Biological and Biochemical Studies of African Green Monkey Lymphotropic Papovavirus

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The growth of African green monkey lymphotropic papovavirus (LPV) in human lymphoblastoid cell line BJA-B was found to be slow and inefficient due to the accumulation of defective particles. An analysis of molecularly cloned LPV DNAs showed that 3 of 19 clones had DNAs that were longer (5.1 kilobases) than the DNAs of the other clones. The 5.1-kilobase DNA was infectious for BJA-B cells, whereas the shorter (4.8-kilobase) molecules were defective. Unlike the wild-type virus, stocks of LPV made from cloned, infectious DNAs were homogeneous and had higher titers. Using stocks of nondefective LPV, we investigated other biological properties. LPV replication in another human B-lymphoblastoid cell line was observed. The virus did not cause tumors when it was inoculated into newborn hamsters. Serological surveys of human and nonhuman primate sera indicated that virtually all primates, including humans, show evidence of infection by viruses antigenically related to LPV.

The isolation of a newly recognized papovavirus from a B-lymphoblastoid cell line derived from an African green monkey was reported by zur Hausen and Gissmann (28). Electron microscopic observations of the virions, together with the reactivity of lymphotropic papovavirus (LPV)-infected cells with antiserum prepared against disrupted simian virus 40 (SV40) virions, proved that this virus was a member of the *Polyomavirus* genus.

LPV is an interesting virus from a number of standpoints. Thus far, replication of LPV has been limited to continuous lines of B lymphoblasts of monkey or human origin; therefore, it has been referred to as a B-lymphotropic papovavirus (3, 4). Serological studies (3) have shown that about 30% of the humans examined have antibody to LPV, thus providing strong evidence for the existence of a similar virus in humans.

We made similar observations on the prevalence of antibody to LPV in humans; furthermore, an extensive serological survey of other primates indicated the widespread occurrence of related viruses in many other monkeys and apes. These and other studies on the biological and biochemical characterization of LPV are described here.

MATERIALS AND METHODS

Virus and antiserum. Early-passage LPV was obtained from H. zur Hausen, Institute for Virology, Freiburg, Germany. Initially, virus growth was assayed by indirect immunofluorescence, using a broad-

ly reacting anti-SV40 hamster serum which reacted against cells infected with all members of the *Polyomavirus* genus. In later experiments, specific anti-LPV serum made in hamsters was used.

Cells and medium. Human BJA-B cells, a B-lymphoblastoid line (14), were obtained from C. Yee, National Cancer Institute, Bethesda, Md. The cells were grown in RPMI 1640 medium supplemented with 5% newborn calf serum and antibiotics. After infection with LPV, the cells were maintained in the same medium containing 2% newborn calf serum.

FA procedure. Fluorescent antibody (FA) tests were performed as follows. Infected cells were centrifuged at 2,500 rpm for 5 min. The pelleted cells were suspended in a small volume of phosphate-buffered saline (pH 7.2) and smeared onto cover slips. The air-dried cover slips were then fixed in acetone-methanol (1:1) for 3 min and dried. The indirect FA procedure with fluorescein-conjugated goat anti-hamster immunoglobulin was used.

Preparation of LPV DNA. Viral DNA was extracted from infected BJA-B cells by the method of Hirt (12). Supercoiled form I DNA was separated from viral form II DNA and cellular DNA by the ethidium bromide buoyant density method (20).

Cleavage of LPV DNA with restriction endonucleases. Restriction endonucleases *EcoRI*, *BamHI*, *HindIII*, *HincII*, *HhaI*, *HpaII*, *PstI*, and *XbaI* were purchased from Bethesda Research Laboratories, Inc., Rockville, Md., and reactions were carried out according to the instructions of the manufacturer. The undigested and digested DNA samples were adjusted to 8% sucrose and subjected to electrophoresis in horizontal agarose slab gels. The running buffer was either Tris-borate buffer (pH 8.2; 89 mM Tris, 89 mM boric acid, 2.5 mM EDTA) or E buffer (pH 7.9; 40 mM Tris-acetate, 5 mM sodium acetate, 1 mM EDTA). DNA bands were stained with ethidium bromide (0.5 µg/ml

for 30 min) and photographed on UV transilluminator. SV40 DNA (*EcoRI*, *HindIII*, *HinfI*, *HaeIII*, *PstI*, and *HhaI* digests), BK virus (BKV) DNA (*BamHI*, *HindIII*, *BglII*, and *PvuII* digests), and λ DNA (*HindIII* digests) were used for size comparisons. The sizes of the LPV DNA fragments were determined mainly with reference to SV40 DNA (5,226 base pairs) and its fragments, the sizes of which were based on previously compiled data (5), and to BKV DNA (5,196 base pairs) and its fragments (27).

