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LYMPHOTROPIC POLYOMAVIRUS IS DETECTED IN PERIPHERAL BLOOD FROM IMMUNOCOMPROMISED AND HEALTHY SUBJECTS

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Abstract

Background—*Lymphotropic Polyomavirus* (LPV) was isolated from a B-lymphoblastoid cell line of an African green monkey. This virus shares some characteristics with human polyomaviruses, but it is antigenically distinct from *BK Virus* (BKV) and *JC Virus* (JCV). Seroepidemiological studies revealed that human sera react in the presence of LPV antigens, and, recently, the viral genome was amplified in the peripheral blood from patients affected with HIV-related leukoencephalopathies.

Objectives—the aims of the study were to investigate and compare the presence of LPV DNA with that of JCV and BKV in different biological samples and patient groups.

Study Design—LPV, JCV and BKV DNA were searched and quantified in peripheral blood and CSF from HIV+ patients and in peripheral blood from healthy subjects.

Results—The LPV genome was detected in peripheral blood of both HIV+ patients and healthy subjects, with a prevalence of 7.2% and 4.7% respectively, but not in CSF. However, its presence was less frequent than that of JCV and BKV.

Conclusions—The amplification of LPV genome from human peripheral blood confirms the fact that LPV can infect the human population. LPV DNA was amplified from patients affected with HIV-related leukoencephalopathies but also from HIV patients without neurological disorders and from healthy subjects. Therefore, the results do not support the hypothesis of an association between

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LPV infection and any neurological disease. However, given their high similarity, it is possible that LPV, as well as BKV and JCV, could establish latency in humans and cause disease only in rare circumstances.

Keywords

LPV; Polyomavirus; peripheral blood; HIV patients; healthy subjects

1. Background

Lymphotropic Polyomavirus (LPV) was first isolated from a B-lymphoblastoid cell line of an African green monkey ¹. This virus belongs to the *Polyomaviridae* family, that consists of small, non-enveloped DNA viruses. LPV shares morphologic features with the other known human polyomaviruses, *BK Virus* (BKV) and *JC Virus* (JCV), but it is antigenically distinct from them ^{2,3}. Seroepidemiological studies revealed that human sera react in the presence of LPV antigens ^{4,5}, and, recently, the viral genome was amplified in the peripheral blood from patients affected with HIV-related leukoencephalopathies ⁶. However, an extensive investigation of the presence of LPV genome in human clinical specimens from different clinical settings is still lacking, whereas the epidemiology of BKV and JCV has been widely described.

It is well known that BKV and JCV are widespread among the healthy human population, since about 90% and 80% of adults have antibody to BKV and JCV, respectively ^{5,7,8}. The primary infection of both viruses is usually asymptomatic and is followed by a lifelong persistence, occurring mainly in the kidney ⁹. Lymphoid cells have been proposed as another site of latency, since polyomaviruses genome sequences have frequently been detected in blood cells from both immunocompromised and healthy subjects ¹⁰.

Reactivation of JCV in immunocompromised patients may cause a demyelinating disease of the Central Nervous System (CNS), named Progressive Multifocal Leukoencephalopathy (PML), whereas reactivation of BKV may cause haemorrhagic cystitis and Polyomavirus Associated Nephropathy (PVAN) in renal transplant patients ^{11,12}.

The other Polyomaviruses that can infect the human population are the newly discovered *WU* and *KI* viruses, that have been mainly isolated from respiratory specimens¹³. To date, there are no published data regarding the relation between KI, WU and LPV, which, furthermore, has been never found in respiratory samples.

So far, the potential pathogenetic mechanism of LPV during the primary infection or during reactivation is unknown. Therefore, the distribution of LPV in the human population and its possible association with a specific disease need to be elucidated.

2. Objectives

The aims of the study were to examine and compare the presence of LPV, that was recently isolated from the human specimens, with that of JCV and BKV in the peripheral blood and CerebroSpinal Fluid (CSF) from HIV+ patients with and without neurological disorders and in the peripheral blood from healthy subjects.

3. Study Design

3.1 Study populations and samples

HIV+ patients—Peripheral Blood was collected from 83 HIV+ patients, referring to the Unit of General Neurology at the Mondino Hospital, Pavia, Italy and to the Department of Infectious Diseases at the Policlinico San Matteo, Pavia, Italy, for clinical care. The subjects included 61 males and 22 females, with a mean age of 49 years, which were enrolled into one of the four following disease groups: 11 patients were affected with PML, 16 patients were affected with Not Determined Leukoencephalopathy (NDLE), 11 patients were affected with Other Neurological Diseases (OND) and 45 patients did not suffer of any neurological disorders (NND).

