INFECTION OF SAND FLIES BY HUMANS COINFECTED WITH LEISHMANIA INFANTUM AND HUMAN IMMUNODEFICIENCY VIRUS

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Abstract. To determine the role that Leishmania infantum/human immunodeficiency virus (HIV) coinfected patients could play in the epidemiology of visceral leishmaniasis (VL), we applied direct xenodiagnosis of VL in this study to test the infectivity of six coinfected patients to colonized Phlebotomus perniciosus. All patients proved to be infective for the sand flies. The infectivity of patients who had still not received specific treatment for VL was inversely proportional to their absolute CD4+ T lymphocyte cell count. It has been proven that P. perniciosus can acquire and allow the development of L. infantum by feeding on L. infantum/HIV coinfected patients. Since this sand fly is an important vector of VL in southern Europe, a new natural anthropotonic cycle could be considered in the epidemiology of L. infantum/HIV coinfection. The design of leishmaniasis control programs and the management of coinfected individuals should take these findings into account.

The rapid spread of Leishmania infantum/human immunodeficiency virus (HIV) coinfection throughout the western Mediterranean basin has attracted the interest of a number of scientists and clinicians. As an immediate consequence, various immunologic, pathologic, clinical, and epidemiologic studies have been carried out during the last few years with the common aim of identifying the reasons for the high incidence of visceral leishmaniasis (VL) among HIV-infected individuals. The accumulated data have given rise to the postulation of new alternative cycles of L. infantum different from the known cycles of this flagellate. In the simplest case, the syringes shared by coinfected intravenous drug users (IVDU) would replace the anthrophilic sand flies in the transmission of the parasite. This would signify a confrontation with an artificial epidemic anthropotonic completely independent of the participation of infected dogs maintaining the domestic cycle of the disease.

The usefulness of indirect xenodiagnosis for the detection of L. infantum in the blood of HIV-infected patients has suggested that such immunocompromised persons could potentially serve as urban or periurban secondary reservoirs in the natural cycle of VL. Thus, a natural endemic anthropotonic could be established in which the sand fly Phlebotomus perniciosus plays its role as vector of L. infantum. This hypothesis can partly be supported if the ability of the coinfected patients to transmit the parasites to the sand flies that fed on them can be proven. Direct xenodiagnosis is the essential method to investigate this fully innovative epidemiologic aspect.

Here we report the application of the direct xenodiagnosis of VL to study the infectivity of six L. infantum/HIV coinfected subjects to P. perniciosus.

PATIENTS AND METHODS

Patients. The HIV positivity of patients was established by ELISA and confirmed by Western blot. The Centers for Disease Control and Prevention (CDC) (Atlanta, GA) classification system for HIV infection was used to classify the patients. Diagnosis of leishmaniasis of all patients was carried out in our laboratory using at least one of the following methods: indirect fluorescent-antibody testing (IFAT), bone marrow culture, and peripheral blood monocyte culture. Sera were tested by IFAT with a cut-off titer of 1:80. Mononuclear cells, obtained by Ficoll gradient separation of peripheral blood or bone-marrow aspirates, were cultured in NNN medium to reveal the presence of parasites. The following data of the patients were also recorded: age, CDC HIV group, associated infections, fever of unknown origin for at least two weeks, splenomegaly, leukocyte count, CD4+ cell count, hemoglobin level, platelet count, and sand fly infection rates. The Pearson correlation test was used to analyze if there was a significant association between the percentage of sand flies infected by L. infantum and the CD4+ cell count of the patients. All patients gave informed consent to participate in the study. The experiments were approved by the Ethical Committee of the Instituto de Salud Carlos III, Madrid, Spain.

Sand flies. Phlebotomus perniciosus sand flies from an autochthonous colony established in our laboratory were used in this study. Flies were reared in an environmental cabinet at 28°C in a relative humidity that ranged from 95% to 100%, and under a 17:7 hr (L:D) photoperiod.

Direct xenodiagnosis. The infectivity of coinfected patients was tested by putting the hand of each patient into a cage with 25, seven-day-old, laboratory bred, female P. perniciosus together with a similar number of males for 15 min. After feeding, blood-engorged females were held for at least 72 hr in the environmental cabinet at 28°C and 95–100% humidity with a 17:7 hr (L:D) photoperiod. Sand fly midguts were drawn out in a drop of sterile phosphate-buffered saline and examined under the microscope for the presence of promastigotes. All surviving flies were dissected to determine the infection rates. The dissectors wore protective gloves and used electric aspirators. Details on the handling of infected sand flies have been reported previously.

RESULTS

Six coinfected male IVDU patients were studied. All were infective to the sand flies (Table 1). Case four was a 38-year-
Diagnostic leishmaniasis methods

<table>
<thead>
<tr>
<th>Cases</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6†</th>
</tr>
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<tbody>
<tr>
<td>Age (years)</td>
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<td>27</td>
<td>29</td>
<td>38</td>
<td>27</td>
<td>32</td>
</tr>
<tr>
<td>CDC HIV group</td>
<td>A2</td>
<td>C3</td>
<td>B3</td>
<td>C3</td>
<td>B3</td>
<td>B3</td>
</tr>
<tr>
<td>Associated infections</td>
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<td>PCP</td>
<td>OC</td>
<td>T, MAI</td>
<td>OC</td>
<td>No</td>
</tr>
<tr>
<td>Fever</td>
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<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>Leukocytes (× 10⁹/L)</td>
<td>2,200</td>
<td>3,910</td>
<td>2,400</td>
<td>4,450</td>
<td>980</td>
<td>720</td>
</tr>
<tr>
<td>CD4+ (10⁹/L)</td>
<td>120</td>
<td>4</td>
<td>45</td>
<td>12</td>
<td>39</td>
<td>28</td>
</tr>
<tr>
<td>Hemoglobin (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>Platelets (× 10⁹/L)</td>
<td>125</td>
<td>300</td>
<td>72</td>
<td>56</td>
<td>125</td>
<td>70</td>
</tr>
</tbody>
</table>

