Leishmania infection can hamper immune recovery in virologically suppressed HIV-infected patients

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SUMMARY

An HIV-infected patient started combination antiretroviral therapy with 13 CD4+ cells/μL. Despite sustained virological suppression over the following four years, the anemia did not resolve, and the CD4+ cell counts always remained below 200/μL until co-infection with Leishmania was diagnosed in October 2006 when the patient started complaining of persistent mild fever and asthenia. Once treatment for leishmaniasis was started with miltefosine, CD4+ cell count rose above 400/μL. A new drop in CD4+ cell count was observed when Leishmania DNA turned out again to be positive, but treatment with liposomal amphotericin-B restored immune recovery.

KEY WORDS: HIV-1, Leishmaniasis, HAART, Immune reconstitution

Received March 28, 2007 *Accepted* May 14, 2008

Visceral leishmaniasis (VL) is endemic in Asia, South America and the Mediterranean basin (Alvar *et al.,* 1997, Murray *et al.,* 2005). The incidence of the disease and the risk of infection are higher in HIV-infected patients, and its clinical course is usually more severe in the case of advanced immune deficiency (Gradoni, *et al.* 1996, Pintado and Lopez-Velez, 2001, Lopez-Velez, *et al.* 2001).

The main reason for the poorer prognosis in co-infected patients is that the immunological control of VL seems to depend on the efficiency of T-helper type 1 (Th-1) cells rather than humoral immunity (Kemp, *et al.* 1993). Th-1 cells are defective during the course of HIV–infection, and HIV-infected patients show a shift from a Th-1 to a Th-2 pattern of response as their immune deficiency progresses (Clerici, *et al.* 1993). It has also been hypothesised that VL can negatively affect the course of HIV-infection: one study found a rapid increase in serum HIV-RNA after the onset of VL in patients not receiving antiretroviral drugs (Cacopardo, *et al.* 1996).

We here describe a case showing that Leishmania co-infection can hinder expected immune recovery during the course of effective antiretroviral therapy.

The patient first presented in October 2002 with weight loss and radiological findings of interstitial pneumonia. At that time, he was 34 years old and had a history of essential hypertension. Soon after admission, he was diagnosed as having advanced HIV-infection (acquired by heterosexual transmission) and *P. jiroveci* pneumonia: his CD4+ cell count was 13/μL and HIV-RNA level 720,000 copies/mL. Standard treatment with i.v. cotrimoxazole (plus methylprednisolone for the first few days) led to prompt recovery from pneumonia and the patient was discharged.

An antiretroviral therapy with zidovudine, lamivudine and lopinavir/r was started soon after; at baseline, Hb was 11.1 g/dL, platelets

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247,000/µL, white blood cells 2,500/µL, and total lymphocytes 1,300/µL.

Figure 1 shows the time trends of the patient's CD4+ cells, HIV-RNA, Hb, total lymphocyte, parasitemia (Leishmania-DNA) and platelet levels over the subsequent five years. He showed a rapid virological response with a more than 2-$\log_{10}$ reduction in viral load after four weeks of treatment and undetectable HIV-RNA levels (i.e. fewer than 50 copies/mL) being reached within six months. The response was sustained, as his HIV-RNA levels remained undetectable thereafter. CD4+ cell counts increased to a maximum of 174 cells/µL during the first year of antiretroviral therapy. Since October 2003, a further small increase in CD4+ cells percentage was observed, but the rise in CD4+ cell counts was blunted because of a decrease in the total number of lymphocytes.

Hb levels slightly improved after the start of antiretroviral treatment, but always remained below normal.

The antiretroviral regimen has been changed twice since November 2002: lopinavir/r was changed to nevirapine in August 2003 because of hypertriglyceridemia and hypercholesterolemia and, subsequently, zidovudine was replaced by