CSF was also collected from all the patients affected with neurological diseases (PML, NDLE and OND) for diagnostic purpose and part of the CSF sample was stored at -80°C for research use. An informed consent form was signed by each patient at the time of collection of the clinical specimens.

Healthy subjects—Peripheral Blood was collected from 105 healthy subjects, matched for age (mean age: 42 years) and all tested HIV negative, referring to the Center for Translational Research, Saint Joseph Hospital, Milano, Italy, for routine blood testing and part of the sample was used for research purposes. The subjects included 50 males and 55 females, and were enrolled into one of the following age groups: 22 individuals in the <9 years group, 17 in the 9–19 years group, 26 in the 20–49 years group, 19 in the 50–69 years group and 21 in the over 70 years group. An informed consent form was signed by each patient at the time of collection of the clinical specimens.

3.2 Detection of the polyomaviruses genome

Human polyomaviruses loads were quantified after DNA extraction from 0.2 ml of peripheral blood with QIAmp blood mini kit (Qiagen, USA), and from 0.15 ml of CSF with Nucleospin RNA Virus kit (Macherey Nagels, Germany), following manufacturer's instructions. To minimise the risk of contamination, DNA was isolated in a laminar air flow using aerosolresistant, sterile and disposable tips. In addition, a separate room was used to perform the Real Time PCR (Q-PCR) assays.

The Q-PCR protocol for the detection of JCV DNA has been described elsewhere¹⁴, whereas the protocols for the detection of BKV and LPV DNA were newly designed. The primers and probes used are listed in table 1.

Briefly, the Q-PCR reactions were performed in a final volume of 25 μl containing 1X Taqman Universal PCR Master Mix (Applied Biosystems, USA), 0.4 μM primer Forward, 0.4 to 0.9 μM primer Reverse, 0.2 μM probe, and 250 ng of extracted nucleic acid. Thermal cycling was carried out according to the following steps: an initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min, at the end of which the fluorescence was read. The limit of detection was 2 copies/reaction for JCV assay, and 5 copies/reaction for BKV and LPV assays. Each sample was analyzed in triplicate, and each run contained a negative control constituted by the reaction mixture without the DNA template. Standard curves for quantification of viral genome were constructed using serial dilutions of a plasmid containing the entire JCV, BKV and LPV genome (range: 10 to 10^6 plasmid copies). As shown in figure 1, the three Q-PCR assays showed high efficiency, being the slope values -3.445 (JCV), -3.408 (BKV) and -3.453 (LPV) and the correlation coefficient 0.999 for all the three assays.

Viral loads were expressed as copies/ug of DNA extracted from the peripheral blood. In addition, the presence of beta-globin gene was tested in each sample, as a control to confirm the presence and quality of DNA, using primers and probe previously published¹⁵. Amplicons for the beta-globin control gene were detected in all the analyzed samples.

It should also be underlined that the laboratory successfully participated to the external quality control programs for the quantitative assays of JCV and BKV, provided by the Quality Control for Molecular Diagnostics (QCMD) organisation.

3.3 Data analysis

Statistical evaluations were performed using EpiInfo 6.0. Data were analyzed by using Fisher's 2 sided exact test and $p < 0.05$ was considered statistically significant.

4. Results

4.1 Presence of LPV genome

The prevalence of LPV DNA among the HIV+ population was 7.2% (6 out of 83), with no significant differences in the distribution among PML, NDLE and NND patients, whereas the prevalence of LPV DNA among the healthy subjects was 4.7% (5 out of 105). Among NND patients two were co-infected with LPV and BKV (Table 2, panels A and B). Among OND patients, none was infected with LPV (table 2). In addition, LPV was not detected in any of the CSF samples (data not shown).

4.2 Presence of JCV and BKV genome

JCV DNA was found in 13 out of 83 HIV+ patients, with a significant higher prevalence (15.7%, $p < 0.0001$) than in the healthy subjects (1.9%). JCV DNA was also found in all the CSF collected from the 11 PML patients (data not shown).

