HIV and the transmission of *Leishmania*

R. MOLINA*, L. GRADONI† and J. ALVAR*

*WHO Collaborating Centre for Leishmaniasis, Servicio de Parasitología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Carretera Majadahonda–Pozuelo Km 2, 28220 Majadahonda, Madrid, Spain

†Laboratorio di Parassitologia, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161, Rome, Italy

Received and accepted 30 May 2003

In many countries, *Leishmania*/HIV co-infection is now changing the epidemiology of visceral leishmaniasis. The relative transmission of the parasites causing such leishmaniasis were previously dependent on the conventional zoonotic cycle, in which sandflies transmitted the parasites from infected canids to other canids or humans. The co-infection, however, has led not only to marked increases in the sandfly transmission of the parasites from immunocompetent individuals directly to other humans but also, probably, to artificial transmission between immunodepressed intravenous-drug users, as a result of needle sharing.

The epidemiology of human leishmaniasis and the biology of the phlebotomine sandflies that transmit the causative parasites are naturally closely linked (Killick-Kendrick, 1990). There is little evidence to indicate that immunocompetent humans can develop leishmaniasis except as a consequence of being bitten by infected sandflies, although the parasites may occasionally be transmitted as the result of blood transfusions (Cohen et al., 1991; Grogl et al., 1993; Singh et al., 1996; Kubar et al., 1997; Luz et al., 1997; Le Fichoux et al., 1999; Otero et al., 2000). The results of recent xenodiagnostic tests with sandflies indicate that the amastigotes in samples of blood (taken from cases of *Leishmania*/HIV co-infection) remain viable for at least 8 days after the blood has been collected (R. Molina, unpubl. obs.).

From an epidemiological standpoint, three main forms of human visceral leishmaniasis (VL) have been identified: zoonotic, anthrozo–zoonotic and anthroponotic.

The zoonotic form of VL is caused by *Leishmania infantum* (= *Leishmania chagasi*), with infection predominantly in wild and domestic canids and occasionally in humans. This form, which is mainly endemic, extends from the Mediterranean through Central Asia into China and also exists in Latin America. Several species of the genera *Phlebotomus* (Old World) and *Lutzomyia* (New World) have been incriminated in the transmission of *Le. infantum*.

The anthropo–zoonotic form, caused by *Le. donovani* or *Le. infantum*, occurs in East Africa and the south–west of the Arabian peninsula. It is normally endemic but can cause extensive epidemics in humans. Transmission of the parasites that cause this form of VL is predominantly human to human via non-synanthropic vectors such as *P. martini* and *P. orientalis*, although there may also be other mammals acting as ‘reservoir’ hosts.

The anthroponotic form, caused by *Le. donovani*, occurs on the Indian sub-continent. It is normally endemic but severe epidemics can develop among humans. The parasite is
transmitted from human to human by the bite of a species of sandfly that is strictly peridomestic, *P. argentipes*.

Since the global epidemic of HIV infection began, there has been a steady rise in the annual number of reported new cases of VL associated with the virus. *Leishmania*/HIV co-infection is emerging as a new and worrying disease, particularly in south-western Europe (Desjeux, 1998). In Spain — the country reporting the highest incidence of the co-infection — 68% of the cases are intravenous-drug users (Desjeux and Alvar, 2003). In France, Italy, Portugal and Spain, the outbreak of HIV infection has changed the epidemiology of VL, from a disease predominantly found in children to one more common in adults (WHO, 1999). HIV co-infection may re-activate latent leishmanial infections, increase the level of transmission of *Leishmania* (particularly human–human transmission) by phlebotomine sandflies, and facilitate artificial transmission of *Leishmania*, via the sharing of contaminated syringes and needles, from one intravenous-drug user (IVDU) to another (see below).

**NEWMELY ACQUIRED VERSUS RE-ACTIVATED VISCERAL LEISHMANIASIS: THE ITALIAN EXAMPLE**

It is estimated that only one in every five to 10 immunocompetent individuals who have been infected with *Le. donovani* or *Le. infantum* ever develops clinical VL (Desjeux, 1992; Badaró et al., 1996). Since a T-lymphocyte-mediated immune response is necessary to control *Leishmania* infection, however, those residents of endemic areas who have been made immunodeficient as the result of HIV infection are far more prone to VL than their immunocompetent neighbours. Strains of *Leishmania* that are usually non-pathogenic in humans, and even lower trypanosomatids (Chicharro and Alvar, 2003), may all cause illness in HIV-positive individuals. As many asymptomatic individuals in Italy, France and Spain give positive results in leishmanin skin tests (Pampiglione et al., 1975, 1976; Gramiccia et al., 1990; Meller-Melloul et al., 1991; Marty et al., 1992; Arbaji et al., 1993; Acedo-Sánchez et al., 1996; Alvar et al., 1996; Morillas et al., 1996), many detected cases of *Leishmania*/HIV co-infection in these countries are probably the result of latent *Leishmania* infections that have been activated or re-activated as the later HIV infection causes immunosuppression. Many now think that *Leishmania* spp. should be considered as opportunistic pathogens in HIV-infected patients (Alvar et al., 1992). Even in the absence of HIV, *Le. infantum* infections may be re-activated following immunological distress (Ma et al., 1979; Kubar et al., 1998).