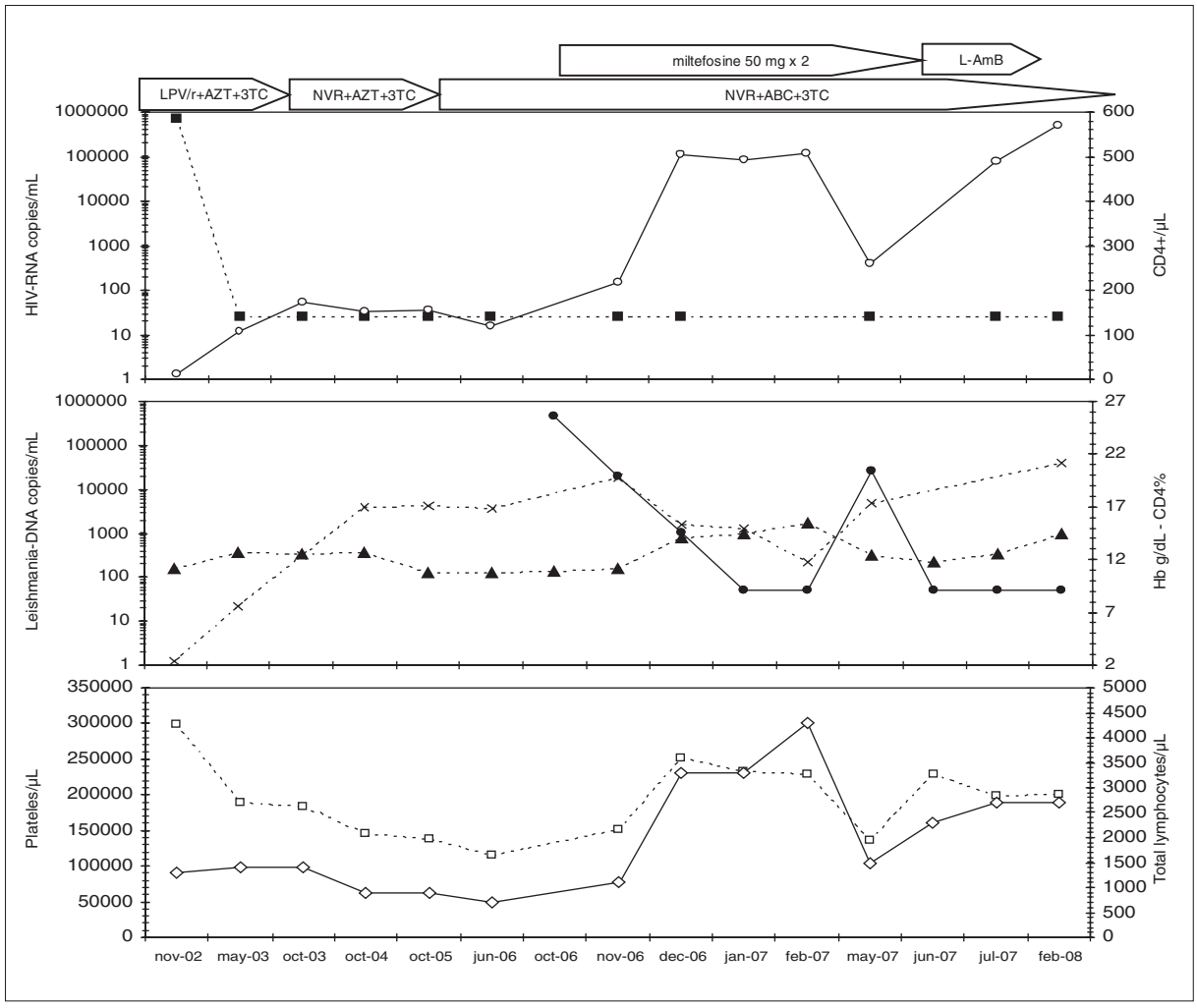

FIGURE 1 - *Dotted line and black squares (■) = HIV-RNA (copies/mL); solid line and white circles (○) = CD4+ cell/µL; solid line and black circles (●) = Leishmania-DNA (copies/mL); dotted line and crosses (X) = CD4 percentage; dotted line and black triangles (▲) = Hb (g/dL); dotted line and white squares (□) = platelets/µL; solid line and white diamonds (◇) = total lymphocytes/µL; AZT = zidovudine; 3TC = lamivudine; LPV/r = lopinavir-ritonavir; NVR = nevirapina; ABC = abacavir; L-AmB = liposomal amphotericin-B*

abacavir because of the persistent anemia, although Hb levels continued to worsen.