Molecular cloning and preparation of recombinant DNA. The procedure used was essentially the same as the procedure of Israel et al. (13). A mixture of LPV DNA and bacterial plasmid pBR322 DNA (2) was digested with *BamHI*. The linear DNAs were incubated at 12°C for 12 h with T4 ligase (Bethesda Research Laboratories) in a solution containing 66 mM Tris-hydrochloride (pH 7.6), 6.6 mM MgCl₂, 0.4 mM ATP, and 10 mM dithiothreitol. The product of the ligation reaction was used to transform *Escherichia coli* K-12 strain HB101 bacteria under the conditions described by Cohen et al. (7), and ampicillin-resistant and tetracycline-sensitive transformants were selected. Selected colonies were grown in 10 ml of L broth, and plasmid DNA was isolated by the rapid isolation method (8). Colonies which contained plasmid DNA with the recombinant size (LPV DNA plus pBR322 DNA) were then selected. For further characterization, plasmid DNA was prepared from chloramphenicol-amplified bacterial cultures (6). Form I DNA was extracted by a modification of the clear lysate procedure (10) and was purified by the dye buoyant density method (20). To isolate linear LPV DNA, the recombinant DNA was cleaved with *BamHI* and subjected to electrophoresis in low-melting-point agarose (Bethesda Research Laboratories) slab gels. The agarose was removed from DNA bands by phenol extraction according to the instructions of the manufacturer.

Assay of infectious LPV DNA. BJA-B cells were infected by the DEAE-dextran method (16), with LPV DNA obtained after cleavage of the plasmid DNAs with *BamHI*. Infected cells were dried on cover slips, fixed in acetone-methanol (1:1), and examined by the indirect FA procedure.

RESULTS

Initial observations on LPV infection of BJA-B cells. In early passages of LPV in BJA-B cells, only a few positive cells were detected 5 days after infection, and the number of positive cells increased very slowly thereafter. After 2 weeks, approximately 10% of the cells were positive for viral antigen. The cells were centrifuged at 2,500 rpm for 5 min, and supernatant fluids were used to infect fresh BJA-B cells. The number of positive cells did not increase appreciably through five passages, but at the sixth passage the percentage of antigen-positive cells increased to about 35% by 8 days. Further passages did not result in a higher percentage of positive cells, and most of the cells appeared to be normal. Similar observations have been reported recently by Brade et al. (4).

Characterization of LPV DNA. The slow

growth of LPV in BJA-B cells suggested the possibility that defective virus particles in the population were interfering with viral replication. Therefore, we performed experiments to analyze the DNA of LPV. Viral DNA was extracted from infected cells and subjected to electrophoresis in agarose slab gels. A small amount of LPV DNA moved slightly slower than SV40 form I DNA, but the majority of the LPV molecules migrated much faster than SV40 DNA (data not shown). These results indicated marked heterogeneity of the DNA of wild-type LPV. Similar results have been reported by Brade et al. (4).

LPV DNA appeared to be cleaved once and linearized after digestion with *BamHI* (data not shown). Cleavage of two samples of LPV DNA prepared at different passage levels with *BamHI* did not indicate that *BamHI*-resistant molecules were present. Digestion with *HindIII* showed that some of the viral DNA had two *HindIII* sites and some had only one site (data not shown). LPV DNA was not cleaved with *EcoRI*.

Molecular cloning of LPV DNA. Since the experiments described above showed that the LPV stock contained a heterogeneous population, which probably accounted for the delayed growth of the virus in BJA-B cells, the viral DNA was molecularly cloned by inserting *BamHI*-cleaved linear DNA into plasmid pBR322 at the *BamHI* site. This was done to characterize the various species of LPV DNA and, in addition, to test for the infectivity of cloned DNA.

