Novel Polyomavirus Detected in the Feces of a Chimpanzee by Nested Broad-Spectrum PCR

Reimar Johne,¹* Dirk Enderlein,¹ Hermann Nieper,² and Hermann Müller¹

Institute for Virology, Faculty of Veterinary Medicine, University of Leipzig,¹ and Landesuntersuchungsanstalt für das Gesundheits und Veterinärwesen Sachsen,² Leipzig, Germany

Received 12 October 2004/Accepted 2 November 2004

In order to screen for new polyomaviruses in samples derived from various animal species, degenerated PCR primer pairs were constructed. By using a nested PCR protocol, the sensitive detection of nine different polyomavirus genomes was demonstrated. The screening of field samples revealed the presence of a new polyomavirus, tentatively designated chimpanzee polyomavirus (ChPyV), in the feces of a juvenile chimpanzee (*Pan troglodytes*). Analysis of the region encoding the major capsid protein VP1 revealed a unique insertion in the EF loop of the protein and showed that ChPyV is a distinct virus related to the monkey polyomavirus B-lymphotropic polyomavirus and the human polyomavirus JC polyomavirus.

Members of the family Polyomaviridae are nonenveloped icosahedral viruses with a circular double-stranded DNA genome approximately 5,000 bp in size (6). Early genes encode two or three regulatory proteins, designated tumor antigens (T-Ag), and late genes encode the structural proteins VP1, VP2, and VP3 (7). With the exception of avian polyomavirus (APV) (14), polyomaviruses are highly species specific (6). Until now, 13 polyomaviruses infecting humans, monkeys, cattle, rabbits, rats, mice, hamsters, geese, and various bird species have been identified (6, 15). Most mammalian polyomaviruses cause subclinical infections with lifelong persistence in their natural nonimmunocompromised hosts (20), whereas polyomaviruses of birds are causative agents of acute disease with high mortality rates (22, 23, 26). The inoculation of mammalian polyomaviruses into newborn laboratory rodents induces multiple-tumor growth (7, 16, 35). It is still a matter of debate whether polyomaviruses of animals can be transmitted to humans and thereafter cause disease (5, 11, 29, 37).

The monkey polyomaviruses simian virus 40 (SV40), B-lymphotropic polyomavirus (LPyV), simian agent 12 (8), and baboon polyomavirus 2 (12) and bovine polyomavirus were originally identified as contaminants of tissue cultures (7). An unrecognized contamination of rhesus monkey kidney cell cultures used for the production of the Salk poliovirus vaccine from 1955 to 1963 lead to the exposure of an estimated 100 million people to SV40 (11). To avoid further risk of infection with unidentified polyomaviruses and to investigate their involvements in disease, broad-spectrum PCRs for the detection of thus far unknown polyomaviruses were established in this study.

For the identification of conserved regions, the genome sequences of 10 polyomaviruses (9, 10, 15, 17, 21, 24, 25, 27, 28, 32) were aligned and 12 primers (Table 1) with binding sites within short regions with high similarity were constructed. Three different nested broad-spectrum PCRs were performed with a PTC-200 Peltier thermal cycler (MJ Research; contributed by Bio-Rad, Munich, Germany) using 100 pmol of primers and 2.5 U of *Taq* DNA polymerase with buffer Y (PeqLab, Erlangen, Germany) in 50- μ l reaction mixtures. The optimized cycling protocol included 5 min of incubation at 95°C, followed by 45 cycles each of 94°C for 30 s, 46°C for 1 min and 72°C for 1 min, and 72°C for 5 min. For nested PCR, 4 μ l of the first

TABLE 1. Oligonucleotides used in polyomavirus broad-spectrum PCRs

PCR	Primer designation	Sequence ^{a} (5'-3')	Product length (bp)
VP1 specific			
1st PCR	VP1-lf	CCAGACCCAACTARRAATGA RAA	829–1137
	VP1-1r	AACAAGAGACACAAATNTTT CCNCC	
Nested PCR	VP1-2f	ATGAAAATGGGGTTGGCCCN CTNTGYAARG	249–273
	VP1-2r	CCCTCATAAACCCGAACYTC YTCHACYTG	
VP3-VP1 specific			
1st PCR	VP3-1f	CTCCAGGAGGTGCAMABC AAMG	562-631
	VP3-1r	TGCAATTCCAGAGGTTCNCC NCCNAC	
Nested PCR	VP3-2f	ACTGGATGCTGCCTYTAMTT YTAGG	394–459
	VP3-2r	TCAGTTTTTACAGTTACWGC YTCCCACAT	
T-Ag specific			
1st PCR	T-1f	GATGTTTCCTTTCTARRTT NAC	536–551
	T-1r	GCAAAGATCAAAAAAGCATH TGYCA	
Nested PCR	T-2f	AAATGATCTCTCAAGTTATC NARRTT	208–235
	T-2r	AAAGGTCCAGTTAATAGTGG NAARAC	