If the natural history of VL in HIV-positive individuals is to be fully understood, long-term, longitudinal, immunological and parasitological investigations on cohorts of individuals at risk of both HIV and *Leishmania* infection will have to be conducted. As the incidence of leishmaniasis in the areas most affected by the co-infection is relatively low, the cohorts studied will have to be large. The relevant epidemiological data already available may be sufficient, however, to estimate the probabilities that clinical VL in a HIV-positive individual is (1) the result of a newly acquired leishmanial infection or (2) the result of a re-activated latent infection. In 1998, L. Gradoni and A. Scalone attempted to estimate these two probabilities in Sicily, by assimilating and updating the relevant epidemiological-surveillance data and then using the resultant data-set in an epidemiological model (unpubl. obs.). Their analysis of the natural history of the *Leishmania*/HIV co-infection is described below, *in extenso*.

**Surveillance Methodology**

In 1989, a programme based on the active detection of *Leishmania*/HIV co-infection was implemented, in collaboration with some 148 infectious-disease units sited in universities and hospitals throughout Italy (Gradoni et al., 1996). The units selected, from the 260
clinical diagnostic centres which were then recording AIDS cases, were chosen because they were not only reporting relatively high numbers of HIV-positive patients but were also in regions where VL was endemic. Collaborators were asked to provide serum, bone-marrow, peripheral-blood or skin-biopsy samples from any patient who, when sero-positive for HIV or suffering from AIDS, developed an infection that was clinically suspected to be VL. One or more of the following clinical and laboratory findings were considered indicative of VL: fever of unknown origin, splenomegaly, hepatomegaly, hypergammaglobulinaemia, and pancytopenia. The presence of single or multiple nodular/ ulcerative skin lesions was considered indicative of cutaneous leishmaniasis (CL). A retrospective survey was conducted to identify any cases of co-infection that had presented at the collaborating units before 1989; confirmatory diagnosis was then based on the laboratory examination of stored sera and bone-marrow smears. Possible Leishmania infections diagnosed in AIDS patients were routinely reported, through the national system of AIDS-case notification, with other associated pathologies (i.e. those not indicative of leishmaniasis) to the National AIDS Registry.

The Situation in Italy
Between 1985 and 1997, 183 cases of Leishmania/HIV co-infection were recorded in Italy. Most (68%) of the co-infections were in patients who fulfilled the criteria for AIDS. In areas of Italy where leishmaniasis was endemic, the incidence of VL among HIV-positive residents (1.6 cases/100) was about 500 times higher than that among their HIV-negative neighbours. In four distinct ‘hotspots’, 12%–100% of the HIV-positives developed VL. The annual number of co-infection cases showed a sharp increase in 1991 but then stabilized, at about 21 cases/year (Fig. 1).

Epidemiological Model
A model was constructed to test two alternative hypotheses: that the VL seen in HIV-positives represented (1) newly acquired infections, or (2) re-activation of latent leishmanial infections.

To test the first of these hypotheses, the number of expected co-infections was derived by applying a known force of leishmanial infection ($p$; see below) to an estimated HIV-infected population exposed to Leishmania during each Leishmania-transmission season. It was assumed that the level and mode

![FIG. 1. Temporal distribution of the cases of Leishmania co-infection among those with AIDS (■) and other HIV-positive individuals (□) recorded during epidemiological surveillance in Italy.](image-url)
of *Leishmania* transmission to and among HIV-positive individuals were the same as those to and among the HIV-negatives, that each HIV-positive individual infected with *Leishmania* developed clinical VL, and that \( p \) had been constant during the 10-year study period (1985–1994).

To test the second hypothesis, the number of expected co-infections was calculated by applying an estimate of the annual incidence of HIV infection to a population that included many individuals — the number being indicated by the results of leishmanin skin tests (LST) — who were asymptomatic carriers of *Leishmania*. It was assumed that each individual found positive in an LST had a latent leishmanial infection that would develop into clinical VL following that individual’s infection with HIV, and that the age-related prevalence of LST positivity was constant over the study period.

**Sources of Data**

**PREVALENCE AND INCIDENCE OF HIV INFECTION**

The prevalence and incidence of HIV infection in Italy in 1994 were estimated using mathematical models, ‘back-calculation’, the annual numbers of AIDS cases notified, and the results of pilot studies on HIV seroconversion (Pezzotti et al., 1995; Rezza, 1998). Prevalence was estimated by subtracting the number of HIV-attributable deaths prior to 1994 from the accumulative incidence of HIV infection. Annual incidence represented all the new HIV infections that occurred in 1994.

