiological risk that coinfected individuals pose for the general population remains to be clarified, however, particularly that associated with asymptomatic and symptomatic patients who are treated, since they are carriers of circulating parasites and are not hospitalized. We have carried out six direct xenodiagnoses in coinfected patients, and all of them were positive (200, 201). It is of interest to point out the parallelism with kala-azar in India, also an anthroponosis. In that country, 70 to 80% of the infected patients show parasites in peripheral blood films, and high infection levels can be achieved in *Phlebotomus argentipes* fed on patients (288). We found that experimental feeding of *Phlebotomus perniciosus* with blood from some asymptomatic, coinfected VL patients resulted in relatively high infection levels in these insects (202).

The potential transmission risk through transfusion of *Leishmania*-parasitized blood from asymptomatic immunocompetent carriers should also be considered (64), because the parasite can remain alive in these blood specimens for at least 8 days after collection if the sample has been kept at 4°C (125, 201).

Finally, according to the anthroponotic transmission hypothesis, the decrease seen in the number of IVDUs during the last 2 years should, in the medium term, bring about a decrease in the number of cases of coinfection.

## MICROBIOLOGICAL MARKERS

### Variability of *Leishmania infantum* among HIV-Positive Patients

The most common method used to study the variability of *Leishmania* spp. in general and of *L. infantum* in particular is enzyme analysis. This technique has permitted the description

TABLE 3. *L. infantum* zymodemes in HIV-positive patients

| Zymodeme (%) | Country | Reference(s) |
|---|---|---|
| MON-1 (61)[a] | Spain | 16, 139, 140, 232, 233, 245, 272 |
| | France | 34, 178, 233 |
| | Italy | 121, 122 |
| | Portugal | 233 |
| | Algeria | 233 |
| | Greece | 233 |
| MON-24 (10) | Spain | 13, 245, 272 |
| | France | 176, 254 |
| | Italy | 114, 121, 122 |
| | Portugal | 48 |
| | Algeria | 233 |
| MON-28 (2) | Spain | 13, 245, 272 |
| MON-29 (4) | Spain | 139, 245 |
| | France | 233 |
| | Italy[b] | 122 |
| MON-33 (7.5) | Spain | 66, 139, 203, 232, 233, 245 |
| | France | 233 |
| MON-34 (2) | Spain | 13, 139, 245 |
| | Italy | 122 |
| MON-77 (0.7) | Spain | 100, 245 |
| MON-78 (2) | Italy | 121, 122 |
| | Algeria | 233 |
| MON-80 (0.7) | Italy | 122 |
| MON-136 (0.7) | Italy | 121, 122 |
| MON-183 (5) | Spain | 139, 141, 233, 234 |
| MON-185 (0.7) | Italy | 122 |
| MON-188 (0.7) | Italy | 122 |
| MON-190 (0.7) | Italy[b] | 122 |
| MON-198 (0.7) | Spain | 139, 141 |
| MON-199 (0.7) | Spain | 139, 141 |
| MON-201 (0.7) | Italy | 122 |

[a] MON, nomenclature proposed by Laboratoire d'Ecologie Médicale, Montpellier, France; percentages of each zymodeme were calculated from 147 characterized stocks.
[b] Infection was probably acquired in Spain.

of zymodemes and constitutes an extremely useful taxonomic tool that has contributed in large measure to the improved comprehension of leishmaniasis epidemiology (159). Enzymatic characterization of *Leishmania* isolates from HIV-positive individuals has shown the extreme variability of *L. infantum* in such patients from the Mediterranean basin. Approximately 150 *Leishmania* isolates obtained from coinfected individuals have been characterized, and a total of 17 zymodemes have been described in accordance with the nomenclature proposed by the Laboratoire d'Ecologie Medicale, Montpellier, France (247) (Table 3). MON-136, MON-183, MON-185, MON-188, MON-190, MON-198, MON-199, and MON-201 are new zymodemes which to date have been encountered only in HIV-positive individuals. This epidemiological pattern, which includes the new zymodemes and greater variability in coinfected patients, is common in Spain (141) and Italy (122) and to a lesser degree in France. It extends to other Mediterranean countries such as Portugal, Greece, and Algeria, although the number of isolates characterized in these countries is not very large (233).

The greater variability among *L. infantum* isolates from HIV-positive individuals has been described in Italy, where 11 zymodemes have been identified in 38 isolates characterized. Five of them are new enzyme variants that have not been described to cause either VL or cutaneous leishmaniasis (CL) in immunocompetent individuals. The heterogeneity is even greater in more southern zones, although a complete ecoepidemiological study has not been carried out. In any case, six different zymodemes were identified in 12 isolates characterized in Sicily (122). In Spain, the typing of 46 *Leishmania* isolates from HIV-positive patients has permitted the distinction of nine zymodemes in these individuals (139); among them, three new zymodemes, MON-183, MON-198, and MON-199, have appeared in HIV-positive individuals (141). The zymodeme MON-183, previously described in two cases of VL in IVDU HIV-positive individuals who appear to have contracted the infection in Spain (234), is confirmed as a new zymodeme responsible not only for VL but also for CL in coinfected patients (139).

Several hypotheses might explain the high variability of *L. infantum* isolates from HIV-positive subjects, as well as the appearance of new zymodemes in these patients. First, these new zymodemes could be dermotropic variants in the immunocompetent subject, which visceralize in HIV-positive individuals because of their anergic state. This hypothesis is supported by the fact that these zymodemes share electrophoretic mobilities with zymodemes responsible for CL in immunocompetent subjects. In addition, patterns are observed which in some cases correspond to a combination of characteristic electrophoretic mobilities of cutaneous zymodemes. This hypothesis does not exclude the existence of some type of recombinative phenomenon between different zymodemes, which would give rise to virulent zymodemes (122, 139).

To date, no correlation has been found between zymodemes and clinical expression of leishmaniasis in HIV-positive individuals or among zymodemes and $CD4^+$ levels. Since the number of strains isolated in each risk group is not yet representative, no data are available on whether this zymodeme variability is exclusive to IVDU or whether it is also found in other groups. Characterization of sequential isolates from the same individual has shown that they belong to the same zymodeme, indicating that infections are due to relapses and not to reinfections (138, 233).

### Visceral Localization of Cutaneous Zymodemes

In the Mediterranean basin, 16 *L. infantum* zymodemes causing leishmaniasis in immunocompetent individuals have been described within 230 *Leishmania* isolates characterized. While some are responsible exclusively for VL (MON-27, MON-28, MON-72, MON-77, MON-98, and MON-187) or for CL (MON-11, MON-24, MON-29, MON-33, MON-78, and MON-111), others cause both VL and CL (MON-1, MON-34, MON-80, and MON-189). MON-1 was responsible for VL in 90% of patients and for CL in 20%. Other zymodemes such as MON-27, MON-72, MON-111, MON-187, and MON-189 have been found in lower proportions in immunocompetent individuals and have not yet been found in HIV-coinfected patients (42, 119–122, 208).

It is common to isolate both visceral and cutaneous zymodemes from the bone marrow of HIV-positive patients (121, 122, 139, 140, 176). Thus, the zymodeme MON-24 is dermotropic and widely distributed throughout the Mediterranean area, having been described in Italy (36), Algeria (29), and Tunisia (117); MON-29 was isolated from cutaneous lesions in southwestern France (208, 232, 246) and Spain (114); MON-33 was also found in patients from these two countries (208, 232);

and MON-78 was isolated in Malta (114). These four zymodemes have been isolated from the bone marrow of coinfected patients (48, 113, 121, 122, 140, 176, 232, 233, 254). The anergic state of HIV-positive subjects permits parasite dissemination, regardless of the isolate's zymodeme (11, 140).

In addition, in patients with *Leishmania*-HIV coinfection, new zymodemes that have not been previously described in immunocompetent individuals with VL or CL appear and infect their viscera (121, 122, 139, 141, 233, 234). In the immunocompetent subject, these low-virulence variants would be eliminated, simplifying the spectrum of zymodemes known to be capable of causing disease. The tropism of *L. infantum* for skin or viscera and thus its virulence would be influenced by the immunological state of the host (11, 140, 141). The theoretical development of this hypothesis was elegantly performed by Gradoni and Gramiccia (112).

In HIV-positive individuals, few cases of VL are caused by *Leishmania* species other than *L. infantum*; among them are *L. braziliensis* (71, 88, 132, 168), *L. aethiopica* (32), *L. tropica* (54, 170), and *L. major* (106); one case has also been caused by a new *Leishmania* variant which shares kinetoplast sequences with *L. mexicana* and *L. braziliensis* (133).