Of 24 tetracycline-sensitive colonies, 19 contained recombinant plasmids which released linear viral DNA after digestion with *BamHI*. The released linear LPV DNA consisted of three different sizes. Three colonies had recombinant plasmids (pL02, pL16, and pL24) which contained the longest viral DNA insertions (about 5.1 kilobases [kb]) (Fig. 1). This DNA had two *HindIII* sites (Fig. 2, lane 3). A total of 15 colonies had the shortest DNA, which was cleaved once with *HindIII* (data not shown). Cleavage of these 15 recombinant DNAs containing shorter LPV DNA insertions with *BamHI* and *HindIII* (alone or combined) indicated that these LPV-cloned DNAs were similar to each other (data not shown). One representative DNA (pL04) released 4.8-kb LPV DNA upon digestion with *BamHI* (Fig. 1, lane 6). There was a third recombinant plasmid class (pL05), which contained an LPV DNA insertion whose size was intermediate between the sizes of the two classes described above. This LPV DNA had two *HindIII* sites (data not shown).

Infectivity tests of cloned LPV DNA. Two representative recombinant DNAs from the longest (pL02) and shortest (pL06) DNAs were

FIG. 1. Electrophoresis of linear LPV DNA. Linear LPV DNA was released from recombinant plasmids pL02 and pL04 by digestion with *Bam*HI. Electrophoresis was done in 0.6% agarose in E buffer at 100 V for 3 h. pJC(1-4) DNA is pBR322 plasmid containing a JCV(Mad-1) DNA insertion at a *Bam*HI site and was a generous gift from P. Howley, National Cancer Institute, Bethesda, Md. Lane 1, *Hind*III digests of λ DNA; lane 2, *Bam*HI cleaved BKV DNA; lane 3, *Eco*RI cleaved SV40 DNA; lane 4, *Bam*HI-digested pL02 DNA (linear LPV and linear pBR322 DNAs); lane 5, *Bam*HI-digested pJC(1-4) DNA (linear JCV and linear pBR322 DNAs); lane 6, *Bam*HI-digested pL04 DNA (linear LPV and linear pBR322 DNAs); lane 7, *Hha*I digests of SV40 DNA (the smaller, 490-base pair fragment ran off the gel and is not shown); lane 8, *Pvu*II digests of BKV DNA. SV40 and pBR322 DNAs are 5.23 (5) and 4.36 (23) kb long, respectively.

cleaved with *Bam*HI and tested for infectivity with BJA-B cells. BJA-B cells were centrifuged and suspended at a concentration of 10^6 cells per ml in serum-free RPMI 1640 medium containing 100 μ g of DEAE-dextran per ml. Viral DNA (1 to 2 μ g/ 10^6 cells) was added to the cells, and the preparation was shaken and allowed to incubate at room temperature for 30 min. The cells were then centrifuged and suspended in RPMI 1640 medium containing 2% serum. At 3 days after infection, low numbers of FA-positive cells were detected in the cells infected with pL02 DNA; the cells infected with pL06 DNA were negative. The number of positive cells in the culture infected with pL02 viral DNA continued to increase, and at 12 days after infection more

than 50% of the cells stained for viral antigen. The culture infected with pL06 DNA remained negative throughout this period. The culture infected with pL02 DNA which contained more than 50% positive cells was centrifuged, and the supernatant fluid was removed for further testing. Infection of fresh BJA-B cells with this stock of virus (designated LPV-02) resulted in the appearance of virus-positive cells much sooner, and a higher percentage of positive cells were detected. Infection of 10^6 BJA-B cells per ml with undiluted LPV-02 resulted in detection of positive cells as early as 2 days; by day 6 most of the cells were positive, and cytopathic effects were observed.