Gradoni et al. (1996) used maps of the distribution of leishmaniasis in Sicily (based on accurate locality data for all cases of human and canine leishmaniasis that had been recorded over the previous 18 years) to estimate the size of the human population at risk of *Leishmania* infection on the island. They then used the same maps and the relevant AIDS-case notification reports, stored at the National AIDS Registry, to estimate the number of HIV-positive residents of Sicily who lived in areas where leishmaniasis was endemic.

**FORCE OF INFECTION (\( p \))**

The force of infection (Lysenko and Beljaev, 1987) was estimated, as the mean number of times a resident of Sicily was inoculated with *Leishmania* over a year, from LST data indicating the prevalence of leishmanial infection among Sicilians aged 0–15 years, who were assumed to be non-immune. Surveys based on LST have been carried out in Sicily since 1975. Their results indicate that the force of infection in Sicily vary little from year to year and is similar to those in other leishmaniasis-endemic areas of southern Italy (Pampiglione et al., 1975; Gradoni et al., 1993; unpubl. obs.).

**THE POPULATION POTENTIALLY HARBOURING Leishmania**

The results of the LST surveys in Sicily were also used to estimate the number of individuals on the island who were LST-positive and therefore possibly carrying latent *Leishmania* infections. Particular attention was paid to the LST results for residents of Sicily who were aged 20–30 years, this age-group being considered the one at greatest risk of HIV infection.

**REPORTED INCIDENCE OF THE Leishmania/HIV CO-INFECTION**

The annual numbers of cases *Leishmania*/HIV co-infection detected among Sicilian residents during epidemiological surveillance between 1985 and 1994 were compared with those predicted using each of the two mathematical models.

**Evaluation of the Models**

**HYPOTHESIS 1 (‘NEW INFECTIONS’)**

In Sicily, the number of HIV-positives living in areas where VL was endemic ranged from...
approximately 800 in 1985 to approximately 4200 in 1994 (Fig. 2). The corresponding numbers of AIDS cases showed the same trend (Fig. 2). The mean force of leishmanial infection in these areas was calculated to be 0.003 infection/person-year, indicating that the areas are only hypo-endemic for VL. By applying this \( p \)-value to the number of HIV-positives present in the areas of the island where VL is endemic, the annual numbers of cases of co-infection were predicted to increase from two in 1985 to 12 in 1994, with a total of 74 cases over the study period.

**Hypothesis 2 (‘re-activations’)**
The estimated incidence of HIV infection in Sicily peaked at about 600–700 cases/year between 1986 and 1989 and then decreased to about 200–300 cases/year (Fig. 2). Most of these cases were aged 20–30 years. A mean of 8.0% of the Sicilian residents aged 20–30 years who were checked in LST surveys were found LST-positive. By applying this proportion to the size of the HIV-positive population, the numbers of cases of co-infection were predicted to decrease from 48–56/year in the period 1986–1989, to 16–24 cases/year, with a total of 376 cases over the study period.

**Observed HIV/VL Cases**
The actual recorded numbers of HIV-positive individuals found to have VL in Sicily increased from nil in 1985 to nine in 1994, with a total of 35 HIV/VL cases over the study period. The trend in the numbers of reported cases was similar to that predicted when the ‘new-infection’ hypothesis was applied, and differed significantly from that predicted when the ‘re-activation’ hypothesis was assumed to be correct (\( P < 0.01; \) Fig. 3).

**Discussion**
In Italy, the problem of HIV/VL co-infection is no longer as alarming at it appeared to be in the early 1990s, when the number of cases was showing dramatic increases from one year to the next. Although, in some areas of the country, the incidence of VL does appear to have increased recently, this upward trend...
appears to be unrelated to HIV (Gradoni et al., 1996). It seems that the predicted flood of HIV/VL cases has been stemmed by the largely successful introduction of anti-retroviral treatment for those found sero-positive for HIV. The quarterly incidence of AIDS in Italy peaked at 10 cases/100,000 inhabitants in 1995, falling to six cases/100,000 in the first quarter of 1998.

The results of the epidemiological modelling discussed above indicate that, in Sicily at least, most cases of HIV/VL co-infection are the result of individuals who are already HIV-positive being newly infected with Leishmania. Had such co-infection been predominantly the result of those with (latent) leishmanial infection being infected with HIV, then many more cases should have been detected in the first few years of the present study period, as HIV swept through Sicily. Such large numbers of cases were not observed at this time in Sicily, in other areas of the Mediterranean basin where VL is endemic, or in the young people of northern Europe who have frequently taken their vacations in regions of the Mediterranean littoral where the disease is endemic.

The epidemiological models have to be treated with some caution as they may be too simplistic. Although the two hypotheses were assumed to be mutually exclusive, both new infections and re-activations may contribute to the actual number of clinical HIV/VL cases. Although the predicted number of cases for each year was compared with the actual number of cases diagnosed in the same year, an incubation period — the length of which is difficult to estimate — will have separated co-infection from diagnosis. Some of the assumptions and estimates made appear more reasonable than others. The estimates of $p$ and of the proportion of the population who may have latent Leishmania infections, for example, were both based on LST data that showed consistency over a long period. These estimates were supported by a stable incidence of recorded VL among the immuno-competent population of Sicily during the
study period, in the absence of measures to control the canine reservoir or phlebotomine vectors and of dramatic changes in local environmental conditions (Cascio et al., 1997). The possibility that HIV infection has led to, or is associated with, unusual routes of leishmanial infection (see below) was not considered in the epidemiological models.