Diagnostic leishmaniasis methods

1. IFAT
2. Bone marrow culture
3. Peripheral blood monocyte culture
4. Direct xenodiagnosis

% of infected sand flies (n)

- CDC = Centers for Disease Control and Prevention; HIV = human immunodeficiency virus; PCP = Pneumocystis carinii pneumonia; OC = oral candidiasis; T = toxoplasmosis; MAI = Mycobacterium avium infection; IFAT = indirect immunofluorescent antibody test; ND = not done; n = number of sand flies dissected.

† Relapse after treatment for leishmaniasis. = negative.

old man in whom parasites were accidentally detected in a sample from a cutaneous lesion during a microscopic screening for Mycobacterium avium. Case six was a 32-year-old man, originally diagnosed with leishmaniasis by hepatic biopsy culture, who had received seven courses of treatment for VL and relapsed after each of them. At the time when direct xenodiagnosis was applied to this patient, none of the conventional diagnostic techniques had been able to detect the presence of the parasite.

In the patients who had not received a specific leishmanial treatment (cases 1–5), there was a significant negative association between their infectivity to sand flies and their absolute CD4+ T lymphocyte cell count (product-moment correlation coefficient of Pearson, r = −0.909, P < 0.05). This indicates that the lower the CD4+ cell count of the patient, the higher the patients infectivity for the sand flies (Figure 1).

**FIGURE 1.** Infectivity of patients coinfected with *Leishmania infantum* and human immunodeficiency virus before treatment for leishmaniasis in relation to their CD4+ lymphocyte cell counts.

**TABLE 1**

Direct xenodiagnosis of leishmaniasis in coinfected patients: clinical and parasitologic data

**DISCUSSION**

The direct evidence we have obtained for the incrimination of HIV/Leishmania coinfected subjects in the transmission of *L. infantum* to the sand flies validates the data obtained by indirect xenodiagnosis in previous studies.4,5,8 This situation would in some aspects be similar to Indian kala-azar caused by *L. donovani* in which the disease is strictly anthroponotic and high infection rates among the sand flies could be observed when the insects were fed on the infected persons.8 Consequently, besides the conventional zoonotic cycle (canids-sand flies-canids and humans), there would exist two hypothetical alternative anthropoontic cycles of this parasitosis that fit into the broader concept of VL: 1) the artificial one (immunodepressed IVDU-syringes-immunodepressed IVDU), and 2) the natural one (immunodepressed persons-sand flies-immunodepressed persons or indeed susceptible infants and adults who have not acquired immunity to *Leishmania*). The following example demonstrates the epidemiologic implications that such an anthropoontic cycle would raise.

Although the prevalence of human coinfection in the larger population is low, there is a high probability of encountering *L. infantum/HIV* coinfected subjects in institutions that maintain detoxification programs for IVDU or in special health care centers for patients with acquired immunodeficiency syndrome (AIDS). The majority of such installations in Spain are situated in isolated places, mostly periurban or rural, close to the natural habitat of the sand flies. Consequently, the possibility of a contact between the patients and the sand flies would be increased so that the appearance of new cases of VL could be expected.

It is important to remember that the initiation of the kala-azar outbreak in West Bengal, India in 1980 was attributed to a case of post-kala-azar dermal leishmaniasis.10 However,
the demonstration of the existence of a natural anthroponotic cycle in an environment in which humans are also at risk from zoonotic transmission is very difficult. Proving such transmission will require cases in non-IVDU persons in areas with coinfected persons, but without infected canids.

Some isoenzymatic phenotypes of *L. infantum* seem to be linked to the immunodepression since they could not be found in immunocompetent persons or dogs until now. According to previous results using indirect xenodiagnosis some of these isoenzymatic variants can develop in *P. perniciosus* females. The presence of these zymodemes in the wild sand fly populations would support anthroponotic transmission. On the other hand, epidemiologic analysis of the populations at risk, case distribution, incidence, and prevalence will be necessary.

The results have also revealed a strong association between the decrease in the number of CD4+ T lymphocytes and the increase in infectivity of coinfected persons to the sand flies. The CD4+ cell count should therefore be useful for determining the infectivity of coinfected subjects. The CD4+ T lymphocytes and their induced Th1 and Th2 cell subsets play an important role controlling the spread of *Leishmania* infection. At the same time, the CD4+ T lymphocytes are the primary target for HIV, increasing the risk of infection with opportunistic diseases such as VL due to *L. infantum*. Since the CDC AIDS surveillance case definition includes all HIV-infected persons who have less than 200 CD4+ T lymphocytes/µL, most coinfected patients with AIDS could serve as secondary reservoirs of *L. infantum*.

Recently, the World Health Organization estimated that in southern Europe 25–70% of adult cases of VL are related to HIV and 1.5–9% of AIDS cases have newly acquired or reactivated VL (World Health Organization, 1994, unpublished data). Since the coinfected subjects and the sand flies could be involved in an anthroponotic cycle of leishmaniasis, we would be confronted with a more complex situation. The epidemiologic implications of this possible anthroponosis could be involved in an anthroponotic cycle of leishmaniasis, and insecticide spraying might be necessary, especially at the detoxification and treatment center.

#### REFERENCES