### *Leishmania*-Like Flagellates in AIDS Patients ("Lower Trypanosomatids")

Within the family Trypanosomatidae, the genera *Leishmania* and *Trypanosoma*, both digenetic, are pathogens in humans and some animals. In Brazil, some cases of *Trypanosoma cruzi* infection with development of encephalitis have been detected in patients who contracted HIV through transfusion (248). *Phytomonas* and *Endotrypanum* spp. are also digenetic parasites of lactiferous plants and some mammals, respectively. Other trypanosomatids, *Herpetomonas*, *Crithidia*, *Blastocrithidia*, *Leptomonas*, and *Sauroleishmania* spp., are monogenetic, parasitize insects, and are known as "lower trypanosomatids" (184). None of these trypanosomatids have been confirmed as human pathogens.

Nevertheless, the literature describes a possible *Herpetomonas* infection in a woman from Texas (184), and some observations have been made of flagellates isolated from individuals in Kenya which, by enzyme analysis or kDNA flotation density, showed a greater similarity to *Herpetomonas* and *Crithidia* spp. than to *Leishmania* spp. (107, 186). Finally, a case of atypical VL was described in a 10-year-old HIV-2-seropositive girl from Guinea-Bissau (an infection possibly contracted through vaccination), which was unlikely to have been caused by a *Leishmania* sp., because leishmaniasis has not been previously described in this country (300). The authors attribute the infection to some reptile trypanosomatid (256).

A case of a diffuse nodular cutaneous syndrome has recently been described in an HIV-positive individual from Martinique. Enzyme characterization confirmed that it was not caused by an Old or New World *Leishmania* species or by *Trypanosoma* or *Sauroleishmania* spp. Ultrastructural analysis of the parasite indicated that the infection could have been produced by a lower trypanosomatid not belonging to the genera *Blastocrithidia* or *Crithidia* but possibly to *Leptomonas* or *Herpetomonas*, although the authors do not assign it definitively to either of these genera. The immune system depression in the patient might explain the parasitization by this trypanosomatid (79).

A similar case of an unusual, *Leishmania*-like parasite was found in a patient in Madrid who was HIV positive and a member of the IVDU risk group and who showed typical symptoms of VL (142). Biochemical characterization with specific gDNA and kDNA probes ruled out *Leishmania* spp. When *P. perniciosus* and *Lutzomyia longipalpis* were fed experimentally with the flagellate, the infection was not reproduced, and electron microscopy furnished no help in its classification. Comparison of enzyme patterns of this trypanosomatid with those of *Crithidia fasciculata*, *Herpetomonas muscarum*, *Leptomonas ctenocephali*, *Blastocrithidia* spp., and *Sauroleishmania* spp. did not permit its assignment to any of these genera.

The finding of these monoxene trypanosomatids in HIV-positive individuals suggests that these patients may be vulnerable to other trypanosomatid parasites of insects, which might give rise to a clinical pattern similar to *Leishmania* infection. It is difficult to speculate about the possible transmission mechanism of these trypanosomatids, although it must be recalled that they are in general subject to fecal transmission. Some of them, like the "water strider" parasites *Blastocrithidia gerridis* and *Crithidia flexonema*, can survive in water for 48 and 30 h, respectively, under experimental conditions (289). Other authors have also shown in the laboratory that transmission of *Crithidia fasciculata* in *Culiseta incidens* occurs in the water in which the flagellates live in their free form, although this is only an approximation of the possible situation in nature (61). Since the patient cited above was an IVDU patient the infection might have been contracted by using syringes washed in water contaminated with these flagellates, and her anergic state would have facilitated visceral dissemination.

## PATHOGENESIS AND IMMUNOLOGICAL ASPECTS

### Analogies and Differences in the Immune Response in *Leishmania* and HIV Infections

Infection by HIV and infection by *Leishmania* spp. each induces important changes in the immunological status of affected individuals. HIV infection is a complex process in which the nature of the virus itself intervenes, as does its capacity to infect and destroy the cells required for the production of an effective immune response. *Leishmania* infection gives rise to a range of diseases, depending on the species involved and the efficiency of the individual's immune system response to the parasite. Nonetheless, although numerous cases have been reported, little work has been done on the immunological status induced by *Leishmania*-HIV coinfection.

The immunological disturbances produced by both infections have similarities that can enhance their effects if they occur concomitantly. This has been observed by Cenini et al. (53) in peripheral blood mononuclear cells. The authors also observed that when the effects are not synergistic, those of the viral infection predominate over those of the parasitic infection. The result is a considerable heterogeneity of immune cell phenotypes in coinfected patients. These alterations are related to the stage of HIV infection, being more evident in patients with AIDS than in patients with AIDS-related complex (53). The predominance of HIV-induced changes is also seen in the results obtained in our laboratory from coinfected individuals treated for visceral leishmaniasis, in whom the percentages of peripheral blood mononuclear cell populations showed no differences from those obtained in HIV-positive individuals without VL, except for $CD8^+$ $CD57^+$ cells whose percentage is significantly lower in coinfected patients than in non-*Leishmania*-infected HIV-positive patients (209). A decrease in the number of $CD8^+$ $CD11b^+$ suppressor cells was also reported in coinfected patients after therapy (53), although the significance of the decrease of T-cell subsets with inhibitory activities after treatment remains obscure.

### Humoral and Cellular Responses

The predominance of the immunological state induced by the viral infection determines the immune system response to the parasitic infection. In fact, although VL is characterized by a strong humoral response (130), a large proportion of coinfected individuals (over 40%) have no detectable specific antibody levels against *Leishmania* spp. and the remainder have varying titers (11, 116, 188, 204). This is probably a consequence of an oligoclonal B-cell response due to the absence of T cells that can recognize specific *Leishmania* antigens or stimulate the B cells. In cases in which a high titer is found, it has been proposed that the *Leishmania* infection occurred prior to the HIV infection (116), as occurs in areas of high prevalence of leishmaniasis, where practically all coinfected patients have high antibody titers (33). A several-year-long prospective serological study of 20 coinfected patients shows, however, that the humoral response is capricious, and can be present in situations of total lymphocyte depletion, absent in patients with more than 500 lymphocytes/$mm^3$, or even intermittent (188).

HIV-induced immunodepression also prevails over the cellular response that the parasite provokes. Whereas in patients with CL and mucocutaneous leishmaniasis (MCL) the cellular response appears from the onset of the disease and patients with VL develop it after successful chemotherapy (160), 1 of 2 patients reported with CL and AIDS showed an in vitro lymphoproliferative response to *Leishmania* antigens after treatment (71), as did 4 of 14 treated patients with VL and AIDS (209).

A number of studies involving the murine experimental leishmaniasis model have shown that protective immunity to the parasite is determined by an adequate Th1 response whereas susceptibility is mediated by a Th2 response (reviewed in reference 242). This finding is also applicable to human CL (146) and VL (147, 196), in which marked hypergammaglobulinemia produced by polyclonal B-cell activation and high interleukin-4 (IL-4) levels is associated with the Th2 response (304) while the cellular response and gamma interferon (IFN-γ) production are associated with the Th1 response (149). It has been proposed that in individuals who have been in contact with HIV, a Th1 response would be immunoprotective and would help to avoid infection by the virus and/or AIDS progression while seroconversion and AIDS progression would be correlated with a Th2 response (62). The study of cytokines during development of the disease (27) and the natural protection against the virus shown by some individuals (274) seem to confirm this hypothesis. Although findings obtained by others authors do not support this proposal (124, 169), it is clear that altered cytokine production in HIV-infected, antigen-presenting cells may favor the expression of Th2 cytokines. In fact, low IL-12 production (55), as well as a decrease in costimulatory molecule expression (41), has been observed in HIV-positive patients. As the anti-*Leishmania* response depends in large measure on the cytokines present during early T-cell activation (268), the absence in coinfected individuals of a protective response against the parasite is understandable. The parasite finds the immune status of the HIV-positive patient altered in a direction particularly favorable for its uncontrolled replication. For patients in whom *Leishmania* infection occurred before viral infection, the virus-induced Th2 state would also explain the reactivation of leishmaniasis (32). Although coinfected patients can respond to treatment initially (80% of cases) by generating a specific cellular antiparasite response (214), the frequent percentage of relapses observed in these patients indicates that this response is overcome by the overall Th2 state of the patients (47).

An interesting aspect that may have considerable importance in the effective control of leishmaniasis in individuals who, like HIV-positive patients, have compromised CD4-cell functions, is the importance of $CD8^+$ cells in response to the infection. $CD8^+$ cells have traditionally been associated with resistance to viral infections, but it is now thought that they may play an important role in immunity to intracellular microorganisms, acting as much by cytokine production as by direct lysis of infected cells. Immunocompetent patients with CL have $CD8^+$-cell clones that proliferate in response to the parasite and are associated with recovery from the disease (70, 192). Coutinho et al. (67) reported the case of a patient with CL and AIDS in whom the majority of reactive T cells after treatment were $CD8^+$ cells; in addition, this cellular response was accompanied by IFN-γ production. In our laboratory, we observed that in four patients with AIDS and VL who showed a cellular response, two had a majority of $CD8^+$ cells (209).

