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Rapid development of subacute myelopathy in three organ transplant recipients after transmission of human T-cell lymphotropic virus Type I from a single donor

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Abstract

Background.

Human T-cell lymphotropic virus type I (HTLV-I) causes a subacute myelopathy in less than 5% of chronic carriers. However, the risk of neurologic disease appears to increase in persons infected through blood transfusion.

Methods.

We report three recipients of solid organ transplants who developed a subacute myelopathy within 2 years after becoming infected with HTLV-I from a single asymptomatic HTLV-I donor. Genetic studies were performed in *LTR* and *tax* sequences in proviral DNA, and HTLV-I proviral load was measured by real-time quantitative polymerase chain reaction.

Results.

HTLV-I sequences were obtained in two of these individuals, and they were almost identical and clustered within the Cosmopolitan A HTLV-I subtype, which indicates a common source. All typical changes in Tax amino acid sequence of the HTLV-I Cosmopolitan A were identified, plus two additional changes were noted. Although *tax* A has been associated with a greater risk of neurologic disease, both patients were positive for human leukocyte antigen-A*02, which is considered a protective factor.

Conclusion.

Rapid development of subacute myelopathy may occur in recipients of organ transplants from asymptomatic HTLV-I donors. A particular virulence of the virus strain, the large size of the virus inoculum, and the immunosuppressed condition after transplantation may have contributed to produce this unusual rapid development of HTLV-I associated myelopathy.

Human T-cell lymphotropic virus type I (HTLV-I) is the causative agent of a subacute myelopathy termed tropical spastic paraparesis (TSP)/HTLV-I associated myelopathy (HAM). The majority of HTLV-I-infected persons do not manifest any clinical symptoms throughout their lives, and only a minority (<5%) develops TSP/HAM after a long-term asymptomatic period ([1]). The mechanisms by which HTLV-I causes disease have not been fully elucidated, although environmental, viral, and host factors have been implicated. With regard to viral factors, the proviral load is almost always elevated in patients with TSP/HAM compared with asymptomatic carriers of HTLV-I ([2]). Moreover, some HTLV-I subtypes are more prone to produce HTLV-I-related disease than others. For instance, Cosmopolitan A viruses carrying the *tax* A gene have been linked to a greater risk of TSP/HAM development ([3]). Host factors, such as the human leukocyte antigen (HLA) haplotype, may also influence the outcome of HTLV-I infection, and the class I allele HLA-A*02 seems to be strongly protective against TSP/HAM ([4]).

Environmental factors can also determine the outcome of HTLV-I infection. When HTLV-I infection is acquired through blood transfusion, TSP/HAM is more frequent and the latency period is often shorter ([5]). The large size of the virus inoculum may explain this unusual poor outcome. Herein, we report three cases of HTLV-I infection acquired through organ transplantation from a single donor. All three developed TSP/HAM shortly after transplantation. Genetic analyses performed in two of them proved that viral factors could be involved in the rapid course of the disease, although environmental circumstances, primarily the immunosuppressive status of the recipients, could equally play a role.

Patients

In 2000, three white patients presented with clinical manifestations of subacute myelopathy. All of them had received an organ transplant from the same donor less than 2 years earlier. The first was a 44-year-old woman who underwent a liver transplant (case 1) after developing alcoholic cirrhosis ([6]). The second was a 53-year-old woman who received a kidney (case 2). The third was a 55-year-old man who received the second kidney (case 3). In all cases, cyclosporin A (15 mg/kg per day) was the only immunosuppressive agent used to prevent organ rejection. Fresh blood samples from the second and third patients were available for serologic and genetic analyses. Only stored sera were available from the donor and one of the recipients.

Serology

Serum and cerebral spinal fluid (CSF) specimens from patients were tested for antibodies to HTLV-I/II using an enzyme linked immunoabsorbent assay (Abbott HTLV-I/II; North Chicago, IL). Repeated reactivity was confirmed by Western blot (Bioblot HTLV, Genelabs, Singapore). The HTLV European Research Network criteria ([1]) were used for interpreting Western blot patterns.

