MOLECULAR DIAGNOSIS AND TYPING OF TRYPANOSOMA CRUZI POPULATIONS AND LINEAGES IN CEREBRAL CHAGAS DISEASE IN A PATIENT WITH AIDS

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Abstract. Trypanosoma cruzi DNA was amplified from an intracranial biopsy and peripheral blood of an HIV patient with encephalitis; this episode was indicative of AIDS and congenital Chagas disease. The analysis of a microsatellite locus revealed a multiclonal parasite population at the brain lesion with a more complex minicircle signature than that profiled in blood using restriction fragment length polymorphism (RFLP)-PCR and low stringency single primer (LSSP) PCR. Interestingly, different sublineages of T. cruzi II were detected in blood and brain by means of spliced-leader and 24s ribosomal-DNA amplifications. Quantitative-competitive PCR monitored the decrease of parasitic load during treatment and secondary prophylaxis with benznidazole. The synergy between parasiticidal plus antiretroviral treatments probably allowed the patient a longer survival than usually achieved in similar episodes. This is the first case report demonstrating a differential distribution of natural parasite populations and sublineages in Chagas disease reactivation, showing the proliferation of cerebral variants not detectable in peripheral blood.

INTRODUCTION

Due to the increasing control of transmission routes mediated by vector, blood transfusion, and organ transplant, congenital transmission of Trypanosoma cruzi has emerged in terms of public health. Congenital cases are frequently asymptomatic, passing unnoticed unless specific tests are made. The acquired immuno deficiency syndrome (AIDS) pandemic, with 1.4 million infected people in endemic regions for Chagas disease, may lead to reactivation of quiescent chronic T. cruzi infections.

Natural T. cruzi populations are composed of multicolonial strains with different biological properties such as replication rates, drug susceptibility, virulence, and tissue tropism, which may be implicated in the clinical forms of the disease. These strains cluster within two major phylogenetic lineages, T. cruzi I and T. cruzi II, with 5 lesser subdivisions within T. cruzi II, whose phylogenetic relationships are still under debate. The high sensitivity of PCR-based typing strategies provides direct assessment of parasite genetic diversity in clinical specimens without the need of culture isolation, which may underestimate natural population complexity due to strain selection during culture expansion. Herein, we report the differential diagnosis of chagasic encephalitis in a patient with presumptive cerebral toxoplasmosis due to AIDS, applying PCR in a surgical sample of brain tissue and peripheral blood specimens, not detectable using current parasitological methods. Furthermore, PCR-based identification of T. cruzi lineages and profiling of microsatellite and minicircle signatures were assessed directly from the clinical specimens, providing evidence of a differential host tissue distribution of natural parasitic populations.

CASE REPORT

A 29-year-old HIV-positive Argentinian man, born in Buenos Aires, was hospitalized in February 2003 with left hemiparesis and a history of protracted headaches of 1 month of evolution. Magnetic resonance imaging showed multiple masses on the left brain hemisphere (Figure 1A) and anti-toxoplasma IgG serodiagnosis was positive, although near the cutoff line (6 IU/mL). Consequently, treatment of presumptive cerebral toxoplasmosis was implemented. As the patient did not respond to the specific treatment, an intracranial biopsy guided by CT-scan was indicated to determine the etiology of the encephalitis. Histologic analysis exhibited edema and high lymphocyte counts but did not reveal any protozoan forms. Thus, we applied PCR procedures targeted to T. gondii B1 and T. cruzi minicircle (kDNA) and satellite (sat-DNA) sequences from DNA extracts of the brain tissue specimen, which only revealed T. cruzi DNA, indicative of cerebral Chagas disease. Microhematocrit analysis did not show any bloodstream forms, but the high sensitivity of kDNA-PCR (0.4 log parasite genome equivalents/mL) allowed detection of T. cruzi DNA in peripheral blood. Besides, anti-T. cruzi serodiagnosis was also resulted positive (ELISA titer R: 2.7, cutoff < 1, and indirect hemaglutination titer 1/256, cutoff < 1:32). Thus, on the basis of T. cruzi DNA amplification and seroreactivity, the patient received a final diagnosis of chagasic encephalitis. Accordingly, trypanocidal therapy with benznidazole (5 mg kg−1 day−1) was administered. Treatment (tmt) response was monitored by competitive-qPCR (Q-PCR) on peripheral blood, which initially revealed approximately 280 parasites genome equivalents/mL of blood (2.4 log pge/mL) (Figure 1B; April 2003). Treatment regimen was modified 25 days later because the patient presented polymorphemic erythema. At that time, the parasitic load had decreased to 1.4 log pge/mL (Figure 1B; May 2003). After the remission of symptoms, tmt was re-started for 60 days leading to further decrease of parasitemia to undetectable loads (Q-PCR below 1.09 log, and kDNA-PCR below 0.4 log pge/mL; Figure 1B, August 2003). There-
after, secondary prophylaxis with benznidazole was initiated (5 mg kg$^{-1}$ day$^{-1}$ twice a week) with persistently negative PCR findings (Figure 1B; August 2003 to February 2005) accompanied by a good clinical evolution without neurologic symptoms. Magnetic resonance imaging showed a reduction of perilesional edema although brain mass did not remit (not shown); two cerebral spinal fluid samples withdrawn during post-treatment follow-up were negative when examined by microscopy and kDNA-PCR (not shown). The anti- T. cruzi immunologic response persisted positive throughout follow-up. Antiretroviral tmt (ART) promoted a decrease of HIV viral load from > 500,000 copies/mL to undetectable levels (< 1.7 log HIV copies/mL), accompanied with an increase of CD4+ cell counts from 22/mm$^3$ to 194/mm$^3$ (Figure 1B). After 30 months of survival, the patient continues under anti- T. cruzi prophylaxis, ART, and anticonvulsive tmt. This study was approved by the hospital review board and ethical committees of the institutions; written informed consent was required in agreement with the guidelines of the International Conference on Harmonization.