### Virus-Protozoan Interaction

Another important question to be considered about the immunological status of coinfected patients is the possible influence of the parasitic infection on AIDS development. Any stimulus that induces a Th2 state will act as a cofactor for AIDS, so that an individual whose immune system is brought to this state by a non-HIV signal will be more susceptible to HIV infection and/or AIDS progression, resulting in increased virus expression and its spread throughout the body. The two infections, in addition to inducing similar changes in the host, share the same target cell. *Leishmania* spp. are obligate intracellular parasites which infect and replicate within macrophages, whereas HIV can invade and replicate within macrophages in addition to $CD4^+$ T cells (210). The presence of both microorganisms in the same cell type could have important implications in their expression and extension (291). Thus, Bernier et al. (35) have shown in vitro that *L. infantum* promastigotes can induce virus expression in monocyte lines latently infected with HIV-1 and that this expression is mediated by the lipophosphoglycan, a basic component of the parasite membrane. Treatment of monocyte cell lines with lipophosphoglycan induces the secretion of tumor necrosis factor alpha, a cytokine that has previously been reported to induce HIV-1 expression in T cells and monocytes (95, 96). It has also been observed that in vitro, lipophosphoglycan can inhibit syncytium formation, viral infection, and production of the protein antigen p24 by the virus (87). Although it is possible that leishmaniasis induces viral expression in coinfected patients, to date only the data of Cacopardo et al. (47) are available. Over a 4-month period, they observed an increase in the viral RNA level in the serum of three coinfected patients at the same time as an increase in IL-4, IL-6, and IL-10 levels. This led them to propose that the switch in the response from Th1 to Th2 is irreversible. Nonetheless, it is necessary to obtain more data on the immunological status of coinfected individuals for a better understanding of the synergistic effect of both infections and to determine the best procedures for treatment and control. In this sense, it would be very useful to establish an experimental model of coinfection, such as that developed for *Trypanosoma cruzi* and murine leukemia virus (283), which would permit an improved approach to the problem of coinfection.

## LABORATORY DIAGNOSIS

### Serological Diagnosis

Over the years, a number of serological techniques have been developed to show the humoral immune response to

infection by *Leishmania* spp. The serological methods are simple, noninvasive procedures that are particularly useful for diagnosing VL, MCL, and diffuse cutaneous leishmaniasis, although not for CL, in which only a weak immune response is established (145, 160). During the acute phase of VL, a strong humoral response appears, with marked hypergammaglobulinemia and the presence of specific antiparasite antibodies and nonspecific immunoglobulins as a consequence of polyclonal B-cell activation (101). Serological diagnostic techniques take advantage of this characteristic of VL and determine specific anti-*Leishmania* antibodies both in immunocompetent VL patients and in immunodepressed individuals not infected with HIV (22, 44, 93).

Most of the cases of *Leishmania*-HIV coinfection described in the literature are cases of VL (11, 78), although more than 40% of the coinfected patients show no detectable levels of anti-*Leishmania* antibodies (11, 301). The most frequently used VL serological diagnostic methods in coinfected individuals are indirect immunofluorescence (IFAT), dot enzyme-linked immunosorbent assay (ELISA), and Western blotting (WB).

Commercial IFAT kits, which usually show low sensitivity, were used for the diagnosis of VL in some of the published series of clinical cases of coinfection (10, 31). In recent studies involving an experimental IFAT test, significant anti-*Leishmania* titers were detected in 40, 82, and 90% of the patients analyzed (82, 103, 116). A sensitivity level between 72% (103) and 78% (301) is claimed for dot ELISA. In a study of seven Ethiopian VL patients coinfected with HIV, high antibody titers were detected in all of them by the ELISA method (33). Finally, the antibody response to *L. infantum* antigens in HIV-coinfected individuals has been analyzed by WB in three recent studies. Mary et al. (180) observed an antigen recognition pattern identical to that of immunocompetent VL patients in 11 coinfected subjects. Although the 14-kDa band was not found in some patients, recognition of the 16-kDa band, to which a greater diagnostic value is ascribed, was constant. By IFAT and ELISA, 7 of the 11 patients studied (64%) gave negative results. In another study in southern France, taking the presence of the 14- and/or 16-kDa band as the criterion for positivity, 83% sensitivity was observed (103). Cardeñosa et al. (51) studied the antibody response of four VL- and HIV-positive patients by WB and detected a 17-kDa band in all of them, which also appeared in immunocompetent VL patients and disappeared after chemotherapy; therefore, this band may have predictive value. In a study in which several diagnostic methods were compared (228), 20% of coinfected patients gave negative results in all serological techniques tested (counterimmunoelectrophoresis, IFAT, ELISA, and WB).

In spite of these data, little is known about the pattern of the humoral immune response to *Leishmania* spp. in coinfected patients. We recently studied 212 sequential serum samples from 20 coinfected patients (188). For 18 of the 20 patients, a sample was available from the time of parasitological diagnosis of VL, and the serological follow-up before and after the acute phase ranged from several months to more than 7 years. All sera were analyzed by IFAT and by an ELISA which uses recombinant rK-39 antigen (45). Sera that were positive by at least one technique and all acute-phase sera were studied by immunoblotting. In the analysis of acute-phase sera, IFAT and rK-39 ELISA showed very low sensitivity (11 and 22%, respectively), even though the rK-39 ELISA had been shown to be a very sensitive technique for VL diagnosis in immunocompetent individuals from various geographical areas (45, 236). When these samples were analyzed by immunoblotting, 78% were reactive, although the banding pattern was quite variable and appreciably weaker than in nonimmunocompromised VL patients. The humoral immune response throughout the life of coinfected patients is extremely variable and apparently independent of the total number of $CD4^+$ lymphocytes. In two individuals who suffered relapses of VL, a positive serological response was found to be permanent by IFAT and rK-39 ELISA; nonetheless, a similar response pattern was observed in apparently cured individuals. Thus, these techniques seem to have no predictive value for the clinical course of VL in coinfected patients. Nevertheless, progressive disappearance of the bands recognized is observed by WB in apparently cured individuals and a more persistent antibody response is seen in individuals who suffered relapse.

Although more sensitive techniques have recently been applied to the serological diagnosis of VL in persons coinfected with HIV, it is clear that a percentage of patients cannot develop a humoral immune response to the parasite. It has also been estimated that specific anti-*Leishmania* antibody levels in AIDS patients are 50 times lower than in those with an intact immune system (180). Gradoni et al. (116) suggested that the serological response could be related to the sequence of temporal acquisition of the infectious agents. Seropositivity would represent a reactivation of latent infection before the immune depression caused by the viral infection (asymptomatic carriers), while seronegativity would result from primary *Leishmania* infections after viral infection. However, the serious depletion of memory cells, which have CD4 molecules on their surfaces, in patients with advanced stages of AIDS could also explain the existence of seronegative individuals among asymptomatic carriers. Likewise, the severe dysfunction of T and B lymphocytes in HIV-infected individuals would explain the decrease in specific antibody production, as occurs for other infections (38, 298).

Among the possible mechanisms responsible for failure of the immune response in these patients could be an alteration in antigen presentation by macrophages (167) or in T- and B-lymphocyte cooperation (156). To understand this phenomenon, further basic research on the pathogenesis of coinfection is necessary.

## Etiological Diagnosis

Demonstration of the parasite is considered the diagnostic criterion par excellence and is a requisite for evaluation of treatment in any of the disease forms (299). The sensitivity limits of conventional diagnostic techniques make advisable the use of at least one isolation method (culture or inoculation in experimental animals) with microscopic examination of prints, smears, or histological sections of biological material (stained with Giemsa, hematoxylin-eosin, or other stains). Of the nucleic acid-based methods, PCR is the most promising alternative for parasite detection, although its role in epidemiology, prevention, and treatment of parasitic diseases remains to be determined.