ALTERNATIVE ANTHROPONOTIC CYCLES FOR Leishmania infantum

In the predominant, natural cycle of transmission, an individual, whether HIV-positive or HIV-negative, becomes infected with Le. infantum when he or she is bitten (usually in rural or peri-urban settings) by a sandfly that has already been infected when taking a bloodmeal from an infected canid. The possibility of other routes of transmission, such as needle-mediated infection, should not, however, be ruled out (Alvar et al., 1992). Direct human–sandfly–human transmission may also occur, particularly from HIV-positives, who carry particularly high numbers of leishmanial amastigotes in their peripheral blood.

An Artificial Cycle of Transmission

It seems likely that not only HIV but also Leishmania may be transmitted, on and in shared needles and syringes, among IVDU. The observation that mammals may be infected with Leishmania, either accidentally (Owens et al., 2001) or experimentally (Palatnik de Sousa et al., 1996), by transfusions of blood from infected hosts underlines the possibility of this mode of transmission. There has also been at least one case of laboratory-acquired Le. donovani infection as the result of a needlestick injury (Freedman et al., 1987). Although there is, as yet, no direct evidence of the spread of Leishmania through the sharing of syringes among IVDU, there is much indirect evidence indicating that this happens. AIDS increases the risk of VL by 100- to 1000-fold in areas where VL is endemic (WHO, 1999). In western Europe, IVDU who share syringes form the main population at risk of HIV infection and account for 44% of the HIV/AIDS cases (Desjeux, 1998). In a joint consultative meeting on the Leishmania/HIV co-infection, held in September 1998, it was established that IVDU had a 2.5- to 3.6-fold higher risk of VL/AIDS than other AIDS cases (WHO, 1998). In Spain and Italy, biochemical variants of Le. infantum that rarely if ever cause leishmaniasis in immuno-competent patients have been recovered from cases of the HIV/Leishmania co-infection (Gramiccia et al., 1995; Jiménez et al., 1995; Pratlong et al., 1995; Agostoni et al., 1998). Amastigote-infected macrophages can be found in approximately 50% of bloodsmears from co-infected patients (Martinez et al., 1993; Medrano et al., 1993), and promastigotes can be found in 67% of NNN cultures ofuffy coats from such patients (López-Vélez et al., 1995).

The AIDS-case surveillance system that covers the Madrid region of Spain has also provided data indicating that needle-sharing carries a risk of HIV/Leishmania co-infection (Amela et al., 1996). As this system only records AIDS cases, VL diagnoses in any HIV-infected individuals who fail to meet the diagnostic criteria for AIDS are not included. In total, 6652 cases of AIDS were diagnosed between 1982 and 1993 and recorded by the Madrid system. Only 166 (2.5%) of these cases developed VL, and the dates of diagnosis of both the AIDS and VL were available for only 137 of the co-infection cases. The VL was diagnosed before the AIDS in 33 cases, after the AIDS in 65 cases, and at the same time as the AIDS in 39 cases. The prevalence of VL among the AIDS cases who were IVDU was significantly higher than that in any of the other exposure-groups (relative risk = 2.57; 95% confidence interval = 1.64–4.01).

In Italy, the unexpected occurrence of small hotspots of HIV/Leishmania co-infection, in which every detected case of HIV infection
may have VL, also indicates that transmission is not restricted to the normal, canid–sandfly–human route. The observations that IVDU represent 84% of the Italian cases of HIV/Leishmania co-infection but only 64% of Italian AIDS patients without leishmaniasis (64%) and that the zymodeme spectrum of the Le. infantum from the co-infected cases differs from that of the parasitic isolates from HIV-negative adults, and the distances between the hotspots, also support the view that leishmanial parasites can be transmitted on needles and syringes (Gradoni et al., 1996). In northern Italy, 17 (77%) of the 22 co-infected patients investigated by Agostoni et al. (1998) were IVDU.

Pineda et al. (1998) investigated the factors associated with VL in individuals infected with HIV-1 who lived in southern Spain. When Giemsa-stained smears of bone-marrow aspirates from 291 HIV-1 carriers were examined, 45 were found positive for amastigotes. Thirty-two of the amastigote-positive carriers of HIV-1 had symptomatic VL, the other 13 having subclinical infections with Leishmania. Symptomatic VL again appeared particularly common among the HIV-positive IVDU included in the study. Although this association was not quite found to be statistically significant, when adjustments were made for clinical category and gender in a multivariate analysis, Pineda et al. (1998) thought that Leishmania transmission through the sharing of needles was very probable. The isolations of the MON-18 zymodeme of Le. donovani from a Portuguese drug addict with clinical VL and AIDS (Campino et al., 1994), and of the MON-253 zymodeme of Le. infantum from a cluster of three co-infected patients, all IVDU, living in the same town in north–eastern Spain (Chicharro et al., 1999), also support the view that leishmanial amastigotes are being transmitted on shared needles. When Cruz et al. (2002) checked syringes discarded by IVDU in Madrid, they found 34%–52% to be PCR-positive for leishmanial DNA.

