Inability to Detect Human T Cell Lymphotropic Virus Type 2-Specific Antibodies in a Patient Coinfected with HIV-1, Human T Cell Lymphotropic Virus Type 1, Human T Cell Lymphotropic Virus Type 2, and Hepatitis C Virus

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Abstract

HIV-1, human T cell lymphotropic virus type 1 and type 2 (HTLV-1 and HTLV-2) and hepatitis C virus (HCV) are common among intravenous drug users (IDUs) and can cause chronic infections in the host. Usually, the diagnosis of such viruses employs serological assays; however, some difficulties in confirming HTLV-2 infection have been reported in high-risk populations in Brazil. We present data of an unusual case of coinfection with HIV-1, HTLV-1, HTLV-2, and HCV in a male IDU in which HTLV-2 was detected only by molecular assays. Comparative analysis of retroviruses from 2002 and 2012 showed identical HTLV-1 and HTLV-2 sequences (LTR, *env*, and *tax*), and a change in HIV-1 tropism from CXCR4 to CCR5. No mutation was detected in the hot points of the *env* region of the HTLV-2 isolate that justified the lack of rgp46-II-specific antibodies. These data emphasize the need for molecular assays to diagnose HTLV-2 in high-risk populations in Brazil.

HIV/AIDS IS CONSIDERED ENDEMIC in Brazil, with a prevalence rate between 0.3% and 0.6%.¹ Brazil also has the highest number of human T cell lymphotropic virus type 1 and type 2 (HTLV-1 and HTLV-2)-infected individuals in the world, estimated at 2.5 million.^{1–3} HTLV-1 infection is prevalent in northeastern Brazil, most likely as a consequence of the slave trade of the seventeenth to nineteenth centuries.⁴ HTLV-2 is endemic among the indigenous populations of the northern Amazon region⁵ and also among HIV/AIDS patients and intravenous drug users (IDUs), mostly from southeastern and southern Brazil.⁶ The prevalence of hepatitis C virus (HCV) in Brazil is intermediate, ranging from 1% to 2%, with a higher prevalence rate (2.12%) in the north.⁷

HIV-1, HTLV-1, HTLV-2, and HCV are common among IDUs, and all of these viruses can cause chronic infection in the host. The viruses also share the same routes of transmission, such as blood transfusion, sexual intercourse, intrave-

nous drug use, and others. However, after mandatory predonation screening processes were implemented for blood donation in Brazil, a reduction in the blood-borne transmission of these viruses has been observed (data not shown). At present, HIV is transmitted primarily through sexual contact, HTLV-1 is spread through breastfeeding, and HTLV-2 and HCV are spread by intravenous drug use.^{1–7}

The natural history of HIV infection has changed since the era of highly active antiretroviral therapy (HAART), and understanding the processes that occur during retroviral coinfection presents a challenge. Because HTLV-1 preferentially infects CD4⁺ T cells and HTLV-2 has a preferential tropism for CD8⁺ T cells, their influence on HIV-1 infection and disease progression differs substantially.⁸

Coinfections of HIV-1/HCV and HIV-1/HTLV-1 have been associated with a worse prognosis of the diseases,^{8–10} while HIV/HTLV-2 coinfection has been associated with a

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delay in the progression to AIDS.^{8,9} A well-documented effect of HIV-1/HTLV-1 coinfection is the increased CD4⁺ cell count, which has clinical relevance; CD4⁺ cell count is the main surrogate marker used by clinicians to define the most favorable time to begin HAART treatment or to introduce prophylaxis against certain opportunistic infections.⁹ However, as soluble factors produced by HTLV-1-infected cells can either enhance or suppress HIV-1 infection, the effect of HIV-1/HTLV-1 coinfection on HIV-1 pathogenesis is still controversial.⁸ In contrast, several studies have shown that HTLV-2 exerts a protective role, increases patient survival, and delays the progression to AIDS. Particularly, cytokine and chemokine network modulation by HTLV-2 has been proposed to help maintain CD4⁺ and CD8⁺ T cell counts, thereby lowering HIV replication and promoting immune activation.⁸

Little is known about disease progression and clinical outcomes in individuals coinfecting with HIV-1, HTLV-1, and HCV. One study conducted in Brazil suggested that triply coinfecting patients would have better outcomes, with respect to the HCV infection, than their dually infected counterparts.¹⁰ Indeed, to our knowledge, no clinical or laboratory studies on patients with a quadruple coinfection of HIV-1, HTLV-1, HTLV-2, and HCV have been published previously.