^{*a*} B,C+G+T; H,A+C+T; M,A+C; N,A+C+G+T; R,A+G; W,A+T; Y,C+T.

^{*} Corresponding author. Mailing address: Institute for Virology, Faculty of Veterinary Medicine, University of Leipzig, An den Tierkliniken 29, D-04103 Leipzig, Germany. Phone: 49(0)341-9738204. Fax: 49(0)341-9738219. E-mail: johne@vetmed.uni-leipzig.de.



FIG. 1. Testing the VP1-specific nested broad-spectrum PCR (A) and the ChPyV-specific PCR (B) with different polyomavirus genomes. Genomic DNA of SV40, BKPyV, JCPyV, LPyV, MPyV, MPtV, HaPyV, goose hemorrhagic polyomavirus (GHPV), and APV was excised from plasmids and circularized by use of T4 DNA ligase. For the ChPyV plasmid, DNA carrying the cloned secondary PCR product of sample 12 was used. A total of 1 ng of DNA was used as the template for each PCR. PCR products were separated on ethidium bromide-stained 2% agarose gels. (–) Ctrl, negative control; M, DNA ladder mix (Fermentas, Vilnius, Lithuania).

PCR product was used as the template in a similar reaction at 95°C for 5 min, 45 cycles of 94°C for 30 s, 56°C for 30 s and 72°C for 30 s, and 72°C for 5 min. PCR products were visualized by ethidium bromide-stained 2% agarose gel electrophoresis.

The primer combinations were tested with genomic DNA of SV40, BK polyomavirus (BKPyV), JC polyomavirus (JCPyV), LPyV, murine polyomavirus (MPyV), mouse pneumotropic polyomavirus (MPtV), hamster polyomavirus (HaPyV), goose hemorrhagic polyomavirus, and APV that was rescued from plasmids and circularized by using 3 U of T4 DNA ligase (Promega, Madison, Wis.) in an overnight reaction at 16°C. A total of 1 ng of DNA was used as the template for the PCR. In all cases, secondary PCR products of the expected lengths were amplified with each of the three protocols (data not shown). The highest intensity of specific bands, however, was obtained with the VP1-specific protocol (Fig. 1A).

When this protocol was applied to 17 samples derived from different animal species with various clinical problems (data not shown), a specific PCR product was detected only in the case of sample 12 (Fig. 2A). This sample was derived from the feces of a juvenile chimpanzee (*Pan troglodytes*) that had severe diarrhea, previously tested positive for rotaviruses and *Salmonella* spp., and was kept in captivity with two other chimpanzees The amount of polyomavirus DNA in the sample was small, as a specific band was detected only after nested PCR but not after the first or the second PCR alone (Fig. 2B). After the cloning and sequencing of the PCR product, a similarity search with BLAST 2.1.3 (1) revealed a relationship with but no identity to VP1-encoding sequences of polyomaviruses. The suspected new virus was designated chimpanzee polyomavirus (ChPyV).



FIG. 2. Detection of DNA sequences of a new polyomavirus in clinical sample 12 derived from a chimpanzee. (A) PCR products of seven field samples (#11 to #17), SV40-infected Vero cells (SV-40), and the negative control [(-) Ctrl] were separated on ethidium bromide-stained 2% agarose gels. (B) Analysis of products amplified with primers VP1-1f and VP1-1r (1st PCR) and primers VP1-2f and VP1-2r (2nd PCR) with single PCR protocols or with the first PCR followed by the second PCR in a nested PCR protocol by ethidium bromide-stained 2% agarose gel electrophoresis. The template DNA was derived from sample 12, SV40-infected Vero cells (SV-40), or the negative control [(-)-Ctrl]. M, DNA ladder mix (Fermentas). (C) Phylogenetic relationships of the new polyomavirus (ChPyV) with 10 other polyomaviruses, based on the nucleotide sequences of the whole VP1-encoding region, aligned by the ClustalW method.