In a retrospective analysis of 965 cases of co-infection reported in south–western Europe between 1990 and 1998, the predominance of IVDU, who were identified as the main population at risk, was clear (WHO, 1999).

**Natural Human–sandfly–human Transmission**

Most humans are infected with Le. infantum when they are bitten by sandflies that have already been infected when they fed on dogs or other canids harbouring the parasite. As uninfected sandflies are not frequently infected with Le. infantum as they feed on immunocompetent humans carrying the parasite, human–human transmission of Le. infantum, via a sandfly, is rare. There is, however, considerable evidence indicating that uninfected sandflies feeding on individuals co-infected with Le. infantum and HIV are quite likely to become infected with the parasite. Transmission of leishmanial parasites from a HIV-positive human is therefore more likely than transmission from a HIV-negative individual. Most of the data indicating that sandfly-mediated transmission of Le. infantum from co-infected HIV-positive individuals occurs have been collected during xenodiagnostic tests with sandflies.

**INDIRECT XENODIAGNOSIS**

The confirmation of a suspected diagnosis of leishmaniasis in immunodepressed patients frequently requires the use of several techniques. Although the examination of bone-marrow aspirates for amastigotes has been proposed as the best technique, it requires an invasive and painful procedure and is not highly sensitive. In the search for a diagnostic test that is less invasive and less painful, for patients who may already be seriously ill, the use of indirect xenodiagnosis (IXD) — that is, the feeding of uninfected (usually laboratory-bred) sandflies, through a membrane, on a sample of venous blood from the suspected case of leishmaniasis — has been considered. Molina et al. (1992) found IXD with Phlebotomus perniciosus, an important vector of Le. infantum in the south–western
Mediterranean region, very useful in confirming that an AIDS patient had VL. Subsequently, Molina et al. (1994) investigated the potential usefulness of IXD in the routine detection of leishmaniasis in those co-infected with *Le. infantum* and HIV, and attempted to establish a standard protocol. The study, designed to investigate the infectivity to *P. perniciosus* of 10 HIV-positive individuals who had symptoms indicative of VL (*N* = 9) or had duodenal amastigotes detected during routine endoscopy (*N* = 1), also served to compare the IXD with the more conventional methods of diagnosis. The other methods, tested in parallel with the IXD, were IFAT for the detection of anti- *Leishmania* antibodies, the microscopical examination of Giemsa-stained smears of bone-marrow aspirates and peripheral blood, and NNN cultures of bone-marrow aspirates and peripheral mononuclear cells. Records were made, for each patient, of associated infections, fever (for >2 weeks), splenomegaly, treatment for the leishmaniasis and the response observed, the sandfly-feeding procedure, and other information (Table 1).

The *P. perniciosus* used came from a local laboratory colony kept at 27 ± 1°C and 90%–100% relative humidity, with a 17-h-light:7-h-dark photoperiod (Molina, 1991). Batches of 30–150 female *P. perniciosus* collected 4–21 days post-eclosion were fed, in the presence of some males, through a membrane on anticoagulated peripheral blood from each patient. The bloodfed sandflies were dissected 2–7 days after taking their bloodmeals so that their guts could be checked for promastigotes. All 10 of the patients were found positive by the IXD but only eight, seven, five and five, respectively, had positive cultures of bone-marrow aspirates, positive cultures of peripheral blood monocytes, amastigotes detected in their bloodsmears, or were found seropositive in the IFAT (Table 1). Even in the absence of cutaneous macrophages carrying amastigotes, most (up to 93%) of the sandflies taking a blood-meal were infected. [The results of studies conducted on *Le. infantum*-related VL in Pakistan have led to the hypothesis that skin parasites, rather than parasites circulating in blood monocytes, might be the principal source of infection for sandflies in areas where anthroponotic leishmaniasis is found (Rab et al., 1992).]

Using IXD, parasites can be clearly observed 48 h after sandfly feeding; parasites in cultures of aspirates and blood usually take longer to multiply sufficiently for them to be detected. Although IXD is very useful for detecting *Le. infantum* in HIV-positive patients, its application is, for the moment, largely restricted to those cases who have given negative results in other diagnostic tests even though there is strong clinical evidence indicating that they have leishmaniasis. IXD is a highly sensitive technique when used on immunodepressed patients. Molina et al. (1998), for example, used IXD to demonstrate the presence of viable parasites in 21 (95%) of 22 blood samples from cases of *Le. infantum*/HIV co-infection and in blood samples from two other patients who, though HIV-negative, were immunodepressed as the result of acute lymphoblastic leukaemia.