Usually, HIV-1, HTLV-1/2, and HCV infections are diagnosed by serology; however, in the high-risk populations of Brazil, the commercial Western Blot (WB) confirmatory assay (HTLV Blot 2.4, Genelabs, Singapore) is not able to detect all of the HTLV-2-infected individuals.^{11,12}

Here, we report an unusual case of quadruple coinfection of HIV-1, HTLV-1, HTLV-2, and HCV in a white IDU male from the southeast region of Brazil; at the beginning of this study, the patient was 37 years of age, and HTLV-2 infection could be detected only by using molecular assays.

In 1997, the patient was diagnosed with AIDS because he presented with *Pneumocystis jirovecii* pneumonia and seropositivity for HIV. He began HAART therapy with didanosine, lamivudine, and zidovudine. In 2002, he participated in an epidemiological survey of HCV and HTLV-1/2 infections at an AIDS Reference Center in Londrina, Paraná state, Brazil. After obtaining a signed informed consent, a blood sample was collected and analyzed for the presence of specific antibodies. The results obtained confirmed the HCV and HTLV-1 infections using serology; anti-HCV antibodies were assessed using a microparticle enzyme immunoassay version 3.0 assay (AxSYM System, Abbott GmbH & Co. KG, Wiesbaden, Delkenheim, Germany), and anti-HTLV-1/2 antibodies were assessed using two EIA (Vironostika HTLV-I/II, Microelisa System, Biomerieux, Durham, NC; Abbott-Murex HTLV-I + II GE80/81, Murex Biotech Limited, Dartford, Kent, UK). WB analyses confirmed the HTLV-1 infection (HTLV Blot 2.4, Genelabs, Singapore).¹¹ Surprisingly, the polymerase chain reaction (PCR) test detected DNA segments of the *env* and LTR regions of both HTLV-1 and HTLV-2, resulting in a positive identification of HTLV-2 infection, but not HTLV-1 infection.¹¹ At that time, we hypothesized that a mutation in the *env* region of HTLV-2 was responsible for the discordant results between the serology and PCR tests.

In March 2005, the patient presented with lipodystrophy and peripheral vasculitis. In July 2007, the patient's HAART therapy was changed to didanosine, lamivudine, and lopinavir/ritonavir, and the patient is currently still on this regimen.

During follow-up examinations, the CD4⁺ and CD8⁺ cell counts were evaluated using flow cytometry (FACSCount, Becton Dickinson, San Jose, CA), and the HIV viral load was assessed using RT-PCR and bDNA assays (Cobas Amplicon Monitor HIV-1 version 1.5, Roche Diagnostic Systems, Brachburg, NJ; Versant HIV-bDNA 3.0 Assay, Siemens, Berkeley, CA). The results obtained showed a mean CD4⁺ cell count of 287/ μ l (range 170 to 441), a mean CD8⁺ cell count of 805/ μ l (range 481 to 1090), a mean CD4⁺/CD8⁺ of 0.39 (range 0.2 to 0.6), and an HIV viral load generally under the limit of detection of the assay.

In October 2012, using PCR protocols optimized for Brazilian isolates,^{13–15} we retested the patient's blood sample from 2002 and compared it with a new blood sample from 2012; both samples were positive by PCR for HTLV-1 and

FIG. 1. Pattern of human T cell lymphotropic virus (HTLV) reactivity from the WB 2.4 analysis (HTLV Blot 2.4, Genelabs, Singapore) obtained from serum samples of a patient coinfecting with HIV-1, human T cell lymphotropic virus type 1 (HTLV-1), human T cell lymphotropic virus type 2 (HTLV-2), and hepatitis C virus (HCV). Lanes 1, 2, and 3 denote HTLV-1, HTLV-2, and a mixture of HTLV-1 and HTLV-2 positive controls. Lanes 4 and 5 correspond to serum samples from 2002 and 2012, respectively.