**Skin biopsy and culture.** The sensitivity of direct examination or culture of skin biopsy specimens varies according to the form of CL, the characteristics of the lesion, and the method of obtaining the biological material (144). It has been accepted traditionally that biopsy specimens and skin splits should be taken from the indurated border of the lesion (300), but some authors find greater sensitivity when using aspirates or scrapings even from the center of the ulcers, without this posing a risk of secondary contamination (215, 297). Microscopic examination of cutaneous biopsies has permitted the detection of CL-AIDS coinfections in the Old World, which are produced presumably by *L. infantum* and *L. major* (226, 227). This

method, together with culture in axenic media, confirms the production of completely tegumentary leishmaniases with no evidence of hematogenic dissemination by *L. infantum* in these patients (264). *Leishmania aethiopica* has been identified by these methods in HIV-positive patients, causing recurrences in healed lesions (32). In the New World, the AIDS-associated cases of CL described can have a clinical appearance similar to that of the diffuse cutaneous disease (69, 249), and although the presence of *L. braziliensis* has been confirmed in a similar case (71), the existence of mucocutaneous forms produced by the same parasite (88, 168) appears to confirm reactivation of the disease by persistent parasites, as has been found in immunocompetent patients (262).

*Leishmania infantum* has been cultured from apparently healthy skin of three Pakistani patients (237), and *L. donovani* has been isolated under the same circumstances from at least one patient in India (261). The existence of amastigotes in skin suggests that they could be the most important source of infection for sand flies in areas of anthroponotic *L. donovani* VL (238). In coinfected patients, *L. infantum* amastigotes have also been detected in apparently healthy skin by histological section or in culture (225, 278, 284), making the systematic search for the parasite advisable in this tissue (78).

**Spleen and lymph node aspirate and liver biopsy staining and culture.** Microscopic analysis of stained spleen aspirate preparations is considered the most sensitive method for parasite detection in suspected kala-azar patients. However, obtaining spleen aspirate and liver biopsy specimens entails serious risk to patients; the use of lymph node or bone marrow aspirates is therefore recommended. The sensitivity obtained with different biological materials varies according to the researcher; comparative studies indicate sensitivities of 94.7 to 96.4% for spleen aspirates, 76.9 to 91% for liver biopsy specimens, 52.6 to 70.2% for bone marrow aspirates, and 52.6 to 58.8% for lymph node aspirates (276, 303). In patients with generalized lymphadenopathy, microscopic examination of lymph node aspirates has a sensitivity similar to that for bone marrow aspirates (90, 222, 276).

In patients with VL-HIV coinfection, diagnosis by microscopic examination of spleen or lymph node aspirate preparations is usually limited to those in countries with endemic kala-azar and a tradition in obtaining these samples. One of the few published studies from these countries, describing seven coinfected patients, showed high sensitivity, with amastigote detection in the spleen aspirate of six patients examined as well as in the two lymph node aspirates done (33). Treatment was evaluated by determining parasite density by the method of Chulay and Bryceson (57). In Europe and North America, spleen and lymph node aspirates and liver biopsy, especially indicated when there is a high transaminase level, are used as alternatives for confirmatory diagnosis and, generally, following a negative result with a bone marrow aspirate (10, 205, 225, 243).

**Bone marrow aspirate staining and culture.** Bone marrow aspiration is considered the simplest procedure and that which entails least risk to the patient. It is recommended that stained smears of this material be examined in parallel with isolation in culture. Of 45 VL patients in India, 18 (40%) were negative in microscopic examination and 39 were diagnosed by culture (277), while microscopic examination in pediatric kala-azar patients in southern France showed 93% sensitivity (179). In VL patients, the sensitivity of bone marrow aspirate cultures and smears depends largely on the parasite burden. The culture medium used is also critical, and NNN medium is recommended. According to several authors, the sensitivity of bone marrow cultures can range from 40 to 95% (240, 260, 273). Culture of biopsy specimens from MCL and CL patients shows sensitivities from 70 to 89.8%, respectively, or even much lower (215, 258).

In the Mediterranean area, confirmatory diagnosis of VL-AIDS coinfection is done by visualization of *L. infantum* amastigotes in stained bone marrow aspirate preparations. Several authors recommend the examination and culture of bone marrow aspirates in HIV-positive patients with fever, visceromegaly, or hematological abnormalities (10, 66). Sensitivities of microscopic examination vary from 78 to 94% for the first episode and 64% for relapses, with the possibility of increasing sensitivity by culture in NNN medium (10, 11, 82, 205, 301).

**Blood staining and culture.** In zones of endemic VL infection such as India, Kenya, China, and Sudan, it is common to detect the presence of the parasite in blood by conventional parasitological techniques. The parasitemia corresponds to the density of parasites observed in spleen aspirates (58, 261). In one study carried out in Kenya with 20 VL patients, *L. donovani* was detected in blood in 11, 10, and 7 patients, by direct examination, culture, and isolation in hamsters, respectively (58).

Massive parasitemias, uncommon in the Mediterranean countries where infection is endemic, should raise a suspicion of associated immunodepression (94). Direct observation of amastigotes in peripheral blood preparations is considered a simple, rapid, noninvasive method which permits diagnosis in infected patients. In Spain, microscopic examination of peripheral blood permits diagnosis in a high proportion (50 to 53%) of VL-HIV-coinfected patients (174, 190). Culture of buffy coats from these patients in NNN medium permits an increase in sensitivity to 67% (163).

**Diagnosis of leishmaniasis in unusual locations.** Between 10 and 30% of coinfection cases are found in unusual locations for primary or secondary leishmaniasis (78, 254). Following histological examination, the parasite has been found in the lungs (181, 253), larynx (109), gastrointestinal tract (115, 129, 152, 153, 182, 294), and rectum (255); one case of disseminated multivisceral leishmaniasis has also been published (204). Finally, *L. infantum*-parasitized macrophages were isolated from the cerebrospinal fluid of one patient with *Candida albicans* meningitis (151). Frequently the diagnosis of leishmaniasis is fortuitous, since it coexists with other opportunistic infections or tumors which dominate the clinical symptoms. Particularly for the digestive tract, several authors have indicated the importance of routine gastrointestinal biopsy in HIV-positive patients who have lived in or traveled to zones of endemic VL infection, even when endoscopy shows no abnormalities (115, 153).

*Leishmania* antigens have been detected in urine by agar precipitation techniques in VL diagnosis (148). In this and other body fluids such as nasal and pharyngeal secretions, *L. donovani* amastigotes have been isolated with an efficiency of 33 to 51.6% from immunocompetent patients in Kenya (185). In urine from *L. infantum*-infected immunocompetent and coinfected patients, two diagnostic antigens of 123 and 72 to 75 kDa are found. The efficiency of the method is high (nine of nine HIV-positive and five of six HIV-negative VL patients), so that it may be a noninvasive technique which assists in the diagnosis when bone marrow aspirates are not feasible (65).

## PCR

PCR has been applied to the detection and identification of *L. donovani* (89, 193, 279), *L. infantum* (241), and, in the New World, to species of the subgenera *Viannia* (76, 77, 162) and *Leishmania* (91), all in immunocompetent patients. The joint

use of PCR with specific probes permits discrimination of the causative species of CL (157, 250).

Diagnosis of VL by bone marrow aspirate is an invasive technique, which should be replaced by an equally sensitive method, such as PCR, for the detection of parasites in blood. The results obtained with peripheral blood samples appear to demonstrate the utility of this "noninvasive PCR" in early diagnosis and in patients with asymptomatic cases of VL. Nevertheless, serial studies are necessary to determine whether the parasitemia is continuous (2, 216, 265). Both, false-positive and false-negative cases have been reported by PCR (229).

Most of the previously described assays have not been used for diagnosis in patients with *Leishmania*-HIV coinfection and should be evaluated through extensive use, clinical observation, and comparison with other methods whose characteristics are known. It must also be determined whether these methods can provide a solution to the problem of diagnosis in patients with persistent infections. The comparative study in *Leishmania*-HIV-coinfected patients has confirmed the greater sensitivity of PCR over conventional parasitological methods and has shown the difficulty in establishing the significance of positive results in the presence of negative serological results, history of infection, or patient treatment (228). A set of carefully-chosen criteria must accompany PCR diagnosis. When the clinical history does not concur with the PCR result, confirmatory parasitological diagnosis must be established before treatment is initiated, even when a new sample must be obtained.

The use of PCR together with species-specific probes has permitted the identification of *L. braziliensis* and a variant that shares kinetoplast sequences with *L. braziliensis* and *L. mexicana* as being responsible for two cases of VL-HIV coinfection in which the parasite could not be isolated in bone marrow culture (131, 133).

PCR has not yet been applied to solving the problems associated with the diagnosis of coinfection and will probably permit an evaluation of the true extent of this coinfection in regions of endemic infection. PCR is especially indicated in the early diagnosis of VL, to establish the diagnosis of diffuse CL (clinical or asymptomatic) or of post-kala-azar dermal leishmaniasis when the parasite is found in unusual organs, in typical MCL in HIV-positive patients in whom *Leishmania* spp. isolation is difficult, and in the treatment follow-up in relapses.