B	
M	Consens us
MAP TKRKGS CPGAAP KKPKEP VQVPRL VIKGGI EVLGVK TGVDSF TEVECF LNPQMGNPD EHQKGLS KSLA	SV-40
MAP TKRKGE RKDP VQVPKL LIRGGV EVLEVK TGVDSI TEVECF LTPEMG DPD EHLRGFS KSIS	JCPYV
MAP TKRKGE CPGAAP KKPKEP VQVPKI.LIKGGV EVI.EVK TGVDAI TEVECF I.NPENG DPD EN I.RGFS I.KI.S	вкруу
MAP QRKRQD GACK KTCPIP APVPRL LVKGGV EVLEVK TGPDAI TQIEAY LNPKNGNN-IPS ED LYGIS NSIN	LPYV
MSR MIKKNMIN PERKGLAGQE SEVERL TINGGI EVIGUE IGEDSI TITELE LINERAIQESTESETIGIS DAQA	вруч
MAP VVKKT SQNQGLSP QKSQNS VVVGGI QVLDVK TGPDSI TQIEAF LNPKNG APV DS DFIGFS DNIT MAP VVKKT SQNQGLSP QKSQNS VVVGGI QVLDVK TGPDSI TQIEAF LNPKNG ADETDE SITERCOVCOVC GAS GAS	MPUV
MAR KRKSOV SRCETA	H e Dury
MAP KKNSGA SSKCAN PFFFCA PPCPKP ANVENT IMKGGE EVI NV IGEDSTITTERVI NVENIG UNFFKEGIGT DGF QTTGES QSTK MSN KKKSS	h aryv A pv
MAP KVKRPR N GFN UVERLIVKGGI EVLGVE TGPDST TTTEAY INPRMG TNN	GHPV
MAD BEKEAR CUSTET KUKCUE KKOUD TEURKI. LUKGU FUINIT TOEDST TETELY LEDEMA IN-SET OKKEWYGYS FUIH	ChPvV
	0.111
C S LP. N. T. MWEA TE. C H. C E C H.	Consens us
AEK OFTDDS PDKDOL PCNSVA RIPLPN LNEDLT CGNILM WEAVTV KTEVIG VTAMIN LHSGTO KTHENG -AGKPICG SNFH	S V-40
TSD TFESDS PURDML PCNSVA RTPLPN INEDLT CONTIM WEAVTL KTEVIG VTSLMN VHSNGO ATHONG -AGKPVCG TSFH	JCPVV
AEN DESSDS PERKML PCYSTA RIPLPN LNEDLT CGNLLM WEAVTY OTEVIG ITSMIN LHAGSO KVHEHG -GGKPICG SNEH	BKPVV
TAF SKASDT PNKDTL PCYSVA VIKLPL LNEDMT CDTILM WEAVSV KTEVVG ISSLVN LHQGGK YIYGSS -SG CVPVG5 TTYH	LPyV
GST SRTDED LISAEL PRYSLG VVQLPL LNEKLT DDVLLM WEAVSC KTEVVG VNTLTT CHGYKK RYSPSA GQGS AMPIEG INYH	BPyV
VSA DYTODM PRIKEL PCYSMA KISLPM LNEDMT CDTILM WEAISC KTEVVG VSSLTN CHSAVK RLYDNE GAG FPVCG LNFH	MPtV
LAT SDTDDS PGNNTL PTWSMA KLOLPM LNEDLT CDTLOM WEAVSV KTEVVG SGSLID VHGFNK FTDTVN TKGI STPVEG SQYH	MPyV
VNS SLTADE VKANQL PYYSMA KIQLPT LNEDLT CDTLQM WEAVSV KTEVVG VGSLIN VHGYGS RSETKD - IGI SKPVEG TTYH	Ha₽yV
VQA EGYQDA PHSTEV PCYSCA RIPLPT INDDIT CPTLLM WEAVSV KTEVVG VSSIIN MHSGAF RAFNGY GGGFTICG PRIH	APV
VAT SLNPDV PPKAEL PCYSCA RIGLPM LNEDMT TPEILM WEAVSV KTEVVG VTTMON VHSASI RMNGGY GVGRPIES LNCH	GHPV
HAD GYDNNL LN-IOM POYSCA RVOLPM LNTDMT CDTLMM WEAVSC KTEIVG IGSLI <mark>S VHLLEA KMAAKE -GGDGP SOPIE</mark> G MNYH	ChPyV
ा स	
.F. \\$G. L. Q Y	Consens us
FFA VGGEPL ELQGVL ANYRTK Y PAQTVTP KN ATVDS QQMNTD HKAVLD KDNAYP VECWVP DPSKNE NTRY	SV-40
FFS VSGEAL ELQGVL FNYRTK Y POTIFP KN ATVQS QVMNTE HKAYLD KNKAYP VECWVP DPTRNE NTRY	Ј СРУV
FFA VSGEPL ENQGVL MNYRSK Y PDGTITP KN PTAQS QVMNTD HKAYLD KNNAYP VECWVP DPSRNE NARY	в круи
MFA VGGHPL ELQGLV ASSTAT Y PDDVVAI KN MKPGN QGLDPK AKPLLD KDGNYP VEVWCP DPSKNE NTRY	LPyV
FFA VGGEPL ELQFIC EDFKAP YHPT ETIVPP KDKLSNKS QVLDPT LKGILD KDGVYP VECWCP DPSKNE NTRY	B P Y V