Viral DNA was extracted from infected cells

FIG. 2. Comparison of nondefective LPV DNA isolated from recombinant plasmid pL02 and LPV DNA extracted from BJA-B cells infected with a nondefective LPV-02 stock. pL02 DNA was digested with *Bam*HI and subjected to preparative electrophoresis, and *Bam*HI cleaved LPV DNA was isolated from gels. Electrophoresis was performed in 1.2% agarose slab gels in Tris-borate buffer at 100 V for 160 min. The difference in the mobilities of the reference DNA fragments in gels A and B was due to the difference in room temperature when electrophoresis was done. Lane 1, BKV DNA digested with *Hind*III; lane 2, λ DNA digested with *Hind*III; lane 3, *Bam*HI linear LPV DNA (from pL02) digested with *Hind*III; lane 4, BKV DNA digested with *Hind*III; lane 5, λ DNA digested with *Hind*III; lane 6, circular LPV DNA (from infected cells) digested with *Hind*III; lane 7, circular LPV DNA (from infected cells) digested with *Bam*HI and *Hind*III. The arrows indicate the positions of three LPV DNA fragments generated by *Bam*HI and *Hind*III digestion.

TABLE 1. Sizes of LPV DNA fragments generated by restriction endonucleases

Enzyme(s)	Fragment	Length (kb) ^a
<i>Hind</i> III	A	3.98
	B	1.10
<i>Pst</i> I	A	2.75
	B	2.35
<i>Hha</i> I	A	3.90
	B	1.16
<i>Xba</i> I	A	3.15
	B	1.95
<i>Hpa</i> II	A	2.07
	B	1.59
	C	0.78
	D	0.62
<i>Hind</i> III + <i>Bam</i> HI	A	3.99
	B1	0.78
	B2	0.32
<i>Pst</i> I + <i>Bam</i> HI	A1	1.70
	A2	1.04
<i>Hha</i> I + <i>Bam</i> HI	B	2.36
	A	3.89
	B1	0.59
<i>Xba</i> I + <i>Bam</i> HI	B2	0.59
	A	3.13
	B1	1.04
<i>Hpa</i> II + <i>Bam</i> HI	B2	0.92
	A	2.07
	B1	1.07
	B2	0.51
<i>Hinc</i> II + <i>Bam</i> HI	C	0.78
	D	0.62
	A	3.76
	B	1.33

^a The sizes of the fragments were estimated from the mobilities of DNA bands in 0.6, 1.2, or 1.4% agarose (depending on the size of the DNA to be determined) slab gels, compared with the mobilities of SV40 DNA, BKV DNA, and the fragments of these DNAs (4, 27).

and compared with the LPV DNA isolated from recombinant pL02. The cleavage pattern obtained after digestion with *Bam*HI and *Hind*III (Fig. 2, lane 7) was identical to that of cloned LPV DNA (Fig. 2, lane 3). Thus, the LPV-02 stock appeared to be much more homogeneous than the parental wild-type virus. These results showed that the 5.1-kb DNA, which constituted a minor fraction of the DNA extracted from infected cells, was nondefective and that the 4.8-kb DNA was defective.

Physical map of nondefective LPV DNA. We constructed a restriction endonuclease cleavage map of LPV DNA by using two different DNA samples, the linear DNA isolated from recombinant plasmid pL02 and form I DNA extracted from BJA-B cells infected with nondefective LPV-02 stock. Table 1 summarizes the sizes of the DNA fragments generated by *Hind*III, *Pst*I, *Hha*I, *Xba*I, *Hpa*II, *Hinc*II, and *Bam*HI digestions. From these results and those shown in

TABLE 2. Susceptibilities of human T, B, and null cells to LPV infection

Cells ^a	Type	% Positive ^b	Reference
Ball-1	B	3	18
WIL-2	B	0	1
TL-1	B	12 ^c	11
HPB-ALL	T	<1	19
MOLT-4	T	2	22
HPB-MLT	T	0	19
CCRF-CEM	T	0	9
HPB-NALL	Null	0	Morikawa ^d
K-562	Null	1	15
NALL-1	Null	0	18

^a CCRF-CEM cells were obtained from the American Type Culture Collection, Rockville, Md. All other cell lines were kindly provided by Yorio Hinuma, Kyoto University, Kyoto, Japan.

^b Percentage of positive cells detected by FA 6 days after infection.

^c At the second passage, 55% of TL-1 cells were positive 9 days after infection.

^d S. Morikawa, unpublished data.

