Frequency of p12K and p12R Alleles of HTLV Type 1 in HAM/TSP Patients and in Asymptomatic HTLV Type 1 Carriers

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ABSTRACT

HTLV-1 has a complex genome, and contains four open reading frames (ORFs) in the 3' region encoding viral and cellular regulatory proteins. p12 is a small, ORF I-encoded hydrophobic protein, the function of which is not well understood. It has been shown that p12 enhances the E5-transforming ability of bovine papillomavirus; and binds to the 16-kDa subunit of the vacuolar ATPase pump, immature forms of the β and γc chains of the interleukin 2 receptor, and the free chain of MHC I. p12 carrying a lysine residue (p12K) at position 88 of its sequence may be rapidly degraded in the cell via proteasome, whereas p12 with an arginine residue (p12R) at the same position is severalfold more stable. These alleles are found in proviral DNA of HTLV-1-infected individuals and it was previously observed that the p12K allele was more frequent in HAM/TSP (HTLV-1-associated myelopathy/tropical spastic paraparesis) patients and was not found at all in asymptomatic carriers, whereas patients with adult T cell leukemia/lymphoma (ATLL) carry the p12R allele. To extend these observations and verify whether the p12K mutation could be used as a marker of progression to HAM/TSP, we analyzed 37 HAM/TSP patients and 40 asymptomatic carriers at different stages of infection. In our cohort, only one HAM/TSP patient carried the p12K phenotype, which accounted for a frequency of 2.7% (1 of 37). We also found, among the 40 asymptomatic HTLV-1 carriers, one who presented the p12K phenotype, contrasting with previous publications. Thus, p12K does not seem to be universally diagnostic for HTLV-1-associated neurological disease. Further screening of HTLV-1-infected individuals in other populations may elucidate this observation.

INTRODUCTION

HTLV-1 is associated with adult T cell leukemia/lymphoma (ATLL) and with a myelopathy (HTLV-1-associated myelopathy/tropical spastic paraparesis [HAM/TSP]), besides other conditions such as uveitis, poliomyositis, and infective dermatitis.1 HTLV-1 has a complex genomic structure, with a pX region in the C terminus that encodes regulatory proteins affecting viral and cellular gene expression. Tax is considered the major viral trans-activating protein, and has been implicated in cellular transformation processes. Another protein from the pX region is p12, which is codified by open reading frame (ORF) I.2 Although the function of p12 is not well defined, it has been suggested that p12 may play a role in cellular transformation. It may be a substrate for ubiquitination and degradation by proteasome, because p12 with lysine in the C-terminal region (position 88) has a half-life significantly shorter than that of p12 with arginine in the same position.3 It has been postulated that the destabilization of the viral proteins via proteasome may be a mechanism for intracellular defense against viral infections and that p12 is essential for persistent infection by HTLV-1.4 This protein can bind to the 16-kDa subunit of the H+-ATPase proton pump5 and to the β and γc chains of the interleukin 2 (IL-2) receptor,6 consequently inducing activation of STAT5,7 and can also enhance the transforming ability of the bovine papillomavirus E5 oncoprotein.5 More re-

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cently, p12 has also been demonstrated to bind the free MHC I heavy chain and preferentially target it for degradation. As a result, there is a decrease in MHC I levels at the cell surface. Previous data concerns the distribution of the natural alleles of p12 (p12K and p12R), determined on the basis of a small number of ex vivo samples from HTLV-1-infected subjects, show that p12K was detected less frequently and was present mainly in HAM/TSP patients. Conversely, p12R was found in all ATLL and healthy carrier samples of the group studied, suggesting that a selective pressure over p12 might be occurring in the host. In the present work, we analyzed sequential blood samples from 40 HTLV-1-infected asymptomatic blood donors from Fundação Hemominas and 37 HAM/TSP patients from Hospital Sarah Kubistchek (both of Belo Horizonte, Brazil) to verify whether the detection of one or the other allele of p12 could be associated with progression to HAM/TSP. Our cohort included 77 individuals: 37 with diagnosed HAM/TSP and 40 healthy carriers. The samples were collected at different times postinfection, to make up 60 samples from each group analyzed.

