Mutation in the VP-1 Gene Is Responsible for the Extended Host Range of a Monkey B-Lymphotrophic Papovavirus Mutant Capable of Growing in T-Lymphoblastoid Cells

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African green monkey B-lymphotropic papovavirus (LPV), isolated from and capable of growing only in B-lymphoblastoid cells (18), has a number of characteristics common to polyomaviruses. Antiserum against sodium decyl sulfate-disrupted simian virus 40 virions reacts with LPV-infected cells (16, 19), and, as in simian virus 40, three virion proteins (VP-1, VP-2, and VP-3) and a large T antigen have been identified (1, 14). The replication of the closed circular 5.1-kilobase genome DNA (16) initiates at a specific origin, and the resulting replication forks proceed bidirectionally at the same rate (7). The genomic organization, deduced from the location of the replication origin (4, 7) and the partial DNA homology to simian virus 40 DNA (7), is similar to those of the other polyomaviruses. Hamster embryo cells can be transformed in vitro by LPV (17). The prevalence of antibody against African green monkey LPV among primates suggests the existence of related, as yet unidentified, primate LPVs (2, 16, 19). Further investigations are necessary to assess the pathogenicity of LPV in primates.

Despite features shared with other polyomaviruses, LPV has a special host range. LPV grows only in some of the continuous lines of B-lymphoblastoid cells, for example, lines derived from human malignant B-lymphomas (BJA-B [8] and T1-1 [5]) and Epstein-Barr virus-transformed B-lymphocytes of human and African green monkey origins (3, 16, 19). LPV did not grow in any of the T-lymphoblastoid cell lines tested (3, 16, 19). Recently, an LPV mutant (LPV-76) capable of growing in both B-lymphoblastoid cells (BJA-B) and T-lymphoblastoid cells (MOLT-4 [15]) has been isolated from extrachromosomal LPV genomes in LPV-transformed hamster cells (6).

From the analyses of the constructed recombinant viruses between wild-type LPV and LPV-76, the mutation responsible for the extended host range of LPV-76 has been located within the PstI-B segment, which constitutes 46% of the 5.1-kilobase LPV DNA and includes the VP-1 and VP-3 coding regions and the C-terminal portions of VP-2 and

![Diagram](http://jvi.asm.org/ on August 27, 2017 by guest)

FIG. 1. Physical and functional maps of LPV DNA. Restriction sites and open reading frames, indicated by thick arrows with orientation of 5' to 3' direction of mRNA, are based on the total nucleotide sequence of the wild-type LPV-02 of 5,102 base pairs (unpublished data). The circular DNA has been cut open at one of the HaeIII sites at the center of the symmetrical region of the DNA replication origin, which has been arbitrarily designated as nucleotide 0 (4). From nucleotide 0, numbering increases toward the early genome and decreases with minus sign toward the late genome (4). The orientation of the maps here has been chosen so that the sequence (Fig. 2) can readily be correlated to the late mRNA. The coding regions were well conserved, except for some single-base substitutions in the VP-1 gene (Fig. 2). The transcriptional control region is variable in structure between the two wild-type viruses (4, 12). The PstI-BamHI A1 and A2 fragments of LPV-76 are shorter than the counterparts of LPV-02, but these changes are not related to the altered host range of LPV-76 (6).

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FIG. 2. Nucleotide sequences of PstI-B segments of LPV-02 and LPV-76. For mutant LPV-76, only the nucleotides differing from those of wild-type LPV-02 are shown and the identical nucleotides are indicated by dashes. The sequence represents the 5′ strand of LPV DNA and has the same polarity as the late mRNA in the 5′ to 3′ direction. Nucleotides are numbered by the system of Furuno et al. (4) as in Fig. 1. Small solid-line boxes indicate the initiation codons (at nucleotides −728 to −730 for VP-3, and at −1,320 to −1,322 for VP-1) and the stop codons (at −1,439 to −1,441 for VP-2 and VP-3, at −2,424 to −2,426 for VP-1, and at −2,490 to −2,492 for large T). The nucleotide marked with a star in the LPV-02 sequence indicates a single-base substitution in the LPV-K38 sequence (12); A for G at −1,570. T for G at −2,158, C for A at −2,391, A for C at −2,411, G for T at −2,438, and G for A at −2,488. The first G at −683 and the last A at −3,036 correspond to nucleotides 1,015 and 3,368, respectively, in the numbering system for K38 (12).
large-T antigen genes (Fig. 1) (6). In this study we compared the nucleotide sequences of PsI-B segments from LPV-76 and wild-type virus LPV-02 (17) to find the gene determining the tropism for B- and T-lymphoblastoid cells.