Phylogenetic Analysis of the HTLV-I LTR Gene

The long terminal repeat (LTR) region of HTLV-I was amplified by nested polymerase chain reaction (PCR) from proviral DNA, using primers and conditions described elsewhere ([7]). The 720-

base pair (bp) amplified product was used for phylogenetic analysis. Sequences were aligned with ClustalW, and a pairwise distance matrix was generated with the DNADIST program, available with the PHYLIP package. The phylogenetic tree was constructed using Kimura's 2-parameter method and inferred by the Neighbor-Joining method. Its robustness was measured by bootstrap analysis using 1,000 replicates.

DNA Sequence Analysis of the HTLV-1 *tax* Gene

Nearly the whole HTLV-I *tax* gene was amplified (1,061 bp) by nested PCR from proviral DNA, as previously described ([3]). Amplified products were sequenced in an ABI 310 genetic analyzer using the Dye Terminator DNA sequencing kit (Applied Biosystems, Foster City, CA). HTLV-I *tax* nucleotide and predicted amino acid sequences were aligned using the ClustalW software with BOI and ATK HTLV-I reference strains.

Human Leukocyte Antigen Typing

PCR sequence-specific primer reactions using the Dynal classic SSP HLA-A2 kit (Dynal Biotech, Oslo, Norway) were performed to characterize the HLA-A*02 allele in DNA extracted from peripheral blood mononuclear cells (PBMCs) from all TSP/HAM samples.

Quantification of HTLV-I Proviral Load

A quantitative real-time PCR method was developed to measure HTLV-I proviral load in PBMCs. The PCR reaction was performed using LightCycler technology. The Fast Start DNA Master Mix Hybridization Probe Kit (Roche, Basel, Switzerland) and primers SK432 and SK111 matching the *pol* region of HTLV-I were used for genetic amplification ([7]). The SK111 primer was internally labeled at residue 22 (T) with fluorescein. A specific HTLV-I probe (CCACCTGAATGTGTTAACCAACTGCCAC) close to primer SK111 labeled with LC-640 in its 5′ end was designed to allow its detection using fluorescence resonance energy transfer technology.

Three-hundred nanograms of genomic DNA extracted from each patient's PBMCs were analyzed. The PCR mixture, with a final volume of 20 μL, contained 2 μL DNA master mix, 2.8 μL 25 mM $MgCl_2$, primers SK111 and SK432 (0.125 μM each), HTLV-I-640 probe (0.2 μM), β-globin primer mix (0.25 μM each), β-globine-LC705 probe (0.2 μM), and water. The reaction conditions were as follows: denaturation at 95°C for 10 min, followed by 45 cycles of 95°C for 15 sec, 60°C for 5 sec, and 72°C for 10 sec.

For each run, a standard curve was generated using 60 to 6×10^4 copies of a recombinant HTLV-I plasmid DNA. An amplified HTLV-I *pol* fragment (198 bp) was cloned into a pPCR-Script plasmid (Stratagene, La Jolla, CA). Recombinant plasmids were purified using the QIAfilter Plasmid Maxi Kit (Qiagen, Hilden, Germany) and quantified by A_{260} measurements. The copy number in each clinical sample was estimated by interpolation from the plasmid regression curve. Amplification of β-globin gene was used as an internal control and to correct for sample DNA quality differences. All samples were run in duplicate. Results were expressed as copies per 10^4 PBMCs.

The donor and the three recipients of his organs were seropositive for HTLV-I. The donor was a young white man born in Spain who suffered a fatal car accident in 1999. He showed no apparent risk factors for HTLV-I infection; however, a close interview with his relatives revealed that his mother was originally from Venezuela, where HTLV-I is endemic ([8]). All three organ recipients developed TSP/HAM with an unusually short asymptomatic period after acquiring HTLV-I infection through solid organ transplantation. The diagnosis of the neurologic condition was

performed according to the standard criteria ([8]), which included typical findings in the nuclear magnetic resonance of the spine, the recognition of HTLV-I antibodies in both CSF and sera, and the exclusion of other common causes.

As expected, the phylogenetic analyses of the virus *LTR* region revealed that HTLV-I proviruses from two of these TSP/HAM patients (cases 2 and 3), from whom PBMCs were available, were closely related, being the comparison of their genetic distance was almost zero. This verifies that both patients were infected from a common source. The analysis also showed that the viruses infecting these patients belonged to the HTLV-I Cosmopolitan A subtype (Fig. 1).