The observation that blood from cases of *Leishmania*/HIV co-infection remains infective to the sandflies used in IXD even if used after storage for at least 8 days (unpubl. obs.) adds support to the idea that leishmanial infections can be transmitted by blood transfusion.

IXD has uses in the field of epidemiology other than the demonstration of leishmanial co-infection in those who are HIV-positive. It has been used, for example, to show the importance of haematogenic dissemination in the development of mucosal leishmaniasis and other metastatic manifestations of *Le. braziliensis* infection (Martinez et al., 1992). Da-Cruz et al. (1992) used IXD to study the failure of T-cell-mediated immune responses to prevent diffuse cutaneous leishmaniasis developing in HIV-positives infected with *Le. braziliensis*. As the result of using *P. argentipes* in IXD, Addy and Nandy (1992) concluded that the skin parasites in
<table>
<thead>
<tr>
<th>Case</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>Age (years)</td>
<td>36</td>
<td>33</td>
<td>35</td>
<td>25</td>
<td>38</td>
<td>29</td>
<td>22</td>
<td>28</td>
<td>28</td>
<td>37</td>
</tr>
<tr>
<td>Exposure group for HIV infection</td>
<td>IVDU</td>
<td>IVDU</td>
<td>IVDU</td>
<td>IVDU</td>
<td>HS</td>
<td>IVDU</td>
<td>IVDU</td>
<td>IVDU</td>
<td>IVDU</td>
<td>IVDU</td>
</tr>
<tr>
<td>Associated infection(s)</td>
<td>PTB, OC</td>
<td>CMV</td>
<td>PCP</td>
<td>EC</td>
<td>PTB, EC</td>
<td>OC</td>
<td>EPTB</td>
<td>No</td>
<td>No</td>
<td>PCP</td>
</tr>
<tr>
<td>Fever?</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Splenomegaly?</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Leucocyte count (leucocytes/µl)</td>
<td>2700</td>
<td>2400</td>
<td>NA</td>
<td>2500</td>
<td>4300</td>
<td>3600</td>
<td>2400</td>
<td>1600</td>
<td>1900</td>
<td>5900</td>
</tr>
<tr>
<td>CD4 + count (CD4 + cells/µl)</td>
<td>40</td>
<td>10</td>
<td>68</td>
<td>56</td>
<td>48</td>
<td>51</td>
<td>144</td>
<td>20</td>
<td>148</td>
<td>ND</td>
</tr>
<tr>
<td>Haemoglobin (g/l)</td>
<td>8.4</td>
<td>8.3</td>
<td>NA</td>
<td>7.2</td>
<td>8.4</td>
<td>9</td>
<td>10.1</td>
<td>10</td>
<td>6.5</td>
<td>7.4</td>
</tr>
<tr>
<td>Platelet count (platelets/ml)</td>
<td>71</td>
<td>164</td>
<td>NA</td>
<td>84</td>
<td>75</td>
<td>98</td>
<td>73</td>
<td>179</td>
<td>131</td>
<td>170</td>
</tr>
<tr>
<td>Amastigotes in bloodsmear?</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Amastigotes in smear of bone-marrow aspirate?</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Serology (IFAT titre)</td>
<td>1:160</td>
<td>&lt;1:40</td>
<td>1:80</td>
<td>&lt;1:40</td>
<td>&lt;1:40</td>
<td>&lt;1:40</td>
<td>1:160</td>
<td>1:320</td>
<td>1:40</td>
<td>&lt;1:40</td>
</tr>
<tr>
<td>Bone-marrow culture</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Peripheral-blood-monoocyte culture</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Treatment for leishmaniasis (days)</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Relapse</td>
<td>Relapse</td>
<td>Relapse</td>
<td>0</td>
</tr>
<tr>
<td>Result of indirect xenodiagnosis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Blood storage at 4°C prior to xenodiagnosis (h)</td>
<td>6</td>
<td>6</td>
<td>24</td>
<td>6</td>
<td>36</td>
<td>6</td>
<td>24</td>
<td>48</td>
<td>72</td>
<td>24</td>
</tr>
<tr>
<td>% of bloodfed sandflies found infected</td>
<td>22.8</td>
<td>37.5</td>
<td>14.2</td>
<td>62.7</td>
<td>92.9</td>
<td>48.3</td>
<td>8.0</td>
<td>10.7</td>
<td>13.8</td>
<td>53.3</td>
</tr>
</tbody>
</table>

M, Male; F, female; IVDU, intravenous-drug user; HS, heterosexual; PTB, pulmonary tuberculosis; OC, oral candidiasis; CMV, cytomegalovirus infection; PCP, *Pneumocystis carinii*; EPTB, extrapulmonary tuberculosis; NA, not available; ND, not done.
a patient with nodulo-ulcerative post-kala-azar dermal leishmaniasis represented a source of infection in a VL focus in West Bengal.