Fig. 1, the nondefective LPV DNA was estimated to be 5.1 kb long. Figure 3 shows the cleavage patterns generated after digestion with the various restriction endonucleases (alone or appropriately combined). From these results, a physical map of nondefective LPV DNA was constructed (Fig. 4). The physical map of the LPV genome determined by Brade et al. (4) is slightly different from our map. This may be due to differences in the methods used to propagate LPV.

Biological studies with nondefective LPV-02. (i) Infectivity assay of LPV-02. An assay was developed as follows. BJA-B cells were dispensed

TABLE 3. LPV antibody in humans and nonhuman primates^a

Serum	No. positive/ no. tested	% Positive
Human	13/49	27
African green monkey	63/130	48
Rhesus monkey	6/63	10
Baboon	0/28	0
Chimpanzee	15/50	30
Gibbon	12/49	24
Gorilla	10/13	77
Orangutang	7/12	58
Marmoset ^b	8/30	27
Owl monkey ^b	2/19	11
Spider monkey ^b	1/12	8
Squirrel monkey ^b	5/14	36

^a All sera were tested by the FA procedure, using LPV-02-infected BJA-B cells.

^b New World monkeys.

into tubes at a concentration of 10^6 cells per ml in RPMI 1640 medium supplemented with 2% serum. The cells were inoculated with 0.5-ml volumes of serial 10-fold dilutions of LPV-02 stock, using two tubes per dilution. The tubes were incubated in an upright position in racks. Since the cells settled to the bottoms of the tubes, the medium was easily removed by careful suction and replaced with fresh medium every 2 days. Beginning on day 4 after infection, after the medium was removed, a small sample of cells was smeared onto cover slips, air dried, fixed in acetone-methanol (1:1), and examined for virus-positive cells by the FA test. At the lower virus dilutions, positive cells were detected as early as 4 days, whereas at the higher

dilutions (from 10^{-3} to 10^{-6}) there was a progressive delay in the appearance of positive cells. The test was ended on days 12 to 14. Based on this assay, the infectious titer of LPV-02 of various stocks was from 10^5 to 10^6 /ml.

(ii) **Tests for susceptibility of other human lymphoblastoid cell lines to LPV-02.** Thus far, the only human lymphoblastoid cell line which has been found to support LPV replication has been the BJA-B cell line. A number of other cell lines were tested by zur Hausen et al. (29) and Brade et al. (4). Although two other cell lines showed a low percentage of positive cells, the infection was abortive. We also tested a number of lymphoblastoid lines which were B, T, or non-B, non-T (null) cells. These cells (kindly provided

FIG. 3. Cleavage of nondefective LPV DNA with restriction endonucleases. For alignment of the cleavage sites with various enzymes, LPV DNA was digested with two enzymes simultaneously or consecutively and subjected to electrophoresis in a 1.4% (A and C) or 1.2% (B) agarose slab gel in E buffer (A and C) or Tris-borate buffer (B) at 100 V for 100 min (A), 140 min (B), or 130 min (C). Digestion of LPV DNA with *HpaII* was often incomplete. Partial digests (fragments A and D) formed a band behind the fragment A band, and the amount of these digests varied from experiment to experiment (lanes 6, 13, and 16). Lane 1, SV40 DNA digested with *PstI*; lane 2, LPV DNA digested with *PstI*; lane 3, LPV DNA digested with *PstI* plus *HindIII*; lane 4, LPV DNA digested with *HindIII*; lane 5, LPV DNA digested with *HindIII* plus *HpaII*; lane 6, LPV DNA digested with *HpaII*; lane 7, LPV DNA digested with *HpaII* plus *PstI*; lane 8, BKV DNA digested with *HindIII*; lane 9, SV40 DNA digested with *HaeIII*; lane 10, λ DNA digested with *HindIII*; lane 11, LPV DNA digested with *BamHI* plus *HindIII*; lane 12, LPV DNA digested with *HpaII* plus *HincII*; lane 13, LPV DNA digested with *HpaII*; lane 14, SV40 DNA digested with *HindIII*; lane 15, SV40 DNA digested with *HinfI*; lane 16, LPV DNA digested with *HpaII*; lane 17, LPV DNA digested with *HpaII* plus *HhaI*; lane 18, LPV DNA digested with *HpaII* plus *XbaI*; lane 19, SV40 DNA digested with *HindIII*.