FFS VGGEAL DLQWLW KNYRCN Y PAGVAAL QA APKAA QVLDPK LKAKLT ADGKFP IEAWSP DPAKNE NTRY	MPtV
VFA VGGEPL DLQGLV TDARTK YKE EGVVTI KTITKK DMVNKD QVLNPI SKAKLD KDGMYP VEIWHP DPAKNE NTRY	MPYV
MFA VGGEPL DLQGLV QNYNAN Y EAAIVSI KTVTGK AMTSTN QVLDPT AKAKLD KDGRYP IEIWGP DPSKNE NGRY	НаРуV
FFS VGGEPL DLQACM QNSKTV Y PAPLIGP GEG ERRETA QVLDTG YKARLD KDGLYP IECWCP DPAKNE NTRY	A PV
MFA VGGEPL ELQGCV QNWSTT Y P SGVVAP PL KDAKA QVLDPG LKARLD KDGAYP VECWCP DPSRNE NTRY	GHPV
MFA V <mark>GGEPL DLOGIE SNALTK YASAIP PKTIHP NDIAKL AEEEKPOL OGLVPK AKARLD KDGFYP IEEWSP GPSRNE N</mark> SRY	Ch₽yV
H I	
	Consens us
FGT YTGGEN VPPVLH ITNTAT TVLLDE OGVGPL CKADSL YVSAVD IGLFT NTS-GT OQWKGL PRYFKI TLRRRS VKNPYP ISFL	SV-40
FGT LIGGEN VEPVLATINTATIVELDE FGVGEL CAUDAL LEGAND VGMET ANS 35 QQWAGL SKIFAV QLEARAKA VANDEP LSEL	J CPYV
FOT FTGGEN VEPVLH VINTAT IVLEDE OGVOEL CKADSE IVSAAD IGELFT NSS - ST QUWKEL AKTEKI KERKS VINNEPT ISEL	BKPYV
TRAFTOGAL TERVING TINST I VILLE NOVOEL CREDIN ELSCAD TAGVITINTS EL QUE LA LINE TRANCI UNIVER SAL	L F Y V
FOT ITGOVS TEVELO TINITI TILLEDE NOVOEL CKADAL ITTAAD IQE LI QAN DU QUEKOL EKIMSV TEKKE VANPIP IASI EGY VIGGI OTDOVLO TINITI TILLEDE NOVOEL CKADAL VI SON TILGED ONLA MUNICI DEVEST UIDKG. CANDVU VISI	BPYV MD+V
TGT FIGGET A PPULO FINITI TVILLE NGVGEL CKGEGV VISCUD FIGTKI TRIMA VHMRGL PRYFKI TLERKW VKNPVP MASI.	MPVV
YON FTOGTE TO PAY THIS TO LLDE NOVOEL CKODEL YISAD VNGWY FYNGAG WHWRGI DRYPNY TI RKWW YKNDYD WTSI.	HaPvV
IGN LIGGPE TPEVLA FINITI TILLE NGVGEL (KGDGL FLSAD VAGTY DOR - GROYNGL PRYSI OLRKIN VRNPY VSGL	APV
PGT YTGGOO TPDVI D FTNTVT TVI I DF NGVGDI. CKGDGI. YI SCUD I CGFYS FOYSOK OHFRGI DEVESV SLEKRI. VENDVD VSSI.	GHPV
FGS FVGGIN TPPNLO FTNAVT TVILLE NGVGEL (KGDGI, FVSAAD I GSVMV KADNEA IRYRGI, SRYFKV TLRKRA VKNPYP ITSI.	ChPvV
	· ···· · ·
	Consens us
LSD LINRRT ORVDGO PMIGMS SOVEEV RVYEDT EELPGD PDMIRY IDEF-G OTTTRM O	SV-40
LTD LINRRT PRVDGO PMYGMD AOVEEV RVFEGT EELPGD PDMMRY VDKY-G OLOTKM L	JCPvV
LSD LINRRT QRVDGQ FMYGME SQVEEV RVFDGT ERLPGD PDMIRY IDKQ-G QLQTKM L	BKPyV
LNT FFSGLM POIORO PIERVS GOVEEV RIFOGT EGLPGD PDLNRY VDKF-C OHOTVL PVSNDM	LPVV
LTS LFTNSL PPVTSQ EMD KQVEEV RIYQGV EGLPGD PDMVRY INKF-G QEETCI PK	BPyV
LNT FSSEMM PLNSWM LOVEEV RIYDGV ERLPGD PDMIRY RIIWPG RLLSLI FPAMRH KHLYFF VMOAFT VI.	MPtV
ISS LFNNML PQVQQQ PMEGEN TQVEEV RVYDGT EPVPGD PDMTRY VDRF-G KTKTVP FGN	MPyV
LAS LYNNML PTIEGQ PMEGEA AQVEEV RIYEGT EAVPGD PDVNRF IDKY-G QQHTKP PAKPAN	HaPyV
LNS LFNDLM PRMTGQ SMQGSD AQVEEV RVYEGM EGLAPE IDMPPK APR	APV
LTS LFTSII PKMQGQ PMVGDN SQVEEV RVYEGV EPLPGD PDISRF VDRF-G EQHTHM PSN	GHPV
LGS LFTGLM PKMDGQ PMTGPD AQVEEV RIYQGK EGLPAD PDMKRY IDQF-G QEQTPT PTPAAP AA	C hPyV