After several years of using IXD to test blood samples from Spanish cases of Le. infantum/HIV co-infection, Molina et al. (1996) developed a standard protocol. Samples of peripheral blood are collected in tubes with anticoagulant (usually heparin, although EDTA or sodium citrate are equally satisfactory) and brought to the laboratory refrigerated, at 4°C. Each is tested by offering 50, 7-day-old, laboratory-bred, female P. perniciosus a 1.5-ml sample of blood, held at 37°C, for 1 h in a sterile membrane-feeding apparatus, using the skin of a 3-day-old chicken as the membrane. Any unfed flies are then removed with an electrical aspirator and killed with chloroform or CO2.

The fed flies are maintained on 30% fructose solution, replaced daily, for 48–72 h before the dissections begin. Each fly to be dissected is anaesthetized with CO2, placed in a drop of sterile phosphate-buffered saline (PBS) on a sterile microscope slide, and decapitated with sterile needles. The midgut is then drawn out, transferred to another drop of sterile PBS, covered with a sterile coverslip and examined under the microscope for promastigotes. If a gut is found to be heavily infected it is gently ruptured, by pressing on the coverslip, and used to inoculate NNN medium so that the Leishmania strain can be isolated and typed by iso-enzyme analysis. If a gut is found to contain only a few promastigotes 48 h after the infective feed, the surviving flies that had fed on the same sample are maintained until at least day 7 post-bloodmeal, before they too are dissected in the hope that, by then, some will be heavily infected and of use for parasite isolation.

DIRECT XENODIAGNOSIS
The ease with which sandflies can be infected with leishmanial parasites by feeding them, through a membrane, on blood from a HIV-positive individual who is co-infected with Le. infantum, raises the possibility that the cases of co-infection (and any other immunocompromised cases of VL) may act as secondary reservoirs of VL, in a natural but anthroponotic cycle of the disease. Direct xenodiagnosis (DXD) — in which uninfected sandflies are allowed to feed directly on humans — has recently been used to explore this possibility (Molina et al., 1999). Each of six co-infected patients was asked to place one of his or her hands into a small cage containing 25, 7-day-old, laboratory-bred, female P. perniciosus (and a similar number of male flies) for 15 min (Fig. 4; Table 2). The unfed flies were carefully removed with an electrical aspirator and killed, while the blood-fed females were kept in the cage for at least 72 h before being dissected (Molina et al., 1996). All six patients were found to be infective to the sandflies, their infectivity being negatively correlated with their CD4+ cell counts (Fig. 5). Counts of CD4+ cells may therefore be a useful indicator of the infectivity of a co-infected patient.

To summarize, the results of xenodiagnoses have indicated that: (1) vector sandflies are readily infected by feeding on immunodepressed cases of Le. infantum/HIV co-infection, whereas immunocompetent individuals infected with Le. infantum are poorly infective to such insects; (2) CD4+ cell counts are useful indicators of the infectivity of co-infected patients; and (3) at least some of the Le. infantum in the blood of those co-infected with HIV remain infective to sandflies for at least 8 days after the blood has been collected.

Epidemiological Implications
The ease with which sandflies may be infected when fed, directly or indirectly, on the blood of those with Le. infantum/HIV co-infection has considerable epidemiological implications, particularly as the cases of co-infection may remain asymptomatic, the VL in HIV-positive individuals often responds
FIG. 4. Direct xenodiagnosis of a suspected case of Leishmania infantum/HIV co-infection.

TABLE 2. Clinical and parasitological data for six cases of Leishmania infantum/HIV co-infection investigated by direct xenodiagnosis

<table>
<thead>
<tr>
<th>Case</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>26</td>
<td>27</td>
<td>29</td>
<td>38</td>
<td>27</td>
<td>32</td>
</tr>
<tr>
<td>Associated infection(s)</td>
<td>No</td>
<td>PCP</td>
<td>OC</td>
<td>T, MAI</td>
<td>OC</td>
<td>No</td>
</tr>
<tr>
<td>Fever?</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Splenomegaly?</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Leucocyte count (leucocytes/μl)</td>
<td>2200</td>
<td>3910</td>
<td>2400</td>
<td>4450</td>
<td>720</td>
<td></td>
</tr>
<tr>
<td>CD4 + count (CD4 + cells/μl)</td>
<td>120</td>
<td>4</td>
<td>45</td>
<td>12</td>
<td>39</td>
<td>28</td>
</tr>
<tr>
<td>Haemoglobin (g/l)</td>
<td>8.6</td>
<td>8.7</td>
<td>10.1</td>
<td>9.6</td>
<td>7.9</td>
<td>8.7</td>
</tr>
<tr>
<td>Platelet count (platelets/μl)</td>
<td>125</td>
<td>300</td>
<td>72</td>
<td>56</td>
<td>125</td>
<td>70</td>
</tr>
<tr>
<td>Serology (IFAT titre)</td>
<td>$1:640$</td>
<td>$&lt;1:40$</td>
<td>ND</td>
<td>ND</td>
<td>1:320</td>
<td>$&lt;1:40$</td>
</tr>
<tr>
<td>Bone-marrow culture</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Peripheral-blood-monocyte culture</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Result of direct xenodiagnosis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>% of bloodfed sandflies found infected</td>
<td>9.1</td>
<td>85.7</td>
<td>37.5</td>
<td>88.9</td>
<td>38.5</td>
<td>18.2</td>
</tr>
</tbody>
</table>

*Relapse after treatment for leishmaniasis. PCP, Pneumocystis carinii; OC, oral candidiasis; T, toxoplasmosis; MAI, Mycobacterium avium infection; ND, not done.