FIG. 4. Physical maps of LPV DNA. Circular LPV DNA was opened at its unique *Bam*HI site. The maps were constructed from the data in Table 1 and Fig. 3.

by Yorio Hinuma, Kyoto University, Kyoto, Japan) were tested with the LPV-02 pool whose titer was 10^6 /ml. For each cell line, 10^6 cells were infected with 1 ml of undiluted virus. At different times after infection, the cells were tested for viral antigen by the FA test. Table 2 lists the cells which were tested and the percentage of positive cells 6 days after infection. Although 5 of the 10 lines showed varying degrees of susceptibility to LPV, only the B-lymphoblastoid line TL-1 showed a fairly high degree of sensitivity. That LPV was replicating and producing infectious virus was proven by the fact that a second passage resulted in the induction of a high percentage of positive cells (55%) in TL-1 cells. These results show that most human T, B, or null cells are resistant to LPV and that the BJA-B and TL-1 cell lines are unusual.

(iii) **Hemagglutination tests with LPV.** Several members of the *Polyomavirus* genus have the capacity to agglutinate erythrocytes. This is a useful property which can be utilized as a simple and rapid method for roughly estimating the amount of virus and as a sensitive method for measuring antibody to the virus. Therefore, erythrocytes from a number of different species of animals were tested for agglutination by LPV. These included human type O, guinea pig, rat, mouse, rhesus monkey, chicken, sheep, rabbit,

horse, and goose erythrocytes. The tests were performed by using phosphate-buffered saline (pH 7.2), and the virus-erythrocyte mixtures were kept at 4°C for 2 h. All tests were negative. Thus, LPV appears to be a non-hemagglutinating virus.

(iv) **Lack of tumor production by LPV in hamsters.** A total of 85 hamsters were inoculated with LPV at birth subcutaneously and intraperitoneally (0.05 ml/animal). All hamsters were observed for 11 months, and no tumors developed. We have also attempted to transform hamster and rat cells, but thus far results have been negative.

(v) **Prevalence of antibody to LPV in primates, including humans.** There is serological evidence that humans are infected with a virus related to LPV (4, 29). We confirmed this and, in addition, examined the sera of other primates for antibody to LPV (Table 3). With the exception of baboons, all primates had antibody to LPV. Both Old World and New World monkeys were positive for LPV antibody. The antibody titers were not exceptionally high, ranging from 1:5 to 1:40. The rhesus and African green monkey sera (obtained from the Bureau of Biologics, Food and Drug Administration, Bethesda, Md.) were from recently caught monkeys; all other sera were from captive animals. It is possible that the virus may spread more readily among captive

animals, which would account for the high percentage of positive sera in gorillas and orangutangs.

Based on these experiments, it is apparent that virtually all primates, including humans, are infected with a virus closely related or identical to the African green monkey LPV.

DISCUSSION

There are now six primate polyomaviruses; they are BKV and JC virus (JCV) (human), SV40 and stump-tailed macaque virus (STMV) (macaque), SA12 (baboon), and LPV (African green monkey). Although LPV shares the common antigenic determinant of all members of the polyoma virus genus, it is immunologically distinct from other members of the group. The T antigens of the primate viruses BKV, JCV, SV40, STMV, and SA12 are immunologically related (21, 24–26). However, the LPV T antigen appears to be different from the T antigens of the other primate viruses since anti-T sera against SV40, BKV, and JCV T antigens failed to react against BJA-B cells infected with LPV, which presumably were synthesizing T antigens (28; unpublished data). Definitive proof of a unique T antigen of LPV must await the production of LPV tumor serum.

The narrow host range of polyomaviruses is well known. LPV, the newest member of the group, displays an even greater restriction since it replicates only in dividing B-lymphoblastoid cells. Nondividing primary lymphocytes appear to be resistant to LPV infection. We have demonstrated that besides BJA-B cells, another B cell line (TL-1) supports the growth of LPV. It is interesting that only certain B cells are susceptible to LPV infection; most of the B, T, and null cells are resistant.