### Xenodiagnosis

Sand flies have been little used in the diagnosis of VL in recent years. This is in part because unsatisfactory results were obtained when this method was used for direct xenodiagnosis in immunocompetent individuals (3, 4). Possible hypersensitivity reactions caused by using these insects directly on patients' skin has also impeded the diagnostic utility of this method, which has therefore been limited to occasional epidemiological studies (1).

The diagnosis of VL in immunodepressed patients is often complex and frequently requires the use of several techniques for confirmation. Bone marrow aspirate culture in NNN medium has been proposed as the diagnostic method of choice, but it is an invasive and painful technique for patients who are, in great part, already in fairly poor clinical condition. PCR must still be standardized for the diagnosis of coinfection and its associated problems. Recent studies evaluating the efficiency of indirect xenodiagnosis of VL by using *Phlebotomus perniciosus* (Diptera: Psychodidae) have shown that it is one of the most efficient techniques for parasite detection in HIV-*L. infantum*-coinfected patients (202, 203). In indirect xenodiagnosis, promastigotes can be detected in the digestive tract of the insect 48 h after it has ingested the blood sample, a considerably shorter period than that needed for conventional isolation techniques. As the method uses peripheral blood previously extracted from the patient, the problems inherent in direct xenodiagnosis are avoided. In posttreatment relapses of the parasite infection, the time at which it is most difficult to demonstrate the presence of the parasites, indirect xenodiagnosis is especially efficient. After more than 3 years of using this technique in our laboratory, an indirect xenodiagnosis protocol for coinfected patients has been defined (201) which can basically be summarized as follows. A 1.5-ml sample of heparinized peripheral blood from a coinfected patient is maintained at 4°C until processing. The blood is used to feed female *P. perniciosus* flies maintained in the laboratory by using a membrane feeding apparatus (296). The sand fly guts are dissected and examined for promastigotes 2 to 7 days after feeding; when a digestive tube is found to be parasitized, its content is seeded in NNN medium. The blood sample can be stored at 4°C for at least 8 days in any anticoagulant and can be sent by mail by following the procedures for shipping contagious samples. However, xenodiagnosis is a diagnostic method whose use should be restricted to qualified laboratories with biosecurity guarantees and to patients for which normal diagnostic techniques have failed despite the suspicion of persistent leishmaniasis. In any case, it is an alternative diagnostic method to VL in coinfected patients which should at least be available in leishmaniasis reference laboratories. The technique can be also used to detect promastigotes of VL-causing *Leishmania* species by using the appropriate species of sand fly. Thus, for example, the need has been mentioned for techniques such as xenodiagnosis that provide more information on the hematogenic dissemination of *L. braziliensis* in the development of mucocutaneous forms and other metastasic manifestations of the disease (173).

## CLINICAL MANIFESTATIONS

### Incubation Period

In immunocompetent subjects, the incubation period is quite variable and is estimated to be 2 to 6 months. It has not been determined in HIV-positive patients but could be quite short, if the time at infection coincides with severe immunodepression, or quite long, if the disease is the result of reactivation of latent infection, clinically flourishing when the cellular immunity becomes sufficiently decreased. Thus, cases of VL in countries where the infection is not endemic can be detected in immigrants, military personnel, or travelers months after returning from a region of endemic infection (25, 54, 170, 285).

### Immunological Status of Patients

The majority of leishmaniasis cases in HIV-positive patients appear in the advanced stages of the disease (Table 4). The number of $CD4^+$ lymphocytes is less than 200/mm$^3$ in 77 to 90% of patients, between 200 and 500/mm$^3$ in 7 to 22%, and greater than 500/mm$^3$ in only 0 to 3%. In general, half of the patients (between 42 to 72%) have AIDS-defining criteria before or during the first VL episode (10, 78, 188, 205, 206, 243, 254, 301).

During any VL episode, other concomitant opportunistic infections are diagnosed in 42 to 68% of HIV-positive patients (189, 254). This finding is not unexpected, since most cases of leishmaniasis appear when the patient is seriously immunode-

TABLE 4. VL in HIV-positive patients: clinical manifestations[a]

| Reference | No. of patients | Risk factor (%) for HIV[c] | $CD4^+$ count/$mm^3$[c] | % of patients with[c]: | | | | | Other clinical features and concomitant opportunistic infections (no. or %)[c] |
|---|---|---|---|---|---|---|---|---|---|
| | | | | AIDS criteria | Fever | Adeno-pathies | Viscero-megalies | Peripheral blood | |
| 31 | 9 | 89 (IVDU), 11 (HD) | ND | 22.2 | 66.6 | 44.4 | 55.5 (HS), 88.8 (H/S) | 77.7 (A), 44.4 (L), 44.4 (T) | DIC (1), diarrhea (1), pulmonary tuberculosis (1) |
| 205[b] | 40 | 92.5 (IVDU), 5 (ST), 2.5 (UK) | <400 = 90%, $X$ = 204 | 47.5 | 95 | 57.5 | 80 (HS), 92.5 (H/S) | 82.5 (P) | Constitutional syndrome (72.5%), cutaneous leishmaniasis (10%) |
| 259 | 12 | 100 (IVDU) | <200 = 66.6% | 25 | 100 | ND | 100 (H/S) | 100 (P) | Cutaneous leishmaniasis (1), rectal leishmaniasis (2) |
| 10 | 8 | 62.5 (IVDU), 25 (ST), 12.5 (HD) | <200 = 62.5% | 37.5 | 100 | ND | 100 (S) | 87.5 (A), 87.5 (L), 87.5 (T) | Oral candidiasis (7), *Salmonella* bacteremia (3), *Pneumocystis carinii* pneumonia (2), pulmonary tuberculosis (1) |
| 46 | 8 | 50 (IVDU), 50 (ST) | <200 = 100%, $X$ = 69.5 | 50 | 87.5 | 100 | 75 (H), 62.5 (S), 87.5(H/S) | 62.5 (A), 100 (L), 71.4 (T) | Pleuropulmonary and mediastinal lymph node leishmaniasis (1), constitutional syndrome (50%), *Rhodococcus equi* lung abscess (1), Hodgkin's lymphoma (1), disseminated cryptococosis (1), CNS toxoplasmosis (1) |
| 189 | 47 | 66 (IVDU), 21.3 (ST), 12.7 (HD) | <200 = 87% | 66 | 87.2 | 44.7 | 74.5 (H), 72.3 (S) | 76.6 (P) | Opportunistic infections (32/47 [68%]), including esophageal candidiasis (17), extrapulmonary tuberculosis (10), *P. carinii* pneumonia (9), disseminated cytomegalovirus (4), CNS toxoplasmosis (2), disseminated cryptococosis (2), *Salmonella* bacteremia (1) |
| 33 | 7 | ND | <200 = 100% | 50 | 100 | 57.1 | 42.8 (H), 85.7 (S) | 100 (A), 85.7 (L) | Diarrhea (71.4%), constitutional syndrome (85.7%), sepsis (42.8%) |
| 82 | 10 | ND | <200 = 100%, $X$ = 57 | 77.7 | 100 | ND | 40 (S) | 100 (A), 100 (L), 90 (T) | ND |
| 243 | 20 | 50 (IVDU), 40 (ST), 10 (HD) | <200 = 90%, $X$ = 69 | 60 | 70 | ND | 85 (H), 90 (S) | 50 (P) | Opportunistic infections (8/20 [40%]), including cutaneous leishmaniasis (1), mucosal leishmaniasis (1), constitutional syndrome (90%), CNS toxoplasmosis (2), cytomegalovirus retinitis (2), *P. carinii* pneumonia (1), disseminated tuberculosis (1), esophageal candidiasis (1), PML (1) |
| 164 | 54 | 79.6 (IVDU), 13 (ST), 3.7 (HD), 3.7 (UK) | <200 = 89.3% | 47.3 | 80 | 11.9 | 80.9 (H), 70.2 (S), 67.8 (HS) | 92.6 (A), 90.7 (L), 92.6 (T) | Opportunistic infections (16%) |

[a] Analysis of more relevant studies.
[b] 15 previously reported cases are included in this study (206).
[c] Abbreviations: CD4, $CD4^+$ lymphocyte count per $mm^3$; X, average; AIDS, patients meeting AIDS criteria at the first diagnosis of VL; H, hepatomegaly; S, splenomegaly; HS, hepatomegaly and splenomegaly; H/S, hepatomegaly or splenomegaly; ST, sexually transmitted (heterosexual or homosexual or bisexual); HD, hemoderivates (blood transfusion, hemophiliacs, etc.); UK, unknown; ND, no data; A, anemia; L, leukopenia; T, thrombocytopenia; P, pancytopenia; DIC, disseminated intravascular coagulation; CNS, central nervous system; MAC, *Mycobacterium avium* complex; PML, progressive multifocal leukoencephalopathy.

pressed, the parasitic disease itself causes associated immune system depression, and a severe decrease in the number of $CD4^+$ lymphocytes has been observed in HIV-negative individuals with VL (68). The clinical symptoms of leishmaniasis can therefore be masked by concomitant opportunistic infections.