Molecular profiling of parasitic populations and lineages involved in Chagas disease reactivation. To characterize the genetic diversity of the patients parasitic populations found in peripheral blood and brain tissue samples, the 330-bp kDNA amplicons were profiled using low stringency single primer (LSSP) and restriction fragment length polymorphism (RFLP) PCR strategies$^{10,11}$ (Figure 2). Interestingly, minicircle signatures from brain tissue parasites exhibited a different profile than that obtained from bloodstream parasites at time of diagnosis (Figure 2A). The minicircle signatures detected in brain tissue showed a higher number of amplicons than those in blood, suggesting a higher diversity of parasite variants. This diversity was further explored by amplification of a microsatellite marker, known as TAC 15,$^9$ which revealed a multiclonal population in the brain chagoma (amplicons of 128, 131, and 134 bp; Figure 2B, br) whereas a homozygous and conserved population was detected in bloodstream at time of diagnosis and during treatment follow-up (amplicons of 131 bp; Figure 2B, bl$_1$ and bl$_{15}$, respectively).

The bloodstream parasite minicircle signatures were characterized during antiparasite treatment (Figures 2A and 2C). The RFLP-PCR and LSSP-PCR profiles obtained from T. cruzi kDNA in blood collected 45 days after initiation of tmt revealed a reduction in the number and/or the relative presence of certain minicircle subclasses compared with the patterns of the pretreatment population (Figures 2A and 2C, lanes bl$_1$ and bl$_{15}$). These findings suggested a differential tmt response of certain parasite variants until the complete clearance of the parasitemia at the end of tmt.

To identify the parasite lineages in peripheral blood and brain chagoma, the intergenic region of the spliced-leader locus (values in the scale given in bp) was amplified by multiplex PCR assay,$^{13}$ revealing T. cruzi II in both clinical specimens (Figure 2D, lanes 1 and 4). Furthermore, heminested amplification of the D7 domain of the 24s ribosomal RNA genes$^{15}$ allowed to distinguish T. cruzi II b/e (rDNA group 2/2) at the brain lesion and T. cruzi IId (rDNA group 1/2) in bloodstream (Figure 2D, lanes 2 and 5, respectively).

**DISCUSSION**

According to Centers for Diseases Control and Prevention, 3 of the 12 case-defining opportunistic infections for AIDS Group IV are parasitoses, namely toxoplasmosis, cryptosporidiosis, and isosporidiosis. However, in endemic regions for Chagas disease, T. cruzi should be included among the potential opportunistic pathogens indicative of AIDS.$^3,^4$ In this scenario, the PCR provided a rapid differential and sensitive diagnosis of T. cruzi reactivation allowing prompt administration of specific chemotherapy, which contributed to the patients' long survival. This case further illustrated the usefulness of Q-PCR to follow-up parasitic response to treatment and secondary prophylaxis. Moreover, prospective Q-PCR could aid in assessing the risk of reactivation to implement primary prophylaxis. It is worth noting that the synergy of parasiticial secondary prophylaxis plus antiretroviral treatment may have allowed the patient a longer survival than usually achieved in similar episodes.$^3,^4$

To date, few cases of chagasic reactivation due to AIDS have been described in detail.$^3,^4$ The typical epidemiologic sequence of events was to acquire T. cruzi infection in rural areas of endemcity and to move to urban centers where HIV
infection was acquired, leading to opportunistic disease as immunosuppression ensued.\textsuperscript{3,4} However, the case reported herein most likely acquired Chagas disease through vertical transmission. This was assumed because a) the patient was born in a nonendemic area, b) did not have any contact with triatomine vectors, did not receive transfusions, did not use intravenous drugs, and c) his mother was seropositive for Chagas disease. Congenital Chagas disease cannot be prevented, but early diagnosis and treatment achieve very high cure rates.\textsuperscript{2,13} This stresses out the public health importance of surveying all newborns to seropositive mothers in nonendemic areas for Chagas disease.

The role of \emph{T. cruzi} parasitemia and genetic make-up on the onset of chagasic reactivation due to AIDS, as well as the impact of HIV infection in \emph{T. cruzi} genetic diversity, are poorly explored fields of clinical parasitology.\textsuperscript{6,17} Comparative zymodeme studies of parasite stocks obtained from HIV-positive patients with \emph{T. cruzi} coinfection did not reveal significant differences regarding the tissue tropism or repartition of the strains.\textsuperscript{16} Remarkably, this case is the first evidence of a differential distribution of \emph{T. cruzi} populations associated with reactivation, showing the proliferation of cerebral parasite multiclonal populations, belonging to a sublineage not detectable in peripheral blood, indicative of lineage-histotropism in Chagas disease.

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