In July 2006, a number of laboratory tests were performed in an attempt to clarify the reason for the persistent anemia, and the results led to the exclusion of hemolytic anemia, thalassemia, iron deficiency, and folate or vitamin B12 deficit. A bone marrow aspirate was not diagnostic and the patient refused a bone marrow biopsy. It must be stressed that the patient denied fever or relevant asthenia or weight loss between November 2002 and September 2006. His liver and spleen remained within normal limits upon physical abdominal examination until Spring 2006, but both were enlarged in July 2006; ultrasonography revealed no focal lesions.

In October 2006, the patient started complaining of persistent mild fever and asthenia; blood cultures (also for mycobacteria species) yielded negative results and Leishmania DNA was sought in the blood by means of real-time PCR (Bossolasco, *et al.* 2003): 453,764 copies/mL were found, leading to a diagnosis of VL. The anti-Leishmania antibody titre was 1:640.

Treatment with miltefosine 50 mg twice daily was started on 26 October 2006: the fever rapidly disappeared and, within a few of months, a dramatic increase in total lymphocyte and CD4+ cell counts (the CD4+ cells percentage showed minor changes) paralleled a rapid decline in parasitemia. By January 2007, Leishmania DNA was undetectable in blood (i.e. fewer than 100 copies/mL). Miltefosine was well tolerated and continued at the same dose. In May 2007, Leishmania DNA turned out again to be positive (26,021 copies/mL), along with an increased anti-Leishmania antibody titre (1:2560) and a new decrease in total lymphocyte and CD4+ cell counts, but not with the re-occurrence of fever.

Resistance to miltefosine was hypothesised and, on 24 June 2007 the treatment was changed to liposomal amphotericin-B 5 mg/kg on days 1-5, and then on days 10, 17, 24, 31 and 38. This led to a new and very rapid decline in parasitemia, once again associated with an increase in total lymphocyte and CD4+ cell counts. The last dose of amphotericin-B was given on 27 July 2007 and Leishmania DNA was still undetectable as of 22 February 2008 (nine consecutive samples from 29 June 2007). On the contrary, the anti-Leishmania antibody titre did not decrease but remained two dilutions higher than at the onset of the full-blown disease.

We hypothesise that the patient acquired Leishmania infection from his dog some years before diagnosis (the existence of a likely infected dog was discovered only at the time of diagnosis).

The treatment of HIV infection with combination antiretroviral therapy usually leads to a substantial increase in CD4+ cell counts (Gras, *et al.* 2007, Moore and Keruly, 2007). In particular, a regimen of lopinavir/r plus stavudine and lamivudine led to ongoing immune reconstitution during six years of therapy in a cohort of HIV–infected, antiretroviral-naïve subjects with suppressed HIV-1 RNA levels, even when baseline CD4+ cell counts were less than 50 cells/μL (Landay, *et al.* 2007).

In our case, CD4+ cell counts did not decrease before or at the time of onset of the clinical symptoms of VL, but as virological suppression was sustained we expected immune recovery to continue over time. However, this only occurred after the Leishmania co-infection had been efficaciously treated. We cannot exclude the possibility that immune reconstitution was interrupted by the switch from a regimen based on a protease inhibitor to one based on a non-nucleoside reverse transcriptase inhibitor, but the time trend of the CD4+ cell counts clearly suggests that it was hindered by leishmaniasis.

It has been observed *in vitro* that *L. infantum* can induce virus expression in monocyte lines latently infected with HIV-1 (Bernier, *et al.* 1995). However, the viral load in our patient did not increase before or during the course of Leishmania infection, probably because of the concomitant effective antiretroviral treatment, which may also have allowed the number of CD4+ cells to increase after the start of therapy against leishmaniasis.

Unlike previously described patients, our patient had antibodies against Leishmania at the time of disease onset, but their titre poorly reflected the clinical course. This confirms that antibody titres poorly reflect the clinical course of the disease and do not seem to be useful in diagnosing and monitoring leishmaniasis in HIV-co-infected patients (Alvar, *et al.* 1997, Pintado and Lopez-Velez, 2001). On the other hand, our case confirms the usefulness of real-time PCR in diagnosing and monitoring the clinical course of Leishmania in-

fection (Bossolasco, *et al.* 2003, Reithinger, *et al.* 2007): the time trend of the parasitic load measured by means of PCR perfectly fitted the clinical picture and the changes in CD4+ cell counts.
Finally, our case suggests that VL should be considered when an HIV-infected patient with full and sustained virological suppression presents consistent signs and symptoms, and does not show the degree of immune recovery expected from an effective antiretroviral therapy.

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