The nucleotide sequences of PsI-B segments of LPV-02 and LPV-76 are shown in Fig. 2. PsI-B fragments (2.4 kilobases) were isolated from recombinant plasmids (pBR322 and LPV DNAs) pL02 (17) and pL76 (6) after cleavage with PsI and were digested further with appropriate restriction endonucleases (Alul, BstNI, DdeI, HaeIII, HinfI, Sau3A1, and Stul). The DNA fragments with appropriate sizes were cloned into bacteriophage M13mplO and M13mpl11 (9) (Amersham International plc, Buckinghamshire, England). All of the enzymes used were obtained from Takara Shuzo Co., Ltd., Kyoto, Japan; Bethesda Research Laboratories, Inc., Gaithersburg, Md.; or New England Biolabs, Inc., Beverly, Mass., and were used as recommended by the suppliers. The nucleotide sequences of the cloned DNA fragments were determined by the dideoxy method (13) under conditions previously described (10) and were aligned to the reported nucleotide sequence of another wild-type LPV K38 (12). In the LPV-76 PsI-B segment, single-base substitutions were found at the five sites indicated in Fig. 2.

The nucleotide changes of LPV-76 in the functional map of LPV are shown in Fig. 3. The nucleotide substitution at nucleotide −2,488 was outside any coding region. The other four substitutions were in the VP-1 coding region. Three of them caused amino acid substitutions: Phe for Ser at nucleotide −1,540, Ser for Tyr at nucleotide −1,738, and Ser for Asn at nucleotide −2,158. How these amino acid substitutions affect the conformation of VP-1, and whether or not all the three changes are necessary for alteration of host range remain to be investigated. Still, the data strongly suggest that a change in VP-1 is responsible for the extension of LPV-76 host range to T-lymphoblastoid cells (MOLT-4).

A previous study of ours has shown that the LPV-76 VP-1 migrates more slowly than the VP-1 of LPV-02 in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (6). The molecular masses of VP-1 estimated from the electrophoretic mobilities are 41 kilodaltons for LPV-02 and 42 kilodaltons for LPV-76 (6). The molecular masses of VP-1 calculated from the deduced amino acid sequences are 39,883 for LPV-02 and 39,926 for LPV-76. Apparently, the difference between the estimated molecular masses does not agree with that between the calculated ones. Perhaps the electrophoretic mobility of LPV-76 VP-1 was decreased by the unidentified structural change caused by the three amino acid substitutions rather than by the slightly increased molecular mass.

Comparison of the nucleotide sequences within the PsI-B segments of LPV-K38 (12), LPV-02, and LPV-76 shows that the base mismatches were in either the VP-1 coding region or the noncoding region between the VP-1 and T antigen genes. The sequences were fully conserved in the coding regions for VP-2, VP-3, and large-T antigen. In the VP-1 genes of LPV-02 and LPV-K38 were three amino acid substitutions, which apparently did not alter the host range, since the two LPVs could grow only in B-lymphoblastoid cells. Other variable regions within LPV genome were the enhancer-promoter and the unique region of the small-T antigen gene (unpublished data). Variation of host-discriminating viral enhancer sequences (11) and virion proteins are likely important in the evolution and adaptation of polyomaviruses in
nature. The extent and nature of changes of VP-1 allowing LPV to survive are unclear.

The data obtained in this study imply that the major capsid protein VP-1 of LPV determines its tropism for B- and T-lymphoblastoid cells and that its special host range is restricted at the level of virus adsorption (and penetration). In addition, the host range of LPV may be affected by its enhancer sequence. The LPV enhancer functions well only in hematopoietic cells including both B- and T-lymphoblastoid cells (11). If the subtle structural change of VP-1 induced by amino acid substitutions successfully complements variation of the surface of various lymphoblastoid cells, the virus penetrates them and then may initiate a productive infection in them. Thus, the tropism of LPV appears to be determined at two levels, at adsorption-penetration-uncoating and at transcription, by its unique structures of capsid protein and viral enhancer, respectively.

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