Poorly to treatment, and VL may develop at any stage of the HIV infection (Pineda et al., 1998). The results of leishmanin skin tests and lymphoproliferative tests in vitro indicate that, in areas where VL is endemic, many individuals, though asymptomatic, may be carrying leishmanial infections (Pampiglione et al., 1975, 1976; Gramiccia et al., 1990; Meller-Melloul et al., 1991; Marty et al., 1992; Arbaji et al., 1993; Acedo-Sánchez et al., 1996; Morillas et al., 1996). Le Fichoux et al. (1999) detected leishmanial DNA in nine blood samples (out of 76 found sero-positive for Leishmania) from the Monaco blood bank, indicating that Le. infantum circulates, albeit perhaps only intermittently and at a low density, in the blood of asymptomatic blood donors.
It seems that some iso-enzymatic phenotypes of *Le. infantum*, which have been detected in HIV-positive individuals but not in immunocompetent humans or dogs, are particularly associated with immunodepression (Gramiccia *et al.*, 1995; Jiménez *et al.*, 1995; Gradoni *et al.*, 1996; Pratlong *et al.*, 1995; Rosenthal *et al.*, 1995; Harrat *et al.*, 1996; Agostoni *et al.*, 1998; Chicharro *et al.*, 1999). Such phenotypes occur among the many zymodemes to be found in wild sandfly populations (Riouillé *et al.*, 1986; Gradoni *et al.*, 1991; Martín-Sánchez *et al.*, 1994, 1995, 1996) and at least some of them can infect laboratory-bred *P. perniciosus* (J. M. Lohse and R. Molina, unpubl. obs.).

In the presence of HIV, *Le. infantum* adapted to the peridomestic environment could evolve without the intervention of any other vertebrate host apart from humans, to a true anthroponotic (Tesh, 1995). In some aspects, this situation would emulate that of the strictly anthroponotic VL, caused by *Le. donovani*, seen in India, where even HIV-negative cases of VL are highly infective to sandflies (Theodor, 1964). As most (91.5%) cases of *Le. infantum*/HIV co-infection detected in south–western Europe have low CD4 + counts, of <200 cells/μl (WHO, 1999), many are probably infective to their local sandflies. The overall prevalence of human co-infection in the VL-endemic areas of Europe is very low when compared with that of canine leishmaniasis. There may be foci, however, in which human–human transmission via sandflies is playing an important part in the local epidemiology of VL. Cases of Leishmania/HIV co-infection may be clustered together, for example, in institutions specializing in the care of AIDS patients or the detoxification of IVDU. In some countries, including Spain, such institutions are usually in peri-urban or isolated, rural settings in which sandflies are...
often common. It may only take one case of Leishmania/HIV co-infection to trigger an outbreak of VL; the 1980 outbreak of VL in West Bengal possibly developed from one case of post-kala-azar dermal leishmaniasis (Addy and Nandy, 1992).

Clearly, the unequivocal demonstration of immunodeficient-human–sandfly–human transmission will be difficult in an environment in which canid–sandfly transmission is relatively common. It may be possible to demonstrate a new leishmanial infection in an individual who is not an IVDU but lives near a case of *Le. infantum*/HIV co-infection and distant from any infected canids. Even then, it would be almost impossible to prove that there are no infected canids nearby, and the possibility of transmission from an immunocompetent human carrier of *Le. infantum* could not be excluded (Le Fichoux et al., 1999).

The World Health Organization estimates that, in south–western Europe, 76.9% of the cases of co-infection are aged 31–50 years, 71.1% of them are IVDU aged >15 years, and up to 9% of people with AIDS suffer from VL (Desjeux, 1998; WHO, 1999). The number of co-infected individuals is expected to fall in Europe, as the result of the widespread use of anti-HIV drugs, and to rise in South Asia and sub-Saharan Africa (Desjeux, 1998; WHO, 1999). Discouraging the practice of syringe-sharing among IVDU may further reduce the incidence of the Leishmania/HIV co-infection, although it will have little if any effect on the leishmanial infection of sandflies feeding on the cases of co-infection. The potential threat posed to the rest of their communities by cases of co-infection, as sources of sandfly infection, will be difficult to reduce. Several control measures might be necessary, such as periodical counts of CD4+ cell counts (allowing the epidemiological risk posed by each case of co-infection to be evaluated), the rapid and prolonged antileishmanial treatment and isolation of co-infected patients, and the use of insecticide-impregnated bednets and indoor insecticide spraying in high-risk settings (such as detoxification and AIDS-treatment centres).

REFERENCES


HIV AND Leishmania TRANSMISSION