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Figure 1:
Phylogenetic tree analysis of human T-cell lymphotropic virus type I (HTLV-I) strains based on long terminal repeat (LTR) sequences. Patients 2 and 3 (*open boxes*) were compared with HTLV-I reference sequences and other HTLV-I Spanish sequences (SP12769, SP7647, SP8172, SP8995, and SP142). The tree was rooted with the Melanesian subtype. Numbers given at branch points represent the bootstrap values.

The analysis of the *tax* gene showed the four specific nucleotide substitutions characteristic of the *tax* A subgroup ([3]). Four additional nucleotide substitutions within the *tax* gene were found, two of them resulting in amino acid changes (Table 1). The *tax* A gene has been associated with a greater risk of TSP/HAM development, probably as a result of an increased transactivation of inflammatory cytokine genes, which shows neurotoxic activities ([2,9]). CD4+ T lymphocytes represent the primary cellular target for HTLV-I infection, but other cell types, such as astrocytes, have been found to be infected with HTLV-I. For instance, *tax* mRNA has been detected in astrocytes in central nervous system lesions in the absence of a high lymphocyte infiltration ([10]). The production of neurotoxic mediators, which may damage the central nervous system, has been postulated as one of the mechanisms by which HTLV-I causes neurologic disease ([11]). The recognition of *tax* A in our patients may explain, at least in part, why they all developed TSP/HAM.

T1-19
Table 1:
Table 1. Nucleotide and amino acid changes in *tax* gene

HLA-A*02 restricted cytotoxic T lymphocytes (CTLs) are efficient at controlling HTLV-I replication. By lowering the HTLV-I proviral load, they appear to reduce the risk of TSP/HAM ([4]). It is noteworthy that although both of our patients presented with this HLA-A*02 haplotype, their HTLV-I proviral load was high: 1,664 and 1,244 copies per 10^4 PBMCs, respectively. As noted before, high HTLV-I proviral load is a common finding in TSP/HAM patients ([2]).

The high proviral load observed in our patients despite the presence of HLA-A*02 could be related to a compromise in the CTL response as a result of the immunosuppressive treatment (cyclosporin A) given to all these patients after transplantation. It is noteworthy that immunosuppression therapy may preferentially limit the cellular response but does not severely affect the humoral response. In fact, anti-HTLV-I antibodies were detected in both blood and CSF in our patients. Thus, deposition of antibodies or complement could have contributed to the neurologic damage in our patients, as previously reported ([10]). Inasmuch as Tax protein is the dominant target antigen for CTL, the amino acid changes we found in the HTLV-I strains infecting our patients could have altered the molecule recognition and favored virus escape from the immune surveillance, producing an increase in the proviral load and, therefore, leading to a rapid disease onset ([2]).

Finally, environmental factors, including the route of transmission, could have played a decisive

role in the outcome of HTLV-I infection in these patients. HTLV-I transmission by blood products or after organ transplantation may result in a shorter latency and a greater risk of TSP/HAM ([5,10]). Transmission by these routes implies exposure to large virus inoculum (which may facilitate the infection of a large number of CD4+ and CD8+ cells), in contrast with infection acquired by mucosal routes such as breastfeeding or sexual contact ([12]). Moreover, in the setting of transplantation, a rapid increase in HTLV-I proviral load may be favored by a lack of CTL response as a result of the immunosuppression status of the transplant recipients.

Given that HTLV antibody screening is not uniformly recommended before transplantation in Western countries ([1]), we stress the public health implications of our study. Routine screening of HTLV-I antibodies in blood donations is not performed in Spain because of the low rate of HTLV-I infection found in sentinel studies conducted in blood donors. However, our findings of a more frequent and rapid disease course of HTLV-I disease in transplant recipients of organs from asymptomatic HTLV-I carriers indicates the need to reconsider the current guidelines. We believe that HTLV-I screening should be mandatory in all organ donors, given the high risk of infection and rapid disease onset in recipients.

GenBank Accession Numbers

HTLV-I *tax* and LTR sequences generated in this study were submitted to the GenBank database. Accession numbers were AF485380 to AF485383.

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