HTLV-2 (LTR, *env*, and *tax* regions). We discarded the possibility of a false-positive result in the PCR assays because neither the HTLV-1/2-seronegative samples nor the water sample scored positive in the PCR assay. To determine whether a period of HTLV-2 seroconversion was responsible for the negative result in the WB 2.4 preliminary analyses, we tested the new blood sample from 2012 and confirmed a single HTLV-1 seropositivity (Fig. 1). We also ran a mix of the HTLV-1 and HTLV-2 positive controls to simulate a dual infection, allowing us to rule out any interference with the gp46-I or gp46-II bands (Fig. 1). The WB analysis confirmed no interference among the antibodies.

To compare the HTLV-1 and HTLV-2 isolates from the two periods, provirus DNA segments of LTR, *tax*, and *env* were sequenced. HTLV-1 subtyping and HTLV-2 subtyping were performed using the NCBI-Genotyping and REGA-Subtyping tools. Phylogenetic analysis of HTLV-1 revealed identical sequences for the *env* [GenBank: JQ435912 and JX280961] and *tax* [GenBank: JN887701, JX280962] segments, and only the LTR sample from 2002 was sequenced [GenBank: JF271840]; these sequences belong to the HTLV-1 cosmopolitan subtype of the transcontinental subgroup A. Phylogenetic analysis of HTLV-2 revealed identical sequences for the LTR, *env*, and *tax* segments; these sequences belong to the HTLV-2a subtype (variant -2c) [GenBank: JQ435902, JQ435911, JN887725 (2002) and JX280963, JX280964, JX280965 (2012)].

To determine why the patient did not produce HTLV-2-specific antibodies, the *env* sequence of HTLV-2 was compared with the sequence used in the preparation of the WB 2.4

test (rgp46-II, K55).¹⁶ Using the www.uniprot.org website, we searched for mutations in glycosylation, disulfide bonds, and fusion sites, and we found no mutations in these hot spots that would justify the negative serological result (Fig. 2). Additionally, real-time PCR (*pol*) to quantify the HTLV-1 and HTLV-2 proviral load was conducted, and the results obtained confirmed that the HTLV-2 provirus was present with a copy number similar to HTLV-1 in both samples (data not shown). Thus, we excluded severe immunosuppression (very low CD4⁺ cell count), antibody masking, mutations in the *env* region, and low HTLV-2 copy number as potential causes of the negative results by WB analyses. Corroborating these data, another study in Brazil showed that the molecular diversity of the envelope, the immunosuppression status, and the proviral load were not related to indeterminate WB results in patients infected with HTLV-2.¹⁷ Interestingly, this study showed a high frequency of HCV coinfection in patients who had indeterminate WB results when compared to patients who had HTLV-2-positive results in the WB 2.4 analysis.¹⁷ We do not know whether there is an association between these infections that could explain the lack of HTLV-2-specific antibody production or detection, but it is interesting to note that the patient in the present study was also infected with HCV. More studies are necessary to confirm or disprove this association.

We established the HIV-1 subtype using a protocol that amplifies only a portion of the *env* region,¹⁸ and we were able to classify the HIV-1 sequences as subtype B according to the NCBI-Genotyping tool [GenBank: KF309371 (2002) and KF309372 (2012)]. Interestingly, when we examined the HIV-1