Only 68 peripheral blood samples from HIV+ patients were analyzed for the presence of BKV DNA, because the nucleic acid extracted from peripheral blood was not sufficient to perform BKV Q-PCR for the remaining 17 samples. It was observed that BKV was the most frequent detected human polyomavirus among both HIV+ patients and healthy subjects, with a prevalence of 25% (17 out of 68) and of 11.4% (12 out of 105), respectively. Among NDLE patients and NND patients, one was infected with both BKV and JCV. Both viruses were detected in all the subgroups of HIV+ patients without any significant difference (Table 2, panels A and B). BKV DNA was not detected in any of the CSF samples (data not shown).

As shown in table 2 (panels A and B), on the whole, the prevalence of the human polyomaviruses genomes among the HIV+ populations (43.4%) was much higher ($p < 0.0001$) than among the healthy subjects (18.1%). In addition, the LPV distribution in both populations appeared to be slightly lower (5.8%) than that of JCV (7.9%) and BKV (16.8%).

4.3 Age dependent prevalence of human polyomaviruses in peripheral blood

When the viral presence was compared in the different age groups of healthy subjects, it was found that the rate of polyomaviruses slightly increased with increasing age, from 13.6% (3 out of 22) in the youngest's group (aged 0–9 years) to 33.3% (7 out of 21) in the oldest's group (aged over 70 years). In regard to the HIV+ patients, the rate of polyomaviruses did not differ with increasing age (data not shown), however the range of age in this group was too narrow (22–58 years) to draw any conclusion.

4.4 Viral load

The LPV median viral load was 1.41E+01 copies/ug of extracted DNA among the HIV+ patients and 4.70E+01 copies/ug of extracted DNA among the healthy subjects. The median viral loads of BKV and JCV did not significantly differ from the LPV median values among both the HIV+ population and the healthy subjects population (figure 2).

5. Discussion

We have investigated the presence of LPV genome in different clinical specimens from immunocompromised and immunocompetent individuals and we have compared it with that of two well known human polyomaviruses (JCV and BKV), since, to date, the percentage of the human population exposed to LPV has not been determined.

A recent study has demonstrated that LPV seroprevalence in a wide human population was about 15% in both adult and pediatric subjects, but no evidence has been shown regarding the presence of the viral genome in biological samples ⁵.

The published reports about the detection of LPV DNA in the humans have shown contrasting results. Delbue and colleagues have observed, by means of nested-PCR, the presence of LPV genome in 6% of the studied populations (3 out of 50 peripheral blood samples), whereas, using a less sensitive standard PCR, Focosi and colleagues, whose case study included 2 peripheral blood and 7 CSF/brain tissues samples, did not find any positive samples ¹⁶.

In contrast to our previous study ⁶, in the current survey we could detect LPV genome not only in the biological samples from immunocompromised patients, but also in those from healthy subjects. The employment of the new designed Q-PCR, that has an higher sensitivity than the previously used nested-PCR, may account for the different results obtained. Thus, it could be possible that all the previously published data were underestimated and, consequently, an association between LPV and HIV-related leukoencephalopathies cannot be confirmed.

In addition, LPV was not detected in the studied CSF, confirming its strict lymphotropism¹ and indicating that probably the virus is not directly involved in the pathogenesis of neurological diseases of immunocompromised patients ¹⁶.

The presence of polyomaviruses in the peripheral blood from immunocompromised patients was confirmed to be higher than that of healthy subjects^{17,18,19} and the overall prevalence of BKV DNA was confirmed to be higher if compared to that of JCV DNA ²⁰.

It is not surprising that LPV DNA was detected with a lower prevalence than that of the other known human polyomaviruses, since LPV was characterized by a lower seroprevalence in humans than JCV and BKV ⁵. In addition, a wide distribution of LPV in the human population was not expected, because LPV has been so far considered a virus with a very narrow host range, limited to the primates.

In contrast with recent seroepidemiologic data ²¹, it could be observed that increasing age may not be a decisive and significant factor accounting for the presence of polyomaviruses DNA in the peripheral blood, since the observed differences in the prevalence were not significant among the groups of age.

To our knowledge, this is the first study that investigated the presence of LPV in a broad cohort of subjects and compared LPV distribution with that of JCV and BKV. This study demonstrates that LPV is present in peripheral blood from both immunocompromised and immunocompetent individuals, but not in the CSF collected from HIV+ patients with neurological diseases. Thus, these results are important because they support the serological evidence of LPV infection in

the human population and reinforce the notion that some human viruses have eluded detection despite many years of virological research.