We have shown that the original stock of LPV grown in BJA-B cells contained heterogeneous DNA molecules, the majority of which were probably defective. Similar findings have been reported by Brade et al. (4). We have reported previously (17) that human polyoma virus JCV DNA was heterogeneous after adaptation to growth in human embryonic kidney cells. These results suggest that certain classes of defective mutants may have a selective advantage during the process of adaptation to growth in different cells.

There is compelling evidence that humans and many nonhuman primates are infected with a polyomavirus closely related to the African green monkey LPV. We have confirmed the reports of zur Hausen et al. (29) and Brade et al. (3) that approximately 25 to 30% of all humans tested have antibody to LPV which can be detected by the FA test. This may be an underestimate of the percentage of the human popula-

tion with LPV antibody since the FA test is not the most sensitive test for antibody detection. The evidence is conclusive that the antibody which is found in human sera is directed against LPV antigens since positive serum neutralizes LPV infectivity and also reacts against a 40,000-dalton protein in LPV-infected cells, which is probably major viral protein VP-1 (3). Thus, besides BKV and JCV, humans are infected with a third polyomavirus, which is still to be discovered.

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LITERATURE CITED

1. Alspaugh, M. A., F. C. Jensen, H. Rabin, and E. M. Tan. 1978. Lymphocytes transformed by Epstein-Barr virus. Induction of nuclear antigen reactive with antibody in rheumatoid arthritis. *J. Exp. Med.* 147:1018–1027.
2. Bolivar, F., R. L. Rodriguez, P. J. Greene, M. C. Betlach, H. L. Heyneker, H. W. Boyer, J. H. Cross, and S. Falkow. 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. *Gene* 2:95–113.
3. Brade, L., N. Muller-Lantzsch, and H. zur Hausen. 1980. B-lymphotropic papovavirus and possibility of infections in humans. *J. Med. Virol.* 6:301–308.
4. Brade, L., W. Vogl, L. Gissmann, and H. zur Hausen. 1981. Propagation of B-lymphotropic papovavirus (LPV) in human B-lymphoma cells and characterization of its DNA. *Virology* 114:228–235.
5. Buchman, A. R., L. Burnett, and P. Berg. 1980. The SV40 nucleotide sequence, p. 799–829. In J. Tooze (ed.), *Molecular biology of tumor viruses*, 2nd ed., part 2. DNA tumor viruses. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
6. Clewell, D. B. 1972. Nature of Col E₁ plasmid replication in *Escherichia coli* in the presence of chloramphenicol. *J. Bacteriol.* 110:667–676.
7. Cohen, S. N., A. C. Y. Chang, and L. Hsu. 1972. Nonchromosomal antibiotic resistance in bacteria: genetic transformation of *Escherichia coli* by R-factor DNA. *Proc. Natl. Acad. Sci. U.S.A.* 69:2110–2114.
8. Davis, R. W., M. Thomas, J. Cameron, T. P. St. John, S. Scherer, and R. A. Padgett. 1980. Rapid DNA isolations for enzymatic and hybridization analysis. *Methods Enzymol.* 65:404–411.
9. Foley, G. E., H. Lazarus, S. Farber, G. G. Uzman, B. A. Boone, and R. E. McCarthy. 1965. Continuous culture of human lymphoblasts from peripheral blood of a child with acute leukemia. *Cancer* 18:522–529.
10. Guerry, P., D. J. LeBlanc, and S. Falkow. 1973. General method for the isolation of plasmid deoxyribonucleic acid. *J. Bacteriol.* 116:1064–1066.
11. Hayashi, Y., Y. Matsumura, T. Nishihira, I. Watanabe, R. Ohi, M. Kasai, K. Kumagai, and N. Kamada. 1980. Burkitt's lymphoma cell line bearing surface IgA and negative for nuclear antigen of Epstein-Barr virus (EBNA). *Jpn. J. Exp. Med.* 50:423–434.
12. Hirt, B. 1967. Selective extraction of polyoma DNA from infected mouse cell cultures. *J. Mol. Biol.* 26:365–369.
13. Israel, M. A., H. W. Chan, W. P. Rowe, and M. A.