MATERIALS AND METHODS

ORF I PCR

p12 amplification was done as previously described, with 0.5–1.0 μg of DNA from peripheral blood mononuclear cells (PBMCs) in 50 μl containing 20 mM Tris-HCl (pH 8.0), 50 mM KCl, 1.5 mM MgCl2, 200 mM deoxynucleoside triphosphates, 2.5 U of Taq DNA polymerase (GIBCO-BRL, Gaithersburg, MD), and 10 pmol each of primers ORF I (5′-CACC-TGCCTTCCAACTG-3′, nucleotides 6768–6785) and PX1AS (5′-GCTGTGCTTGACGGTTTGC-3′, nucleotides 7160–7142). Thirty cycles of 30 sec at 94°C, 15 sec at 58°C, and 30 sec at 72°C were carried out in a thermal cycler (PTC-100; MJ Research, Watertown, MA). Plasmids p12K-HA1 and p12R-HA1, carrying the p12 sequence with lysine or arginine at position 88, respectively, were used as positive controls. The amplified product (18 μl) was digested directly with 10 U of Apal (Pharmacia Biotech, Uppsala, Sweden) at 37°C for 1 hr. The digested samples were electrophoresed on a 2.5% agarose gel and stained with ethidium bromide. The cleaved product corresponds to p12R, whereas the uncut product indicates p12K.

Sequencing of p12K samples

To confirm that p12K samples have a lysine at position 88, we sequenced the uncut products by the Sanger dideoxy method. Sequencing reactions were carried out with the fmol DNA cycle sequencing system (Promega, Madison, WI). The reverse reaction was done with the PX1AS primer.

RESULTS

Low frequency of p12K allele in HAM/TSP patients

We analyzed 60 samples from 37 HAM/TSP patients: 27 female and 10 male, all of them born in Brazil. The majority of them have had HAM/TSP for a long time, with lesions at the thoracic level and with both neurogenic bladder and bowel (Table 1). Only 1 of 37 HAM/TSP patients was p12K (Fig. 1), and all remaining patients were p12R. The p12K subject was a 68-year-old male, who had had HAM/TSP for 12 years, classified as ASIA D, with neurogenic bladder and bowel. DNA sequencing of the PCR product from this patient confirmed the lysine code (AAG) at position 88 (Fig. 2).

Presence of p12K allele in asymptomatic HTLV-1 carriers

We analyzed 60 samples from 40 asymptomatic HTLV-1 carriers and found just 1 p12K phenotype (Fig. 1), which was

<table>
<thead>
<tr>
<th>Asymptomatic</th>
<th>HAM/TSP</th>
<th>positive controls</th>
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<tbody>
<tr>
<td>p12K</td>
<td>p12R</td>
<td>p12K</td>
</tr>
<tr>
<td>p12K88</td>
<td>p12R88</td>
<td>neg</td>
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<tr>
<td>Apal</td>
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FIG. 1. Natural occurrence of p12 alleles in samples from HTLV-1-infected asymptomatic carriers and HAM/TSP patients. Ethidium bromide-stained gel of PCR product from the HTLV-1 ORF I before (−) and after (+) Apal cleavage. Cleaved products indicate p12R and uncut products suggest p12K. neg, Negative control; MW, 100-pb DNA ladder (GIBCO-BRL).
confirmed by DNA sequencing and was positive in two samples collected 2 years apart. This subject was an asymptomatic young female and her follow-up for 5 years did not show clinical signs indicating neurological disease.

**DISCUSSION**

The difference in frequencies of the p12K allele in HAM/TSP patients (2.7%) and in asymptomatic HTLV-1 carriers (2.5%) analyzed in our study was not significant (OR, 0.93; 95% CI, 0.02–35.36), whereas it was relevant in previous studies.\(^3,7\) In these, p12R was found in all 25 ATLL patients and 9 healthy carriers, whereas p12K was found in 5 of 17 HAM/TSP patients (29.4%), a much higher frequency than we found in our cohort. The samples previously analyzed were derived from patients of different geographical origins, and it has been suggested that selective pressure presented by the HTLV-1 carrier rather than random mutation in the p12 sequence could be occurring.

Here we provide evidence for the presence of the p12K allele in an asymptomatic carrier. This subject has been monitored longitudinally to assess whether progression to HAM/TSP would occur, but after 5 years no neurological signals have become evident. However, in two patients diagnosed with HAM/TSP, and in whom a clear progression of disease occurred, no change to the p12K phenotype was observed.

We concluded that in our cohort the p12K phenotype is not a good marker for HAM/TSP. In our study, all individuals were born in the same geographic region (Minas Gerais State, Brazil) and might represent a particular HTLV-1 carrier population in which selective pressure on the p12 sequence would not be occurring. This absence of association could be occurring in selected populations. The previously reported data\(^3,7\) were based on a much lower number of individuals, who also were from different geographic areas. This could explain the difference in prevalences of p12K in HAM/TSP patients.

The significance of natural p12 alleles is unclear, and it has been proposed that because of the significant difference in the *in vitro* stability of the two p12 proteins, the biological effects of the proteins in the host could be different, depending on which allele is present. Because of the p12 interaction with MHC I, further studies will be necessary to establish whether mutation at position 88 of p12 may have a selective advantage in individuals with a certain MHC I.

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**REFERENCES**


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