FIG. 3. Alignment of the deduced amino acid sequences of the VP1 of 11 polyomaviruses. Letters in the consensus sequence (in italics) indicate amino acids that are identical in all sequences; periods indicate positions where amino acids are not identical. Regions of VP1 forming outer loops as determined from the crystal structure of SV40 are boxed and designated BC, DE, EF, and HI loops according to reference 19.

Based on this sequence, a PCR amplifying a 195-bp fragment of the ChPyV VP1 gene was developed by using primers ChPyV-s (5'-TTTCAGCTGCTGATATCTGTGGT-3') and ChPyV-as (5'-TCTGGGCCTGTCATAGGTTGTC-3'). The cycling profile was 95°C for 5 min, 40 cycles each of 95°C for 30 s, 60°C for 30 s, and 72°C for 30 s, and finally 72°C for 5 min. Only the cloned ChPyV sequence, and no DNA of nine other polyomaviruses (Fig. 1B), was amplified by this PCR. Testing fecal samples from the group of chimpanzees mentioned above, derived at different time points, showed that this PCR reacted positively with only one chimpanzee during the period of diarrhea (data not shown). One hundred microliters of a dilution (1:10 in phosphatebuffered saline) of sample 12 was inoculated onto subconfluent monolayer cell cultures of human (293T and HeLa), monkey (MA104, MARC-145, and Vero), and canine (MDCK) origins, respectively. After 1 h at 37°C, the mixture was removed and the respective medium was added. After 6 days at 37°C and three cycles of freezing and thawing, cellular debris was removed by centrifugation and fresh cultures were infected with the supernatant. After five passages and an observation period of 6 weeks, however, no cytopathic changes were observed. A ChPyV-specific PCR with DNA isolated from cells from the fifth passage remained negative (data not shown).