- Martin. 1979. Molecular cloning of polyoma virus DNA in *Escherichia coli*: plasmid vector system. *Science* 203:883-887.
14. Klein, G., T. Lindahl, M. Jondal, W. Leibold, J. Menezes, K. Nilsson, and C. Sundstrom. 1974. Continuous lymphoid cell lines with characteristics of B cell (bone-marrow derived), lacking the Epstein-Barr virus genome and derived from three human lymphomas. *Proc. Natl. Acad. Sci. U.S.A.* 71:3283-3286.
 15. Klein, G., J. Zeuthen, I. Eriksson, P. Terasaki, M. Bernoco, A. Rosen, G. Masucci, S. Poverly, and R. Ber. 1980. Hybridization of a myeloid leukemia-derived human cell line (K562) with a human Burkitt's lymphoma line (P3Hr-1). *J. Natl. Cancer Inst.* 64:725-738.
 16. McCutchan, J. H., and J. S. Pagano. 1968. Enhancement of the infectivity of simian virus 40 deoxyribonucleic acid with diethylaminoethyl-dextran. *J. Natl. Cancer Inst.* 41:351-357.
 17. Miyamura, I., K. Yoshike, and K. K. Takemoto. 1980. Characterization of JC papovavirus adapted to growth in human embryonic kidney cells. *J. Virol.* 35:498-504.
 18. Miyoshi, I., S. Hiraki, T. Tsubota, I. Kubonishi, Y. Matsuda, T. Nakayama, H. Kishimoto, and I. Kimura. 1977. Human B cell, T cell, and null cell leukaemic cell lines derived from acute lymphoblastic leukaemias. *Nature (London)* 267:843-844.
 19. Morikawa, S., E. Tatsumi, T. Harada, and K. Yasuhira. 1978. Two E-rosette-forming lymphoid cell lines. *Int. J. Cancer* 21:166-170.
 20. Radloff, R., W. Bauer, and J. Vinograd. 1967. A dye-buoyant-density method for the detection and isolation of closed circular duplex DNA: the closed circular DNA in HeLa cells. *Proc. Natl. Acad. Sci. U.S.A.* 57:1514-1521.
 21. Reissig, M., T. J. Kelly, Jr., R. W. Daniel, S. R. S. Rangan, and K. V. Shah. 1976. Identification of the stump-tailed macaque virus as a new papovavirus. *Infect. Immun.* 14:225-231.
 22. Srivastava, B. I. S., and J. Minowada. 1973. Terminal deoxynucleotidyl transferase activity in a cell line (MOLT-4) derived from the peripheral blood of a patient with acute lymphoblastic leukemia. *Biochem. Biophys. Res. Commun.* 51:529-535.
 23. Sutcliffe, J. G. 1979. Complete nucleotide sequence of the *Escherichia coli* plasmid pBR322. *Cold Spring Harbor Symp. Quant. Biol.* 43:77-90.
 24. Takemoto, K. K., and M. F. Mullarkey. 1973. Human papovavirus, BK strain: biological studies, including antigenic relationship to simian virus 40. *J. Virol.* 12:625-631.
 25. Valls, J. D., N. Newell, M. Reissig, H. Malherbe, V. Kaschula, and K. V. Shah. 1977. Characterization of SA12 as a simian virus 40-related papovavirus of chacma baboon. *Infect. Immun.* 18:247-252.
 26. Walker, D. L., B. L. Padgett, G. M. ZuRhein, A. E. Albert, and R. F. Marsh. 1973. Human papovavirus (JC): induction of brain tumors in hamsters. *Science* 181:674-676.
 27. Yang, R. C. A., and R. Wu. 1979. BK virus DNA: complete nucleotide sequence of a human tumor virus. *Science* 206:456-461.
 28. zur Hausen, H., and L. Glissmann. 1979. Lymphotropic papovaviruses isolated from African green monkey and human cells. *Med. Microbiol. Immunol.* 167:137-153.
 29. zur Hausen, H., L. Glissmann, A. Mincheva, and J. F. Bocker. 1980. Characterization of a lymphotropic papovavirus, p. 365-373. *In* M. Essex, G. Todaro, and H. zur Hausen (ed.), *Viruses in naturally occurring cancer*, vol. 7a. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.