HTLV-1 [GenBank: JQ435912 (2002), JX280961(2012)]

aa144

EVSRLNINLHFSKCGFPFSLLDVAPGYDPIWFLNTEPSQLPPTAPPLLPHSNLDHI
LEPSIPWKSLLTLVQLTLQSTnYTcIVcIDRASLSTWHVL YSPnVS/PSSSSTPLL
YPSLALPAPHLTLPFnWTHCFDPQIQAI VSSPCHNSLILPPFSLSPVPTLGSRSRRA
VPVAVWLVSALAMGAGVAGGITGSM SLASGKSLLEHVDKDISQLTQAIKVN
KNLLKIA

aa372

HTLV-2 [GenBank: JQ435911 (2002), JX280964 (2012)]

aa163

APGYDPLWFITSEPTQPPPTPPPLVHDSLEHVLTPSTSWTTKILKFIQLTLQSTn
YScMVcVDRSSLSSWHVL YTPnISIPQQTSSRTILFP SLALPAPPSQFPWTHCYQP
RLQAITTDNCnNSIILPPFSLAPVPPATRRRRRAVPIAVWLVSALAAGTGIAGG
VTGSLSLASSKSLLEVDKDISHLTQAIKVNHNILRVAQYAAQNRRLDLLF
WEQGGLcKAIQEQccFLnISNTHVSVLQERPPLEKRVITGWGLNWDGLSQWAR
EALQTGITILALLLVILFGPCILRQIQALPQRLQNRHNQYSLINPETML

aa486

FIG. 2. Amino acid sequences of a partial segment of the *env* region of HTLV-1 and HTLV-2 proviral DNA obtained from two blood samples (years 2002 and 2012); samples were obtained from a patient coinfecting with HIV-1, HTLV-1, HTLV-2, and HCV. The important sites in the envelope region were searched using the www.uniprot.org website. The glycosylation sites are depicted in bold lowercase letter, the C-C disulfide bonds in underlined bold lowercase letter, and the hot points in the fusion site of HTLV-1 in underlined bold capital letters; the whole fusion site of HTLV-2 is marked in underlined bold capital letters. The amino acid (aa) numbering is in accordance with the envelope sequences of the MT-2 isolate [Uniprot P23064 and GenBank X56949] and of the HTLV-2 prototype [Uniprot P03383 and GenBank K02024]. Mutations in relation to the prototypes are depicted in italic (V245I in HTLV-1 and S183P in HTLV-2).

tropism using the HIV-1-binding sites for the chemokine coreceptors CCR5 (R5) and/or CXCR4 (X4), there was a difference between the two isolates. The *env* region containing the third variable region (V3 loop) was amplified in three independent replicates, and the tropism prediction was performed using the Geno2pheno tool with a 10% cut-off.¹⁹ Curiously, the sample from 2002 was predicted to be an X4-tropic variant thought to be related to advanced disease (syncytium-inducing, lymphocyte-tropic strains), and the sample from 2012 was an R5-tropic variant (non-syncytium-inducing, macrophage-tropic strains). We do not know why this patient was identified as having X4 variants at the beginning of the study and R5 variants at present, but we consider that this change in tropism could be due to HAART treatment.

In conclusion, more studies are necessary to understand why this patient did not produce HTLV-2-specific antibodies and to determine whether the current WB analysis used for HTLV-2 detection needs to be improved in Brazil. In this regard, a study that intends to compare the efficiency of the WB analysis and the immunochromatographic assay (INNO-LIA HTLV I/II Ab, Innogenetics, Ghent, Belgium) is in progress in our laboratory. Finally, this study emphasizes the need for molecular assays to confirm HTLV-2 infection in the high-risk populations of Brazil, as this infection could have prognostic value in delaying AIDS progression.

Sequence Data

The GenBank accession numbers of sequences from this patient were as follow: HTLV-1; LTR: BRLO37-02 (JF271840); *env*: BRLO37-02 (JQ435912) and BRLO37-12 (JX280961); tax: BRLO37-02 (JN887701) and BRLO37-12 (JX280962). HTLV-2; LTR: BRLO37-02 (JQ435902) and BRLO37-12 (JX280963); *env*: BRLO37-02 (JQ435911) and BRLO37-12 (JX280964); tax: BRLO37-02 (JN887725) and BRLO37-12 (JX280965). HIV-1; *env* BRLO37-02 (KF309371) and BRLO37-12 (KF309372).

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Author Disclosure Statement

No competing financial interests exist.

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