Virus was concentrated from the supernatant of a suspension of sample 12 by ultracentrifugation. DNA extracted from the resulting pellet was analyzed by the VP3-VP1-specific and T-Ag-specific nested broad-spectrum PCRs, respectively. A polyomavirus-specific product was amplified with the VP3-VP1-specific PCR, whereas the T-Ag-specific protocol revealed only unspecific products. Primers 5'-GTTAAATGGC GCCTCCCAGGAAAAG-3' (delineated from the VP3-VP1specific product) and ChPyV-as amplified the 5'-region of the VP1 gene. Primers ChPyV-s and 5'-GTTTCCCAGTAGGTC TCNAATAAA-3' (containing the putative polyadenylation signal for the early mRNA, which is italicized here) amplified the 3' region of the VP1 gene. These PCRs were performed using the FastStart high-fidelity PCR kit (Roche, Mannheim, Germany). Although many other primers were tested in different combinations, no more polyomavirus-specific PCR products could be amplified from the sample.

Analysis of the assembled complete VP1-encoding nucleotide sequence (GenBank accession no. AY691168) was performed using MegAlign (DNASTAR, Madison, Wis.) with the ClustalW method (34). The lowest percentage of identity (55.8%) was found in comparisons to MPtV and MPyV, whereas LPyV and JCPyV showed the highest percentages of identity (60.5 and 61%, respectively). Phylogenetic trees established using the ClustalW (Fig. 2C), ClustalV, or Jotun Hein method consistently showed that ChPyV branches between LPyV and a group formed by SV40, BKPyV, and JCPyV.

On the deduced amino acid (aa) level (Fig. 3), the highest sequence variability is found within the BC, DE, EF, and HI loops forming the outer surface of the viral particle (19) and in the N- and C-terminal parts of VP1. The outer loops are involved in receptor binding (31) and carry antigenic epitopes together with the C-terminal part of VP1 (3, 13, 30). Phylogenetic analysis shows that these regions of ChPyV VP1 are distinct from corresponding regions of all other polyomaviruses, whereas the remaining part of ChPyV VP1 is closely related to that of LPyV (data not shown). With 395 aa residues, VP1 of ChPyV is the largest among those of the polyomaviruses, mainly due to an insertion of about 10 aa within the EF loop. This loop is located mainly at the vertices of VP1 pentamers and contributes to capsid stability by the binding of calcium ions but also extends to the outer surface of the viral capsid. A putative nuclear localization signal (RKR, aa 5 to 7) and a putative calcium binding domain (E172, D175, and D372) can be deduced from the ChPvV VP1 sequence.

Broad-spectrum PCRs were successfully utilized for the detection of new viruses, e.g., herpesviruses (36) and papillomaviruses (2). A broad-spectrum PCR was also developed for the detection of polyomaviruses (39); however, by testing 32 human tumor samples and 14 cell lines, no polyomavirus-specific sequences were amplified (38). In our investigation, nested PCR protocols developed to increase sensitivity proved to be essential, as none of the single PCRs alone was capable of detecting ChPyV DNA in sample 12. In further studies, this PCR should be applied to the screening of animal and human samples or tissue culture-derived products for polyomaviruses.

Using this approach, DNA of a novel polyomavirus was detected. Attempts to isolate virus from this sample failed, most probably due to the small amounts of virus, the poor quality of the sample, or a nonpermissive tissue culture system. ChPyV DNA could also be present in the feces of the chimpanzee due to nonproductively infected inflammatory cells which invaded the gastrointestinal tissue; hence, no infectious virus could be isolated. It remains unclear whether ChPyV infection may cause disease in chimpanzees. A distinct clinical picture could not be linked to infection with LPyV (4, 18, 33), which turned out to be most closely related to ChPyV. Further investigations should record the prevalence of ChPyV infections in chimpanzees with and without disease. With regard to the close relationship between chimpanzees and humans, the screening of human samples for ChPyV would be of special interest.

We thank Klaus Eulenberger (Zoo Leipzig) for assistance in the sampling of zoo animals, Kristina Dörries (University of Würzburg) for providing the cloned BKPyV, JCPyV, and MPtV genomes, Jitka Forstova (University of Prague) for providing the cloned MPyV genome, Michael Pawlita (German Cancer Research Center, Heidelberg, Germany) for providing the cloned LPyV genome, and Rainer Ulrich (Humboldt-University of Berlin) for providing the cloned HaPyV genome.

This work was funded by a grant from the Deutsche Forschungsgemeinschaft (JO 369/3–1).