### Clinical Forms: Differential Diagnosis

The entire clinical spectrum of leishmaniasis has been described in HIV-positive patients, including asymptomatic or paucisymptomatic cases, cases with enhanced clinical symptoms, and fatal cases. The most frequent clinical form is VL, with bone marrow, liver, spleen, and lymph nodes as the parasite's target organs. The disease can also affect the digestive or respiratory tract or any other organ within a syndrome of generalized parasitization. Cutaneous, mucocutaneous (affecting the pharynx and larynx), and diffuse cutaneous forms have also been described. In the Mediterranean area, where *L. infantum* causes both CL and VL, cutaneous forms can appear occasionally, can precede a visceral form by some months (visceralization of prior cutaneous lesions), can be concomitant with a visceral form, or can even appear after a previously treated visceral form. Infiltrative cutaneous lesions that resembled dermatomyositis have been described (73).

In Mediterranean countries and in other areas of the world, the clinical manifestations of leishmaniasis in HIV-positive patients do not differ notably from those of immunocompetent individuals (136). The "atypical" manifestations described in up to 10% of HIV-positive patients (78) should not be so classified because, although now only rarely observed in immunocompetent patients, the majority have been well known for decades (172, 231).

The classic clinical triad of fever, pancytopenia, and hepatosplenomegaly is found in 75% of all patients, and the clinical characteristics most commonly encountered are fever, which appears in 80 to 95% of patients; a constitutional syndrome with asthenia and weight loss, in 70 to 90% of patients; splenomegaly, generally moderate, in 54 to 90%; hepatomegaly, in 34 to 85%; combined hepato- and splenomegaly, in 68 to 73%; adenopathies, in 12 to 57.5%; anemia, which is usually marked (with hemoglobin values below 10 g/dl), in 49.5 to 100%; leukopenia, which is usually moderate (with leukocyte values below $2.4 \times 10^3$/l), in 56 to 95%; thrombocytopenia, which is generally pronounced (with platelet counts of less than $150 \times 10^3$/liter), in 52 to 93.%; and a combination of anemia, leukopenia, and thrombocytopenia, in 35 to 77% (10, 78, 164, 189, 205, 206, 225, 243, 254) (Table 4).

Gastrointestinal involvement is common, having been described in half of the HIV-negative patients in Sudan (211). In HIV-positive individuals with leishmaniasis, digestive tract localizations are relatively common (254). In fact, 3.2% of HIV-positive patients undergoing endoscopy for undiagnosed digestive symptoms have amastigotes (152). *Leishmania* spp. can invade any part of the digestive tract asymptomatically or can be accompanied by esophageal symptoms, epigastralgia, diarrhea, or rectal discomfort, although on occasion these symptoms are produced by other simultaneously infecting pathogens such as cytomegalovirus or *Candida* spp. (72, 153, 187, 251). Gastrointestinal leishmaniasis should be considered in the differential diagnosis of chronic diarrhea in HIV-positive patients who live or have traveled to countries with endemic leishmaniasis (9, 235). A recent publication on gastrointestinal leishmaniasis in HIV-positive patients, in which 5 cases were described and another 10 were reviewed (153), points out that the patients had a $CD4^+$ lymphocyte count lower than 200/$mm^3$; 12 had been diagnosed with AIDS previously; only 7 had fever and splenomegaly; in 10 the VL diagnosis was made from gastrointestinal biopsy; 13 showed some digestive symptoms (6 with diarrhea, 6 with dysphagia and odinophagia, 2 with abdominal pain, 2 with epigastralgia, 1 with intestinal hemorrhage, and 1 with rectal pain). *Leishmania* spp. were found in the duodenum in 90% and in gastric mucosa in 75%, and endoscopic findings were quite variable, with a normal mucosal appearance in six patients, erosive gastroduodenitis in three, gastric ulcer in three, esophageal ulcer with cytomegalovirus in one, erythematous lesions in rectal mucosa in one, and Kaposi's sarcoma in one.

In HIV-positive patients, involvement of the respiratory tract is not uncommon. In an anatomopathological study of coinfected patients, *Leishmania* amastigotes were found in alveoli and pulmonary septa (shown by immunohistochemistry) in 76.8% (86). Pulmonary clinical manifestations are, however, less frequent. Parasites are usually found when a bronchoalveolar lavage is done on patients with cough and fever who are undergoing diagnostic fibrobronchoscopy (135, 243, 252, 253). In some VL-coinfected patients with respiratory symptoms and pleural effusion, histological examination of serous bloody fluid has revealed amastigotes within histiocytes (56). Patients with pneumonitis associated with pleural effusion (lymphocytic and high protein level) and with mediastinal adenopathies, but no bone marrow involvement, have also been reported (181), as has pulmonary involvement seen at autopsy of a coinfected patient (97).

A case of multiorgan dissemination has also been described at necropsy, with parasitization of liver, spleen, bone marrow, lymph nodes, digestive tract, skin, adrenal glands, and myocardium (204).

In the Mediterranean basin countries, visceral dissemination of an initial cutaneous lesion is uncommon in immunocompetent subjects (266). However, in studies carried out decades ago, up to 15% of patients previously treated for VL presented, some time later, with cutaneous lesions from which *Leishmania* organisms were isolated (52). This situation would be similar to that of post-kala-azar dermal leishmaniasis, which has been reported in a coinfected patient (7). *Leishmania* organisms can also be isolated from the healthy skin of immunocompetent patients in the course of *L. donovani* VL (261).

In HIV-positive patients, exclusive cutaneous involvement is uncommon (227), occurring in 2 to 3% of all patients with HIV-*Leishmania* coinfection (301). The most frequent symptom, in 8 to 12% of patients, is the appearance of cutaneous lesions concomitant with VL (205, 254). *Leishmania* organisms can be found in all areas of healthy skin, as well as in Kaposi's sarcoma lesions (284, 302) and in lesions together with herpes simplex and varicella-zoster viruses (28, 92). Forms of diffuse cutaneous leishmaniasis associated with anergy in patients have been described (30, 302). Dermonodular lesions and dermatomyositis-like lesions have also been reported (73, 263). Reactivation of arthritis, which occurs sometimes due to parasitic infections, has been reported for the first time to be caused by *Leishmania* spp. in a coinfected patient (223).

The majority of cutaneous lesions are caused by *L. infantum*, which also produces the typical cutaneous Oriental sore and can involve the nasal (50, 195) and buccal (26, 194) mucosa and invade the larynx (49, 109).

Diffuse CL with nonulcerated papulonodular lesions has been described, as has VL caused by *L. infantum* in the Caribbean (63) and *L. major* (106). In HIV-positive patients, *L. braziliensis* can produce mucocutaneous involvement exclusively (168) or disseminated disease with visceral, digestive tract, or skin involvement (132). A case in Peru with mucocu-

taneous involvement has also been described (88). *L. aethiopica* can be reactivated in HIV-positive patients years after the cure of localized CL and can reappear as MCL affecting nasal and oral mucosa (32).

The opportunistic infections that most commonly mimic and coexist with VL are disseminated tuberculosis, atypical mycobacteriosis, and lymphoma. In fact, between 7 and 17% of HIV-positive patients with fever of unknown origin have *Leishmania* amastigotes in bone marrow (14, 39, 197). Other diseases to be considered are typhoid fever, bacillary angiomatosis, disseminated cytomegalovirus infection, histoplasmosis, coccidioidomycosis, or disseminated disease caused by *Toxoplasma gondii* or *Pneumocystis carinii*, among others.

### Evolution of Disease and Survival

Estimation of the period of freedom from disease, survival, prognostic factors, and causes of death vary from one study to another and depend on the group of patients studied (189, 205, 225, 301). Unlike in immunocompetent persons, VL in HIV-positive patients usually takes a relapsing course, with the number of relapses and mean time to relapse depending on the patient's immunological status and clinical situation; 60% of patients relapse at 6 to 9 months and 90% relapse at 12 months after a correctly treated first episode.

Mortality during the first VL episode is 10 to 19% and is associated with the toxicity of the antiparasitic treatment used, the complications arising during the episode, and concomitant opportunistic infections; it can be as high as 24% during the month following termination of treatment. In spite of this high initial mortality, long-term survival curves show no significant differences compared with those of other HIV-positive patients without VL (164, 243). The mean survival time varies from 4 to 12 months, and the probability of being alive 12 months after the first episode is approximately 60% (205).

