First identification and molecular characterization of Lymphotropic Polyomavirus in peripheral blood from patients with leukoencephalopathies

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Short title: Lymphotropic Polyomavirus in human blood

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Lymphotropic Polyoma virus (LPV) was first isolated in 1979 from a B-lymphoblastoid cell line of an African green monkey by zur Hausen and Gissman (1). This virus has some characteristics common to human polyomavirus, such as virion morphology, the presence of a closed circular double-stranded molecule of DNA, and in vitro transforming activity (2), but it is antigenically distinct from Simian Virus 40 (SV40), BK Virus (BKV), and JC Virus (JCV) (3). In cell culture, LPV has a highly restricted host range for both human and monkey B-lymphoblasts (4,5) and seroepidemiological studies revealed that, in addition to sera from monkeys, many human sera had strong reactions in the presence of LPV antigens (6).

More recently, Lednicky and colleagues were able to detect a fragment of the LPV genome in the blood of an immunocompromised rhesus monkey (7), but LPV or any other related virus has not yet been isolated from human biological fluids or tissues.

In order to investigate the possible presence of LPV in human specimens, we examined DNA extracted from peripheral blood mononuclear cells (PBMCs) that were collected from Italian HIV positive patients who were affected with different forms of leukoencephalopathies or other neurological diseases (OND), as well as HIV+ subjects without any neurological disorders (NND). We also collected cells from healthy subjects as controls (HC). All the individuals gave written consent for the enrollment in the study.

In particular, the LPV genome was searched by means of nested Polymerase Chain Reaction (n-PCR), targeting the viral transcriptional control region (TCR) (7) in DNA from ten patients with Progressive Multifocal Leukoencephalopathy (PML), ten with Not Determined JCV-negative Leukoencephalopathy (NDLE), ten with OND, ten with NND, and ten HC. Plasmid pLPV-K38, containing a full-length genome of LPV-K38 (8), was used as a positive control template for the amplification assay.
The LPV control region was successfully amplified from three DNA extracts: one from PBMCs collected from a PML patient and two from PBMCs collected from two NDLE patients. However, the bands, created by the inner PCR reaction, were different in size (520 bp and 270 bp). The amplified fragments, together with the positive control, were subjected to direct automated sequencing, according to published procedures (9), and the obtained nucleotide sequences were aligned with the GenBank database. DNA isolation, PCR and sequencing were repeated twice. Cross-referencing with the database showed the expected complete nucleotide identity (100%) of LPV (accession number K02562.1) to the LPV-K38 control, whereas lower nucleotide identities of the LPV genome to the amplified TCR from the PBMCs of the patients were shown.

In particular, the molecular organization of the LPV TCR, amplified from the PML patient, was rearranged as well as the LPV-K38 control, but five nucleotide changes were detected between the two viral strains (nt 110, 222, 335, 394 and 408, numbering relative to strain K38). Two archetypal LPV regulatory regions were identified in the PBMCs from the two NDLE patients. To rule out the hypothesis that a cross contamination between the samples could have happened during the n-PCR procedures, the two archetypal sequences were aligned and differences in six places were discovered between the two strains (nt 126, 129, 133, 201, 269, 555, numbering relative to strain K38). As shown in figure 1, the ori region and the enhancer 63-bp subgenomic element of the rearranged sequence, found in the PML patient, were duplicated in comparison to the archetype sequence, amplified from the two NDLE patients.

In order to confirm these findings, another PCR targeting VP1 region was performed. Specific primers LPV1 (nt 1765-1784) and LPV2 (nt 1966-1985) were used and a 221 bp VP1 fragment was successfully amplified in the PML and in one of the NDLE patients. Lack of material did not allow to perform the PCR in the PBMCs from the third patient (data not shown).
Even if the investigated human population was limited, it is noteworthy that the LPV genome was exclusively found in the peripheral blood of patients with the two different forms of leukoencephalopathies, but not in the OND, NND, or HC cases. Moreover, two different molecular organizations of the viral TCR were detected, and based on what is known about human polyomaviruses (10), we can hypothesize that the archetypal organization is probably the form naturally transmitted (7), whereas rearranged forms, derived from partially duplication of the archetype nucleotide sequence, could be generated as a consequence of highly active viral replication.

To the best of our knowledge, this is the first direct detection of the LPV genome in human peripheral blood and this validates current seroepidemiological evidence suggesting the presence of LPV-like infections in human subjects. Due to the low number of studied subjects, we cannot draw a clear conclusion, but the data suggest at least the possible involvement of LPV in CNS leukoencephalopathies occurring in HIV positive individuals.

Isolation of LPV and/or whole-genome sequencing of the virus will be necessary to additionally support the significance of our results; however the presence of the viral DNA in human blood cells is an important step and warrants further investigations that may correlate LPV infection with specific human diseases.

References:


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Nucleotide accession number

The accession numbers for the two Archetypal and the Rearranged sequences determined in this study are: [GenBank:EU559168, GenBank:EU559169, GenBank:EU559170] respectively.
Figure 1 legend:

Schematic representation of the amplified TCR sequences. Panel A, Comparison between the rearranged TCR form amplified from the PML patient and the one amplified from the plasmid lpvk38. Panel B, comparison between the two TCR archetypal forms amplified from the two NDLE patients. The LPV PCR primer binding sites are indicated with LPR1 and LPR2. The arrows indicated the point mutation positions. The T-Antigen recognition pentanucleotides are boxed with a solid line, while a dashed line boxes the enhancer 63-bp subgenomic elements. The ori region is underlined with a solid line.