REFERENCES

- Altschul, S. F., T. L. Madden, A. A. Schäffer, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25:3389–3402.
- Antonsson, A., and B. G. Hansson. 2002. Healthy skin of many animal species harbors papillomaviruses which are closely related to their human counterparts. J. Virol. 76:12537–12542.
- Babe, L. M., K. Brew, S. E. Matsuura, and W. A. Scott. 1989. Epitopes on the major capsid protein of simian virus 40. J. Biol. Chem. 264:2665–2671.
- Brade, L., N. Muller-Lantzsch, and H. zur Hausen. 1981. B-lymphotropic papovavirus and possibility of infections in humans. J. Med. Virol. 6:301–308.
- Carbone, M., H. I. Pass, L. Miele, and M. Bocchetta. 2003. New developments about the association of SV40 with human mesothelioma. Oncogene 22:5173–5180.
- Carstans, E. B., M. K. Estes, S. M. Lemon, J. Maniloff, M. A. Mayo, D. J. McGeoch, C. R. Pringle, and R. B. Wickner. 2000. Polyomaviridae, p. 241– 246. *In* M. H. V. van Regenmortel, C. M. Fauquet, and H. L. Bishop (ed.), Virus taxonomy. Academic Press, London, United Kingdom.
- Cole, C. N., and S. D. Conzen. 2001. Polyomaviridae: the viruses and their replication, p. 2141–2174. *In* D. M. Knipe and P. M. Howley (ed.), Fields virology. Lippincott-Raven Publishers, Philadelphia, Pa.
- Cunningham, T. P., and J. M. Pipas. 1985. Simian agent 12 is a BK virus-like papovavirus which replicates in monkey cells. J. Virol. 54:483–492.
- Delmas, V., C. Bastien, S. Scherneck, and J. Feunteun. 1985. A new member of the polyomavirus family: the hamster papovavirus. Complete nucleotide sequence and transformation properties. EMBO J. 4:1279–1286.
- Frisque, R. J., G. L. Bream, and M. T. Cannella. 1984. Human polyomavirus JC virus genome. J. Virol. 51:458–469.
- Garcea, R. L., and M. J. Imperiale. 2003. Simian virus 40 infection of humans. J. Virol. 77:5039–5045.
- Gardner, S. D., W. A. Knowles, J. F. Hand, and A. A. Porter. 1989. Characterization of a new polyomavirus (Polyomavirus papionis-2) isolated from baboon kidney cell cultures. Arch. Virol. 105:223–233.
- 13. Jin, L., P. E. Gibson, W. A. Knowles, and J. P. Clewley. 1993. BK virus

antigenic variants: sequence analysis within the capsid protein VP1 epitope. J. Med. Virol. **39:**50–56.

- Johne, R., and H. Müller. 1998. Avian polyomavirus in wild birds: genome analysis of isolates from *Falconiformes* and *Psittaciformes*. Arch. Virol. 143: 1501–1512.
- Johne, R., and H. Müller. 2003. The genome of goose hemorrhagic polyomavirus, a new member of the proposed subgenus Avipolyomavirus. Virology 308:291–302.
- Khalili, K., L. Del Valle, J. Otte, M. Weaver, and J. Gordon. 2003. Human neurotropic polyomavirus, JCV, and its role in carcinogenesis. Oncogene 22:5181–5191.
- Lednicky, J. A., A. S. Arrington, A. R. Stewart, X. M. Dai, C. Wong, S. Jafar, M. Murphey-Corb, and J. S. Butel. 1998. Natural isolates of simian virus 40 from immunocompromised monkeys display extensive genetic heterogeneity: new implications for polyomavirus disease. J. Virol. 72:3980–3990.
- Lednicky, J. A., S. J. Halvorson, and J. S. Butel. 2002. PCR detection and DNA sequence analysis of the regulatory region of lymphotropic papovavirus in peripheral blood mononuclear cells of an immunocompromised rhesus macaque. J. Clin. Microbiol. 40:1056–1059.
- Liddington, R. C., Y. Yan, J. Moulai, R. Sahli, T. L. Benjamin, and S. C. Harrison. 1991. Structure of simian virus 40 at 3.8-A resolution. Nature 354:278–284.
- Major, E. O. 2001. Human polyomaviruses, p. 2175–2196. *In* D. M. Knipe and P. M. Howley (ed.), Fields virology. Lippincott-Raven Publishers, Philadelphia, Pa.
- Mayer, M., and K. Dörries. 1991. Nucleotide sequence and genome organization of the murine polyomavirus, Kilham strain. Virology 181:469–480.
- Miksch, K., E. Groβmann, K. Köhler, and R. Johne. 2002. Nachweis des Goose Haemorrhagic Polyomavirus (GHPV) in Beständen mit hämorrhagischer Nephritis und Enteritis der Gänse in Süddeutschland. Berl. Muench. Tieraerztl. Wochenschr. 115:390–394.
- Müller, H., and R. Nitschke. 1986. A polyoma-like virus associated with acute disease of fledgling budgerigars (Melopsittacus undulatus). Med. Microbiol. Immunol. 175:1–13.
- Pawlita, M., A. Clad, and H. zur Hausen. 1985. Complete DNA sequence of lymphotropic papovavirus: prototype of a new species of the polyomavirus genus. Virology 143:196–211.
- Rothwell, V. M., and W. R. Folk. 1983. Comparison of the DNA sequence of the Crawford small-plaque variant of polyomavirus with those of polyomaviruses A2 and strain 3. J. Virol. 48:472–480.
- Sandmeier, H., H. Gerlach, R. Johne, and H. Müller. 1999. Polyomavirusinfektionen bei exotischen Vögeln in der Schweiz. Schweiz. Arch. Tierheilkd. 141:223–229.