In multivariate analysis studies, the only factors that correlate with mortality are having AIDS criteria at the first diagnosis of VL (205, 225, 243, 301) and thrombocytopenia, such that for each reduction of 10,000 platelets/$mm^3$ of peripheral blood, the risk of mortality increases by 6% (164).

## TREATMENT

The drug choice and dosage for VL treatment in HIV-positive patients are based on data available for VL treatment in immunocompetent individuals; i.e., pentavalent antimonial salts ($Sb^V$), since no controlled clinical surveys have been performed with different drugs in HIV-positive patients. The treatment scheme for CL in immunodepressed subjects should follow the same criteria as for visceral forms, due to the risk of later dissemination. One case of *L. infantum* MCL with no visceral involvement was treated systemically for 28 days with $Sb^V$ (195); this patient suffered no relapses in the following 2 years (164). A patient with clear visceral symptoms infected with a non-*Leishmania* trypanosomatid was treated in the conventional manner with systemic $Sb^V$ and had no relapses 3 years later (142).

### Treatment with Pentavalent Antimonial Salts

The administration of $Sb^V$ still constitutes the treatment of choice for VL. Two antimony salts are currently available: sodium stibogluconate (Pentostan), containing 100 mg of $Sb^V$/ml, and meglumin antimoniate (Glucantime), containing 85 mg of $Sb^V$/ml. The effectiveness of these products is similar, and treatment dosage and duration have varied according to studies performed with immunocompetent patients. The WHO recommends treating VL with doses of 20 mg of $Sb^V$/kg/day to a daily maximum dosage of 850 mg of $Sb^V$ for 3 to 4 weeks. The lack of secondary effects with this dosage and the evidence for a lower cure rate compared with doses equal to or greater than 20 mg/kg/day have led to the suggestion of treatment with 20 mg/kg/day for 4 weeks with no daily dosage limit (134). With the latter treatment, response levels from 80 to 95% are obtained, depending on the country of origin of the infection. Some studies find a similar cure rate with 30 doses of 20 mg/kg/day or doses of 10 mg/kg every 8 hours for 10 days, with a reduction in the number of days of treatment and hospitalization (99).

The severe toxicity of the antimonials centers especially on the pancreas, heart, and kidney. Pancreatic involvement is almost constant during antimonial treatment. Elevation of the pancreatic amylase level was observed in 48 (98%) of 49 patients treated with $Sb^V$ for CL or VL, and clinical pancreatitis was observed in 47% of the patients, although suspension of treatment was not always necessary (104). Fatal cases of pancreatitis have been reported sporadically. Cardiac toxicity is rare and appears only with very high doses or very prolonged treatment; it is characterized by alterations in the T wave and the S-T segment (19). Renal toxicity is less frequent than the other two toxicities, with tubular and renal insufficiency having been described (293).

Although more than 800 patients coinfected with *Leishmania* and HIV have been reported, we do not yet know the treatment of choice, the best dosage, or the duration. This is because treatment dosage and duration have not yet been clearly defined for the drugs used, no random prospective studies have been performed, and it is uncommon to confirm the absence of parasites upon recovery. A large number of patients do not finish the treatment cycle due to early death, toxicity, or loss to follow-up (189, 205). The HIV-positive patient with VL meets the ideal conditions for a poor response to antimonials and thus to have a recurrent course of the disease. It has been shown experimentally that antimonials require an intact immune system to be effective and that athymic mice with VL do not respond to $Sb^V$ treatment (212). Nevertheless, all of the retrospective series and clinical case reports published agree that the clinical response to antimonial treatment is usually good (10, 189, 205, 243). It has been estimated that 83% of the patients who finish the course of treatment undergo an improvement in their symptoms (205). Clinical evaluation is complicated because of the coexistence of other AIDS-related diseases whose symptoms can overlap with those of VL (189) and because clinical improvement does not mean parasitological cure (154). Parasitological cure in patients treated with $Sb^V$ at dosages equal to or lower than 20 mg/kg/day (some with simultaneous allopurinol) for 21 days was achieved in 25 (54%) of 46 patients evaluated parasitologically (102, 165, 189, 243, 254). Treatment with 20 mg of $Sb^V$/kg/day or 850 mg/day for 28 days or more produced parasitological cure in 9 (75%) of 12 patients evaluated (31, 34, 72, 81, 97, 110, 123, 290, 294). With these results and the knowledge that longer treatment periods in the immunocompetent patient tend to lead to a higher cure rate, it can be assumed that HIV-positive patients with VL require $Sb^V$ treatments for a longer period or in higher dosages than an immunocompetent patient to achieve apparent parasitological cure. It is currently advisable to use 20 mg of $Sb^V$/kg/day with no dose limit for at least 28 days. There is currently no noninvasive test that can evaluate the microbiological response to leishmaniasis treatment, although PCR, xenodiagnosis, and measurement of circulating antigens in body fluids are promising tools (see earlier sections).

Antimonial toxicity in HIV-infected patients has not been

evaluated prospectively. Isolated cases of pancreatitis, myocarditis, and renal insufficiency have been described (9, 31, 153, 243, 271), but it is not known whether drug tolerance is poorer than in immunocompetent patients.

### Combined Treatments

Other treatment alternatives combine antimonial compounds with allopurinol, aminosidine, or IFN-γ.

Allopurinol is a hypoxanthine analog that is hydrolyzed to allopurinol riboside and is incorporated into the *Leishmania* RNA, interfering with protein synthesis. Allopurinol and $Sb^V$ have a synergistic effect in vitro against several *Leishmania* species. In human studies with patients who at times did not respond to $Sb^V$, allopurinol given as monotherapy or combined with antimonials produced parasitological responses in 66 to 75% of patients, although dosages and treatment durations were not sufficiently similar to make definitive recommendation (59, 137, 143). A randomized controlled assay with 124 patients later demonstrated that the combination of $Sb^V$ at 20 mg/kg/day together with allopurinol at 21 mg/kg/day, both for 30 days, was not more effective than $Sb^V$ alone (98).

The combination of $Sb^V$ and allopurinol is well tolerated in HIV-positive patients, and, as is the case for treatment with $Sb^V$ alone, the highest percentages of cure can be obtained with treatments of 28 days or more (154). There are no current data to suggest that this combination is better than $Sb^V$ alone. In the patients in whom recovery was observed, the allopurinol treatment was prolonged (81, 207, 278).

Aminosidine is an antibiotic of the aminoglycoside family. In vitro, it is more potent than $Sb^V$, and together there is a synergistic effect. Dosages of 12 to 16 mg/kg/day are given by the intramuscular or intravenous route for 2 to 3 weeks. Several open studies have shown its efficiency and safety as a first-line treatment or after failure of $Sb^V$ treatment. In Kenyan or Indian patients with first VL episodes, combined treatment for 3 weeks produced recovery in 82 to 87% of the patients and was superior to aminosidine or $Sb^V$ monotherapy (60, 287). Combined treatment with $Sb^V$ and aminosidine has permitted the reduction of the treatment period from 30 to 17 days in VL patients from Kenya with no loss in efficiency, which is important for patients living in areas with few health resources (270). The efficiency of aminosidine monotherapy in patients who do not respond to or cannot tolerate $Sb^V$ has also been observed. It is possible that treatment should last for a longer period in these patients (267).

Experience with $Sb^V$ combined with aminosidine or aminosidine alone in HIV-infected patients is insufficient for us to draw conclusions (183, 267).

Patients with VL have a suppression of the T-cell response to *Leishmania* antigens and a decrease in IFN-γ and IL-2 production. In experimental VL, $Sb^V$ efficiency was T-cell dependent, but a response could be obtained in athymic mice by administering either IL-2 or IFN-γ. Nonetheless, later studies on athymic mice have shown that effective recovery was in fact T-cell dependent and IFN-γ independent (212). Preliminary studies in humans led us to believe in the improved efficiency of combined $Sb^V$ and IFN-γ treatment in patients who are unresponsive to $Sb^V$ (24, 280). However, in a 24-patient randomized pilot study, the combination of $Sb^V$ and IFN-γ was only as efficient as $Sb^V$ alone, although the parasitological response was more rapid in the IFN-γ-treated group (280).

As HIV infection produces a marked decrease in the capacity for IFN-γ synthesis, combined therapy was theoretically attractive for use in HIV-positive patients. To date, a limited number of HIV-infected patients with VL who have been treated with $Sb^V$ plus IFN-γ have been reported, although the results have been variable (110, 165, 290). In fact, in two patients with VL and Kaposi's sarcoma, IFN-γ treatment was associated with rapid progression of the tumor (8). With these results, the contribution of IFN-γ to the cure of some cases of leishmaniasis remains unknown (110).