It is now clear that not all virus infections lead to illness and that confirmation of a virus association to a disease is difficult. However, since viruses are probably pathogens, their identification represents an urgent scientific task. It has been suggested that, rather than a single detection, longitudinal studies are essential to confirm causation.

In conclusion, our results do not support an association between LPV infection and the development of HIV-related leukoencephalopathies, but, given the high similarity between LPV and the other human polyomaviruses, JCV and BKV, it is possible that LPV could establish latency in humans, causing disease only in rare circumstances. Therefore, searching for a disease associated with LPV in humans would be challenging and could have important pathogenic implications.

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Abbreviations

LPV	Lymphotropic Polyomavirus
BKV	BK Virus
JCV	JC Virus
HIV	Human Immunodeficiency Virus
CNS	Central Nervous System
PML	Progressive Multifocal Leukoencephalopathy
PVAN	PolyomaVirus Associated Nephropathy
NDLE	Not Determined Leukoencephalopathy
OND	Other Neurological Diseases
NND	Not Neurological Disorders
CSF	CerebroSpinal Fluid
Q-PCR	real-time PCR

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A

B

C

Fig.1.

Charts of the standard calibration curves of the Q-PCR assays for JCV, BKV and LPV (panels A, B and C). The slopes are -3.445 , -3.408 and -3.453 respectively whereas the correlation coefficients (R^2) are 0.999 for the three curves, showing the high efficiency of the three assays.

Figure 2.
Median Viral load of the human polyomaviruses genomes in the peripheral blood from HIV+ patients and from healthy subjects

Table 1

Primers and probes used for the detection of LPV, JCV and BKV in peripheral blood from HIV+ patients and healthy subjects

VIRUS	TARGET REGION	PRIMERS SEQUENCE	PROBE SEQUENCE
JCV	LT-Ag	Forward: GAGTGTGGGATCCTGTGTTTC Reverse: GAGAAAGTGGGATGAAGACCTGTTT	6-FAM- TCATCACTGGCAAAACATTCTTCATGGC-MGB
BKV	VP1	Forward: AGTGGATGGGCAGCCTATGTA Reverse: TCATAATCTGGGTCCCCTGGA	6-FAM-AGGTAGAAGAGGTAGGGTGTGTTGATGGCACAG-TAMRA
LPV	VP1	Forward: TGGCCCTCAAAGAAAAAGG Reverse: GCGGGAATAGGGCATGTTT	6-FAM-AGACGGAGCATGCAAA-MGB

Table 2

panel A: Prevalence of LPV, JCV and BKV DNA sequences in peripheral blood from HIV+ patients, divided on the basis of the neurological disease.
 Panel B: Prevalence of LPV, JCV and BKV DNA sequences in peripheral blood from healthy subjects, divided into ages groups.

A	HIV+ PATIENTS				
	PML	NDLE	OND	NND	TOTAL
LPV	1/11 (9.1%)	2/16 (12.5%)	0/11	3/45 [#] (6.7%)	6/83 (7.2%)
JCV	2/11 (18.2%)	2/16 [*] (12.5%)	3/11 (27.2%)	6/45 [*] (13.3%)	13/83 (15.7%)
BKV	1/7 (14.2%)	3/11 [*] (27.3%)	2/5 (40%)	11/45 ^{*#} (24.4%)	17/68 (25%)
TOTAL	4/11 (36.4%)	7/16 (43.7%)	5/11 (45.4%)	20/45 (44.4%)	36/83 (43.4%)
B	HEALTHY SUBJECTS				
	< 9	9 – 19	20 – 49	50 – 69	> 70
LPV	1/22 (4.5%)	0/17	1/26 (3.8%)	1/19 (5.2%)	2/21 (9.5%)
JCV	0/22	0/17	0/26	0/19	2/21 (9.5%)
BKV	2/22 (9.1%)	1/17 (5.8%)	3/26 (11.5%)	3/19 (15.7%)	3/21 (14.3%)
TOTAL	3/22 (13.6%)	1/17 (5.8%)	4/26 (15.38%)	4/19 (21.05%)	7/21 (33.3%)

* : one patient had a double infection JCV+BKV;

: two patients had a double infection LPV+BKV