- Schuurman, R., C. Sol, and J. van der Noordaa. 1990. The complete nucleotide sequence of bovine polyomavirus. J. Gen. Virol. 71:1723–1735.
- Seif, I., G. Khoury, and R. Dhar. 1979. The genome of human papovavirus BKV. Cell 18:963–977.
- 29. Shivapurkar, N., T. Takahashi, J. Reddy, Y. Zheng, V. Stastny, R. Collins, S. Toyooka, M. Suzuki, G. Parikh, S. Asplund, S. H. Kroft, C. Timmons, R. W. McKenna, Z. Feng, and A. F. Gazdar. 2004. Presence of simian virus 40 DNA sequences in human lymphoid and hematopoietic malignancies and their relationship to aberrant promoter methylation of multiple genes. Cancer Res. 64:3757–3760.
- Siray, H., C. Frommel, T. Voronkova, S. Hahn, W. Arnold, J. Schneider-Mergener, S. Scherneck, and R. Ulrich. 2000. An immunodominant, crossreactive B-cell epitope region is located at the C-terminal part of the hamster polyomavirus major capsid protein VP1. Viral Immunol. 13:533–545.
- Stehle, T., Y. Yan, T. L. Benjamin, and S. C. Harrison. 1994. Structure of murine polyomavirus complexed with an oligosaccharide receptor fragment. Nature 369:160–163.
- 32. Stoll, R., D. Luo, B. Kouwenhoven, G. Hobom, and H. Müller. 1993. Molecular and biological characteristics of avian polyomaviruses: isolates from different species of birds indicate that avian polyomaviruses form a distinct subgenus within the polyomavirus genus. J. Gen. Virol. 74:229–237.
- Takemoto, K. K., A. Furuno, K. Kato, and K. Yoshiike. 1982. Biological and biochemical studies of African green monkey lymphotropic papovavirus. J. Virol. 42:502–509.
- 34. Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22:4673–4680.
- Tognon, M., A. Corallini, F. Martini, M. Negrini, and G. Barbanti-Brodano. 2003. Oncogenic transformation by BK virus and association with human tumors. Oncogene 22:5192–5200.
- VanDevanter, D. R., P. Warrener, L. Bennett, E. R. Schultz, S. Coulter, R. L. Garber, and T. M. Rose. 1996. Detection and analysis of diverse herpesviral species by consensus primer PCR. J. Clin. Microbiol. 34:1666–1671.
- Vilchez, R. A., and J. S. Butel. 2003. SV40 in human brain cancers and non-Hodgkin's lymphoma. Oncogene 22:5164–5172.
- Völter, C., H. Hausen, D. Alber, and E. M. de Villiers. 1997. Screening human tumor samples with a broad-spectrum polymerase chain reaction method for the detection of polyomaviruses. Virology 237:389–396.
- Völter, C., H. zur Hausen, D. Alber, and E. M. de Villiers. 1998. A broad spectrum PCR method for the detection of polyomaviruses and avoidance of contamination by cloning vectors. Dev. Biol. Stand. 94:137–142.