### Treatment with Amphotericin B

Amphotericin B has high anti-*Leishmania* activity through its union with the membrane ergosterol precursors, followed by membrane rupture and death of the parasite. Experimentally, it is a much more potent drug than $Sb^V$ and kills both extra- and intracellular forms. In athymic mice, amphotericin B was superior to pentamidine in the treatment of experimental leishmaniasis, showing that its activity is T-cell independent (213).

Amphotericin B has been used as an alternative to antimonials, but its use is limited by its toxicity, especially fever, chills, phlebitis, anemia, hypokalemia, hypomagnesiumemia, and, above all nephrotoxicity (292). Comparative prospective studies with Indian patients showed that low-dose amphotericin B (0.5 mg/kg on alternating days, for a total of 14 doses) was superior to $Sb^V$ (20 mg/kg/day for 40 days) as a first-line treatment of VL (199) and superior to pentamidine (4 mg/kg on alternating days, 20 doses) (198) in patients who are unresponsive to $Sb^V$, with no significant toxicity.

Its greater in vitro anti-*Leishmania* activity compared with antimonials, its greater experimental effectiveness in T-cell-deficient hosts, and its higher in vivo activity than $Sb^V$ or pentamidine make amphotericin B a very adequate drug for treatment of VL in HIV-positive patients. However, experience in this situation is limited. Rosenthal et al. treated 12 patients with amphotericin B at a total dose of 20 mg/kg and showed parasitological cure in all of them (254). Amphotericin B is usually not used as a first-line drug against VL at present. It has been used as a second-line treatment for only a limited number of HIV-positive patients, and no conclusions can yet be drawn (17, 175, 183).

### Other Treatments

Pentamidine is an effective drug in VL treatment, but its toxicity limits its use to patients who are resistant to or intolerant of antimonials (286). Few pentamidine-treated HIV-positive patients have been reported (81, 129, 165, 278).

The azoles inhibit ergosterol synthesis in the *Leishmania* cell membrane and can produce cell death. They are less potent than amphotericin B but have the advantage that they can be administered by the oral route and are only weakly toxic. Ketoconazole has been shown to be effective in CL treatment, and its efficiency varies according to the *Leishmania* species treated. Experience of VL treatment is limited. In two small groups of Indian patients, ketoconazole treatment has given varied results (281, 295). One HIV-positive patient with VL was asymptomatic for 1 year after treatment with ketoconazole (17).

Itraconazole has been tested in immunocompetent patients with CL but has shown poor results (6, 40). In HIV-positive patients with VL (72, 239), itraconazole treatment was ineffective as well, and results in the few reported patients with CL have been discrepant (204, 227). There are currently no data that support the usefulness of azole treatment in HIV-positive VL patients.

Another drug tested in nonimmunodepressed Kenyan VL patients is an 8-aminoquinolone (WR6026). In a phase II study, it was used on eight patients at a dosage of 0.7 to 1

mg/kg/day for 14 days and cured only one patient; however, at a dosage of 1 mg/kg/day for 28 days, it cured four of eight patients (275). Studies with higher doses and longer treatment periods are needed to find optimal treatment doses. Its efficiency in HIV-positive patients is unknown.

Amphotericin B incorporated in liposomes is a very attractive treatment choice, because it combines the efficiency of amphotericin B with excellent tolerance to the preparation and a selective deposit of the drug in the organs of the mononuclear phagocytic system in which the parasite is found. A group of immunocompetent patients, some with VL relapses, were treated with liposomal amphotericin B (AmBisome) at dosages of 1 to 1.38 mg/kg/day for 21 days (10 patients) or 3 mg/kg/day for 10 days (10 patients), and they all recovered (74). In a later study, several therapeutic protocols with liposomal amphotericin B were tested in patients living in areas of endemicity who were intolerant of or resistant to antimonials. Six intermittent doses of 4 mg/kg brought about recovery in 88% of those treated (269). Other lipid formulations containing amphotericin B also show a high efficiency and good tolerance in the treatment of immunocompetent VL patients (85, 282).

### Treatment of Relapses

Experimental studies have shown that after effective treatment, the parasite remains quiescent in several organs (5); after induction of immune system depression in the host, the parasite can be reactivated and cause new disease (21). These data can explain the persistence of *Leishmania* DNA in the blood many years after recovery (128) and the recrudescence of the infection in immunodepressed patients following effective treatment (23). In addition, the parasite can develop drug resistance after noncontinuous exposure or inadequate dosage. Anti-*Leishmania* susceptibility tests are not standardized, nor are they available in the majority of clinical laboratories. In addition, in vitro tests differ from the in vivo situation, as there is no concomitant immunological pressure, a critical factor in *Leishmania* control. However, in a study of CL, there was good correlation between in vitro susceptibility test results and the in vivo response (126).

It is possible to cure VL relapses in immunocompetent patients with antimonial treatment, sometimes administered for longer periods than usual. Another safe and effective alternative is to use low-dose amphotericin B (198) or pentamidine, although their toxicity is high (286).

HIV-positive patients with VL have very frequent recurrences, probably due to the reactivation of the process caused by the inability of the immune system to help control the infection. Recurrences of VL can be treated de novo with $Sb^V$ (10, 20, 243), for which reason an increase in the treatment time has been suggested. Nevertheless, the efficiency of this drug can decrease (81, 113), perhaps because the parasite develops resistance to the drug (16, 165). The efficiency of second-line treatments under these circumstances has not been adequately explored. Treatment with liposomal amphotericin B has shown high effectiveness with no relevant toxicity (74, 158, 161, 183, 290). In a prospective study, 11 immunodepressed patients (7 of them HIV positive) were treated with doses of 1.37 to 1.85 mg/kg/day for 21 days, with parasitological cure in all of them (74). Based on the long tissue half-life of liposomal amphotericin B, intermittent high-dose treatment was used in 15 HIV-positive patients with VL and found to be equally efficient (75, 155). In spite of the high recovery level, recurrences were not avoided (74, 75, 155). In the future, liposomal amphotericin B could become a first-line drug for treatment of VL, although at present its high cost limits its use.

### Secondary Prophylaxis

The opportunistic infections that appear in HIV-positive individuals are recurrent, and highly potent primary or secondary prophylactic treatments are available. It has not been possible to show the effectiveness of any treatment in the prevention of recurrence of HIV-related VL, although pentamidine in dosages once every 3 to 4 weeks (105, 181, 224), liposomal amphotericin B in fortnightly doses (158, 161, 183), allopurinol (243), and itraconazol (150, 239) have been used. Recently, it has been shown in a nonrandomized, retrospective, and open trial with 37 patients that pentavalent antimony given monthly prevents relapse in 93% of the patients during the first year, but if allopurinol is given alone the protection against relapse occurs only in 21% (244).

## CONCLUSIONS

*Leishmania* spp. are opportunistic pathogens and, particularly in the case of *L. infantum* in the Mediterranean basin, should be considered emerging parasites. A complementary anthroponotic cycle among IVDU can be juxtaposed to the zoonotic transmission cycle, so that the problem of coinfection with HIV has been most prominent in areas in which intravenous drug abuse is a social problem.

Strains of *L. infantum* isolated from HIV-positive individuals show greater zymodeme variability than do those from immunocompetent patients or from dogs, probably due to immune selection and the specific mechanical transmission cycle of syringe sharing. Anergy in these patients favors visceral dissemination of the cutaneous variants and permits the establishment of other, normally nonpathogenic flagellates.

A close correlation is found between $CD4^+$ cell levels, which conditions the clinical presentation and the evolution of disease. The majority of cases are classical VL, but CL, MCL, diffuse cutaneous and post-kala-azar dermal leishmaniasis, as well as unusual locations, combinations of the above, and even asymptomatic cases are also seen. The clinical presentations can be masked by concomitant opportunistic parasitic diseases. The most relevant event is the chronic course of VL with frequent relapses.

Serological testing shows low sensitivity (of these techniques, WB is the most sensitive), but it is quite common to observe parasitized macrophages in peripheral blood, which helps to avoid bone marrow biopsy. However, this latter technique and subsequent culture in NNN medium are still the diagnostic methods of choice.

Until randomized prospective treatment trials are available, coinfected patients should be treated following the conventional schedules used for immunocompetent patients. However, both the side effects and relapses seen with antimonials or amphotericin B are more frequent than are the ones in immunocompetent VL patients. As is the case with other parasitic diseases associated with HIV infection, secondary prophylaxis could be helpful to prevent relapses.

Almost one-fourth of coinfected patients die during or within 1 month of treatment due to their severe state of immune system depression, but the high toxicity of the drugs used in AIDS therapy must also be considered. Evaluation of first-line treatments and secondary prophylaxis in the VL associated with HIV infection is needed. Finally, it is urgent to stimulate research into the search for and design of new alternative drugs for treatment.

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